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UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

School of Medicine

Infection, Inflammation and Immunology Division

Epidemiology of Adolescent Asthma: Risk and Prognosis in a Birth
Cohort over Adolescence

By

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Thesis for the degree of Doctor of Philosophy

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ABSTRACT

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES

SCHOOL OF MEDICINE

Doctor of Philosophy

EPIDEMIOLOGY OF ADOLESCENT ASTHMA:
RISK AND PROGNOSIS IN A COHORT FROM BIRTH TO ADOLESCENCE

By Abid Raza

Epidemiologic evidence suggests that a significant proportion of childhood asthma remits during adolescence and that early life environment plays a significant role towards the development of asthma in the adolescent period. Wheeze over adolescence is considered to represent asthma unless proven otherwise.

This thesis explores the impact of early life and adolescent specific factors on adolescent asthma and wheeze, in a prospective birth cohort. Adolescent wheeze was characterised at 18 years. Atopy, physical measures of height, weight, spirometric pulmonary function, bronchodilator response, fractional exhaled nitric oxide (FeNO) and bronchial reactivity were assessed in all those that visited in person, whilst information on wheeze and diagnosed asthma was obtained using validated ISAAC and 1989 Isle Of Wight birth cohort study specific questionnaires from all study participants that consented for the 18 year follow-up. Follow-up of 90% (1313/1456) was achieved at 18 years, with 66 % (864/1313) study participants visiting the research centre. Asthma was defined as having doctor diagnosed asthma with either wheeze or whistling in the chest in the last 12 months or on current use of asthma medications.

We observed prevalence of physician diagnosed asthma increased from the age of 10, with continued significance of atopy retained over the adolescent period. A significant proportion of childhood asthma remitted over the adolescent period fulfilled the criteria for complete remission. Remission was observed to be seen largely favouring male gender with greater improvement in remittent asthmatics with significant improvement of pulmonary function compared to asthmatics observed to be associated with this change. However, there was a lack of net remission of asthma during adolescence due to more adolescents developing asthma over 10 to 18 years; Genetic predisposition of family history and environmental factors of paracetamol use were associated with both wheeze and asthma at 18. We did not find a significant contribution of early life factors for asthma over adolescence. Adolescent environment rather than early life factors are associated with increased risk for adolescent onset asthma suggestive of a true adolescent onset condition in genetically predisposed adolescents.

Non-asthma-wheeze, a distinct wheeze phenotype that lacks physician diagnosis of asthma, was considered to represent undiagnosed asthma. This phenotype is associated with increased tobacco smoke exposure, poor lung function, non-atopy and female gender. A significant proportion of un-diagnosed wheeze is misclassified as adolescent asthma. Whilst Non-asthma-wheeze is largely non-atopic, atopy continues to retain its significance over the adolescent period for asthma. Smoking was observed to be associated with Non-asthma-wheeze but not with asthma.

CONTENTS

Chapter 1: Introduction.....	1
1.1 Asthma.....	1
1.2 Asthma Prevalence: Past and Current Trends	2
1.3 Phenotypes of Asthma.....	7
1.4 Contribution of Asthma and Allergic Cohort Studies	12
1.5 Immuno-Pathology of Atopy.....	20
1.6 Hygiene Hypothesis	21
1.7 How Much of Adolescent Wheeze is Asthma?.....	21
1.8 Concept of Early Life Origins and its Significance for Adolescent Asthma	22
1.9 Atopy and its Significance for Adolescent Asthma.....	22
1.10 Asthma and Rhinitis: One Airway Phenomenon	23
1.11 Risk Factors for Adolescent Asthma.....	24
1.12 Unanswered Questions about Asthma	31
1.13 Summary of the Specific Aims of this Thesis	32
 Chapter 2: Methodology.....	 37
2.1 Study Design.....	37
2.2 Study Centre	37
2.3 Selection of Study Participants.....	37
2.4 Ethics.....	37
2.5 Subject Recruitment.....	38
2.6 Clinical Assessment	38
2.7 Questionnaires	38
2.8 Study Definitions.....	39
2.9 Physical Examination	40
2.10 Laboratory Investigations.....	44
2.11 Data Management.....	46
2.12 Data Cleaning Process	47
2.13 Statistical Methods.....	47
 Chapter 3: Demographics and Characteristics of 18 Year Follow-up.....	 53
3.1 Assessment of 18 Year Participation.....	53
3.2 Socio-economic Status of Cohort at 18 Years	58
3.3 Physical Measures	62
3.4 Spirometry Results	63
3.5 Smoking and Alcohol Consumption	64
3.6 Effect of Social Class on Smoking Pattern.....	67
3.7 Discussion	67

Chapter 4: Characteristics of Undiagnosed Wheeze at 18; is this Asthma?	73
4.1 Introduction.....	73
4.2 Results	74
4.3 Discussion	90
 Chapter 5: Risk Factor Profiles for Asthma and Non-asthmatic-wheeze in 18 Year Olds: Two Distinct Wheeze Illnesses	 97
5.1 Introduction.....	97
5.2 Results	97
5.3 Discussion	108
 Chapter 6: Do Children Grow out of Asthma? Transitions of Asthma over Adolescence.....	 113
6.1 Introduction.....	113
6.2 Results	113
6.3 Discussion	124
 Chapter 7: Remission Compared to Persistent Asthma	 131
7.1 Introduction.....	131
7.2 Results	132
7.3 Discussion	148
 Chapter 8: Early Life versus Adolescent Risk Factors for New Adolescent Onset Asthma	 155
8.1 Introduction.....	155
8.2 Results	156
8.2.1 Comparison of New Adolescent Onset Asthma with Persistent Asthma.....	156
8.3 Anthropometric Measures	160
8.4 Comparison of Lung Function between New Adolescent Onset and Persistent Asthma	161
8.5 Combined Model.....	174
8.6 Discussion	175

Chapter 9: Discussion	181
9.1 Follow-up at 18 Years and Pattern of Changing Gender Prevalence	181
9.2 Non-asthma-Wheeze a Distinct Wheeze Phenotype	181
9.3 Composition of Asthma at Age 18 Years	182
9.4 Prospective Transitions of Childhood Asthma at 10 by 18 Years	182
9.5 Adolescence and Remission of Asthma	182
9.6 Characteristics of Adolescent Onset Asthma	183
9.7 Fundamental Assumptions for Characterising Asthma	183
9.8 Characteristic of Non-asthma Wheeze	184
9.9 Defining Asthma over Adolescence	186
9.10 Example of Impact of Childhood Wheeze Heterogeneity on Asthma over Adolescence	187
9.11 Growth and Remission of Asthma over Adolescence	187
9.12 Lack of Evidence for Role of Obesity	188
9.13 Evidence for the Single Airway Hypothesis	188
9.14 Rhinitis as a Risk Factor for Asthma	188
9.15 Significance of Early Life Factors?	189
9.16 Relevance of Atopy	189
9.17 Tobacco Smoke Exposure and Asthma	191
9.18 Paracetamol and Asthma	192
9.19 Strengths and Limitations	192
9.20 Unanswered Research Questions and the Need for Future Studies	193
References	197
Appendices	214

INDEX OF TABLES

Table 1.1 Classification of Asthma Phenotypes (50)	7
Table 1.2 Summary of the Isle of Wight Birth Cohort	18
Table 1.3 Summary of the Isle of Wight Birth Cohort	19
Table 2.1 Which Subjects will Undergo which Study Procedures	46
Table 3.1a Gender-wise Reasons for Non-enrolment at 18 Years	54
Table 3.1b Gender-wise Reasons for Dropout at 18 Years	54
Table 3.2 Status of Study Enrolment.....	55
Table 3.3 Mode of Data Collection.....	56
Table 3.4 Characteristics of Children Seen at 18 Years by Participation	57
Table 3.5 Characteristics of Adolescents not seen at 18 Years	58
Table 3.6 Type of Residence.....	59
Table 3.7 Social Factors of Accommodation, Involvement in Education and/or Work ..	59
Table 3.8 Family Income	60
Table 3.9 Social class	61
Table 3.10 Adolescent Pet Ownership	62
Table 3.11 Anthropometric Measures	63
Table 3.12 Gender-wise Spirometric Pulmonary Function Measures	64
Table 3.13a Alcohol Consumption	65
Table 3.13b Tobacco Smoking	65
Table 3.14 Current Tobacco Smoke Exposure by Amount of Tobacco Smoked	66
Table 3.15 Smoking by Social Class.....	67
Table 4.1 Characteristics of Wheeze Phenotypes at Age 18 Years.....	75
Table 4.2 Comparison of Clinical Features of Wheeze Phenotypes	76
Table 4.3 Pattern of Symptom Distribution for Asthma	77
Table 4.4 Asthma Treatment at Age 18 Years	78
Table 4.5a Cross-sectional Lung Function and Height at 18 Years	79
Table 4.5b Cross-sectional Lung Function and Height at 10 Years	80
Table 4.6 Gain in Lung Function from 10 to 18 Years of Age	82
Table: 4.7 Additional Lung Function Tests.....	83
Table 4.8 Environmental Exposures and Wheeze Phenotypes	86

Table 5.1 Univariate Analysis of Risk Factors for Asthmatic Wheeze at 18 years (Early Life Factors)	98
Table 5.2 Univariate Analysis of Risk Factors for Asthmatic Wheeze at 18 years (Adolescent Factors).....	99
Table 5.3: Univariate Analysis of Paracetamol/NSAID use for Asthmatic Wheeze at 18 years (Adolescent Factors).....	100
Table 5.4 Univariate Analysis of Risk Factors Non-Asthmatic Wheeze at 18 Years (Early Life Factors)	102
Table 5.5 Univariate Analysis of Risk Factors for Non-Asthmatic Wheeze (Adolescent Factors).....	103
Table 5.6: Univariate Analysis of Paracetamol/NSAID use for Non - Asthmatic Wheeze at 18 years (Adolescent Factors)	104
Table 5.7 Multivariate Analysis of Factors Associated with Asthmatic-Wheeze Compared to Non-wheeze	106
Table 5.8 Multivariate Analysis of Factors Associated with Non-Asthmatic-Wheeze Compared to Non-Wheeze at 18-Years	107
Table 6.1 Asthma Prevalence from Age 4 to 18 Years	116
Table 6.2 Gender Wise Difference in Atopic to Non-atopic Proportions of Asthma	118
Table 6.3 Asthma Transition Phenotypes from Age 4 to 18 Years	120
Table 7.1: Symptoms & Treatment of Asthma at Age 10 Years between Participants whose Asthma Remitted and Persisted between 10 and 18 Years.....	132
Table 7.2 Recognised Triggers as Reported by Study Participants at 10 Year Follow-up by Remission or Persistence of Asthma Over Adolescence	133
Table 7.3 Allergen Sensitization Pattern at 10 Years for Remission In Relationship Persistence of Asthma.....	134
Table 7.4 Allergen Sensitization Pattern at 18 Years for Remission in Relation to Persistence of Asthma.....	135
Table 7.5a Males: Height Adjusted Pulmonary Function from Age 10 to 18 Years by Asthma Remission or Persistence	136
Table 7.5b Female: Height Adjusted Pulmonary Function from Age 10 to 18 Years by Asthma Remission or Persistence	137
Table 7.6a Methacholine Lung Challenge Test, Bronchodilator Reversibility and FeNO for Remission in Relation to Persistence of Asthma	140
Table 7.6b Methacholine Lung Challenge Test, Bronchodilator Reversibility and FeNO	141
Table 7.7 Inflammatory Cell Type from Induced Sputum	142
Table 7.8 Allergic Sensitization, Early Life Wheeze/Asthma, Longitudinal Information on Allergic Diseases Rhinitis and Eczema	143
Table 7.9 Family History of Asthma/Rhinitis Determined at Birth to Age 10.....	144

Table 7.10 Early Life Factors towards Remission of Asthma	145
Table 7.11 Adolescent Factors and Their Relation to Remission of Asthma	146
Table 7.12 Adolescent & Childhood Factors (Final Model).....	147
Table 8.1 Symptoms and Treatment of Asthma between New Adolescent Onset Asthma and Persistent Asthma	156
Table 8.2: Asthma Triggers Comparison; New Adolescent Onset Asthma to Persistent Asthma	157
Table 8.3 Allergen Sensitization Pattern at 10 Years	158
Table 8.4 Allergen Sensitization Pattern at 18 Years	159
Table 8.5 Gender Wise Physical Measure Assessment	160
Table 8.6a Males: Growth Parameters with Height Adjusted Pulmonary Function from Age 10 To 18	162
Table 8.6b Females: Growth Parameters with Height Adjusted Pulmonary Function ..	163
Table 8.7a Additional Lung Function Test at 10 Years.....	164
Table 8.7b Additional Lung Function Tests at 18 Years.....	165
Table 8.8 Inflammatory Cell Type from Sputum Induction	167
Table 8.9 Allergic Sensitization, Early Life Wheeze/Asthma, Longitudinal Information on Allergic Diseases	168
Table 8.10 Family History of Asthma/Rhinitis Determined at Birth	169
Table 8.11 Early Life Factors towards Adolescent Asthma	170
Table 8.12 Adolescent Factors and their Relation to New Adolescent Onset Asthma ..	172
Table 8.13 Multivariate Early Life Model for New Adolescent Onset Asthma	173
Table 8.14 Multivariate Adolescent Model for New Adolescent Onset Asthma	174

INDEX OF FIGURES

Figure 2.1 Overview of 18-year follow up study of 1989/1990 Isle of Wight Cohort	45
Figure 4.1 Triggers for Wheeze Onset	77
Figure 5.1 Time in Years for Wheeze Onset and its Relation to Cigarette Smoking	105
Figure 6.1 Transition of different asthma groups over 10 to 18 years.....	121
Figure 7.1 Bronchial Reactivity for Adolescent Asthma Transition Phenotypes (Remission to Persistent and Never Asthma) At 10 Years	138
Figure 7.2 Bronchial Reactivity for Adolescent Asthma Transition Phenotypes (Remission to Persistent and Never Asthma) At 18 Year	139
Figure 8.1 Bronchial Reactivity at 10 Years in Adolescent Asthma (Adolescent Onset)	166
Figure 8.2 Bronchial Reactivity at 18 Years in Adolescent Asthma (Adolescent Onset)	167

INDEX OF APPENDICES

Appendix 1: Participant Information Sheet, Version 4	214
Appendix 2: Consent Form	220
Appendix 3: Postal Questionnaire Male	222
Appendix 4: Postal Questionnaire Female	224
Appendix 5: ISAAC Questionnaire	226
Appendix 6: Food Allergy Symptom Questionnaire	230
Appendix 7: Asthma Symptom Questionnaire	230
Appendix 8: Rhinoconjunctivitis Symptom Questionnaire	232
Appendix 9: Eczema Symptom Questionnaire	232
Appendix 10: Juniper's Asthma Quality of Life Questionnaire	234
Appendix 11: Additional 17 year questionnaire	234
Appendix 12a: Male Section of (PDS)	236
Appendix 12b: Female Section of (PDS)	236
Appendix 13: Dermatology life quality index	237
Appendix 14: Height and Weight Measurement Protocol	239
Appendix 15: Sputum Processing Protocol	241
Appendix 16: Sputum Induction protocol	243
Appendix 17: Methacholine Test Protocol	245
Appendix 18: R&D Approval Letter	247
Appendix 19: Ethical Committee Approval	249
Appendix 20: Correction of Study Document Numbers Letter from Ethics	251

DECLARATION OF AUTHORSHIP

I, Abid Raza declare that the thesis entitled “Epidemiology of Adolescent Asthma: Risk and Prognosis in a Birth Cohort over Adolescence”

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- I was jointly responsible for collection of data and performing the bulk of the methacholine lung challenge tests, in addition to collecting all the sputum samples at the 18 year follow-up of this birth cohort.
- The data cleaning, analysis and interpretation represents my own work. The National Institutes of Health (USA) paid for my salary and tuition as a Clinical Research Fellow.
- this work was done wholly or mainly while in candidature for a research degree at this University;
- 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;
- where I have consulted the published work of others, this is always clearly attributed;
- where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- 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;
- none of this work has been published before submission

Signed:

DATE:

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ABBREVIATIONS

ACTH	Adreno-corticotrophic hormone
ANOVA	Analysis of variance
APC	Antigen presenting cell
ATS	American Thoracic Society
BAL	broncho-alveolar lavage
BHR	Bronchial hyper-responsiveness
BMI	Basal metabolic index
COPD	Chronic Obstructive Pulmonary Disease
cs IgE	Cord serum immunoglobulin E
DHAARC	David Hide Asthma and Allergy Research Centre
DMHDS	Dunedin Multidisciplinary Health and Development Study
DNA	Deoxy-ribonucleic acid
ECRHS	European Community Respiratory Health Survey
EAACI	European Academy of Allergology and Clinical Immunology
ECP	Eosinophil cationic protein
ELISA	Enzyme-linked immunosorbent assay
FeNO	Fractional exhaled Nitric Oxide
FEF _{25-75%}	Forced expiratory flow 25 – 75 percent
FeNO	Fractional expired Nitric Oxide
FEV ₁	Forced expiratory volume in the first second
FVC	Forced vital capacity
HDM	House dust mite
HPA	Hypothalamic pituitary axis
IDA	Investigator diagnosed asthma
IgE	Immunoglobulin E
IOW	Isle of Wight
ISAAC	International study on asthma and allergy in childhood
NHS	National Health Service
NOCA	New onset childhood asthma
OR	Odds ratio
PC ₂₀	Provocative concentration of a substance (methacholine) causing a 20% fall in FEV1
pbs	phosphate base saline
PDS	Pubertal developmental scale
PEFR	Peak expiratory flow rate
PS	Persistent SPT positivity
RR	Relative risk
RSV	Respiratory Syncytial Virus

SD	Standard Deviation
SPT	Skin Prick Test
TCRS	Tucson Children's Respiratory Study
TGFβ ₁	Transforming growth factor beta 1
TH-1	Type 1, Helper- T Cell
TH-2	Type 2, Helper- T Cell
TNF-α	Tumour necrosis factor alpha
NAW	Non-asthmatic-wheeze

DEDICATION

I would like to thank God almighty for allowing me to complete my project. Confucius once said, "A journey of a thousand miles begins with a single step", I cannot but thank my parents who encouraged me through those early moments in life to what I am today, particularly my father who supported me in all of my endeavours. My work would not be complete if I did not have the care and unflinching backing of my family particularly my wife and sister. It is said that "best friends listen to what you don't say*" and I have been blessed with colleagues at the David Hide Research Centre who did just that, without whose shoulder this work would not have been possible. I would finally like to thank my supervisors who were always there like a beacon to guide me through the tides.

(*Samantha Norman)

*"Dedicated to my dear wife Eram
and
lovely daughters Aleena and Menaal who missed their dad very much"*

CHAPTER 1: INTRODUCTION

1.1 Asthma

Asthma is a chronic reactive airway disease, with an element of airway inflammation. It has varied presentation and affects 30 million inhabitants of Western Europe and approximately 300 million people globally (1). At present fifteen percent of the UK population suffers from asthma (2). The study of asthma is complex as there is no “Gold Standard” definition. Asthma being a heterogeneous disease that presents as reversible airway obstruction characterised by symptoms of wheeze and chest tightness along with a spectrum of other respiratory symptoms (3).

There has been disagreement over the pathophysiology of asthma due to lack of direct causality and clarity in the sequence of events leading to the outcome that is characterized by airway inflammation and reversible bronchoconstriction. The definition that has commonly been used in epidemiological studies has been based on symptoms and investigations linked with the diagnosis of asthma. In order to consolidate the diagnosis now some definitions also incorporate the phrase “use of asthmatic medication” (4). The current evidence to-date confirms the chronic inflammatory nature of this disease affecting the lining of the airways of the lungs (5). Localised inflammation in airways is known to spread both proximally and distally affecting small airways with increasing severity of the disease and in some instances involving adjacent alveoli (6).

Inflammatory process in asthma involves various types of inflammatory cells. In a significant proportion of asthma cases eosinophils form the main inflammatory cell type, infiltrating the air passages with the subset of CD4+ T-helper Th-2 lymphocytes taking responsibility for activating an immune response whilst in the remaining neutrophilic infiltration is predominant (7-9). The heterogeneity at cellular level extends into clinical presentation with non-eosinophilic asthma implicated to represent a severe asthma phenotype (phenotype: category) (10) which is less steroid responsive compared to eosinophilic asthma (11).

The imbalance between the CD4+ Th-2 and CD4+ Th-1 activation is considered to account for asthma and other allergic disorders (9). However this imbalance only represents an explanation for the allergic eosinophilic asthma, for neutrophilic asthma the immunologic process is still not clear.

1.2 Asthma Prevalence: Past and Current Trends

1.2.1 Trends in Asthma Prevalence: the International Study of Asthma and Allergies in Childhood (ISAAC)

One of the most significant developments in the early 1990s was realisation of a global survey on asthma and allergic diseases by the International Study of Asthma and Allergies in Childhood (ISAAC). The first survey was carried out to determine prevalence of a global disease of epidemic proportions in a standardized manner. It created collaboration by linking countries not just from developed nations but by including developing ones that lacked resources and technical support. A standardised written and video questionnaire was developed for self-completion by teenage children. The specific aims of this survey were to describe the prevalence and severity of asthma, rhinitis and eczema in children living in different countries and to make comparisons within and between countries.

The ISAAC design comprised of three phases. Phases one and three used simple core written questionnaires for two age groups, 6-7 year old and 13-14 year old children. The main aim for these two phases was to accurately document prevalence and severity of asthma, rhino-conjunctivitis and eczema. The repeat of phase I after 5 years as phase III was to allow assessment of time trends for prevalence and severity of these diseases. ISAAC phase II was performed in children aged 9-11 years in smaller sample to that of phase I and III. The aims for this phase were to assess determinants for the differences observed in prevalence and severity of allergic conditions.

ISAAC phase one was completed over 5 years (1992-1996) with an enrolment of 463,801 13-14 year old adolescents from 56 countries whilst a smaller number of 257,800 participated of ages 6-7 year olds from 38 countries. There were 15-fold differences in prevalence of asthma symptoms, reported between countries. The prevalence of wheeze over the last 12 months varied from 2.1% to 32.2% whereas it was from 4.1% to 32.1% in the adolescent age group. Wheeze prevalence and reports of diagnosed asthma prevalence both demonstrated wide variations. As expected the prevalence of asthma in English speaking countries along with Latin American region were amongst the highest. Countries in the developing part of the world reported lower than 10% prevalence of current wheeze symptoms whereas those considered belonging to the developed regions of the globe were likely to have more than 10% prevalence rate. However there was no indication from the results obtained that countries with a high prevalence had more severe asthma symptoms than those with lower prevalence. The prevalence of asthma symptoms in relation to gender was

observed to vary considerably with proportion of males ranging from 34.1% to 69.9%. On average the males were more likely to report symptoms of asthma than females in the younger age group; conversely a greater proportion of females suffered from asthma symptoms over adolescent period although there were considerable number of countries with mixed picture (12). A general observation in this study was that younger children had lower prevalence of asthma symptoms in comparison to the adolescent group (12).

Phase Three (2000–2003) essentially represented a repeat of Phase One ISAAC reporting results on time trends for asthma prevalence. This third phase revealed increasing asthma prevalence in countries that had very low asthma prevalence at phase one while those with high prevalence were demonstrating levelling out of asthma prevalence or some countries even starting to observe declining trends (13). In addition to time trends phase III asthma cross-sectional analysis demonstrated a higher proportion of severe asthma symptoms in low prevalence region, compared to more affluent English speaking countries with highest prevalence (14). The lower proportion of severe asthma symptoms in developed countries have been suggested to be due to better asthma care or to lack of awareness for wheeze as a symptom of asthma in lower income countries (14). The reports of stabilizing trends for asthma prevalence in children and adolescents as reported by ISAAC study validated other recent reports from European countries (15-18).

1.2.2 Asthma Prevalence in Early Adulthood: European Community Respiratory Health Survey (ECRHS)

The European Community Respiratory Health Survey (ECRHS) was a similar survey to ISAAC study but conducted in adults of 20- 44 years old in predominantly Western part of Europe (19). This study was conducted with the aim of assessing the prevalence and management of asthma, and identifying its determinants.

The prevalence of all symptoms between the countries varied widely just as observed in ISAAC study. Generally highest prevalence were observed in English speaking countries (British Isles, New Zealand, Australia and the United States) with lower prevalence of asthma symptoms being observed in northern, central and southern Europe. Conversely to phase I of ISAAC study, wide variations were present within some countries.

Increased frequency of asthma attacks were observed to be associated with increased prevalence of night time symptoms as well as shortness of breath along with greater proportion of nasal allergies. Greater utilization of asthma medication was observed with increased frequency of symptoms. More than 80% medication use was observed in those with diagnosed asthma in The Netherlands, Sweden, New Zealand and the United Kingdom whereas it was lower in Italy, France and Spain being less than 70%. No definite conclusions could be drawn on the relationship of asthma symptoms to age and sex due to considerable variation between countries therefore no clear pattern emerged in ECRHS.

The prevalence of asthma symptoms in ECRHS suggest adults generally tend to have higher prevalence of these symptoms compared to children. Both ISAAC and ECRHS reported highest prevalence of these symptoms to be present in UK population. Both children and adults were observed to have higher proportion of severe asthma symptoms in accordance with higher prevalence of asthma in general. Morbidity and mortality data compiled in a report for Asthma UK by Lung and Asthma Information Agency demonstrated children in comparison to adults were more likely to seek medical attention and receive hospital admission for asthma symptoms despite adults having not just higher prevalence of asthma symptoms but having greater proportion of severe asthma as well with 330 per 100000 boys compared to 60 per 100,000 men admitted. Gender-wise distribution of hospital admission was suggestive of more boys whereas in adults more women were being admitted for asthma symptoms (20). This pattern was observed in all of the European countries studied (Austria, Finland, Germany, Ireland, Lazio in Italy, Luxembourg, Netherlands, Portugal, Valencia in Spain, Sweden, Switzerland, United Kingdom).

1.2.3 Asthma Prevalence: Age and Cohort Effects

When studying prevalence of diseases such as asthma, in addition to period effects it is important to consider age and cohort effects. The increasing trends for the prevalence of asthma in past, appears to have stabilised and this is largely based on evidence obtained from cross-sectional studies (14, 21). We also need to look at what is the pattern of this condition with increasing age (age effect) and between generations (cohort effect). A review of school health data on asthma prevalence in a group of successive cohorts (30 birth cohorts) of school children of age 6 to 17 years in Japan from 1984 to 2004, observed that in their population, asthma prevalence increased by a non-linear equation with a peak prevalence of asthma in at 13 year for boys and a year later at the age of 14 in girls, which was followed with a marked decline (22). In all the 30 birth cohorts (1968 – 1997) that were studied, mean asthma prevalence demonstrated an increasing trend, on the contrary to increasing prevalence

observed for age and cohort prevalence, period prevalence was observed to demonstrate a declining pattern (22).

Asthma related mortality rates from Australia (1920-1994), revealed increased mortality with advancing age, with period prevalence demonstrative of a decline after peaking in mid-sixties and late eighties. The declining period prevalence was attributable to improvement in asthma management and availability of preventative medications, however a continued increase of asthma between successive cohorts (cohort effect), that has not demonstrated a decline is of concern (23).

If we consider period prevalence of ISAAC and ECRHS reports, they are reassuring that trends of UK are levelling off and maybe even be on the decline (13). This has also been reported for other parameters such as that of hospital admissions, primary care prescriptions and surveys (24). However comparison of asthma prevalence in the ISAAC and ECRHS for the question “ever had asthma” in adolescents and adults revealed differences suggestive of cohort effect, with adults reporting 10.8% and adolescents of 13-14 age with 20.8%. Differences in exposure during early life are speculated to be relevant, with different environmental exposure between generations to account for this observation (19). Despite a pattern of declining period prevalence that has been reported, this evidence of increasing cohort effects is disconcerting.

When we review the pattern of increasing asthma prevalence between children of different age groups, we see increasing prevalence in adolescents (13-14 year) compared to children (6-7 year). This contrasts to that observed in the 1958 British Birth Cohort Study. The highest age wise prevalence in the British Birth Cohort study was observed for children at age 7 (25). Similar observations were also present in the Tucson Children Respiratory Study (26). Prospective review of asthma symptoms provide us a clue as to age effect, however they are also likely to be influenced by cohort effect with significant early life environment interaction with genetic predisposition. Therefore prevalence data that are controlled for cohort effect need to be reviewed in order to actually determine the true current status of asthma and its prognosis.

The Australian review of mortality data and observation of increasing cohort prevalence over time demands increased efforts for identifying risk factors that are contributing to increasing asthma symptoms between successive generations despite apparent stability in asthma prevalence that is observed. This is suggested to be largely accounted for by better management rather than addressing the root cause of the disease; good cohort studies are essential for this.

1.2.4 Gender Reversal in Asthma

Male predominance is seen throughout childhood for both asthma and atopy (27). Gender reversal is observed during adolescence, with asthma prevalence in females surpassing that in males (25, 28, 29). This reversal occurs at the same time that asthma seems to remit in a subset of children. However the dynamics of the change are still not clear (30). In the Melbourne cohort, there was less remission in girls with asthma symptoms seen to improve in boys over the adolescent period (31). In another study childhood asthma improved equally in both sexes, but there was significantly more new onset asthma in girls during adolescence (32). A large, though retrospective, study from Italy supports the second possibility, suggesting that asthma presents in two forms; early-onset asthma occurred in boys during childhood and late-onset asthma mainly affected women (33). Another study showed that reduced growth in lung function was observed in asthmatic females, but not males with asthma, during the teen years (34). Gender reversal was also evident from data collected for all hospital admissions due to asthma in Scotland (35), suggestive of increasing severity of asthma cases amongst females in the adolescent group. We do not know the respective contribution of the new onset asthma, as opposed to persistence of childhood asthma in girls, as the reason for gender reversal observed at this age. The causes of persistence or new occurrence of asthma in females are also not known, but may be related to pubertal/hormonal changes, BMI or lung development during the adolescent period.

1.2.5 Relapse and Remission in Adolescence

We are reviewing Isle of Wight Birth Cohort in a very important period of adolescence. This phase of life is associated with huge physical, hormonal and behavioural changes. During the transition from childhood to adulthood, asthma symptoms often decrease and in many cases a cure is presumed (36). Unfortunately, this good news is dampened by the fact that there is recurrence of asthma symptoms in adulthood in many of those who were presumed cured (37). Population based studies over successive decades have reported remission of asthma symptoms to take place during the adolescent period (25, 31, 38). In the Dunedin study of childhood asthma, remission was observed during adolescence in nearly 50% of subjects, however, almost half of these relapsed in adult life. Similarly, in the Melbourne study of childhood asthma 50% remission was observed during adolescence but nearly half of those in remission relapsed in early adult life (39). Other studies confirm that nearly 50% of childhood asthmatics lose symptoms as they go through adolescence (28, 40, 41). Despite the absence of symptoms during remission, airway obstruction and BHR

could be demonstrated in 50-60% of these subjects (28, 39, 42-44). Furthermore, recent studies have suggested that some children, who had apparently outgrown their asthma, continued to have persistent airway inflammation (45, 46). Van den Toorn et al. (47) demonstrated significantly increased exhaled nitric oxide (a marker of airway inflammation) and BHR were present in young adults that were in clinical remission of asthma. Obase et al. (45) studied 23 young adults with remission of asthma; in 20 of 23 subjects, they were able to detect signs of airway inflammation in the induced sputum cells (eosinophils), toxic mediator (eosinophil cationic protein) and cytokine (TNF- α), similar to those with current asthma. A large proportion of those in remission of asthma with on-going underlying inflammation are speculated to have a potential for relapse of their asthma symptoms (48).

1.3 Phenotypes of Asthma

In order to gain a better understanding of the heterogeneous nature of asthma, various approaches have been applied. Efforts have been made through characterizing asthma into different phenotypes in a hope that this will guide asthma management. “Phenotype” is defined as “visible characteristics of an organism that result from interaction between its genetic makeup and the environment” (49). A number of different ways of characterisation of phenotypes have been proposed over recent decades to tackle the variability in the presentation of asthma. Some of the commonly employed approaches are given in table 1.1.

Table 1.1 Classification of Asthma Phenotypes (50)

Developmental state based	Early onset, Late onset (Cohort Studies)
Symptom-based	Mild, Moderate, and Severe (Clinical Guidelines based)
Response to treatment	Well controlled (Asthma control based on guidelines and cross-sectional clinic based studies)
Pathological tests based	Eosinophilic
Statistically determined	Using Latent Class Analysis

1.3.1 Phenotypes Based on Time

Most of the cohort studies observe asthma or, to be more precise, symptom of wheeze prospectively over time. They adopted a natural history approach identifying

different asthma/wheeze phenotypes based on time of onset, reporting persistence of wheeze or its transience (26, 51). The challenge faced by cohort studies in early childhood stems from the lack of understanding of what constitutes asthma in early life. These studies, including ours, therefore relied on characteristic symptoms of asthma, in particular wheeze, to determine the risk and predictive factors for these symptoms (52, 53). However an alternative approach uses both wheeze and asthma to determine risk for these two outcomes in some of these studies (26, 51, 54, 55). Tucson Children's Respiratory Study is considered a land mark study as it laid the foundations for the concept that asthma has its origins early in childhood (56). In this prospectively followed study, asthma over adolescence and young adulthood was characterised based on early life wheeze. This view was supported by objective evidence of tracking of lung function established in the early years of life into adolescent period (26) and was shared by two other prospective cohort studies from Dunedin (New Zealand) (51) and Melbourne (Australia)(31). This study identified four distinct wheeze phenotypes based on the early life origins concept (57, 58):

1. Transient early wheeze
2. Late-onset wheeze (non-atopic wheeze)
3. Persistent wheeze
4. Never wheeze

Transient wheeze, as the name suggests is limited to first three years of a child's life and is not present thereafter. Wheeze was observed with respiratory tract infection in the first three years of life. This phenotype is not associated with family history of asthma or atopy but a greater proportion of these children are reported to have mothers that smoked during pregnancy (57). These children present with lower level of lung function as early as first year of life, which is observed to track into adolescence (26). However this group of individuals are not observed to have other features of asthma such as airway lability (57). The transient group was not observed to have increased risk of asthma but is speculated to be at risk of developing obstructive airway disease such as COPD later in life, as they are disadvantaged with lower lung function to begin with (59, 60). Therefore poor lung function is unlikely to have been due to wheeze illness or respiratory infection.

Late onset wheeze was assigned based on absence of wheeze with lower respiratory tract illness in the first 3 years of life, but which was present at the age of 6 years (61). This group was largely made up of atopic children with lung function comparable to non-wheeze children at the ages of 11 and 16 years (26). Bronchial hyper-responsiveness (BHR) at the age of 11 years was seen more with atopy (62). The comparison of differences for the three wheeze phenotypes in relation to BHR are not

presented by the authors as these wheeze groups are heterogeneous themselves with respect to atopy, BHR and peak flow variability (63).

This leads us to the most studied group of wheeze, that is persistent wheeze, characterized by high IgE from early life, persistence of lower lung function, increased peak flow variability, bronchial responsiveness and severity of disease (26, 57). In this group more than 50% of children start wheezing before 3 years of age and there is significant genetic predisposition to allergen sensitization (57). This group has been further stratified by atopy and two additional wheeze phenotypes were studied namely atopic and non-atopic wheezers (60% atopic with 40% non-atopic children at the age of 6 years). Non-atopic wheezers demonstrated two different wheezes based on bronchodilator response. Those children that had lower respiratory infection with respiratory syncytial virus (RSV) were more likely to demonstrate significant bronchodilator response compared to those that did not have this infection (63). This group had a better prognosis with greater remission of symptoms after age of 13 years (57, 63).

However none of the outlined characteristics for each wheeze phenotype is exclusive to that group. These 3 different phenotypes demonstrate that there is considerable variability within each phenotype whether it is early life infections, atopy or pulmonary function markers.

1.3.2 Phenotypes Based on Severity

Another commonly employed approach for classifying asthma is to base it on clinical severity such as mild, moderate or severe form of disease; this has more relevance to managing asthma with similar format to that of asthma management guidelines (64). This also has the benefit of direct translation of findings into clinical management. Melbourne cohort study adapted this methodology by characterizing severity based wheeze phenotypes. Study participants were assigned five grades depending on frequency of wheeze episodes and asthma symptom severity (65). Persistence of asthma in this was predicted by severity of asthma symptoms, with an additional risk of developing hay fever and eczema in later life. The transitions between persistent asthma and no asthma were observed on fewer instances than movement from intermediate categories of asthma severity into either severe asthma or to no asthma groups (66, 67).

Although severity has good clinical correlation in relating research findings to clinical application, the current trend is towards using the term “control” in clinical management of asthma as this incorporated the seriousness of condition and how

well it is managed (3). Recent asthma studies have assessed control, they are largely clinic or hospital based studies looking at cross-section of the population for evaluating prevailing practices and their effectiveness in managing asthma. Two recent European surveys, one looking at children and adolescents (68) and the other adults (69) both arrived at the conclusion that there is less than satisfactory levels of control.

1.3.3 Phenotypes Based on Cell Type

A third commonly used form of classifying asthma phenotype is based on the composition of inflammatory cell type, with eosinophilic, non-eosinophilic and neutrophilic assignments (8). Laboratory based studies looking at cells and tissues obtained by bronchial biopsies, broncho-alveolar lavage (BAL), sputum inductions and breath condensate are utilized in studying patho-physiology of samples obtained from asthmatic and control subjects both from clinical or community environments.

Eosinophilic asthma is a distinct entity that is observed to be associated with thickening of sub-epithelial basement membrane of bronchial airways and this phenotype is responsive to steroid therapy, conversely non-eosinophilic asthma is characterised by severe disease, being steroid resistant and lacking thickening of basement membrane (10), equally common is neutrophilic asthma however there is less clear understanding of this phenotype, (7, 70) but both cell types are not mutually exclusive, as neutrophilic cells are observed to populate eosinophilic asthmatic passages in severe asthmatics (8). Furthermore evidence suggests that these phenotypes are not stable (71). Eosinophilic asthma associated with changes in epithelial basement membranes is considered to signify airway remodelling and this has been observed in children as young as in the first year of life (72). Degree of airway remodelling is a harbinger of asthma severity (73). Presence of eosinophilic inflammation, in asthmatics with remission of asthma symptoms are speculated to be at risk of recurrence (45). Eosinophilic inflammation is observed in children despite good asthma control (74). With increasing information on the nature of underlying airway inflammation in asthma, different patterns of inflammation are observed within the other phenotypes of asthma, as severe form of asthma is observed to differ from mild-moderately severe asthma by demonstrating mixed cellular pattern instead of eosinophilic or neutrophilic predominance (75).

1.3.4 Phenotypes based on Atopy

Wheeze has also been phenotyped on the basis of atopy, with atopic wheeze observed to be associated with bronchial hyper-responsiveness and airways

obstruction in school age children with atopic wheeze, these children were also more likely that non-atopic wheeze phenotype to receive treatment or have asthma diagnosis, whereas morbidity between them did not differ (76). The finding of atopic wheeze receiving a diagnosis of asthma has been consistent and seems to be age independent (77). As atopy in turn is associated with airway eosinophilia (78), and eosinophilia associated with BHR and FeNO (79). Atopic assignment in most of the epidemiologic studies has been based on either by documenting concurrent presence of allergic disease in asthmatic individuals (80), by a positive wheal obtained on skin prick testing to a common panel of allergens (81) or by the use of raised serum specific immunoglobulin E levels (IgE) (82). Atopic asthma is a distinct phenotype and has been in the centre stage of any debate on asthma, it has consistently been associated with persistence of asthma (83). However both phenotypes based on atopy for wheeze/asthma share a similar morbidity (76, 84). Atopy is also suggested to be causally associated with asthma, as it often is observed to predate asthma occurrence (83), however not all those who are atopic go on to develop asthma therefore countering this view. A general observation in epidemiological studies is of changing pattern of asthma in relation to atopy, with childhood asthma which has male dominated pattern and associated with atopy that becomes less atopic with female preponderance with increasing age (85, 86).

1.3.5 Phenotypes by way of Statistical Categorisation

Phenotyping is not just limited to conducting the study but advanced statistical methods have been applied for dealing this complex nature of this disease that has various components which vary within each asthmatic individual and over time. Some of the recent methods adapted have used advanced statistical models of cluster, latent class and principal component analyses overcome investigator bias in determining phenotypes of asthma. These models are attractive from conceptual point of view as they remove the investigator's control by defining the number and structure of the classes but they have a subjective element of operator defining number and nature of variables used for running the exploratory model in identifying the different classes (87).

The aim of phenotyping wheeze illness is to understand disease mechanisms and to optimize management of a labile airway condition that has significant morbidity. Epidemiologic and laboratory based studies have proposed there are multiple asthma phenotypes, reflecting a heterogeneous group of conditions that follow a common final pathway that presents as recurrent airway obstruction.

1.4 Contribution of Asthma and Allergic Cohort Studies

Our understanding of asthma and allergic diseases has improved considerably over a period of last 5 decades due to a global effort, not just by investigators but by each study participant visiting the study centres and providing their precious time and effort. We will begin our review of contribution made by major studies beginning with the earliest ones. However prior to our review it is imperative to note that cohort studies have reported these results from various perspectives, e.g. some of the studies have reported persistence of asthma and risk factors for persistence, keeping remission as reference, whilst others have reported remission and risk factors accounting for only this phenotype. We will highlight only significant findings of major cohort studies leading up to the time of Isle of Wight birth cohort study.

1.4.1 British National Cohort (1958)

The 1958 British National Cohort study included 17,414 children born over one week in March across UK. The study group was followed at 7 year intervals, with first visit taking place at age 7 years. The asthma related component of this study was only a minor segment of a wider purpose of health and nutritional data collection of this national cohort. Despite the limitations of the study in relation to addressing asthma parameters, it has contributed significantly towards our understanding of this disease in relation to early incidence and prevalence in the context of atopy and gender. Importance of this study is also magnified by the fact that UK has consistently reported highest prevalence of asthma and allergic diseases in multiple surveys over past couple to decades. The significant findings of this study are as given below:

1. The annual period prevalence reported for asthma was observed to be highest at the age of 7 years of 8%, which it remained at a constant level around 3 - 4% in the successive follow-ups (25).
2. Male dominant asthma prevalence was reported up to the age of 16 that reversed to show female prevalent pattern at the age of 23 years (25).
3. Males were also observed to be more atopic during childhood but with increasing age females also start to match male atopic proportions (25).
4. Identified that atopy rather than inhaled precipitants such as aeroallergens, were associated with the development of asthma during childhood and adolescent period (25).
5. Asthmatics in remission of childhood wheeze were more likely to relapse if they were atopic and or exposed to cigarette smoking (88).

6. Incident wheeze from age 17 onwards was associated with cigarette smoking and history of hay fever (88).

1.4.2 Tasmania Asthma Study (TAS) (1968)

This study began as a survey enrolling 8583, 7-year-old school children. Two additional surveys were performed, at the ages of 13 and 44. Spirometric lung function was obtained at baseline.

1. The results of this study suggested that genes determining asthma were unlikely to have a single major locus influencing asthma susceptibility (89).
2. Childhood eczema was observed to be associated with persistence and new onset asthma into middle age (90).
3. Children with allergic rhinitis were at an increased for both risk new-onset and persistent asthma from childhood into middle age (91).
4. Young females with higher body mass index (BMI) at 7 years of age had higher risk of developing asthma in adult life (92).

1.4.3 Tucson Epidemiologic Study of Obstructive Lung Diseases (1972) (63)

A multistage stratified cluster sampling of Anglo-white Tucson households was used in this longitudinal study that enrolled 1655 households. The study sample included 3805 of a wide age range i.e. from childhood to >65 year old. The selection was representative and covered the household structures, including the age and sex, of the Tucson Anglo-white population. The study enrolment began in March 1972 and was completed in April 1973. Significant findings from this study are given below:

Severity of asthma and impaired FEV_1 function were associated with persistent asthma, and recurrence was more likely in past asthmatics with on-going respiratory symptoms. It also highlighted that adolescent is the period where most remission was seen (38).

Longitudinal population data that was used to describe the development and decline with age of the peak expiratory flows (PEF). The PEF data from the 6th-11th surveys (1979-1989) were used to study the pattern of airway function over entire age. Gender was observed to influence the age at which changes in growth rates of PEF occurred. The comparison of cross-sectional to prospective data for adults revealed a less steep decline with age. Individuals with respiratory diseases did not reach the

same maximum level of lung function and had a steeper decline throughout adult life. Asthmatics of both genders had slightly greater declines compared to the other respiratory conditions (93).

This study identified that respiratory symptoms reported by parents very early in life were not associated with development of asthma at 11 years; however asthma symptoms that present at the ages 3 to 4 years were more likely to predict asthma into early adolescent period (94).

Prior episodes of asthmatic symptoms of wheeze and shortness of breath, along with atopy (SPT or IgE) were independently predictive asthma in young adults by age 21, however spirometric testing was not (95).

Children of mothers of lower socioeconomic who smoke more than 10 cigarettes per day were observed to be at considerable risk of developing asthma (96).

Blood eosinophils were observed to decrease with age in men but not in women. Increased percentage of blood eosinophils were observed during the months of February through May, coinciding with the Tucson region's pollen season. Blood eosinophils were also related to allergy skin test reactivity, circulating IgE concentrations, and reduced ventilatory function (97).

1.4.4 The Dunedin Multidisciplinary Health and Development Study (1975)

This study was a prospective study addressing health and behaviour characteristics in a complete birth cohort. The study was conducted in Dunedin, New Zealand, between April 1972 and March 1973 and enrolled 1037 of the 1139 children born during that period at the age of three years. The families of this cohort were primarily of European origin. The cohort was observed at 2 years intervals between the ages of 3 and 15 years with additional follow-ups at the ages of 18, 21, and 26 years. Although this study has contributed immensely to improve our understanding of asthma and allergic diseases, however there is one major flaw in that limits the interpretation of early life events, i.e. the data collected on respiratory symptoms for these children in relation to early life was collected retrospectively at the age of 9 years. Skin prick tests (SPT) (13 and 21 years), IgE levels (11 and 21), bronchodilator response (18 and 26 years) and airway reactivity to methacholine (9, 11, 13, 15 and 21 years). The strength of this cohort is its objective assessment of asthma related measures over the adolescent period. Important findings and characteristics particular to this cohort are given below:

1. A large proportion (72.6%) of individuals in this cohort had wheezed at some point in first 26 years of their life (51).
2. Early age at onset of wheeze was a risk for relapse in early adult life in addition to allergy to house dust mites and increased bronchial reactivity (51).
3. Persistently impaired lung function and airway hyper-responsiveness was observed in persistent asthmatics despite treatment with inhaled corticosteroids (51).
4. Smoking was a risk factor for persistence but not for relapse of wheeze at 21 years, along with increased bronchial reactivity, allergy to house dust mites, and female sex (51).
5. This study supported the concept of tracking of lung function between different wheeze groups from early life into early adulthood (51).
6. This study supported the view that childhood wheeze is largely seen in males with females demonstrating adolescent onset wheeze. History of maternal atopy (hay fever or asthma) was observed to differ between the gender with age, being a significant risk factor for both boys and girls during childhood however demonstrated significance only for females that developed adolescent wheeze. Conversely paternal history of atopy was significant only for boys during childhood only (98).
7. The other significant information gained from this cohort was that overall bronchial responsiveness was observed to decrease with age (99).
8. The degree of skin test reactivity and level of IgE at age 9 was predictive of lung function, bronchial reactivity and severity of symptoms at the age of 15 years (99).

1.4.5 Tucson Children's Respiratory Study (1980) (63)

This is one of the most active birth cohort studies to-date that has continually updated and reassessed the original cohort in the light of new information gained and tested new hypotheses. This cohort was established in Tucson Arizona, with the focus of studying the impact of early life factors towards development of asthma later in life. A large number of infants were enrolled soon after birth, with total of 1246 infants enrolled over a period of four and half years (1980-1984). The cohort was reviewed at the ages of 2, 3, 6, 8, 11, 16 and 22 years of age. The salient findings in relation to asthma by this cohort are as given below:

1. Majority of early life wheeze was a transient condition in infants which was associated with diminished airway function at birth, however these babies did not have an increased risk of asthma or allergies later in life with the exception of a few (61).

2. Infant with early life transient wheeze are more likely to be non-atopic and be exposed to maternal smoking but without maternal history of asthma (63).
3. Children with persistent wheeze were more likely to be atopic, with a maternal history of asthma and lower lung function (62).
4. Non-atopic early life wheeze (a heterogeneous group with 60% non-atopic and 40% atopic) was identified to have better prognosis and was observed to be associated with early life respiratory syncytial virus (RSV) infection (63).
5. A higher proportion of lower respiratory infections were observed in children exposed to cigarette smoking, attending day care centres (100) and of younger mothers (61) while breast feeding of at least one month duration was found to have a protective effect from these infections (63).
6. Having an older sibling and attendance at day care centre accorded protection from developing asthma, wheeze and atopy in school aged children (100).
7. Obesity was reaffirmed to be a risk just as other studies had done earlier (58).
8. All of the analyses in the TCRS failed to observe relationship of IgE in the causal path for asthma development and have suggested it to be asthma that affects IgE levels, different from mechanism observed in non-asthmatic populations (63).
9. Lower lung function was present in infants prior to infections and was predictive of lower respiratory infection (101) in infants (63).
10. Asymptomatic bronchial reactivity in childhood was associated with asthma and atopy, later in life (102).

1.4.6 Southampton Cohort (1987) (52)

This cohort study was established in the Southampton area to study history of atopic disorders (asthma, hay fever, and eczema), relevance of family history of asthma and environmental factors (presence of smokers, pets; use of gas cookers and stoves within the household); impact of birth weight for development of asthma. This cohort was established as a questionnaire survey assessing respiratory symptoms in children aged 6–8 years, who were born between July 1, 1978, and June 30, 1980. The study successfully enrolled 3,542 children that were registered with 86 family practitioners in the Southampton. In December 1994, this survey was repeated as a postal questionnaire. Review of the earlier survey of this cohort identified increase in prevalence of wheeze in this cohort, with age, as higher prevalence of wheeze was noted at the age of 14–16 year children compared to at the ages of 6–8 year, with 50% of this wheeze was due to persistence of wheeze that was present at earlier follow-up, suggestive of increasing prevalence of wheeze with age. This study also noted that adolescent onset wheeze was more likely to affect females, thus may contribute to gender reversal pattern observed in adolescent asthma. Personal history of smoking

was associated with late onset of wheeze but not to doctor diagnosed asthma, whereas passive paternal smoking was. Symptoms of active wheeze and cough in addition to late onset wheeze demonstrated associations to passive smoke exposures. This study was able to demonstrate increased risk for developing adolescent wheeze following exposure to tobacco smoke however it was not able to account for developing asthma. History of atopy (defined by having an allergic condition such as asthma, hay fever or eczema) was associated with current wheeze and asthma at 14-16 years. It was also significant for persistence of wheeze that was present at age 6-8 years and for late onset wheeze observed at the age of 16 years (52).

1.4.7 The Isle of Wight Birth Cohort Study (1989)

The Isle of Wight (IOW) birth cohort study provides us the ideal epidemiological study environment, with its well defined geographical location, that has easy access to the UK mainland through a short boat ride. Absence of direct land links, resisting free flow movement of its residents assures a reasonably stable population. The Isle of Wight (IOW) Birth Cohort was established as an unselected whole population birth cohort in 1989. Based on the last census of 2001 the island has 132,000, residents, with 0-15 year age group making a little over 18% of this population. The ethnic background of Island residents is mainly Caucasian.

Table 1.2 Summary of the Isle of Wight Birth Cohort

Birth (January 1989 – February 1990): Family history of allergy, household pets, parental smoking, socioeconomic status, gestational and parturient factors, cord blood IgE, maternal IgE	N = 1,536
Age 1 year: Asthma and other allergic manifestations (physician diagnosis, symptoms and treatment), chest infections, household pets, parental smoking, method of feeding	86.9% (1,316/1514)
Age 2 years: Asthma and other allergic manifestations, chest infections, household pets, parental smoking, method of feeding	79.8% (1,205/1510)
Age 4 years: Asthma and other allergic manifestations, skin prick test, chest infections, household pets, parental smoking, housing characteristics (age, dampness, method of heating)	83.7% (1,218/1456)
Age 10 years: Asthma and other allergic manifestations (additional: ISAAC questionnaire), skin prick test (n = 1,036), serum IgE and blood sampling for gene analysis (n = 945), baseline spirometry (n = 981), bronchial provocation test (n = 784), household pets, parental smoking	94.3% (1,373/1456)

Percentage determined with N = Total study participants available for follow-ups, n = number of participants enrolled at each follow-up.

Drs Arshad and Hide at the David Hide initiated The IOW cohort study. This cohort was established to prospectively study a whole population cohort for the development of asthma and allergic diseases. Over time the contribution of this cohort has added towards identifying important genetic and environmental risk factors relevant to these conditions (6, 53, 103-107). The parents of all babies born on Island between January 1, 1989 and February 28, 1990 were approached and informed about the study. There were 1,536 babies born over this period. Enrolment of these babies took place at the time of birth. Consent was taken from the parents of these children. There have been four follow-ups over this time, at the ages of 1, 2, 4 and 10 years, details of which are as given in Table 2. Information was collected on heredity and

environmental exposures from birth and has been updated at each of the follow-ups. At these follow-ups, the detailed questionnaires were completed with the parents, regarding asthma and allergy prevalence in their children.

Table 1.3 Summary of the Isle of Wight Birth Cohort

Some basic characteristics of the cohort:	% (n/N)
Maternal smoking during pregnancy	26.0 % (393/1509)
Wheezing at age 1-year	12.2% (164/1339)
Wheezing at age 4-years	21.4% (261/1218)
Wheezing at age 10-years	18.9% (259/1373)
Skin prick test positive at age 4-years	19.7% (193/980)
Skin prick test positive at age 10-years	26.9% (279/1036)

Basic characteristics presented as percentages with (n) = number of study participants with condition, with (N) = total number of study participants that provided information

In the earlier follow-ups, information on symptoms of asthma, in particular wheeze was collected along with further inquiries were made into asthma morbidity and medication requirement. Skin prick testing was performed to 14 common food and aero-allergens at 4 and 10 years. The study at 10 year follow-up utilised standardised International Study of Asthma and Allergy in Childhood (ISAAC) questionnaires (61). Spirometry and bronchial provocation tests were performed, blood was collected for genetic studies, and serum was used for IgE measurements and stored for future analysis (Table 1.2).

A combination of ISAAC questionnaire fields along with our study tools i.e. symptom questionnaire, skin prick and lung challenge tests were used to develop the criteria for diagnostic labels. Definitions for the diagnosis of various allergic manifestations in the IOW cohort, are given in the definitions section.

The majority of early childhood wheeze in the IOW birth cohort was transient and showed improvement before the age of 10 years. However, a significant sized group of early wheezers (30%) continued to have persistent wheeze and developed asthma. The 10 year follow up allowed the opportunity to study the established asthma group with respect to their symptoms, co-morbidities and lung function tests such as spirometry and BHR (108).

The advantage of this cohort is that it provides a whole population outlook limiting sample selection bias although this study design is sensitive to attrition. We have been successful to have good follow-up rates with last follow-up at 10 year of age of this group recording 94.3%. The follow-up rates at each of the assessments are given in table 2. Results of earlier follow-up at the ages 1, 2, 4 and 10 years have been published (6, 53, 76, 103-105, 108-116).

We will now review important concepts and associations that have been observed with asthma. Some of the associations are considered to be causally related. In order to gain a better understanding of these factors we will review important concepts that are linked to asthma before we proceed to those factors that are of interest for our current analyses.

1.4.8 Summary

The birth cohorts have considerably enhanced our understanding by allowing us to understand the natural history of childhood asthma and wheeze. These studies have provided us the opportunity to assess impact of various factors that contribute toward asthma occurrence. New factors demonstrating associations for asthma such as paracetamol are being further assessed for their merit and relevance towards asthma. Prospective studies by their design allow us to follow individuals over time to understand sequence of events leading to outcome of interest i.e. asthma. We still have various unanswered questions on causality of identified factors. Our understanding of mechanism of asthma development has many more questions that need to be answered. We still need to learn why do boys tend to grow out of asthma over the adolescent period? What predisposes girls to asthma over the same period? How is smoking associated with asthma? What is the mechanism of gender switch from male to female preponderance? These are only a few of the many questions that emerge when we review the present literature, that appear as gaps in our knowledge of asthma.

1.5 Immuno-Pathology of Atopy

Human body defends itself from infection by various mechanisms one of which is by mounting an immunologic response that counters the offending organism by a process of inflammation. Innate part of the immune system comprises of macrophages and dendritic cells (also referred to as antigen presenting cells (APC)) which act as front line non-specific immune cells that engage offending pathogens or allergens that manage to enter the human tissues through either breaking or via damaged epithelial

barrier (117). These cells then present processed fragments of offending agents to our immune regulatory cells (adaptive immune system) i.e. CD4 +ve T-helper (Th) lymphocytes, that recognise and direct a specific immune response. Generally Th1 sub-set of immune cells are activated and direct specific cellular and humoral inflammatory response via B cells (by producing antibodies e.g. IgM, IgG or IgE) in order to eliminate the pathogens. However in the absence of infection activated Th2 sub-set of CD4+ helper T-cells are speculated to cause the culmination of IgE on exposure to allergens (118). Atopy or allergic sensitization is characterized by raised IgE levels and is associated with allergic diseases such as asthma, rhinitis and eczema (119).

1.6 Hygiene Hypothesis

Prevalence studies have consistently observed increased burden of allergic diseases in the industrialized countries in contrast to lower proportions of such conditions in the developing part of the world (12, 14). This has been attributed to declining childhood infections from better sanitary conditions accorded by higher standards of living (120). This concept is supported further by lack of immunological mechanisms proposed for the allergic phenotype with an imbalance between the T helper 1 (Th1) and T helper 2 (Th2) cells. It is hypothesised on the basis that absence of bacterial and viral infections during early life predispose the maturing immune system toward Th2 pathway. This results in a weak Th1 profile, leading to an increase in allergic response. However this debate is challenged by observations that parasitic (helminth) infections are also associated with Th2 response that is largely present in the developing part of the world (117). Autoimmune diseases are associated with Th1-response, therefore it was considered plausible for individuals with atopy to be protected, by nature of their matured immune system favouring Th2 pathway, however this association is not observed, thus counters the evidence for hygiene hypothesis (121).

1.7 How Much of Adolescent Wheeze is Asthma?

Wheeze as a symptom in childhood is not specific to asthma. Particularly in early life wheeze is known to be a transient condition, with a low probability for developing asthma in later life (26, 51). Prospective studies have assessed risk that are largely based on early life phenotyped wheeze (26) or determined risk for adolescent wheeze rather than asthma (51). Wheeze over adolescence has not been characterised, and is considered to represent asthma unless proven otherwise, with asthma and wheeze terminology being used interchangeably over the adolescent period (51, 122, 123). This has also led a number of studies, labelling un-diagnosed wheeze as under

diagnosed asthma (122, 124, 125). Wheeze that is not diagnosed as asthma over adolescence is attributed to risk prone adolescent behaviour rather than to our lack of understanding of this wheeze phenotype (126). This presumption that undiagnosed wheeze as asthma is likely to lead to misclassification unless this wheeze is better characterized.

1.8 Concept of Early Life Origins and its Significance for Adolescent Asthma

Another generally accepted concept linked to asthma over adolescent period that is rigidly adhered to by most of the prospective studies is the early life origins of adult asthma. An in-depth review of results from Tucson Children's Respiratory Study (TCRS), reveals that this view may not be true for a significant proportion of young adult asthmatics, as newly diagnosed asthma in TCRS largely comprised of 60% of asthmatics at 22 years that never had asthma or wheeze illness as a child (123). Two of the well-studied cohort studies, the TCRS and the Dunedin Multidisciplinary Health and Development Study (DMHDS), have focussed on early life origins of wheeze and characterized early life asthma and wheeze phenotypes into young adults restricting their analysis based on the concept of all childhood wheeze has a potential to develop into asthma later in life, with one study using interchangeably the term of wheeze with asthma over adolescent period (51), whilst in the other true adolescent onset asthma was not used (group included recurrence of childhood persistent wheeze) as a phenotype for risk determination (123). The resistance to the view of moving away from early life origins to adolescent period of susceptibility as confirmed by the investigators of TCRS themselves appears to be self-defeating; it is apparent from the current evidence that not all wheeze develops into asthma (26). Risk determination for such asthma groups that includes recurrent asthma from childhood when clumped with pure adolescent onset asthma is unlikely to improve our understanding of true adolescent onset asthma.

1.9 Atopy and its Significance for Adolescent Asthma

Association of atopy and childhood asthma has been established with consistent reports in multiple studies (61, 83, 127) particularly in boys (25). However the contribution of atopy towards adolescent asthma has been debated, with studies reporting changing pattern of from male dominant to female dominant pattern with atopy losing its significance (85). Questions have been raised about the research communities' pre-occupation with atopic asthma and lack of attention given to a significant proportion of non-atopic asthma for which our knowledge is lacking (128).

A recent review of asthma and atopy challenged the causal relation of atopy to asthma and considered the two as independent conditions (129). However few prospective studies have observed whether atopy continues to pose a significant risk over the adolescent period (52) with females eventually acquiring male atopic proportions (25). Clear evidence is still missing as atopy has been determined by parental history of hay fever or eczema, which may or may not have an allergic basis, rather than utilization of quantitative measure such as skin prick test or IgE level. Some of the prospective studies have failed to identify atopy as a risk towards new onset asthma over adolescent period (85, 123).

In the Isle of Wight birth cohort, at 10 years of age, atopy was observed to be associated with a higher proportion of wheeze that received a diagnosis of asthma compared to non-atopic wheeze in our study (76). Also atopy led to more clinically significant wheezing illness as observed in children of 10 year age (111). Our cohort has been characterised in terms of allergic diseases (asthma, hay fever and eczema) from birth to 10 year-old children, identifying significant association of familial and environmental factors with consistent strong associations with atopy to all of these disease states (53, 105, 108, 109, 112). Characterisation of atopic children showed predominance of aero-allergens when compared to food allergens from birth to 10 year of age (112). Chronic atopic children were more likely to have persistent wheeze, eczema, rhinitis and greater cord IgE when compared to those that were never atopic (112). Other cohort studies have also demonstrated similar associations though to different specific allergens, for example *Alternaria*, pollens, cat and rye grass (51, 58, 66). Most of the cohort studies have demonstrated House Dust Mite (HDM) as a common allergen to be associated with persistent asthma (53) (51, 66). The contribution of atopy towards adolescent wheeze and diagnosed asthma (wheeze and asthma at 18 years), remains unresolved with conflicting evidence.

1.10 Asthma and Rhinitis: One Airway Phenomenon

Historically, the diagnosis and treatment of the upper airway (from the nose down to the vocal cords) has been managed separately to that of the lower airway (below the vocal cords) (130). Anatomy, patho-physiology and management of rhinitis was considered independently to that of asthma with two specialities, otolaryngology dealing with upper airway and pulmonology concerned with lower airway conditions (130). However consistent epidemiological evidence repeated over time has demonstrated that one part of this airway is often observed to affect the other (80, 105). Rhinitis has been observed not only to co-exist but also precede development of asthma (80, 91). This increased risk of rhinitis is not confined to allergic rhinitis (131). Therefore, rhinitis is not just the outcome but a risk factor as well. Physiologic basis

for link between the two conditions has been forthcoming, as both upper and lower airway demonstrates significant correlation of eosinophilic infiltration (132). Evidence suggests that individuals with rhinitis that do not report any asthma symptoms also demonstrate airway responsiveness methacholine (133, 134). Common triggers are observed for both upper and lower airway responsiveness, however the actual mechanisms for this association are not clearly understood (134). Clinically significant rhinitis is observed to affect asthma symptoms that are associated with severity, such as increased frequency of wheeze (134). Further evidence supporting one airway concept is afforded by comparable response has been observed for symptoms of rhinitis and asthma with anti-inflammatory treatment e.g. effect of montelukast, locally acting corticosteroids, and recent immunological therapy of omalizumab, suggestive of common mechanisms at work, at least for the allergic phenotypes of asthma (135). There is reasonable evidence that control of asthma is not going to be sustained unless rhinitis is concomitantly addressed (136).

1.11 Risk Factors for Adolescent Asthma

Adolescent asthma is a term that encompasses those children that have on-going asthma symptoms (persistent asthma) as well as ones that develop asthma over the adolescent period (adolescent onset asthma). The period of adolescence has been defined differently in different studies, some have used 10 to 18 years (137), while others have used 12 to 18 (122). In the next section we would examine various factors that have been associated with asthma from childhood into the young adulthood. We have divided these factors into early life and adolescent, although segregation of some of the factors may be arbitrary based on time only, for example passive tobacco smoke exposure.

1.11.1 Early Life Factors

Chest Infections

In the early childhood years, respiratory tract infection is usually associated with wheeze. Only small proportions of children continue to wheeze and are diagnosed with asthma in later childhood (53, 138). Hospital admissions due to bronchiolitis have also been associated with persistence of wheeze in the first five years of a child's life, however the long term consequences of these infections lacks clarity (139). Repeated viral infections in early life, with the exception of those affecting lower respiratory tract, have been observed to offer a protective effect from development of asthma in the 7 year olds while chest infections were associated with persistence of early life

wheeze (140). A study assessing the impact of early life, questionnaire-based, surrogate factors for infections could not account for an increased risk of asthma (141). In addition to chest infections, increased use of antibiotics in early life has also been observed to be associated with childhood asthma (140, 142). A higher risk for asthma with antibiotic use has been suggested to represent a proxy marker for increased severity and number of infections and not true association of antibiotic use being a risk (143). TCRS supports the view of lower subsequent asthma/wheeze development in individuals that are exposed to infection early on in their lives (63). Contradictory evidence on contribution of early life chest infections and development of asthma particularly its relevance over the adolescent period requires additional studies to assess the true nature of this relationship.

Breast Feeding

There are conflicting observations with regards to effect of breast feeding and risk of asthma. Some of the studies have observed breast feeding offers protective effect in children that are breast fed (143) whilst in another study a longer duration of breast feeding was associated with increased risk of asthma by the age of 18 years (141). In our birth cohort, beneficial effect of breast feeding led towards improved lung function in infants that were breast fed for at least 4 months duration compared to those that were not (144). There is biological plausibility both in favour and against these contrasting observations on effects breast feeding. The evidence from well characterised and prospective study is required for assessment of long term effect of breast feeding.

Birth Weight and Type of Delivery

Low birth weight, in particular very low birth weight, babies have greater risk of suffering from chronic disease states in adult life (145). A maternal national health survey assessing effect of low birth weight in a large population demonstrated association to asthma diagnosis by age 3 years (146). Low birth weight (<2500g) was associated with higher risk of wheeze in Jewish teenage males of 17 years (147). Further support for this was provided by a study reporting low birth weight and lower weight gain in early childhood with reductions in adult lung function (148). However these associations have not been reported in prospective studies of TCRS or by the Dunedin Cohort. A twin cohort study from Sweden was also able to demonstrate significant association between early life low birth weight and asthma but only for monozygotic twins (149). Emergency caesarean section and pregnancy related complications have also been implicated to demonstrate higher risk of development

asthma (143, 150, 151). However most of these studies have observed these associations in children, and evidence for adolescent period is lacking.

Early Life Smoke Exposure, Smoke Exposure During Pregnancy and Social Class

Effect of maternal smoking during pregnancy and in infancy has been considered by most of the surveys and cohort studies. Cigarette smoke exposure from pregnancy onwards is known to affect the unborn child by raising the likelihood of developing childhood asthma (103). There is reasonable evidence with regards to early life tobacco smoke exposures whether intra-partum, or over early childhood for wheeze and asthma (152-154) however the effect of this on adolescent onset asthma and early adulthood has not been reported. One prospective hospital based study on children <2 years, has reported indirect association of early life smoke exposure both active and passive for asthma at age 17 – 20 years (155). The association of smoke exposure in TCRS was observed only in children of mothers with lower education, but not in mothers of higher social stratum (96), whereas in another study this was association was significant only for the non-atopic asthma phenotype (81).

Pet Cat or Dog

The ownership of pets with indoor exposures has also been observed to be associated with conflicting evidence for asthma. TCRS reported a protective effect of indoor exposure to dog but not for cats towards frequent wheeze only in those children that did not have parental history of asthma (63). Recent review of all of the studies reporting exposure to furry pets and asthma revealed that the overall risk was higher for asthma but was lower for cat exposure (156). We have in our cohort reported protective effect of early life cat exposure (115), but dog exposure was associated with increased risk for childhood wheeze (111).

1.11.2 Adolescent Factors

Smoking and Asthma: Is the Association Real?

Active tobacco smoking is reported to be associated with incident asthma over adolescent period into adulthood (153, 157). However, current evidence suggesting increased risk of active tobacco smoke exposure for adolescent onset asthma should be interpreted with the caution as early life asthma status was determined

retrospectively for incident asthma at the age of 8 - 10 years by parental report of wheeze (153). Both active and passive smoking have been shown to be associated with asthma (158, 159). Bronchial hyper-responsiveness and strength of association of incident asthma is proportional to amount of cigarette smoke exposure (160, 161). Higher IgE levels were observed in asthmatics that were active smokers compared to never smokers (162). Interaction between atopy and smoking were seen in those with onset of current wheeze during adolescence with decreasing plasma levels of alpha-1-anti-trypsin levels (160). Some suggest active smoking is relevant for only asthma severity and not for developing asthma over adulthood (163). A large cross-sectional survey of teenagers in US identified a significant proportion of wheezy adolescents female smokers of low socio-economic status were lacking asthma diagnosis (122). TCRS also only observed the association of maternal smoke exposure to be a risk only in a sub-set of children of lower social class and that too for wheeze and not asthma (164). The evidence for increased risk of active smoking and adolescent onset asthma is lacking from cohort studies.

Paracetamol Use

Evidence suggests that consumption of paracetamol increases risk of developing asthma in all ages (165-167). Paracetamol as a medication is commonly used in children as young as neonates for the control pain and fever. However this drug is known to affect the antioxidant glutathione thereby predisposing individuals to oxidative tissue injury (168). Paracetamol has not just been observed to be associated with risk of developing asthma but has also been observed to be significant for COPD along with reduced spirometric lung function (169). Use of paracetamol may predispose the lung to environmental insults.

Physical and Lung Growth

One of the most significant changes taking place during puberty is the acceleration in lung function (170). Increase in lung function initially follows a steady increase in height. This linear association with height is observed in both male and females until puberty (171, 172). The young female child has a higher FEV1/FVC than the male child, (173) demonstrating the fact that males have smaller airways than females. This condition reverses and airways become larger in boys than in girls from puberty (174). A discontinuous pattern of increase in lung function with increasing age is observed with the onset of puberty in males with linear increases followed by a sudden pubertal rise and a further increase with height which was more marked than before puberty. The non-linear pattern of lung function was eliminated after correcting for varying thoracic dimensions in males (175). This demonstrates that there are

differences between male and females in the physical development affecting lung function during this period of life. Lung growth and development in females ceases immediately after menarche whereas in males it continues until late into puberty (176). Most of these findings have been observed in cross-sectional studies that were conducted to provide pulmonary function reference data for the adolescent age group.

Tanner and colleagues have been studying the impact of puberty on various aspects of growth and development (177, 178). They have developed models for staging of puberty with respect to chronological age taking gender into consideration, along with variations observed within these categories. This switching phenomenon of puberty occurs earlier in girls at skeletal age of 11 years and in boys two years later at the age of 13 years (179). Growth in height from birth follows a curvy-linear relationship, with rapid rise in height and weight for the first two years of life, followed by deceleration around the age of toddler. A steady growth both for male and female takes place until the growth spurt is reached, heralded by the onset of puberty (180). There is considerable variability in the timing of puberty, several factors are known to affect onset of pubertal changes, these include “genetic, general health, nutritional, environmental and socio-economic relate”(179).

The onset of sexual maturation in girls marked by thelarche, followed by adrenarche and pubertal growth spurt coincides with breast stages of 2-4, which peaks just before menarche that occurs at average age of 13.5 years in Caucasian female (177). Menarche marks the deceleration of growth, averaging at the skeletal age of 15 years and signalling the completion of pubertal development (179). There is close correlation of maximal deceleration in height growth and menarche (177). The two events, dates of which can reliably be ascertained are menarche and breast development (177). The age of onset of menstruation as a reliable time point of puberty in females that is considered to be resistant to recall bias (181). In boys onset of pubertal development occurs two years after the average time of onset in the female (178). The sequence of events in boys begins with testicular enlargement followed by adrenarche, growth spurt and other events, such as facial hair growth (178). Change in voice and development of genitalia with sexual hair growth continue simultaneously (179). In boys genitalia begin to develop before the appearance of pubic hair and attainment of peak height velocity. The peak height velocity follows pubic hair development and corresponds to Tanner stage G4 in boys (178, 182). Puberty follows specific sequence of events and to document the timing of these events we are using pubertal developmental scale (PDS), a validated questionnaire that is considered appropriate for a rough assessment of pubertal status. The validation study for PDS reported a strong correlation of self-reported pubertal status for the peak growth (182).

The ages of peak weight velocity to peak height are closely related, being higher for boys than girls (183). The relationship that is observed for the maximum height velocity to age is not repeated for weight (183). There is also difference in the pattern of weight gain between the gender and this is suggested to be governed by fat gain in girls, and by muscle in boys (183). The spirometric lung function is known to be proportional to height (173, 184). There is a sudden increase of this linear growth in lung function that is seen during puberty that coincides with pubertal growth spurt (173) .

Obesity and Asthma

Obesity has been observed to be associated with asthma across various study designs, with cross sectional studies (185, 186) demonstrating a parallel increased prevalence with asthma and further causal relationship being identified across longitudinal studies (58, 187). Obesity has been keenly studied with respect to asthma pathogenesis. Both asthma and obesity share underlying inflammation as an essential pathological entity, (188) providing support for the argument for its consideration as a potential factor in the causality pathway for asthma. Some studies have shown that this association is stronger in females than in males (55, 186) whereas others have refuted this finding in their study (189). The majority of the studies have utilized body mass index as a marker for obesity with values of $>25 \text{ Kg/m}^2$ to class overweight and $>30 \text{ Kg/m}^2$ as the level of obesity. Some of the studies have demonstrated the strength of association of asthma proportional to the body mass index in a dose dependent manner (186). Evidence implicating obesity is further substantiated by an increase in incident asthma with an increase in body mass index (187), although this was only observed in the females in the group. A prospective study has shown inverse relationship of BMI to FEV1/FVC ratio (55). There is lack of data on airway responsiveness and not many longitudinal studies have demonstrated this effect with the exception of a few, (185, 189) but this association has not been consistent (190). As the body of evidence implicating obesity to be a significant contributory factor for the development of asthma has been growing we will assess its association with the phenotypic groups in our birth cohort by reviewing data at 10 years of age and documenting changes such as body mass index during adolescence.

1.11.3 Airway characteristics

In addition to early life and adolescent factors, we will also study two important characteristic those are attributed to asthma although not exclusive to it. Inflammation and bronchial hyper reactivity also contribute to heterogeneity observed in asthmatics.

1.11.4 Fractional Exhaled Nitric Oxide (FeNO) and its Relation to Asthma

There has been increasing interest in fractional exhaled nitric oxide (FeNO) as a potential non-invasive marker for asthma, after reports suggesting its link with chronic inflammatory diseases of the lungs, particularly those that involve the airways such as asthma and COPD (191, 192). Studies have demonstrated highest levels of FeNO to be associated with steroid naive, mild asthmatics and those having difficult asthma (193, 194). Asthmatics on oral steroid treatment have higher FeNO levels than patients on inhaled corticosteroids (193). Further evaluation into effects of asthma on FeNO levels are related to symptom frequency and with increased requirement for rescue beta agonist use (193, 195). Studies have supported FeNO as a sensitive marker for the management and monitoring of asthma but recent studies have suggested otherwise (196). It has also been suggested to aid in the diagnosis of cough variant asthma (197-200). The FeNO varies with pollen season in allergic asthmatic patients, with raised values corresponding with increase in asthma symptoms but without changes in lung function (195). Eosinophils and neutrophils are the two main types of cells seen in the asthmatic airway (70). The link between raised FeNO levels with sputum eosinophil counts in asthmatic patients has been observed (201) and is associated with a phenotype characterized by atopy with increased bronchial hyper-responsiveness but with little contribution of asthma (202). Large values of FeNO have been observed in patients with asthma irrespective of atopic status whereas modest levels were seen with airway eosinophilia (203). Gender difference has also been noted with high FeNO values in the females in childhood, this is reversed in adulthood with male predominance. This gender reversal contrasts with asthma prevalence (202). There has been considerable interest in FeNO, with studies identifying various associations. Majority of these findings have emerged from clinical setting or through community based studies with relatively small samples that are prone to lack sufficient power to assess the various interactions, between factors associated with FeNO. One study, that was community based with reasonable sample size, did find atopy to be highly significant as a confounder for raised FeNO in asthmatics, highlighting the atopic undertones that were identified by earlier clinical studies (204). This current evidence is suggestive of close link of FeNO to allergic status and thereby with asthma.

1.11.5 Bronchial Reactivity and its Relation to Asthma over Adolescence

Bronchial responsiveness is a non-specific characteristic of bronchial smooth muscle to contract in response to a variety of stimuli. Bronchial hyper-responsiveness (BHR) is a term used to describe excessive response of airway smooth muscles to various stimuli. Studies have observed a consistent association of BHR with asthma and wheeze (52, 205, 206) during childhood. Those with moderate to severe BHR have clinically significant morbidity (207, 208). However not all asthmatics have BHR, furthermore BHR is also observed in healthy children without asthma. Therefore, the association of this characteristic or lack of it has not been clearly understood. In a general population survey of ECRHS, prevalence of BHR varied from 3 to 28% (209, 210) and was not restricted to English speaking countries, which has been a consistent finding in questionnaire based asthma prevalence studies (14, 24).

The non-specific nature of BHR, along with poor reproducibility over short periods (206) limits its value as a diagnostic clinical tool for asthma. Epidemiological studies have reported BHR to reliably predict persistence of asthma (211) and have used the feature of excessive responsiveness of airway smooth muscle to characterize asthma phenotypes (212). Individuals with rhinitis have also demonstrated increased bronchial responsiveness in the absence of asthma (213, 214). BHR is also observed in patients following respiratory tract infections (215, 216) and in smokers (217), suggestive of its association to inflammation of airways. Sputum (213, 218) and blood eosinophilia (214) are both associated with BHR and so is atopy (206, 217, 219). Increased bronchial responsiveness has been demonstrated to be correlated with sub-epithelial fibrosis (airway remodelling) (220) this may partly explain the observed association severe BHR to persistence of asthma (211). Bronchial responsiveness is observed to decrease over time, which is highest during early childhood and decreases over adolescent period (99). There is conflicting evidence with regards to non-asthmatic children with BHR for developing asthma later in life (221, 222). Only few prospective of the cohort studies have performed repeated bronchial challenge tests (99, 223) and relation of bronchial responsiveness for adolescent onset asthma is not reported in the current literature.

1.12 Unanswered Questions about Asthma

Evidence from earlier cohort studies suggest influence of early life factors for asthma and wheeze into adolescent period. The bulk of evidence has been observed from two prospective studies that have largely focussed on factors for early life wheeze into adolescence and abstained from characterizing adolescent onset asthma or rather restricting it based on early life wheeze pattern. Whereas the contribution of early life

factors by other longitudinal studies is inconsistent. Intrigued prospectively collected information on wheeze and asthma with the dynamics of adolescent period, and prospect of impact of metamorphosis of children into young adults, we wanted to explore role of adolescent factors of growth and environment accounting for the phenomenon of growing out of childhood asthma that is observed over adolescence. We want to investigate what constitutes and predicts adolescent asthma and wheeze. In addition our aim was to assess the airway characteristics for adolescent asthma phenotypes and determine tracking of lung function over this period as suggested by earlier cohort studies.

Specific areas for clarification are

- Characterize and determine risk profile for adolescent wheeze phenotypes
- Document the transition patterns of asthma in the context of gender and atopy over 10 to 18 years determining trends from birth
- Determine the characteristics of adolescent onset asthma and assess risk pattern in particular relevant contribution of early life and adolescent risks
- Explore the characteristics of remission of childhood asthma and identify prognostic markers for this phenotype based on significance of early life and adolescent period.

This thesis draws upon data gathered from birth well into young adulthood. The characteristics of this cohort has been presented as given in table 1; in brief, children were recruited at or right after birth, enabling detailed prospective characterisation of early life factors and documentation of physical measures. The children were regularly followed up and characterised over first few years of life up to age 4 and then at 10 years. Availability of comprehensive data over the entire cohort in particular over the adolescent period enhances our ability to determine relevant contributions of each individual component contributing towards asthma over this period, including early life factors.

1.13 Summary of the Specific Aims of this Thesis

This thesis seeks to explore the relevant influences of childhood and adolescent factors towards adolescent asthma phenotypes; with a focus on early life factors (pregnancy to first 4 years of life) and contribution of adolescent factors with a focus on physical and pulmonary growth and impact of adolescent environment (personal tobacco smoking, alcohol consumption, level of activity and paracetamol consumption). The study participants were teenagers of an unselected, prospectively recruited birth cohort. This cohort was first established to study atopic diseases of

asthma, rhinitis and eczema. Information on the cohort was collected with recruitment at birth and yearly follow-up in the first 2 years of life, with additional follow-ups at 4, 10 and 18. The main body of this study uses more detailed data collected from a subset of these children at 10 and 18-year follow-ups and seeks to characterize adolescent wheeze and determine risk patterns for adolescent onset asthma and remission of childhood asthma.

The specific objectives are to:

1. To characterise and quantify adolescent wheeze phenotypes and determine risk pattern profile of un-diagnosed asthma in relation to diagnosed asthma with a hypothesis that factors which lead to under-diagnosis may differ based on severity and nature of risk (early life and adolescent).
2. To test the hypothesis that the early life factors continue to influence asthma and wheeze at 18 (considering relevant contribution of atopy at different time points and effect of gender)
3. To examine the hypothesis that physical and pulmonary growth affects remission of asthma symptoms over adolescent period, particularly in boys, whilst lack of atopy favours remission.
4. To test the hypothesis that adolescent onset asthma is a different phenotype not only with respect to clinical severity but also with respect to risk factor profile from that of childhood persistent asthma (particular focus on adolescent specific factors in relation to those that are observed in childhood mostly).

CHAPTER 2: METHODOLOGY

2.1 Study Design

At an 18 year follow-up of a prospectively studied whole population birth cohort based on the Isle of Wight, the clinical phase was undertaken during the first 29 months with methacholine bronchial challenge tests and sputum inductions completed over an additional 12 months.

2.2 Study Centre

The clinical part of the study was based at the David Hide Asthma and Allergy Research Centre at St Mary's hospital on the Isle of Wight. The Isle of Wight is an island, 23 miles by 13 miles, off the south coast of England. It has a population of 132,731 according to the 2001 census.

2.3 Selection of Study Participants

Inclusion Criteria: All children (1536) that were enrolled at birth in the Isle of Wight birth cohort in 1989/1990.

Exclusion Criteria: There were no exclusion criteria except those who declined to participate in the study.

2.4 Ethics

This protocol and amendments were submitted to an independent Ethics Committee for formal approval of the study conduct (appendix 19). Approval was granted on 21 June 2006 from the (REC 06/Q1701/34) with first study participant enrolled on 4 July 2006.

Before the adolescents agreed to participate in the trial, all subjects were provided with sufficient information in the participant information sheet (Appendix 1) to allow them to give informed consent. The participant information sheet document was approved by the Ethics Committee along with the study protocol.

2.5 Subject Recruitment

The majority of the subjects in the 1989/90 cohort remain on the Isle of Wight or in the United Kingdom. Contact details of the entire cohort are held at The David Hide Asthma and Allergy Research Centre (DHAARC). Letters of invitation for participation in the study were sent out to all study subjects for whom contact information was available on NHS data base and at DHAARC. They were given details of the proposed study and with their consent, we arranged a suitable time for them to be seen at the Centre. If a subject consented to take part in the study but was unable to attend the clinic, the questionnaires were completed over telephone. Where a subject did not respond, one further letter was sent out. Where a subject had provided us with their telephone details, we made one final attempt to contact them by telephone before labelling them as missing for the follow-up at 18 years.

2.6 Clinical Assessment

Subjects were assessed in a standardised order: questionnaires, physical examination, skin prick testing, exhaled nitric oxide measurement, spirometry, blood samples and methacholine challenge test. A number of subjects were asked to return at a later date for methacholine challenge test and sputum induction (Figure 2.1).

2.7 Questionnaires

Up to five short questionnaires were completed. All of these were interview based questionnaires, with the exception of an additional 17 year questionnaire (appendix 11 that was self-administered. Participants were asked to complete some with their parents as adolescents at this age tend to underestimate or understate their symptoms.

ISAAC questionnaire (interviewer based): These were completed by all enrolled participants. This is a standardised questionnaire, which has been widely used in prevalence studies of asthma and allergy across the globe (12) (see appendix 5). This questionnaire was also used at the 10 year follow-up; this was filled in by research nurse/research fellow on an Access (Microsoft Office, 2007) data base. All nurses and the research fellows familiarised with the format of administering the questionnaire by mock sessions arranged at the Centre by the senior research nurses (Sharon Matthews and Bernie Clayton for standardised way of registering the response fields for the questionnaires).

Symptom questionnaire (interviewer based): This was only completed by participants with allergic problems since 10 years of age. This questionnaire (see appendices 6 to 9) has been used throughout this study since the first follow-up at age 1 year. This is a more detailed questionnaire, seeking information on not only presence, but also morbidity in terms of symptoms, level of medication, and number of exacerbations for these diseases. The purpose of using this questionnaire was to be able to compare prevalence and severity of these diseases at different ages in this cohort. Again, it was completed in an Access database.

Additional 17 year questionnaire (Self-administered): This was completed by all participants. After completing the quality of life questionnaire, adolescents were given the opportunity, away from their parents, to complete a confidential questionnaire about their own smoking habits (Appendix 11). They were also asked to complete the validated Pubertal Development Scale (PDS) (182). Both of these questionnaires were filled in a written format on a paper form and were then separately entered in the data base after the completion of the study visit. The PDS scale was modified by addition of a question on the year of onset for each item (appendix 12).

Paediatric asthma quality of life questionnaire (interviewer based) (224): This was completed by subjects with asthma. This was a validated questionnaire (see appendix 10) to determine how much asthma interferes with daily life activities such as education, physical activities. This was completed on the Access database.

Dermatology Life Quality Index (interviewer based) (225): This was only completed by subjects with active eczema (symptoms over last week). This was a short validated questionnaire (see appendix 13) to determine how much eczema interferes with daily life activities such as education and physical activities. Participants were asked to complete this without help from their parents. This was completed on the Access database.

2.8 Study Definitions

2.8.1 Cross-sectional Definitions

Asthma: Defined based on study participants answering yes to question 'Have you ever had asthma' and either 'have had wheeze or whistling in the chest in the last 12 months' or 'current use of asthma medication'.

Non-asthma-wheeze (NAW): Included all study participants that could recall frequency of wheeze over last 12 months and responded positively to 'have had

wheeze or whistling in the chest in the last 12 months' but did not have asthma diagnosed by a doctor.

Rhinitis: Defined based on a yes response to 'have you ever had a problem with sneezing or a runny or a blocked nose when you did not have a cold' and 'In the past 12 months have you had a problem with sneezing or a runny or blocked nose when you did not have a cold or the flu'.

Eczema: Present if study participant responded positively to 'have you ever had eczema' and to 'have you had this rash at any time in the last 12 months'.

2.8.2 Prospective Asthma Categories

Persistent asthma: A study participant was considered to have persistent asthma if he had asthma at both 10 and 18 years.

Remission asthma: Participant having asthma at 10 but not at 18 years.

Adolescent onset asthma: Those fulfilling the definition of asthma at 18 but not at 10 year.

New adolescent onset asthma: asthma present only at 18 but not at any previous follow-up.

2.9 Physical Examination

2.9.1 Height, Weight and Blood Pressure Measurements

Subjects were examined by a trained research nurse/clinical research registrar to detect the presence of eczema or wheeze. A stadiometer (PM Supplies, health care services, Rookley IW 721400) was used to measure height and weight was measured using by electric scale (Seca alpha Model 770 Germany) by research nurse/clinical research registrar using standard protocol (appendix 14).

2.9.2 Skin Prick Tests

Skin prick testing (SPT) was performed by experienced nurses using standard technique and the same protocol was used at the 10 year follow-up (108). A panel of 14 common allergens were tested comprising house dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix, tree pollen mix, cat and dog epithelia, *Alternaria alternata*, *Cladosporium herbarum*, milk, hen's egg, soya, cod, wheat and peanut plus histamine and physiological saline was used to act as positive and negative controls, respectively (Alk-Abello, Horsholm, Denmark). Allergen skin test reaction with a mean wheal diameter of at least 3 mm greater than the negative control was regarded as positive after 15 minutes (10 minutes for positive control) and the subject defined as atopic. Atopy was defined by at least one positive reaction to the panel of allergens tested.

2.9.3 Blood Samples

If requested, a local anaesthetic cream (EMLA) was applied before taking blood. A total of 20 ml of blood was taken for the following tests:

- Serum total IgE
- Serum leptin

Ethical approval was obtained for any future study using these specimens. These samples were stored at -70°C at the research centre for the duration of the study and following completion were transferred to the tissue bank at University of Southampton.

2.9.4 Urine Collection

Similar to blood samples storage, urine sample was stored at the DHAARC at -20°C.

2.9.5 Exhaled Nitric Oxide Measurement

Exhaled nitric oxide levels were measured using the single expiratory breath method using the ATS/ERS guidelines (226). Measurements were made in a standardised manner using a hand held Nitric Oxide analyser (227, 228) with the

subject standing without a nose clip, with continuous steady blow recorded by the hand held device for 6 seconds following a standard procedure of inspiration through the device followed by expiration through it for a duration of 6 seconds with steady flow of air regulated by the analyser. If a steady flow for this duration was not recorded the device registered error and this test was repeated till two standard readings were obtained for each study participant. These readings were then entered into the Access data base. All measurements were undertaken before spirometry. The measurements of exhaled nitric oxide from the Niox-Mino hand held analyser is comparable to the conventional standard stationary chemiluminescence unit (228).

2.9.6 Spirometry

We followed American Thoracic Society guidelines to ensure spirometry validity and reproducibility (229). As recommended, the highest of three FEV_1 measurements within 5% of each other were used. The Koko system (Koko Spirometer manufactured by nSpire Health, Inc. 1830 Lefthand circle, Longmont, CO 80501 USA) was used. To perform this test the study participants were required to be free from respiratory infection for 14 days, not taking short acting β_2 -agonist medication for 6 hours, long acting β_2 -agonist medication for 12 hours and abstain from caffeine intake for at least 4 hours. We recorded forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), mid expiratory flow (MEF), peak expiratory flow (PEF). Percent predicted for age, height, sex and ethnic origin were calculated for the above data and forced expiratory ratio (FEV_1/FVC). If subjects did not have a methacholine challenge, spirometry was repeated 10 minutes after 600mcg salbutamol, inhaled via a large volume spacer to document reversibility. Reversibility was defined as $\geq 12\%$ increase in FEV_1 .

2.9.7 Methacholine Test

The methacholine test protocol (Appendix 17), previously utilised for the 10 year follow-up of this cohort, was followed. This was a standardised protocol, as recommended by the American Thoracic Society, (230). This was undertaken by a trained, experienced research nurse and registrar. A computerised dosimeter system (Koko Digidoser, PDS Instrumentation, Louisville, USA) was used with compressed air source at 8 L/minute and nebuliser output of 0.8 L/minute. A pre-test spirometry reading was obtained to ensure an FEV_1 of above 70% predicted for age and height. Initial inhalation of 0.9% saline was followed 1 minute later by spirometry recording to obtain a baseline value. If the FEV_1 dropped by more than 10% with saline, the challenge was to be deferred for an hour or rescheduled for another day; if it dropped

by more than 15%, the test was postponed. Subsequently, incremental concentrations from 0.062 mg/mL to 16 mg/mL of methacholine were serially administered using the methods of Chai and co-workers (231).

To perform this test, adolescents were required to be free from respiratory infection for 14 days, not taking a course of oral steroids, not taken short acting β_2 -agonist for 6 hours, and long acting β_2 -agonist for 12 hours, and needed to have abstained from caffeine intake for at least 4 hours.

We used both categorical and continuous measures for evaluating bronchial responsiveness. The categorical measure was used to define bronchial hyper-responsiveness (BHR). A concentration causing a 20% fall in FEV_1 from the post-saline value was interpolated and expressed as $PC_{20} FEV_1$ (provocative concentration causing a 20% fall in FEV_1). The concentration of $\leq 8\text{mg/mL}$ was used as a cut-off for defining bronchial hyper-responsiveness (BHR) based on earlier population based studies including our intervention cohort study (51, 55, 232).

A continuous measure of dose-response-slope (DRS) was also applied so that all adolescents could be included in the analysis even if their FEV_1 did not drop by at least 20% with the final dose of methacholine; details of determination of DRS are given in the statistical analysis section.

2.9.8 Sputum Induction

At a separate appointment, we aimed to collect induced sputum from twenty subjects from 4 clinical groups as defined below:

1. Persistent asthma group with asthma at ages 10 years and 18 years
2. Asthma remission, having asthma at age 10 years, but no symptoms, medication use at 18 years
3. Adolescent onset asthma, asthma at age 10 years with no symptoms or treatment used for 12 months at 18 years
4. Control group who have never wheezed

An appointment letter and instructions were sent out to the subjects selected for sputum induction. The visit lasted approximately 40 minutes and no food was permitted to be eaten for 1 hour beforehand, and participants had to be free from respiratory infection and not taking any oral steroids. A standard protocol was used (233)(Appendix 16). After informed consent and explanation of the procedure,

baseline exhaled nitric oxide, spirometry and peak-flow was performed, followed by administration of 400mcg salbutamol with spirometry repeated after 10 minutes. The research registrar supervised sputum induction. If FEV₁ was $\geq$ 60% predicted, participants received serial nebulisation of hypertonic saline (4.5%) for 5 minutes up to a maximum of 20 minutes (5 x 4). Subjects were asked to reschedule their appointment if they develop an infection within 2 weeks of the date of their appointment, if the post bronchodilator FEV₁ was below 60% predicted or if induction is unsuccessful. If FEV₁ remains below 60% predicted on a second visit or the induction unsuccessful a second time, the subject was to be replaced within the same group. Samples were immediately placed on ice and processed within 2 hours for cytology. Total and differential cell counts (epithelial cells, lymphocytes, eosinophils, basophils, macrophages and neutrophils) were recorded. Supernatant was stored at -70°C for later analysis for mediators and cytokines.

Sputum results are presented as continuous variables. The cellular composition are presented as percent of inflammatory cell type (n) to that of total cell count (N), Eosinophilic component has been given in both continuous % as well as categorical data with 3% used as a cut-off for defining eosinophilia. There was considerable variation in threshold for eosinophilia, we reserved a value of >3% cell count of eosinophils as a cut-off based on greater observation of symptomatic asthma (234).

2.10 Laboratory Investigations

2.10.1 Urinary Cotinine

For further confirmation of exposure to tobacco smoke, cotinine was measured in urine samples using ELISA (ELx 800 BKT Biotek absorbance microplate reader, USA).

Blood samples were stored at human tissue bank at University of Southampton for later use.

2.10.2 Sputum Cell Count

Sputum slides that were prepared at the DHAARC were transported to Southampton University for cell counting on Nikon Multi-head microscope for cell counting by two research fellows simultaneously performing cell count. This process was performed manually and calculations were made on cell counter by one observer with the other observer noting and identifying the cell types. Cell types that were not

mutually identified by both observers were not included in the total cell counts.
Protocol given in appendix 15.

Figure 2.1 Overview of 18-year follow up study of 1989/1990 Isle of Wight Cohort

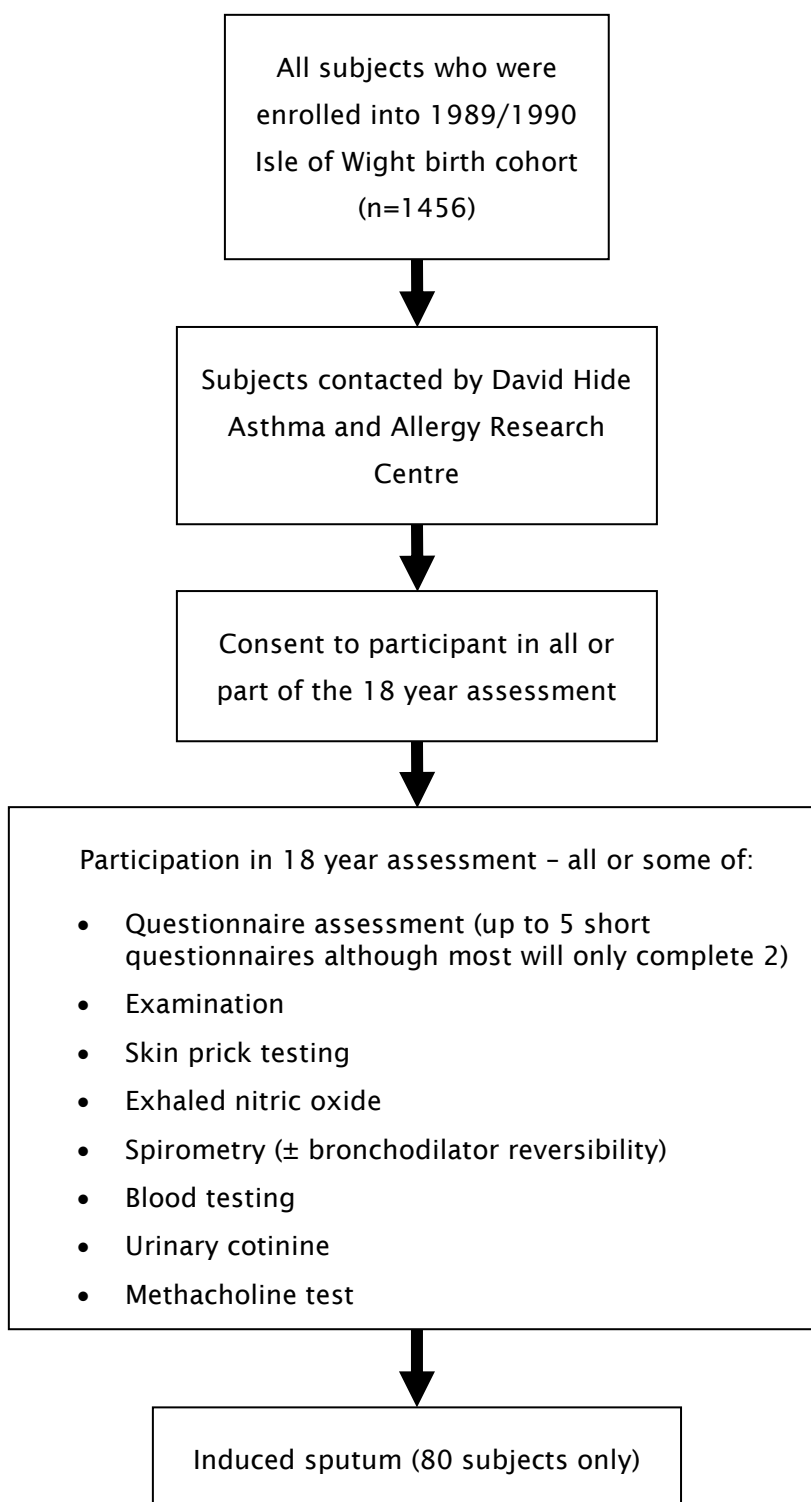


Table 2.1 Which Subjects will Undergo which Study Procedures

Study produces	Subject Categories	
	Participants able to visit the DHAARC	Participants unable to travel to the DHAARC
Informed Consent	✓	✓
Questionnaire	✓	✓(telephone)
Examination	✓	×
Skin Prick Testing	✓	×
Exhaled Nitric Oxide	✓	×
Spirometry (± bronchodilator reversibility)	✓	×
Non-genetic Blood Test (serum IgE, leptin)**	✓	✓/× (may not be possible to obtain blood from subject)
Genetic Blood Test**	✓/× (not all will wish to donate a blood specimen)	✓/× (may not be possible to obtain blood from subject)
Saliva or Buccal Swab for Genetic Tests (if unable to obtain blood sample)	✓/× (Only if unwilling to provide blood specimen)	✓/× (Only if unwilling or unable to provide a blood specimen)
Urinary Cotinine	✓	×
Methacholine test	✓	×
Induced Sputum (only 80 subjects)	✓/×	×

2.11 Data Management

A large volume of data that was generated in this project was managed by automated data management. Data management follows a similar pattern to that was successfully used previously. Current name and address was entered into an Excel database on the password protected NHS server. An Access database (Microsoft office 2007, USA), using study number only for identification, was set up. Confidentiality of all samples, interviews, and medical records was assured by a) keeping all records under lock and key, b) separating data from names, c) keeping the linkage study numbers under lock and key, d) allowing only study staff members to have access to

the data, e) keeping identifiers of individuals out of public material and reporting only aggregated data.

Research participants were seen at The David Hide Asthma and Allergy Research Centre where all data was gathered, processed and stored. During the study visit, the research staff reviewed completed paper records to resolve any discrepancies. All digital forms were printed following completion of the study visit. Computer printouts were produced for test results. The paper records and printouts are maintained within the Centre together with those from the 1, 2, 4 and 10 year follow-up and are instantly available as reference for any queries.

Results of biochemical analyses produced in the laboratory were entered into Excel data sheet, including study and laboratory number and date, and transferred to The David Hide Asthma and Allergy Research Centre. The data was double entered and checked for consistency. This was then linked to the anonymised data set for future statistical analyses.

2.12 Data Cleaning Process

The data cleaning process began with visual consistency check on the Access database by Dr Graham Roberts and Dr Ramesh Kurukulaaratchy. They had examined 400 hundred study cases before the task was assigned to Dr. Abid Raza. A further review of the remaining 913 study cases was performed. All inconsistencies were noted and discussed on fortnightly basis with Dr Roberts. The database directly from Access database was exported into SPSS by export function. An analysis plan was developed after the visual consistency check was done to evaluate all of the dependent fields to see appropriateness of the response. Frequency checks for all of the variable fields were done. Cross tabs for all of the dependent field responses were performed. Any discrepancy noted, was reviewed and all queries were discussed with the concerned person responsible for filling in the form. Any information that was not clear was documented as a "Missing response" and changes were noted in an excel sheet.

2.13 Statistical Methods

In our preliminary descriptive analyses we assessed characteristics for the outcome variables such as asthma and wheeze at 18. All of the outcome variables were categorical. Independent variables were either continuous or categorical. For continuous independent variables such as immunoglobulin (IgE) and force expiratory volume in first second (FEV₁) sample data distribution was assessed in order to use appropriate statistical for significance at level of $p < 0.05$. Data mean with standard

error (S.E.) are given for normally distributed data, whereas data that is skewed, median with 25th and 75th percentile or geometric mean with standard deviation (SD) has been reported, e.g. number of times per month usage of paracetamol/NSAID has been reported with median values accompanied by 25th and 75th centiles. For categorical data percentage proportions are provided where n = participants with exposure and N= total participants that had provided the information on the variable.

For binary variables, for proportions, z-test of proportions was used and for independent sample categorical data such as proportions of atopy or exposure to tobacco smoke between two groups, Chi-square test was used. This test is based on observed verses expected counts of membership to each cell of a 2x2 contingency table. Membership to each cell was exclusive. Validity of the test was affected if any of the expected cell counts of the 2x2 contingency table has counts of <5. Fischer's Exact Test was utilized where Chi-square test was limited by cell count. Independent samples t-test was used for normally distributed data, with significance determined at $p < 0.05$. Where distribution of normality was violated non-parametric tests were applied (For binary variables Mann-Whitney-U test and Kruskal Wallis test for multi-nomial data).

For prospective groups, where multiple groups were assessed for between group effects for continuous variables, one-way analysis of variance (235) test was used. This test is based on ratio of two variances. Bonferroni's multiple comparison correction was applied to adjust for by chance occurrence of statistical significance in multiple comparison testing.

Odds ratio have been reported for effects of exposure variables towards the outcome categorical data of asthma and non-asthma-wheeze at 18 years, and for prospective asthma groups of remission of childhood asthma and new adolescent onset asthma by applying Multinomial logistic regression models with significance determined at $p < 0.05$. Odds ratio represents probability of an exposure influencing an outcome in relation to probability of similar outcome in un-exposed individuals. A value of 1, is indicative of non-difference in outcome based on exposure. An odds ratio of 2 is suggestive of increased probability of an exposure leading to a specific outcome e.g. asthma.

A linear line (least square regression line) was obtained by linear regression analysis for spirometric FEV₁ drop in lung function from baseline with each successive incremental dose of methacholine administered. The gradient of linear regression line that was obtained is referred to as dose-response-slope (DRS). A DRS was obtained for each study participant and was plotted to assess normality and homoscedacity of distribution. Lack of normality that was observed required transformation. A transformation of Log₁₀ (DRS+ 10), was required to satisfy the distributional

assumption of normal data. A higher positive value of this transformation signifies greater bronchial reactivity.

Generalized estimating equation model was used to determined prospective data on asthma prevalence over time as well as atopic sub-sets of asthma with significance determined at $p < 0.05$.

CHAPTER 3: DEMOGRAPHICS AND CHARACTERISTICS OF 18 YEAR FOLLOW-UP

Isle of Wight birth cohort is comprised of 1536 children that were born on the island between 1 January 1989 and 28 February 1990. At the age of 18 year, 1456 teenagers were available for follow-up. There were 80 study participants that were not available. Of the 1456 that were available, 1313 (90%) participated in the 18 year follow-up (Table 3.1a). The cohort has been followed up at the ages of 1, 2, 4 and 10 years with 1316 (86.9%), 1205 (79.8%), 1218 (83.7%), and 1373 (94.3%) study participants enrolled respectively.

3.1 Assessment of 18 Year Participation

Of the 90% (1313/1456) adolescents providing at least questionnaire based information, 65% (864/1313) visited the research centre for full-study related procedures with exception of sputum induction and methacholine challenge tests. A total of 64% (842/1313) skin prick tests (SPT) were performed, while fractional exhaled nitric oxide was measured in 64% (834/1313) individuals. Spirometry with bronchodilator reversibility, in those who were eligible and consented, was performed in 64% (839/1313) subjects. At a separate visit methacholine lung challenge test was performed in 45% (585/1313) study participants. A subset of 6% (80/1313) adolescents participated in sputum induction procedure.

Original Cohort (1536) ---- Dropouts over 18 years (80) ---- Total Available study participants at 18 year (1456)

The total number of study participants “enrolled” at 18 years was 1313. A total of 1456 were available for follow-up at 18 years from a birth cohort of 1536, after exclusion of 80 study participants that dropped out of the study. Therefore a follow-up of 90.2% (1313/1456) was achieved at 18 year. Details on factors accounting for non-participation are given in table 3.1a. Table 3.1b further elaborates reasons for 80 study participants that dropped out of the study. The distribution of data by gender on participation is presented in order to assess selection bias.

Table 3.1a Gender-wise Reasons for Non-enrolment at 18 Years

Gender-wise distribution on status of participation	Overall	Male % (n)*	Female % (n)*
Contacted but failed to take part	1 (19)	1 (9)	1 (10)
Not traceable	9 (124)	10 (75)	7 (49)
Continued participation	90 (1313)	89 (653)	92 (660)
Total available for 18 year follow-up (N)	100 (1456)	100 (737)	100 (719)

Gender-wise comparison were made using z-test of proportions with significance determined at $p < 0.05$. Total numbers of participants available at 18 year were 1456.

* n = number of participants in each group with (%) = percentage given for each group total represented by N

Table 3.1b Gender-wise Reasons for Dropout at 18 Years

Gender wise reasons for Dropout	Overall	Male % (n)*	Female % (n)*
Died	29 (23)	27 (13)	32 (10)
Refused 18 year follow-up	29 (23)	27(13)	32 (10)
Adopted and drop out	6 (5)	8 (4)	4 (1)
Consent withdrawn - drop out	36 (29)	38(19)	32(10)
Not- available, %(N) †	100 (80)	100(49)	100 (31)

* n = number of participants in each group with (%) = percentage given for each group total represented by N

† Dropout comprises of all those that died, refused follow-up, withdrew consent or dropped out. Total numbers of participants that dropped out at 18 year were 80.

Details on dates of enrolment are provided in table 3.2. The overall duration for the first phase of enrolment lasted 29 months which consisted of collection of questionnaire based data, blood samples, spirometry with reversibility and skin prick testing. The second phase began on 10th of March 2007, this phase comprised of methacholine challenge testing and induced sputum.

Table 3.2 Status of Study Enrolment

Date of First Participant Enrolled (first phase)	4 th July 2006
Date of Last Participant Enrolled (first phase)	29 th November 2008
Mean age of study participants	18 years (SD: 0.63)
Duration of first phase of enrolment	29 months
Total number of study participants enrolled	1313
Number participants visiting in person	864
Number of participants over telephone	421
Number of participants filling postal questionnaire	28
Number completed exhaled nitric oxide	834
Number completed spirometry	839
Number of skin prick test	842
Methacholine lung challenge tests (Second phase):	10 th March 2007
Planned date of study enrolment closure (Second phase):	25 th October 2009
Number of methacholine lung challenge tests:	585
Number of sputum inductions	100 (80 good samples)

The first phase activities ended with last participant being enrolled into the study on 29th November 2008. Males were more likely to provide questionnaire based information whereas a higher proportion of females participated by visiting the research centre, however there was no gender related difference in participation at 18 years (table 3.3).

We also assessed 11 characteristics of the study participants, who participated in this study by visiting the centre and completing all the study related procedures and compared these to those that were only able to provide questionnaire based information (table 3.4).

Table 3.3 Mode of Data Collection

How visit/questionnaire took place	Overall n (%) [*]	Male n (%) [*]	Female n (%) [*]	p-value [†]
In person	66(864)	63(410)	69(454)	0.04
By telephone	32(421)	35(231)	29(190)	0.006
By post	2(28)	2(12)	2(16)	0.42
Total (N)	100 (1313)	100(653)	100(660)	0.82

^{*} n = number of participants in each group with (%) = percentage given for each group total represented by N

[†] Chi-square test for difference between males and females with significance at $p < 0.05$

Table 3.4 Characteristics of Children Seen at 18 Years by Participation

	Full Visit %(n/N)	Questionnaire Only %(n/N)	p-value*
Male	48 (410/864)	54 (243/449)	0.023
High social class	51 (410/802)	57 (215/377)	0.06
Smoke exposure inside house	26 (219/852)	31 (138/439)	0.03
Current smokers	25 (212/853)	37 (156/425)	<0.001
Domestic cat	43 (371/859)	44 (194/441)	0.81
Domestic dog	49 (416/856)	49 (219/444)	0.82
Asthma ever	29 (245/853)	29 (128/443)	0.95
Eczema ever	26 (226/856)	22 (96/442)	0.07
Hay fever ever	37 (318/864)	32 (141/444)	0.08
Maternal asthma	17 (144/847)	16 (68/418)	0.61
Paternal asthma	14 (106/782)	13 (47/375)	0.70

% (n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test with significance at $p < 0.05$

From table 3.4, it is observed that males and smokers were more likely to participate by questionnaire rather than by in person visit to the research centre. Similarly those exposed to tobacco smoke were also less likely to participate by in person visit. However when assessed if more male smokers (28%) were less likely than female smokers (30%) to participate by in person visit, this did not demonstrate any difference between the genders ($p=0.48$).

Review of allergic morbidity of earlier follow-ups did not demonstrate bias for study participation at 18 years follow-up (table 3.5).

Table 3.5 Characteristics of Adolescents not seen at 18 Years

	Enrolled at 18 years %(n/N)	Not enrolled at 18 years %(n/N)	p-value*
Asthma at 1 or 2 years	15 (173/1183)	12 (24/194)	0.44
Asthma at 4 years	15 (156/1041)	15 (25/173)	0.91
Asthma at 10 years	15 (172/1178)	15 (29/190)	0.83
Positive skin prick test at 4 years	20 (167/839)	18 (26/141)	0.73
Positive skin prick test at 10 years	27 (238/896)	29 (41/140)	0.54

%(n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between enrolled and not enrolled at 18 years with significance at $p < 0.05$

3.2 Socio-economic Status of Cohort at 18 Years

3.2.1 Residence

Majority (71%) of the study participants reported living in privately owned homes. There were approximately 11% that were living in privately rented accommodation with 16% reported to be living on benefit housing and a very small fraction reported to use an abode that did not fit into the above category. There were no significant gender wise differences noted.

Table 3.6 Type of Residence

House Type	Overall %(n/N)	Male %(n/N)	Female %(n/N)	p-value
Owned privately	71 (908/1280)	73(467/638)	69 (441/642)	0.08
Rented privately	11 (145/1280)	10 (62/638)	13 (83/642)	0.07
Rented council/housing association	16 (209/1280)	16(103/638)	17 (106/642)	0.86
Other	1 (18/1280)	1 (6/638)	2 (12/642)	0.16

%(n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between males and females with significance at $p < 0.05$

3.2.2 Residing with Parents

Based on the information obtained, a vast majority of the study participants were living with parents (90%). Our analysis showed that higher proportion of males 93% (599/642) versus 87% (559/645) adolescent females ($p=0.014$) were still living with their parents.

Table 3.7 Social Factors of Accommodation, Involvement in Education and/or Work

	Overall %(n/N)	Male %(n/N)	Female %(n/N)	p-value
Living with parents/guardians	90 (1158/1287)	93 (599/642)	87 (559/645)	<0.001
Continuing education	70 (898/1286)	71 (454/641)	69 (444/645)	0.44
Involvement in work	68 (876/1287)	65 (415/642)	72 (461/645)	0.009

%(n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between males and females with significance at $p < 0.05$

Over two thirds of the study participants were still in education and a similar proportion was engaged in some form of work. A higher proportion of females were observed to be involved in some form of work compared to males.

3.2.3 Family Income

All of the study participants were asked questions on family income, less than 50% study participants responded to this field.

Table 3.8 Family Income

	Overall %(n/N)	Male %(n/N)	Female %(n/N)	p-value
> £42,000	19 (104/557)	16 (53/324)	20 (67/335)	0.71
£ 30,000 – 41,999	13 (73/557)	13 (41/324)	13 (42/335)	
£ 18,000 – 29,999	25 (142/557)	27 (86/324)	27 (90/335)	
£ 12,000 – 17,999	26 (143/557)	26 (84/324)	25 (84/335)	
Less than £ 12,000	17 (95/557)	19 (60/324)	16 (52/335)	

%(n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between males and females with significance at $p < 0.05$

3.2.4 Social Class

A large section of the adolescents belonged to families that were from higher social classes. As there were 67% that were either engaged in skilled non-manual work or higher category based on social occupational classification 2000 (SOC 2000). The six categories of SOC 2000 were collapsed into binary variable of Higher (SOC categories of I to III NM) and Lower (SOC categories of III M to V). This division was based on skilled manual and non-manual labour.

Table 3.9 Social class

Social occupational class	Overall %(n/N)	Male %(n/N)	Female %(n/N)	p-value*
Professional (I)	5 (55/1179)	5(27/596)	5 (28/583)	0.69
Managerial & technical (I 57)	42 (499/1179)	44 (261/596)	41 (238/583)	
Skilled non-manual (III NM)	20 (236/1179)	19 (111/596)	21 (125/583)	
Skilled manual (III M)	19 (221/1179)	19 (111/596)	19 (110/583)	
Partly skilled (IV)	11 (129/1179)	11 (63/596)	11 (66/583)	
Un-skilled (V)	3 (39/1179)	4 (23/596)	3 (16/583)	

%(n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between males and females with significance at $p < 0.05$

3.2.5 Pet Ownership

Every 3 out of 4 adolescents owned a pet and girls were more likely to keep pets compared to boys. Also girls were more likely to own a cat compared to boys; however there was no gender difference for dog ownership. Overall pet ownership was not influenced by social class. There was no class difference noted within the gender for pet ownership, similar proportion of pet ownership was present in males/females whether they belonged to low or high social class.

Table 3.10 Adolescent Pet Ownership

Pet Type	Overall %(n/N)	Males %(n/N)	Females %(n/N)	p - value
Any Pet	79 (1040/1310)	75 (491/652)	83 (549/658)	<0.001
Cat	43 (565/1300)	39 (254/647)	48 (311/653)	0.002
Dog	49 (635/1300)	48 (312/648)	50 (323/652)	0.62
Other	34 (442/1282)	31 (197/639)	38 (245/643)	0.006

% (n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

* Chi-square test for difference between males and females with significance at $p < 0.05$

Others included birds, fishes, reptiles (snakes & lizards), other large mammals such as cows and horses, and smaller mammals chipmunk, rabbits, squirrels and mice etc., stick insects.

3.3 Physical Measures

Adolescents in lower social classes (defined as those in SOC categories of III M thru V) were observed to have slightly higher BMIs (23.7kg/m^2) to those from higher social classes (23.0 , $p = 0.04$). Overall males at 18 year follow-up were observed to be taller, heavier, and had lower BMI compared to females. When impact of social class was assessed within the gender, for height, weight and BMI, no difference was observed in these measures for males. However within females of lower social class were observed to have lower height (163cm , 165cm ; $p = 0.005$) and trend for greater weight (66.4cm , 63.7cm ; $p = 0.051$), and higher BMI (25kg/m^2 , 23kg/m^2 ; $p = 0.005$) compared females of higher social group.

Table 3.11 Anthropometric Measures

Physical Measures	Overall	Males	Females	p – value*
Mean Height (236)(n)	171 (859)	178 (408)	165 (451)	<0.001
Mean Weight (Kg)(n)	68 (856)	71 (408)	65 (448)	<0.001
Mean BMI (Kg/m ²)(n)	23 (856)	23 (408)	24 (448)	<0.001

* Difference between males & females by independent samples t-test with significance determined at $p < 0.05$

(n) = number of participants in with the characteristic

3.4 Spirometry Results

Gender related differences that were observed for males and females in relation to physical body measures of height, weight and BMI were also observed in pulmonary function test for all of the spirometric measures (table 3.12). However there was no difference observed for FEV₁ bronchodilator response which was 5% for both the genders.

Table 3.12 Gender-wise Spirometric Pulmonary Function Measures

Pre -bronchodilator function	Overall (n = 839)	Males (n = 396)	Females (n = 443)	p - value*
FEV ₁ (L)	4.0	4.6	3.5	<0.001
FVC (L)	4.6	5.4	4.0	<0.001
FEV ₁ /FVC	0.87	0.87	0.88	0.006
FEF _{25-75%} (L/s)	4.4	5.0	4.0	<0.001
Post-bronchodilator function	Overall (n=791)	Males (n =370)	Females (n = 421)	p - value
FEV ₁ (L)	4.2	4.8	3.6	<0.001
FVC (L)	4.7	5.4	4.0	<0.001
FEV ₁ /FVC	0.90	0.89	0.91	0.004
FEF _{25-75%} (L/s)	4.9	5.5	4.4	<0.001
Percentage (relative) change with bronchodilator	Overall (n=791)	Males (n =370)	Females (n = 421)	p - value
FEV ₁ (%)	5	5	5	0.89
FVC (%)	2	2	2	0.95
FEV ₁ /FVC (%)	3	3	3	0.97
FEF _{25-75%} (%)	10	12	13	0.45

Independent samples t-test was used to assess differences between male and female lung function with significance determined at p<0.05

3.5 Smoking and Alcohol Consumption

Both males and females were equally affected by tobacco smoke exposure. Males were more likely to be consuming alcohol and in greater amounts. There was no difference in alcohol consumption as both high and low classes consumed alcohol equally.

Table 3.13a Alcohol Consumption

Alcohol use	Overall	Males	Females	p - value
Any alcohol use in the previous week % (n/N)	73 (889/1224)	78 (474/609)	68 (415/615)	<0.001*
Median units of alcohol Consumed/week (p) (n)	3 (0,8) (1224)	4 (1,10)(609)	2 (0,5)(615)	<0.001#

% (n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

(p) = percentile (25th, 75th centiles) and (n) = number of participants with information on characteristic

* Chi-square test for difference between males and females with significance at p < 0.05

Mann-Whitney U test for difference between males and females with significance at p < 0.05

Table 3.13b Tobacco Smoking

Tobacco smoke exposures	Overall % (n/N)	Males % (n/N)	Females (n/N)	p - value#
Current smoking	29 (368/1278)	28 (176/631)	30 (192/647)	0.48
Mean age smoking started, years (n)	14.6 (564)	14.8 (268)	14.4 (296)	0.002*
Current or ever smoker	48 (608/1271)	46 (287/627)	50 (321/644)	0.15
Passive only exposure	29 (354/1206)	30 (185/613)	29 (169/593)	0.52
Active and passive exposure	43 (562/1312)	42 (274/652)	44 (288/660)	0.56

Chi-square test with significance at p < 0.05 for difference between males and females

* Independent samples t-test with significance at p<0.05 for mean age of tobacco smoke between males and females

% (n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic; (n) = number of participants in with the characteristic

Table 3.14 Current Tobacco Smoke Exposure by Amount of Tobacco Smoked

Amount of tobacco smoke exposure in current smoker	overall % (n/N)	Male % (n/N)	Female % (n/N)	p - value*
<1 per day	4 (14/359)	4 (6/171)	4 (8/188)	0.58
1 - 5 per day	24 (86/359)	20 (35/171)	27 (51/188)	
6 - 10 per day	33 (120/359)	34 (58/171)	33 (62/188)	
11 - 15 per day	23 (83/359)	26 (44/171)	21 (39/188)	
16 or more per day	16 (56/359)	16 (28/171)	15 (28/188)	

* Chi-square test for difference between males and females with significance at $p < 0.05$

% (n/N) = percentage with n = number of participants in with the characteristic

N = Total number of participants that had information on a particular characteristic

3.6 Effect of Social Class on Smoking Pattern

Table 3.15 Smoking by Social Class

Tobacco smoke exposures	Lower social class* % (n/N)	Higher social class* % (n/N)	p - value [#]
Current smoking	36 (136/374)	23 (174/774)	<0.001
Mean age started smoking (n)	14.5 (133)	14.8 (170)	0.22
Current and ever smoker	58 (215/369)	40 (310/772)	<0.001
Passive tobacco smoke exposure	31 (95/308)	31 (188/615)	0.93
Active and passive current exposure	53 (205/388)	34 (270/790)	<0.001

* Lower social class is based on Social Occupation Classification categories (IIIM to V) and high class based on categories (I to III NM) of SOC 2000. (n) number of participants
% (n/N) = percentage with n = number of participants in with the characteristic
N = Total number of participants that had information on a particular characteristic
Chi-square test with significance at $p < 0.05$ for difference between males and females

We assessed influence of social class on tobacco smoke exposure. A higher proportion of active smokers (current smoker), previous smokers, or current and past combined exposures were observed in lower social class. No difference was observed in age at which tobacco smoking was started.

The likely reason for low response may be due to lack of knowledge of their parental income, or they may consider this to be confidential information, which they did not feel comfortable to disclose.

3.7 Discussion

The 18 year assessment of the Isle of Wight 1989 Birth Cohort achieved a 90% (1313/1456) follow-up at 18 years. A higher than expected enrolment was made possible due to inherent characteristics of the research team and of the island. Almost all the requirements for a good cohort follow-up were present: a well-defined geographical location with stable population, a dedicated research team and continued

participation by members of the original research team. Few birth cohorts have been followed up to the adult age. The Isle of Wight Cohort follow-up matches follow-ups reported by Sears group (Dunedin cohort) at age 21 with 957 (92.3%) subjects seen. However they recruited children at age 3 years (not a birth cohort) and they reported a lower follow-up at age 18 years with 868 (83.7 %) subjects (51). Information on participation in Tucson Children's Respiratory Study had not been clearly presented in study publications; however, another cohort from Belmont, Australia (237) reported a lower follow-up of 63% (449/718) at 16 years.

Mean age of follow-up of the cohort was 18 years (SD 0.63; min 16.6 and max 19.7). No gender difference was noted in participants followed-up at age 18. Study participants that took part in full study visit by participation in person i.e. visited the research centre, had lower age (17.7 year) compared to those that responding to either telephone (18.3 year) and postal questionnaires (18.9 year). However this difference was low and its practical significance is questionable. The possible explanation for this observation is based on the fact that all effort was made to invite everyone for in person visit, however it is important to highlight here that telephone questionnaire was also offered if the study participant was not able to attend the research centre at the time of invitation for full-visit. However there were instances at the initial visit where some participants first agreed to visit the research centre but due to their inability to attend for in person visit they were later approached and telephone questionnaire was completed.

Males and smokers were less likely to attend the centre for full centre visit. Lower participation by smokers has previously been reported (238). Reasons for lower participation for in person visit by males was sought analysing attributes such as involvement in continued education, current smoking status and lower social class, however, none of these factors affected males differentially to females. Females in general were more likely to be involved in work compared to men (73% versus 65%, $p = 0.002$).

There was no difference observed between males and females who consented for skin prick test, however, females were more likely to agree for pulmonary function tests. We have accounted for gender in our analyses for possible gender related bias towards measured variables. It is possible that pulmonary function test were performed in disproportionately higher number of healthier/non-smokers subjects (those who attended the Centre for full visit). However, we are not able to adjust for this possible bias.

Because of the very high participation rate and equal proportion of males and females participated (table 3.3); selection bias is less likely to have occurred. However

comparatively more males participated through questionnaire only with a greater proportion of females participating by in person visits. This may have implications for objective measures but overall good participation rate in this study provided meaningful analysis for objective tests and measures.

Gender-wise comparison of those available at 18 years, and those that participated was performed, it is apparent from table 3.1a that overall gender-wise participation difference lacked statistical significance, being $p < 0.05$, the practical relevance of this difference was 3% which is also questionable.

Overall characteristics particular to adolescents were reflective of islands social and economic make up with a large number of families residing in family homes owned by their parents, with majority of the study participants continuing their education and engaged in work.

Majority of the adolescents owned a pet; girls were more likely to own a cat than boys. There was no difference observed in the gender for ownership of dogs. No relationship for pet ownership by social class as that was observed at 10 year follow-up. Social class did have an effect on smoke exposure with lower social class demonstrative of not only increased passive tobacco smoke exposure but also increased active tobacco smoke by adolescents themselves. However this pattern of increased tobacco smoke exposure was not shared with pattern of alcohol consumption as both classes consumed alcohol in similar proportions. However boys were more likely to drink alcohol and in greater amounts compared to females. The differential pattern of social behaviour towards smoking and alcohol consumption for gender are likely to have both short and long-term implications on health.

Social and gender related factors were important for physical measures such as height, weight, BMI and lung function. Males were taller, heavier but proportionally had lower body mass compared to females. This gender related difference in physical attributes was also observed in lung function with males having bigger lung volume, more airflow through large and small airways. Despite having greater flow the ratio of airflow to total volume was marginally lower in males compared to females. This is suggestive of in-appropriateness of air-passages for the volume of air and indication of air trapping. The relevance of this will need to be assessed by further examining sub-populations with wheeze that may account for this finding.

When we stratified physical measures by social class within the gender, females of lower social class were more likely to be smaller and having greater body mass compared to adolescent males. This finding may be indicative of life style and dietary factors may be playing a role with further possible long term health implications.

Overall there was good follow-up achieved at 18 year follow-up to allow for meaningful analysis of the Isle of Wight Birth Cohort Study. Participation did not demonstrate differential social representation or allergic predisposition. There were significantly more healthy participants that visited the research centre to those that responded by telephone questionnaire. However this difference was small and not likely to affect questionnaire based evaluation. The demographic profile of adolescent participants is reflective of clear gender related differences observed for physical measures of height, weight and BMI, in addition to spirometry. However no difference was observed for bronchodilator response. The socio-economic and life-style differences were observed with greater proportion of males consuming alcohol with comparable tobacco smoke indulgence between the genders. As expected those belonging to lower social class were more likely to be smokers than those in higher social class.

CHAPTER 4: CHARACTERISTICS OF UNDIAGNOSED WHEEZE AT 18; IS THIS ASTHMA?

4.1 Introduction

Asthma is a condition that affects the airways that has varied phenotypic forms and clinical presentations (239). It is characterised by an episodic airway obstruction with an element of inflammation (3). Symptom of wheeze is considered one of the cardinal features of asthma, however it is not the only one required for a clinical diagnosis. There is general consensus on inclusion of wheeze in epidemiological studies for defining asthma (37, 240) as large epidemiological studies have based questionnaires on this particular characteristic (241). All past and current asthma studies have used this as a key component to identify individuals likely to be suffering from asthma (57, 88, 127). Childhood wheeze is not sine qua non to asthma as other respiratory illnesses also present with this symptom, especially infant wheeze associated with viral infections (64). However, over adolescence, wheeze as a symptom suggestive of asthma gains more importance (12, 242) as adolescents are considered to have larger sized airways than children and are less likely to present with non-asthmatics wheezing associated with viral and other illnesses (61, 243). Childhood wheeze has received considerable attention with cohort studies teasing out features of early life wheeze, but our understanding of adolescent wheeze lacks clarity due to dearth of prospective studies that have followed and characterised asthma from birth into teenage years with the exception of a few (25, 26, 51). Therefore, adolescents that report wheeze and lack diagnosis of asthma are generally presumed to have un-diagnosed asthma (122). Furthermore, adolescence is a period where teenagers pick up new habits, such as smoking, and become socially active. Both asthma and wheeze over this period have been associated with tobacco smoke exposure and with poor lung function (137, 242). However, the association with tobacco smoke exposure has been more consistent with un-diagnosed adolescent wheeze (122, 244, 245) than with diagnosed adolescent asthma (163).

We aimed to assess and characterise adolescent wheeze and asthma in our whole population birth cohort at 18 years. Our secondary aim was to determine the severity and control of respiratory symptoms in teenagers with diagnosed asthma and those individuals with un-diagnosed wheeze. Based on earlier reports we hypothesised that there would be a significant number of teenagers with un-diagnosed wheeze and expected to find poor control in individuals having a diagnosis of asthma.

4.2 Results

Asthma was observed in 17.9% (234/1306) of the population at 18-years whereas wheeze that was not diagnosed as asthma (termed as non-asthma-wheeze from here onwards) was observed in 4.9% (64/1306); the remainder of subjects were defined as non-wheezers at 18-years. Most cases of asthma (71%), demonstrated wheezing at assessments in the 1st decade of life suggesting a persistent or recurrent phenomenon. However, only 25% of non-asthma-wheeze had evidence of wheezing at earlier assessments, which was the same as non-wheezers at 18. Thus, non-asthma-wheeze represents more of an adolescent onset condition.

Table 4.1 Characteristics of Wheeze Phenotypes at Age 18 Years

Demographics	Non-wheeze	Non-asthma-wheeze	Asthma
Female gender; % (n/total)	49 (493/1008)	59 (38/64)	55 (128/234)
p-value	Ref.	0.10	0.11
Low social class; * % (n/total)	32 (292/909)	42 (22/53)	35 (74/212)
p-value	Ref.	0.16	0.44
Family history of asthma; [Ⓚ] % (n/total)	43 (419/966)	61 (36/59)	62 (139/226)
p-value	Ref.	0.008	<0.001
Still in education at 18; % (n/total)	71 (699/990)	59 (36/61)	70 (161/230)
p-value	Ref.	0.06	0.86
In work at age 18; % (n/total)	68 (675/991)	66 (40/61)	69 (158/230)
p-value	Ref.	0.68	0.86
Atopy and airway inflammation			
Atopy at 18 years; % (n/total)	34 (221/643)	38 (17/45)	69 (114/165)
p-value	Ref.	0.64	<0.001
Atopy at 10 years; % (n/total)	20 (145/731)	23 (11/47)	57 (104/184)
p-value	Ref.	0.55	<0.001
Atopy at 4 years; % (n/total)	14 (99/703)	25 (10/40)	43 (68/159)
p-value	Ref.	0.06	<0.001
FeNO ppb [€] at 18; geometric mean (n)	17 (425)	18 (24)	31 (107)
p-value	Ref.	0.70	<0.001

Comparison of wheeze groups by Chi-square test for categorical variables determined at $p < 0.05$, group wise with non-wheeze as the reference group. For FeNO, ANOVA with Bonferroni's correction was applied.

* Lower social class categories (IIIM to V).

[Ⓚ] Parent or sibling history of asthma recorded at birth.

[€] FeNO = Fractional exhaled Nitric Oxide measured in parts per billion (ppb).

We assessed demographic characteristics for the two wheeze phenotypes (Table 4.1), however no statistically significant difference was observed for gender, social class, education, involvement in work, between the two wheeze phenotypes but both the diagnosed asthmatics and non-asthma-wheezers demonstrated a higher proportion of family history of asthma ($p<0.001$) and ($p=0.008$) respectively to the non-wheeze group.

Table 4.2 Comparison of Clinical Features of Wheeze Phenotypes

Clinical Features	Asthma % (n/total)	Non-Asthma- Wheeze % (n/total)	p-value*
Wheeze frequency >4 attacks/12 months	31 (72/230)	34 (22/64)	0.64
Wheeze with exercise	72 (167/232)	70 (45/64)	0.79
Sleep affected by wheeze	46 (102/223)	46 (28/61)	0.98
Dry cough at night	47 (108/230)	38 (23/61)	0.20
Speech affect by wheeze	15 (34/224)	23 (14/61)	0.15
Rhinitis	66 (154/233)	53 (34/64)	0.06
Mean self-rated health status; † visual analogue score (S.E.) (n)	75 (0.5)(216)	72 (2.2)(60)	0.20

* Comparisons were made using Chi-square test for categorical variables determined at $p<0.05$, for the difference between asthma and non-asthma-wheeze.

† For self-rated health status, Low scores infer lower self-rated health status
(n): number of participants

Adolescents with diagnosed asthma were more likely to be atopic ($p<0.001$) with significantly higher FeNO ($p<0.001$), while there was no difference in atopy prevalence or FeNO values between non-asthma-wheeze and non-wheezers (Table 4.1).

Interestingly, when we assessed the self-reported health status, of asthmatic and non-asthma-wheeze individuals, while both of these reported equally poor health state, these wheeze phenotypes had lower self-rated health state compared to non-wheeze individuals ($p<0.001$) (data not shown). In order to assess difference in symptoms and severity we compared asthma symptoms and rhinitis between the two wheeze groups (table 4.2). There was no difference noted for any of these symptoms with the exception that a trend for lower proportion of rhinitis was observed in those

with non-asthma-wheeze. Wheeze frequency also was observed to be similar between those with asthma and non-asthma-wheeze (table 4.3).

Table 4.3 Pattern of Symptom Distribution for Asthma

Frequency of wheeze/12 months Counts n (%)	1-3	4-12	>12	Total = N
Asthma	118 (62%)	48 (25%)	24 (13%)	190
Non-asthma-wheeze n (%)	42 (57%)	15 (20%)	7 (9%)	64
p - value*	0.61	0.77	0.72	-

* Chi-square test was performed to test the significance of difference between asthma and

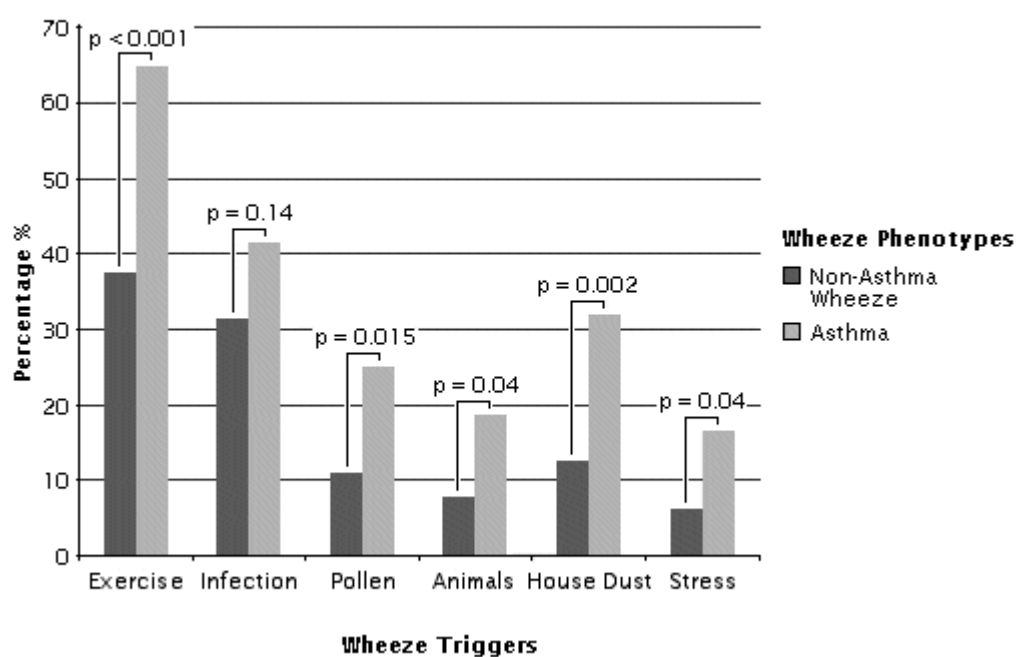
non-asthma-wheeze groups determined at $p < 0.05$

n = number of study participants with condition

N = Total number of participants with information

On examining the asthma and non-asthma-wheeze no difference was observed for frequency of wheeze between the two wheeze phenotypes at 18 years (table 4.3).

Figure 4.1 Triggers for Wheeze Onset



Trigger for wheeze are presented as percentage of each group with information on 64 participants with NAW and 234 with asthma.

Typical asthma triggers, especially allergenic, were reported significantly more commonly for asthma than non-asthmatic-wheeze, with the exception of chest infections (Figure 4.1).

Table 4.4 Asthma Treatment at Age 18 Years

Asthma Treatment (SIGN/BTS)	%	n
Step I: short acting bronchodilator (SAB)	34	53
Step II: inhaled steroid +/- SAB	35	54
Step III or IV: SAB + inhaled steroid + LABA*	8	13
Step V: oral corticosteroids use with any step:	23	36
Total = N:	100	156
Frequency of oral steroid use from age 10		
< 5 episodes of steroid use	78	28
≥ 5 episodes of steroid use	22	8
Total = N:	100	36

* Step III includes short acting bronchodilator (SAB) + inhaled steroid with long-acting bronchodilator (LABA) or a third anti-asthma medication use such as monteleukast or theophylline

n = number of participants, N = total number of study participants

Amongst asthmatics, 70 % (156/223) were on current asthma treatment while remainder had asthma treatment in the past only. Of those that were receiving treatment, two thirds were on steps I or II of BTS guidelines. If we consider >5 episodes of steroid use since last follow-up at 10 years as a marker of severe form of asthma, then 5% of those with asthma diagnosis were observed to have severe asthma.

Pre-bronchodilator spirometry was available at both 10 and 18-years. At both assessments, females had significantly lower lung function than males, therefore

cross-sectional analysis of lung function for wheezing phenotypes were stratified by gender (Table 4.5).

Table 4.5a Cross-sectional Lung Function and Height at 18 Years

Lung Function ^Y	Overall	Non-wheeze	NAW	Asthma
Male (n)	327	248	16	63
FEV ₁ (L) (S.E)	4.62	4.66 (0.03)	4.54 (0.13)	4.43 (0.07)
p-value		Ref.	0.94	0.002
FVC (L) (S.E)	5.36	5.34 (0.04)	5.47 (0.15)	5.43 (0.08)
p-value		Ref.	0.41	0.28
FEV ₁ /FVC (S.E)	0.86	0.88 (0.005)	0.86 (0.02)	0.82 (0.01)
p-value		Ref.	0.32	<0.001
FEF _{25-75%} (L/s)(S.E)	4.98	5.14 (0.07)	5.00 (0.28)	4.35 (0.14)
p-value		Ref.	0.50	<0.001
Height (cm)(S.E.)	178 (0.4)	178 (0.4)	175(0.7)	178 (0.8)
p-value		Ref.	0.27	1.00
Female (n)	372	273	23	76
FEV ₁ (L) (S.E)	3.47	3.53 (0.02)	3.35 (0.08)	3.29 (0.04)
p-value		Ref.	0.027	<0.001
FVC (L) (S.E)	3.97	4.00 (0.03)	3.78 (0.09)	3.91 (0.05)
p-value		Ref.	0.024	0.14
FEV ₁ /FVC (S.E)	0.88	0.89 (0.004)	0.89 (0.01)	0.84 (0.01)
p-value		Ref.	0.90	<0.001
FEF _{25-75%} (L/s)(S.E)	3.95	4.08 (0.05)	3.93 (0.18)	3.48 (0.10)
p-value		Ref.	0.42	<0.001
Height (cm)(S.E.)	165 (0.4)	165(0.4)	164(1.0)	164 (0.7)
p-value		Ref.	0.22	1.00

Comparison between groups was performed using General Linear Model (GLM) adjusted for height with significance at p < 0.05.

^Y Height adjusted mean lung function values of FEV₁ & FVC in litres (L) and FEV₁/FVC, FEF_{25-75%} litres/second(L/s) and standard error of mean (S.E.) are presented.

Table 4.5b Cross-sectional Lung Function and Height at 10 Years

Lung Function ^Y	Overall	Non-wheeze	NAW	Asthma
Male (n)	327	248	16	63
FEV ₁ (L) (S.E)	2.07	2.09 (0.02)	2.06 (0.06)	2.01 (0.03)
p-value		Ref.	0.70	0.02
FVC (L) (S.E)	2.37	2.36 (0.02)	2.38 (0.06)	2.39 (0.03)
p-value		Ref.	0.83	0.44
FEV ₁ /FVC (S.E)	0.88	0.88 (0.04)	0.87 (0.01)	0.84 (0.07)
p-value		Ref.	0.27	<0.001
FEF _{25-75%} (L/s)(S.E)	2.39	2.45 (0.03)	2.36 (0.13)	2.13 (0.07)
p-value		Ref.	0.50	<0.001
Height (cm)(S.E.)	139 (0.4)	139 (0.4)	138 (0.8)	139 (0.7)
p-value		Ref.	0.23	0.77
Female (n)	372	273	23	76
FEV ₁ (L) (S.E)	2.00	2.01 (0.01)	1.96 (0.04)	1.97 (0.02)
p-value		Ref.	0.21	0.13
FVC (L) (S.E)	2.25	2.24 (0.01)	2.20 (0.04)	2.27 (0.02)
p-value		Ref.	0.32	0.30
FEV ₁ /FVC (S.E)	0.89	0.90 (0.003)	0.90 (0.01)	0.87 (0.06)
p-value		Ref.	0.74	<0.001
FEF _{25-75%} (L/s)(S.E)	2.48	2.53 (0.03)	2.53 (0.11)	2.27 (0.06)
p-value		Ref.	0.97	<0.001
Height (cm)(S.E.)	139 (0.4)	139 (0.4)	140 (1.4)	138(0.7)
p-value		Ref.	0.77	0.13

Comparison between groups was performed using General Linear Model (GLM) adjusted for height with significance at $p < 0.05$.

^Y Height adjusted mean lung function values of FEV₁ & FVC in litres (L) and FEV₁/FVC, FEF_{25-75%} litres/second(L/s) and standard error of mean (S.E.) are presented.

At 18-years, asthmatic wheeze demonstrated spirometric evidence of airflow obstruction compared to non-wheezers. Non-asthmatic-wheezers on the other hand had a different pattern of altered lung function that affected females with lower FEV_1 and FVC (Forced Vital Capacity) at 18-years in comparison to non-wheezers resulting in normal airflow as demonstrated by (FEV_1/FVC ratio). Impaired smaller airway function ($FEF_{25-75\%}$) was present only in asthmatic wheeze adolescents at 18 years. At the age of 10-years, non-asthmatic-wheeze had normal spirometry, while asthmatics had significantly reduced FEV_1/FVC ratio and $FEF_{25-75\%}$.

Overall and sex stratified differences for pre-bronchodilator lung function gains over 10 to 18-year is presented as gain of mean FEV_1 and FVC in litres (L) and mean $FEF_{25-75\%}$ in litres/second (L/s) plus gain in height in centimetres (cm) with standard error of means (S.E.) (Table 4.6). Lung function gains over adolescence (10 to 18 years) were adjusted for height gain in centimetres. The overall gain in lung function was impaired (FEV_1 and $FEF_{25-75\%}$) for asthma only. Non-asthma-wheeze demonstrated comparative gains to non-wheeze adolescents. After stratifying by gender, females with asthma had lower lung function parameters, whereas those with non-asthma-wheeze demonstrated had lower (FEV_1 and FVC) gain but their small airway function was comparable to non-wheeze individuals. No difference was observed for gain in height between the groups.

In general data on age of start of tobacco smoking demonstrated that girls started tobacco smoking at an earlier age of 14.4 years in comparison to boys 14.8 years ($p = 0.002$). However when we stratified this analysis by wheeze phenotypes no significant gender-wise differences were seen.

Table 4.6 Gain in Lung Function from 10 to 18 Years of Age

Lung function ^y and height	Non-wheeze #	NAW	Asthma
All participants (n)	521	39	139
FEV ₁ (L) (S.E.)	2.01 (0.02)	1.95 (0.07)	1.82 (0.04)
p-value	Ref.	0.36	<0.001
FVC (L) (S.E.)	2.33 (0.02)	2.28 (0.08)	2.28 (0.04)
p-value	Ref.	0.58	0.34
FEF _{25-75%} (L/s) (S.E.)	2.08 (0.03)	1.96 (0.12)	1.67 (0.07)
p-value	Ref.	0.35	<0.001
Height (236) (S.E.)	32 (0.35)	29 (1.29)	32 (0.69)
p-value	Ref.	0.07	0.89
Males (n)	(248)	(16)	(63)
FEV ₁ (L) (S.E.)	2.58 (0.03)	2.52 (0.11)	2.44 (0.05)
p-value	Ref.	0.60	0.17
FVC (L) (S.E.)	2.98 (0.03)	3.02 (0.13)	3.05 (0.06)
p-value	Ref.	0.75	0.30
FEF _{25-75%} (L/s) (S.E.)	2.67 (0.05)	2.50 (0.20)	2.22 (0.10)
p-value	Ref.	0.39	<0.001
Height (236) (S.E.)	39 (0.27)	37 (1.05)	39 (0.53)
p-value	Ref.	0.21	0.31
Females (n)	(273)	(23)	(76)
FEV ₁ (L) (S.E.)	1.52 (0.02)	1.38 (0.07)	1.31 (0.04)
p-value	Ref.	0.046	<0.001
FVC (L) (S.E.)	1.76 (0.02)	1.57 (0.08)	1.63 (0.04)
p-value	Ref.	0.02	0.01
FEF _{25-75%} (L/s) (S.E.)	1.56 (0.04)	1.40 (0.14)	1.22 (0.08)
p-value	Ref.	0.28	<0.001
Height (S.E.)	26 (0.32)	24 (1.09)	26 (0.60)
p-value	Ref.	0.12	0.83

(#) Univariate ANNOVA test with Bonferroni's correction was used with comparisons of asthma and non-asthma-wheeze against reference of non- wheeze determined at p-value <0.05.

^Y Height gain adjusted mean lung function gain values of FEV₁ & FVC in litres (L) and FEV₁/FVC, FEF_{25-75%} litres/second(L/s) and standard error of mean (S.E.) are presented (n) Represents the number of study participants with available prospective spirometric data.

Table: 4.7 Additional Lung Function Tests

Lung Function ^Y	Overall	Non-wheeze #	NAW	Asthma
18 year (n)	699	521	39	139
BHR DRS (n) *	1.12 (585)	1.07 (447)	1.08 (25)	1.37 (113)
p-value		Ref.	1.00	<0.001
% PC20† (n/Total)	7.5 (44/585)	3.0 (12/447)	4.0 (1/25)	27 (31/113)
p-value		Ref.	1.00	<0.001
% FEV ₁ reversibility (n) ‡	5.0 (790)	4.2 (592)	4.2 (45)	8.5 (153)
p-value		Ref.	1.00	<0.001
10 year (n)	699	521	39	139
BHR DRS	1.47 (723)	1.36 (515)	1.37 (39)	1.84 (169)
p-value		Ref.	1.00	<0.001
%PC20	31 (221/725)	22 (111/516)	23 (9/40)	60 (101/169)
p-value		Ref.	1.00	<0.001

Comparisons of wheeze phenotypes against non-wheeze reference (Ref) group. Chi-square test was used for categorical data and ANOVA with Bonferroni's correction for multiple comparisons with significance determined at p <0.05

* BHR DRS: refers to a continuous dose-response (DRS) measure of bronchial hyper-responsiveness (BHR) expressed as Log10

¶ Represent p-values for comparisons of asthma against reference of non- wheeze (#). (DRS+10); higher values infer greater BHR.

† %PC20: denotes proportion within each group demonstrating BHR at methacholine challenge as defined by PC20 <8mg/ml. Chi-square test was used with significance determined at p<0.05.

‡ %FEV₁ relative reversibility: to 400micrograms inhaled salbutamol.

Methacholine bronchial challenge test was performed at 10 and 18 year follow-ups. At the 18 year follow-up additional tests were performed that included Fractional exhaled Nitric Oxide (FeNO) and bronchodilator response to salbutamol (BDR) (400mcg). Methacholine bronchial challenge test has been reported here in two ways, one as a binary outcome of number of individuals demonstrating PC₂₀ (concentration of methacholine at which a 20% FEV₁ fall is observed from the baseline saline stage, and the cut-off for this to be considered positive was 8mg/ml) and secondly as a standard epidemiological use of continuous measure of methacholine challenge test is to present the bronchial reactivity (BR) (when all FEV₁ values from each stage of lung challenge test are used to obtain a regression gradient of a straight line, reported as, dose response slope, (DRS), with higher values representing increased reactivity of airways). The details of how the DRS was obtained is explained in the statistical methods section. A higher proportion of asthma individuals demonstrated PC₂₀ and BR at both 10 and 18-year assessments but non-asthmatic-wheezers had lower values that were comparable to non-wheeze individuals (Table 4.7). Bronchodilator reversibility at 18-years was significantly greater in asthmatics than non-asthmatic-wheezers and non-wheezers.

Table 4.8 Environmental Exposures and Wheeze Phenotypes

	Non-wheeze	NAW	Asthmatic
Smoke Exposure			
Current personal smoking at age 18; % (n/total)	26 (260/984)	47 (30/64)	34 (75/224)
p-value	Ref.	<0.001	0.10
Mod to high urinary cotinine; % (n/total) ^y	17 (78/467)	57 (16/28)	27 (33/123) ^z
p-value	Ref.	<0.001	0.033
Any tobacco smoke exposure (active or passive) at age 18; % (n/total)	41 (414/1008)	61 (39/64)	45 (105/234)
p-value	Ref.	0.006	0.87
Personal tobacco smoking (current or past); % (n/total)	45 (437/977)	73 (47/64)	54 (120/224)
p-value	Ref.	<0.001	0.06
Mean age started smoking in years	14.7	13.6	14.5
p-value	Ref.	<0.001	0.61
Home tobacco smoke exposure during pregnancy % (n/total)	45 (447/999)	58 (37/64)	46 (106/232)
p-value	Ref.	0.04	0.79

Table 4.8 Environmental exposures and wheeze phenotypes (cont...)

	Non-wheeze	NAW	Asthmatic
Paracetamol/NSAID use			
Median paracetamol use per month* (p)(n)	1 (0,2) (983)	1 (0,3) (63)	1 (0,3) (223)
p-value	Ref.	<0.001	<0.001
Median NSAID use per month (p)(n)	0 (0,1) (983)	0 (0,1.5) (63)	0 (0,1) (223)
p-value	Ref.	<0.001	0.24
Males			
Median paracetamol use per month* (p)(n)	0 (0,1) (534)	1.0 (0,2) (26)	1.0 (0,3) (101)
p-value	Ref.	0.09	<0.001
Median NSAID use per months* (p)(n)	0 (0,0) (508)	0 (0,1) (26)	0 (0,1) (101)
p-value	Ref.	0.009	0.51
Females			
Median paracetamol use per month* (p)(n)	1 (0,2) (480)	2 (1,4) (37)	2 (0,3) (122)
p-value	Ref.	0.024	0.012
Median NSAID use per months* (p)(n)	0 (0,1) (480)	1.0 (0,3) (37)	0 (0,2) (122)
p-value	Ref.	0.06	0.42

Definitions for Table 4.8

Comparisons of wheeze phenotypes against non-wheeze reference (Ref) group. Chi-square test was used for categorical data and ANOVA with Bonferroni's correction for multiple comparisons with significance determined at $p < 0.05$. Mann Whitney U test applied for continuous data not showing normal distribution.

[†] Proportion of study participants having moderate to high urinary cotinine compared to total urinary cotinine tests in each wheeze group. Three categories of urinary cotinine were obtained by using optimal binning process in SPSS with current smoking status was the optimizing variable.

[‡] p- value for difference between non-asthma-wheeze and asthma $p = 0.002$

[§] Median values with (p) = percentiles (25th, 75th percentiles) are provided for the number of times paracetamol/NSAID use per month

(n) represents number of participants that provided information

To understand the nature of non-asthmatic wheeze, we investigated the role of common environmental risk factors of tobacco smoke exposures and the number of times adolescents reported monthly use of paracetamol and NSAIDs (Table 4.8). Major differences were observed for exposure to cigarette smoke and paracetamol between wheeze phenotypes, however for monthly use of NSAID, there were more males with non-asthmatic-wheeze. Gender-wise comparison for personal smoking at 18-years was similar for females (50%) and males (46%; $p = 0.15$) in our population. Non-asthmatic-wheeze had significantly greater domestic exposure to smoking during pregnancy than non-wheezers, whereas asthmatics showed no difference versus non-wheezers (Table 4.8). Significantly more non-asthmatic-wheezers were smokers at 18-years compared to non-wheezers and asthmatics ($p = 0.029$). Current or past personal smoking was seen in 75% of non-asthmatic-wheeze with no significant gender difference ($p = 1.0$). Current tobacco smoke exposure in non-asthmatic-wheezers was validated by greater prevalence of cotinine values in the moderate-high category than non-wheezers (Table 4.8) and compared to asthmatics ($p = 0.002$).

Non-asthmatic-wheezers also had earlier smoking onset than non-wheezers (Table 4.8) and asthmatics ($p = 0.016$). Non-asthmatic-wheezers reported higher per month paracetamol and NSAID use than non-wheezers with similar consumption to asthmatics. Females reported higher consumption of both paracetamol and NSAID's ($p < 0.001$). After gender stratification we observed that male non-asthmatic-wheezers had significantly higher NSAID intake than male non-wheezers. Conversely female non-asthmatic-wheezers had significantly higher paracetamol intake than female non-wheezers. This gender related pattern differed from asthmatics where both males and females demonstrated higher paracetamol (rather than NSAID) use than non-wheezers.

4.3 Discussion

A first glance at the results of adolescent wheeze, it is tempting to consider that a significant proportion of teenagers with non-asthma-wheeze have undiagnosed asthma, as suggested by earlier reports of under diagnosis of asthma in teenagers. However, when we study this group in a bit more detail, we observe these teenagers are different from those with diagnosed asthma. They differ in terms of objective parameters such as lung function, atopy and FeNO plus they have higher smoke exposure both at birth and subsequently over adolescence. Therefore, at least two possibilities exist with this wheeze that is not diagnosed as asthma; a) this is an asthma phenotype characterised by airway remodelling resulting in partially irreversible airway disease and second, that this is a distinct pulmonary condition affecting teenagers, associated with smoking that is not responsive to asthma treatment and possibly represents early origins of an adult pulmonary condition.

Teenagers with diagnosed asthma not only had similar symptoms to those with non-asthma-wheeze, but there was no difference observed in severity between the two wheeze phenotypes. However non-asthma-wheeze individuals were observed to lack reversibility and had less atopy with lower FeNO (246) and also bronchial responsiveness. A possible explanation for the lack of atopy and reversibility observed in this wheeze phenotype was to consider this to be a non-atopic asthma phenotype with early childhood un-recognised asthma that had undergone airway remodelling. If this were the case, then we expected to find at least a comparable baseline lung function in these teenagers at 10 year of age to those with asthma, but this was not observed, non-asthma-wheeze group demonstrated lung function parameters that were similar to non-wheeze group. Another feature that was different in non-asthma-wheeze was that this wheeze seemed to be largely of adolescent onset with only 30% (15/50) wheezing at earlier follow-ups, in comparison to ones with asthmatic wheeze at 18 was largely composed of persistent of their wheeze (75%, 158 /212).

In order to avoid any misclassification for adolescent asthma we characterized all wheeze that did not meet the study definition of asthma as non-asthma-wheeze (NAW) at 18 years and characterised their lung function at 18 years. Significant differences were observed between the two wheeze phenotypes with asthmatics demonstrating a clear obstructive pattern of lung function with reduced FEV_1/FVC to non-wheeze individuals, whereas females, but not males of the non-asthma-wheeze group demonstrating a lower FEV_1 and FVC pattern, thereby lacking the typical obstructive lung pattern that is observed in asthma. The difference in pulmonary function patterns and objective tests of bronchial reversibility and bronchial reactivity

are suggestive of a distinct wheeze illness that is not asthma and less likely to respond to normal asthma treatment modalities.

On further examining the impact of environmental factor of smoking towards the wheeze groups at 18, exposure to tobacco smoke was observed to be greater and earlier in the non-asthma-wheeze than the non-wheeze, whereas asthmatic individuals failed to demonstrate significant difference to tobacco smoke exposure either active or passive that differed from non-wheeze. A group of adolescent that present with wheeze has been reported previously (242), and they have been considered to represent un-diagnosed asthma (122). However, there is no report of characterization of adolescent onset wheeze to-date. Link of tobacco smoking does raise our suspicion towards early COPD but based on lung function parameters, non-asthma-wheeze with a pattern suggestive of restrictive lung disease rather than that observed in asthma or COPD. It is too early in life to predict long-term consequence of this wheeze. The vast majority of the non-asthma-wheeze did not have asthma or wheeze at 1, 2, 4 and 10 years (data not shown); therefore it is unlikely that the absence of reversibility could be attributable to remodelling in children with under-diagnosed asthma. The characteristics and clinical picture of non-asthma-wheeze has some resemblance to respiratory bronchiolitis interstitial lung disease (RB-ILD)(247-251), which is a less well recognised condition presenting as wheeze in adolescent period, with history of smoke exposure. There is lack of data on the origins of this condition, therefore, we cannot rule out the possibility of RB-ILD in our differential diagnosis for non-asthma-wheeze.

Based on self-perception of health through visual analogue scale (249), a validated instrument that is applied to assess a general health status, it was observed that those with non-asthma-wheeze had significantly lower self-rated health status compared to those with non-wheezers and was similar to asthma. This is suggestive of presence of significant impairment of health in non-asthma-wheeze.

We also assessed use of analgesic (paracetamol) and anti-inflammatory (general NSAIDs) medication use for the wheeze phenotypes at 18 years. Overall females were observed to use more paracetamol ($p < 0.001$) with males taking NSAIDs ($p < 0.001$), but as expected asthmatic wheeze individuals demonstrated increased use of paracetamol rather than using NSAIDs (Table 4.8). Overall non-asthma-wheeze individuals reported higher use of both paracetamol and NSAIDs in comparison to the non-wheeze group. After gender stratification, a pattern of preference for paracetamol use in females was observed in comparison to males that had reported higher consumption of NSAIDs within the non-asthma-wheeze group. This finding of greater paracetamol use has been reported in an older population in the National Health and Nutritional Examination Survey in an adult population, however, in their survey, paracetamol was studied separately from use of aspirin and in combination with other NSAIDs (252).

Independent significance of increased paracetamol use needs to be prospectively assessed for non-asthma-wheeze after taking other adolescent factors into consideration, whereas most studies have reported this either retrospectively as in a case-control study or in a cross-sectional study (167, 253, 254).

In summary, two distinct wheeze phenotypes were observed at 18 years, firstly, non-asthma-wheeze associated with personal tobacco smoke exposure and lacking objective evidence of asthma and secondly asthmatic wheeze. Our study suggests we need to question the diagnosis of asthma in adolescents with clinical features suggestive of asthma when there is significant history of tobacco smoke exposure and if it fails to respond to treatment. This non-asthma-wheeze should not be labelled as un-recognised asthma without objective spirometric data. Prospective studies are required to assess the impact of childhood tobacco smoke exposure into adulthood and to assess the outcome and prognosis of non-asthma-wheeze. Epidemiologic studies over adolescent period for asthma need to take into account this phenotype in order to avoid any misclassification.

CHAPTER 5: RISK FACTOR PROFILES FOR ASTHMA AND NON-ASTHMATIC-WHEEZE IN 18 YEAR OLDS: TWO DISTINCT WHEEZE ILLNESSES

5.1 Introduction

Wheeze at 18 years is often considered to represent asthma, unless proven otherwise, introducing heterogeneity and misclassification. Hence, previous studies have reported inconsistent patterns of risk factors for adolescent wheeze (153, 158, 163). There are few studies that have documented risk factors prospectively (26, 51, 80). In this chapter we have assessed the impact of early life and adolescent risk factors for asthma and non-asthmatic-wheeze at 18 years after characterizing wheeze in the previous chapter. At the 10 year follow-up, characterization of wheeze revealed different risk patterns for atopic and non-atopic wheeze (76). Atopy, over adolescence, has been suggested to play diminished role (52, 85). Another generally observed finding is of gender reversal is observed with non-atopic females gaining higher prevalence of asthma in comparison to males (85, 255). Risk of tobacco smoke has been observed to be a risk factor for adolescent asthma but there are inconsistent results with studies reporting variable significance towards asthma occurrence (160), while others limiting this significance to asthma severity (163). Rhinitis, has historically been used as a marker of allergic disease (127) and is also considered a risk factor for asthma (256), whereas the concept of one airway disease suggests that asthma and rhinitis should be thought of as one disease (135). To our knowledge there are no studies that have characterized risk factor pattern for non-asthmatic-wheeze at 18. Our aim was to identify risk factor profiles for adolescent asthma and non-asthma-wheeze at 18.

5.2 Results

Amongst all of the early life factors evaluated for asthma at 18, univariate significance was observed for atopy from age 4 to 18, family history of asthma and rhinitis at 10 and 18 year follow-ups but not at 4 (tables 5.1 & 5.2). Adolescent factors that were observed to be associated with asthmatic-wheeze were personal tobacco smoking (this included all those reporting current or past smoking status), family history of asthma (determined at birth from mother) and higher monthly consumption of paracetamol (table 5.3). Early life asthma or wheeze and early life chest infections at the ages of 1 & 2 years demonstrated significance at the univariate analysis for asthma at 18. Non-significant trends for asthma at 18 were observed fewer days of vigorous exercise at 18.

Table 5.1 Univariate Analysis of Risk Factors for Asthmatic Wheeze at 18 years (Early Life Factors)

Early life factors	Asthmatic Wheeze % (n/total)	Non-Wheeze % (n/total)	OR	95% Confidence Interval	p- value
Environment tobacco smoke exposure first 4-years of life [¥]	57 (101/176)	53 (402/761)	1.20	0.86 – 1.67	0.28
Maternal smoking during pregnancy	22 (51/233)	24 (235/994)	0.91	0.64 – 1.28	0.57
Home tobacco smoke exposure during pregnancy ^χ	46 (106/232)	45 (447/999)	1.04	0.78 – 1.38	0.79
Chest infection at 1 or 2-years	22 (42/190)	16 (134/839)	1.49	1.01 – 2.20	0.04
Breast feed > 3 months	44 (91/209)	47 (434/919)	0.86	0.64 – 1.17	0.34
Lower Social Class during pregnancy*	53 (74/141)	54 (321/593)	1.07	0.74 – 1.54	0.73
Pet cat 0 – 4 years	50 (115/232)	42 (421/1000)	1.35	1.01 – 1.80	0.04
Pet dog 0 – 4 years	50 (117/233)	48 (477/997)	1.10	0.83 – 1.46	0.51
Rhinitis at 1 or 2-years	20 (38/192)	17 (144/860)	1.23	0.82 – 1.83	0.31
Asthma/Wheeze 1 or 2 year	20 (42/212)	13 (120/939)	1.69	1.14 – 2.49	0.008
Atopy at 4-years [⊕]	43 (68/159)	14 (99/703)	4.56	3.12 – 6.66	<0.001

Comparisons between asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

¥ Environment tobacco smoke exposure is obtained from smoke exposures of follow-ups 1, 2, and 4 years.

χ Tobacco smoke exposure inside the house by either parents or any other family member during pregnancy.

⊕ Atopy defined by at least 1 skin test wheal response ≥ 3 mm diameter greater than control

* Lower social class is based on Social Occupation Classification categories (IIIM to V) determined at pregnancy

Table 5.2 Univariate Analysis of Risk Factors for Asthmatic Wheeze at 18 years (Adolescent Factors)

Adolescent factors	Asthmatic Wheeze % (n/total)	Non-Wheeze % (n/total)	OR	95% Confidence Interval	p- value
Home tobacco smoke exposure at 18-years (active and passive) ^z	45 (105/234)	41 (414/1008)	1.17	0.88 – 1.56	0.29
Current or past self-smoke exposure ^a	54 (120/224)	45 (437/977)	1.43	1.07 – 1.91	0.017
Family history of asthma ^d	62 (139/226)	43 (419/966)	2.01	1.55 – 2.81	<0.001
Number of days exercise/wk. at 18 [#]	2.7 (175)	3.08 (746)	0.90	0.80 – 1.01	0.07
Lower social class at 18-years*	35 (74/212)	32 (292/909)	1.13	0.83 – 1.55	0.44
Lower social class at 10-years*	51 (98/193)	50 (428/864)	1.05	0.77 – 1.44	0.76
Rhinitis at 10-years	46 (100/216)	18 (171/955)	3.95	2.89 – 5.41	<0.001
Atopy at 10-years ^Δ	57 (104/184)	20 (145/731)	5.25	3.73 – 7.41	<0.001
Rhinitis at 18-years	66 (154/233)	28 (278/1006)	5.11	3.77 – 6.92	<0.001
Atopy at 18-years ^Δ	69 (114/165)	34 (221/643)	4.27	2.95 – 6.17	<0.001

Definitions for Table 5.2

Comparisons between asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

χ Tobacco smoke exposure inside the house by either parents or any other member including self.

ϖ Atopy defined by at least 1 skin test wheal response ≥ 3 mm diameter greater than control.

α Past or current tobacco smoking status determined at age 18

δ Family history of asthma, determined at birth from parents

Geometric mean of the number of days of vigorously exercise per week

* Lower social class is based on Social Occupation Classification categories (IIM to V)

Table 5.3: Univariate Analysis of Paracetamol/NSAID use for Asthmatic Wheeze at 18 years (Adolescent Factors)

	Asthmatic Wheeze	Non-Wheeze	OR	95% Confidence Interval	p- value
Paracetamol use per month ^γ	1.0 (0,3) (223)	1.0 (0,2) (983)	1.12	1.06 – 1.17	<0.001
NSAID use per month ^γ	0 (0,1) (223)	0 (0,1) (983)	1.02	0.97 – 1.08	0.42

Definitions for Table 5.3

Comparisons between asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

γ Median (25th, 75th) percentile reported for number of times paracetamol or NSAID use/month

Amongst all of the early life factors evaluated for non-asthma-wheeze, univariate trends for significance were observed for only tobacco smoke exposure during pregnancy (Table 5.4). Adolescent factors that were observed to be associated with non-asthma-wheeze were personal tobacco smoking (this included all those reporting current or past smoking status), family history of asthma (determined at birth from mother), higher consumption of paracetamol and or suffering from rhinitis at age 18 but not at any of the earlier follow-ups (Tables 5.5 & 5.6). There was no association to atopy for this wheeze phenotype at any time point; however trends for significance were present at the age of 4 years.

Table 5.4 Univariate Analysis of Risk Factors Non-Asthmatic Wheeze at 18 Years (Early Life Factors)

	Non-Asthmatic-Wheeze % (n/total)	Non-Wheeze % (n/total)	OR	95% Confidence Interval	p - value
Environment tobacco smoke exposure first 4-years of life [¥]	57 (25/44)	53 (402/761)	1.18	0.64 – 2.17	0.61
Maternal smoking during pregnancy	34 (22/64)	24 (235/994)	1.69	0.99 – 2.89	0.06
Home tobacco smoke exposure during pregnancy ^χ	58 (37/64)	45 (447/999)	1.69	1.02 – 2.82	0.04
Chest infection at 1 or 2-years	19 (9/48)	16 (134/839)	1.21	0.58 – 2.57	0.61
Breast feed > 3 months	43 (23/53)	47 (434/919)	0.86	0.49 – 1.50	0.59
Lower social class during pregnancy [*]	55 (23/42)	54 (321/593)	0.98	0.52 – 1.83	0.94
Pet cat 0 – 4 years	41 (26/64)	42 (421/999)	0.94	0.56 – 1.57	0.81
Pet dog 0 – 4 years	56 (36/64)	48 (477/997)	1.40	0.84 – 2.33	0.19
Rhinitis at 1 or 2-years	16 (8/51)	17 (144/860)	0.93	0.43 – 2.01	0.84
Asthma/wheeze 1 or 2 year	18 (10/55)	13 (120/939)	1.52	0.75 – 3.09	0.25
Atopy at 4-years [⊕]	25 (10/40)	14 (99/703)	2.03	0.96– 4.29	0.06

Comparisons between non-asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

¥ Early life tobacco smoke exposure is obtained from smoke exposures of follow-ups 1, 2, and 4 years.

χ Tobacco smoke exposure inside the house by either parents or other family members.

* Lower social class is based on social occupation classification categories (IIIM to V).

⊕ Atopy defined by at least 1 skin test wheal response >3mm diameter.

Table 5.5 Univariate Analysis of Risk Factors for Non-Asthmatic Wheeze (Adolescent Factors)

Adolescent factors	Non-asthmatic-wheeze % (n/total)	Non-Wheeze % (n/total)	OR	95% Confidence Interval	p -value
Home tobacco smoke exposure at 18-years (active and passive) ^χ	61 (39/64)	41 (414/1008)	2.24	1.33 – 3.76	0.002
Current and past self-smoke exposure ^α	73 (47/64)	45 (437/977)	3.42	1.93 – 6.03	<0.001
Family history of asthma ^ϑ	61 (36/59)	43 (419/966)	2.04	1.19 – 3.50	0.009
Number of days exercise at 18/wk. [#]	3.21 (53)	3.08 (746)	0.94	0.84 – 1.04	0.22
Lower social class at 18-years*	42 (22/53)	32 (292/909)	1.50	0.85 – 2.64	0.16
Lower social class at 10-years*	58 (29/50)	50 (428/864)	1.41	0.79 – 2.51	0.25
Rhinitis at 10-years	17 (10/58)	18 (171/955)	0.96	0.47 – 1.93	0.90
Atopy at 10-years ^Δ	23 (11/47)	20 (145/731)	1.17	0.61 – 2.49	0.55
Rhinitis at 18-years	53 (34/64)	28 (278/1006)	2.97	1.78 – 4.94	<0.001
Atopy at 18-years ^Δ	38 (17/45)	34 (221/643)	1.16	0.62 – 2.16	0.64

Definitions for Table 5.5

Comparisons between non-asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

χ Tobacco smoke exposure inside the house by either parents or any other member including self.

⌚ Atopy defined by at least 1 skin test wheal response ≥ 3 mm diameter.

α Past or current tobacco smoking status determined at age 18

∅ Family history of asthma, determined at birth from parents

Geometric mean of the number of days of vigorously exercise per week

* Lower social class is based on Social Occupation Classification categories (IIM to V)

Table 5.6: Univariate Analysis of Paracetamol/NSAID use for Non – Asthmatic Wheeze at 18 years (Adolescent Factors)

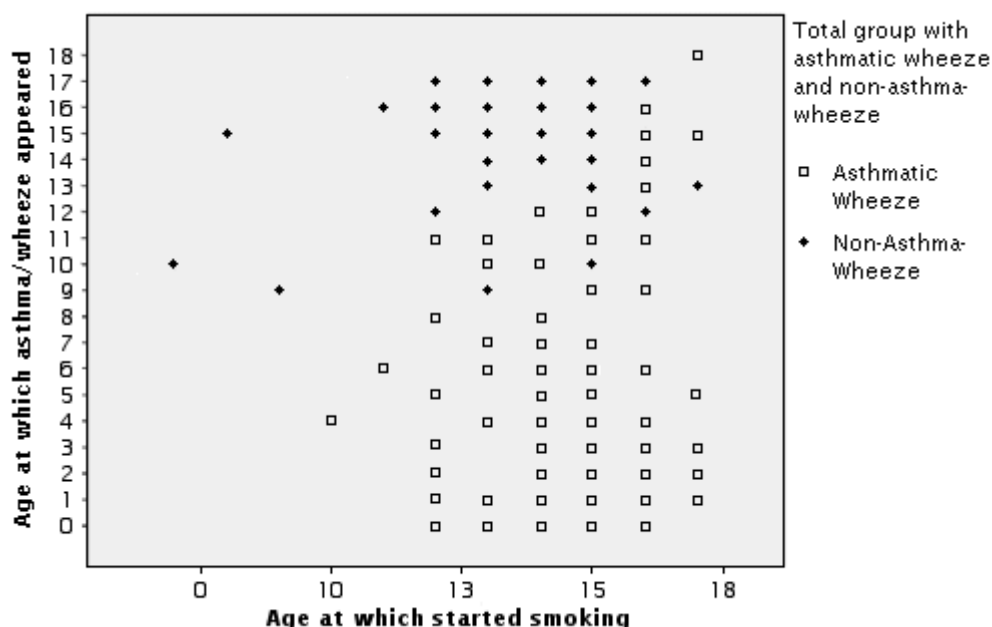
	Asthmatic Wheeze	Non- Wheeze	OR	95% Confidence Interval	p- value
Paracetamol use per month ^y	1.0 (0,3) (63)	1.0 (0,2) (983)	1.13	1.06 – 1.20	<0.001
NSAID use per month ^y	0 (0,1.5) (63)	0 (0,1) (983)	1.07	1.00 – 1.14	0.056

Definitions for Table 5.6

Comparisons between asthmatic-wheeze and non-wheeze groups were made using logistic regression for single variables. Odds ratios represent probability of demonstrating asthmatic wheeze in relation to non-wheezers with significance determined at $p < 0.05$.

y Median (25th, 75th) percentile reported for number of times paracetamol or NSAID use/month

Figure 5.1 Time in Years for Wheeze Onset and its Relation to Cigarette Smoking



The mean age of wheeze onset was retrospectively inquired at the age of 18, with non-asthma-wheeze teenagers reporting mean age onset of wheeze symptoms 14.5 years (95% confidence interval (CI) [13.8 – 15.2]) compared to asthmatic wheeze with 6.3 years (95% CI; 5.3 – 7.3). Similarly mean age of active smoking for non-asthma wheeze was 13.5 years (95% CI; 12.8 -14.1) and that of asthmatic wheeze was at 14.4 (95%CI; 14.1 – 14.7).

In figure 5.1, the pentagon/star markers represents the age at which children started to wheeze or were diagnosed with asthma. The x-axis represents the age at which smoking was started and y-axis represents age at which wheeze was experienced. It was observed that most of the children with diagnosed asthma well in advance of starting tobacco smoking, whereas majority of the Non-Asthmatic Wheeze children developed wheeze after starting tobacco smoking.

Table 5.7 Multivariate Analysis of Factors Associated with Asthmatic–Wheeze Compared to Non–wheeze

Risk Factor	Odds ratio	95% Confidence Interval	p - value
Gender (female)	1.48	0.96 – 2.29	0.08
Family history of asthma ^Q	1.74	1.14 – 2.66	0.01
Asthma/wheeze at 1 or 2 years	1.46	0.75 – 2.84	0.26
Chest infection at 1 or 2-years	1.14	0.60 – 2.15	0.69
Teenage smoking (past or current)*	1.35	0.88 – 2.08	0.17
Atopy at 4 year [△]	3.98	2.52 – 6.28	<0.001
Rhinitis at 10 years	3.97	2.57 – 6.14	<0.001
Paracetamol use at 18 years ^γ	1.10	1.03 – 1.17	0.005
Number of days exercise at 18 [#]	1.05	0.96 – 1.15	0.33

Factors for asthmatic wheeze showing significance at univariate analysis ($p < 0.1$) were entered en-bloc into logistic regression to assess independent effect of each factor. Multivariate logistic model was controlled for gender. Odds ratios represent probability of asthmatic wheeze to non-wheeze determined at significance of $p < 0.05$

^QFamily history of asthma, determined at birth from parents

* Self smoking status current or past was determined at the 18 year follow-up.

[△]Atopy defined by at least 1 skin test wheal response > 3 mm diameter.

^γ Average number of times paracetamol use/month

[#] Geometric mean of number of days per week vigorous exercise is done

Factors demonstrating independent significance towards asthmatic-wheeze at 18 year were a) family history of asthma, b) rhinitis at 10, c) atopy at 4 and d) higher average number of times paracetamol usage per month. This model was controlled for gender of teenagers.

Table 5.8 Multivariate Analysis of Factors Associated with Non-Asthmatic-Wheeze Compared to Non-Wheeze at 18-Years

Risk Factor	Odds ratio	95% Confidence Interval	p - value
Gender (female)	1.47	0.72 – 3.00	0.29
Rhinitis at 18 years	2.82	1.38 – 5.73	0.004
Atopy at 4 year [△]	1.90	0.82 – 4.38	0.13
Teenage smoking (past or current)*	2.54	1.19 – 5.41	0.02
Paracetamol use at 18 years [∇]	1.11	1.01 – 1.23	0.03
NSAID use at 18 years	1.01	0.90 – 1.12	0.89
Family history of asthma ^ℓ	2.26	1.10 – 4.63	0.03
Home tobacco smoke exposure during pregnancy [⌘]	1.57	0.77 – 3.18	0.22

Factors for non-asthmatic wheeze showing significance at univariate analysis ($p < 0.1$) were entered en-bloc into logistic regression to assess independent effect of each factor. Multivariate logistic model was controlled for gender. Odds ratios represent probability of non-asthmatic wheeze to non-wheeze determined at significance of $p < 0.05$

[△] Atopy defined by at least 1 skin test wheal response ≥ 3 mm diameter.

* Self smoking status current or past was determined at the 18 year follow-up.

[∇] Number of times paracetamol or NSAID use/month.

^ℓ Family history of asthma, determined at birth from parents.

[⌘] Tobacco smoke exposure inside the house by either parents or any other member.

In a multivariate logistic regression model controlling for gender, independent significance for non-asthmatic-wheeze was observed for having rhinitis at 18 years, asthmatic family history, higher use of paracetamol and current smoking in comparison to non-wheeze adolescents (Table 5.8).

5.3 Discussion

Wheeze at age 18, demonstrated two distinct phenotypes, asthmatic and non-asthmatic-wheeze, with overlapping features for family history of asthma and increased use of paracetamol, but differing with respect to greater atopic predisposition along with earlier presentation of upper airway involvement (i.e. rhinitis) for asthma, whereas non-asthmatic wheeze was associated with personal tobacco smoking and delayed onset rhinitis.

There are not many studies that have characterized wheeze from birth to adolescence (51), especially whole population studies. The Isle of Wight birth cohort provides a rare opportunity to study a cross-section of teenagers, who have been studied at six time points and allows us to address the heterogeneous nature of wheezing illness. Not much is known about wheeze phenotypes over adolescence, and any significant wheezing identified in teenagers by observational studies is generally considered to represent asthma unless proven otherwise (241, 257, 258). Wheezing illness that goes un-recognised over adolescent period has largely been regarded as un-diagnosed asthma (122, 124, 244). Although it is possible that some of the un-recognised wheeze over adolescence is under-diagnosed asthma, but teasing out non-asthma-wheeze from asthmatic wheeze should be possible in a country where health care access is considered good. This needs further investigation into the causes of this lack of recognition other than implicating adolescent behaviour to account for it (126).

In one of the largest adolescent asthma surveys in the United States, frequent wheeze was observed to be associated with smoking and considered to be under diagnosed asthma (122). Both passive (244) as well as active smoking (122) has been linked to asthma/wheeze over the teenage years. We observed that smoking was associated with non-asthmatic-wheeze, a distinct adolescent wheeze phenotype, but not to asthma at 18. Additional review of information on timing of wheeze demonstrates almost all of non-asthmatic-wheeze presented after initiation of smoking and as expected there was no such relationship for asthmatic wheeze. This was not observed for asthmatic wheeze. Majority of non-asthma-wheeze followed rather than preceded the age at which smoking was taken up whereas those with asthma were more likely to have had wheeze before they took up tobacco smoking themselves (figure 5.1). Personal smoking demonstrated significance only at univariate analysis for asthma, and this was not observed in the multivariate model after adjustment for other factors such as atopy and gender. The association of smoking and asthma over adolescence has been inconsistent, with some demonstrating significant links to active tobacco smoking (160, 256) whilst others have suggested that it is relevant to asthma severity and persistence rather than being a risk factor for its development (158, 163).

It is interesting to note that the association to smoking has been quite consistent for non-atopic group of wheezy individuals over adolescent period, whether diagnosed as asthma or undiagnosed (76, 81, 259) and even in those studies that have reported smoking to be associated with adolescent asthma, they have reported a stronger association of smoking to non-atopic wheeze (160). The studies that have reported an association of tobacco smoke to asthma, in addition to having diagnosis of asthma by a doctor, used other parameters such as two events of spastic bronchitis or different parameters leading to variation in results (160).

Rhinitis at age 18 was observed to be associated with non-asthma-wheeze whereas asthmatic wheeze demonstrated significance for rhinitis at an earlier age of 10 years. The relevance for different time points and rhinitis becomes apparent when we consider the one airway one disease concept, i.e. rhinitis (upper respiratory tract) and wheezing illness (lower respiratory tract) are functionally part of one airway disease, with an arbitrary anatomical separation (135, 260). This hypothesis explains earlier association of rhinitis with asthma as onset of asthmatic wheeze was reported at an earlier age compared to non-asthmatic-wheeze.

In addition to smoking, higher average monthly paracetamol use was also associated with wheeze in teenagers (261). Paracetamol use predisposes lungs to oxidative injury by blocking glutathione reductase enzyme (254), we speculate that coupled with tobacco smoke, this insult is likely to lead to damaging impact on not just lungs but the complete respiratory system as observed in our study with onset of wheeze and rhinitis by the age of 18 in those with non-asthma-wheeze. There is only one earlier report of dual risk factors of smoking in conjunction with higher paracetamol use leading to wheeze and rhinitis, however the authors observed those associations to be weak (262). Passive tobacco smoke exposure during early life was not observed to be significant in the multivariate model for non-asthmatic wheeze. However increase in susceptibility of pulmonary system results by either a breach in protective mechanism such as glutathione enzymes by paracetamol use as mentioned earlier or alpha-1- anti-trypsin deficiency acting as dual risk factors as has been observed in a recent study in Germany that was part of the ISAAC study group (160).

Family history of asthma was associated with both wheeze as well as asthma and this is a consistent finding amongst most of the asthma studies (98, 263, 264). This underscores significance of genetic predisposition for environmental influences; however this interaction is complex, and required further investigations.

Although early life atopy at the age of 4 was associated with asthma at 18, but atopy at all the other follow-ups was significant at the univariate level. On the other hand, non-asthma-wheeze did not demonstrate association to atopy at any time. The different risk profiles for asthma and non-asthma-wheeze has been overlooked in the majority of earlier studies investigating adolescent asthma and wheeze (122). We report a distinct wheeze phenotype that shares some basic risk features of genetic predisposition with asthma and to environmental exposures of higher paracetamol use but differs by way of lacking atopy and an important effect of active tobacco smoke exposures was observed.

CHAPTER 6: DO CHILDREN GROW OUT OF ASTHMA? TRANSITIONS OF ASTHMA OVER ADOLESCENCE

6.1 Introduction

Adolescence is characterised by profound changes in gender-related asthma prevalence. A gender switch in the prevalence of asthma is a recognized phenomenon (25, 33), describing a change from male towards female predominance. However the mechanism of this change has not yet been understood with studies attributing this transition to either higher persistence (51, 265) or the new-onset of asthma in females (85). Earlier cohort studies have demonstrated that half of childhood asthma enters clinical remission during this time (39, 266). This is reflected in clinical practice where it is not uncommon for adolescents and young adults to say that they have “grown out” of asthma.

The association between atopy and new onset asthma in adolescence is controversial. Some studies observed a rise in non-atopic asthma with increasing age (85, 267), but other studies suggested that atopy continues to be an important risk factor for teenage asthma (98, 237). We prospectively collected information from a whole population birth cohort established on the Isle of Wight in 1989. We assessed the prevalence, remission, and incidence of asthma over adolescence and examined the influence that gender and atopic status may have for developing asthma.

6.2 Results

We investigated the transition of asthma over adolescence by evaluating asthma status in the study participants from 10 to 18 years. A significant rise in the overall prevalence of asthma was observed from 10 to 18 years (14.7% to 17.9%, $p = 0.027$) (Table 6.1). At 10 years, there was predominance of asthma in males with prevalence of 17% (118/696) compared with 12.4% in females (83/672, $p = 0.018$), however at 18 years, this male dominant pattern disappeared with more females with asthma (19.5% versus 16.4 % of males) but this difference did not reach statistical significance ($p = 0.18$) (Table 6.1).

We studied the influence of atopy in the context of changing gender prevalence for asthma during adolescence (10 to 18 years), an overall rise in prevalence of atopic

asthma was observed from 9.7% (100/1034) at 10 years to 13.4% (114/852, $p=0.014$) at 18 years (Table 6.1). This increment was observed in both males and females but was statistically significant for females only with an increasing prevalence from 7.1% (37/518) at age 10 to 11.7% (52/446, $p = 0.021$) at age 18.

This was further supported by loss of statistical significance for the difference in prevalence for atopic asthma between the genders 18 years, which at an earlier follow-up of 10 years, was demonstrative of a higher prevalence in boys – 12.2% versus 7.1% in girls, $p=0.008^{**}$. The overall prevalence of non-atopic asthma did not change from age 10 to 18. Gender wise comparison for non-atopic asthma revealed there were more females with non-atopic asthma compared to males ($p=0.02$) at 18 years, whilst at 10 years there was no difference. Gender wise atopic proportion of asthma at the ages of 10 and 18 years, demonstrated increased ratio of atopic asthma at 18 years in males compared to females (Table 6.2). The proportion of atopic asthma in females remained stable.

In order to better understand the changes taking place over adolescence, data on asthma at age 4 (Early life) was reviewed. Asthma prevalence at age 4 was similar to that at age 10 years (Adolescent), however with equal gender ($p = 0.47^{**}$) and atopic ($p = 0.13$) proportions (Table 6.1).

Patterns of gender and atopy were examined prospectively in context of asthma prevalence from age 4 to 18 years that demonstrated a stable prevalence over the childhood period with significant increase in the second decade of life. When we assessed prevalence of atopic asthma a consistent rising pattern was noted from age 4 to 18.

Gender distribution fluctuated from age 4 to 18 years. Asthma prevalence was equal between the genders at age 4, which demonstrated a male dominant pattern at age 10 and by 18 years was again demonstrative of non-dominant pattern.

The non-atopic asthma had highest prevalence of 9.2 at age 4, with lower but stable prevalence over 10 and 18 years. Gender wise distribution of asthma in the non-atopic phenotype was only demonstrative of female dominant pattern at age 18 with equal prevalence at both age 4 and 10 years. Atopic to non-atopic proportions of asthma showed a greater proportion of atopic asthma compared to non-atopic phenotype with ($p=0.004$) and ($p<0.001$) from age 10 to 18 years respectively.

Table 6.1 Asthma Prevalence from Age 4 to 18 Years

Asthma 12-month Period Prevalence % (n/total)		Prevalence % (n/total)			
		Early Life	Adolescence		Time Trends § (p – value)
			Age 4	Age 10	
All	Both Males Females p-value**	14.9 (181/1214) 15.7(97/619) 14.1 (84/595) 0.47	14.7 (201/1368) 17.0 (118/696) 12.4 (83/672) 0.018	17.9 (234/1306) 16.4 (106/646) 19.4 (128/659) 0.18	<0.001 [§]
Atopic*	Both Males Females p-value**	7.2 (71/980) 8.7 (43/492) 5.7 (28/488) 0.09	9.7 (100/1034) 12.2 (63/516) 7.1 (37/518) 0.008	13.4 (114/852) 15.3 (62/406) 11.7 (52/446) 0.15	<0.001 [‡]
Non-Atopic*	Both Males Females p-value**	9.2 (90/980) 9.2 (45/492) 9.2 (45/488) 0.94	6.2 (64/1034) 6.6 (34/516) 5.8 (30/518) 0.69	5.8 (49/852) 3.9 (16/406) 7.9 (35/446) 0.02	
Atopic to non-atopic proportions †	p-value†	0.13	0.004	<0.001	

Definitions for Table 6.1

* Atopic status at 18 years from all study participants that had a skin prick test at 18 years

** The p-value represents the significance of difference between males and females in asthma and atopic groups

† The p-value represents the significance of difference between atopic and non-atopic asthma groups

§ Trends over 4 to 18 years for asthma prevalence in different subgroups were determined using generalized estimating equations with significance at $p < 0.05$

¶ The p-value represents significance of trends asthma prevalence over 4 to 18 years

‡ The p-value represents significance of trends of atopic to non-atopic proportion of asthma over 4 to 18 years

Table 6.2 Gender Wise Difference in Atopic to Non-atopic Proportions of Asthma

Gender	Age 10	Age 18
Male	Atopic/non-atopic ratio: $63/34 = 1.9$	Atopic/non-atopic ratio: $62/16 = 3.9$
Female	Atopic/non-atopic ratio: $37/30 = 1.2$	Atopic/non-atopic ratio: $52/35 = 1.5$
p-value*	0.21	0.006

*Difference between male to female atopic to non-atopic asthma proportions by chi-square test.

Information on 94% (220/234) asthma patients was available to determine asthma transition patterns. Persistent asthma comprised 56% (125/220) of all asthma cases at 18 years with remaining 44% (95/220) representing adolescent onset asthma (Table 6.3). A large proportion of adolescent onset asthma comprised new asthma that developed over 10 to 18 years, which was termed as new adolescent onset asthma. This new onset asthma was observed for the first time with only 23% (17/73) of these cases having had diagnosed asthma at an earlier follow-up at ages of 1, 2 or 4 years (Table 6.3).

Asthma at 18 years, demonstrated equal gender prevalence, brought about by greater proportions of non-atopic females 5.5% (21/380) developing asthma (males 1.9% (6/322), $p = 0.021$), reversing the male dominant pattern observed at 10 years of age (Table 6.3). Contrary to this, new onset childhood asthma (NOCA) from 4 to 10 years exhibited a male dominance with increasing number of atopic males effecting change from equal gender prevalence at age 4 towards higher male prevalence of asthma.

Allergic sensitization was also found to be associated with a significant risk for adolescent onset asthma with relative risk (RR) of 2.5 (1.6 - 4.0) with atopy determined at age 18 years, and RR of 2.6 (1.7 to 4.0) with atopy at age 10 years (Table 6.3 & 6.4). This effect remained significant after gender stratification.

A trend towards greater remission in boys was observed with 38 of 104 asthmatics at 10 years experienced relief of asthma symptoms, in comparison to only 18 out of 77 girls with asthma at 10 years (boys: 36.5 %, girls: 23.4%; $p = 0.08$). In addition, when we assessed non-atopic groups from 10 to 18 years, we observed remission in teenagers lacking allergic sensitization; [RR: 0.4, (0.2-0.7)]. However, when stratified by gender this was significant for males only (Table 6.3).

Table 6.3 Asthma Transition Phenotypes from Age 4 to 18 Years

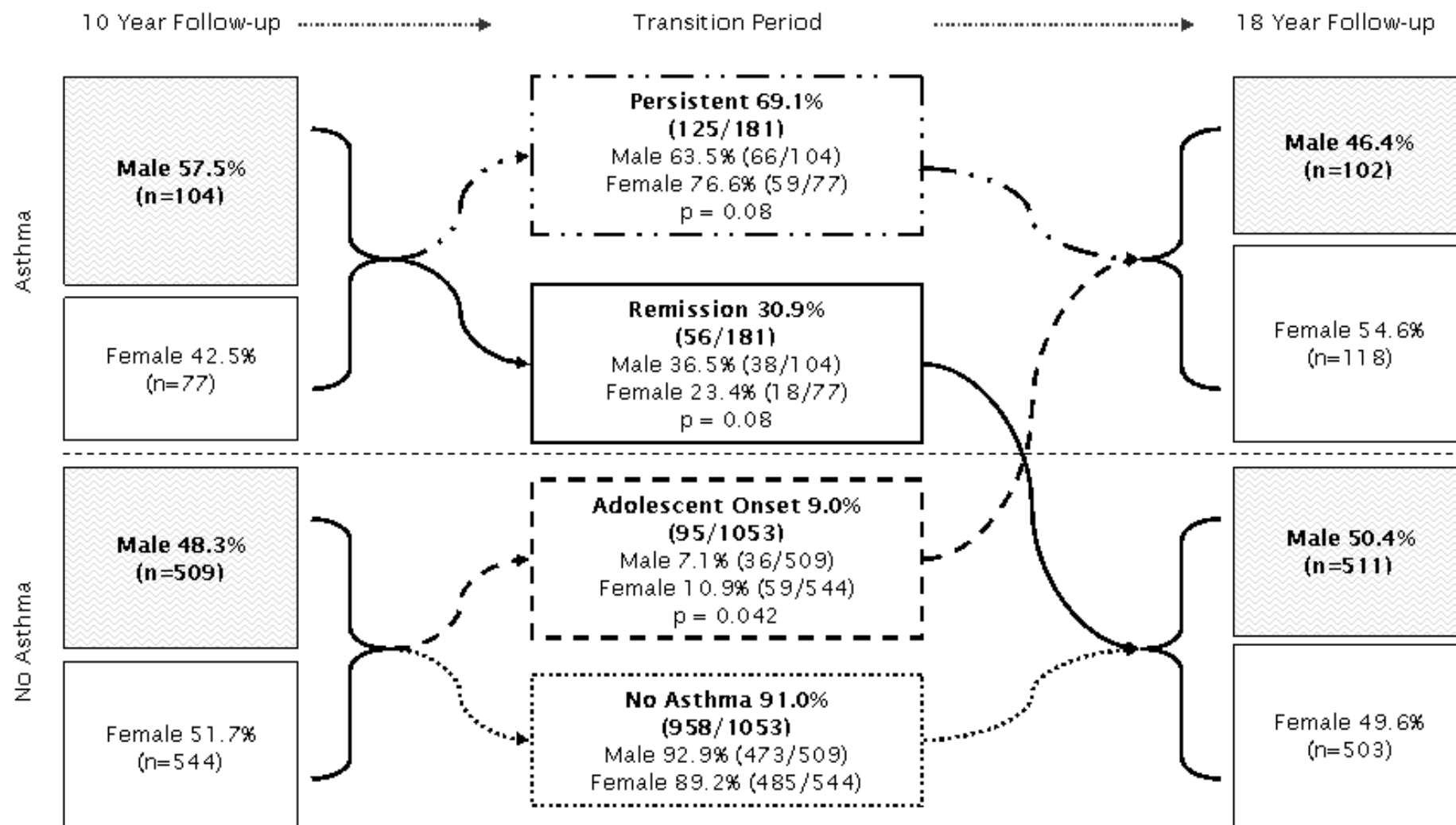
Asthma 12-month period prevalence % (n/total)		New onset childhood asthma % (n/total)	Remission % (n/total)	Adolescent onset asthma % (n/total)	Remission % (n/total)
		Age 4 - 10	Age 4 - 10	Age 10 - 18	Age 10 - 18
All	Both Males Females p-value**	8.0 (78/981) 10.5 (52/497) 5.4 (26/484) 0.004	47.7 (84/176) 47.4 (45/95) 48.2 (39/81) 0.96	9.0 (95/1053) 7.1 (36/509) 10.9 (59/544) 0.042	30.9 (56/181) 36.5 (38/104) 23.4 (18/77) 0.08
Atopic*	Both Males Females p-value**	5.5 (43/783) 8.3 (32/387) 2.8 (11/396) 0.001	14.2 (21/148) 16.5 (13/79) 11.6 (8/69) 0.54	5.4 (38/702) 6.5 (21/322) 4.5 (17/380) 0.30	10.2 (13/127) 12.9 (9/70) 7.0 (4/57) 0.43
Non-Atopic*	Both Males Females p-value**	3.4 (27/783) 3.9 (15/387) 3.0 (12/396) 0.65	31.8 (47/148) 29.1 (23/79) 34.8 (24/69) 0.57	3.8 (27/702) 1.9 (6/322) 5.5 (21/380) 0.021	13.4 (17/127) 15.7 (11/70) 10.5 (6/57) 0.55
Relative risk of new onset asthma or remission by atopy (95% CI)**	Both Males Females	5.2 (3.3 - 8.1) 5.5 (3.2 - 9.7) 3.9 (1.8 - 8.3)	0.3 (0.1 - 0.5) 0.5 (0.3 - 0.9) 0.4 (0.2 - 0.7)	2.5 (1.6 - 4.0) 4.6 (1.9 - 11.1) 1.9 (1.04 - 3.4)	0.4 (0.2 - 0.7) 0.4 (0.2 - 0.7) 0.4 (0.1 - 1.1)

* Atopic status at based on atopy determined at 10 and 18 years for 4-10 and 10-18 years respectively for all study participants

** The p-value represents the significance of difference between males and females in asthma groups

** Relative risk of asthma was calculated by risk of asthma in atopic to that in the non-atopic group

Figure 6.1 Transition of different asthma groups over 10 to 18 years



An increase in prevalence of asthma over the adolescent period was observed with a low remission rate of 30.9 % (56/181) from 10 to 18 years, coupled with a high adolescent onset asthma of 9% (95/1053), resulting in absence of net remission over adolescence as only 56 adolescents outgrew their asthma with 95 developing asthma over the same period (Figure 6.1).

Table 6.4 New Adolescent Onset Asthma and its Comparison to Adolescent Onset Asthma

Adolescent onset asthma % (n/total)		Adolescent onset asthma (Age 10 – 18)	Relapse as a % of adolescent onset asthma (Age 10 -18)	New adolescent onset asthma (Age 10 -18)
All	Both	9.0 (95/1053)	23 (17/73)	9.2 (56/611)
	Males	7.1 (36/509)	19 (5/27)	7.0 (22/313)
	Females	10.9 (59/544)	26 (12/46)	9.6 (34/354)
	p-value**	0.042	0.65	0.23
Atopic* (prior)	Both	3.8 (31/809)	19 (5/26)	3.8 (21/555)
	Males	4.0 (15/379)	17 (2/12)	4.0 (10/250)
	Females	3.7 (16/430)	21 (3/14)	3.6 (11/305)
	p-value**	0.99	0.84	0.99
Non-Atopic* (prior)	Both	5.7 (46/809)	26 (10/39)	5.2 (29/555)
	Males	3.7(14/379)	27 (3/11)	3.2 (8/250)
	Females	7.4(32/430)	25 (7/28)	6.9 (21/305)
	p-value**	0.032	0.79	0.08
Relative risk of asthma by atopy** (95% CI)	Both	2.6 (1.7 to 4.0)		3.4 (1.9 – 6.2)
	Males	3.7 (1.8 to 7.2)		5.4 (2.1 – 14.1)
	Females	2.2 (1.3 to 3.8)		2.7 (1.2 – 6.0)

* Atopic status at 10 years from all study participants that had a skin prick test at 10 years

** The p-value represents the significance of difference between males and females in asthma groups

** Relative risk of asthma was calculated by risk of asthma in atopic to that in the non-atopic group

However when we restricted our analysis to include only those cases with new adolescent onset asthma after excluding any early life diagnosed asthma, our results did not change with regards to risk of atopy towards adolescent onset asthma however difference between gender did not reach statistical significance as sample lacked power to detect the difference between the genders.

6.3 Discussion

It is generally assumed that many children with asthma outgrow their disease during adolescence. Contrary to this belief (39, 40, 51, 85), we failed to observe net remission over this period. Indeed, there was a significant increase in asthma prevalence from 10 to 18 years due to a lower than expected remission (30.9 % in our cohort as opposed to ~50% reported by several previous studies (39, 85, 266) during this period, and a higher than expected adolescent onset of asthma of 1.1%/year (total 9%) compared to 0.71%/year observed by the UK national birth cohort study. There was only a 23% recurrence of outgrown childhood asthma observed in the adolescent onset asthma group. This pattern of low remission coupled with an increase in adolescent onset asthma was particularly evident in girls, resulting in a changing gender ratio from male towards female dominance of asthma over adolescence. In addition, we also observed, state of allergic sensitization continues to be a significant factor in both the onset and remission of asthma, during adolescence.

The absence of net remission over adolescence was unexpected; this has not been previously reported by earlier population-based prospective studies. The UK National Birth Cohort (25) and the Tucson Children's Respiratory study (58) have both demonstrated positive net remission of asthma over adolescence, as have others (38, 85). It is possible that this could be reflective of a change in trend over time as the Tucson study that enrolled new born children in early 1980's, they demonstrated low remission rate but the new asthma cases were lower in comparison to those growing out of asthma over the teenage years, therefore this resulted in positive net remission (58). We applied standardized ISAAC questionnaires both at 10 and 18 year follow-ups and the overall remission was assessed in 84% of the birth cohort, with more than 90% enrolment being achieved at both time points, thereby minimizing the sample selection bias. Study participants that were in education were more likely to attend the centre than those that answered telephone or postal questionnaires, however there was no difference with regards to prevalence of allergic disease morbidity, social class or gender in those that visited the research centre. Thus, it is unlikely to have affected our analysis as we have assessed the dynamics of gender and atopy towards occurrence of asthma over adolescence. Based on the use of standard epidemiological

procedures and definitions, we are confident in the validity of our findings in reporting the lack of net remission over the teenage years.

The lower remission of 30.9 % (56/181), accompanied with a higher incidence of 9% (95/1053), has resulted in an increasing prevalence of asthma over adolescence (from 14.7% to 17.9%). In the UK national birth cohort study, the prevalence of asthma was stable at 13.7% over 11 to 16 years. This is suggestive of two possibilities, one is a change in trend of asthma over adolescence i.e., a period effect, or the second possibility is that this finding could be due to characteristics shared by a group bonded by time of birth, i.e. a cohort effect (23). A recent report of increasing prevalence of asthma amongst the teenagers in the UK (268) provides support to the former view. It can be argued that parents tend to under-report (269) the symptoms that may lead to apparently lower remission and this needs to be taken into account, however this not likely to have affected our results relative to other cohorts studies.

Prospective studies have documented higher prevalence of asthma in boys during childhood, with transition over adolescence in which girls make up a larger proportion of the asthma cases (25, 85, 270). In our cohort, there was no significant gender difference in the asthma prevalence at 18 years, but a trend towards shift in asthma prevalence from boys to girls was observed between 10 and 18 years. Nicolai and colleagues studied changing gender prevalence and attributed this to increased incidence of asthma in girls (85), however the prevalence in their cohort was still male dominated at the age of 16 years and they were only able to follow-up a select sample of the original study population. Guerra et al suggested increased persistence of asthma in girls as a possible explanation for the changing gender difference in asthma prevalence (58). In our cohort, we observed a significant difference between the genders with females demonstrating a higher adolescent onset asthma (new and recurrence of early life asthma) compared to males but also trends towards higher persistence of asthma or in other words lower remission if we focus on the transition groups, thus affecting a change of gender ratio for asthma prevalence over adolescence (figure 6.1).

Few prospective studies have utilized objective tests for confirmation of atopic status by SPT, and have relied upon the use of questionnaire based information by using atopic disease conditions as proxy for this assignment (25, 52). We used SPT for the assessment of atopy at both 10 and 18 years allowing us to objectively assess changes in allergic sensitization over this period, particularly in relation to the transition of gender prevalence in asthma. Where previous studies have reported that childhood asthma is largely atopic with higher prevalence in boys, and teenage asthma is non-atopic and more common in the girls (85, 88, 266). Our study consolidates on

these earlier findings and suggests that in addition to higher risk of adolescent onset asthma in females lacking allergic sensitization, there is a continued role of atopy over adolescent period that is not well documented (98, 237). Another cohort study has highlighted the contribution of atopy during adolescence with particular reference to the association of new onset asthma in boys (98); however its role during this period continues to be questioned (128). Contrary to other studies, we observed an increase in the prevalence of atopic asthma over adolescence for both boys and girls; in addition to this loss of gender difference in asthma prevalence at 18 was present, which had demonstrated a male dominant pattern at the age of 10. Furthermore, there was a higher risk of developing asthma at 18 years if children demonstrated allergen sensitization at the age of 10 years (table 6.4). The association of atopy in girls has not been shown by earlier cohort studies and is likely to be accounted for by higher prevalence of atopy in their study populations at an earlier age of 10 years compared to our study. The prevalence of atopy in our cohort at 18 years of 41.3% was similar to the prevalence of atopy in the Dunedin cohort (44.7% at age of 13 years), and German MAS study (45.6%) at age 10, however overall prevalence of atopy at 10 years in our cohort was only 26.9%. The higher risk observed for both boys and girls are accounted for by differences in overall allergen sensitization status of the community at different time points. Since we are determining risk of adolescent onset asthma it is important to consider prior atopic status of those developing asthma. In addition to atopy, gender-specific factors play an important role over adolescence, increasing both persistent and adolescent onset asthma in female that is largely non-atopic. Current body of evidence suggests important role of gender in conjunction with genetic and environmental factors over the teenage years affecting clinical expression of asthma with predisposition of female gender towards asthma occurrence in the second decade of life (271).

Information from early life asthma was available to determine recurrence of childhood asthma that was in remission at 10 years, however this contributed to only 23% of adolescent onset asthma cases with no gender-related difference at 18 year follow-up. In the Isle of Wight birth cohort the majority of the adolescent onset asthma was due to new cases that had not been diagnosed as having asthma previously (Table 6.4). This is against the accepted dogma that nearly all asthma starts in childhood and adult asthma is generally a recurrence of childhood asthma. In the German cohort study, half of the asthma in adolescence was due to recurrence of childhood asthma with higher contribution by relapse of asthma in females (85). Very few studies have characterized asthma from birth into adolescence. Definitions used for new onset asthma over this period has varied; from those that consider recurrence and pure adolescent onset groups together to classify adolescent onset asthma (256), to those that exclude any prior asthma (153) while others remove any early life wheeze as well as diagnosed asthma (92). To provide an overall view of adolescent onset asthma in

this chapter we selected any new diagnosed asthma at 18, all of these participants did not have asthma at the age of 10 years. In order to quantify any prior asthma diagnosis we performed a sub-analysis to see the impact of considering exclusion of any prior asthma diagnosis from birth to 10 year follow-up (Table 6.4). Restricting our analysis to those with no history of early life asthma did not affect the risk of atopy for adolescent onset asthma. However due to smaller sample size, we lacked the power to comment on gender related difference between the non-atopic true adolescent onset asthma in females which was observed higher in adolescent onset group (Table 6.4), comprised of true as well as recurrence of childhood asthma.

An improved understanding of the dynamics and risk factors of asthma during adolescence is necessary before strategies can be devised to influence this change from persistent and new onset asthma towards remission. Gender-specific and puberty-related factors such as growth and hormonal effects might interact with immunological influences such as atopy to bring about this change.

CHAPTER 7: REMISSION COMPARED TO PERSISTENT ASTHMA

7.1 Introduction

Few birth cohorts have followed childhood asthma into adolescence (52, 58, 85, 127). All of the earlier studies have tried to address remission either directly; or indirectly by enumerating factors that are associated with persistence. We have demonstrated, lower than expected remission over adolescence with trends towards the changing gender difference shared by only a few previous cohorts (58) as asthma is assumed to go through remission over adolescence (38, 39, 85). In this chapter, we focus on characteristics of remission group and factors that are associated with this change. There is considerable variation in definition of remission of asthma. Some consensus exists on absence of asthma symptoms for over 12 months i.e. clinical remission (46, 256). Studies have shown evidence of underlying airway inflammation in a proportion of apparently remittent asthmatics has a potential for relapse in later in life (37, 46, 48). If clinical remission is supported by absence of airway inflammation on objective tests then true remission is presumed.

Adolescence is a period that is characterized by pubertal changes taking place in the body that coincides with the phenomenon of remission of asthma in a proportion of subjects with childhood asthma. The clinical remission during adolescence is associated with gain in lung function in combination with other significant pubertal changes that take place over this period of rapid growth and physical development. Amongst factors that have been studied for remission of asthma, tobacco smoke exposure and atopy are the ones that have been reviewed by most with inconsistent results (41, 52, 58, 158).

We hypothesised that growth of lungs over adolescence which is associated with pubertal growth spurt, is likely to account for clinical remission observed in asthmatic children. We investigated pubertal, as well as genetic and environmental factors that may account for remission of asthma over 10 to 18 years.

7.2 Results

Table 7.1: Symptoms & Treatment of Asthma at Age 10 Years between Participants whose Asthma Remitted and Persisted between 10 and 18 Years

Asthma symptoms & treatment	Remission % (n/N)	Persistent % (n/N)	Odds Ratio	95% Confidence Interval	p -value
Wheeze frequency >4 /year	38 (17/45)	50 (54/108)	0.61	0.30- 1.23	0.17
Exercise induced wheeze	57 (32/56)	70 (87/125)	0.58	0.31 – 1.11	0.10
Any sleep affected	57 (28/49)	48 (52/108)	1.44	0.73 – 2.82	0.02
≥1 times per week	4 (2/49)	16 (17/108)	0.23	0.06 – 0.93	0.04
Speech affected	13 (7/55)	15 (18/122)	0.84	0.34 – 2.11	0.72
Dry cough at night	54 (30/56)	65 (80/124)	0.64	0.34 – 1.20	0.16
Current treatment	76 (42/55)	93 (114/123)	0.23	0.10 – 0.63	0.002

Univariate logistic regression model with odds ratios for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)

We compared asthma related symptoms at 10 years in individuals experiencing remission of their asthma symptoms to those that had on going asthma over adolescence. There were no differences observed between these two groups with the exception of higher proportion of children with sleep related symptoms in the remission group ($p = 0.02$). However, when this was further investigated, persistent asthma had greater frequency of affected sleep ($p = 0.04$). A higher proportion of children who persisted with their asthma during adolescence were on current treatment at 10 years ($p = 0.002$).

Interestingly there was difference noted between recognised triggers for asthma at the age of 10 years, with lower proportion of remission group exhibiting asthma symptoms on exposure to house dust and animals.

Table 7.2 Recognised Triggers as Reported by Study Participants at 10 Year Follow-up by Remission or Persistence of Asthma Over Adolescence

Asthma triggers at 10 years	Remission % (n/N)	Persistent % (n/N)	Odds Ratio	95% Confidence Interval	p-value
House Dust	13 (6/47)	28 (33/116)	0.37	0.14 – 0.95	0.04
Pollen	15 (7/47)	25 (29/116)	0.53	0.21 – 1.30	0.16
Animals	2 (1/47)	16 (18/116)	0.12	0.02 – 0.91	0.04
Infection	62 (29/47)	64 (74/116)	0.91	0.45 – 1.84	0.80
Exercise	68 (32/47)	61 (71/116)	1.35	0.66 – 2.77	0.41
Stress	17 (8/47)	26 (30/116)	0.59	0.25 – 1.40	0.23

Univariate logistic regression model with odds ratios for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)

In order to objectively determine relevance of clinical triggers for asthma symptoms at age 10, we matched this with each group's specific allergic sensitization status at 10 and 18 years. It was noted that in relation to the identified trigger factors for persistent to remission groups (table 2), higher proportion of persistent asthma had house dust mite sensitization at age 10 (table 3) that was also present at age 18 (table 4), compared to remission group. The association between animal triggers at age 10 year and persistence equated with a significant association with atopic sensitization to dog at 18 years ($p = 0.04$) but not for cat at either 10 or 18 years.

Table 7.3 Allergen Sensitization Pattern at 10 Years for Remission In Relationship Persistence of Asthma

Allergens	Remission % (n/N)	Persistent % (n/N)	Odds Ratio	95% Confidence Interval	p-value
House Dust Mite	34 (15/44)	55 (59/107)	0.42	0.20 – 0.87	0.02
Grass	25 (11/44)	36 (38/107)	0.61	0.28 – 1.32	0.21
Tree	2 (1/44)	4 (4/107)	0.60	0.09 – 4.15	1.00
Cat	18 (8/44)	30 (32/107)	0.52	0.22 – 1.23	0.14
Dog	7 (3/44)	2 (22/107)	0.28	0.09 – 0.94	0.04
Cladosporium	2 (1/44)	2 (2/107)	1.22	0.16 – 9.63	1.00
Alternaria	2 (1/44)	6 (6/107)	0.39	0.06 – 2.58	0.67

Univariate logistic regression model with odds ratios for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)

Physical measures of height and weight were not differentially distributed between the remission and persistent asthma groups (data not shown). Pulmonary function data for remission and persistent asthma groups were examined at both 10 and 18 years (table 5a and 5b). As there were significant gender related differences in these measures at 18 years, we stratified this analysis by sex of the study participants.

There were no differences observed for height, weight, or change in these measures over adolescent period between remission and persistent asthma groups for males or females. However spirometric review revealed interesting results that is presented in the tables 5a and 5b.

Table 7.4 Allergen Sensitization Pattern at 18 Years for Remission in Relation to Persistence of Asthma

	Remission % (n/N)	Persistent % (n/N)	Odds Ratio	95% Confidence Interval	p-value
House Dust Mite	33 (10/30)	63 (61/97)	0.30	0.13 – 0.69	0.004
Grass	20 (6/30)	53 (51/97)	0.23	0.09 – 0.59	0.002
Tree	3 (1/30)	11 (11/97)	0.27	0.04 – 1.72	0.29
Cat	20 (6/30)	35 (34/97)	0.46	0.18 – 1.22	0.12
Dog	23 (7/30)	32 (31/97)	0.65	0.26 – 1.64	0.37
Cladosporium	7 (2/30)	8 (8/97)	0.80	0.18 – 3.55	1.00
Alternaria	10 (3/30)	20 (19/97)	0.46	0.13 – 1.57	0.28

Univariate logistic regression model with odds ratios for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)% (n/N) = Numbers are percentages (number/total number)

There was no difference between persistent and remission groups for pulmonary function (FEV1, FVC, FEV1/FVC or FEF25-75%) in males at 10 years (table 5a). At age 18, individuals in remission had better small airway function (FEF25-75%, $p = 0.009$), along with a trend towards improved air flow in larger airway (FEV1, $p = 0.09$) to those with persistent asthma. Persistent asthma also demonstrated lower FEV1/FVC ratio compared to remission group ($p = 0.04$) indicating significant airway obstruction at age 18. This ratio was observed to be preserved in the remission group.

The remission group improved more in their lung function from 10 to 18 years compared to persistent asthmatics with significant increase in both small ($p = 0.04$) and large airway functions ($p = 0.005$) (table 5 a). The changes observed in males were not accompanied by similar improvements in pulmonary function of remittent females.

Although there was a trend toward remittent females having better small airway function ($p = 0.09$) at the age of 10.

Table 7.5a Males: Height Adjusted Pulmonary Function from Age 10 to 18 Years by Asthma Remission or Persistence

	Remission (n = 17)	Persistent (n = 40)	β	95% Confidence Interval	p-value
Spirometry at 18 years*					
FEV ₁ (L) (S.E.)	4.65 (0.12)	4.41(0.77)	0.24	- 0.04 to 0.52	0.09
FVC (L) (S.E.)	5.39 (0.15)	5.45 (0.10)	- 0.06	- 0.41 to 0.29	0.72
FEV ₁ /FVC (S.E.)	0.87 (0.02)	0.82 (0.01)	0.05	0.15 to 0.10	0.04
FEF _{25-75%} (L/s) (S.E.)	5.21 (0.28)	4.29 (0.18)	0.92	0.24 to 1.60	0.009
Spirometry at 10 years*					
FEV ₁ (L) (S.E.)	2.05 (0.06)	2.02 (0.04)	0.03	- 0.11 to 0.17	0.68
FVC (L) (S.E.)	2.36 (0.05)	2.39 (0.04)	- 0.02	- 0.15 to 0.11	0.75
FEV ₁ /FVC (S.E.)	0.87 (0.02)	0.85 (0.01)	0.02	0.01 to 0.06	0.22
FEF _{25-75%} (L/s) (S.E.)	2.32 0.13)	2.19 (0.08)	0.14	- 0.18 to 0.45	0.39
Gain in Spirometric function from 10 to 18 years*					
FEV ₁ (L) (S.E.)	2.61(0.09)	2.38 (0.06)	0.23	0.02 to 0.45	0.04
FVC (L) (S.E.)	3.01(0.13)	3.07 0.08)	- 0.07	- 0.37 to 0.24	0.67
FEV ₁ /FVC (S.E.)	-0.0002 (0.02)	-0.03 (0.01)	0.03	- 0.01 to 0.07	0.10
FEF _{25-75%} (L/s) (S.E.)	2.69 0.17)	2.09 (0.11)	0.61	0. 19 to 1.03	0.005

*General Linear Model (GLM) for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals. Estimated marginal means used for determination of height adjusted means.

(n) represents number of participants that provided information

FEV₁ = Forced expiratory volume in first second in litres (L) with standard error (S.E.)

FVC = Forced vital capacity in litres (L) with standard error (S.E.)

FEV₁/FVC = ratio of FEV1 to FVC

FEF_{25-75%} = Forced expiratory flow 25 to 75% in litres per second (L/s) with standard error (S.E.)

Table 7.5b Female: Height Adjusted Pulmonary Function from Age 10 to 18 Years by Asthma Remission or Persistence

	Remission (n = 7)	Persistent (n = 45)	β	95% Confidence Interval	p-value
Spirometry at 18 years*					
FEV ₁ (L) (S.E.)	3.52 (0.16)	3.29 (0.06)	0.23	- 0.12 to 0.57	0.20
FVC (L) (S.E.)	4.08 (0.15)	3.96 (0.06)	0.12	- 0.20 to 0.43	0.46
FEV ₁ /FVC (S.E.)	0.87 (0.03)	0.83 (0.01)	0.03	-0.04 to 0.10	0.37
FEF _{25-75%} (L/s) (S.E.)	3.89 (0.35)	3.47 (0.14)	0.42	- 0.35 to 1.18	0.28
Spirometry at 10 years*					
FEV ₁ (L) (S.E.)	2.09 (0.09)	1.96 (0.04)	0.13	- 0.07 to 0.25	0.20
FVC (L) (S.E.)	2.33 (0.08)	2.26 (0.03)	0.07	- 0.011 to 0.25	0.44
FEV ₁ /FVC (S.E.)	0.90 (0.03)	0.87 (0.01)	0.03	- 0.02 to 0.08	0.27
FEF _{25-75%} (L/s) (S.E.)	2.62 (0.21)	2.22 (0.08)	0.40	- 0.06 to 0.85	0.09
Gain in Spirometric function from 10 to 18 years*					
FEV ₁ (L) (S.E.)	1.43 (0.12)	1.33 (0.05)	0.10	- 0.16 to 0.37	0.43
FVC (L) (S.E.)	1.77 (0.13)	1.69 (0.05)	0.08	- 0.19 to 0.35	0.57
FEV ₁ /FVC (S.E.)	- 0.03 (0.02)	- 0.03 (0.009)	0.000 1	- 0.05 to 0.05	1.00
FEF _{25-75%} (L/s) (S.E.)	1.29 (0.23)	1.25 (0.09)	0.05	- 0.46 to 0.55	0.86

* General Linear Model (GLM) for remission of asthma to persistent asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals. Estimated marginal means used for determination of height adjusted means.

(n) represents number of participants that provided information

FEV₁ = Forced expiratory volume in first second in litres (L) with standard error (S.E.)

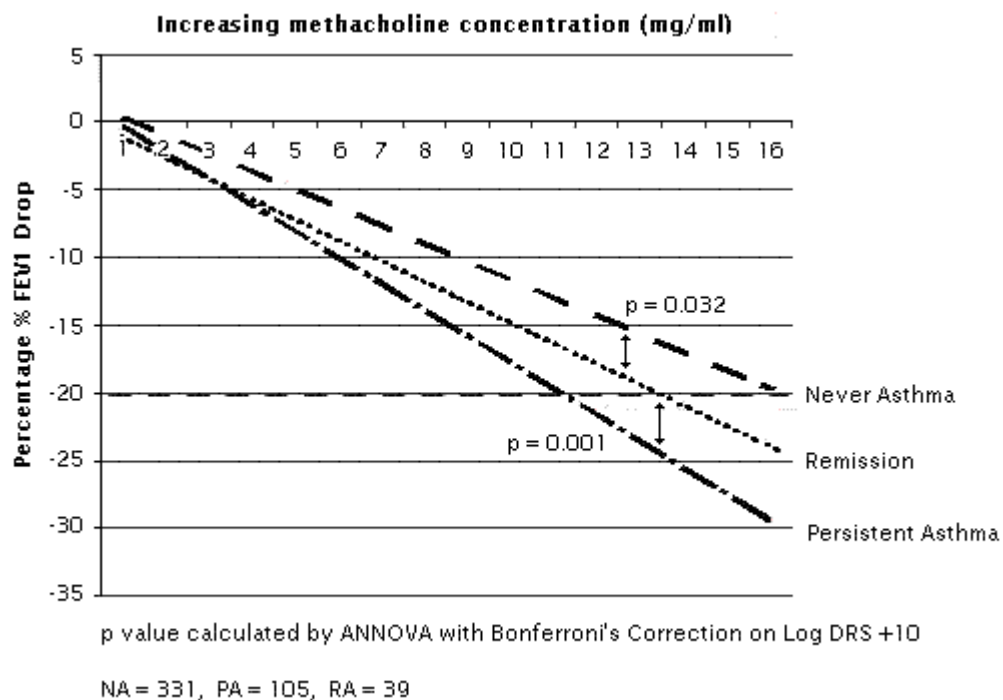
FVC = Forced vital capacity in litres (L) with standard error (S.E.)

FEV₁/FVC = ratio of FEV₁ to FVC

FEF_{25-75%} = Forced expiratory flow 25 to 75% in litres per second (L/s) with standard error (S.E.)

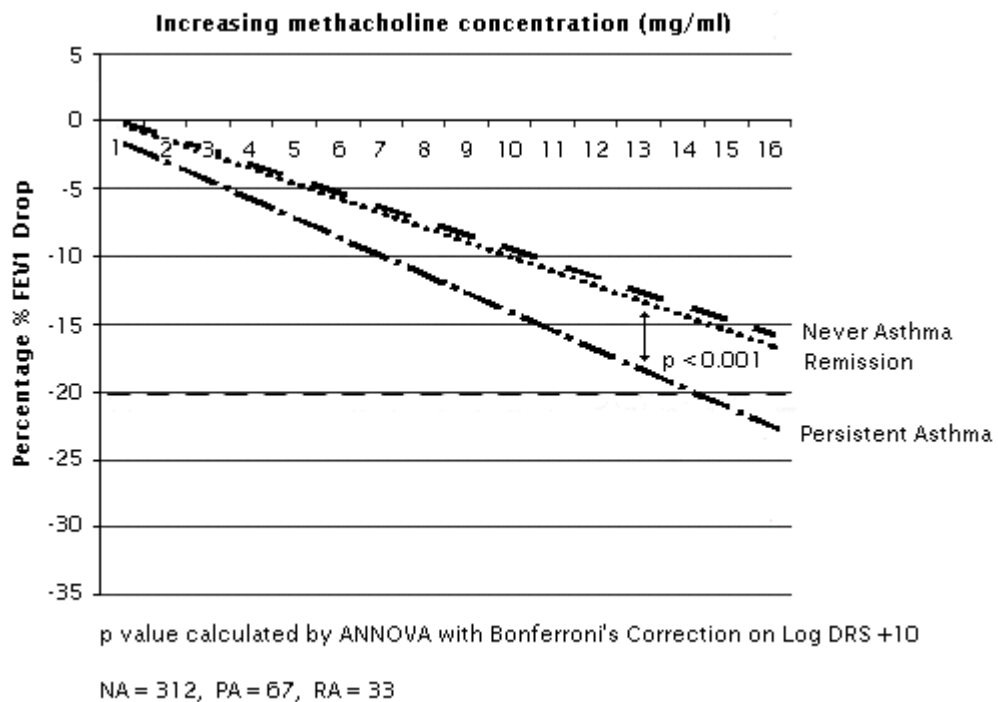
Methacholine bronchial challenge tests were performed at both 10 and 18 years. Additional data on reversibility and fractional exhaled nitric oxide was also collected at 18 years. We assessed bronchial responsiveness in remission group and compared it to both persistent asthma and those that never had asthma from birth. Remission group demonstrated significantly greater proportion of PC_{20} compared to never asthma group (table 6b) but lower to that of persistent asthma at both 10 and 18 years (table 6a). Bronchial reactivity, a continuous measure of bronchial airway responsiveness was used to determine differences between remission, persistent and never asthma groups (tables 6a & b). We evaluated difference of reactivity within gender; however both male and female had similar bronchial airway responsiveness (data not shown).

Figure 7.1 Bronchial Reactivity for Adolescent Asthma Transition Phenotypes (Remission to Persistent and Never Asthma) At 10 Years



Mean dose response slopes demonstrating bronchial reactivity for asthma phenotypes obtained by linear regression equation for each of the asthma phenotypes

Figure 7.2 Bronchial Reactivity for Adolescent Asthma Transition Phenotypes (Remission to Persistent and Never Asthma) At 18 Year



Mean dose response slopes demonstrating bronchial reactivity for asthma phenotypes obtained by linear regression equation for each of the asthma phenotypes

Bronchial reactivity (BR) in remission group was compared with persistent and never asthma groups, from age 10 to 18 years. Mean bronchial reactivity as determined by mean value of DRS using ANOVA with Bonferroni's correction was obtained for remission, persistent and never asthma groups. Mean DRS slopes for the three phenotypes are plotted in Figures 1 & 2. An overall decline in BR was observed over adolescence; however the pattern of BR was preserved with highest BR in persistent and lowest in never asthma.

At age 10, the remission group had higher bronchial reactivity (as shown by steeper gradient of dose response slope (DRS)) compared to never asthma but lower than those with persistent asthma, despite the fact that both group of children had asthma at age 10 had similar symptomatic morbidity and lung function. By age 18 years, the airway reactivity of remission group had further shifted towards the DRS of never asthma to attain similar bronchial responsiveness to that of never asthma.

Table 7.6a Methacholine Lung Challenge Test, Bronchodilator Reversibility and FeNO for Remission in Relation to Persistence of Asthma

Bronchial challenge	Remission	Persistent	Odds Ratio	95% CI	p-value
10 year					
PC ₂₀ [#] % (n/N)	41 (16/39)	66 (69/105)	0.36	0.17 – 0.77	0.008
Bronchial reactivity [†] (S.E.)	1.55 (0.08)	1.94 (0.06)	0.27	0.13 – 0.57	0.001
18 Year					
Positive PC ₂₀ % (n/N)	9 (3/33)	31 (21/67)	0.22	0.06 – 0.80	0.021
Bronchial reactivity [†] (n) (S.E.)	1.10 (33) (0.04)	1.42 (67) (0.06)	0.08	0.01 – 0.44	0.004
Reversibility § % (n) (S.E.)	4.03 (26) (0.58)	9.32 (91) (0.84)	0.86	0.78 – 0.94	0.001
Log ₁₀ (FeNO) [‡] (n) (SD)	1.24 (33) (0.06)	1.53 (65) (0.02)	0.05	0.01 – 0.24	<0.001

Univariate logistic regression model for remission of asthma to persistent asthma group determined at alpha level of p<0.05 with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)

(n) represents number of participants that provided information

(SD) standard deviation

[#] PC₂₀ is concentration of methacholine eliciting a drop of 20% of FEV₁ from baseline saline. Challenge test was considered positive, with a PC₂₀ of methacholine of 8mg/ml or less. Number of study participants with positive PC20 given as percentage % (n/N)

§ Relative percent reversibility of bronchodilator response following administration of 600 mcg of salbutamol

† A high value represents increased bronchial reactivity; obtained by log₁₀ (DRS+10) transformation of dose response slope (DRS)

‡ Log₁₀ transformation FeNO value as data was not normally distributed

Table 7.6b Methacholine Lung Challenge Test, Bronchodilator Reversibility and FeNO

	Remission	Never Asthma	Odds Ratio	95% CI	p-value
10 year					
PC ₂₀ [#]	41 (16/39)	20 (65/332)	2.86	1.43 – 5.72	0.003
Bronchial reactivity [†] (S.E.)	1.55 (0.08)	1.34 (0.02)	3.23	1.57 – 6.65	0.001
18 year					
Positive PC ₂₀ [#] % (n/N)	9 (3/33)	2 (7/312)	4.36	1.07 – 17.73	0.04
Bronchial reactivity [†] (n) (S.E.)	1.10 (0.04)	1.06 (0.01)	2.37	0.42 – 13.53	0.33
Reversibility § % (n) (S.E.)	4.03 (26) (0.58)	4.05(427) (0.24)	1.00	0.92 – 1.09	0.99
Log ₁₀ (FeNO) [‡] (n) (S.E.)	1.24 (33) (0.06)	1.27 (292) (0.05)	0.67	0.18 – 2.55	0.56

Univariate logistic regression model for remission of asthma to persistent asthma group determined at alpha level of p<0.05 with 95% confidence intervals.

% (n/N) = Numbers are percentages (number/total number)

(n) represents number of participants that provided information

(SD) standard deviation

PC20 is concentration of methacholine eliciting a drop of 20% of FEV1 from baseline saline. Challenge test was considered positive, with a PC20 of methacholine of 8mg/ml or less.

§ Relative percent reversibility of bronchodilator response following administration of 600 mcg of salbutamol

† A high value represents increased bronchial reactivity; obtained by log10 (DRS+10) transformation of dose response slope (DRS)

‡ Log10 transformation FeNO value as data was not normally distributed

Remission group was observed to have similar response to inhaled short acting bronchodilator (salbutamol: 600mcg) to that of never asthma group and lower compared to those with persistent asthma (table 6a &b). Fractional exhaled nitric oxide (FeNO) was used to determine atopic airway inflammation. Remission group had lower values of FeNO compared to persistent asthma and this was similar to the never asthma group (table 6).

Table 7.7 Inflammatory Cell Type from Induced Sputum

Induced Sputum	Remission (N = 14)	Persistent (N = 23)	Never asthma (N = 30)
Eosinophils, median (%) (IQR)	0.12 (0.88)	1.50 (4.5)	0.25 (1.25)
p - value	Ref.	0.11	0.53
Eosinophils $\geq 3\%$ (n/N)	7 (1/14)	35 (8/23)	13 (4/30)
p - value	Ref.	0.11	0.55
Neutrophils, median (%) (IQR)	27.8 (30.6)	9.8 (16.5)	12.0 (34.5)
p - value	Ref.	0.02	0.15
Epithelial cells, median (%) (IQR)	5.0 (10.6)	8.0 (10.0)	4.5 (9.3)
p - value	Ref.	0.29	0.91

Remission compared to persistent and never asthma, Mann-Whitney U test applied with significance determined at $p < 0.05$. Chi-square test applied for categorical eosinophils variable with significance determined at $p < 0.05$

Ref.: Remission used as reference group to which others compared

Values are medians with inter-quartile range (IQR)

Percentage (%) with n=number of individual with variable and N= total number of individuals that provided sputum sample

We invited all of the study participants from remission and persistent asthma groups to provide a sample of sputum. Remission group had a non-significant trend for less sputum eosinophils than persistent group (remission 7% (1/14) vs. persistent (35%, $p = 0.11$). This lack of significance may be due to small numbers. The remission group however, had a significantly higher neutrophil count.

Table 7.8 Allergic Sensitization, Early Life Wheeze/Asthma, Longitudinal Information on Allergic Diseases Rhinitis and Eczema

	Remission (n/N)%	Persistent (n/N)%	Odds Ratio	95% Confidence Interval	p-value
Atopy at 4,	42 (16/38)	58 (54/94)	0.54	0.25 – 1.16	0.112
Atopy at 10	43 (20/47)	70 (73/104)	0.39	0.19 – 0.80	0.01
Atopy at 18	43 (13/30)	75 (73/97)	.0.25	0.11 – 0.59	0.002
Wheeze at 1 or 2	46 (19/41)	37 (36/98)	1.49	0.71 – 3.11	0.29
Wheeze at 4	63 (32/51)	60 (64/107)	1.13	0.57 – 2.25	0.72
Wheeze at 10	89 (50/56)	88 (110/125)	1.14	0.42 – 3.10	0.80
Asthma at age 4	52 (26/50)	56 (59/106)	0.86	0.44 – 1.69	0.67
Rhinitis at 1 or 2	25 (11/44)	19 (19/98)	1.03	0.43 – 2.47	0.95
Rhinitis at age 4	15 (8/53)	17 (17/103)	1.00	0.40 – 2.50	1.00
Rhinitis at age 10	46 (25/55)	53 (64/121)	0.74	0.39 – 1.41	0.36
Rhinitis at age 18	34 (19/56)	74 (92/125)	0.18	0.09 – 0.36	<0.001
Eczema at 1 or 2	26 (11/43)	34 (34/100)	0.67	0.30 – 1.49	0.32
Eczema at age 4	24 (12/51)	31 (33/106)	0.68	0.32 – 1.47	0.33
Eczema at age 10	9 (5/56)	30 (36/122)	0.23	0.09– 0.64	0.004
Eczema at age 18	13 (7/56)	20 (25/124)	0.57	0.23 – 1.40	0.21

Univariate logistic regression analysis with odds ratios for remission of asthma to persistent asthma determined at alpha level of <0.05.

Numbers are percentages (number/total number)

Children destined to grow out of asthma were less likely to have atopy and eczema (and also BHR) at age 10 years. However, no significant differences were found in early childhood wheeze, asthma or eczema prevalence in the two groups.

Table 7.9 Family History of Asthma/Rhinitis Determined at Birth to Age 10

	Remission % (n/N)	Persistent % (n/N)	Odds Ratio	95% Confidence Interval	p-value
Family History of Asthma					
Maternal	13 (7/55)	15 (19/124)	0.81	0.32 – 2.05	0.65
Paternal	13 (7/55)	15 (18/123)	0.85	0.33 – 2.17	0.74
Parental	22 (12/55)	28 (35/124)	0.71	0.34 – 1.50	0.37
Sibling	21 (6/29)	17 (13/77)	1.28	0.44 – 3.88	0.65
Family History of Rhinitis					
Maternal	22 (12/55)	26 (32/124)	0.80	0.38 – 1.71	0.57
Paternal	13 (7/55)	20 (24/123)	0.90	0.45 – 1.79	0.27
Parental	31 (17/55)	39 (48/123)	0.70	0.36 – 1.38	0.30
Sibling	22 (12/55)	26 (32/124)	0.80	0.38 – 1.71	0.57

Univariate analysis by logistic regression was used for determining odds ratios for remission to persistent asthma, 95% confidence interval and p -value with significance at $p < 0.05$.

% (n/N) = Numbers are percentages (number/total number)

No difference was observed in family history of asthma and rhinitis as both remission and persistent asthma groups had similar proportions. However lower proportion of family history of hay fever in the siblings at the age of 10 year was associated with remission of asthma but there was no effect noted for family history of siblings with asthma for the same follow-up. Remission of asthma was not observed to be influenced by the early life events (table 10).

Table 7.10 Early Life Factors towards Remission of Asthma

	Remission	Persistent	Odds Ratio	95% Confidence Interval	p-value
Birth Weight (Kg)(SD)(n)	3.3 (0.57)(53)	3.3 (0.50)(123)	1.05	0.58 – 1.90	0.87
Pre-term delivery* % (n/N)	2 (1/53)	2 (2/123)	1.16	0.10 – 13.09	0.90
Post-term delivery ^Ω % (n/N)	4 (2/53)	5 (5/123)	0.93	0.17 – 4.95	0.93
Recurrent chest infections Birth to 2 years† % (n/N)	37 (15/41)	32 (31/98)	1.25	0.58 – 2.68	0.57
Less than 4 months breast feeding % (n/N)	78 (36/46)	65 (72/111)	1.95	0.88 – 4.35	0.10
Formula Feeding in first 4 months of life ^α % (n/N)	83 (38/46)	73 (80/109)	0.58	0.24 – 1.39	0.22
Maternal Smoking during pregnancy % (n/N)	27 (15/55)	19 (24/124)	1.56	0.74 – 3.28	0.24
Pet cat (0-4) %(n/N)	85 (34/40)	84 (77/92)	1.10	0.39 – 3.09	0.85
Pet dog (0-4) %(n/N)	40 (16/40)	32 (29/92)	1.45	0.67 – 3.13	0.35

Univariate analysis by logistic regression was used for determining odds ratios for remission to persistent asthma, 95% confidence interval and p -value with significance at $p < 0.05$.

General Terms

%(n/N) percentage determined from number of participants with condition/total number of participants with available information on the variable

(SD) = standard deviation (n) = number of participants

Specific Terms

* Pre-term delivery was defined as delivery of child in less than 37 complete weeks of gestation

^Ω Post-term delivery was defined delivery of child after 42 complete weeks of gestation

† Recurrence of chest infections reported by parents from birth to 2 years

^α Infants starting formula milk in first 17 weeks after birth

^χ Regular exposure to tobacco smoke inside the house reported at any time from birth to age 4 years

Table 7.11 Adolescent Factors and Their Relation to Remission of Asthma

	Remission	Persistent %	Odds Ratio	95% Confidence Interval	p-value
Current cigarette smoking at 18 years % (n/N)	31 (16/51)	39 (47/120)	0.71	0.35 – 1.42	0.34
Current and past cigarette smoking [#] %(n/N)	43 (22/51)	58 (70/120)	0.54	0.28 – 1.05	0.07
Mean age of tobacco smoke initiation in years (n)(SD)	14.5 (20)(1.5)	14.7 (67) (1.5)	0.92	0.66 – 1.27	0.60
Tobacco smoke exposure inside the house at 10* %(n/N)	53 (28/53)	43 (52/121)	1.49	0.78 – 2.84	0.23
Tobacco smoke exposure inside the house at 18* %(n/N)	26 (12/46)	26 (25/98)	1.03	0.46 – 2.29	0.94
Median number of days exercise/week (n) (p)	2.0 (55)(0,7)	2.0 (124) (2,5)	1.12	0.98 – 1.27	0.11
Mean number of hours watch TV/computer weekday (n)(SD)	3.9 (55)(3.0)	3.6 (124) (2.5)	1.07	0.94 – 1.18	0.32
Mean number of hours watch TV/ computer weekend day (n)(SD)	3.5 (55)(2.9)	3.2(124) (2.7)	1.04	0.92 – 1.18	0.52
Farm living [‡] %(n/N)	4 (2/55)	9 (11/123)	0.38	0.16 – 2.27	0.22
Annoyed by pollution at least once a week %(n/N)	9 (5/55)	14 (17/122)	0.62	0.22 – 1.77	0.37
Car Passing house at least 1 per hour %(n/N)	67 (37/55)	70 (86/123)	0.88	0.45 – 1.75	0.72
Heavy Vehicles at least 1 per hour %(n/N)	36 (20/55)	42 (52/123)	0.78	0.41 – 1.50	0.46
OCP use in girls§ % (n/N)	27 (4/15)	50 (28/56)	0.36	0.10 - 1.28	0.12
Paracetamol use/month	0 (0, 1)	2 (0,4)	0.84	0.71 – 0.99	0.04
NSAID use/month	0 (0, 0)	0 (0, 1)	0.93	0.87 – 1.13	0.93

Definitions for Table 7.11

Univariate logistic regression analysis was used to determine odds ratios for remission to persistent asthma, 95% confidence intervals and p-values with significance determined at $p < 0.05$.

General Terms

% (n/N) percentage determined from number of participants with condition/total number of participants with available information on the variable; (SD) = standard deviation (n) = number of participants (p) = 25th and 75th percentiles

This includes both current and those who have ever smoked cigarettes at any time in the past

* Tobacco smoke exposure inside the house excluding self-smoking

‡ Study participants that reported positively to “Have you ever lived on a farm”

§ n = number of girls reporting oral contraceptive use (OCP)

Similar to early life factors, no clear indicators favouring remission emerged at univariate level. However a trend was noted in asthmatic individuals that smoked less were more likely to remit than those that continued to smoke. Along with smoking, lower use of paracetamol also showed trend supporting growing out of asthma.

Table 7.12 Adolescent & Childhood Factors (Final Model)

Remission of asthma to Persistent asthma	Odds Ratio	95% Confidence Interval		p - value
Sensitization to house dust mite & / or dog at 10 years	1.34	0.41	4.43	0.63
Number of times paracetamol used per month	0.41	0.21	0.79	0.008
Rhinitis at age 18	0.19	0.06	0.59	0.004
Gain in small to mid-sized airway function (FEF _{25-75%})	1.74	1.03	2.94	0.039
Bronchial reactivity at 10 years	0.30	0.10	0.90	0.032

Odds ratios for remission to persistent asthma are presented by backward stepwise multivariate logistic regression analysis was used with significance determined at $p < 0.05$

The final regression model included all factors that demonstrated significance at $p < 0.1$, for remission asthma. In addition to the generic selection of variables demonstrating significance for remission of asthma, available information on atopy was assessed at 10 and 18 years for independent effect in the final model, both of which did not demonstrate significance towards the final model. Atopic status was further screened to match objective skin prick test to most significant identified clinical trigger, therefore allergic sensitization for house dust mite and/ or dog at 10 years were selected for the final model.

For lung function, gain in $FEF_{25-75\%}$ (gain in small airway function) demonstrated significance in the final model. Other factors that were assessed were gender, use of paracetamol, and tobacco smoking, active or past. In the backward stepwise removal process, gender and tobacco smoking status were removed. This model demonstrated independent significance for lower paracetamol use and lower bronchial reactivity at 10 years. The final combined model demonstrated significant associations for remission of asthma with lower bronchial reactivity at the age of 10 (predicting remission), along with reported lower use of paracetamol during adolescence. However, neither atopy nor tobacco smoke exposure were observed to play a role during adolescence.

7.3 Discussion

The findings from our study support the view that remission of asthma is not only associated with better growth in lower airway airflow but also accompanied by significant improvement of upper airway symptoms of rhinitis, thus validating one airway hypothesis. In addition to this level of bronchial reactivity at 10 years was observed to be predictive of remission state at 18 years. Amongst the factors lower use of paracetamol was the single most important factor that demonstrated significant advantage in growing out of asthma.

Characteristics of those in clinical remission of asthma was assessed through symptom questionnaire and objective tests of lung function, sputum cells, FeNO, methacholine bronchial challenge tests. Although our definition of remission was based on absence of wheeze symptoms for at least 12 months, however the additional question on age at which asthma stopped revealed each individual experienced remission for a minimum duration of 3 years at the 18 year follow-up. Remission group was not different in terms of severity of asthma at 10 years of age, when compared to the persistent group.

Eosinophilic infiltration of the airways is a prominent feature of asthma (272, 273). Eosinophilic count (EC) in the induced sputum correlates with asthma severity in both treated and untreated subjects (75). We therefore selected EC as the biomarker to estimate sample size for sputum studies. Several studies (272-274) have shown that children with asthma have an increased EC in sputum, however the proportion of eosinophils differs widely (1-4% vs. 18-41%). Given these differences and taking the skewed distribution of eosinophils into account, we required a sample of at least 15 children in four groups (based on asthma status over 10 to 18 years; Persistent, Never, Adolescent onset and Remission) to show a difference with a power of 80%. We were able to obtain 14 samples from Individuals in remission that demonstrated lower eosinophilic proportions of inflammatory cells on sputum samples to that of persistent asthma, and this difference failed to reach statistical significance as the sample size lacked sufficient power to detect this difference, however the remission asthmatics had similar and possibly lower proportions to those with never asthma.

Also bronchial reactivity at age 18 in remission group was similar to no asthma and lower in comparison to persistent asthma. FeNO, a marker for atopic inflammation was also lower in remission to those with persistent asthma. Largely the characteristic of remission group does satisfy the requirements for complete remission of asthma and representative of true remission.

Adolescents with persistent asthma more often implicated house dust and animal exposures as trigger factors for their symptoms in comparison to those that experienced remission of asthma at the baseline i.e. at 10 year follow-up. Allergen sensitization pattern at baseline conformed to participant's responses to the questionnaire on trigger factors. This allowed us to select specific atopic groups to test for atopy in the multivariate model; however the univariate significance of specific allergen sensitization towards persistence of asthma was not observed in the final model. We have previously highlighted atopy predisposing to persistence of early childhood wheezing and asthma at 10 years of age (53). Others have demonstrated atopy towards both persistence and relapse of asthma over adolescence (37, 58) and beyond into adulthood (158). However our findings are in line with an earlier study over adolescence demonstrating lack of atopic association towards persistence of asthma (52).

Adolescence is the second most important period in each individual's life after the early childhood, where considerable developmental changes are taking place that are not restricted to overt physical features but one that is accompanied by internal physiological changes. This period is characterised by rapid growth in lung that is concurrently taking place along with pubertal changes in preparation for adaptation of human body for its adult functions (173). We observed better growth of airway airflow

in individuals experiencing remission of asthma symptoms. This was similar to those with no asthma (data not shown), and was significantly greater in comparison to that experienced by those with persistent asthma. This improvement in the lower airway function was seen to be accompanied by loss of upper airway symptoms of rhinitis. At 10 years there was no difference in either lung function or rhinitis between those destined for remission to the ones that were likely to keep their asthma. Similar findings were reported by Groningen study where individuals with atopic asthma over adolescent period gained better lung function resulting in remission of asthma symptoms (275). However the of gain in lung growth independent of atopy could not be ascertained in that cohort, which in our study demonstrated significance after adjusting for gender, atopy and smoking. In relation to role of lung growth velocity, the cause and effect is not clear. Thus, it is not known if better lung growth and larger airway leads to remission of symptoms or asthma remission, driven by some other mechanisms (possibly inflammation), results in a larger than average improvement in lung function. Rapid increase in lung function have been documented by Rosenthal and others during adolescence (170, 173, 175) and this coincides with the age at which majority of teenagers reported losing their asthma symptoms i.e. 13 – 15 years. Another hospital out-patient unit based study in Groningen comprising of 15-21 year atopic asthmatic teenagers attaining better growth of large airway function over adolescence leading to remission of their asthma symptoms (275). However, a well characterized community based study has identified that a 1/3 of individuals experiencing remission have a relapse of their asthma symptoms that had apparently outgrown asthma during adolescence (37). This argues against lung growth driven asthma remission as recurrence should not be common. This is suggestive of the fact that clinical remission in our cohort was driven by improvement in airway inflammation which led to better lung growth and function. A subset of (14 of 56) remission group subjects were further characterised for underlying airway inflammation using sputum induction and FeNO. They had little or no evidence of active inflammation. This persistent airway inflammation has been speculated to account for future relapse and thus we hope that our remission group is at lower risk of future recurrence.

On the other hand our finding of better growth of airway function was independent of atopy, which in an earlier study was not distinguishable as all of the study participants were atopic (275). The gain in large to mid-airflow function retained independent significance for remission in the multivariate model after controlling for height gain, atopy, gender, smoking and paracetamol usage. This in turn is suggestive of a combined effect of growth and diminished inflammation favouring remission. This aspect of growth in lungs needs to be further assessed for its role in remission, possibly by regular serial pulmonary function in a prospective cohort along with inflammatory markers.

We examined early life and adolescent specific environmental and genetic factors (family history of allergic disease). The other factor that emerged was lower use of paracetamol in the remission group. This observation is of significance as there is evidence that higher serum concentrations of dietary antioxidant vitamins and selenium is beneficial to lung health resulting in improved FEV₁, i.e. large airway airflow (276). Higher use of paracetamol is detrimental to oxidative protective mechanism of lungs affecting glutathione enzymes thereby predisposing to insults not only to lower but also upper airways (254). At a stage of rapid development of lung, this may have long lasting effects that are likely to persist beyond adolescent period.

We observed lower bronchial reactivity in asthmatic individuals who were destined for remission of their asthma during adolescence. The level of bronchial reactivity was significant in the final multivariate model after adjusting for all of the univariate factors that demonstrated significance at $p < 0.1$. This has only been observed in one earlier Australian cohort studies that severity of bronchial hyper-responsiveness was more useful predictor for asthma prognosis compared to the prevailing current application for the evaluation of asthma (207). This characteristic of bronchial airway has been deemed fit for only research purpose, and not reliable enough to be used as a prediction tool for children with asthma (223). However our finding contradicts this and proposes further evaluation of bronchial reactivity for its prognostic value in determining remission or persistence of childhood asthma.

CHAPTER 8: EARLY LIFE VERSUS ADOLESCENT RISK FACTORS FOR NEW ADOLESCENT ONSET ASTHMA

8.1 Introduction

Information on childhood asthma has been studied in considerable detail over last couple of decades with asthma cohort studies contributing valuable data by looking at various aspects of this heterogeneous disease. However, only a few of these prospective cohorts have been successfully reviewed beyond the childhood and into adolescent years (26, 51, 80). The natural history of asthma over adolescence differs from that of childhood. There is a change in the pattern of risk for asthma, with diminishing role of atopy and reversal of gender (35, 85) along with increasing importance of environmental factors such as that of personal tobacco smoking (153), and greater exposure to environmental pollutants playing their individual roles in genetically predisposed individuals (153, 259, 277). On the other hand, there is evidence to suggest a role of early life factors in adolescence asthma (51, 123). This is supported by suggestions of that, with the establishment of lung growth path that each individuals lungs are programmed in early life to attain over the adolescent period (26).

A significant change over the teenage years is that of puberty with changing dimensions of human body in preparation for adult roles it needs to play over life time. The growth spurt that is experienced over adolescent years is accompanied by significant increase in lung growth with attainment of maximal lung function (172, 176). Disproportionate gain in weight and height may lead to obesity and this has been identified as playing a significant role for new adolescent onset asthma (278). Our aim was to determine the impact of early life factors in comparison to adolescent specific factors towards asthma over this age. We hypothesise that based on current evidence; a pattern of early life factors will demonstrate significant risk towards new adolescent onset asthma.

8.2 Results

8.2.1 Comparison of New Adolescent Onset Asthma with Persistent Asthma

Review of questionnaire data on asthma related symptoms revealed that there was no difference between the persistent and new adolescent onset asthmatics (Table 8.1). Gender distribution for this assessment was similar with 39% (22/56) males in new adolescent onset asthma to 53% (66/125; $p = 0.09$) males in persistent asthma. Symptom of speech limitation is considered to represent a marker of severity; however this was also similar between the two groups. Only significant difference noted was for current treatment which was higher in the new adolescent onset asthma compared to persistent asthma.

Table 8.1 Symptoms and Treatment of Asthma between New Adolescent Onset Asthma and Persistent Asthma

Symptoms/Treatment	New adolescent onset asthma % (n/N)	Persistent Asthma % (n/N)	Odds Ratio	95% Confidence Interval	p -value
Wheeze frequency >4 /year	30 (17/56)	34 (42/124)	0.85	0.43 - 1.67	0.64
Exercise induced wheeze	64 (36/56)	77 (96/124)	0.53	0.27 - 1.04	0.07
Sleep affected	45 (25/56)	50 (60/120)	0.81	0.43 - 1.52	0.51
Sleep >1 time per week	13 (7/56)	17 (20/120)	0.71	0.29 - 1.77	0.48
Speech affected	14 (8/56)	18 (22/120)	0.74	0.31 - 1.76	0.51
Dry cough at night	32 (18/56)	45 (55/123)	0.59	0.30 - 1.13	0.11
Current treatment	80 (45/56)	66 (79/120)	2.12	1.00 - 4.48	0.049

Comparisons in this table were made using Chi-square test, with significance at $p < 0.05$

% (n/N) = percentage, where n= number of participants with condition, N = total number of participants that responded in that group

Table 8.2: Asthma Triggers Comparison; New Adolescent Onset Asthma to Persistent Asthma

Asthma triggers at 18 year	New adolescent onset asthma % (n/N)	Persistent asthma % (n/N)	Odds Ratio	95% Confidence Interval	p-value
House Dust	27 (15/56)	39 (48/122)	0.56	0.28 – 1.13	0.11
Pollen	23 (13/56)	26 (32/122)	0.85	0.41 – 1.78	0.67
Animals	16 (9/56)	23 (28/122)	0.64	0.28 – 1.47	0.30
Infection	29 (16/56)	48 (58/122)	0.44	0.22 – 0.87	0.02
Exercise	66 (37/56)	65 (79/122)	1.06	0.54 – 2.06	0.41
Stress	14 (8/56)	17 (21/122)	0.80	0.33 – 1.94	0.6

Odds of new adolescent onset of asthma to persistent asthma group were obtained using univariate logistic regression model for determined at alpha level of $p < 0.05$ with 95% confidence intervals

% (n/N) = Numbers are percentages (number/total number)

On examining the trigger factors for asthma symptoms, fewer teenagers with new adolescent onset asthma implicated infection to act as a precipitant for asthma symptoms.

Table 8.3 Allergen Sensitization Pattern at 10 Years

	New adolescent onset asthma % (n/N)	Persistent Asthma % (n/N)	p-value*	Never Asthma % (n/N)	p-value*
House Dust Mite	34 (17/50)	55 (59/107)	0.014	11 (54/504)	0.13
Grass pollen	16 (8/50)	36 (38/107)	0.012	9 (44/504)	0.02
Tree pollen	6 (3/50)	4 (4/107)	0.52	1 (6/504)	0.04
Cat	8 (4/50)	30 (32/107)	0.002	4 (22/504)	0.25
Dog	4 (2/50)	21 (22/107)	0.007	3 (13/504)	0.56
Cladosporium	0 (0/50)	2 (2/107)	0.33	1 (7/504)	0.40
Alternaria	4 (2/50)	6 (6/107)	0.67	1 (7/504)	0.19

Chi-square test, comparing new adolescent onset asthma to persistent asthma and never asthma groups determined at alpha level of $p < 0.05$ with 95% confidence intervals

% (n/N) = Numbers are percentages (number/total number)

* p-value represents comparisons to remission group

In order to determine the relevance of asthma triggers of pollen, animal and house dust, pattern of aeroallergen sensitization at 10 and 18 years were analysed for adolescent onset to those with never asthma. Four allergens demonstrated differences in sensitisation pattern between prospective asthma groups of new adolescent onset asthma and never asthma over 10 to 18 years whereas mould spores were not different at either assessment between the two phenotypes. Sensitisation of house dust mite and tree was higher in new adolescent onset asthma to never asthma at both 10 and 18 year follow-ups. Cat and dog allergen sensitisation increased over adolescent period (10 to 18 years) in new adolescent onset asthma with more than two fold rise for cat and five-fold for dog while two-fold rise was noted for never asthma for both of these allergens. This resulted in significantly lower prevalence of sensitisation in never asthma in comparison to that observed with new adolescent onset asthma which at 10 year was comparable between the two groups.

Table 8.4 Allergen Sensitization Pattern at 18 Years

	New adolescent onset asthma % (n/N)	Persistent Asthma % (n/N)	p-value*	Never Asthma % (n/N)	p-value*
House Dust Mite	49 (19/39)	63 (61/97)	0.13	21 (96/452)	<0.001
Grass pollen	31 (12/39)	53 (51/97)	0.02	19 (87/452)	0.09
Tree pollen	13 (5/39)	11 (11/97)	0.81	4 (20/452)	0.02
Cat	15 (6/39)	35 (34/97)	0.02	7 (30/452)	0.04
Dog	21 (8/39)	32 (31/97)	0.18	6 (27/452)	0.001
Cladosporium	3 (1/39)	8 (8/97)	0.45	2 (10/452)	0.89
Alternaria	8 (3/39)	20 (19/97)	0.09	6 (28/452)	0.71

Chi-square test, comparing new adolescent onset of asthma to persistent asthma and never asthma groups determined at alpha level of $p < 0.05$ with 95% confidence intervals

% (n/N) = Numbers are percentages (number/total number)

* p-value represents comparisons to remission group

In summary, the most significant aeroallergens observed for new adolescent onset asthma were house dust mite and tree pollen at baseline, with cat and dog sensitizations gaining significance at 18 years.

8.3 Anthropometric Measures

Table 8.5 Gender Wise Physical Measure Assessment

	Males (n = 327)	Females (n = 372)	β	95% Confidence Interval	p-value
Height at 18, cm (S.E.)	178 (0.36)	165 (0.32)	13.1	12.2 – 14.1	<0.001
Weight at 18, Kg (S.E.)	71 (0.69)	65 (0.72)	6.3	4.3 – 8.3	<0.001
Height at 10, cm (S.E.)	139 (0.34)	139 (0.32)	0.18	- 0.9 – 0.9	0.97
Weight at 10, Kg (S.E.)	34 (0.41)	36 (0.38)	-2.0	-3.1 - -0.9	<0.001

Definitions for Table 8.5

Gender-wise comparisons were made by General Linear Model with significance determined at $p < 0.001$, value of β representing the difference between the genders. Measures for height and weight are average height and weight measures along with their standard errors of mean (S.E.)

There were significant differences observed between the genders at 18 years, with boys demonstrating greater height than girls (table 8.5) which at the age of 10 years were comparable. Boys at the age of 10 years had lower weight compared to their female counterparts, by 18 years, they surpassed their female colleagues and on an average had higher weights.

When differences in the anthropometric measurements of height, weight and BMI were assessed for new adolescent onset asthma and teenagers with never asthma, there was no difference observed in physical measures of size, weight or height or any change in these measures taking place over adolescence (data not shown).

8.4 Comparison of Lung Function between New Adolescent Onset and Persistent Asthma

In addition to physical measures of height and weight, spirometric information was collected at 10 and 18 years. Adolescents with available data on spirometric lung function at 10 years were compared to their 18 year pulmonary function. We stratified lung function by sex, as boy and girls have different lung functions and growth patterns over adolescence.

At 10 years, boys who developed new adolescent onset asthma demonstrated lower airway airflow (both for FEV_1 & $FEF_{25-75\%}$) along with a lower FEV_1/FVC ratio compared to never asthmatics. However, at the age of 18, no difference was observed in the lung function, with the exception of a trend towards lower FEV_1/FVC ratio ($p = 0.07$) for adolescent males with asthma compare to never asthma. No difference was noted for gain of lung function values over this period between new adolescent onset asthma and those with never asthma.

Table 8.6a Males: Growth Parameters with Height Adjusted Pulmonary Function from Age 10 To 18

	New adolescent onset asthma (n = 15)	Never Asthma (n = 171)	β	95% Confidence Interval	p- value
Spirometry at 18 years*					
FEV ₁ (L) (S.E.)	4.55 (0.14)	4.70 (0.04)	- 0.16	- 0.46 to 0.14	0.29
FVC (L) (S.E.)	5.41 (0.15)	5.37 (0.05)	0.04	- 0.28 to 0.36	0.80
FEV ₁ /FVC (S.E.)	0.84 (0.02)	0.88 (0.005)	- 0.03	-0.002 to 0.001	0.07
FEF _{25-75%} (L/s) (S.E.)	4.69 (0.28)	5.18 (0.08)	-0.49	-1.06 to 0.09	0.10
Spirometry at 10 years*					
FEV ₁ (L) (S.E.)	1.97 (0.06)	2.11 (0.02)	- 0.14	- 0.26 to - 0.02	0.03
FVC (L) (S.E.)	2.36 (0.06)	2.39 (0.02)	- 0.03	- 0.15 to 0.10	0.70
FEV ₁ /FVC (S.E.)	0.83 (1.40)	0.89 (0.42)	- 0.05	- 0.08 to - 0.02	0.001
FEF _{25-75%} (L/s) (S.E.)	2.10 (0.14)	2.49 (0.04)	- 0.40	- 0.68 to - 0.11	0.006
Gain in spirometry from 10 to 18 years*					
FEV ₁ (L) (S.E.)	2.54 (0.12)	2.60 (0.03)	- 0.51	- 0.29 to 0.19	0.67
FVC (L) (S.E.)	3.01 (0.13)	2.98 (0.04)	0.03	- 0.23 to 0.30	0.81
FEV ₁ /FVC (S.E.)	0.008 (0.02)	-0.009 (0.005)	0.02	- 0.01 to 0.05	0.27
FEF _{25-75%} (L/s) (S.E.)	2.43 (0.21)	2.69 (0.06)	- 0.26	- 0.70 to 0.18	0.25

*General Linear Model (GLM) for the difference (β) between new adolescent onset asthma to never asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals. Estimated marginal means used for determination of height adjusted lung function means.

(n) represents number of participants that provided information

FEV₁ = Forced expiratory volume in first second in litres (L) with standard error (S.E.)

FVC = Forced vital capacity in litres (L) with standard error (S.E.)

FEV₁/FVC = ratio of FEV₁ to FVC

FEF_{25-75%} = Forced expiratory flow 25 to 75% in litres per second (L/s) with standard error (S.E.)

Table 8.6b Females: Growth Parameters with Height Adjusted Pulmonary Function

	New adolescent onset asthma (n = 19)	Never Asthma (n = 213)	β	95% Confidence Interval	p- value
Spirometry at 18 years*					
FEV ₁ (L) (S.E.)	3.33 (0.09)	3.53 (0.03)	- 0.20	- 0.38 to - 0.02	0.03
FVC (L) (S.E.)	3.85 (0.10)	3.99 (0.03)	- 0.014	- 0.35 to 0.07	0.19
FEV ₁ /FVC (S.E.)	0.86 (0.02)	0.89 (0.004)	-0.026	- 0.06 to 0.005	0.10
FEF _{25-75%} (L/s) (S.E.)	3.69 (0.20)	4.09 (0.06)	- 0.42	- 0.82 to - 0.02	0.041
Spirometry at 10 years*					
FEV ₁ (L) (S.E.)	1.96 (0.05)	2.02 (0.01)	- 0.06	- 0.15 to 0.04	0.25
FVC (L) (S.E.)	2.20 (0.05)	2.25 (0.01)	- 0.05	- 0.15 to 0.05	0.34
FEV ₁ /FVC (S.E.)	0.89 (1.25)	0.90 (0.37)	- 0.35	- 0.02 to 0.02	0.79
FEF _{25-75%} (L/s) (S.E.)	2.41 (0.12)	2.54 (0.04)	- 0.13	- 0.38 to 0.12	0.30
Gain in spirometry from 10 to 18 years*					
FEV ₁ (L) (S.E.)	1.36 (0.07)	1.52 (0.02)	0.16	- 0.31 to - 0.006	0.042
FVC (L) (S.E.)	1.62 (0.09)	1.74 (0.03)	0.13	- 0.31 to 0.05	0.16
FEV ₁ /FVC (S.E.)	- 0.03 (0.02)	- 0.01 (0.01)	0.02	- 0.05 to 0.01	0.26
FEF _{25-75%} (L/s) (S.E.)	1.27 (0.16)	1.55 (0.05)	0.2	- 0.61 to 0.05	0.10

*General Linear Model (GLM) for the difference (β) between new adolescent onset asthma to never asthma group determined at alpha level of $p < 0.05$ with 95% confidence intervals. Estimated marginal means used for determination of height adjusted lung function means.

(n) represents number of participants that provided information

FEV₁ = Forced expiratory volume in first second in litres (L) with standard error (S.E.)

FVC = Forced vital capacity in litres (L) with standard error (S.E.)

FEV₁/FVC = ratio of FEV₁ to FVC

FEF_{25-75%} = Forced expiratory flow 25 to 75% in litres per second (L/s) with standard error (S.E.)

The spirometric function of females had a different pattern to that of males, with lower large and mid to small airway functions at 18 years with no difference at the baseline of 10 years. Adolescent girls demonstrated lower gain of large airway function over 10 to 18 years. A trend of obstructive airway (lower FEV₁/FVC ratio) similar to that of male lung function was present in female adolescent asthma group (p = 0.1) at the age of 18 years.

Table 8.7a Additional Lung Function Test at 10 Years

	New adolescent onset asthma (n = 19)	Never Asthma (n = 213)	Odds Ratio	95% Confidence Interval	p-value
PC ₂₀ [#] % (n/N)	52 (22/42)	20 (65/332)	4.52	2.33 – 8.77	<0.001
Bronchial reactivity [†] (S.E.)	1.66 (0.08)	1.34 (0.02)	5.41	2.68 – 10.93	<0.001

Odds ratio of new adolescent onset asthma to never asthma at univariate level determined by logistic regression with significance at p<0.05

[#] PC₂₀ is concentration of methacholine eliciting a drop of 20% of FEV₁ from baseline saline.

Challenge test was considered positive, with a PC₂₀ of methacholine of 8mg/ml or less. Number of study participants with positive PC20 given as percentage % (n/N)

[†] A high value represents increased bronchial reactivity; obtained by log₁₀ (DRS+10) transformation of dose response slope (DRS)

Methacholine bronchial challenge test was performed at both 10 and 18 years. New adolescent onset asthma at both time points demonstrated higher bronchial reactivity compared to never asthma (p<0.001).

Table 8.7b Additional Lung Function Tests at 18 Years

	New adolescent onset asthma (n = 19)	Never Asthma (n = 213)	Odds Ratio	95% Confidence Interval	p-value
Positive PC ₂₀ (8mg/ml) # % (n/N)	25 (7/28)	2 (7/312)	14.52	4.66 – 45.28	<0.001
Reversibility § % (n) (S.E.)	7.80 (35) (1.20)	4.05 (427) (0.24)	1.12	1.05 – 1.18	<0.001
Bronchial reactivity † (n) (S.E.)	1.32 (28) (0.08)	1.06 (312) (0.01)	8.48	2.84 – 25.27	<0.001
Geometric Mean FeNO‡ (n) (S.E.)	29 (25) (1.15)	19 (292) (1.05)	7.37	2.08 – 26.12	0.002

Odds ratio of new adolescent onset asthma to never asthma at univariate level determined by logistic regression with significance at $p < 0.05$

PC₂₀ is concentration of methacholine eliciting a drop of 20% of FEV₁ from baseline saline.

Challenge test was considered positive, with a PC₂₀ of methacholine of 8mg/ml or less. Number of study participants with positive PC20 given as percentage % (n/N)

† A high value represents increased bronchial reactivity; obtained by $\log_{10}$ (DRS+10) transformation of dose response slope (DRS)

§ Relative percent reversibility of bronchodilator response following administration of 600 mcg of salbutamol

‡ $\log_{10}$ transformation FeNO value as data was not normally distributed

Methacholine bronchial challenge test was performed at both 10 and 18 years. New adolescent onset asthma at both time points demonstrated higher bronchial reactivity compared to never asthma ($p < 0.001$). Additional tests at 18 years of bronchodilator reversibility and FeNO were also significantly higher for new adolescent onset asthma to never asthma.

Figure 8.1 Bronchial Reactivity at 10 Years in Adolescent Asthma (Adolescent Onset)

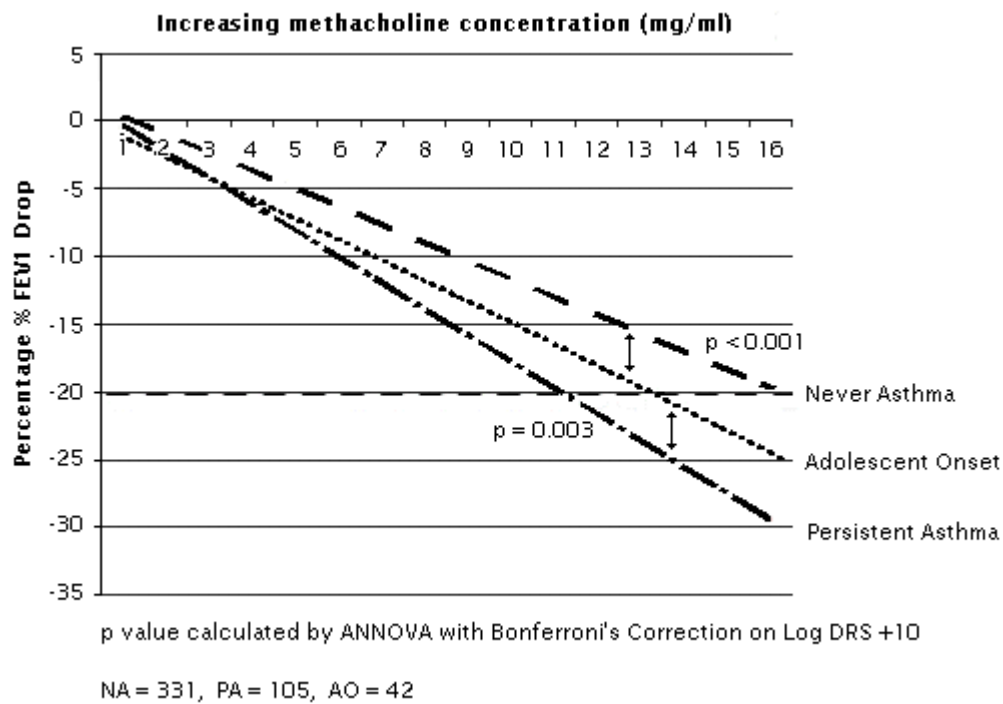
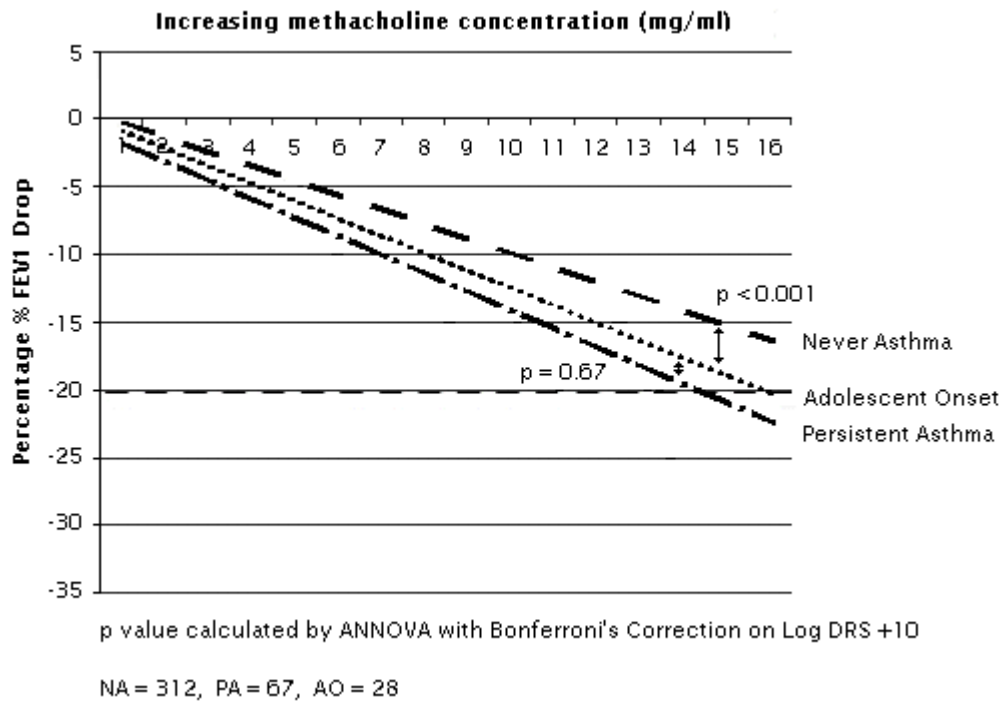


Fig 8.1 The mean gradient of dose response slope obtained for each of 3 prospective asthma groups.

Figure 8.1 demonstrates new adolescent onset asthma at the age of 10 was midway between never asthma and persistent asthma. At age 18 years Fig 8.2 exhibits the shift of this gradient for new adolescent onset asthma that was significantly lower than persistent asthma at 10 years but at the age of 18 years did not demonstrate any significant difference.

Figure 8.2 Bronchial Reactivity at 18 Years in Adolescent Asthma (Adolescent Onset)



The mean gradient of dose response slope obtained for each of 3 prospective asthma groups.

Table 8.8 Inflammatory Cell Type from Sputum Induction

Induced Sputum	New adolescent Onset* (N = 12)	Never asthma (N = 30)	Odds Ratio	95% Confidence Interval	p -value
Eosinophils† (%), median (IQR)	2.4 (5.8)	0.3 (1.3)	1.35	1.02 – 1.78	0.023
Eosinophils > 3% % (n/N)	50 (6/12)	13 (4/30)	6.50	1.46 – 29.05	0.01
Neutrophils † (%), median (IQR)	11 (22)	12 (35)	0.98	0.95 – 1.01	0.22
Epithelial cells† (%), median (IQR)	6 (13)	4.5 (9)	1.01	0.95 – 1.08	0.34

Odds ratio of new adolescent onset asthma to never asthma at univariate level determined by logistic regression with significance at $p < 0.05$

† Median values of percent cells reported for eosinophil, neutrophil and epithelial cells with (IQR) inter-quartile range; ppb = parts per billion

When we sampled sputum induction from the prospective asthma groups, no difference was observed between proportion of boys and girls by participation, although almost all of the sputum samples from new adolescent onset asthma were from the atopic individuals 92% (11/12) with 40% (12/30) never asthma group having atopy ($p = 0.002$). New adolescent onset asthma demonstrated a higher proportion of increased eosinophils to never asthma both as continuous percentage ($p=0.023$) as well as when cut-off of 3% or more was used ($p = 0.01$). The fractional exhaled nitric oxide levels in new adolescent onset asthma was also higher than the never asthma ($p = 0.004$).

Table 8.9 Allergic Sensitization, Early Life Wheeze/Asthma, Longitudinal Information on Allergic Diseases

	New adolescent onset asthma (n/N)%	Never asthma (n/N)%	Odds Ratio	95% Confidence Interval	p-value
Atopy at 4,	19 (9/48)	13 (67/519)	1.56	0.72 – 3.36	0.26
Atopy at 10	42 (21/50)	18 (89/505)	3.39	1.85 – 6.21	<0.001
Atopy at 18	64 (25/39)	34 (153/452)	3.49	1.76 – 6.91	<0.001
Rhinitis at 1 or 2	14 (8/56)	10 (62/611)	1.48	0.67 – 3.26	0.34
Rhinitis at age 4	5 (3/56)	3 (17/593)	1.97	0.56 – 6.96	0.29
Rhinitis at age 10	43 (24/56)	16 (97/609)	3.96	2.23 – 7.01	<0.001
Rhinitis at age 18	60 (33/55)	30 (182/611)	3.54	2.01 – 6.23	<0.001

Univariate logistic regression analysis for calculation of odds of new adolescent onset asthma to never asthma group determined at alpha level of $p<0.05$ with 95% confidence intervals.

Numbers are percentages (number/total number)

New adolescent onset asthma was found to be associated with atopy and rhinitis at the ages of 10 and 18 years, but not when these conditions were present at the 4 year assessment. No significant difference was observed for New adolescent onset asthma for eczema at any of the follow-ups. There was no difference in proportion of atopic rhinitis in New adolescent onset asthma 80% (19/24) compared to never asthma 65 % (92/141); $p = 0.18$.

Table 8.10 Family History of Asthma/Rhinitis Determined at Birth

	New adolescent onset asthma% (n/N)	Never asthma % (n/N)	Odds Ratio	95% Confidence Interval	p-value
Family History of Asthma					
Maternal History	16 (9/56)	8 (49/610)	2.19	1.01 – 4.74	0.04
Paternal History	7 (4/56)	8 (51/608)	0.84	0.29 – 2.42	0.75
Parental History	23 (13/56)	16 (96/608)	1.61	0.84 – 3.11	0.15
Family History of Rhinitis					
Maternal History	23 (13/56)	20 (123/610)	1.20	0.62 – 2.30	0.59
Paternal History	14 (8/56)	15 (93/607)	0.92	0.42 – 2.01	0.84
Parental History	34 (19/56)	32 (193/607)	1.10	0.62 – 1.97	0.74

Univariate analysis by logistic regression was used for determining odds ratios of new adolescent onset asthma to never asthma, 95% confidence interval and p -value with significance at $p < 0.05$.

% (n/N) = Numbers are percentages (number/total number)

Amongst the hereditary factors only maternal history of asthma was observed to be associated with increased risk of new adolescent onset asthma. Early life factors did not demonstrate significance towards new adolescent onset asthma. Adolescent specific environmental factors also did not reveal a higher risk, with the exception of paracetamol ($p = 0.03$) along with earlier age ($p = 0.02$) and increase amount ($p = 0.03$) of self-tobacco smoking.

Table 8.11 Early Life Factors towards Adolescent Asthma

	New adolescent onset asthma	Never asthma	Odds Ratio	95% Confidence Interval	p-value
Birth Weight, Kg (SD)(n)	3.4 (0.54) (56)	3.5 (1.39) (595)	0.77	0.44 – 1.32	0.34
Pre-term delivery* % (n/N)	2 (1/56)	0.5 (3/597)	3.65	0.37 – 35.75	0.27
Post-term delivery ^Ω % (n/N)	4 (2/56)	2 (13/597)	1.69	0.37 – 7.67	0.50
Recurrent chest infections Birth to 2 years† % (n/N)	4 (2/55)	6 (35/597)	0.61	0.14 – 2.59	0.50
Less than 4 months breast feeding % (n/N)	64 (35/55)	60 (361/600)	1.16	0.65 – 2.06	0.62
Formula Feeding in first 4 months of life ^α % (n/N)	74 (40/54)	70 (414/595)	0.80	0.43 – 1.51	0.49
Maternal Smoking during pregnancy % (n/N)	11(6/56)	18 (111/607)	0.54	0.22-1.28	0.16
Early life tobacco smoke exposure (Birth to 4 years) ^χ %(n/N)	57 (32/56)	49 (294/597)	0.73	0.42 – 1.27	0.26
Lower Social Class at birth ^Ω	36 (14/39)	50 (180/360)	0.56	0.28 – 1.11	0.10
Pet Cat ownership (age 4) %(n/N)	45 (25/56)	37 (228/611)	1.36	0.78 – 2.35	0.28
Pet cat (0-4) %(n/N)	91 (50/55)	84 (512/604)	0.56	0.22 – 1.43	0.23
Pet dog (0-4) %(n/N)	46 (25/55)	37 (226/604)	0.72	0.41 – 1.25	0.24

Definitions for Table 8.11

Odds ratio for new adolescent onset asthma to never asthma determined by univariate logistic regression with significance at $p < 0.05$

General Terms

%(n/N) percentage determined from number of participants with condition/total number of participants with available information on the variable

(SD) = standard deviation (n) = number of participants

Specific Terms

* Pre-term delivery was defined as delivery of child in less than 37 complete weeks of gestation

^Q Post-term delivery was defined delivery of child after 42 complete weeks of gestation

† Recurrence of chest infections reported by parents from birth to 2 years

^a Infants starting formula milk in first 17 weeks after birth

^z Regular exposure to tobacco smoke inside the house reported at any time from birth to age 4 years

^Ω Lower Social Class represents classes I to III of Registrar General's Classification at birth.

Table 8.12 Adolescent Factors and their Relation to New Adolescent Onset Asthma

	New adolescent onset asthma	Never asthma	Odds Ratio	95% Confidence Interval	p-value
Current cigarette smoking at 18 years % (n/N)	15 (8/53)	23 (140/604)	0.59	0.27 – 1. 28	0.18
Current and past cigarette smoking* %(n/N)	42 (22/53)	42 (251/598)	0.98	0.56 – 1.74	0.95
Tobacco smoke exposure inside the house at 10* %(n/N)	36 (20/56)	36 (211/595)	1.01	0.57 – 1.79	0.97
Tobacco smoke exposure inside the house at 18* %(n/N)	30 (13/43)	31 (144/462)	0.96	0.49 – 1.89	0.90
Median number of days exercise/week (n) (p)	3.0 (54)(1,4)	2.0 (597)(1,5)	1.12	0.98 – 1.27	0.11
Mean number of hours watch TV weekday (n)(SD)	3.9 (54)(2.6)	3.7 (597) (2.3)	1.07	0.94 – 1.18	0.32
Mean number of hours watch TV/ Computer weekend day (n)(SD)	3.5 (54)(2.6)	3.2 (596)(2.3)	1.04	0.92 – 1.18	0.52
Farm living [†] %(n/N)	7 (4/56)	7 (42/601)	1.02	0.35 – 2.97	0.97
Annoyed by pollution at least once a week %(n/N)	16 (9/56)	10 (58/602)	1.80	0.84 – 3.85	0.13
Car Passing house at least 1 per hour %(n/N)	68 (38/56)	70 (422/602)	0.90	0.50 – 1.62	0.73
Heavy Vehicles at least 1per hour %(n/N)	45 (25/56)	36 (219/602)	1.41	0.81 – 2.45	0.22
OCP use in girls§ %(n/N)	38 (12/32)	48 (148/310)	0.66	0.31 - 1.39	0.27
Paracetamol use/month (n)(p)	1 (0,3)	1 (0,2)	1.10	1.03 – 1.17	0.03
NSAID use/month (n)(p)	0 (0,1)	0 (0,1)	1.01	0.92 – 1.12	0.81

Definitions for Table 8.12

Odds ratio for new adolescent onset asthma to never asthma determined by univariate logistic regression with significance at $p < 0.05$

General Terms

% (n/N) percentage determined from number of participants with condition/total number of participants with available information on the variable

(SD) = standard deviation (n) = number of participants (p) = 25th and 75th percentiles

This includes both current and those who have ever smoked cigarettes at any time in the past

* Tobacco smoke exposure inside the house excluding self-smoking

‡ Study participants that reported positively to “Have you ever lived on a farm”

§ n = number of girls reporting oral contraceptive use (OCP)

Early life factors (maternal smoking during pregnancy and social class at birth along with their interaction term) were assessed along with genetic factor of maternal history of asthma was added to backward stepwise multivariate logistic regression model. Family history of maternal asthma was the only significant factor for new adolescent onset asthma. Maternal smoking was not significant for new adolescent onset asthma.

Table 8.13 Multivariate Early Life Model for New Adolescent Onset Asthma

	Odds Ratio	95% Confidence Interval		p - value
Maternal history of asthma	2.62	1.11	- 6.20	0.03
Lower social class at birth	1.83	0.92	- 3.66	0.09

Backward stepwise multivariate logistic regression model, significance determined at $p < 0.05$.

All adolescent specific factors demonstrating significance < 0.1 over teenage years were included in the final second multivariate model. In both early life and adolescent models, the term for sex was entered, but was removed in the backward elimination. Although personal tobacco smoking variable did demonstrate significance at $p < 0.2$, its addition to the model for mean age or median number of tobacco use severely limited the model restricting new adolescent onset asthma to only 10

individuals. Therefore this variable was not included in the final model. In addition to active and past smoking status failed to demonstrate biological or statistical relevance to be added to the final model.

Table 8.14 Multivariate Adolescent Model for New Adolescent Onset Asthma

	Odds Ratio	95% Confidence Interval		p - value
Paracetamol.18	1.10	1.01	1.19	0.027
Atopic at 10 year	2.35	1.08	5.09	0.031
Rhinitis at 10 year	2.35	1.11	5.01	0.027
Bronchial reactivity at 10	3.42	1.55	7.59	0.002

Odds ratio for new adolescent onset asthma to never asthma determined by backward stepwise multivariate logistic regression with significance at $p < 0.05$

Adolescent model demonstrated increased risk of new adolescent onset asthma for individuals having atopy, rhinitis and bronchial reactivity at the age of 10 years that were atopic, and had reported higher consumption of paracetamol.

8.5 Combined Model

We performed a final combined analysis by restricting our analysis, including only those variables that demonstrated significance of $p < 0.1$ similar to our analysis for the remission of asthma, with backward stepwise multinomial regression analysis was used. However this model did not result in a change from that of adolescent model (Table 8.14), as removal of sex and maternal history of asthma resulted in the backward stepwise elimination process with removal of factors with $p < 0.1$.

8.6 Discussion

Our review of Isle of Wight birth cohort at 18 years, for new adolescent onset asthma, demonstrated genetic predisposition to family history of asthma with amongst the early life factors with environmental influence of increased paracetamol use at 18 years as a significant adolescent associated factor. Atopic individuals at the age of 10 continue to remain at higher risk for the occurrence of asthma. We did not observe gender to contribute differentially towards new adolescent onset asthma. Children having rhinitis with increased reactivity of airways at age 10 were more likely to develop asthma at the age of 18 years. We failed to observed relevance of early life risk factors towards new adolescent onset asthma.

Before we embark on elaborating on the details of our findings it is important to highlight that on further analysing the new adolescent onset asthma phenotype, we observed that overall 17 of 56 New AOA had wheezed at the age of 10 years, which were not diagnosed as asthmatic. This may raise few questions in relation to the term of new adolescent onset asthma. However if we consider that not all wheeze equates to asthma, particularly in view of childhood wheeze phenotypes as described by Tucson and Dunedin cohort studies(51, 57). Viral wheeze is observed well into adolescent period of age 13 years(239). Further to the complexity that exists with regards to the nature of wheeze, our assessment of wheeze at 18 years also provides additional evidence that not all wheeze can be regarded as asthma.

It has consistently been observed that not all wheezing illness in early life is asthma (61, 110, 279) and we have demonstrated the same is true for wheeze at 18 (chapters 4 & 5), where a fifth of wheezing lacks the objective features of asthma (spirometric function, bronchial responsiveness, bronchodilator response, allergic sensitization, FeNO). For our definition of new adolescent onset asthma we used standard definition of asthma diagnosed for the first time between 10 and 18 years of age and excluded those who have been diagnosed to have asthma, using similar definitions at earlier follow-ups.

It has been suggested that new adolescent onset asthma is largely observed in non-atopic individuals (52). We characterised the asthmatics by objective measures of skin prick tests and questionnaire data on allergic conditions such as rhinitis. Evidence gathered from the Isle of Wight birth cohort study does not support the lack of association of atopy to new adolescent onset asthma, and this finding has also been observed in other earlier studies(52, 237).

Contrary to our expectation, the severity of new adolescent onset asthma was similar to that of persistent asthma. Indeed, a lower proportion of those with persistent asthma were on active treatment for control of asthma symptoms in comparison to those with new adolescent onset asthma. There is evidence to suggest that adherence to regular asthma treatment is poor over adolescence, particularly in those that have long standing disease (280). Trigger factors for asthma symptoms were similar for both persistent and new adolescent onset asthma. Exception to this was a lower proportion of infection being reported as a precipitant for asthma in new adolescent onset asthma compared to persistent asthma. The likely explanation for this finding is increased chances of occurrence of infection over longer duration of disease, as infections are known causes of asthma exacerbations (281).

We observed univariate significance for atopy at 10 but not at 4 year, for new adolescent onset asthma. In addition to this rhinitis at 10 also demonstrated increased risk for new adolescent onset asthma. However rhinitis at age 4 was not associated with increased risk even at univariate level. Although both atopy and rhinitis are known risk factors for asthma (131, 136) however the timing or duration of exposure lacks clarity. Lack of association to early childhood atopy and rhinitis by age 4 years coupled with absence of significance to all of the early life factors in our study for new adolescent onset asthma, is suggestive of a true adolescent onset condition independent of early life impact and is in contrast to the view that all asthma in early adult life has its origin in early life (56).

We wanted to determine the difference in lung function between true new adolescent onset asthma to those with never asthma stratified by gender. On examining the spirometric function, different pattern of lung function were observed for boys and girls over adolescence. At the age of 10 years, both girls and boys had comparable lung function, however, boys tended to grow taller by the age of 18 years (table 8.5) and attain higher lung function, in comparison to girls over this period (table 3.12). We stratified the spirometric analysis by gender as complex models on lung function that are adjusted for gender, results in controlling of the model for sex but the estimates of parameters of interest are not readily apparent. An example is that for lung function FEV_1 , when adjusted for gender will provide an odds ratio between new adolescent onset asthma to never asthma, however gender wise estimates of difference between boys and girls will not be available. The disadvantage of stratifying is that it reduces power to detect differences between the outcome groups. We proceeded with our present analysis with some interesting results despite compromising statistical power, as boys and girls behave differently during adolescence. Boys demonstrated lower forced airway airflow (FEV_1 & $FEF_{25-75\%}$) and obstructive pattern prior to developing symptoms of asthma at the age of 10 years. However this sequence of events was not shared by females with new adolescent onset

asthma, to those with never asthma, as at baseline both had comparable lung function and by 18 years, girls with new adolescent onset asthma demonstrated lower forced airway airflow (FEV_1 & $FEF_{25-75\%}$). Greater bronchial airway reactivity was observed for both boys and girls with new adolescent onset asthma at age 10 in comparison to those with never asthma prior to experiencing symptoms of asthma. Increased bronchial reactivity retained independent significance for predicting asthma at age 18 in the multivariate model for new adolescent onset asthma (table 13).

Lower forced airway air-flow (FEV_1 & $FEF_{25-75\%}$) was observed for females at the age of 18 years with new adolescent onset asthma but not for males. Also growth of pulmonary function over this period demonstrated lower gain of FEV_1 for females, along with trends for $FEF_{25-75\%}$. The pattern of lower gain of lung function was not observed in males with new adolescent onset asthma over 10 to 18 years. A likely explanation for the lack of difference in lung growth and exhibiting a lower lung function at the age of 18 in females but not in male new adolescent onset asthmatics may be due to difference in timing of pubertal growth spurt as well as gender related differences in growth pattern of lungs over adolescence. Males have a prolonged growth phase in comparison to females who reach a plateau earlier for lung function gain following menarche (176). We struggled to observe any tracking of early life lung function at 10 years in females going on to develop asthma over adolescence, however we did observe lower lung function in males prior to developing asthma symptoms at the age of 10, but this difference in lung function between the new adolescent onset asthma to never asthma was not present at the age of 18 years even in males, as suggested by the Tucson cohort study (26). Lack of concurrence between the two studies might be due to different selection criteria. We studied diagnosed asthmatics while Tucson study included all wheezy children. Also the model they selected to examine lung function was corrected for gender effect, masking the effects of pubertal growth that is known to affect lung function over this period. Therefore the concept that lung function is already established in early life as suggested by Martinez and colleagues (26, 278) for those with new adolescent onset asthma, was not observed in our study.

We did not observe increased risk of smoking for new adolescent onset asthma in our study, which has been reported by some studies (51, 153, 158, 160), with conflicting results from others (163, 282, 283). Early life as well as adolescent exposures was assessed for significance, in addition to personal smoking. None of these factors were observed to demonstrate any significance. The only difference observed between never asthmatics to new adolescent onset asthma was earlier age of starting tobacco smoking with increased number of cigarettes smoked. But due to only few of asthmatics reporting number of cigarettes smoked we did not have enough sample size for its inclusion in the multivariate model. However if we consider

proportion of those with tobacco smoke exposure whether active or passive there was no significant difference noted between the never asthma and new adolescent onset asthma groups. Earlier results from prospective studies have demonstrated link with tobacco smoke exposure to incident asthma over adolescence (153), however in our study after characterizing non-asthma-wheeze and differentiating this from adolescent asthma wheeze, we did not observe smoking to be of relevance towards new adolescent onset asthma.

In addition to rhinitis and atopy, a larger proportion of new adolescent onset asthmatics reported annoyance to pollution and report of number of cars passing by the house or heavy vehicle passage were both significant at the univariate level. This finding may be due to the fact that asthmatics are likely to notice air pollution more often than those that do not have asthma (284). Finally the one important factor, over adolescence that demonstrated independent significance for new adolescent onset asthma was higher use of paracetamol in children that develop asthma from 10 to 18 years. Although this question was asked at the 18 year follow-up but has been consistently observed not just for new adolescent onset asthma but for remission as well, demonstrating lower use associated with remission of asthma over adolescence. Other studies both cross-sectional (167) and prospective (253) have identified this as an important factor towards incident asthma. We identified increased use of paracetamol associated with non-asthma-wheeze as well as asthma at 18 years. The global effect of paracetamol towards different wheeze phenotypes is suggestive of its role in affecting pulmonary function that is independent of phenotype. Studies have demonstrated that this effect of paracetamol is independent of age and is observed from pre-natal period for early childhood asthma into late adulthood (165-167, 285).

There are few reports on risk factors on new adolescent onset asthma (80, 91, 153, 286), however studies suggesting early life origins of incident asthma over adolescent period have largely based their reports on adolescent wheeze rather than diagnosed asthma (26, 51, 98), therefore the conclusion that all asthma has early life origin undermines the importance of adolescent period, which is a period of susceptibility, where teenagers undergo dramatic physical and physiological changes and factors specific to this age are likely to play an important role towards new adolescent onset asthma. In summary our findings challenge the conventional view of early life origins for new adolescent onset asthma; however we do acknowledge that overall asthma observed at 18 is due to persistence of early life asthma and therefore this concept may overshadow the small but significant proportion of true adolescent onset phenotype.

CHAPTER 9: DISCUSSION

9.1 Follow-up at 18 Years and Pattern of Changing Gender Prevalence

We have been fortunate to have an excellent overall follow-up of 90.2 % (1313/1456) at 18 years that took 2 years and 5 months to complete, with a mean age of 18 years and a standard deviation of 0.6 years. The mean age of Tucson Children's Respiratory Study in their young adults assessment was 22 years (SD = 1.2) with 68% (849/1246) follow-up (123). Prevalence of asthma from the of age 4 to 10 years within the cohort remained unchanged; however a significant increase of asthma was observed over the adolescent period (10 to 18 years) from 14.7 % to 17.9% ($p=0.027$). A good follow-up rate over 10 and 18 years of >90% allowed us to study the dynamics of changing gender difference in asthma prevalence over adolescent period. We did not observe a statistically significant gender switch at 18 years of age in our birth cohort; however review of information from earlier follow-ups was suggestive of trends towards transition of asthma from male predominant asthma at 10 years which demonstrated equal dominance at the age of 18 to that of female prevalence. The mechanism of this transition was observed to be driven by more females developing asthma with a trend towards a higher proportion of boys experiencing remission. Some studies have reported this change is brought about by higher persistence of asthma in females (31) whereas others have suggested both genders undergo remission but there is greater proportion of females developing asthma over this period (32, 85).

9.2 Non-asthma-Wheeze a Distinct Wheeze Phenotype

Our aim was to characterise and quantify adolescent asthma by examining wheeze phenotypes and to determine risk pattern profile of wheeze that was not diagnosed as asthma in relation to diagnosed asthma. We observed 17.9% (234/1306) asthmatic wheeze was present with a significant burden [4.9% (65/1306)] of non-asthmatic-wheeze (NAW), a distinct wheeze phenotype characterised by tobacco smoke related wheeze in non-atopic young adults with increased paracetamol consumption. The symptom profile and severity was not different to that of asthma but objective parameters of such as atopy was observed to be associated with asthmatic wheeze rather than NAW, similarly lung function for asthmatic wheeze demonstrated obstructive pattern whereas that of NAW exhibited a restrictive profile. Bronchial reactivity and FeNO were also significantly greater for asthmatic wheeze in comparison to NAW.

9.3 Composition of Asthma at Age 18 Years

The cross-sectional asthma at 18 years comprised of more than 56% of childhood persistent asthma, with 34% of true adolescent onset with remaining 10% of early childhood recurrent asthmatics. This finding is in line with studies suggestive of majority of asthma in young adults has its origin early in life (26, 51); however it does also highlight the fact that a third of asthma in late teenage is of true adolescent onset.

9.4 Prospective Transitions of Childhood Asthma at 10 by 18 Years

Most children who had asthma at age 10 still had it at age 18 (69.1%) but 30.9% experienced remission of their asthma symptoms". Lack of atopy was associated with remission of asthma in males whilst atopy continued to be a risk for both genders towards asthma at 18 years. However, a higher proportion of non-atopic females observed new onset of asthma.

9.5 Adolescence and Remission of Asthma

In contrast to earlier studies we did not observe any net remission over the adolescent period. Lack of remission over adolescent is likely to account for increasing asthma prevalence with age. This age effect of increasing prevalence is in agreement with earlier report of a cohort study from Southampton that also reported matching wheeze prevalence figures (52). However the difference between our studies is that we reported increasing prevalence for asthma which forms four fifths of all observed wheeze over the adolescent period. We also observed an increase of asthma from the age of 10 to 18 years whereas the prevalence of wheeze in Southampton Cohort study was assessed between the ages of 6-8 to 14-16 years of age. If we compare prevalence of any wheeze; in the Isle of Wight birth cohort it increased from 18.9% (259/1373)(53) to 23% (296/1306), compared to 14.7% to 18% in the adolescent in the Southampton Cohort Study (52). This is suggestive of two possibilities, that trend for wheeze prevalence between the two cohorts has been stable or it may be on the rise if we are to discount the difference in age at which the prevalence of wheeze was evaluated between the two cohort studies. In either case, it is certainly not suggestive of a downward trend as suggested by an earlier report (24).

Majority of the remission group satisfied the requirements for complete remission of asthma. Remission was associated with better growth in lower airway

airflow and accompanied by improvement of upper airway symptoms of rhinitis supporting the one airway hypothesis. A lower level of bronchial reactivity at 10 years in asthmatic children was predictive of remission at 18 years, with lower use of paracetamol, emerging as the single most important factor. An increase in growth of the airway correlated with remission; however causality could not be ascertained.

9.6 Characteristics of Adolescent Onset Asthma

The genetic predisposition of asthmatic family history with environmental influence of increased paracetamol use and annoyance to air pollution was observed to be associated with new adolescent onset asthma. This association was one of cross-sectional relationship and future studies will be needed to determine nature of this relationship, whether it is one of cause or effect. Increasing evidence is accumulating suggestive of this to be causal rather than that of effect (287). Atopic individuals in our cohort continued to retain higher risk for the occurrence of asthma. Gender did not contribute differentially towards adolescent onset asthma after recurrent asthma was taken into account. Rhinitic children with increased bronchial reactivity at age 10 were more likely to develop asthma at 18 years. We failed to observe relevance of early life risk factors towards new adolescent onset asthma. Contrary to our expectation, the severity of new adolescent onset asthma was similar to that of persistent asthma.

9.7 Fundamental Assumptions for Characterising Asthma

The evaluation of wheeze disorder at 18 years was performed using objective physiological measures such as lung function and methacholine bronchial challenge tests that were also performed at 10 years of age. The different wheeze phenotypes were formulated based on questionnaire information obtained by the study participants. Ability to characterize different wheeze phenotypes on the objective measures of reversibility, bronchial responsiveness with FeNO and induced sputum differential cell counts provided us with the opportunity to identify undiagnosed asthmatic wheeze at 18 years. Diagnosis of asthma is largely clinical and therefore it has been acceptable to define asthma based on questionnaire information on asthma symptoms in epidemiological studies (288), to the extent that some studies have only used postal questionnaires (52) to study risk factors for asthma without any objective measures. This approach gains support from reports in which use of questionnaire based information on asthma symptoms e.g. wheeze in comparison to objective measures such as bronchial reactivity has demonstrated a higher predictive value for doctor's diagnosis of asthma (288). However it is important to note that in childhood

the positive predictive value of asthma symptoms is lower compared to that observed for adults, which is suggestive of the fact that not all wheeze is asthma.

Wheeze over adolescent period has not been studied in detail, however a higher predictive value of wheeze for asthma diagnosis in adults leads most studies to equate adolescent wheeze with asthma which has a potential for misclassification and account for the discrepant associations for asthma related risk factors such as smoking (163, 256) and atopy (85, 237) between studies. We characterized asthma on questionnaire based information and compared this to wheeze that lacked asthma diagnosis (non-asthma-wheeze), which has not previously been reported. Although both phenotypes of wheeze were quite similar in their questionnaire based responses making it difficult to distinguish between them, on further assessments with objective measures of spirometric and inflammatory markers, it was evident that the two conditions were quite distinct from each other. Therefore, without objective measures of pulmonary functions and allergen skin prick test, it is impossible to be confident that non-asthma-wheeze is different from asthma solely on the basis of questionnaire based information alone.

9.8 Characteristic of Non-asthma Wheeze

In our cohort non-asthma-wheeze comprised of approximately 5% of participants in a cross- section of the 18 year old population and a fifth of all wheeze. This wheeze phenotype demonstrated close correlation to age of initiation of cigarette smoking and wheeze onset in comparison to asthmatic wheeze. Non-asthma-wheeze was largely non-atopic having trends for less rhinitis ($p=0.06$), lacking bronchodilator reversibility, bronchial responsiveness and lower FeNO when compared to individuals with asthma. Asthmatic individuals lacked association to either active or passive and current or past tobacco smoke exposures. It is possible that misclassification of non-asthma-wheeze phenotype as asthma when considering risk factors for adolescent wheeze is likely to account for the observed associations of tobacco smoke to these states in some previous studies (122). Similarly discrepancies reported for adolescent asthma in relation to atopy may in part be accounted for by lack of recognition of non-asthma-wheeze phenotype. We are aware of the fact that some of those individuals with wheeze lacking asthma diagnosis may truly be asthmatics, however the cohort study design is more likely to favour earlier asthma diagnosis based on greater awareness amongst its participants for asthma symptoms and signs than the general population. Therefore, under-diagnosis is more likely to account for undiagnosed asthma cases in surveys such as that reported by Kaur (124). In their study they have previously reported significant proportion of 13-14 year-olds lacking asthma diagnosis. In view of the above findings it is important to consider that such reports are likely to

over-estimate under-diagnosis of asthma by assigning non-asthma wheeze phenotype into undiagnosed asthma category. The non-asthma wheeze phenotype should be considered separately as it has distinct characteristics, risk factor and possibly prognosis compared to asthma and hence its identification has great public health relevance for planning and implementing any broad based interventions for improving lung health in teenagers.

Although our focus was to study asthma but we were surprised by finding of this distinct wheeze phenotype and tried to review and match the characteristics of this group to current literature to determine what is known in relation to undiagnosed wheeze over adolescent period. A large cross-sectional survey comprising of 12 to 18 year-olds that enrolled 122, 829 teenagers reported 6% of undiagnosed frequent wheeze cases, which closely approximates our results. The risk factor profile for this undiagnosed frequent wheeze had demonstrated significant association to active and passive tobacco smoke exposures, female gender and lower social class along with increased prevalence of allergies in this group (122). However this study is likely to have a mixed wheeze population of both non-asthma-wheeze and true adolescent undiagnosed asthma. The population characteristics was suggestive of a social disadvantaged background that was not present in our study furthermore health system in United States is largely based on individually financed health care unlike government supported health system of United Kingdom. It is therefore, surprising that despite significant differences in the population characteristics, similar proportion of undiagnosed wheeze was identified. The conclusion in the study by Yeatts and colleagues was that greater efforts were required by health care professionals in proactively asking wheeze related questions and identifying these individuals. We would support this approach but suggest that if these teenagers demonstrate lack of broncho-dilator response in addition to significant history of tobacco smoke exposure than these are less likely to represent asthma but possibly non-asthma-wheeze. However the final phenotypic form or the prognosis of this group needs further exploration. We are tempted to speculate non-asthma-wheeze as possible early presentation of COPD, with its association to tobacco smoking as an important factor however the objective parameters of spirometric lung function at this time point are of a restrictive impairment rather than that of obstructive patterns of lung function.

9.9 Defining Asthma over Adolescence

A recent review of 122 cohort studies noted 66 different definitions were used for defining asthma (289). Four most frequently used definitions were applied to a single cohort by the authors to test variations in prevalence and prediction model performances. The results of this study observed considerable variations for both predictive model and asthma prevalence. The most conservative asthma prevalence was reported for the definition that used physicians diagnosed asthma in conjunction with symptom of wheeze over last 12 months, which also had least number of cases with clinical indecision as highest probability represented by area under curve (290) value of 0.76 (289). At 10 year follow-up a similar definition was used as above for predictive modelling (53). At the 18 year follow-up, we additionally incorporated the use of asthma medication for defining asthma to a physician diagnosis if these individuals demonstrated good control of their wheeze. This definition was applied to both 10 and 18 year asthma groups to determine transition of asthma over the adolescent period. As presented earlier, not only the risk profile but objective measures between the wheeze phenotypes differed significantly between the wheeze groups at 18 years. The continued observation of wheeze heterogeneity at 18 years which is also observed during childhood (63) is suggestive of complex nature of this symptom that is closely associated with asthma and is used as an important surrogate marker in epidemiological studies. Therefore, in order to determine true adolescent onset asthma and its characteristics, we restricted our adolescent onset asthma group to those with asthma diagnosed for the first time during adolescence (between 10 and 18) thus excluding any child that had received asthma diagnosis before age 10 but did not exclude early life wheeze. The argument against our approach to exclude only early life diagnosed asthma instead of early life wheeze could be that we are likely to include any potential undiagnosed asthmatic child in our adolescent onset asthma group thereby ending up with a group that was effectively not true adolescent onset as the name implies. However, this approach is likely to affect our adolescent onset asthma group non-differentially as the early life wheeze were distributed proportionally between the never asthma and those with true adolescent onset asthma (data not shown) as far as risk factor determination is concerned. Lack of significance of early life factors for adolescent onset asthma also provided support to our phenotype determination of a true adolescent onset phenotype.

9.10 Example of Impact of Childhood Wheeze Heterogeneity on Asthma over Adolescence

The Tucson Children's Respiratory Study observed 22% prevalence of asthma at the age of 22 years of which 37% of cases had never wheezed or had wheezed only transiently in early life, which is similar to that reported by our study (123). Martinez has quite comprehensively characterised early life wheeze phenotypes (61), which was further studied at the ages of 11 and 13 years (62, 63). Over this entire period early life transient wheeze retained its phenotypic identity and was resistant to asthma diagnosis. We need to be careful in labelling wheeze as asthma, as this in itself is likely to lead to misclassification. Therefore, to label all early life wheeze as asthma may not be appropriate and is likely to lead to under-estimation of true adolescent onset asthma.

9.11 Growth and Remission of Asthma over Adolescence

The hallmark of adolescent period is the onset of puberty, marked by physical changes in secondary sexual characteristics that accompany rapid growth and development (291). Unfortunately we were unable to report the exact time points of the specific age related pubertal changes in our study as all of these events were captured retrospectively at the age of 18 years. However, we were able to document changes in physical measures of height, weight and spirometric lung function over this period for all consenting study participants that visited the research centre. The overt physical growth is accompanied by drastic increase in lung function to cope with increasing size of the human body (172). We observed remission of asthma to be characterised by significantly improved lung function over the adolescent period although this increase was significant only for boys (173). It could not be conclusively confirmed in our study whether this improved lung function contributed to remission or whether it was the result of remission of asthma in boys. It has been hypothesised that growth of lung may be a contributory factor for driving remission of asthma over adolescent period (33). All children in remission of asthma recalled outgrowing their symptoms the age at which pubertal changes are observed. This is suggestive of contributory effect of lung growth which may partly account for remission of asthma over the adolescent period particularly for boys. Lack of similar lung growth advantage in remittent females is likely to be accounted for by earlier onset of puberty in comparison to males, in addition to the fact that males continue to benefit from longer duration of lung growth in comparison to their female counterparts (176). Therefore, we speculate that mechanisms other than growth are likely to have greater influence on females in remission of their asthma symptoms.

9.12 Lack of Evidence for Role of Obesity

Obesity has been implicated to increase risk for asthma, particularly in girls over the adolescent period (58). A high body mass index (BMI) of >25 represents above average weight and individuals with a BMI of >30 are considered to be obese (2004 #489). There was no difference in BMI observed between various wheeze phenotypes or for adolescent onset asthma phenotype. The reason for lack of such finding in our cohort is not clear. We speculate Island living may have some role with active life style of adolescents, as there was no difference observed for children who exercised between asthmatic and non-asthmatic individuals; similarly number of hours these adolescents were involved in watching television or working on computers was comparable.

9.13 Evidence for the Single Airway Hypothesis

The one airway hypothesis states that, both upper and lower airways act as a unit (260), which has been observed to hold true when we consider rhinitis and its association to both wheeze and asthma at 18. Furthermore, lower proportion of rhinitis was observed in remittent asthmatics compared to those with persistent asthma, thus our study contributes towards growing evidence that proposes a greater need to approach both upper and lower airway management together rather than treating them as separate entities.

9.14 Rhinitis as a Risk Factor for Asthma

When we considered adolescent onset asthma, rhinitis at the age of 10 emerged as a risk factor for asthma over this period. It is equally possible that rhinitis may be the initial presentation of progressive upper airway pathology that over time involves the lower airway passages. The exact pathogenesis of association between asthma and rhinitis for adolescent onset asthma is yet to be determined. In earlier studies, history of hay fever or eczema were used for atopic assignment (88), however we are now able to apply more objective tests such as skin prick test to common allergens, and distinguish independent contribution of atopy to that of rhinitis.

9.15 Significance of Early Life Factors?

Hygiene hypothesis proposes exposures to infections, particularly early in life, provide protection from developing allergic conditions by favouring predominance of Th1 type immune response (non-allergic) to develop in place of Th2 that is associated with allergic disease states. However, we were unable to observe this association towards wheezing illness over the adolescent period when we examined effect of early life chest infections to adolescent asthma or wheeze. Having other siblings and attending day care centres are considered proxy for exposures to infection. Early life pet ownership which may be considered a source of infection, also did not demonstrate any effect towards asthma. We did not find having additional sibling offered any protection nor did being the only child increase the risk of developing asthma over adolescence.

In general, when we review relative contributions of early life in comparison to adolescent factors, asthma risk profile seems to be associated more with adolescent exposures rather than to early life events. There were some exceptions to this as non-asthma-wheeze individuals did exhibit significance of greater exposure to tobacco smoke and trend towards significance with greater proportion of smoking in pregnant mothers at univariate level. But it is equally likely that this could just be a confounding as children of parent who smoke were more likely to take up smoking as young adults themselves. Similarly breast feeding was not observed to offer protection from developing wheeze over the adolescent period, as it had done over the childhood (111).

9.16 Relevance of Atopy

Atopy has also been a matter of debate for its significance beyond the childhood with lack of significance towards occurrence of asthma (85). We not only observed greater proportion of boys with asthma were atopic 80%, but were also able to demonstrate that females that go onto develop asthma over this period develop both atopic and non-atopic asthma contrary to the general perception that it is largely non-atopic asthma in females. Our results are supported by the earlier British National Cohort study that observed that in girls atopic influence continues to pose increased risk of asthma over the adolescent period (80). The relevance of atopy at age 10 demonstrated greater significance towards adolescent onset asthma rather than early life atopy at 4. This and other factors combined are suggestive of a true adolescent onset condition that is largely influenced by factors over adolescent period. Highest atopic proportions were noted for persistent asthmatic individuals (75%) followed by

64% for adolescent onset asthma individuals, whereas those experiencing remission of asthma had 43% atopy and few of non-asthmatics were atopic (34%). This gradient of atopic proportions is demonstrative of close relationship that exists between atopy and asthma.

We compared wheeze and adolescent asthma phenotypes to atopic inflammatory marker of FeNO (246). The atopic assignment was in concordance with levels of FeNO observed for adolescent asthma phenotypes. Information on asthmatics with FeNO at 18 years comprised 69% atopic individuals with a geometric mean value of 31 ppb of FeNO, whereas non-wheeze and non-asthma-wheeze had FeNO of 17 and 18 ppb for 34% and 38% atopic proportions. This pattern was maintained for both adolescent onset asthma and those with remission of childhood asthma over the adolescent period. Therefore, we were able to test our atopic groups by an internal validation of their atopic status through this airway inflammatory marker.

The atopic disease state has been known to demonstrate bronchial responsiveness (219, 292). Both asthma and rhinitis have close associations with increased bronchial reactivity (212, 214). Similarly allergen sensitization leads to greater bronchial responsiveness (293). Sears and colleagues have previously demonstrated that bronchial reactivity tends to be higher in children and decreases with age (99). BHR has been shown to increase with allergy, respiratory infections and cigarette smoking (292). Our study consolidates the finding of Dunedin cohort of decrease in bronchial reactivity with increasing age. We observed increased bronchial responsiveness of the airway for both persistent and adolescent onset asthma phenotypes. The persistent asthma phenotype demonstrated the highest with non-asthma group demonstrative of least bronchial reactivity. Transitions in bronchial responsiveness were seen from age 10, in those individuals that were to grow out of their asthma or to in those that were to develop it over adolescent period. Both of these had similar bronchial reactivity which was lower than that of persistent asthmatics at the age of 10 years, and had significantly greater than that of non-asthma individuals. Bronchial responsiveness of remittent asthmatics was comparable to non-asthma individuals at 18 years, with adolescent onset asthmatics having similar reactivity to persistent asthma. Therefore, bronchial reactivity at the age of 10 may have a predictive role for asthma prognosis later in life. Further work needs to be undertaken in developing predictive models based on bronchial responsiveness taking into account all determinants such as atopy, tobacco smoking and co-morbid allergic conditions.

9.17 Tobacco Smoke Exposure and Asthma

In contrast to some studies that have demonstrated association of tobacco smoke exposures to adolescent asthma (160), we did not observe this association. Instead active tobacco smoking at 18 years was associated with wheeze that lacked an asthma diagnosis which we termed non-asthma-wheeze. The cross-sectional asthma versus non-asthma-wheeze at 18 years that we have characterised strives to address the discrepant findings of tobacco smoke exposure and its risk for asthma (163). Non-asthma-wheeze individuals demonstrated a close association to tobacco smoke exposure early in life with greater proportion of pregnant mother and early life passive exposures. These children were then observed to take up smoking themselves as teenagers and exhibit wheeze following their active tobacco smoking. This pattern of tobacco smoke exposure is suggestive of early life influence playing a crucial role towards the development of non-asthma-wheeze. This will need epigenetic review of the cohort and determination of prospective impact of early life environmental as well as genetic factors towards disease occurrence for both asthmatic and non-asthmatic wheeze phenotypes. This finding of wheeze associated with active tobacco smoking has previously been documented and suggested, but such large population based studies did not characterise these individuals on objective pulmonary function parameters and considered these adolescents to have undiagnosed asthma (122, 242). When we modelled early life as well as active tobacco smoke exposures at 18, the non-asthma-wheeze was demonstrative of association with active tobacco smoke more than early life exposure. We cannot conclusively state whether early life tobacco smoke exposure had direct effect on lung function or indirectly via conditioning habit of children of parents who smoke. But the information from spirometric data from 10 year is supportive of the latter view as only females having non-asthma-wheeze had comparable lung function at 10, with diminished FEV1 and FVC by the age of 18. Furthermore, non-asthma-wheeze phenotype largely comprised of new wheeze cases with no previous occurrences in early life. The current evidence is suggestive of the fact that adolescent period is prone to environmental insults such as tobacco smoke exposure on the changing physiology including pulmonary functions. However, smoking did not demonstrate significance towards asthma or any of the prospective asthma phenotypes over the adolescent period. We speculate that this lack of association of tobacco smoke with asthma is likely to be explained by exclusion of non-asthma-wheeze that may have been encompassed in other studies in the adolescent asthma groups (52, 160, 256).

9.18 Paracetamol and Asthma

Amongst the information that was gathered on environmental exposures at the 18 year follow-up, information on consumption of paracetamol and non-sedative anti-inflammatory medications was also collected. Paracetamol is implicated to affect pulmonary function by inactivating the glutathione buffer system that prevents oxidative injury to the tissues (254). Although the information of paracetamol use at an earlier follow-up may have yielded greater benefit, but despite the natural limitations we observed increased use of paracetamol to affect all wheeze phenotypes. Its higher use was associated with non-asthma-wheeze, asthma at 18 and adolescent onset asthma while lower consumption favoured remission. Our study adds to the accumulating evidence that cautions use of paracetamol (167), particularly in children, with lower use of paracetamol may offer advantage for remission; however further prospective studies are needed to confirm this.

9.19 Strengths and Limitations

We evaluated questions on pubertal status and age at which tobacco smoking was started. It was observed that both of these were likely to have some recall bias. All efforts were made to avoid any such variable that may bias interpretation of risk factor analysis. The strength of our study is the prospectively collected information on variables including allergic conditions such as hay fever, eczema, wheeze, environmental exposures such as passive tobacco smoke exposures, and objective tests such as skin prick tests collected from the age of 4. Family history of asthma was observed to be associated with not just asthma but also to non-asthma-wheeze phenotype. This is suggestive of essential role of genetic predisposition as a pre-requisite for environmental factors to have an effect. We speculate that beyond early life period, adolescence is the next most significant time where rapid changes are observed in the human body, both anatomic and physiological, that may render them susceptible to environmental insults or risk taking behaviours with adverse outcomes in genetically predisposed individuals.

9.20 Unanswered Research Questions and the Need for Future Studies

- The group of individuals with non-asthmatic-wheeze that have significant morbidity needs further assessment
- The long term prognosis of non-asthmatic-wheeze needs to be followed up
- Although lung function improvement in adolescents with remission of asthma has been observed in our study, the relevance of this finding to cause and effect needs further investigation, particularly in relation to effect of exercise
- We did not observe smoking to be associated with either cross-sectional asthma or to new adolescent onset asthma, however the association of non-asthmatic-wheeze and its significant association to tobacco smoking needs further assessment
- Paracetamol has emerged as a non-specific environmental factor that is not only associated with asthma occurrence and with non-asthmatic-wheeze but its lower use was associated with remission of asthma. Prospective studies evaluating impact of paracetamol needs to be developed to assess the impact and usage of this important medicament.
- Further work needs to assess relevance of bronchial reactivity and factors that influence this characteristics of the airway as it may play important prognostic marker for remission or persistence of asthma
- Gender plays an important role over adolescent period and differential growth patterns between boys and girls need in-depth study understanding role of genetic and hormonal influences that are likely to contribute towards their pulmonary growth and function
- Fractional exhaled nitric oxide is an important marker of atopic airway inflammation, and its associations to bronchial reactivity needs further review, which we are likely to work on and review its association with asthma in particular the atopic asthma.

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APPENDICES

Appendix 1: Participant Information Sheet, Version 4

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

St Mary's Hospital, Newport, Isle of Wight, PO30 5TG. Tel: 01983 534898

PARTICIPANT INFORMATION SHEET - Isle of Wight Birth Cohort study- 17 year follow-up, 9 May 2008

We would like to thank you for your involvement so far in the 1989/1990 birth cohort. The data we have been able to collect from you has been tremendously helpful in advancing our understanding of asthma and other allergic diseases.

You are now being invited for a follow-up as part of the 1989/1990 birth cohort study. Before you decide to participate it is important for you to understand why the research is being done and what it will involve.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?

Over the last few decades there has been a dramatic increase in the number of teenagers with asthma, eczema, hay fever and food allergy. We still do not know why people develop these diseases. The purpose of this study is to understand how changes in asthma and other allergic diseases relate to the other changes that occur during adolescence. We know that many teenagers outgrow their asthma and allergies but we do not know why this happens. If we can understand this, we will be able to predict who will outgrow their asthma and it may also provide us with new ideas for treating or even curing asthma and allergic disease.

We are also continuing our work to understand how our genes are involved in the development of asthma and other allergic conditions. Certain genes have been linked with the development of asthma and other allergic diseases. We need to undertake further research work to understand this link. If we are to maximise our chance of discovering how our genes are involved in the development of asthma and other allergic conditions, we need to look at the genes of as many of the group as possible. We can do this by taking a small amount of blood. Alternatively we could collect a small amount of saliva from you or swab the inside of your mouth.

Why have I been chosen?

You have been chosen because you are part of the Isle of Wight 1989/1990 birth cohort. This is a birth cohort of nearly one-and-a-half-thousand young people all born on the Isle of Wight in 1989 and 1990.

What will happen to me if I take part?

We will arrange for you to visit the David Hide Research Centre at a convenient time. We will reimburse your local travel expenses. We will repeat many of the procedures that you may have been asked to do at previous visits. Even if you have not visited us before we would like you to participate this time by undertaking all or part of the following:

- Questionnaire about your current health
- Weight, height and blood pressure, and check whether you have signs of eczema or asthma
- Collect a small amount of your breath to measure the amount of nitric oxide it contains. This tells us if you have inflammation in your lungs which will affect how they work.
- Spirometry – we will ask you to blow into a computer to check how well your lungs are working
- Methacholine test – this is a special breathing test to ascertain how sensitive your lungs are. We will ask you to breathe in a mist containing methacholine and blow into a computer
- Skin prick test – this is a safe, standard medical test for allergies (eg house dust mite); a drop of the liquid will be put on the skin and gently scratched; the test is positive if a small wheal develops after 15 minutes.
- We would like to take a small amount of blood using anaesthetic cream to numb the skin before hand

- If you are not happy to allow us to take some blood, we will ask for either a saliva sample (we will ask you to spit into a small cup) or buccal swab (the inside of your cheek is gently swabbed with a cotton swab).
- Urine – we would ask you to provide us with a small urine sample

We will invite a few (80) teenagers back to collect some sputum from them; using standard technique where we will ask you to breathe in a little salty air, to will help you to cough up some sputum.

What do I have to do?

We will ask you to come to the David Hide Asthma and Allergy Research Centre, at a convenient time, for up to a 2 hour visit. About 1 in 12 teenagers will also be asked to return for one shorter 1 hour visit. Before each visit we will ask you not to drink or eat any caffeine (eg coffee, tea, coke, chocolate) for 4 hours; if you take asthma medication please do not use your reliever inhaler (eg ventolin, salbutamol, terbutaline, bricanyl) for 6 hours; long acting inhaler (eg salmeterol, serevent, seretide, eformoterol, oxis) for 12 hours; and antihistamines for 72 hours. If you have had a respiratory infection in the previous 2 weeks or are taking oral steroids we will rebook your visit at a convenient time for you.

What are the possible disadvantages and risks of taking part?

We will minimise any discomfort from taking blood with local anaesthetic cream to numb the skin. For some people the methacholine challenge or sputum collection may cause slight wheeze but this can be easily treated with an inhaler. In the highly unlikely event of any injury caused to you, medical care through the National Health Service will be available to you. St. Mary's Hospital NHS Trust has indemnified this study.

What are the possible benefits of taking part?

Your participation in this study is very important as it will allow us to see how asthma and allergies affect children and teenagers as they grow up. The information we learn will help us to understand why children and teenagers develop asthma and other allergic problems. It may also point us towards new treatments for these medical problems in the future. This is critically important as more of us are developing these illnesses.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. The first part of the consent form (Part A) asks for your consent to participate in the study. The second part (Part B) asks for permission to store any unused blood or other samples for use in future research into allergic diseases. The samples will only be used for studies approved by the Local Research Ethics Committee. The samples will be fully anonymous to the researchers who use them but contain codes that would allow the clinical study team who collected them to link them back to you. You are free to choose to just sign Part A and not sign Part B. You will receive a copy of the signed consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the medical care you receive.

What if there is a problem?

If you have any questions or concerns, please contact Dr Graham Roberts or Sharon Matthews at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534897. Email: IOWstudy@iow.nhs.uk.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential.

Contact for further information

Sharon Matthews or Graham Roberts, The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight. Telephone: 01983 534897. Email: IOWstudy@iow.nhs.uk.

This completes Part 1 of the Information Sheet. Part 2 will give you more detailed information about the conduct of the study.

Part 2

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions (Sharon Matthews 01983 534897). If you still have questions or concerns, you can contact Alex Punter (Lead for Research and Development, St Mary's Hospital, Newport, Isle of Wight, PO30 5TG; email alex.punter@iow.nhs.uk).

In the very unlikely event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against St Mary's Hospital but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

The personal information collected in this study will be kept confidential. The data we collect from you will not be labelled with your personal details and will be stored securely. Data collected during the study will be shared with our research collaborators in the USA; however they will not know who the information belongs to as your name and address will not leave The David Hide Asthma and Allergy Research Centre. Only the study personnel will have access to your personal details. You will not be individually identified in any reports or publications resulting from the study. We will keep your data on file for use in future studies approved by the Research Ethics Committee.

Involvement of the General Practitioner

We would like your permission to notify your General Practitioner (GP) of your participation in this study. With your permission we would send your GP the results of your allergy and methacholine tests as they may be useful for your future medical care. We would not send your GP any other results from the study.

What will happen to any samples I give?

Blood: we will use this to check how allergic you are and measure the level of a hormone called leptin that is related to growth during adolescence. Additionally we will extract genetic material from the sample. This will only be used to look for genes such as leptin that may be involved in the development of asthma and allergies.

Urine: we plan to measure the level of cotinine in this sample, this increases if you have been exposed to cigarette smoke, passively or otherwise. The result will not be released to your parents or doctors.

Sputum: we will use this to see if there is inflammation in your lungs as seen in asthma.

Samples will be stored securely at the David Hide Asthma and Allergy Centre until they are analysed. Only the researchers at the centre will have access to them. Some of these blood, saliva, buccal or sputum samples will be analysed outside of the St Mary's Hospital (including in the USA). The samples will not be labelled with your name or address so that the researchers analysing them will not know that the sample belongs to you. With your permission, we would like to store some blood for use in further studies into asthma and allergic disease. We will only use these stored samples for studies reviewed and approved by the Local Research Ethics Committee. If we collect saliva or a buccal swab from you, genetic material would be collected from these and stored for use in further studies into asthma and allergic disease.

Will any genetic tests be done?

As we explained in the "What is the purpose of the study?" section above, we are looking at which of our genes are involved in the development of asthma and other allergic diseases. For this work we can use blood, saliva or buccal swab samples. The results we obtain will help us to understand why some people develop asthma and allergies. The results will not directly help you and will not have any individual significance to you so we will not be able to give you your individual results.

What will happen to the results of the research study?

We aim to publish the results of the study in medical journals so that other doctors and researchers can make use of them. This is likely to be accompanied by an article in the local press on the Isle of Wight. It will not be possible to identify any individual teenager involved in the study from these published results.

Who is organising and funding the research?

The researchers at The David Hide Asthma and Allergy Research Centre are organising and carrying out this study. They are being helped by a group of experts from the Michigan State

University and University of South Carolina in the United States of America, who will provide the expertise to analyse the blood, saliva and buccal swab samples. The study is being supported by the David Hide Asthma and Allergy Research Centre Trustees.

Who has reviewed the study?

This study was given a favourable ethical opinion for conduct in the NHS by the Portsmouth and Isle of Wight Local Research Ethics Committee.

How long do I have to decide whether I should take part?

Your decision to participate in this study is entirely voluntary. You should take as much time as you need.

Thank you for taking time to read this information sheet.

ISLE OF WIGHT BIRTH COHORT STUDY – 17 YEAR FOLLOW-UP

PARTICIPANT INFORMATION SHEET CONTINUATION
(FEMALE PARTICIPANTS ONLY)

Part 3

What is a Lung Challenge Test?

This is a special breathing test to ascertain how sensitive your lungs are. We will ask you to breathe in a mist containing Methacholine and blow into a computer.

Are there any particular requirements for proceeding with a Lung Challenge Test?

Although there are no known adverse effects of a Lung Challenge Test using Methacholine on pregnancy, as an additional safety measure, it is standard for all female study participants to first undergo a pregnancy test. This will just involve a quick test on a small sample of your urine. We understand that at this time you may not be sexually active or that you may be using a reliable birth control measure. However, as we do not have enough information about the effect of the mist on the unborn child, we are obliged to routinely undertake a pregnancy test before the Lung Challenge Test.

If you do not wish to undertake a pregnancy test you can still take part in all other aspects of this study or we can schedule the Lung Challenge to be performed during your period.

What are the implications of undergoing a pregnancy test?

Before you consent to having a pregnancy test, we would like you to take time to consider the implications of taking the test and the result you may receive.

A urine pregnancy test is a screening test only and you may require further testing to confirm your pregnancy.

If you test positive and you were planning to be pregnant then this may be good news for you. However, if you were not expecting to test positive, we understand that you might have mixed feelings about being pregnant.

Unplanned pregnancies happen and every woman has the right to decide for herself how to deal with the situation. The following options are available to you: (i) continue with the pregnancy and keep the baby; (ii) continue the pregnancy and place the baby for adoption or fostering; (iii) end the pregnancy by having an abortion, if you are legally able to do so.

Prior to proceeding with the pregnancy test, there are some questions you may find helpful to ask yourself as given below:

What are my plans and hopes for the future?

How would I feel about achieving those plans if I become pregnant?

What are the two or three things that matter most to me in my life at the moment? Will I have to lose or give up something if I am pregnant?

How would other people who matter to me react if I am pregnant?

Considering my own values and beliefs how would I feel if I am pregnant?

What are the two or three things that I hope to achieve in the next 5-10 years if I am pregnant?

What would I lose or give up in the next 5-10 years if I am pregnant?

What support is available in the event of a positive pregnancy test?

Trained staff will be available at the Allergy Centre to discuss, in confidence, the implications of your pregnancy test and direct you to the appropriate sources of further confidential guidance and professional support available through the Sexual Health Service, St Mary's Hospital (Tel:

01983 534202) or your GP. Alternatively The British Pregnancy Advisory Service (BPAS) website www.bpas.org.uk offers valuable information.

Do I have to take part in the Lung Challenge Test?

It is your decision whether or not you wish to take part. If you do, you will be asked to sign a consent form before commencement of the challenge. A decision to withdraw at any time, or a decision not to take part, will not affect the medical care you receive.

What if there is a problem or require further information?

If you have any questions or concerns, please contact Dr. Abid Raza or Sharon Matthews at The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Newport, Isle of Wight PO30 5TG. Telephone: 01983 534897 or e-mail: IOWstudy@iow.nhs.uk.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential.

This completes Part 3 (Female Participants) of the Information Sheet.

Appendix 2: Consent Form

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE
St Mary's Hospital, Newport, Isle of Wight PO30 5TG. Tel: 01983534373

Study No

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CONSENT FORM - Isle of Wight Whole Population Birth Cohort – 17 year follow up

Name of Researcher: Dr Graham Roberts and Dr Hasan Arshad

PART A

Please initial box if you agree with each section.

1. You confirm that you have read and understand the information sheet dated 9th May 2008 (Version 4) for the above study and have had the opportunity to ask questions: ☐
2. You understand that your participation is voluntary and that you are free to withdraw at any time, without giving any reason, without your medical care or legal rights being affected: ☐
3. You understand that sections of any of your medical notes may be looked at by responsible individuals from The David Hide Asthma and Allergy Research Centre. You give your permission for these individuals to have access to your records: ☐
4. You give your permission for your General Practitioner to be informed of your decision to participate in this study and receive the results of your allergy and methacholine tests: ☐
5. You agree to provide samples of blood / saliva / swab from the inside of your mouth / urine for use only in our research into asthma and allergy (cross out all that are not applicable). You understand that some samples will be analysed in the United States of America. ☐
6. I give permission for the samples to be used for genetic research aimed at understanding the genetic basis of allergic diseases (eg food allergy, asthma, eczema, hay fever) ☐
7. You voluntarily agree to participate in the above study: ☐

PART B: Linked anonymised samples gifted for storage and use in future studies:

You give permission for your samples (which may include blood, buccal swab, urine, saliva and/or sputum) to be stored (potentially for many years) for possible use in future research studies. **Future studies will be reviewed and approved by a Research Ethics Committee prior to your sample being used.** You understand that these studies may not directly benefit your health.

You can alter these decisions at any stage by letting the researcher know.

- a) You give permission for the sample to be used for investigations of medical conditions relating to allergic diseases (eg food allergy, asthma, eczema, hay fever). ☐
- b) You give permission for the sample to be used for genetic research aimed at understanding the genetic basis of allergic diseases (eg food allergy, asthma, eczema, hay fever). You understand that some samples will be analysed in the United States of America. ☐
- c) You give permission for a member of the research team to look at your medical records, to obtain information on allergy. You understand that the information will be kept confidential. ☐

Name of Participant

Date

Signature

Name of Person taking consent
(if different from researcher)

Date

Signature

Researcher

Date

Signature

1 for participant; 1 for researcher; 1 to be kept with hospital notes

Appendix 3: Postal Questionnaire Male

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

Initials				1989 STUDY 17 YEAR FOLLOW UP	Date of Birth			
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Please tick all relevant boxes Date questionnaire completed

The following questions are about your home and family

P1 Does anyone smoke inside your house?

Mother	Yes	☐	No	☐
Father	Yes	☐	No	☐
Other	Yes	☐	No	☐

P2 Have you had a pet in the last 2 years?

Cat	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐
Dog	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐
Other	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐

If other pet, please give details

P3 Have you had any of the following medical problems since you were 10 years of age?

Asthma or wheezing episodes	Yes	☐	No	☐
Eczema	Yes	☐	No	☐
Year round or summer rhinitis (Hay fever)	Yes	☐	No	☐
Food intolerance or food allergy	Yes	☐	No	☐

If yes, what food

P4 The following questions are about your chest

a. Have you had wheezing or whistling in the chest in the last 12 months? Yes ☐ No ☐

b. How many attacks of wheezing have you had in the last 12 months?
None ☐ 1 - 3 ☐ 4 - 12 ☐ More than 12 ☐

c. Have you ever had asthma? Yes ☐ No ☐
If yes, was it diagnosed by a doctor? Yes ☐ No ☐ Don't know ☐
What age was it diagnosed? Years

d. In the last 12 months, has your chest sounded wheezy during or after exercise? Yes ☐ No ☐

P5 These questions are about your nose and eyes

a. In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu? Yes ☐ No ☐

b. Have you ever had hay fever? Yes ☐ No ☐

P6 These questions are about your skin

a. Have you ever had an itchy rash which was coming and going for at least 6 months? Yes ☐ No ☐

b. Have you had this rash at any time in the last 12 months? Yes ☐ No ☐

c. Have you ever had eczema? Yes ☐ No ☐

Please turn over...

P7 The following questions are about any animals you might have contact with

Do you react when near an animal?

Yes ☐ No ☐

If yes, which animal(s)?

If yes, how?

Itchy skin / hives /
angiodema (swelling)

☐ Rhinoconjunctivitis
(itchy eyes and nose)

☐ Wheeze /
cough

☐

P8 The following question is about exercise and leisure activities

a. On average how many days a week do you exercise vigorously (so that you get out of breath or sweat)?

b. Does anything stop you from exercising?

Yes

☐

No

☐

Don't know

☐

If yes, please specify

c. How many hours do you spend watching TV or using the computer or playing electronic games on:

a weekday

 hours

a weekend day

 hours

P9 The last few questions are about you

a. Do you currently smoke?

Yes

☐

No

☐

If no, go to question 9b

If yes, how many cigarettes do you smoke a day on average?

Less than 1

☐

1 – 5 / day

☐

6 – 10 / day

☐

11 – 15 / day

☐

16 or more

☐

b. Have you ever smoked?

Yes

☐

No

☐

If no, go to question 10

If yes, how many cigarettes did you used smoke a day on average?

Less than 1

☐

1 – 5 / day

☐

6 – 10 / day

☐

11 – 15 / day

☐

16 or more

☐

P10 How old were you when you noticed that you started to spurt in height, grow body or facial hair, develop skin changes or notice a deepening of your voice?

Years

P11 Are there any comments you would like to make about your state of health?

Appendix 4: Postal Questionnaire Female

THE DAVID HIDE ASTHMA AND ALLERGY RESEARCH CENTRE

Initials					1989 STUDY 17 YEAR FOLLOW UP	Date of Birth			
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Please tick all relevant boxes Date questionnaire completed

The following questions are about your home and family

P1 Does anyone smoke inside your house?

Mother	Yes	☐	No	☐
Father	Yes	☐	No	☐
Other	Yes	☐	No	☐

P2 Have you had a pet in the last 2 years?

Cat	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐
Dog	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐
Other	Yes, including bedroom	☐	Yes, not bedroom	☐	Yes, not in house	☐	No	☐

If other pet, please give details

P3 Have you had any of the following medical problems since you were 10 years of age?

Asthma or wheezing episodes	Yes	☐	No	☐
Eczema	Yes	☐	No	☐
Year round or summer rhinitis (Hay fever)	Yes	☐	No	☐
Food intolerance or food allergy	Yes	☐	No	☐

If yes, what food

P4 The following questions are about your chest

a. Have you had wheezing or whistling in the chest in the last 12 months? Yes ☐ No ☐

b. How many attacks of wheezing have you had in the last 12 months?
None ☐ 1 - 3 ☐ 4 - 12 ☐ More than 12 ☐

c. Have you ever had asthma? Yes ☐ No ☐
If yes, was it diagnosed by a doctor? Yes ☐ No ☐ Don't know ☐
What age was it diagnosed? _____ Years

d. In the last 12 months, has your chest sounded wheezy during or after exercise? Yes ☐ No ☐

P5 These questions are about your nose and eyes

a. In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you DID NOT have a cold or the flu? Yes ☐ No ☐

b. Have you ever had hay fever? Yes ☐ No ☐

P6 These questions are about your skin

a. Have you ever had an itchy rash which was coming and going for at least 6 months? Yes ☐ No ☐

b. Have you had this rash at any time in the last 12 months? Yes ☐ No ☐

c. Have you ever had eczema? Yes ☐ No ☐

Please turn over...

P7 The following questions are about any animals you might have contact with

Do you react when near an animal? Yes ☐ No ☐

If yes, which animal(s)?

If yes, how? Itchy skin / hives / ☐ Rhinoconjunctivitis ☐ Wheeze / ☐
angiodema (swelling) (itchy eyes and nose) cough

P8 The following question is about exercise and leisure activities

a. On average how many days a week do you exercise vigorously (so that you
get out of breath or sweat)?

b. Does anything stop you from exercising? Yes ☐ No ☐ Don't know ☐

If yes, please specify

c. How many hours do you spend watching TV or using the computer or playing electronic
games on:

a weekday hours

a weekend day hours

P9 The last few questions are about you

a. Do you currently smoke? Yes ☐ No ☐

If no, go to question 9b

If yes, how many cigarettes do you smoke a day on average? Less than 1 ☐
1 – 5 / day ☐
6 – 10 / day ☐
11 – 15 / day ☐
16 or more ☐

b. Have you ever smoked? Yes ☐ No ☐

If no, go to question 10

If yes, how many cigarettes did you used smoke a day on average? Less than 1 ☐
1 – 5 / day ☐
6 – 10 / day ☐
11 – 15 / day ☐
16 or more ☐

P10 Have you begun to menstruate (started to have your period)? Yes ☐ No ☐

If yes, how old were you when you started to menstruate? Years

P11 Are there any comments you would like to make about your state of health?

**Thank you very much for completing this questionnaire.
Please send it back to us in the envelope provided.**

Appendix 5: ISAAC Questionnaire

Summary Screen Sheet

Study database view - Microsoft Access

ID: 0 DOB Gender Name ISAAC SPT, eNO, LFT, etc Meth+ Sputum 17y questions

Summary Comments

SUMMARY How questionnaire completed Anticipated visit date: Status:

Questionnaires Status
ISAAC
Food allergy questions
Asthma questions
Asthma QoL
Rhinitis questions
Eczema questionnaire
Eczema QoL
Scored
17y questionnaire
Investigations
Skin prick testing
Exhaled nitric oxide
Spirometry
Blood sample
Saliva sample
Buccal swab
Urine cotinine

Date Completed by Double checked by
Symptoms
Asthma QoL
Eczema QoL
Investigations

Suitable for Nicki's study

Show all study numbers

Please note, this sheet will not update until it has been closed and reopened!

REMOVE NAMES WHEN DOUBLE CHECK DATA!

Print all (except 17y) Print summary form Print ISAAC form Print first half of extra questions (ISAAC and symptoms) Print second half of extra questions (ISAAC and symptoms) Print SPT, eNO, LFT, etc Print 17y Qu - boy Print 17y Qu - girl Print SPT only

Record: 1 of 1543 Unfiltered Search

Comments Page

Study database view - Microsoft Access

ID: 0 DOB Gender Name ISAAC SPT, eNO, LFT, etc Meth+ Sputum 17y questions

Summary Comments

Note
ISAAC form:
Symptoms questionnaire:
Asthma QoL questionnaire:
Eczema QoL:
17y questionnaire:
Skin prick testing:
Exhaled nitric oxide:
Spirometry:
Bloods:

Print all (except 17y) Print summary form Print ISAAC form Print first half of extra questions (ISAAC and symptoms) Print second half of extra questions (ISAAC and symptoms) Print SPT, eNO, LFT, etc Print 17y Qu - boy Print 17y Qu - girl Print SPT only

Record: 1 of 1543 Unfiltered Search

ISAAC Questionnaire Page 1

Study database view - Microsoft Access

Home Create External Data Database Tools

ID: 0 DOB Gender Name: Save Summary SPT, eNO, LFT, Bloods, etc

ISAAC Questionnaire 1 ISAAC Questionnaire 2 ISAAC Questionnaire 3 ISAAC Questionnaire 4

ISAAC QUESTIONNAIRE
All subjects should complete this form

Date completed: Today's date

Initials of person completing forms:

1. Is your house: Rented-council/housing

2. Do you cook Gas Electricity Oil
no yes no

3. Are you still living with your parents/guardians?: Yes

4. Are you still in education?: Yes

4a. If yes, are you in:

5. Do you work: No

5a. If yes, please specify:

6. What is your father's (or mother's partner's) occupation:

7. What is your mother's occupation?:

8. Your birth order in terms of full sibs: child: of

9. Birth order in terms of all mother's child of

10. What is your family's annual income (estimate): Not known

11. Are there any damp areas on walls or ceiling: yes

12. Does your home have visible mould or fungus on walls, ceiling or windows: don't know

13. Does your mother smoke: yes Where: How many per day: 2

14. Does your father smoke: Where: How many per day:

15. Does any one else smoke: Where: How many per day:

16. Are you regularly exposed to smoking outside home:

17. In the last 2 years have you had a pet
Cat
Dog
Other pet
Describe other pets:

18. In the last 2 years have you had regular (more than once a week) exposure to a pet elsewhere:
Cat
Dog
Other pets
Describe other pets:

Now Save and go to ISAAC 2

Where does your mother smoke Filtered

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ISAAC Questionnaire Page 2

Study database view - Microsoft Access

Home Create External Data Database Tools

ID: 0 DOB Gender Name: Save Summary SPT, eNO, LFT, Bloods, etc

ISAAC Questionnaire 1 ISAAC Questionnaire 2 ISAAC Questionnaire 3 ISAAC Questionnaire 4

19. How frequently are you annoyed by outdoor air pollution (from traffic, industry etc) in your home if you keep the window open:

20. How often do cars pass your house or on the street less than 100 metres away:

21. How often do heavy vehicles (eg trucks/buses) pass your house or on the street less than 100:

22. What term best describes the place you lived most of the time when you were under the age of five years:

23. Did your mother work on a farm while she was pregnant with you:

24. Have you ever lived on a farm:

If yes 24a. How old were you when you started living on a farm (years):

24b. How old were you when you finish living on a farm (years):

If you have lived on a farm, did you have contact with live anima

24c. Dairy cattle: 24f. Beef cattle:

24d. Poultry: 24g. Sheep:

24e. Pigs: 24h. Horses:

24i. Other animals: 24j. Please specify:

24k. Did you spend your 1st year of life on a farm:

24l. Is it an organic farm:

25. Atopic disease since review at age 10 years

25a. Asthma/wheezing episodes:

25b. Night time/recurrent cough (>3 weeks):

25c. Eczema:

25d. Year round/seasonal rhinitis (hay fever):

25e. Urticaria (rash like nettle rash):

25f. Food intolerance or allergy:

If have food allergy (ie answer yes to 25f). please SAVE and go to extra food allergy questions

The following questions are about your chest

26. Have you ever had wheezing or whistling in the chest at any time in the past:

31. Have you ever had asthma:

31a. If yes, was it physician diagnosed:

31b. What age was it diagnosed (years):

33. In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection:

If have asthma (ie answer yes to 26 or 31). please SAVE and go to asthma specific questions

Lastly Save and go to ISAAC 3

22. What term best describes the place you lived most of the time when you were under the age of five years Filtered

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ISAAC Questionnaire Page 3

Study database view - Microsoft Access

Home Create External Data Database Tools

ID: 0 DOB Gender Name: Save Summary SPT, eNO, LFT, Bloods, etc

ISAAC Questionnaire 1 ISAAC Questionnaire 2 ISAAC Questionnaire 3 ISAAC Questionnaire 4

The following question is about your nose

34. Have you ever had a problem with sneezing, or a runny or a blocked nose when you did not have a cold?

39. Have you ever had hay fever?

If have rhinoconjunctivitis (ie answer yes to 34 or 39), please SAVE and go to extra nose questions

These questions are about your skin

40. Have you ever had an itchy rash which was coming and going for at least 6 months?

46. Have you ever had eczema?

If have had eczema since 10 years of age (ie answer yes to 40 or 46), please SAVE and go to extra eczema questions

The following questions are about any animals you might have contact with

47. Do you react when near an animal?

47a. Which animal specify 1:

47b. Which animal specify 2:

47c. If yes, have itchy skin / hives / angioedema:

47d. If yes, have rhinoconjunctivitis:

47e. If yes, how wheeze / cough:

48. Has anybody else in the family ever had the following:

Asthma

Mother: Father: How many sibs with asthma:

Night time/recurrent cough when you have not had a cold (>3w)

Mother: Father: How many sibs with cough:

Eczema

Mother: Father: How many sibs with eczema:

Rhinitis/Hay fever

Mother: Father: How many sibs with rhinitis:

Urticaria

Mother: Father: How many sibs with urticaria:

Food allergy

Mother: Father: How many sibs with food allergy:

49. Have you had the following immunisations since you were 10 years old

49a. BCG (10-14 y):

49b. Polio (about 16 y):

49c. Tetanus (about 16 y):

49d. Diphtheria (about 16 y):

49e. Other vaccinations:

49f. Other vaccinations what 1:

Now Save and go to ISAAC 4

40. Have you ever had an itchy rash which was coming and going for at least 6 months

Filtered

ISAAC Questionnaire Page 4

Study database view - Microsoft Access

Home Create External Data Database Tools

ID: 0 DOB Gender Name: Save Summary SPT, eNO, LFT, Bloods, etc

ISAAC Questionnaire 1 ISAAC Questionnaire 2 ISAAC Questionnaire 3 ISAAC Questionnaire 4

50. On average how many days a week do you exercise vigorously (so that you get out of breath or sweat)?

51. Does anything stop you from exercising?

If yes, please specify:

52. How many hours do you spend watching TV or using the computer or playing electronic games on a weekday?

53. How many hours do you spend watching TV or using the computer or playing electronic games on a weekend day?

The following questions ask about missing school / work due to asthma / allergies (please leave blank if not applicable)

1. How many days of school/work have you missed due to your asthma in the last year?

2. How many days of school/work have you missed due to your allergies in the last year?

The following questions are about medicines that you may use

56. On average, how many times a month do you use paracetamol?

57. On average, how many times a month do you use non-steroidal anti-inflammatory drugs (eg aspirin, brufen, ibuprofen)?

The following questions are about your use of a mobile phone

58a. Have you ever had a mobile phone?:

If NO, go to question 59

58b. What age (years) did you first have a mobile phone:

58c. Did you have a mobile phone in the last year:

If NO 58d. What age (years) did you stop having a mobile phone:

58e. How many minutes a week did you talk on your mobile in the last year / last year you had one:

59. Indicate how good or bad your health is today on a scale 0-100:

Open 0-100 picture

Height, weight and blood pressure

60. Height (cm):

61. Weight (kg):

62a. Blood pressure systolic:

62b. Blood pressure diastolic:

If have eczema, please SAVE and go to scored form

ISAAC completed ☐

Any comments about ISAAC

Please Save and go to SPT, eNO, etc

Initials of person double checking form:

56. On average, how many times a month do you use paracetamol

Filtered

Appendix 6: Food Allergy Symptom Questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

Symptoms questionnaire - food allergy

For participants with food allergy

29. Have you had a food allergy since 10 years of age: If no, go to end

29a. Suspected food 1:

29b. How many reactions have you had to this food since age 10 y:

29c. What type of reaction have you had to this food previously:

Urticaria/Hive ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐
 Wheezing/SOB ☐ Vomiting ☐ Diarrhoea ☐ Throat tightness ☐
 Colic ☐ Systemic ☐

29d. How quickly does the reaction occur after contact with the food:

29a. Suspected food 2:

29b. How many reactions have you had to this food since age 10 y:

29c. What type of reaction have you had to this food previously:

Urticaria/Hive ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐
 Wheezing/SOB ☐ Vomiting ☐ Diarrhoea ☐ Throat tightness ☐
 Colic ☐ Systemic ☐

How quickly does the reaction occur after contact with the food:

29c. Suspected food 3:

29b. How many reactions have you had to this food since age 10 y:

29c. What type of reaction have you had to this food previously:

Urticaria/Hive ☐ Eczema ☐ Angio-oedema ☐ Oral symptoms ☐
 Wheezing/SOB ☐ Vomiting ☐ Diarrhoea ☐ Throat tightness ☐
 Colic ☐ Systemic ☐

29d. How quickly does the reaction occur after contact with the food:

Food allergy symptoms questionnaire completed:

Comments about symptoms (food allergy) questionnaire:

Once completed, click to go back to ISAAC questionnaire

Symptoms questionnaire double checked by:

Record: 1 of 1 Filtered Search

29. Have you suffered from food allergy since 10 years of age

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Appendix 7: Asthma Symptom Questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

Symptoms questionnaire - asthma **Asthma quality of life questionnaire 1** **Asthma quality of life questionnaire 2**

Only for participants with asthma symptoms since age 10 years

27. Have you ever had wheezing or whistling in the chest in the last 12 months:

28. How many attacks of wheezing have you had in the last 12 months:

29. In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing:

30. In the last 12 months, has wheezing ever been severe enough to limit your speech to one or two words at a time between breaths:

32. In the last 12 months has your chest sounded wheezy during or after exercise:

4. At what age (years) did asthma/recurrent wheeze first appear:

5. Have you had asthma/recurrent wheeze in the last 12 months:

6. If no, at what age (years) did it stop:

7. Number of overnight hospital admissions since aged 10 years:

8. Number of ED/ward attendances since aged 10 years:

9. Asthma cared for by:

9a. If other, specify:

10. Asthma treatment:

For questions 11-14 below, please give details of treatments used in last year (please given details of any other asthma treatment that have used since aged 10 years in comments section below)

11. Bronchodilators:

11a. Bronchodilators what:

12. Does it help:

13a. Inhaled steroids:

13b. Inhaled steroid type, device and dose:

13c. Montelukast: 13d. Cromoglycate:

13e. LABA: 13f. Theophylline:

13g. Other:

14a. Oral steroids: 14b. Number of courses since aged 10 yr:

15. Recognised triggers:

Exercise ☐ Infection ☐ Pollen ☐
 Animals ☐ House dust ☐ Stress ☐
 Other ☐ Other triggers specify:

Asthma symptoms questionnaire completed:

Comments about questionnaire:

Please and go to asthma quality of life questionnaire

Record: 1 of 1 Filtered Search

27. Have you ever had wheezing or whistling in the chest in the last 12 months

Transfer Thesis... Additional doc... EC Sputum pro... Appendices 6 t... Data cleaning f... Transfer Thesis... Microsoft Acce...

Appendix 8: Rhinoconjunctivitis Symptom Questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

Symptoms questionnaire - rhinoconjunctivitis

Only for participants with rhinoconjunctivitis symptoms since 10 years of age

35. In the past 12 months have you had a problem with sneezing, or a runny or a blocked nose when you did not have a cold or the flu:

36. In the past 12 months, has this nose problem been accompanied by itchy/watery eyes:

37. In which of the past 12 months did this nose problem occur- check all that apply
 January ☐ February ☐ March ☐ April ☐ May ☐ June ☐
 July ☐ August ☐ September ☐ October ☐ November ☐ December ☐

38. In the past 12 months, how much did this nose problem interfere with your daily activities:

21. At what age (years) did the rhinoconjunctivitis first appear:

22. If you no longer have rhinoconjunctivitis at what age did it stop:

23. Timing:

24. In the past 12 months, how many times have these symptoms occurred:

25. In the past 12 months, have these nose problems been accompanied by sleep disturbance:

26. In the past 12 months, how much has this nose problem interfered with your daily activities, and/or school, and/or work, and/or leisure, and/or sport:

27. Recognised triggers: Yes ☐
 Infection ☐ Animals ☐ Pollen ☒ Dust ☐
 Other ☐ Other specify:

28. Treatment: Yes ☐
 Antihistamines ☐ Nasal steroids ☐ Cromoglycate ☐ Other ☐

Symptoms questionnaire (rhinoconjunctivitis) completed:

Comments questionnaire

Once form completed click to go back to ISAAC questionnaire

Record: 1 of 1 Filtered Search

35. In the past 12 months have you had a problem with sneezing, or a runny or ... Filtered

Appendix 9: Eczema Symptom Questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

Symptoms questionnaire - eczema **Quality of life questionnaire - eczema**

For participants with eczema since age 10 years

41. Have you had this itchy rash at any time in the last 12 months:

42. Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees,
 in front of the ankles, under the buttocks or around the neck, ears or eyes:

43. At what age did this itchy rash first occur:

44. Has this rash cleared completely at any time during the last 12 months:

45. In the last 12 months how often, on average, have you been kept awake at night by this itchy rash:

17. At what age (years) did the eczema first appear:

18. Have you had eczema in the last 12 months:

19. If no, what age did it stop:

Eczema symptoms questionnaire completed:

Comments about questionnaire:

Record: 1 of 1 Filtered Search

Form View Num Lock Filtered

Appendix 10: Juniper's Asthma Quality of Life Questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

0 Symptoms questionnaire - asthma Asthma quality of life questionnaire 1 Asthma quality of life questionnaire 2

List three activities during which your asthma bothers you. Possible activities you might choose are hockey, rounders, baseball, basketball, dancing, football, playing with pets, playing with friends, riding a bike, running, sleeping, swimming, volley ball, walking, walking up hill, laughing, studying, helping around the house, singing, gymnastics, rollerblading, skateboarding, athletics and climbing. You are welcome to choose others.

Activities 2: 3:

DURING THE PAST WEEK:

15. How much have you been bothered by your asthma in activity 1 during the past week:

16. How much have you been bothered by your asthma in activity 2 during the past week:

17. How much have you been bothered by your asthma in activity 3 during the past week:

18. How much did COUGHING bother you in the past week:

19. How much did your ASTHMA ATTACKS bother you in the past week:

20. How much did WHEEZING bother you in the past week:

21. How much did TIGHTNESS IN YOUR CHEST bother you in the past week:

22. How much did SHORTNESS OF BREATH bother you:

23. Think about all the activities you did in the last two weeks. How much were you bothered by your asthma during these activities:

Asthma QoL complete? Comments about question

Once both parts complete go to back to ISAAC questionnaire

AQOLQ double checked:

Record: 1 of 1 Filtered Search

Activities 1 Num Lock Filtered

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Appendix 11: Additional 17 year questionnaire

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

0 17 year questionnaire MALE FEMALE

17 YEAR QUESTIONNAIRE

The answers to these questions will be kept completely confidential

Date seen:

1. Do you currently smoke:

If yes, how many cigarettes do you smoke a day:

Now skip the next question

2. Have you ever smoked:

If no, go to question 6

If yes, how many cigarettes did you used to smoke a day on average:

3. What age did you start smoking: years

4. What age did you stop smoking: years

5. What made you stop smoking:

6. How much alcohol do you drink a week: units
(1 unit equals approximately 1 glass wine, 1/2 pint beer or a measure spirit)

You only need to answer the following question if you have asthma and are being treated with regular preventative medication (in addition to your blue reliever)

7. How often do you forget your preventative asthma medication:

8. Why do you forget your preventative asthma medications:

Comments 17y questionnaire:

Date seen Num Lock Filtered

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Appendix 12a: Male Section of (PDS)

Study database view - Microsoft Access

Subject ID: 0 DOB: Gender: Name: Save Summary

17 year questionnaire MALE FEMALE

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. Please do your best to answer them carefully. If you do not understand a question or do not know the answer, just mark "I don't know"

1. Would you say that your growth spurt in height:

If yes, how old were you when you noticed that you started to spurt in height?: years

2. How about the growth of your body hair? ("Body hair" means hair in many place other than your head, such as under your arms):

If yes, how old were you when you noticed that your body hair started to grow: years

3. Have you noticed any skin changes, especially pimples:

If yes, how old were you when you noticed changes in your skin: years

4. Have you noticed a deepening of your voice:

If yes, how old were you when you noticed your voice deepening: years

5. Have you begun to grow hair on your face:

If yes, how old were you when you noticed your facial hair starting to grow: years

Comments:

Completed ☐

Appendix 12b: Female Section of (PDS)

Study database view - Microsoft Access

Subject ID: 0 DOB: Gender: Name: Save Summary

17 year questionnaire MALE FEMALE

The next questions are about changes that may be happening to your body. These changes normally happen to different young people at different ages. Please do your best to answer them carefully. If you do not understand a question or do not know the answer, just mark "I don't know"

1. Would you say that your growth spurt in height:

If yes, how old were you when you noticed that you had started to spurt in height?: years

2. How about the growth of your body hair: ("Body hair" means hair in any place other than your head, such as under your arms)

If yes, how old were you when you noticed that your body hair started to grow: years

3. Have you noticed any skin changes, especially pimples:

If yes, how old were you when you noticed changes in your skin: years

6. Have you noticed that your breasts have begun to grow:

If yes, how old were you when you noticed your breasts beginning to grow: years

7. Have you begun to menstruate (started to have your period):

If yes, how old were you when you started to menstruate: years

8. How heavy are your periods:

9. Are you on the oral contraceptive pill:

9a. When did you start it?: year

Please enter as age of subject when commenced.

Comments:

Completed ☐

Microsoft Access - Main 17 year follow up 040806: Replica (Access 2000 file fo

Appendix 13: Dermatology life quality index

Eczema Life Quality Index

Study database view - Microsoft Access

Home Create External Data Database Tools

Subject ID DOB Gender Name:

Symptoms questionnaire - eczema **Quality of life questionnaire - eczema**

Eczema quality of life questionnaire

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK.
Please tick one box for each question.

1. Over the last week, how itchy, sore, painful or stinging has your skin been:

2. Over the last week, how embarrassed or self conscious have you been because of your skin:

3. Over the last week, how much has your skin interfered with you going shopping or looking after your home or garden:

4. Over the last week, how much has your skin influenced the clothes you wear:

5. Over the last week, how much has your skin affected any social or leisure activities:

6. Over the last week, how much has your skin made it difficult for you to do any sport:

7. Over the last week, has your skin prevented you from working or studying:

7a. If "No", over the last week how much has your skin been a problem at work or studying:

8. Over the last week, how much has your skin created problems with your partner or any of your close friends or relatives:

9. Over the last week, how much has your skin caused any sexual difficulties:

10. Over the last week, how much of a problem has the treatment for your skin been, for example by making your home messy, or by taking up time:

Eczema QoL completed: Double checked:

Comments eczema QoL:

Once complete both eczema pages, go back to ISAAC

Record: 14 1 of 1 Filtered Search

1. Over the last week, how itchy, sore, painful or stinging has your ...

Num Lock Filtered

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Appendix 14: Height and Weight Measurement Protocol

Author: G.C. Roberts

Height measurement:

A fixed wall mounted stadiometer was used for measuring height.

Study participant was asked to stand facing forwards on the base-plate on the floor of stadiometer

All the subjects were required to remove shoes

Ensure all subjects had their back to the tape

All were instructed to stand as tall and straight as possible with feet together and arms held loosely at the side and shoulders relaxed (to avoid lordosis).

It was ensured that position of the tape was inserted correctly into the base plate.

The head plate was raised vertically along the tape and placed on the top of the subject's head.

The head was assured to be placed in the Frankfurt Plane, an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal.

If a short person is measuring a very tall person, considerable error can be introduced when reading off the height scale: their eyes will read the scale at an angle (parallax effect).

Measurers should be aware of this and aim to read the scale from as level a position as possible. If there is a lot of height disparity the measurer should try to get level with the scale, by standing on a step stool and read the height to the nearest 0.1 cm. Only one measurement of height was to be recorded.

Weight measurement:

Take time to place the weighing scales on a level surface.

Ensure the scales are at zero when they are switched on.

Weigh without shoes.

Aim for subject to be wearing shirt and trousers/skirt only, and to remove heavy items of clothing and heavy jewellery if possible.

Weight to nearest 0.1 kg

One measurement of weight was taken

Reference:

- 1 MRC anthropometry protocol, by S. Duggleby
- 2 Protocol and Guidelines, Countrywide Integrated Non-communicable Diseases Intervention (CINDI) Programme 1995; EUR/ICP/CIND 94 02/PB04

Appendix 15: Sputum Processing Protocol

Process the sample as soon as possible (within 3-4 hours)

1. Pre-weigh 50ml falcon tube
2. Separate sputum from saliva in Petri dish, divide and put into falcon tube
3. Weigh tube and calculate weight of sputum
4. Add x 4 weight of 0.01 ml DTT and 22.5 μ L/mL Protease inhibitor
(Easiest method is to weigh it out in a spare falcon tube)
5. Cell rocker for 30 min
6. Filter through 100 μ m filter (encourage with cell scraper)
7. Centrifuge 1500 rpm (400g) for 10 minutes (**BALANCE CENTRIFUGE**)
8. Aliquot supernatant into labelled tubes (study id No) freeze -80
9. Mix cell pellet with 1ml pbs (phosphate base saline)
10. Remove 10 μ L of the suspension and mix with 90 μ L Trypan blue, mixing well
11. Place in manual haemocytometer and count respiratory cells and squamous cells, viable and dead (<30% squamous cells probably from the lower airways) – 25 squares from central grid on both sides
12. Work out total cell count per ml (dilution factor = 10 (10+90))
Total cell count _____ x 10 (dilution factor) = _____ x 10⁴
Convert to Total cell count _____ x 10⁶ (divide by 100)
Need to have 1 x 10⁶ cells so subtract 1 and add what is left as mL pbs
(E.g. TCC = 1.6 x 10⁶, therefore subtract 1 = 0.6ml is the amount of pbs to add to get 1 x 10⁶)
13. Assemble cytopins and add 70 μ L of diluted cell suspension to 4 slides
14. Spin at 400rpm for 5 minutes, check cell quality and allow to dry (24 hours)
15. Staining
 - a) Once dry, using Rapi-diff II stain pack
 - b) Fix by immersion in solution A for 1 minute
 - c) Transfer without rinsing to solution B for 5 seconds and agitate, drain excess stain onto absorbent paper
 - d) Transfer slide to solution C for 5 seconds and agitate drain onto absorbent paper
 - e) Rinse slide briefly in water
 - f) Leave at least 2 hours before putting on coverslip
 - g) Post pertex and coverslip leave (horizontally for at least 24 hours)
 - h) Cell count
 1. 400 inflammatory cells
 2. Plus squamous cells

Appendix 16: Sputum Induction protocol

Induced sputum protocol – (Standard Protocol).

Standard Sputum induction procedure:

Check with the participant that they have not had symptoms of a lower respiratory tract infection, and/or have not had oral steroids in the previous 14 days.

Consider whether to use the Modified Protocol for the “at risk” participant

There is no simple and reliable way to identify participants who are at risk of developing excessive and sudden broncho-constriction during sputum induction. ERS guidelines state that caution should be exercised in the case of participants with severe asthma, highly reactive airways, participants in exacerbation and those using increasing doses of β_2 agonists. It is always better to err on the side of caution and use the modified protocol whenever clinical judgement suggests that a participant/volunteer is at risk.

1) Give detailed information and clear instructions to the patient prior to the procedure.

Inhalation of a salty solution for up to 20 minutes, with a break every 5 minutes to cough up sputum, or whenever the subject feels able to cough.

The aim is to get samples from deep in the lungs, rather than saliva – so we can look at the different cells in the lungs.

Saliva can be spat into the bowl provided at any time during the procedure

Sputum can be coughed up at any time during the procedure

Remind the subject that there are two separate pots one for saliva and one for sputum!

2) Check safety equipment and set up ultrasonic nebuliser (output ~1 mL/min-1).

3) Measure pre-bronchodilator FEV1 using Koko as per ATS guidelines.

4) Administer four puffs of salbutamol (400 μ mg) via a spacer before commencing.

5) After 10 minutes, measure post-bronchodilator FEV1.

6) Use 40mls of sterile saline solution (4.5%); remember to set the nebuliser at 1ml/min.

7) Perform induction at 5-min intervals for ~20 min

8) Measure FEV1 at the end of each induction interval.

Stop induction if there is a fall in FEV1 of 20% compared with the post-bronchodilator value or, if symptoms occur, or the participant does not wish to continue.

Possible Adverse Effects:

Please observe throughout for any of the following

- Hyperventilation
- Dyspnoea
- Dizziness
- Nausea
- Wheeze

Stop the procedure immediately if the participant expresses any concerns regarding the procedure or if you notice symptoms occurring. It is vitally important to record lung function between each 5 minute nebulisation period to monitor for any changes.

All participants should be assessed by the study doctor before discharging from the unit.

Please remember to record any medication given i.e. Salbutamol onto dispensing logs.

9) Ask the patient to cough and spit after 5, 10, 15 and 20 min of induction or whenever they get the urge to do so. At the end of 20 minutes the induction must be stopped, whether there is a sufficient sample or not.

Remember to put the Petri dish on ice between expectoration!

10) At the end of the procedure if the final FEV₁ is within 10% of the initial reading the subject is free to go. If the FEV₁ is less than this administer two puffs of salbutamol via the spacer and FEV₁ rechecked at 10 minutes.

11) Discard the saliva pot, and ensure that the sputum pot is placed on ice prior to testing.

Appendix 17: Methacholine Test Protocol

Baseline Pulmonary Function

Baseline FEV₁ values will be obtained from lung function performed on KoKo spirometer prior to inhalation of saline. A baseline will be established by having the patient perform spirometry. The objective is to obtain three FEV₁ values within 5% of each other. If three values are obtained within 5% of each other, then the highest FEV₁ will be recorded. If three FEV₁ values are not within 5%, then spirometry will be performed until three values within 5% are obtained, or a total of five spirometry are performed. Based on the results of the baseline spirometry, the patient will receive either the bronchodilator or methacholine challenge.

Bronchodilator Challenge

If a subject's baseline FEV₁ is less than 70% of predicted, reversibility testing will be administered instead of a methacholine challenge. Each subject will be asked to demonstrate reversibility following administration of 600mcg of Salbutamol by MDI (metered dose inhaler) using the following protocol:

1. Assure that the patient is in a seated and upright position
2. Shake the MDI vigorously for several seconds and fit into spacer device
3. Have the patient breathe out to the end of normal expiration (end tidal volume)
4. Have the patient place the spacer mouthpiece into the mouth in an upright position and close lips around it
5. At the start of breathing in, with a slow deep breath, the MDI canister should be actuated (one puff) and the patient should continue to slow, deep breath for four seconds
6. At the end of four seconds, have the patient hold their breath for 10 seconds, then breathe out
7. Wait one minute before repeating steps 1-6
8. Begin pulmonary function testing 15-20 minutes following administration of the 600 mcg of Salbutamol

Methacholine Test

If the baseline FEV₁ is at least 70% predicted the subject can perform the bronchial challenge. Five breaths of 0.9% saline followed by spirometry at 1 minute is performed for control (post-saline) value. If post-saline drop (from the baseline) is <10% FEV₁ subsequent methacholine dilutions (Dose Levels 1 – 9) will be administered with increasing two-fold dilutions until at least a 20% reduction (PD₂₀ FEV₁) from the patient's control (saline) FEV₁ is reached or there is no 20% reduction with dose 9. Spirometry will be performed as described above. If two of the three FEV₁ values are within 5% of each other, then the highest value will be recorded. If two of the three FEV₁ values are not within 5%, then Spirometry will be performed until two values within 5% are obtained, or a total of 5 Spirometry are performed. If none of the five FEV₁ values

are within 5% of each other, the highest FEV₁ value will be recorded. If post-saline drop >10% FEV₁ the methacholine challenge will be rescheduled.

Methacholine dilutions (Dose Levels 1-9) will be administered on continuous 5 min cycles.

All patients will be seated and wear nose clips during the challenge.

Stages of Challenges	Mg/ml of Methacholine
Saline	0.0
1	0.06
2	0.12
3	0.25
4	0.50
5	1.0
6	2.0
7	4.0
8	8.0
9	16.0

A computerised dosimeter system (KoKo DigiDoser) with a fixed straw and baffle position Devilbiss 646 nebuliser will be used to ensure reproducibility.

The Devilbiss nebulisers (calibrated to deliver approximately 1.0 mL/min) to be used in conjunction with the KoKo DigiDoser have been characterised to deliver 5 breaths of Methacholine with firing times of 0.6 seconds, with 3 mL of solution in the nebuliser bowl, compressed air at 30 psi, and a constant inspiratory flow rate of 0.5 litre/sec.

Nebulisers will be numbered and the same nebuliser will be used for all challenges on a given patient. During the challenge, FEV₁ values will be obtained starting 1 minute after completing dosing with each Methacholine solution (Dose Levels 1-9).

After the end of the study subjects will be given a bronchodilator (e.g. 600mcg salbutamol via large volume spacer) and observed until their FEV₁ has returned to their baseline level.

Appendix 18: R&D Approval Letter

Isle of Wight Healthcare **NHS**
NHS Trust

Governance & Assurance Department
St Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Direct Tel No (01983) 534098
Direct Fax No (01983) 53407
Email: johnalex.punter@iwh.nhs.uk

1 June 2006

Dr Graham Roberts
Director
David Hide Asthma & Allergy Research Centre
St Mary's Hospital

Dear Dr Roberts

Epidemiology of asthma and allergic disease: risk and prognosis in a cohort from birth to adolescence

I am writing formally to confirm that the Joint R&D Committee gave organisational approval to the above project on 25 May 2006. This approval was subject to:

- Written confirmation of ethical approval from IOW, Portsmouth & South East Hampshire LREC

In accordance with our Trust Policy for R&D, I draw your particular attention to the following:

- In the event of a serious adverse event, which is linked to your research study, you must report any occurrence using the Trust's Incident Reporting Procedure.
- You will be required to provide a periodic report of progress with your research to the R&D Committee. Such progress reports should include details on any research outputs as well as current participant numbers, project start and end dates and account for all research income and expenditure.
- The National Research Register (NRR) is a database of ongoing and recently completed research projects funded by, undertaken within or of interest to the NHS, including charity-funded and commercially funded projects. Records submitted to the NRR will be published in the public domain and will be free to view by anyone with access to the World Wide Web. As a recipient of NHS R&D support funding, the Trust is required to make regular data submissions to the NRR.

I look forward to hearing from you in due course and wish you every success with your study.

Yours sincerely



for

Alexandra Punter
Lead R&D Officer

Appendix 19: Ethical Committee Approval



21 June 2006

ISLE OF WIGHT, PORTSMOUTH & SOUTH EAST HAMPSHIRE RESEARCH ETHICS COMMITTEE

Dr Zyed Hassan Arshad*
Keele University
Department of Respiratory Medicine
Newcastle
Stoke on Trent
ST4 6QG

1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
Hampshire
SO16 4RJ

Tel: 023 8036 2863
Fax: 023 8036 4110

Email: G.M.E.hio-au.SEHREC@nhs.net

Dear Dr Zyed Hassan Arshad*

Full title of study: Full Title : Full Title Epidemiology of Asthma and allergic disease: risk and prognosis in a cohort from birth to adolescence
REC reference number: 06/Q1701/34

Thank you for your letter of , 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 Committee held on 16 June 2006. 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.

Ethical review of research sites

The favourable opinion applies to the research sites listed on the attached form.

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	5.1	24 March 2006
Investigator CV		25 March 2006
Protocol		22 March 2006
Protocol		
Covering Letter		02 June 2006
Covering Letter		21 March 2006
Summary/Synopsis		01 February 2006
Summary/Synopsis		
Questionnaire	V1	22 March 2006
Letter of invitation to participant	1	22 March 2006
GP/Consultant Information Sheets	1	22 March 2006
Participant Information Sheet	1	24 March 2006

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

Participant Consent Form: PCF specifically for this study	1	02 June 2006
Participant Consent Form	2	24 March 2006
Response to Request for Further Information		
Summary on how funding will be used		02 June 2006
Summary of what is happening to cohort		02 June 2006
non-validated questionnaire	1	22 March 2006

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.

06/Q1701/34	Please quote this number on all correspondence
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With the Committee's best wishes for the success of this project

Yours sincerely



Chair

Email: GM.E.hio-au.SEHREC@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:

St Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Ms Kate Greenwood
R + D Office
Gloucester House
Queen Alexandra Hospital
Cosham
PO6 3LY

SF1 list of approved sites

Appendix 20: Correction of Study Document Numbers Letter from Ethics



National Research Ethics Service

MFK

ISLE OF WIGHT, PORTSMOUTH & SOUTH EAST HAMPSHIRE
RESEARCH ETHICS COMMITTEE

29 May 2008
(Revised 13.11.08)

1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
Hampshire
SO16 4RJ

Dr Syed Hasan Arshad
Reader in Allergy and Hon Consultant Physician
St Mary's Hospital
Newport
Isle of Wight
PO30 5TG

Tel: 023 8036 2863
Fax: 023 8036 4110

Email: scsha.SEHREC@nhs.net

Dear Dr Arshad

Study title: Epidemiology of asthma and allergic disease: risk and prognosis in a cohort from birth to adolescence.
REC reference: 06/Q1701/34
Amendment number: 3
Amendment date: 9 May 2008

The above amendment was reviewed at the meeting of the Sub-Committee of the REC held on 23 May 2008.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Questionnaire	1 Male	13 March 2008
Questionnaire	1 Female	13 March 2008
Participant Information Sheet	4	09 May 2008
Participant Consent Form: Female	2	09 May 2008
Covering Letter for Postal Questionnaire	1	09 April 2008
Appointment Letter	1	11 March 2008
Covering Letter		12 May 2008
Participant Information Sheet	2 Lung Challenge	07 May 2008
Participant Consent Form: Male	1	09 April 2008
Notice of Substantial Amendment (non-CTIMPs)	3, 9 May 2008	13 May 2008

Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.

This Research Ethics Committee is an advisory committee to South Central Strategic Health Authority

The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Isle of Wight, Portsmouth & South East Hampshire Local Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 23 May 2008

Name	Profession	Present	Capacity
Mr David Carpenter	Clinical Scientist	Yes	Chair
Mrs Jayne Tyler	Senior Fire Control Operator	Yes	Lay Member



National Research Ethics Service

ISLE OF WIGHT, PORTSMOUTH & SOUTH EAST HAMPSHIRE RESEARCH ETHICS COMMITTEE

MFK

13 November 2008

Dr Syed Hasan Arshad
Reader in Allergy and Hon Consultant Physician
St Mary's Hospital
Newport
Isle of Wight
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1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
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SO16 4RJ

Tel: 023 8036 2863
Fax: 023 8036 4110

Email: scsha.SEHREC@nhs.net

Dear Dr Arshad

Study title: Epidemiology of asthma and allergic disease: risk and
prognosis in a cohort from birth to adolescence.
REC reference: 06/Q1701/34

Thank you for your letter dated 21 October 2008, informing Mr David Carpenter, Chair of the IOW, Portsmouth & South East Hampshire Research Ethics Committee of a number of typographical errors in your study's ethical approval letter dated 21 June 2006 and approval letter dated 29 May 2008 for your substantial amendment number 3 (dated 9 May).

As these were purely administrative errors, Mr Carpenter did not need to view the letter and I have made the amendments and attach two revised letters dated 29 May 2008 (*Revised 13 November 2008*) and 21 June 2006 (*Revised 13 November 2008*) for your files.

The documents associated with the letter of the 29 May 2008 were not actually enclosed as stated in your letter; however our file for your study verified the dates and therefore I have amended these accordingly.

I hope this all meets with your approval.

Apologies for any inconvenience the errors may have caused you.

06/Q1701/34:	Please quote this number on all correspondence
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Yours sincerely

Mrs Melodie Kreindler
Committee Co-ordinator

E-mail: scsha.SEHREC@nhs.net

Copy to: Mrs Alexandra Punter, Isle of Wight Healthcare NHS Trust

This Research Ethics Committee is an advisory committee to South Central Strategic Health Authority

*The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England*

Study Team:**Supervisors:**

Prof. S.H. Arshad, Dr. G.C. Roberts, Prof. S. Holgate

Respiratory/ Allergy Consultant:

Ramesh Kurukularaatchy

Research Fellow:

Martha Scott

Research Staff:

Sharon Matthews, Bernie Clayton, Linda Terry, Stephen Potter, Jane Grundy, Paula Williams, Frances Mitchell

Acknowledgement:

I would like to thank Prof. Arshad, Drs. Roberts, Kurukulaaratcy for their invaluable support in their guidance and in analysis of the data for my thesis.

