LONG-TERM FOLLOW UP OF INFANTS AT HIGH RISK OF ASTHMA FROM A DEPRIVED COMMUNITY IN SOUTH WALES

Thesis submitted to Cardiff University for the Degree of Doctor of Medicine

January 2015

Dr Sadiyah Hand

Dedicated to

Richard, Shara, Danya and Jimahl

for their love and support

Acknowledgements

I would like to thank my supervisors, Professor Frank Dunstan, Dr Iolo Doull and Professor Ken Jones for giving me the opportunity to undertake this higher degree and for their guidance through the process.

I would like to thank Dr Mike Burr, who, 30 years ago, conceived this project, and tirelessly worked on it until the end. I would also like to thank Sharon Rolf, without whom my involvement in the project may not have happened. Sadly, both will never see the final drafts of this thesis.

Abbreviations

ALSPAC	Avon Longitudinal Study of Parents and Children
BAMSE	Swedish longitudinal study (Bambse is Norwegian for teddy bear)
BHR	Bronchial Hyper-reactivity
BMI	Body Mass Index
CAPS	Childhood Asthma Prevention Study
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Disease
DPT	diphtheria, pertussis and tetanus
ECRHS	European Community Respiratory Health Survey
German MAS	German Multicentre Allergy Study
ISAAC	International Study of Asthma and Allergy in Children
IUATLD	The International Union against Tuberculosis and Lung Disease
LOW	Late onset wheeze (childhood phenotype)
MAAS	Manchester asthma and allergy study
MAPS	Merthyr allergy prevention study
NHANES	National Health and Nutrition Examination Survey
NSHG	National Study of Health and Growth
NW	Never wheeze (childhood phenotype)
OR	Odds Ratio
PIAMA	the Prevention and Incidence of Asthma and Mite Allergy
PPV	Positive predictive value
PW	Persistent Wheeze (childhood phenotype)
RCT	Randomised control trial

SIDRIA	Italian Studies of Respiratory Disorders in Childhood and the Environment
SPT	Skin prick test
TAS	Tasmanian Allergy Study
TCRS	Tucson Children's Respiratory Study
TESAOD	Tucson epidemiological study of airway obstructive diseases
TEW	Transient early wheeze (childhood phenotype)

Summary

Asthma is a chronic respiratory disease with a prevalence that has increased worldwide over the past 40 years. Longitudinal cohort studies have been designed to determine associations between early life events and asthma prevalence. One such cohort is the Merthyr Allergy Prevention Study (MAPS).

MAPS recruited high risk subjects, before birth, from a deprived population in South Wales. The original study was a randomised controlled trial (RCT) of either normal diet for the first four months of life, or a cows' milk protein exclusion diet with soya formula milk supplementation if subjects were not breast fed. Subjects were subsequently followed up as part of a cohort study. The findings presented are based on the final follow up at age 23 years.

While there was a significant protective effect of breast feeding on wheeze at age 1, there was no evidence of an association with wheeze at age 23 years. The intervention arm of the RCT was associated with an increased risk of asthma and sensitisation at age 23 years.

There was tracking of both total serum IgE and positive skin prick test results over the years but there was no clear relationship between these two measures of allergy. Although the prevalence of atopy was low in childhood, there was still a clear association with this and wheeze later in life. Wheeze at age 3 years or older was an important determinant of asthma at age 23 years. There was a significant association between those who wheeze from age of 3 to age 23 years and atopic status at age 7 years.

In conclusion we have investigated a birth cohort from a relatively deprived area of South Wales and found characteristics in the first 7 years of life are critical in determining if asthma develops in early adulthood.

DECLARATION

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

Signed …… (candidate) Date ……

STATEMENT 1

This thesis is being submitted in partial fulfillment of the requirements for the degree of ……(insert MCh, MD, MPhil, PhD etc, as appropriate)

Signed …… (candidate) Date ……

STATEMENT 2

This thesis is the result of my own independent work/investigation, except where otherwise stated.
Other sources are acknowledged by explicit references. The views expressed are my own.

Signed …… (candidate) Date ……

STATEMENT 3

I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loan, and for the title and summary to be made available to outside organisations.

Signed …… (candidate) Date ……

STATEMENT 4: PREVIOUSLY APPROVED BAR ON ACCESS

I hereby give consent for my thesis, if accepted, to be available online in the University's Open Access repository and for inter-library loans **after expiry of a bar on access previously approved by the Academic Standards & Quality Committee.**

Signed …… (candidate) Date ……

Chapters and Sections

Index of tables

Index of figures

Chapter One

Background

1.0 General introduction

Asthma is an inflammatory disease of the airways that may commence at any age, but most often does so in childhood[1]. Before puberty it is more likely to affect boys than girls, with a gender ratio of approximately 2:1, male:female. Following puberty, asthma incidence is greater in females[2]. It may become quiescent in adolescence but can then recur in later life. It is common and approximately 5.4 million individuals (8%) in the UK are receiving treatment for asthma[3]. Asthma has a small but significant mortality; 20 per million population died from asthma in England and Wales in 2012[4]. It is also a significant cause of disability and a history of childhood asthma is now known to be a risk factor for chronic obstructive pulmonary disease (COPD) in adults[5]. In addition to these factors the prevalence of asthma has increased worldwide in recent decades, especially in developing countries[6,7]. There is a considerable economic burden, in both health service resources and days of work lost. The annual cost associated with asthma in the UK is estimated at £2.5 billion, of which £900 million is directly related to the money spent in the National Health Service (NHS). In addition twenty million working days are lost per year due to illness from asthma[8].

Despite the worldwide significance of asthma, there is still no single test to diagnose it. Asthma is defined by the Global Initiative for Asthma (GINA) as: *'...a heterogeneous disease, usually characterised by chronic airway inflammation. It is defined by the history of respiratory symptoms such as*

wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation.'[9]

This clinical definition, however, is not used to define asthma in epidemiological studies. As yet there is no consensus on asthma diagnosis in research. Indeed, one study found that there were 60 different definitions of asthma across 122 research papers on paediatric asthma[10]. Over the past 20 years two groups have sought to standardize the methodology on the study of asthma: the International Study of Asthma and Allergy in Children (ISAAC)[11] and the European Community Respiratory Health Study (ECRHS)[12]. They have produced validated questionnaires, standardized practical procedures such as skin prick testing and facilitated international collaboration. The aim has been to develop an epidemiological definition of asthma that has international validity.

1.1 Validation of asthma definition in epidemiological questionnaires

Wheeze is the sound made when there is turbulent air flow through an airway, and is usually described as a high pitched sound coming from the chest. Wheeze is therefore a condition that can be used to compare many different cohorts. The International Union against Tuberculosis and Lung Disease (IUATLD) found that questions related to wheeze in the past 12 months and asthma ever or asthma in the past 12 months were repeatable and up to 96% sensitive in predicting a positive bronchial provocation test for bronchial hyper-reactivity (BHR) with histamine (proxy for asthma) in European subjects[13]. These questions therefore became part of the core questions used for defining asthma for the different phases of the ISAAC study. However the

way that they are formulated is not always consistent. Van Wonderen et al showed that in epidemiological studies there were at least 60 different definitions of asthma in 122 studies. They also found that where doctor diagnosis of asthma has been used to define asthma, 19% have used current doctor diagnosis of asthma, while 28% have used ever doctor diagnosis of asthma and the rest have not specified when the doctor diagnosis was made[10].

The validated ISAAC phase II questionnaire which was also used in phase III studies defined current asthma as a previous doctor diagnosis of asthma ever and symptoms of asthma in the preceding 12 months. Further development from this questionnaire has led to the core questions in the ISAAC manual which are related to asthma symptoms in the past twelve months and ever having had asthma (see appendix 10). In this way epidemiological studies have addressed content, construct and predictive validity with regards to the appropriate questions for questionnaires defining asthma in epidemiological studies.

It is important to note that this definition will not identify subjects who have only a cough or chest tightness as their symptom of asthma, but it should include the majority of cases and gives a clear guide for international comparison. Wheeze is a distinct entity from asthma and is not interchangeable with asthma, but it is not open to different interpretations as the diagnosis of asthma is[10].

Bronchial provocation testing for bronchial hyper-reactivity (BHR) is also undertaken in many studies to define asthma[10]. Generally it is agreed that in field work BHR is consistent and gives good specificity[14] but validated

questionnaires give the best sensitivity for identifying asthma, without losing specificity, compared to a questionnaire in combination with measured BHR[15].

ISAAC state that two key concepts guided the development of these specific questions. Firstly, the principle of using symptoms rather than diagnosis for international comparisons, and secondly, the recognition that there were several dimensions to asthma severity: frequency of attacks, intensity of attacks, and persistence of symptoms[16]. The phase three ISAAC questionnaire is given in the appendix for both age 6-7 and 13-14 years and is sourced at the following website http://isaac.auckland.ac.nz/resources/tools.php?menu=tools1. As can be seen ISAAC only considers symptoms and not current diagnosis as there is evidence that symptoms rather than diagnosed and treated asthma gives a clearer view of prevalence as suggested in one adult study undertaken by ECRHS[17]. A number of studies have used these validated ISAAC questionnaires to undertake epidemiological studies on asthma.

1.2 Epidemiology of asthma

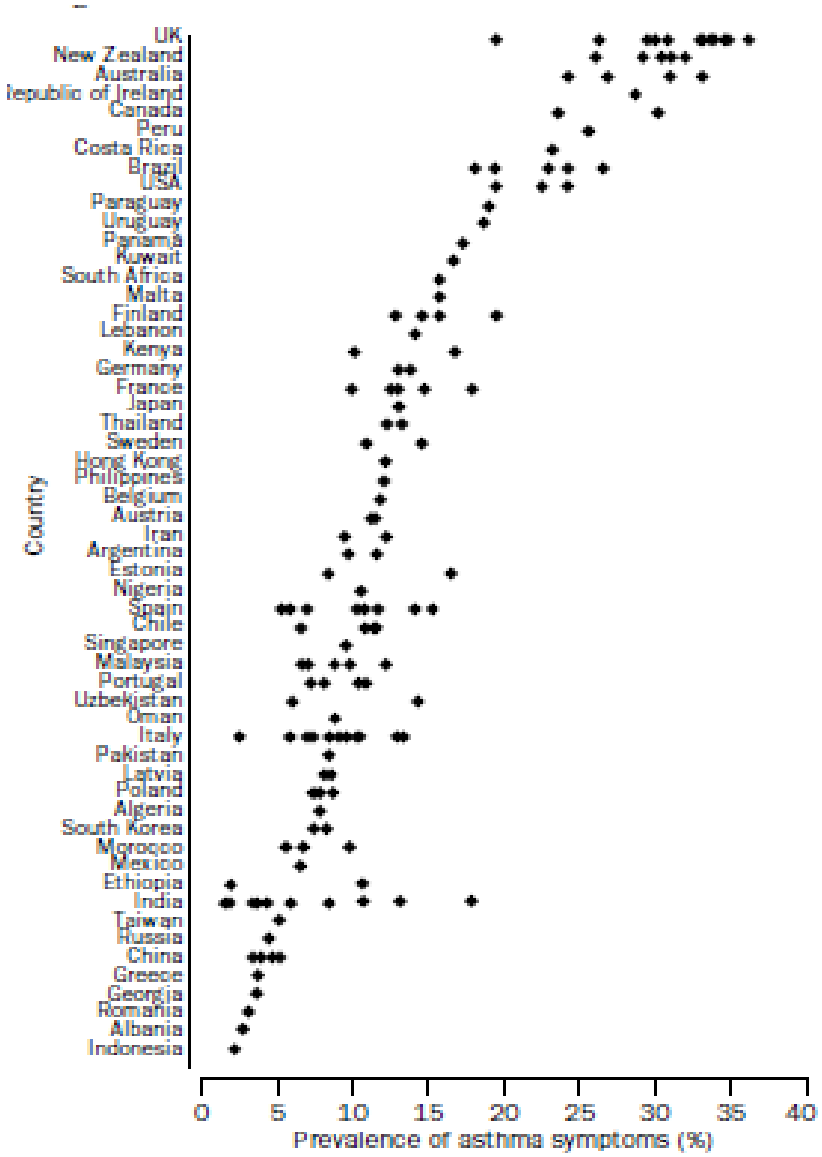
ISAAC and ECRHS have undertaken multicentre international cross-sectional studies which have led to a greater understanding of asthma in both children and adults. They have reported on international prevalence[18], late onset of asthma[19], and the role of certain environmental factors on the development of asthma, including breast feeding[20], early Paracetamol use[21], the presence of siblings and early day care[22] and pet keeping[23]. ISAAC and ECRHS have each developed systems, techniques and international collaboration through 3

phases of studies spanning approximately 5 year periods, from the 1990s to the current day. The focus of ISAAC is on children worldwide, while ECRHS is primarily concerned with adults in Europe.

ISAAC has undertaken worldwide cross-sectional studies on children age 6-7 years and 13-14 years. In early ISAAC studies, countries that had a prevalence of wheeze in the past 12 months (current wheeze) of less than 10% were mainly in Asia, Northern Africa, Eastern Europe and the Eastern Mediterranean regions. Those with wheeze rates greater than 20% were mainly in the UK, Australasia, North America and Latin America[11]. As part of an international ISAAC study between 1996-8, centres worldwide were recruited by professional collaboration provided the researchers agreed to use the ISAAC protocol[24]. The twelve month prevalence of self-reported asthma symptoms from written questionnaires in different centres, during this period, is shown by the points on the graph in figure 1.1. Using similar methods and criteria for definition, there was great variation between countries, and also there was great variation within some countries. Lai et al, reported on the 2003 phase III ISAAC study, that the prevalence of current wheeze ranged from 2.4% in Jodhpur (India) to 37.6% in Costa Rica in 6–7 year olds, and from 0.8% in Tibet (China) to 32.6% in Wellington (New Zealand) in 13–14 year olds[18]. Asthma symptoms became more common in children from 1993 to 2003 in many low- and middle-income countries which previously had low levels. However, in most high-prevalence countries, the prevalence of asthma changed little and even declined in a few countries. What has happened to the prevalence and severity of asthma since 2003 is not clear as there have been

no further worldwide studies in children[25]. However a European Community Respiratory Health Survey (ECRHS) 10 year review of 11168 European subjects suggested that there was a plateauing of symptoms up to 2003 in young adults (age 20-44 years), although there was evidence of an increase in treated asthma[17].

Figure 1.1 Asthma prevalence in centres around the world



Taken from Beasley, worldwide variation in twelve month prevalence of self-reported asthma symptoms from written questionnaires: ISAAC 1996-8[24]. Results from different centres in the same country are marked separately.

1.3 Changes in asthma prevalence

In 1997 Woolcock and Peat reviewed data from a wide range of countries where cross-sectional studies have taken place on more than one occasion and found the prevalence of asthma had risen significantly all over the world. In Australia the prevalence of 'asthma ever diagnosed' in 8-11 year olds increased from 12.9% in 1982 to 29.7% in 1992 and Taiwan's rate of current asthma in 7-15 year olds increased from 1.3% in 1974 to 5.8% in 1991[26]. In a second large cross-sectional postal survey among young adults in Melbourne in 1998, compared to a previous survey that had taken place in 1990, there was a significant rise in doctor diagnosed asthma from 12% to 17% $p<0.001$ over the 8 year period[27]. In cross-sectional studies of 11-12 year olds, in New Zealand in 1989 and again in 2000, Wickens et al showed a similar increase in asthma ever being diagnosed from 17% to 37% between the two studies[28]. Asthma prevalence has increased in countries considered to have a low prevalence as well. Haahtela et al investigated the records of serial military recruits in Finland including records of call up examinations and records of conscripts discharged because of poor health between 1966 and 1989, and compared them to records collected between 1926 and 1961[29]. During 1926-61 the prevalence of asthma remained steady at between 0.02% and 0.08%. However, between 1966 and 1989, there was a continuous, linear increase in prevalence, from 0.29% in 1966 to 1.79% in 1989- a six fold increase or a twenty fold increase compared to 1926[29]. Brogger et al undertook two cross-sectional studies of subjects aged 15–70 years in Oslo, Norway, in 1972 and again in 1998–1999. The prevalence of ever having had a doctor's diagnosis of asthma increased from 3.4 to 9.3% and the prevalence of wheezing

increased from 17.8 to 25.8%[30]. ISAAC studies suggest that the rise in asthma prevalence continues but is relatively greater in those countries that had low prevalence in earlier phases (and would be considered developing countries). The prevalence in those countries where it was previously highest may have now plateaued[7]. However, this is not the experience in the UK where the prevalence appears to continue to rise.

1.4 The prevalence of asthma in the UK

Anderson et al[31] showed that in the UK the prevalence of asthma rose from 1965 through to the 1990s with a plateauing in prevalence from the 1990s. However, Figure 1.2 (taken from Anderson et al) does show a rise in prevalence of asthma diagnosis ever (shown on a log scale), among children in studies in the UK over the past 50 years and suggests that the rate of increase in prevalence was still increasing up to 2004.

Figure 1.2 Trends in the prevalence of a diagnosis of asthma ever in children in the UK, presented on a log scale[31].

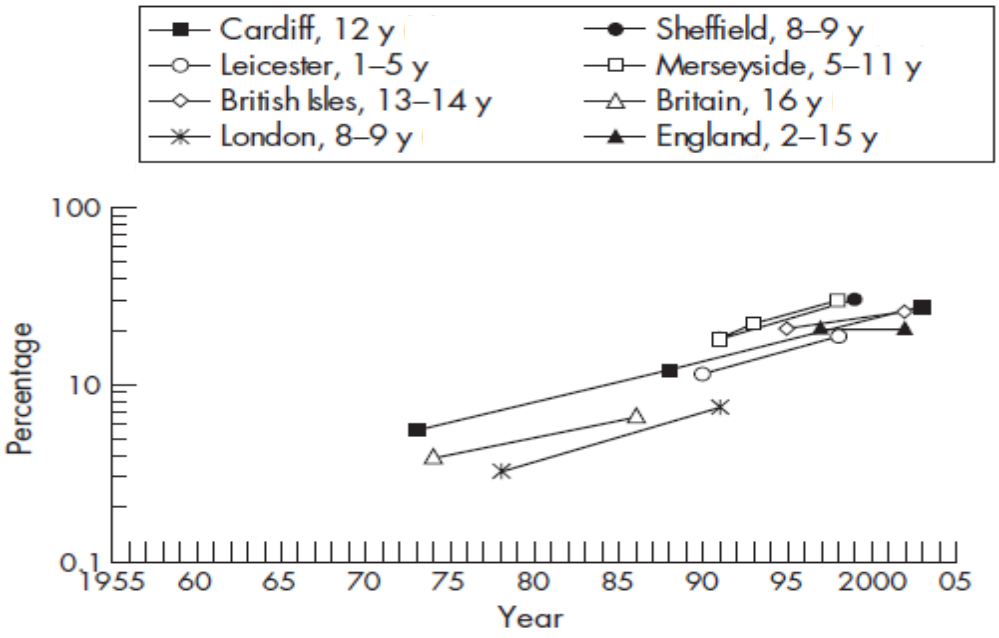
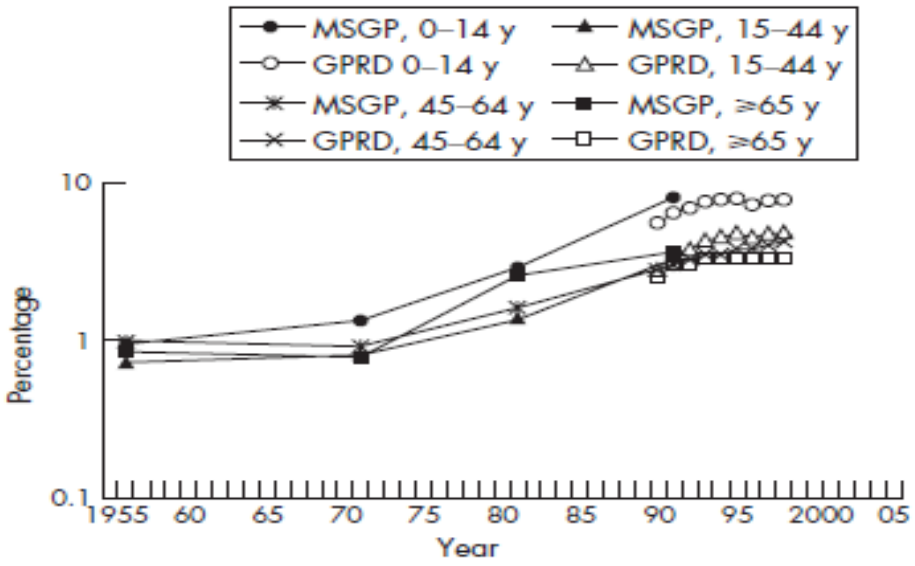


Figure 1.3 is also taken from Anderson et al[31] and shows that there has been an increase in the rate of patients consulting their general practitioners for asthma over the past 50 years in the UK, although this does appear to be levelling off in the 21st century.

Figure 1.3 Patients consulting general practitioners for asthma per 10 000 population, England and Wales 1955-1998. Data from the General Practice Database (GPRD) and Morbidity Statistics in General Practice (MSGP)[31]



1.4a The prevalence of asthma in Wales

The ECRHS reported in 1996 that the highest adult worldwide prevalence of wheeze in the preceding year (used as a proxy for current asthma) was in Wales[12] where there are currently 10% asthma sufferers compared to 9% in the rest of the UK[3]. The phase III ISAAC study showed that the prevalence of asthma symptoms in children in Wales was still among the highest in the world[18]. Studies by Burr in schoolchildren aged 11 years in South Wales showed that the percentage reporting they had ever suffered from asthma

increased from 5% in 1973 to 12% in 1988 and 27% in 2003[32]. Findings of the Welsh Health Survey (2009) state that there were 4,000 hospital admissions for asthma (1.3 per 1000 population per year) a rate which is nearly 30% higher than the rest of the UK[31]. The evidence suggests that the worldwide burden of asthma continues to rise, although in the West there may be a plateauing of this rise. However, Wales may be an exception and there still may be a rising prevalence here.

1.5 Atopy and atopic diseases

The term "atopy" has been applied to allergic conditions that occur in families (hay fever, asthma, and atopic dermatitis). Since immunoglobulin E was first discovered in the 1960s[33], the understanding of its importance in allergic disease has grown and now we describe atopy as *'a genetic propensity to develop immunoglobulin E antibodies in response to exposure to allergens'*[34], This is known as sensitisation[35], which is usually defined on the basis of a positive skin prick test[36, 37] or a raised level of specific IgE[38, 39] to a perennial allergen such as dust mites, pet dander, pollen or mould. A portion of the allergen called the antigen combines with specific IgE on mast cells to cause the release of histamine. Atopy is known to be associated with eczema[40], asthma[41] and hay fever[42]. When investigating the rising prevalence of asthma we must consider atopy since it too is rising in prevalence[43] and there is much evidence that atopy is associated with asthma. However, sensitisation to an allergen does not necessarily mean disease, since up to 40% of children[44] and adults[45] who are sensitised may have no evidence of clinically relevant symptoms. There is evidence that atopy defined as either a positive skin prick

test or raised specific IgE, is associated with asthma in children[46], airway hyper responsiveness in children[47] and asthma in adults[38, 48]. Asthma is often found in association with atopy and there is evidence that an association with atopy can increase the likelihood that asthma becomes chronic[49].

1.6 Theory to explain the increased prevalence of asthma and other atopic diseases -The Hygiene Hypothesis

In 1989 Strachan reported that hay fever was less likely in 11 year olds if they had siblings, especially older ones[50]. He found that the prevalence of hay-fever at ages 11 and 23 years was inversely related to the number of children in the household at age 11, with older siblings having a greater influence on this relationship than younger siblings. His hypothesis was that early life infections, to which subjects with siblings were more likely to be exposed, formed some sort of protection against hay-fever and other allergic diseases. The biological basis for this, the hygiene hypothesis was founded on murine models and suggested the Th1/Th2 paradigm i.e. two mutually exclusive T helper cell phenotypes develop from a common ancestor cell and one type of cell (T-helper type 1, Th1) produces chemical mediators and fights infections while the other cell type (T-helper type 2, Th2) produces another set of chemical mediators and these induce B cell clones to produce IgE [51, 52].

Matricardi et al showed that compared to non-atopic Italian cadets (not sensitised on skin prick testing and specific IgE tests), atopic Italian cadets (sensitised) were significantly less likely to have antibodies to orofeacal and food borne infections (*Toxoplasma gondii, Helicobacter pylori and* Hepatitis

A virus)[53]. Shirakawa et al showed that among Japanese schoolchildren, there was a strong inverse association between delayed hypersensitivity to '*Mycobacterium tuberculosis*'and atopy (sensitised). Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels, and cytokine profiles biased toward Th1 type response[54]. These and other subsequent clinical studies have reinforced the hygiene hypothesis, making it a plausible explanation for why there has been an increase in asthma over the past few decades worldwide.

1.7 Other theories linked to the rise in prevalence of asthma

The rise in asthma prevalence has been linked to the change in diet over the past 30 years, mainly due to the 'fast food' culture in modern Western society. A Mediterranean diet in preschool children has been found to protect against wheeze[55]. A large ISAAC study undertaken in New Zealand showed that those children who ate hamburgers regularly were more likely to suffer with wheeze than those who never did[56]. In a recent international ISAAC study there was an increased risk of severe asthma in adolescents and children associated with the consumption of fast food ≥3 times per week[57]. Fast food has a higher salt content and is depleted in important trace elements and vitamins. High salt intake may be one factor that is increasing the prevalence of asthma and a reduction of salt intake for as little as two weeks significantly improved pulmonary function tests in subjects with exercise induced asthma[58]. A greater imbalance of antioxidants has also been found in asthmatic children compared to normal children[59]. Allen et al undertook a meta-analysis of 40 studies investigating antioxidants in diet and found that relatively low dietary

intakes of vitamins A and C were associated with increased odds of asthma and wheeze[60]. A diet high in Vitamin E which has antioxidant properties was protective against developing asthma in adult women[61]. These studies are consistent with the assertion that changes in diet may also be a factor in the rise in asthma prevalence.

Poor diet can also lead to obesity which has increased in the Western world over the past few decades. The speculation that the growth in the prevalence of obesity may be the cause for the growth in prevalence of asthma has been fuelled by the finding that prevalence of both have increased at a similar rate in the USA as shown in figure 1.4 taken from Sin et al[62].

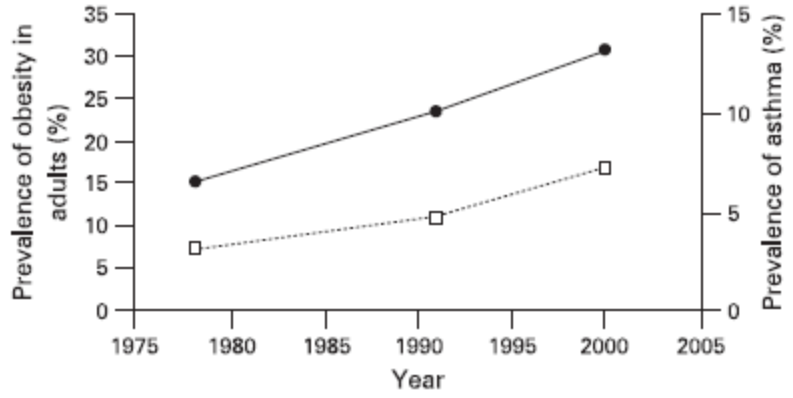


Figure 1.4 Temporal trends in the prevalence of obesity in adults and asthma in the USA. The dotted line represents the prevalence of asthma while the solid line represents the prevalence of obesity.

1.8a) Aetiological factors associated with asthma but not clearly linked to a rise in prevalence

Some longitudinal aetiological factors have not increased in prevalence in line with asthma but there is evidence that they may be associated with asthma inception. Those factors include pollution and viral infection and will be briefly discussed here.

It has been known for many decades that asthma exacerbations are more frequent when the concentration of outdoor pollutants such as nitrogen dioxide, sulphur dioxide and particulate matter in the air increases. One study from Seattle USA found that the risk of a visit to an emergency room for asthma was associated with mean PM_{10} levels (particulate matter up to 10μm) over the four days preceding the emergency visit[63]. There is a perception that pollution in cities is increasing although this is not true in the West. Since 'Clean air legislation' was passed in 1970 in the USA[64] and in 1956 in the UK, there have been lower levels of pollution, especially over the past 30 years[65]. Unfortunately in developing countries outdoor pollution continues to increase[66] but asthma prevalence has not yet been linked to this.

1.8b) Respiratory Syncytial Virus (RSV)

The largest body of evidence regarding viral infections and association with asthma inception exists for Respiratory Syncytial Virus (RSV). RSV is a common childhood respiratory infection in Europe. Many children suffer from it and premature babies are at increased risk of more severe RSV infections which require hospitalisation[67]. There has been no evidence of an increase in prevalence of RSV infection over the past 10-15 years and therefore this cannot explain the rising prevalence of asthma[67]. However, there is evidence of a link between RSV infection and asthma inception. One study from the Avon Longitudinal Study of Parents and Children (ALSPAC) showed there was a positive association between being admitted to hospital with bronchiolitis with a positive test for RSV before the age of 12 months, and having asthma at the age of 7 years[68]. A further longitudinal study from

the Tucson Children's Respiratory Study (TCRS) also showed that children who had an RSV-confirmed infection before the age of 3 years were at increased risk of frequent wheeze up to the age of 11, though the odds ratios decreased from 4.3 at age 6 to 2.4 at age 11; by age 13 the increased risk was small and not statistically significant[69].

A further study reported on RSV infection in 46 infants who were admitted to hospital with the illness and 92 controls followed up to the age of 18. They found there was an association of infant RSV and atopic asthma[70]. Evidence from these studies supports the hypothesis that RSV is associated with childhood asthma, but causation cannot be proven. RSV is the most common cause of hospital admission in the winter season during the first year of life, and severe RSV bronchiolitis has been shown to be associated with an increase in subsequent rates of early wheezing[71]. However, although these studies may show an association, the questions still remains: Is RSV infection the cause of asthma or does it just occur in lungs that later go on to develop asthma. In an attempt to answer this question Blanken et al undertook a randomised controlled study of Palivizumab in healthy preterm babies, who were at increased risk of RSV infection. Palivizumab is a monoclonal antibody which has already been shown to have efficacy in preventing severe RSV infection in high risk infants. At 1 year, treatment with Palivizumab was associated with a reduced risk of wheeze[72]. These one year results suggest that preventing RSV infection may reduce the risk of wheeze and therefore asthma, suggesting that RSV may be a cause of asthma. However follow up results are awaited.

1.9 Longitudinal studies and the aetiology of asthma

Longitudinal studies give the clearest evidence on aetiological factors in asthma, especially if data on risk factors are gathered prospectively. This is supported by one study that showed that recall bias can account for large differences between prospectively collected data and retrospectively collected data (for example 40% of parents who agreed that their child had doctor diagnosed asthma, denied this when asked retrospectively)[73]. A number of longitudinal studies have been established over the past 30-40 years to try to answer some basic questions related to the inception of asthma. Some of the longest running studies are the Dunedin study, Tucson Children Respiratory Study (TCRS), the German Multicentre Allergy Study (German MAS), Tasmanian Allergy Study (TAS), Avon Longitudinal Study of Parents and Children (ALSPAC). Table 1.1 gives a brief overview of each study, and others not mentioned here. Some have recruited at birth (or pre-birth)[74], some in childhood[75]. Some longitudinal studies are based on unselected population studies[76] while others are taken from high risk populations[77].

Table 1.1 shows that a number of early life factors are associated with asthma and allergy. There are some factors that are associated with increased risk in some cohorts but not in others. This disparity may be due to varied definitions of asthma, or it may be related to confounding factors associated with differing environmental conditions as yet unidentified, or due to the ways in which the risk factors were measured. Nevertheless prospective cohort studies have provided a useful tool for the investigation of the natural history of

asthma, and have identified a large number of credible associations between environmental variables and asthma onset in childhood. However as yet we have been unable to identify modifiable causal risk factors that are amenable to intervention for primary or secondary prevention of disease[78].

Table 1.1 Longitudinal studies regarding the inception of asthma

Study Name (Sample studied)	Country	Sample at recruitment	When subjects recruited	Age of subjects at entry	Important findings
TAS (general population)	Australia	8,583	1968 Born - 1961	7 years of age	Childhood eczema increases risk of adult asthma[79]
Dunedin Study (general population)	New Zealand	1037	1972-73	3 years of age	Remodelling, defined as loss of reversibility of obstruction, begins in childhood and continues into adulthood[80] Breast feeding associated with increased risk of asthma[75]
TCRS (HMO†=general population)	USA	1246	1980-84	Birth	Breast feeding no effect unless mother asthmatic, then increases risk[81] Day-care protects[82]
German MAS (general population with nested high risk)	Germany	1314	1990	Birth	House dust mite exposure not related to development of asthma[83] Eczema in infancy is associated with wheeze age 7 years[84] Repeated upper respiratory tract infections associated with a reduced risk of asthma[85]
ALSPAC	UK	11 534	1991-92	Birth	Maternal use of Paracetamol[86] increases risk Breast feeding[87] and mode of delivery[88] no effect on development of asthma
MAAS (unselected population)	UK	1085	1995	Birth	Latent class analysis of sensitisation phenotypes- multiple early sensitisations strong association with asthma[89].
PIAMA (unselected population)	Netherlands	4146‡	1996/7	Birth	Breast feeding protective[90]. Consumption of food containing milk fat reduces risk of asthma[91]

†Health Maintenance Organisation-only subjects with health insurance included

‡ Approximately 50% of the invited women (n=4146) agreed to participate and gave written informed consent. The mothers were allocated to the intervention arm (n=855) or to the ‘‘natural history’’ arm (n=3291) of the PIAMA study. Only allergic mothers were included in the intervention study, while in the natural history study allergic (n=472) and non-allergic (n=2819) mothers were included.

Abbreviations TAS= Tasmanian Allergy Study. TCRS= Tucson Children’s Respiratory Study, German MAS= German Multicentre Allergy Study, MAAS= Manchester Asthma and Allergy Study, ALSPAC= Avon Longitudinal Study of Parents and Children, PIAMA= the Prevention and Incidence of Asthma and Mite Allergy

1.10 Natural history of asthma and childhood wheeze phenotypes

Since airways are small in childhood, wheeze is more common in children and not every wheeze in childhood is the beginning of asthma. The TCRS reported that 30% of children wheeze before the age of 3 years, showing that early wheeze is common[92]. Later the ALSPAC study reported that over 70% of children who wheeze in the first 6 months of life do not wheeze 3 years later, suggesting that early wheeze may not be a prequel to childhood asthma[93]. It is important to classify these early patterns of wheezing, in order to understand the natural history of asthma. However, the question of how to categorize early wheeze remains controversial. In 1995 the TCRS published on the first 6 years follow up of subjects and their patterns of wheeze. They categorised subjects based on wheezing in the first 3 years of life, and at age 6 years, into

- those who did not wheeze at any time point up to age 6 years (No wheeze NW),
- those who wheezed up to age 3 but not in the year of 6th birthday (transient early wheeze TEW),
- those who wheezed in the year of 6th birthday but not up to age 3 (late onset wheeze LOW) and
- those who wheezed up to age 3 years and in the year of 6th birthday (persistent wheeze PW).

Since this publication, many longitudinal studies have used modified TCRS phenotypes to define their study group with respect to early wheeze as shown

in table 1.2. The rationale is usually similar to that of Tucson but modified to take account of ages at which data were collected in each study.

Not all longitudinal cohorts have used these four categories. One study categorised wheeze with infection as wheezy bronchitis[94], while ALSPAC defined 6 categories of childhood wheeze phenotype using latent class analysis[76].

Table 1.2 Early wheeze phenotypes categories used in named longitudinal studies

Study	No wheeze (NW)	Transient Early Wheeze (TEW)	Late onset wheeze (LOW)	Persistent Wheeze (PW)
BAMSE[95] seen age 2months 1 year, 2 years and 4 years	No wheeze age 2 months to 4 years	Three episodes of wheeze between 3 months and 2 years	No wheeze 2 months to 2 years and wheeze at age 4	Wheeze 2 months to 2 years and wheeze at age 4 years
German MAS[96]§	Never wheeze up to age 7	Wheeze at least once before 3 and not in past year age 7	No wheeze before 3 but wheeze in the past year age 7	Wheeze at least once before age 3 and in past year age 7
Jamtland Sweden[97]	No wheeze age 0-4 years	Wheeze 0-1 years only	Wheeze 2-4 only	Wheeze age 1 and age 4
MAAS[98] seen at age 3 years and 5 years	No wheeze up to 3 years and no wheeze in past year at age 5	Any wheeze up to age 3 years but none in past year at age 5	No wheeze up to age 3 years and wheeze in past year at age 5	Wheeze up to age 3 years and wheeze in past year age 5
SIDRIA[99] seen age 7 years	No wheeze between 0-7 years by parental recall	Wheeze 0-2 years by parental recall	Wheeze 2-7 years and not before by parental recall	Wheeze present at age 7 and parental recall for wheeze before age 2
TCRS[92] seen 0-3 then at age 6	0-6 years, no wheeze up to age 3 and no wheeze age 6	Any wheeze up to age 3 none at age 6	Wheeze only at age 6	Wheeze up to 3 and at age 6

Key to studies- TCRS- Tucson Children's Respiratory Study, SIDRIA (Italian Studies of Respiratory Disorders in Chidhood and the Environment), Jamtland-Swedish cohort, BAMSE-Swedish no translation, MAAS-Manchester Asthma and Allergy study

§ German MAS were reviewed at ages 1, 3, 6, 12 and 18 months, then yearly, within 3 months of their birthday, up to 7 years of age

Although there have been a number of longitudinal studies, a number of questions still remain regarding the natural history of asthma. It was therefore considered appropriate to investigate the natural history of a cohort from a

deprived area in the South Wales Valleys who could provide a relatively stable population, since movement of subjects is not as great as in a city. The incidence and prevalence of asthma are known to be higher in deprived areas and therefore the end point of asthma was considered to be more likely to occur in this study group. In addition they were high risk compared to subjects from the general population.

1.11 The Merthyr Allergy Prevention study (MAPS)

The Merthyr Allergy Prevention study (MAPS) aimed to investigate which environmental features, especially those in early life, predispose high risk individuals to develop asthma. In the 1980s there had been 3 randomised trials investigating the withholding of cows' milk and replacing it with soya-based feed in order to reduce the development of allergy in children. All three studies had methodological deficiencies; one was too small, another was invalidated by noncompliance and the third was not a double blind study in that the investigator was aware of which children had which diets[100]. Burr et al recruited pregnant women between 1982 and 1984, with a history of atopy in either themselves, their partner or older siblings of the unborn infant. They established a randomised controlled trial (RCT) to investigate if avoidance of cows' milk during infancy could be a useful strategy to avoid allergy and asthma in later life, in these high risk infants[100]. The main outcome measure in the first years of life was wheeze in the preceding year and doctor diagnosed asthma. The RCT turned into a cohort study over the years, with the last follow-up at age 23. The purpose of this thesis is to use data from this study to examine associations between wheeze, asthma and atopy in early

adulthood and a number of predictors in childhood. The hope is that finding such early life factors may lead to prevention rather than treatment of asthma.

1.12a) Hypothesis and aims

The hypothesis of this cohort study is that early life exposure is associated with asthma, wheeze and atopy in early adulthood.

The aim of this study was to investigate the association of wheeze and other outcomes at age 23 with inherited, immunological, early life environmental and host factors. Special interest is paid to the natural history of childhood wheeze and the development of wheeze and asthma in early adult years.

1.12b) Objectives of thesis

To describe the methods, and review the main findings from previous publications on this study with particular emphasis on those matters that are relevant to the follow up of subjects at age 23 years.

To describe the demographics of the population with a discussion on the representativeness of the follow up cohort at age 23 with respect to the original birth cohort. Associations between the outcomes of wheeze and asthma at age 23 years and family history and childhood and adult BMI will be investigated.

To investigate the relationship between atopy, defined using the results of skin prick tests, and outcomes at age 23. We will also investigate the utility of total IgE as a measure of atopy and its association with skin prick test results. Longitudinal relations between atopy and both wheeze and asthma will be estimated.

To analyse outcomes at age 23 years in relation to the randomisation groups in the original randomised controlled trial. We will investigate the association of neonatal diet, including breast feeding, with adult outcomes.

To investigate the associations between early environmental factors and outcomes at age 23 years.

To investigate the natural history of wheeze and explore the use of different phenotypes to classify children.

To summarise the findings and reflect on how these findings contribute to our understanding of how asthma develops in children and young adults..

Chapter Two

Description of original cohort and methods

2.0 Original study design

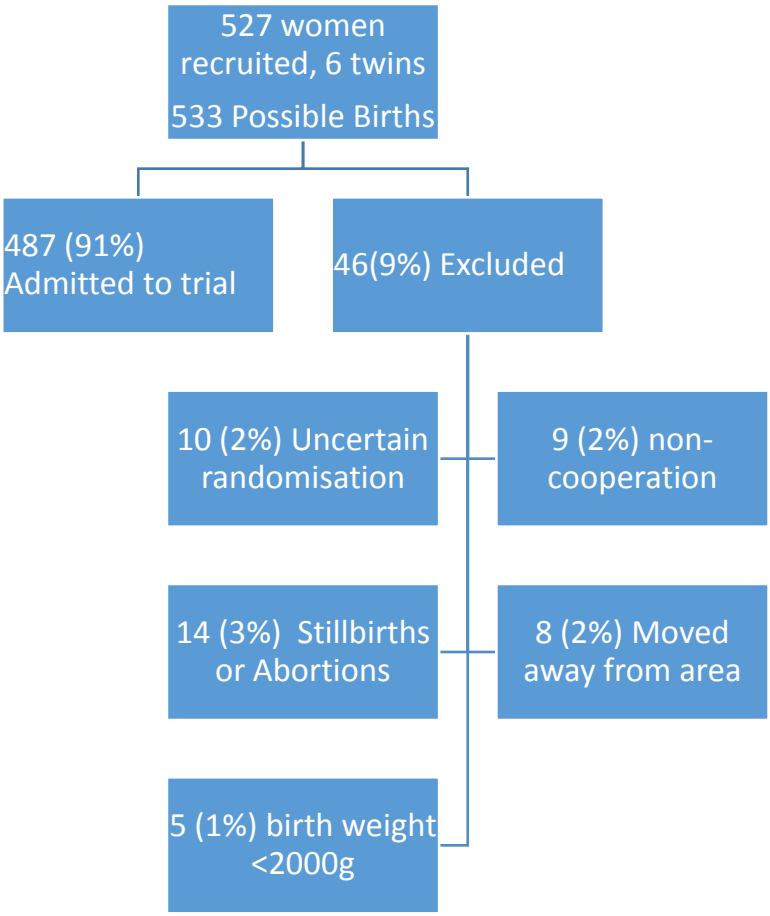
The Merthyr allergy prevention study (MAPS) was designed as a randomised controlled trial (RCT) to test the hypothesis that infants who do not receive cow's milk during the first four months of life are less likely to develop allergic diseases in early childhood than those who receive a normal diet. Participants were recruited from a socially deprived community in the South Wales valleys because there was evidence that asthma prevalence[101, 102] and severity[103] were directly associated with poorer economic circumstances and therefore it was thought that asthma incidence and prevalence would be greater in this population. The subjects were infants born within a defined geographical area (the catchment area of Prince Charles Hospital, Merthyr Tydfil) and they had to have a first-degree relative with a history of eczema, asthma, or hay fever. Their mothers were identified between 1982-1984 at their first antenatal clinic appointment at Prince Charles Hospital, Merthyr or Aberdare General Hospital, Aberdare.

Written informed consent was obtained from pregnant women before entering into the study. Those who gave consent had their new-borns randomised to the intervention group or the control group. The intervention group had a cows' milk exclusion diet with soya milk substitute. Randomisation was undertaken by a computer-generated list that was inserted into numbered envelopes and each envelope was opened in turn on recruitment of a pregnant woman. Pregnant women in the intervention group were asked to restrict their

own cows' milk intake to half a pint (284 ml) daily during the remainder of the pregnancy and while they breast fed. They were also asked not to give cow's milk, or any food made from cows' milk, to the child for at least the first four months of the child's life. A soya preparation, as an alternative to cows' milk or cows' milk based formula, was supplied for mothers in the intervention arm who did not wish to breast feed or who wanted to supplement breast feeding. Those in the control group who did not wish to breast feed were given the cow's milk formulation normally supplied while in hospital, after which they purchased their preferred formulation.

A total of 527 pregnant women consented to participate in the study. As there were 3 sets of twins, there were 533 possible births. Of these 46 (9%) did not participate for the following reasons: eight were born outside the study area; five had a birth-weight less than 2kg (a stated exclusion criterion); 9 pregnant women were unwilling to cooperate in the study despite giving consent; 14 babies were either still born or aborted; and errors occurred in the randomisation of 10 babies and consequently they were excluded[100]. A total of 487 children, 238 (119 male) in the intervention group and 249 (137 male) in the control group entered the RCT. The recruitment process is summarised in figure 2.1.

Figure 2.1 Subjects entered into study and those excluded.



2.1a) Follow up age 0-1 year

On recruitment of a pregnant woman, baseline information was gathered including the woman's smoking habits during the pregnancy, the type of housing the family occupied and features of the housing, such as visible mould on walls and damp. Information on mode of delivery of the infant, was taken from the hospital notes. The data collection forms used at the initial interview and before birth are shown in Appendix 2a, 2b and 2c. These questionnaires were based on validated ISAAC questionnaires as were all the questionnaires in this study. Evidence of validation of the ISAAC questions used to define

asthma compared to a respiratory physician diagnosis of asthma was given in 1996 by Jenkins and Clarke[15]. In this way content, construct and predictive validity have been addressed with regards to the appropriate questions for questionnaires defining asthma in epidemiological studies.

During the first 6 months of life, a dietitian visited families weekly at home and examined diaries in which details of the infants' diets were recorded by the mother. A doctor, who was blind to the randomisation, examined the infants at 3 months of age (see appendix 3a/b), 6 months of age (see appendix 4a/b) and 1 year (see appendix 5a/b). Blood was taken for immunoglobulins at birth from the umbilical cord (cord blood) and at 3 months. Skin prick testing was undertaken at age 6 months and 1 year to validated standards that were developed in the 1980s-1990 by the ISAAC team of which Dr Burr was a member. The full methods including validation for included centres is given in the phase II module handbook published in 1998. The relevant pages are pages 37-45 and the manual can be found at http://isaac.auckland.ac.nz/phases/phasetwo/phasetwomodules.pdf. The relevant section is copied and given in appendix 11.

2.1b) Follow up of the cohort age 7 years

The subjects were followed up as they grew older, creating a cohort. Each year from age 2 to 6 years, around their birthday, a short questionnaire was sent to the mother asking whether the child had wheezed or been treated by a doctor for eczema during the preceding year (appendix 6).

Parents of subjects were invited to bring their child to a research clinic at age 7. At this clinic subjects were reviewed by a consultant paediatrician who was unaware of the children's allocation in the randomised trial. A structured history was taken and a clinical examination conducted; details of the data recorded are shown in appendices 7a/b/c/d. Blood was taken for measurement of total IgE and an assay comprising specific IgE to 12 aero-allergens (AlaTOP). Skin prick tests were performed for common allergens. An exercise challenge test was conducted by measuring peak expiratory flow rate (PEFR) five times at the beginning of the study, then asking the child to run for 6 minutes. After 5 minutes of rest the PEFR was measured again. Further information was obtained about housing conditions.

2.1c) Follow up of the cohort age 15 years

At age 15, subjects were sent a detailed postal questionnaire on current symptoms and treatment, severity of wheezing and active and passive smoking (appendix 8a/b), but subjects were not clinically assessed. A total of 363 subjects completed and returned the questionnaire.

Table 2.1 Summary of investigations undertaken at different ages.

Age	(N -% of total)	Questionnaire	Diet	Examination	Total I gE	Skin prick test
Antenatal		Baseline information, Appendix 2a			Mother	
At birth	487-100%	Birth and perinatal period Appendix 2b, 2c	Weekly visits from dietitian. Diet Diary		Cord blood	
3 months	482-99%	Appendix 3a, b	Weekly visits from dietitian. Diet Diary	By paediatrician	Yes	Yes
6 months	476-98%	Appendix 4a, b	Weekly visits from dietitian. Diet Diary	By paediatrician		Yes
1 year	475 - 98%	Appendix 5a, b		By paediatrician	Yes but too few samples to analyse	Yes
2 years-6 years	468-96%	Birthday card and short questionnaire Appendix 6				
7 years	(453 - 93%)	Full questionnaire and results of examination. Appendix 7a, b, c, d		By paediatrician Exercise test undertaken	Yes	Yes
15 years	(363 - 75%)	Postal questionnaire only Appendix 8a, b				

2.2 Findings at one year

Evidence of compliance with the randomised intervention was provided by the mothers' weekly diet diaries and confirmed by serum IgG4 antibodies at 3 months: cows' milk antibodies were detected in 37% of the intervention group and 70% of the controls at 3 months[100]. This suggested that at least 37% of the intervention group had had some exposure to cows' milk at age 3 months. There was an association between the randomisation group and the prevalence of breast feeding with 32.8% ever breast fed (breast fed for even one week) in

the intervention group and 41.8% ever breast fed in the control group. During the first year of life, 34% of the infants wheezed at some time. There was no association between wheeze in the first year and randomisation to the intervention group or the control group. Napkin rash, diarrhoea and oral thrush were more common in the intervention group, especially during the first three months. The incidence of wheeze during the first year of life in subjects who were breast-fed for any length of time was about half that in those who were never breast fed (22.0% and 41.9% respectively –see chapter 5). It was concluded that withholding cow's milk conferred no clear benefit during infancy. Breast feeding, however was associated with a significant reduction in wheeze at age 1[104]. The incidence of wheeze was higher in boys than in girls, and in babies born during the autumn; it was also higher with a greater number of siblings[105]. Levels of der p 1 in the samples of dust from the child's mattress, the mother's mattress, and the carpet on which the child was most frequently placed were not associated with wheeze at age 1[105].

2.3 Findings at age 7 years

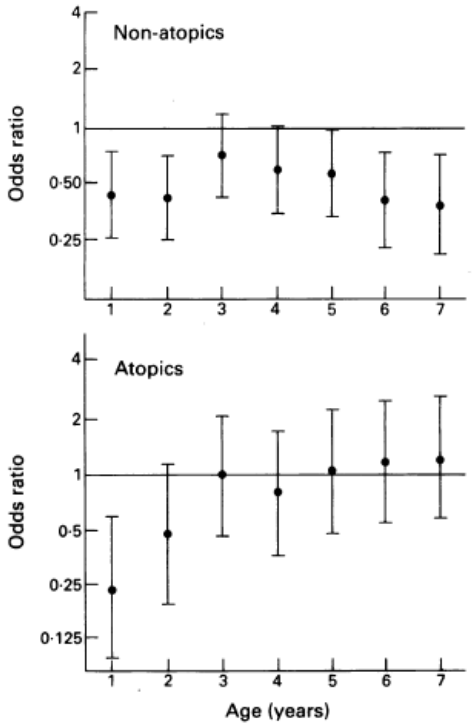
When the subjects were 7 years old, 30% had wheezed during the preceding year, and 32% were considered by the paediatrician to have had asthma at some time over the past 7 years. In 25% of the children at least one skin prick test was positive; and about half of the atopic subjects had wheezed in the preceding year, suggesting a strong association between wheeze and atopy. At

age 7 years there was no association between wheeze and the original randomisation.

Mean PEFR adjusted for height[106] was significantly lower in subjects with wheeze compared to those with no wheeze in the preceding year. The PEFR after exercise as a percentage of the initial value was lower in those who were atopic with wheeze compared to those who were not atopic, whether they had a wheeze or not. Pet ownership in infancy was negatively associated with atopy at age 7. Wheeze in the non-atopic subjects was associated with passive smoking (especially when the mother was a smoker). In addition being from an employed family compared to being from an unemployed family reduced the risk of wheeze in non-atopic subjects: OR 0.38 (95% Confidence Interval 0.21 to 0.70)[107].

Subjects who had ever been breast fed had a lower risk of wheeze at age 7 but only if they were not atopic. Figure 2.2 shows graphs of odds ratios of the association of breast feeding and wheeze at ages 1-7 years. Atopic and non-atopic subjects are plotted separately (taken from Burr et al[107]). There is no clear association between breast feeding and wheeze in subjects who are atopic, but in the non-atopic group there is a clear association with an OR less than 1 at age 6 and 7 years, suggesting protection against wheeze at these ages.

Figure 2.2 Association between wheeze and breastfeeding, by age and atopic status (odds ratios, with 95% CIs, for the)



2.4 Findings at age 15 years

At 15 years of age wheeze was present in 31% of those who responded to the postal questionnaire. There was no association between wheeze and the original randomisation. A third of those with wheeze had severe wheeze, defined by ISAAC criteria: i.e. the symptoms were sufficiently severe to restrict speech to one or two words on occasion or to disturb sleep at least once a week. Half the subjects in the severe wheeze subgroup were receiving inadequate treatment (no inhaled corticosteroids during the past 12 months) or no treatment at all. Many wheezy children, including some with severe symptoms, were unaware that they had ever suffered from asthma. About a quarter of those taking inhaled corticosteroids used them only when they were wheezy rather than at regular intervals. In this cohort it was common for

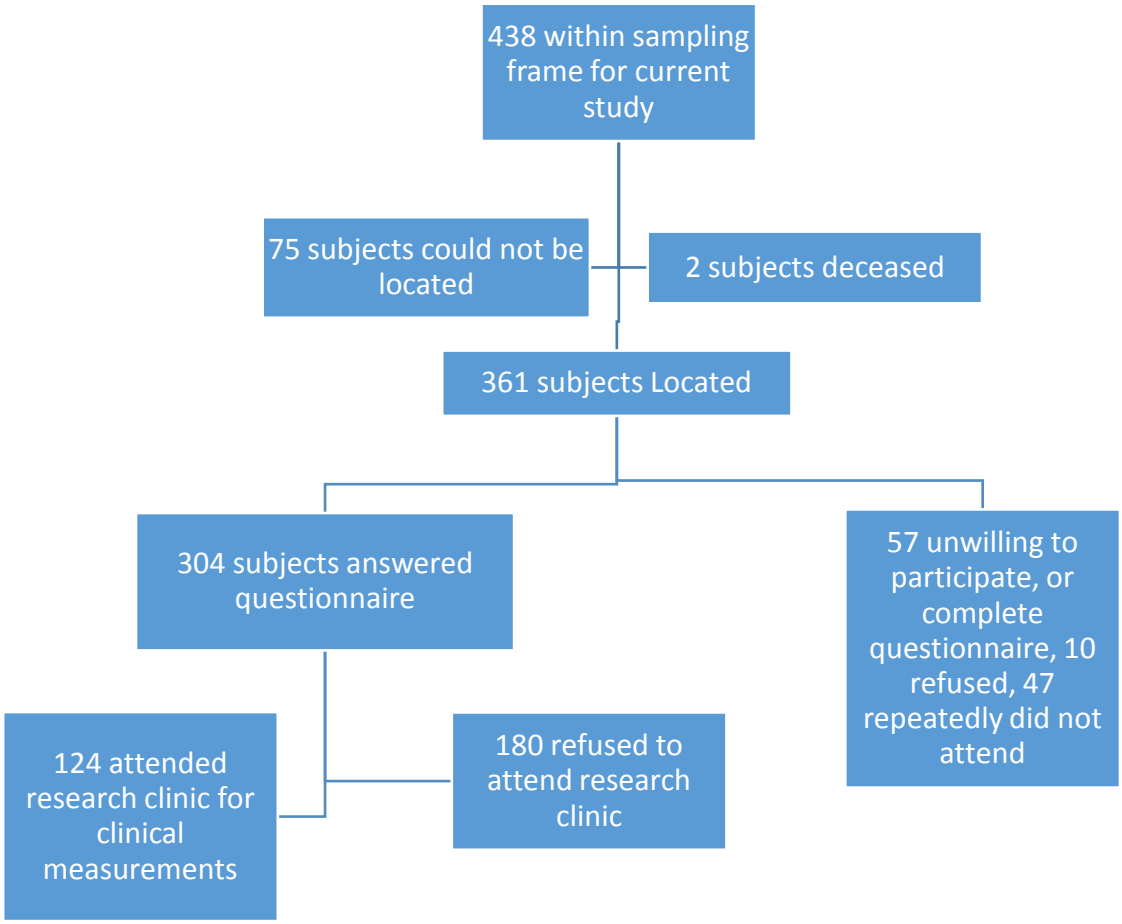
asthma to be undiagnosed in adolescence and treated inadequately or not at all[108].

2.5a) Current study-collection of data at age 23 years

The final phase of follow up was undertaken when the subjects were approximately 23 years of age. This final phase forms the basis of the thesis as outlined below. It was supported by a grant from the Welsh Office of Research and Development in 2006 (now renamed as National Institute for Social Care and Health Research (NISCHR)).

The questionnaire used at age 23 was based on questions from previous ISAAC studies[109] (See appendix 9). Subjects were excluded if they had been unwilling to participate at earlier stages of the study, if they were known to have moved out of the area or were deceased. A total of 438 subjects were eligible to be included in this stage of the study. Figure 2.3 shows the number who entered this follow up phase of the study and the numbers who were excluded, including two further subjects who were later found to be deceased.

Figure 2.3 Flow diagram of subject recruitment at age 23 years



2.5b) Recruitment at age 23 years

Each subject was sent a letter inviting him/her to participate in the follow up study. Those who did not reply were sent a further letter. If they again did not reply, they were contacted by telephone and finally they were approached at home. In this way we were able to ascertain if individuals were still living at their recorded address. Those not living at the recorded address were searched for on the Electoral Roll. We also asked Health Solutions Wales (a provider of information, information technology, telecommunications and consultancy services to the NHS) to help us find addresses using the NHS administrative

register, and finally, we searched death certificate records for Aberdare and Merthyr Tydfil. After four unsuccessful attempts at contact, subjects were excluded from the study population.

Participants were invited to attend a research clinic at Prince Charles Hospital, Merthyr. They were reviewed by a technician who was blinded to the randomisation. If the subjects did not attend at the scheduled time they were contacted and offered a further appointment. Occasionally participants made as many as 5 appointments that they did not attend. Subjects who were contactable and who did not wish to attend the clinic were asked if they would complete the questionnaire by post or over the phone (appendix 9a-e).

2.6 Definitions

Definitions are based on earlier ISAAC studies and are therefore validated, and can be found in the ISAAC phase one manual[110].

2.6a) Atopy

2.6a) Atopy

Assessment of atopy and allergy was made by skin prick testing with commercially prepared antigen extracts (Diagenics U.K.). Atopy was defined as having one or more positive skin prick tests to a set of perennial allergens (house dust mite, grass, tree, moulds, cat, dog, peanut, milk and egg); a positive control (histamine) and a negative control (saline) were also included. The technique used involved puncturing the skin with a calibrated lancet (1 mm) held vertically through a drop of diluted allergen[111]. Weal size was

measured 15 minutes after pricking the subcutaneous layer of skin at 45 degrees through a drop of antigen extract using an ALK lancet. The test was regarded as positive if the skin prick test to the given allergen formed a weal at least 3mm greater than the control (no allergen, just base mixture that carries allergen). Although there is diurnal variation in skin prick reactivity we were unable to keep all skin prick test appointments to the morning and some skin prick testing was undertaken in the afternoon.

2.6b) Wheeze

Wheeze was defined as answering yes to the question "Have you had wheezing or whistling in the chest in the past 12 months?" Subjects were informed that a wheeze was a high pitched sound heard from the chest.

2.6c) Severe Wheeze

Severe wheeze is the symptom considered to signify severe asthma in epidemiological studies. In this study and in line with previous ISAAC studies[18], severe wheeze was defined as wheeze that limits speech or disturbs sleep at least once a week, i.e. answering yes to either of the questions: 'Do you have wheeze that limits speech' or 'Do you have wheeze that disturbs your sleep at least once a week'.

2.6di) Asthma

Asthma at age 23 years was defined as a previous history of asthma, as reported by the subject and answering yes to the question 'Have you had a wheeze in the past year'. These asthma defining questions were based on the core questions developed by ISAAC as shown in appendix 10. The ISAAC phase II manual addressed content, construct and predictive validity of the core questions and Dr Burr in the writing of the questionnaire for the final phase of this study used the ISACC questions on wheeze and severe wheeze (see appendix 9 and 10)

2.6dii) Atopic asthma

It has not been possible to show that asthma is associated with sensitivity to specific allergens, but for the purpose of this thesis we have defined asthma in subjects who are concurrently atopic, as atopic asthma.

2.6e) Social Class

Social class was recorded when the subject was age 1 and age 7 using the Registrar General's classification of occupations (see Appendix 1). It was based on the occupation of the father if he was employed and a member of the household. Otherwise it was based on the occupation of the mother. Those in social class I, II or III non-manual were grouped together as non-manual workers, while those in social classes III manual, IV, V and unemployed were grouped together as manual workers. At age 23 years information on deprivation was based on homeownership compared to rented accommodation

or council housing. In addition information on unemployment was available and used as a proxy for social class.

2.7 Outcome measures

The principal outcome measures for this study were the presence or not of wheeze and of asthma as defined above. Other outcomes were based on physiological measurements at age 23 which included skin prick test results and total IgE.

Lung function was assessed by spirometry. The machine used to undertake the measurement was a Vitalograph model alpha made by Vitalograph, Buckingham, UK. Forced expiratory volume in 1 second (FEV_1) and Forced Vital Capacity (FVC) were both recorded pre and 15 minutes post bronchodilator administration (2 puffs of Salbutamol 100ug via a large volume spacer) as absolute measures and as percentages of change from the baseline value[112]. Reversibility was calculated as the percentage change in FEV_1 between pre- and post-bronchodilator. Participants with an FEV_1 of $\leq$ 80% of predicted[106] with at least 15% reversibility were considered to display features of asthma. Those with an FEV_1 of $\leq$ 80% with limited or no reversibility were considered to display features of chronic obstructive airways disease (COAD). Any individual whose FEV_1 was below 80% predicted or who had significant symptoms was advised to see their GP regarding probable asthma.

2.8 Statistical methods

The majority of the analyses in this work were performed by a small set of standard methods described here; more specialised ones are described in the relevant chapters. The majority of the outcomes are binary and were modelled using logistic regression, with effect sizes summarised by odds ratios, and 95% confidence intervals used to quantify uncertainty. Analyses were adjusted for confounders. It is never simple to choose the set of confounders but those used here generally included the original randomisation, gender, social class, parental allergy and breast feeding history. These were based on evidence in the literature on confounders. Exceptions were required on occasions; for example analyses of breast feeding were not adjusted for breast feeding. The adjustments made are referred to in the results sections of each chapter.

Continuous variables were modelled using linear regression and were compared between two groups using a t-test when the variables were approximately normally distributed, or the Mann-Whitney test when the assumption of normality was not reasonable. Associations were summarised by correlation coefficients. Pearson's correlation coefficient was used where the distribution was normal; otherwise Spearman's coefficient was used. Associations between categorical variables were investigated using the chi-squared test. Time to event data were summarised by Kaplan-Meier estimates of survival and hazard functions and were modelled using Cox-s proportional hazards model. Analyses were carried out using SPSS version 18; the significance level in hypothesis testing was taken as 5%. Representativeness of the population under study will be discussed further in chapter 3.

Chapter 3

Response rate and effect of social class, family history and obesity on asthma, wheeze and atopy, at age 23 years

3.0 Early life events and outcomes in early adulthood

Before examining early life events and associations with specific outcomes, we will investigate the participation rate at age 23 years, and how this may impact on the results of any associations found in this thesis. The prevalence of wheeze, rhinitis and eczema at age 23 years will be examined as well as socio-economic status, familial disposition and raised BMI with reference to wheeze, asthma and atopy at age 23 years.

3.1 Response rate in longitudinal studies

All cohort studies involve a loss to follow-up and this may lead to bias. Cochrane argued that high follow up rates are essential to reduce the risk of bias[113] although these are rarely achievable in a birth cohort study extending to early adulthood[114]. For example, the British 1958 Birth Cohort (National Child Development Study) is a longitudinal study of all people in England, Scotland, and Wales born in one week in March 1958. Of the 18,559 subjects who entered the study, data up to age 16 years was provided by only 29% of the original population[115]. The German MAS study recruited 1314 infants at birth; data up to age 13 years was available for 58% of the original population[49]. A birth cohort of 1,037 born in Dunedin in New Zealand and followed up at regular intervals, had data available up to age 26 for 59% of those who originally entered the study[116] . The TCRS enrolled 1,246 subjects

at birth and there was follow up data at age 22 years for 68% of subjects[117]. Thus it is unrealistic to expect a follow up rate to adulthood much greater than two thirds.

Loss to follow up without differentiation may reduce the power of a study, but differential loss to follow-up can introduce bias and reduce generalisability of the study[118]. Follow up rates are often lower in those who are most troubled by illness[119, 120] and in those of lower socio-economic status[121]. Follow-up at age 6 in the longitudinal birth cohort German MAS study varied with socio-economic status (SES) of the family: low, middle and high SES participation rates, of the original cohort, were 62%, 77% and 80% respectively[122]. In the TCRS those children who had full data collected by age 6 years were more likely to have better educated, non-smoking, non-ethnic, white parents compared to the children with incomplete information[92]. Differential follow-up rates may lead to bias in estimates of prevalence unless estimates are adjusted to take account of this. Estimates of relationships between risk factors and outcomes are less likely to be biased, since for bias to occur the relationship between measured variables would have to be different in the responders than in the non-responders, which is unlikely but could occur. Rothman has argued that lack of representativeness can be an advantage since it is better to *'control skilfully for confounding variables'* rather than to attempt to find the most representative sample and thereby be blinded to any bias that this sample may produce[123].

3.2a) Socio-economic status (SES) and asthma and wheeze

Since the publication of the Black report by the British Government in 1980[124], it has been known that poor SES is associated with a higher risk of ill health. Having a manual occupation, no educational qualifications and living in a deprived area all independently predict lower lung function, even after controlling for smoking habit[125]. In contrast eczema[126], rhinitis[127] and atopy[128] are all more prevalent among subjects from higher social classes. However, asthma is one disease that can be associated with the above three but, itself, has not been shown to be consistently associated with higher social class. Strachan et al found no association of social class with prevalence of wheeze, which was used as a proxy for asthma, among 5-17 year olds in a UK cohort[129], while the European Community Respiratory Health Survey (ECRHS) found that asthma prevalence was higher in lower socio-economic groups, whether defined by educational level or social class, regardless of atopic status[102]. Court et al also reported that, in a large cross-sectional study of 18 434 households, asthma was more common in those of lower social class, determined by the occupation of the head of the household[38]. ALSPAC found that both parents and children who lived in relatively deprived housing (council housing or rented accommodation) were more likely to wheeze than subjects from relatively affluent housing (owner occupier or mortgaged)[130]. Tariq et al, in the Isle of Wight study also showed that asthma is more prevalent in 4 year old children from lower socio-economic groups[101].

3.2b) SES and severe asthma and wheeze

Recent guidelines on the management of severe asthma care have defined severe asthma as asthma that requires high doses of medication to control it[131]. This is not a definition that has been used in epidemiological studies, which tend to use *symptoms*, rather than treatment, to define severe asthma. A recent ISAAC international study of children defines severe symptoms of asthma as severe wheeze which is more than four attacks of wheeze in the last 12 months; or more than 1 night per week of sleep disturbance from wheeze; or wheeze affecting speech in the past 12 months. Severe wheeze was, relatively, more prevalent in less affluent countries, although wheeze as a whole was more prevalent in affluent countries[18]. In the UK-based study considered above, although wheeze increases with higher social class severe asthma symptoms occurred more frequently in those from a lower socio-economic group[129].

3.2c) Temporal changes in the association of SES with the prevalence of asthma

Some evidence that the role of social class may have changed over time is provided by Braback et al who investigated Swedish males born between 1952 and 1977, identified at age 17 years by the Swedish Military Service Conscription Register. This was a repeated cross-sectional study, investigating each birth cohort separately at age 17 years. They found that the prevalence of asthma at age 17 years increased continuously with year of birth during the entire study period, and that subjects of low SES showed the steepest increase in prevalence of asthma compared to subjects from high SES. This increase became progressively greater over the years from earlier birth cohorts to later

ones[132]. A similar chronological change of the association between asthma and social class was shown by Rona et al in a UK wide survey of 5-11 year olds. In 1977 the survey showed no significant association between prevalence of asthma and paternal SES: In the 1993/94 survey children whose fathers had a semi or unskilled manual occupation had a higher prevalence of asthma (16%) than children whose fathers belonged to other social classes (13%)[133]. The German MAS found that, although parental prevalence of asthma was higher in high SES groups, children of high SES parents were no more likely to have recurrent wheeze (used as proxy for asthma) up to the age of 6 than their contemporaries. This finding suggested that high SES was a risk factor for asthma in one generation but not in their children[122]. These studies suggest that the increase in asthma prevalence may be associated with socio economic differences, but that these differences may change over time. This is no surprise as differences in socio-economic status have also changed over the decades. The home environment for lower SES children today may resemble the home environment of higher SES children of 40 years ago more closely than it does the home environment of lower SES children of 40 years ago. For example not every household had a television in the 1960s but this is considered standard now, and a recent study showed that the watching of TV for 5 or more hours compared to less than 1 hour in a 24 hour period was associated with an increased risk of current wheeze in 6-7 year olds and in 13-14 year olds[134].

3.2d) Deprivation in Merthyr Tydfil and surrounding area

In 1980 in England 28% of the population were in social class I or II and the rate increased to 30% by 1984[135]. By contrast, in the cohort under study only 13.5% of subjects' families were in social class I or II in 1983-5 when they were 1 year old and 14.4% when subjects were age 7 (1990-92). At a time when unemployment in the UK was reported in the media as reaching 'an historical high' of 12% in February 1984[136], this cohort had an unemployment rate of 18%. This suggests a high level of deprivation in the cohort under study.

3.3 Familial Disposition

Familial tendency is known to predispose to asthma. However locating the 'asthma gene' has been difficult[137]. A detailed review of genetic associations is beyond the scope of this work, but is given by Vercelli [138] and Kumar[139].

It has been understood for many decades that atopic disease runs in families. Burke et al reviewed more than 30 worldwide, population-based studies that evaluated a family history of asthma in children and found that although a positive family history predicts an increased risk of asthma, it identifies a minority of children at risk, since a large percentage of asthma sufferers have no familial predisposition[140]. Sarafino et al investigated parent and child connections for the atopic illnesses of asthma, eczema, food allergies, and hay fever, in a sample of 325 families[141]. They found that children were more likely to have at least one atopic illness if both parents were atopic than if neither parent was atopic. In addition the number of atopic illnesses in the

children was associated with the number of atopic illnesses in the parents. These findings may support the evidence for genetic factors in the development of atopic illnesses since they suggest that the atopic disease of each parent may contribute to an aggregate risk for their children; however it is not possible to rule out environmental factors, since parents and child will share their environment.

Heritability can be defined as the proportion of observed variation in a particular trait that can be attributed to inherited genetic factors in contrast to environmental ones and can be investigated using twin studies. One study of twin pairs and self-reported asthma, wheezing, and hay fever suggested 60% heritability[142]. If genes influence a particular trait, then because of the greater genetic similarity, monozygotic twins, sharing 100% of genes, should resemble each other to a greater extent than dizygotic twins, who share on average 50% of their genes[143]. Los et al reviewed a number of European twin studies over a thirty year period from 1971 and found the heritability of asthma ranged between 0.36 (monozygotic correlation 0.43; dizygotic correlation 0.25) to 0.79 (monozygotic correlation 0.76; dizygotic correlation 0.45)[143]. Even among monozygotic twins there is incomplete heritability of asthma, which suggests that environmental factors have some influence on the development of asthma.

One American high risk birth cohort study (at least one parent had asthma, rhinitis or eczema) with follow up until a mean age of 3.5 years, showed the odds of having a child with asthma were three times greater in families with

one asthmatic parent and six times greater in families with two asthmatic parents than in families where only one parent had inhalant sensitivity without asthma. Maternal asthma was found to be strongly linked to asthma in the children while paternal asthma was only weakly linked[144]. A further American childhood allergy study reported on a birth cohort recruited between 1987 and 1989 and followed to age 6-7 years. The mother was recruited during antenatal care before the birth of the subject and was asked about her own and the father's history of childhood asthma, adult asthma and persistent asthma (both childhood and adult). Asthma status in the father, whether it was childhood only, adulthood only, or persistent, was associated with current asthma in the children (OR ranged between 4.39-7.36)[145]. By contrast only persistent maternal asthma was associated with current asthma in children at 6-7 years old (OR 2.96). This suggests a stronger relationship with paternal asthma, but since the history of paternal disease was taken from the mother, it is possible that only the more noteworthy and therefore more severe disease was reported by the mother[145]. The Tasmanian Allergy Study (TAS) was a longitudinal study that recruited at the age of 7 and continued follow up to adulthood (age 29 to 32). Both maternal and paternal asthma were associated with asthma in subjects: maternal asthma - OR 1.90; 95% confidence interval (CI) 1.28 - 2 82, paternal asthma- OR 1.61; 95% CI 1.08 - 2.40 [146]. A large German study that investigated children of age 9-11 years found that for paternal asthma the OR was 4.4 (95% CI 2.5 - 7.8), while for maternal asthma the OR was 1.5 (95% CI 0.7 - 2.7)[147]. In summary these studies on familial attributes show that a family history of asthma is associated with the development of asthma. Despite the differences in methodology between

studies, most showed that having a first degree relative with asthma gave the index subject an odds ratio for asthma of between about 1.5 and 4, but there was variation in whether the maternal or paternal history had greater effect. The increase in asthma cases over the past 4 decades cannot be explained by genetics alone and there is likely to be a complex interaction between environmental and genetic factors[148].

3.4a) Weight, BMI and asthma

Over 30 years ago Somerville et al first showed that obesity in children is associated with respiratory symptoms[149]. A number of worldwide cross-sectional studies were reviewed by Ford et al and they found that subjects with asthma were disproportionally obese compared to subjects without asthma[150]. The National Health and Nutrition Examination Survey (NHANES) is a programme of studies designed to assess the health and nutritional status of adults and children in the United States. It combines interviews and physical examinations and began in the early 1960s. It has been conducted as a series of surveys focusing on different population groups or health topics, including NHANES II (1976-80) and NHANES III (1988-1994). In 1999 the survey became a continuous programme. The survey examines a nationally representative sample of about 5,000 persons each year. This study found that children up to the age of 16, who were above the 85th percentile for weight, had a significantly higher risk of asthma than those below the 85th percentile for weight[151]. A large cross-sectional ISAAC study of a total of 76,164 children aged 6–7 years (from 29 centres and 17 countries) and 201,370

adolescents aged 13–14 years (from 73 centres and 35 countries) showed there was an association between current wheeze and being overweight or obese (based on Cole's definitions[152]) in both boys and girls of this age group[134].

Adults with high BMI were found to have a positive association with BHR (used as proxy for asthma) in an ECRHS multi-centre, multinational study and there was a stronger association in males than females[153]. Shaheen et al, in a longitudinal study of 8960 individuals from the 1970 British Cohort Study (BCS70), found that the prevalence of asthma increased with increasing BMI in adults and that the association between being overweight or obese and asthma was stronger in females[154]. A total of 731 (406 male) children were followed from birth and were reviewed at age 3, 5 and 8 years, as part of the longitudinal MAAS study. Higher body mass index (BMI) was associated with an increased risk of wheeze at each of 3, 5 and 8 years[155], although at age 8 years there was a significant association only in girls[155]. The Normative Aging Study is a longitudinal study which recruited men from the Greater Boston area aged between 21–80 years. It showed that both men with a high BMI and also men with a low BMI, had an increased risk of BHR, used as a proxy for asthma in this study[156]. This 'J shaped' relationship has also been shown in a large Chinese study where being either underweight or overweight were associated with asthma in women, and being underweight was associated with asthma in men[157]. In a review and detailed analysis Delgado concludes that most of the epidemiological data indicate that obesity can increase the prevalence and incidence of asthma, although the effect appears modest[158].

In a cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma, obesity was found to be associated with severe asthma[159]. Obesity has been shown to be associated with worse asthma outcomes, especially an increased risk of asthma-related hospitalization[160]. The Severe Asthma Research Programme (SARP) has shown that one phenotype of severe asthma is a group of mostly older obese women with late-onset non-atopic asthma, moderate reductions in forced expiratory volume in one second (FEV_1), and frequent oral corticosteroid use[161] and Dixon et al have shown an improvement in asthma severity after surgery for morbid obesity[162]. These studies suggest obesity is associated with an increased risk of developing severe asthma.

3.4 Aims and objectives:

The aim of this chapter is to describe some features of the MAPS cohort in order to create a context for interpreting results given in later sections of the thesis. In addition we will investigate the association of childhood allergic disease, socio-economic class (SES), family history and BMI with outcomes at age 23 years.

Objectives

To examine how the participation rate at age 23 years varies between subgroups.

To describe the rate of wheeze, rhinitis and eczema at age 23 years and the association of childhood wheeze rhinitis and eczema with asthma and wheeze at age 23 years

To describe the social class of subjects in the MAPS cohort and investigate associations with outcome variables at age 23 years

To investigate the association of family history with asthma, wheeze and atopy at age 23 years

To investigate the association between BMI in both childhood and adulthood, and wheeze at age 23 years.

3.5 Methods

Full methods are given in chapter 2. Here are details of methods specific to this chapter. Subjects at age 15 were asked if they had ever had eczema, rhinitis or a wheeze. At age 23 years eczema was defined as answering yes to the question 'Have you ever had an itchy rash that was coming and going for at least 6 months?' and then answering yes to the question 'Have you had this itchy rash at any time in the last 12 months?' Rhinitis at age 23 was defined as answering yes to the question 'In the last 12 months have you had a problem with sneezing, or a runny or blocked nose, when you DID NOT have a cold or flu?' Wheeze was defined as answering yes to the question "Have you had wheezing or whistling in the chest in the past 12 months?" Severe wheeze was defined as wheeze that limits speech or disturbs sleep at least once a week, i.e. answering yes to either of the questions: 'Do you have wheeze that limits speech' or 'Do you have wheeze that disturbs your sleep at least once a week'.

Social class was categorised using the Registrar General's occupations and social class categories (see appendix 1). At age 23 subjects were asked with reference to their housing if it was owner occupied, rented or council.

Body mass index (BMI) is defined as the weight (in Kg) divided by the square of the height (in metres). Obesity in adults is usually defined as having a BMI of 30 kg/m^2 or greater, while having a BMI of between 25 and 30 is defined as being overweight[163]. In children the definition varies with age. Cole et al[152] have used modelling of data from multi-national studies to derive thresholds for being overweight and obese at different ages, structuring the analysis to ensure continuity with the definitions for overweight and obese in adults at age 18. For example, using Cole's analysis, obesity at age 7 years was defined as a BMI exceeding 20.63 kg/m^2 in boys and 20.51 kg/m^2 in girls. Since the proportion of obese children is small in our cohort, we have also used the actual recorded BMI as a continuous variable in a binary logistic regression to model wheezing status at age 23 years with BMI both at age 23 years and also in childhood. The results are given as odds ratios for a 5 unit difference in BMI (i.e. a change of $5kg/m^2$). The OR for this 5 kg change in BMI is given by taking the OR for a 1 unit change and raising it to the power 5; confidence limits are obtained in a similar way.

3.6 Results

3.6a) Response rate at age 23 years and representativeness of responders compared to original cohort

As in other longitudinal studies, the MAP study has been vulnerable to the loss of subjects over time. Approximately 62% of the original birth cohort of 487 subjects took part at age 23 years. Of the 438 (229 males) subjects deemed contactable at age 23, 304 (69%) participated in the study at age 23. The response rate for males was 65% (n=148) and 75% (n=156) for females. Since it is important to investigate how representative the cohort at 23 years is with respect to the original population, we can consider this statistically. Table 3.1 shows the response rate for groups based on important variables in the study at recruitment and at age 1. Table 3.1 is based on the total population of 487 who were randomised in the initial study rather than the 438 who were deemed contactable at age 23 years, since our aim is to show that those lost to follow up were not significantly different from those that attended. There was no association between the original randomisation and participation at age 23 years, however, participation rates at age 23 years were lower in males than in females, lower in those who wheezed at age 1 year and lower in children from unemployed families.

Table 3.1 Response rate at age 23 years for important subgroups of the study population.

Subgroup	Variable	Response rate	OR (95%CI)	p-value
Gender	Male§	146/257 (56.8%)	1	
	Female	153/230 (66.5%)	1.51 (1.05-2.18)	0.028*
Randomisation group	Control group§	153/249 (61.4%)	1	
	Intervention group	146/238 (61.3%)	1.00 (0.70-1.45)	0.98
Wheeze age 1	No wheeze age 1§	200/303 (66.0%)	1	
	Wheeze age 1	89/158 (56.3%)	0.66 (0.45-0.99)	0.042*
Parental employment age 1	Employed parent§	249/389 (64.0%)	1	
	Unemployed parent	42/88 (47.7%)	0.51 (0.32-0.82)	0.005*

* Significant results

§ reference categories for each analysis

Table 3.2 shows the rates of attendance at the research clinic among those who participated at age 23 years by key characteristics. Rates of attendance at the clinic were higher among females and among those who had wheeze or asthma at age 23 years, suggesting that the population who attended the clinic had higher levels of illness. Table 3.2 shows there was no significant difference between those who attended and those who did not attend with regard to randomisation group, wheeze at age 1 or unemployment at age 1. Although the attendance rate was higher in subjects with symptoms at age 23,

this is unlikely to have a major effect on the analysis of the relationship between variables.

Table 3.2 Clinic attendance rates at age 23 for important sub groups of the study population

Subgroup	Variable	Attendance at research clinic	OR (95%CI)	p-value
Gender	Male§	50/146 (34.2%)	1	
	Female	70/153 (45.8%)	1.62 (1.02-2.58)	0.04*
Randomisation group	Control group§	62/153 (40.5%)	1	
	Intervention group	59/146 (40.4%)	1.01 (0.63-1.60)	0.98
Wheeze age 1	No wheeze§	82/200 (41.0%)	1	
	Wheeze	36/89 (40.4%)	0.98 (0.59-1.63)	0.93
Parental employment age 1	Employed parent§	102/249 (41.0%)	1	
	Unemployed parent	16/42 (38.1%)	0.89 (0.45-1.74)	0.73
Wheeze age 23	No wheeze§	67/201 (33.3%)	1	
	Wheeze	53/98 (54.1%)	2.36 (1.44-3.86)	0.001*
Asthma age 23	No Asthma§	89/244 (36.5%)	1	
	Asthma	31/52 (59.6%)	2.57 (1.39-4.74)	0.003*

* Significant results

§reference categories in the above analysis.

3.7) Prevalence of wheeze, rhinitis and eczema in MAPS cohort

Figure 3.1 shows the prevalence of wheeze, rhinitis and eczema, and their combinations, among the 294 subjects on whom we have complete data at age 23 years. Eczema has the lowest prevalence and rhinitis the highest prevalence in this group. A total of 18 (6.1%) of the population had all three diagnoses.

Figure 3.1 Distribution of allergic disease in the cohort at age 23 years

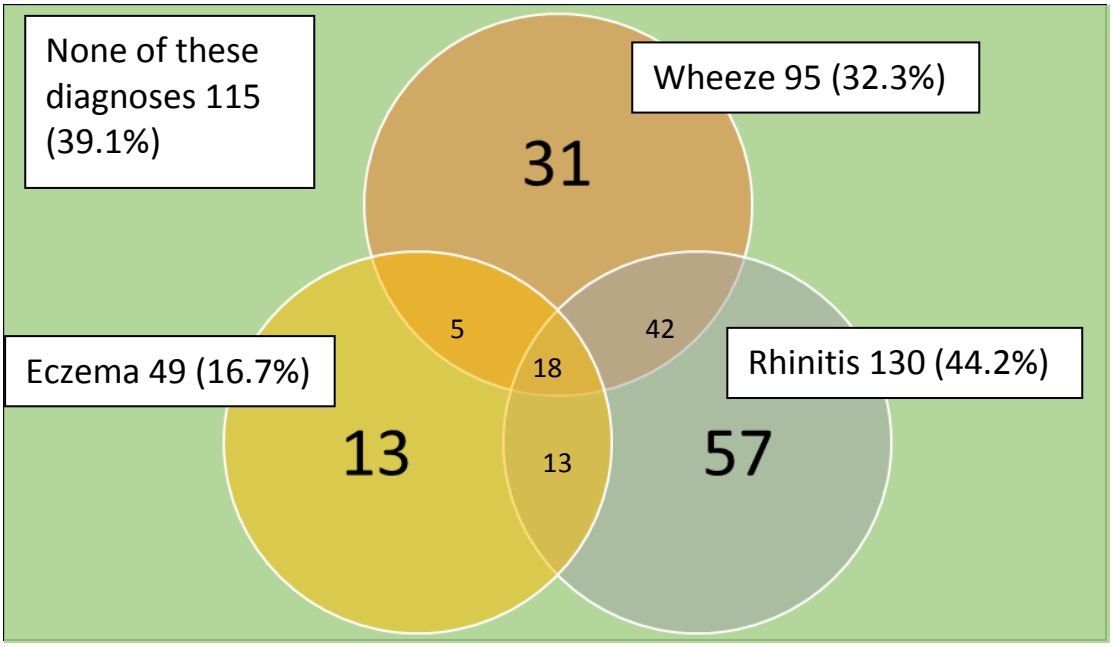


Table 3.3 shows the association of childhood eczema, rhinitis and wheeze and adult eczema and rhinitis with wheeze, asthma and atopy age 23 years based on 294 subjects with complete data. There are a number of positive associations, ever having a wheeze or rhinitis up to age 15 is associated with

asthma or wheeze at age 23 years. Childhood eczema shows the least evidence of an association with adult wheeze or asthma. Adult eczema and rhinitis are both associated with asthma and wheeze at age 23 years.

Table 3.3 Association of wheeze age 23years, asthma age 23 years and atopy age 23 years with atopic symptoms ever in childhood and at age 23 years.

	Wheeze age 23	**No wheeze age 23**	**OR (95%CI) p**
Ever Eczema age 15	28/86 (32.6%)	48/179 (26.8%)	1.32 (0.75-2.31) 0.33
Ever Rhinitis age 15	41/86 (47.7%)	47/179 (26.3%)	2.56 (1.49-4.38) <0.0001*
Ever wheeze age 15	69/83 (83.1%)	113/174 (64.9%)	2.66 (1.38-5.11) 0.003*
Eczema age 23	23/96 (24.0%)	26/198 (13.1%)	2.08 (1.12-3.89) 0.02*
Rhinitis age 23	61/97 (62.9%)	71/201 (35.3%)	3.10 (1.88-5.13) <0.0001*
	Asthma age 23	**No asthma age 23**	**OR (95%CI) p**
Ever Eczema age 15	18/47 (38.3%)	58/218 (26.6%)	1.71 (0.89-3.31) 0.11
Ever Rhinitis age 15	25/47 (53.2%)	63/218 (28.9%)	2.80 (1.47-5.32) 0.002*
Ever wheeze age 15	41/46 (89.1%)	141/211 (66.8%)	4.07 (1.54-10.76) 0.005*
Eczema age 23	16/52 (30.8%)	33/241 (13.7%)	2.80 (1.40-5.61) 0.004*
Rhinitis age 23	37/52 (71.2%)	93/243 (38.3%)	3.98 (2.07-7.65) <0.0001*
	Atopy§ age 23	**No atopy age 23**	**OR (95%CI) p**
Ever Eczema age 15	21/64 (32.8%)	11/46 (23.9%)	1.55 (0.66-3.65) 0.31
Ever Rhinitis age 15	34/64 (53.1%)	6/46 (13.0%)	7.56 (2.81-20.31) <0.0001*
Ever wheeze age 15	46/63 (73.0%)	31/45 (68.9%)	1.22 (0.53-2.83) 0.64
Eczema age 23	18/70 (25.7%)	8/48 (16.7%)	1.73 (0.68-4.38) 0.25
Rhinitis age 23	50/70 (71.4%)	15/49 (30.6%)	5.67 (2.55-12.60) <0.0001*

* Significant result
§ Atopy age 23 years is defined as a positive skin prick test result at age 23 years

3.8) Association between wheeze and social class

Social class was recorded at ages 1 and 7 but at age 23 years the subject was asked if s/he was living in owner occupied, rented or council accommodation and if s/he were employed. A total of 68.3% were both employed and living in owner occupied housing. There was a highly significant weak correlation between social class at age 1 and age 7 years and living in homeowner occupied accommodation at age 23 years (spearman correlation 0.260 $p<0.0001$). Table 3.4 shows the percentages of subjects who wheezed at ages 1, 7, 15 and 23 years by social class at age 1 year. There was a highly significant trend at age 1 showing that children of lower social classes had a higher prevalence of wheeze than those of higher social class. This association was not present at subsequent ages. However if we investigate social class as determined at age 7 years there is a significant association between wheeze at age 7 and social class but not at older ages as shown in table 3.5.

Table 3.4 Proportion with wheeze at ages 1, 7, 15 and 23 in each social class as established age 1 year.

Social Class at age 1 year	Proportion who wheeze at age 1 year (%)	Proportion who wheeze at age 7 years (%)	Proportion who wheeze at age 15 years (%)	Proportion who wheeze at age 23 years (%)
I	2/12 (16.7%)	4/11 (36.4%)	3/8 (37.5%)	4/6 (66.7%)
II	10/49 (20.4%)	6/47 (12.8%)	13/45 (28.9%)	12/36 (33.3%)
III non-manual	12/53 (22.6%)	15/50 (30.0%)	13/43 (30.2%)	8/36 (22.2%)
III manual	51/160 (31.9%)	44/161 (27.3%)	39/130 (30.0%)	34/109 (31.2%)
IV	14/44 (31.8%)	13/43 (30.2%)	12/36 (33.3%)	12/31 (38.7%)
V	24/54 (44.4%)	20/52 (38.5%)	12/40 (30.0%)	10/31 (32.3%)
Unemployed	40/79 (50.6%)	21/72 (29.2%)	20/46 (43.5%)	15/42 (35.7%)
Overall proportion	153/451 (34%)	123/436 (28.2%)	112/348 (32.2%)	95/291 (32.6%)
Chi Squared for trend	p<0.0001*	p=0.109	p=0.226	p=0.836

* significant result

Table 3.5 shows the proportions of wheeze by social class at age 7 years. Wheeze at age 7 years shows a trend in that those with lower social class are more likely to wheeze, but there is little evidence of associations at ages 15 or 23.

Table 3.5 Proportion with wheeze at age 7, 15 and 23 in each social class at age 7.

Social Class at age 7	Proportion who wheeze at age 7 years (%)	Proportion who wheeze at age 15 years (%)	Proportion who wheeze at age 23 years (%)
I	4/18 (22.2%)	6/16 (37.5%)	5/14 (35.7%)
II	22/67 (32.8%)	22/60 (36.7%)	17/47 (36.2%)
III non-manual	10/44 (22.7%)	11/37 (29.7%)	9/31 (29.0%)
III manual	26/133 (19.5%)	30/110 (27.3%)	26/93 (28%)
IV	17/59 (28.8%)	17/49 (34.7%)	19/45 (42.2%)
V	10/32 (31.2%)	7/26 (26.9%)	4/22 (18.2%)
Unemployed	38/92 (41.3%)	21/57 (36.8%)	18/46 (39.1%)
Overall proportion	127/445 (28.5%)	114/355 (32.1%)	98/298 (32.9%)
Chi Squared for trend	p=0.029*	p=0.97	p=0.82

As there was no direct measurement of social class at age 23, we can only use housing tenure and employment status as indicators of socio-economic class. Table 3.6 shows the association between wheeze and accommodation tenure at 23 years while table 3.7 shows the same outcomes and their association with employment status at age 23 years. There is no significant association with

either living in owner occupied accommodation or being employed and wheeze, asthma or atopy at age 23.

Table 3.6 Association of housing tenure age 23 years with wheeze, asthma and atopy at age 23 years.

Age	Living in rented or council housing age 23	Living in owner occupied home age 23	OR (95% CI) p
Wheeze 23	22/72 (30.6%)	70/207 (33.8%)	0. 86 (0.48-1.54) p=0.61
Asthma 23	10/71 (14.1%)	41/207 (19.8%)	0.66 (0.31-1.41) p=0.29
Atopy age 23	16/24(66.7%)	54/95 (56.8%)	1.51 (0.53-3.84) p=0.38

Reference category is living in owner occupied accommodation

Table 3.7 Association of employment status age 23 years with wheeze, asthma and atopy at age 23 years.

Age	Unemployed age 23	Employed age 23	OR (95% CI) p
Wheeze 23	13/39 (33.3%)	83/256 (32.4%)	1.04 (0.51-2.13) p=0.91
Asthma 23	6/39 (15.4%)	46/255 (18.0%)	0.83 (0.33-2.09) p=0. 69
Atopy age 23	6/11 (54.5%)	64/108(59.3%)	0.83 (0.24-2.87) p=0.76

Reference category is employed status in above analysis

3.9a) Association of wheeze, asthma and atopy with parental allergy and asthma

Parental allergy was based on the mothers' responses to questions in the antenatal questionnaire. Pregnant women were asked if there was a history of asthma, rhinitis or eczema in themselves or the unborn child's father.

Table 3.8 shows the frequency of a parental history of asthma and allergy. Fewer than 20% of subjects had parents with no history of asthma, rhinitis or eczema. This was consistent with the admission criteria to the study since subjects were only recruited if a first degree relative had one of these diagnoses, and some of those would be siblings rather than parents. Fewer than 40% of subjects had one or more parent with asthma.

Table 3.8 Frequency of parental history of asthma alone and asthma, rhinitis or eczema.

Attribute	Present in neither parent	Present in father only	Present in mother only	Present in both parents
Asthma	298/486 (61.3%)	94/486 (19.3%)	83/486 (17.1%)	11/486 (2.3%)
Asthma, rhinitis or eczema	95/486 (19.5%)	142/486 (29.2%)	191/486 (39.3%)	58/486 (11.9%)

Table 3.9 shows the association between parental history of allergy and wheeze, asthma and atopy at age 23. Where both parents, and father only had allergy the subject was significantly more likely to wheeze, have asthma or atopy at age 23 years. The associations with maternal allergy alone were not significant.

Table 3.9 Association of parental history of allergy† with wheeze, asthma and positive skin prick test at age 23 years.

Family history of allergy	Wheeze age 23 years	No wheeze age 23 years	OR	95% CI
Neither	10	45	1	
Mother only§	38	80	2.14	0.97- 4.69
Father only§§	33	53	2.80	1.24 – 6.31*
Both††	17	23	3.33	1.31 – 8.42*
	Asthma age 23 years	No asthma age 23 years	OR	95% CI
Neither	5	50	1	
Mother only§	16	100	1.60	0.55-4.62
Father only§§	19	66	2.88	1.01-8.24*
Both††	12	28	4.29	1.37 – 13.42*
	Atopy # age 23 years	No atopy age 23 years	OR	95% CI
Neither	7	14	1	
Mother only§	26	20	2.60	0.88- 7.64
Father only§§	29	13	4.46	1.46 – 13.65*
Both††	8	2	8.00	1.33 – 48.18*

*Significant result †Parental history identified before birth of subject
§Mother only with allergy, exclusion of subjects with both parents who have allergy
§§ Father only with allergy, exclusion of subjects with both parents who have allergy
††Both parents have allergy, exclusion of those who have only one parent who have allergy
Atopy defined as positive skin prick test to one allergen or more at age 23 years

3.9b Association of wheeze, asthma and atopy with parental asthma

Table 3.9 showed the association between the prevalence of wheeze, asthma and atopy at age 23 years and any parental history of allergic disease. Table 3.10 shows comparable results when the parental history is restricted to parental asthma. The number of parents with asthma is fewer and therefore the power of these comparisons is lower and confidence intervals for effect sizes are wider. There are significant associations for father only and both parents having asthma and wheeze and asthma age 23 years. Maternal asthma is not associated with any significant outcomes. There are no significant results between parental asthma and atopy age 23 years.

Table 3.10 Association of parental history of asthma† with wheeze, asthma and positive skin prick test at age 23 years

Family history of asthma	Wheeze age 23 years	No wheeze age 23 years	OR	95% CI
Neither	10	45	1	
Mother only§	12	28	1.93	0.74- 5.05
Father only§§	17	27	2.83	1.13 – 7.08*
Both††	5	2	11.25	1.90 – 66.53*
	Asthma age 23 years	No asthma age 23 years	OR	95% CI
Neither	5	50	1	
Mother only§	4	36	1.11	0.28-4.43
Father only§§	12	32	3.75	1.21-11.65*
Both††	4	3	13.33	2.30 – 77.24*
	Atopy# age 23 years	No atopy age 23 years	OR	95% CI
Neither	7	14	1	
Mother only§	7	9	1.56	0.41- 5.95
Father only§§	13	9	2.89	0.83 – 10.02
Both††	1	0	NA	NA

*Significant result †Parental history identified before birth of subject
§ Mother only has asthma, exclusion of subjects with both parents who have asthma
§§ Father only has asthma, exclusion of subjects with both parents who have asthma
§§§Both parents have asthma, exclusion of those who have only one parent who has asthma
Atopy defined as positive skin prick test to one allergen or more at age 23 years

3.10a) Body Mass Index (BMI) and wheeze age 23 years

Using Cole's cut off points for defining obesity from BMI, (20.63 kg/m^2 in boys and 20.51 kg/m^2 in girls at age 7 years)[152], 10 out of 218 (4.6%) of male children and 9 out of 199 (4.5%) of female children aged 7 in our cohort were obese. At age 23, 18.1% were obese, 15.0% of males and 21.4% of females.

Table 3.11 presents the mean BMI at different ages in childhood, for those who wheeze and those who do not at age 23 years. In addition it gives the OR and 95% confidence interval, with p value, for a 5 unit difference in BMI (5Kg/m^2), calculated using binary logistic regression with BMI as a continuous variable as described in the methods. The results in table 3.11 show in female subjects that wheeze at age 23 is significantly associated with a BMI at ages 1 year, 7 years and 23 years but that is not so for male subjects. The effect size, the OR for a difference of 5kg/m^2 decreases with age but this is largely because the difference of 5kg/m^2 at age 1 is relatively much larger than at older ages. This finding suggests that BMI in childhood may be associated with wheeze in adulthood in female subjects.

Table 3.11 Summary statistics of BMI by wheezing status at age 23 years, and the OR, and 95% confidence interval, for a difference of 5kg/m^2.

Male subjects only				
Age BMI measured	Mean (SD, n) BMI in those who wheeze at 23	Mean (SD, n) BMI in those who do not wheeze at 23	OR for difference of 5kg/m^2	(95% CI) p
1 year	17.94 (1.33, 47)	18.51 (3.03, 88)	0.50	(0.17 to 1.54) 0.22
7 years	16.29 (1.29, 45)	16.32 (2.25, 93)	0.95	(0. 39 to 2.39) 0.93
23 years	26.05 (4.44, 47)	26.02 (4.67, 86)	1.00	(0.70 to 1.47) 0.97
Female subjects only				
Age BMI measured	Mean (SD, n) BMI in those who wheeze at 23	Mean (SD, n) BMI in those who do not wheeze at 23	OR for difference of 5kg/m^2	(95% CI) p
1 year	17.88 (1.41, 43)	17.29 (1.29, 95)	5.19	(1.28 to 21.67) 0.02*
7 years	17.01 (2.85, 49)	16.04 (2.08, 100)	2.29	(1.10 to 4.83) 0.02*
23 years	27.49 (6.73, 40)	25.08 (5.25, 86)	1.40	(1.00 to 1.93) 0.03*

*significant results

3.10b) Severe wheeze and BMI

As cited earlier, there is evidence that severe asthma is associated with obesity, especially in females. For this reason we have investigated the effects of BMI on severe wheeze, as a proxy for severe asthma, in males and females. Table 3.12 demonstrates that severe wheeze is associated with a high BMI at age 23 but only in females. The mean BMI in men with severe symptoms is lower than the BMI of those without symptoms, but the difference is not statistically significant. High BMI is almost certainly associated with severe wheeze in females but not in males.

Table 3.12 Summary statistics of BMI by severe wheeze status at age 23, and the OR, and 95% confidence interval, for a difference of 5kg/m^2.

	Mean (SD, n) BMI in those who have severe wheeze at 23	Mean (SD, n) BMI in those who do not have severe wheeze at 23	OR for difference of 5kg/m^2	(95% CI) p
Total group	27.87 (6.63, 29)	25.70 (4.98, 230)	1.40	(1.00 - 2.01) 0.038*
males	24.13 (3.45,11)	26.19 (4.63,122)	0.52	(0.22 - 1.28) 0.15
Females	30.15 (7.13, 18)	25.13 (5.31, 108)	1.93	(1.28 - 2.82) 0.002*

3.11 Discussion

3.11a) Response rate at age 23 years

In this chapter we have investigated the response rate for subjects at age 23 years and found that subjects who had wheeze at age 1 year, were male or were from unemployed families at age 1 year were significantly less likely to be followed up than those not in these groups. The higher loss to follow-up

from male subjects is consistent with other studies[164], but this effect has not been shown in the MAPS cohort before.

There were significant differences between those who attended the laboratory for physiological measurements and those who did not. This was probably due to self-selection of those who had more symptoms. Sample selection in cohort studies may alter the confounding patterns originally present in the general population, but this does not necessarily introduce selection bias in the exposure-outcome estimates. While there is some evidence that the group under investigation is not completely representative of the original population, this is unlikely to have a major impact on the estimate of the associations between risk factors and outcomes.

3.11b) Rate of atopic disease and association with outcomes

In the MAPS cohort we found high rates of rhinitis (44.4%) and low rates of eczema (16.7%) at age 23. Childhood rhinitis was associated with wheeze, asthma and atopy at age 23 years and childhood wheeze was associated with adult wheeze and adult asthma. Childhood eczema was not associated with any of these attributes at 23 years of age. In the MAPS cohort there was an association of childhood rhinitis and wheeze up to age 15 years with wheeze and asthma age 23 years and childhood rhinitis with atopy age 23 years. Adult rhinitis and eczema were associated with wheeze and asthma at age 23 years and adult rhinitis with atopy at age 23 years. The TAS (now named the Tasmanian health study) found there was an association of childhood rhinitis and eczema with adult atopic asthma, defined as asthma and sensitisation to

four perennial allergens, and that childhood eczema and rhinitis were only associated with atopic asthma but not with non-atopic asthma[165]. Although another GP based study found that eczema followed by asthma followed by rhinitis is the usual allergic march trajectory[166], we found no association of childhood eczema with wheeze, asthma or atopy, in the MAPS cohort, suggesting this was not a common trajectory with our subjects.

3.11c) Socio-economic status and outcomes

We have shown that wheeze at age 1 year was more likely in those from a lower social class at age 1 year. There was also a significant association between wheeze age 7 years and social class at age 7 years, although the association is not as strong as that at age 1 year. At both age 15 and age 23 years wheeze was not associated with social class at age 1 or age 7. Therefore the association of wheeze and social class does not appear to persist after the age of 1. There are a number of possible explanations for this.

We have used housing tenure to indicate social class and there is a precedent for this since Davies et al used data from two well-established sample surveys, the Family Expenditure Survey (FES) 1983 and 1990 and the General Household Survey (GHS) 1984 to estimate associations between housing tenure and income. They showed that living in rented accommodation was significantly associated with low income and living in an owner-occupied dwelling was an important indicator of relative affluence. The effect was greater for data taken in 1990 compare to 1983 in the FES[167]. Therefore we

are left with the question of why we did not show any association with wheeze at age 23 years but did with wheeze at age 1 and social class.

One explanation is that there may be an association between social class and wheeze and asthma age 23 years but the lack of a good assessment of social class at age 23 years has reduced the effect size of this association. Using a poor measure, whether that from an earlier age or a proxy such as housing tenure, is likely to weaken associations. Although Davies et al have shown that housing tenure is usually an effective measure of relative deprivation, the housing stock for our cohort in Merthyr Tydfil may not require such affluence to own. Almost 75% of the population in our cohort were owner occupiers while only 60% in the Davies study were. Over 40% of unemployed subjects in the MAPS cohort were owner occupiers of housing. These facts suggest that housing tenure may not have been such a good indicator of social class in our subjects at age 23 years, and this may be why we found no association between social class and wheeze at this age.

Yet another possible explanation is that there really is no association between wheeze age 23 years and social class. So there is a pattern of associations which weakens with age. This is not consistent with other studies such as that by Braback et al of Swedish recruits. They found that low socio-economic status (SES) was associated with an increased risk of asthma without allergic rhinitis (1.14, 95% confidence interval (CI) 1.11–1.17) but a slightly reduced risk of asthma with allergic rhinitis (0.96, 95% CI 0.93–1.00) [132]. This was a large population study with social class contemporaneously assessed, which may explain the differences between their results and those found in MAPS. However, the German MAS study found that the association of social class

and wheeze changed over generations, with parents in high SES groups more likely to have recurrent wheeze than those in lower SES groups, while in their children's generation there was no such association. Although the MAPS is not completely comparable, as it contains only one generation of subjects, it is possible that the environmental factors that are associated with lower social class subjects and wheeze when they were age 1 year, have changed in some way over the intervening period so that the social class differentiation is lost over time.

A further reason why wheeze at age 1 was associated with social class and later wheeze was not is that early life wheeze may be aetiologically different to the wheeze in later childhood and adulthood. Early wheeze is often considered to be as a result of respiratory infections, which could be more common in lower social class subjects where the environment may be more crowded. In addition not all subjects who wheeze at age 23 years are suffering from the same wheezing disorder and some may have COPD while others have asthma.

Lastly the number of subjects in this study is small at age 23 years and as there are 5 social classes we may not have had the power in our study to show the relationship between social class and outcomes at age 23 years even though a relationship does exist. Hence even though we have not found an association this doesn't mean there isn't one.

3.11d) Familial disposition

Parental allergy, especially allergy in the father of subjects in MAPS, was associated with wheeze and asthma at age 23 years. Our results showed no significant association with maternal asthma or maternal allergy. Although this was similar to the study by Alford et al, which showed that paternal allergy and asthma was more strongly associated with asthma when aged 6-7 years[145], there have been no studies which give similar results to MAPS for subjects in early adulthood. The Tasmanian population-based longitudinal study showed that both maternal and paternal asthma were associated with asthma in the adult offspring but maternal asthma gave a higher OR[146]. It is not clear why our results were so different but one possible explanation is that the history was most often taken from the mother at booking in clinic. This may have introduced bias as the mother would have more information on her previous history than her spouse's history and it is possible that only the more severe asthma and allergy symptoms in her partner were noted by the mother. There is some evidence that mothers may have under reported father's allergic disease since mothers were more frequently affected by allergic symptoms (51% of mothers) than their spouses (41% of fathers). Although this difference is small, there is no reason to suspect that females would be more likely to suffer from atopic disease than males, especially since atopic disease defined by skin prick test is more common in adult males than females[37]. In summary our findings are similar to other published work on parental allergy and asthma although there was a tendency for paternal allergy and asthma to be particularly important in the inheritance patterns of our subjects.

3.11e) BMI in childhood and adulthood and outcomes in adulthood

Our results show a significant association between BMI in females at ages 1, 7 and 23 years and wheeze at age 23 years. A high BMI in childhood is associated with the occurrence of disease symptoms in adulthood, which is evidence against the view that obesity occurs because subjects are unable to exercise (reverse causality), since subjects had higher BMI than their counterparts up to 22 years before the outcome measure. Chen et al found that Canadian female subjects aged at least 12 years with a BMI at least 28 kg/m^2 had a significantly raised risk of asthma compared to those with a lower BMI; there was no similar increased risk in males[168]. However they did not investigate associations between BMI in childhood and wheeze in adults. One study by Castro-Rodriguez et al in USA, investigated obesity in childhood and found females who became overweight or obese between 6 and 11 years of age were at a significantly increased risk of developing new asthma symptoms and increased BHR during the early adolescent period[169]. An RCT of weight reduction in obese adult subjects with asthma showed improved lung function, symptoms, morbidity, and health status as weight was reduced[13]. Our study was not powered to investigate obesity. Are there explanations of this association between BMI in childhood and symptoms in early adulthood? Firstly there may be common risk factors for both asthma and increase in weight. There is evidence that immune responses and metabolic regulation are highly integrated and the proper function of one is dependent on the other[170]. Increase in weight would affect the metabolic regulation of an individual and thereby could have an effect on their immune response. Some have suggested an association between obesity and atopy[171],

but there was no evidence for that in this cohort. However one study from Adelaide showed an increased risk of asthma in females without atopy[172]. Secondly there may be common dietary risk factors for both obesity and asthma. For example, fast food has low levels of antioxidants and vitamins, while hamburger consumption is associated with childhood asthma and take-away consumption is associated with bronchial hyper reactivity[56]. Both excess take-away food and hamburger consumption are associated with an increased risk of obesity. A sedentary life style may also lead to greater time spent at home and therefore greater exposure to aeroallergens that are present in modern homes and this may induce an association between obesity and asthma. However none of these explanations account for the differences between males and females. Adult females have relatively smaller airways for their lung size than do adult males (dysynapsis ratio)[173] and so adiposity and restriction of the chest wall due to adiposity may make obstruction of airways more likely in females.

A further explanation of why women with a higher BMI have an association with wheeze at age 23 years while men do not is provided by the fact that Wheeldon et al have shown that in females during the luteal phase of the menstrual cycle, there is an increase in β2-adrenoceptor density on lymphocytes which is significantly higher than in male subjects[174] and hormonal changes are known to occur in over weight and obese women[175]. The β2 receptors are important targets for many drugs used in the treatment of asthma and it may be that hormone levels in females with a higher BMI interfere with normal cyclical control of β2 receptors.

In summary chapter three has presented that this cohort is representative of the original population. We have also found that there is no significant association between social class and outcomes at age 23 years, although we do not have a good measure of social class at age 23. We found that family history of allergic disease is linked to wheeze and asthma in young adults and that BMI, in childhood as well as adulthood, is associated with wheeze and severe wheeze but only in female subjects. These findings support the view that early life events make an important contribution to risk of adult life outcomes.

Chapter 4

Atopy and Asthma

4.0 The Association of Atopy and Asthma

Atopy is the ability to mount an IgE mediated specific response against an allergen. It is measured by sensitisation on either positive skin prick testing or a raised IgE level in serum to a specific allergen. There is strong evidence of the association of atopy with asthma in both cross-sectional[41, 176] and longitudinal[47] studies. Allergen exposure is known to lead to inflammation in atopic asthma[177, 178] and cause exacerbations of asthma[179], but whether allergen exposure actually causes asthma still remains controversial with some evidence for[180] and other evidence against[83].

The longitudinal Tucson epidemiological study of airway obstructive diseases (TESAOD) study found that sensitisation before the age of 8 years was associated with symptoms of wheeze and shortness of breath in later childhood and children who were sensitised after 8 years of age were no more likely to have wheeze and shortness of breath than children who were never found to be sensitised. Based on these results they concluded that early allergic sensitisation was a significant risk factor for later development of symptoms of wheeze, and late sensitisation was not[181]. Arshad et al studied 4 year old children in a birth cohort. Of those with positive skin prick test reactions to 4 or more allergens nearly 80% had asthma, eczema, and/or rhinitis compared with 20% of those who had no positive skin prick test reactions[34]. The German MAS longitudinal study found that atopy had been

present from an earlier age in subjects with asthma at age 7 years than in those without asthma at this age[182]. They also found that persistent sensitivity to food allergens by the age of 2 years[183] and aeroallergens by age 7[182] was associated with asthma later in life, and that 90% of children who had wheeze but no atopy lost their wheeze and had normal lung function by school age while having atopy by the age of 3 years was associated with loss of lung function at school age[49]. Early sensitisation to a broad panel of allergens was shown to be more strongly associated with asthma at age 10 (Isle of Wight cohort) or 11 (Manchester Asthma and Allergy Study-MAAS) than conventional atopy (sensitisation to one or more allergen)[184]. The MAAS study has shown that multiple early sensitisation, defined by cluster analysis in subjects who were tested at age 1, 3, 5 and 8, was associated more strongly with asthma at age 8 than conventional atopy (positive sensitisation to at least one allergen)[89]. These studies suggest that sensitisation to allergens early in life is often associated with concurrent asthma and also with persisting asthma symptoms later in life. The two phenotypes of early childhood wheeze most likely to develop into asthma at age 16 years, in the TCRS cohort, were the persistent and late onset wheeze phenotypes. Both these phenotypes were associated with sensitisation to common allergens[185].

4.1 Prevalence of sensitisation over time

Sensitisation is the ability of the immune system to mount a response to a specific allergen. The definition of atopy is usually given as the presence of sensitisation to at least one perennial allergen. Sensitisation to at least one allergen[186], and to 5 or more allergens (polysensitisation)[187], has been shown

to be more common in males. The NHANES II cross-sectional study reported that sensitisation peaks in teenagers and young adults[188]. NHANES II also reported that there was an association between skin prick reactivity and socio-economic status with those from a higher social class being more likely to be sensitised[189]. Sensitisation to at least one of the 6 allergens tested in both NHANES II (1976-80) and NHANES III (1988-1994) revealed a large increase in sensitivity between the two studies with 22% of subjects positive in the earlier study, and 42% positive in NHANES III[190]. This echoes the findings in other studies, as reviewed by Eder, that the prevalence of atopy is increasing[6].

TESAOD investigated atopy defined by an allergen skin prick test to 5 allergens, on a population of 1333 subjects aged between 3 years and over 75 years. On the initial review 39.1% of subjects were positive to at least one allergen. Approximately 8.5 years later, at follow up, 50.7% were positive. They found that atopy was more prevalent at younger ages, peaking at age 15-34 and that the greatest increase in prevalence of atopy occurred among those who were children and teenagers (less than 5 to 14 year of age at base line), 22.2% and 19.5% respectively, with only minimal increases after the age of 65 years, 6.0%[191]. A British study divided subjects from a population age 18 to 71 into six 9 year successive birth cohorts and each subject was tested twice once in 1991 and once in 2000. They found that the prevalence of sensitisation was higher in younger subjects in both studies in the initial study 46% of the 18-26 years age group had a positive skin prick test result while only 21% of the 63-71 age group had a positive skin prick test. There was no significant change in

prevalence of sensitisation between the two groups over the 9 year period (46% and 23%) respectively[192].

The prevalence of atopy in childhood increases with age, as shown by the Tucson Children's Respiratory Survey (TCRS) based on an unselected population of children from a Health Management Organisation (HMO) in Tucson, Arizona, who were skin prick tested to aeroallergens. At age 6 years, 38.6% of children were positive while at age 11 years 58.1% were positive[193]. The longitudinal birth cohort study from Stockholm (BAMSE) showed that from age 4 to 8 years, the proportion of children sensitised to any of the aeroallergens tested, increased from 15% to 25%[194]. Although the ages are comparable in the TCRS and BAMSE studies, it is clear that children from Tucson have a higher rate of atopy than those from Stockholm. This is probably due to differing environmental conditions in different countries. Studies have shown that the prevalence of sensitisation varies between countries[195-197], and in different geographical areas within a country[198].

Aslund et al published in 2008 the results of 2 skin prick test examinations, 3 years apart, on subjects from Copenhagen aged 14-44 years, who answered a postal questionnaire reporting they had symptoms suggestive of asthma or allergy. On repeat testing, they found that 22% had developed de novo sensitisation to 1 or more allergens that they were not sensitive to on the original test. A total of 39% had either a gain or a loss of sensitivity and therefore there was a high prevalence of change in sensitisation in this 3 year period[199]. In 2004, Bodgter et al investigated a population of 18–69 year olds

in Copenhagen and their 8-year remission rates for sensitisation to pollen allergens and dust mite/pet allergens based on specific IgE were 6% and 11%, respectively[200]. This suggests that once aeroallergen sensitisation occurs it rarely remits. The German MAS study investigated serum IgE levels to aeroallergens at 2, 5, 7 and 10 years in 273 children and found the prevalence of sensitisation to each allergen increased steadily throughout childhood, and a hierarchy of sensitisation prevalence (grass > birch> house dust mite (HDM)> cat> dog) was maintained from 5 years of age onwards. A mono-sensitisation state was relatively short (measurable half-life = 3 years) as additional sensitisations were acquired frequently, and relatively soon after the first one. Remission of weak sensitisation was also quite frequent, especially before 5 years of age. By contrast, stronger IgE responses were more likely to be persistent. Early sensitisation was associated with a higher tendency for poly-sensitisation at 10 years of age[201].

4.2 Allergen exposure and sensitisation

The German MAS longitudinal study showed that high levels of allergen exposure (defined as Der p 1 and Der f 1 level present in carpet dust, collected at 6 months and again at 18 months by vacuuming undertaken by the subject's family), were associated with sensitisation in the first three years of life[202]. Sporik et al showed a non-significant association between house dust mite exposure in infants (those with the highest quartile of der p1 in their mattresses and carpets) and sensitisation at age 10-11[180]. Cullinan et al found no significant linear association between allergen exposure and sensitisation at age 5 but their results suggested that at low levels of allergen exposure there

was a linear relationship between exposure and sensitisation which was attenuated at higher exposure levels[36]. However, control of the environment, by reducing antigenic load during infant years, did not improve sensitisation rates in the longitudinal MAAS study[203]. The MAPS also found no evidence of an association between the rate of sensitisation, defined by skin prick tests at age 1 year or age 7 years, and the level of house dust allergen[107]. This evidence suggests that the level of exposure may not be associated with the prevalence of sensitisation.

4.3 Studies of total serum IgE and asthma

A decade after IgE was first discovered, Brown et al showed that heightened levels of IgE were associated with allergic respiratory diseases and rhinitis, and that skin prick test reactivity correlated with both total and specific IgE[204]. However, it has been difficult to establish normal values for serum total IgE[205] and subjects who have what is considered to be the non-atopic range for total serum IgE (i.e. below 30iu/ml) may still have a raised positive specific IgE to house dust mite allergen[38]. Burney et al as part of an international European Centre Respiratory Health Study (ECRHS) found that there are substantial variations in the prevalence of positive skin prick test and the level of serum IgE worldwide, and that the variations were independent of each other and likely to be largely environmental in origin[195]. Sears et al reported, in 1991, that asthma risk was related to total IgE in 11 year olds. They found that no child with IgE levels below 32iu/ml had asthma whereas 36% of those with total IgE levels above 100iu/ml did[206]. Burrows et al investigated the association of self-reported asthma with serum total IgE levels and skin-test

reactivity to common allergens in 2657 subjects in a general-population study in Tucson Arizona. They found that regardless of the atopic status of the subject, the odds ratio for asthma increased linearly with the log serum total IgE level after controlling for possible confounders and the degree of reactivity to skin tests ($p< 0.0001$)[41]. An analysis of the Spanish data from the ECRHS confirmed that asthma was associated with serum total IgE independently of specific IgE levels. Serum total IgE above 100iu/l was especially likely to be associated with current asthma in this study[207]. In further studies serum total IgE has been linked to greater BHR[208].

4.4 Exclusion of allergens as prevention of allergic disease

Sporik et al showed that children who had been exposed to high levels of house dust mite in the first year of life were more likely to suffer with asthma at the age of 11[180]. Subsequent research[209, 210] has attempted to exclude allergens, such as house dust mite, that have been thought to pose a risk of atopy and asthma but has found no benefit despite evidence that significant allergen exclusion was achieved. There is no evidence that exclusion of one antigenic substance early in life reduces the risk of asthma[203, 211], although the exclusion of multiple allergens from birth to 12 months of age in the Isle of Wight study has been associated with a reduction in the risk of developing asthma at age 18 years[212].

In conclusion, although controversy remains regarding the causal pathway linking atopy and asthma, the importance of immunological markers of atopy in the asthma syndrome is clear. For this reason it is important for us to

investigate the atopic status of our cohort, how different measures of atopy compare in this cohort, and with what symptoms these markers of atopy are associated.

4.5 Aims and objectives:

The aim is to investigate changes in atopy, defined through both skin prick tests and IgE, from birth to age 23 years and to investigate associations between atopy and outcomes at age 23.

Objectives

- To investigate the changing pattern with age of atopy as assessed by a positive skin prick test and by total IgE
- To compare definitions of atopy based on total IgE and on skin prick tests to assess if a cutpoint can be determined for total IgE, above which atopy can be defined in this cohort .
- To investigate the association of atopy, using both of these definitions, with outcomes at age 23 years
- To investigate the prevalence of atopy among subjects who wheeze in the MAP study cohort
- To investigate the association of total IgE with outcomes at age 23 years after adjustment for atopy (defined as at least one positive skin prick test), as a way of signifying a relationship between total IgE and asthma that is beyond atopic status.

4.6a) Methods

Details of methods for skin prick testing are given in chapter 2. Skin prick testing was undertaken at age 6 months to 12 allergens (cat, egg, grass pollen, tree pollen, milk, soya, house dust mite, feather, fish, flour, *Aspergillus sp*, dry rot) and at 1 year to 14 allergens: the above 12 and also house dust and weed. At age 7 years cat, egg, grass, milk, house dust mite, mould 1 (mixture containing [fungi imperfecti] *Cladosporium sp* and *Alterneria sp*) and mould 2 (*Alterneria sp*) were tested. At age 23 years subjects were tested for the following allergens: house dust mite (HDM), cat, dog, grass, tree, moulds, milk, peanut, eggs. At each age, atopy was defined as having a positive response to at least one allergen.

Total IgE was measured in maternal blood taken during pregnancy. Total IgE was also measured in cord blood, and samples were taken for total IgE at age 3 months, 1 year, 7 years and 23 years. The number of samples taken at age 1 was small (only 13.1% of the cohort) and therefore total IgE results at age 1 have been disregarded.

4.6b) Statistical methods

For basic statistical methods please refer to chapter 2. Total IgE is usually normally distributed after logarithmic transformation[213] and all analyses have used the log of IgE. Linear regression analysis was used to investigate relationships between log total IgE at different ages adjusting for randomisation status and for gender, a known confounder. The results were

summarised using the values of R^2; this measures the square of the multivariate correlation coefficient. This test gives an indication of how much variation in the outcome variable is due to the explanatory variables.

4.6c Defining atopy using total IgE

To define atopy using total IgE we need to define a cutpoint such that any value of total IgE at least equal to this value indicates atopy. This is achieved by relating the total IgE levels to the results of skin prick testing, taking those as the gold standard. There are several criteria for choosing the 'optimal' cutpoint. One approach to establishing a cutpoint for total IgE is to take a fixed threshold based on previous work. This was the approach taken by Sunyer et al in the ECRHS multicentre study in 1996 when they used total IgE above 100iu/l based on previous studies to define a cutpoint for IgE. They found that subjects with an IgE above 100 were more likely to have current asthma even if they were negative for specific IgE[207]. However this approach may not be correct for every population since total IgE is likely to be specific to each population. For the MAPS cohort we will use other methods to define our cutpoint. One is based on sensitivity and specificity of IgE for a positive skin prick. The sensitivity of a diagnostic test is the proportion of subjects, who are truly positive, who are correctly identified by the test. The specificity is the proportion of subjects who are truly negative that are correctly identified as negative by the test. The Receiver Operator Characteristic (ROC) curve is a graphical representation of how well a diagnostic test measures against a 'Gold Standard', such as a clinical diagnosis of a disease. It plots sensitivity

against 1 minus specificity and the area under the curve (AUC) is a measure of the diagnostic ability of the test. An AUC of 1 differentiates perfectly between diseased persons (sensitivity = 1) and healthy persons (specificity = 1)[214]. An AUC of 0.5 means that, overall; there is a 50-50 chance that the biomarker will correctly identify diseased or healthy persons as such. The ideal test would have an area under ROC curve of 1, whereas a random guess would have an area under ROC of 0.5[215].

It is desirable to choose a cutpoint that has high values for both sensitivity and specificity but varying a threshold usually leads to an increase in one at the expense of a decrease in the other. One widely-used criterion is to choose the cutpoint which maximises the sum of the sensitivity and specificity. A second criterion is based on choosing a cutpoint which gives a similar prevalence of atopy based on the total IgE to that using skin prick tests.

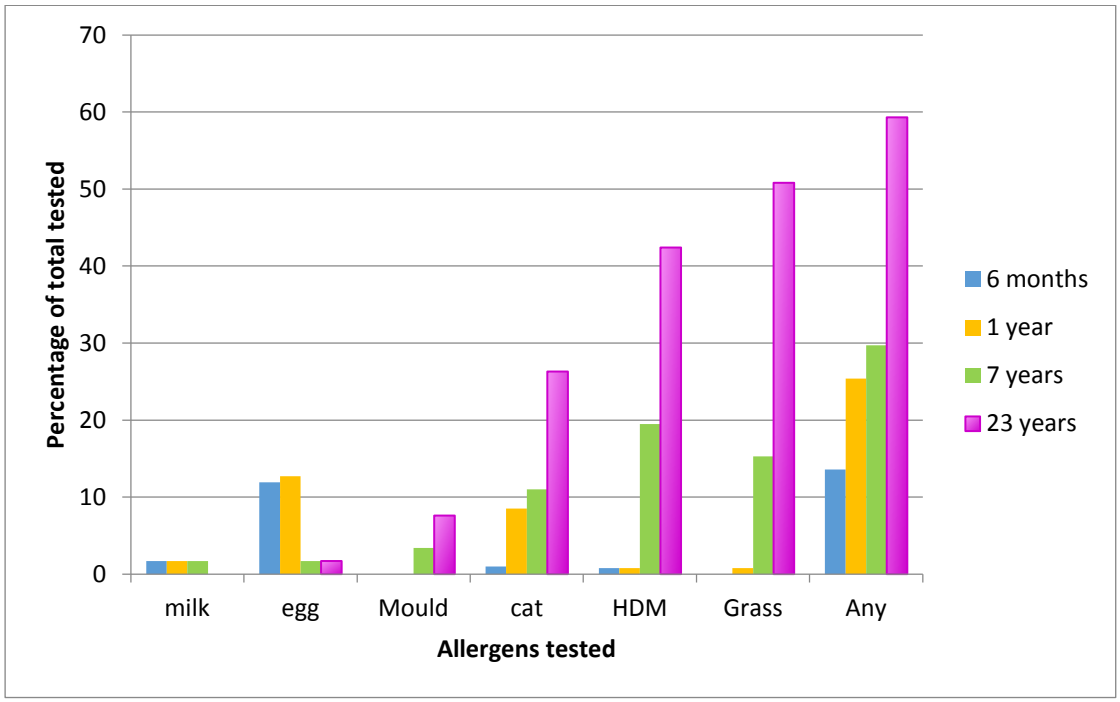
4.7 Results

4.7a Skin Prick Test results

Figure 4.1 shows the prevalence of skin prick positivity to six different allergens at ages 6 months, 1, 7 and 23 years. These are based on the subjects who had skin prick tests at all of these ages. The results show an increase in atopy with age, with a rate of 9% at 6 months of age, 18% at age 1 year, 25% at age 7 years and 59% at age 23 years. Food allergen sensitivity peaked in early childhood and then returned to a low level by age 7 years. The prevalence of HDM sensitivity increased from 0.8% at 6 months to 42.4% at

23 years, while the prevalence of egg sensitivity was 11.9% at 6 months, reached a peak of 12.7% at age 1 year and fell to 1.7% at age 23 years.

Figure 4.1: Positive skin prick test to 6 different allergens over time in those tested on all four occasions.



Changes in positive and negative skin prick tests, over time, for the 118 subjects who had a skin prick test on every occasion, are tracked in Table 4.1 and show that all those who were positive in their first 6 months of life were also positive at age 23 although some had a period of negative skin prick test in their childhood. Of 30 subjects who had a positive skin prick test at age 1 year, 12 were positive at age 6 months and 18 were negative at 6 months. On the whole, the subjects were more likely to become positive later in life if they had been positive earlier in life. Over the course of the study just under a third of subjects who were tested on every occasion were never atopic, while another third were only atopic on one out of the four occasions.

Table 4.1 The time course for the positive skin prick test of the 118 subjects who were tested at all four ages.

Age		6 months		1 year		7 years		23 years	
		positive	negative	positive	negative	positive	negative	positive	negative
6months	posi	16		12	4	12	4	16	0
	neg		102	18	84	23	79	54	48
1 year	posi			30		14	16	21	9
	neg				88	21	67	49	39
7 years	posi					35		34	1
	neg						83	36	47
23 years	posi							70	
	neg								48

Posi= positive skin prick test

neg=negative skin prick test

=positive at earlier age and remains positive

=positive early and negative later

=negative early and then positive

=negative up to this time point

Table 4.2 shows the relationships between atopy at different ages, emphasising this tracking of atopy throughout the study period. A positive skin prick test early in life means that a positive skin prick test later in life is more likely. There is a significant association between an early positive skin prick test and positive skin prick tests later in life. Few subjects had skin prick test

undertaken at age 23 years and therefore the confidence intervals are wide, though the effect sizes are quite striking.

Table 4.2 Association between skin prick testing at different ages for all subjects who underwent skin prick testing at each age

Earlier age	Later age	OR (95% CI)	P value
6 months	1 year	11.19 (5.69-21.99)	<0.0001
	7 years	6.46 (3.27-12.77)	<0.0001
	23 years	NA†	NA†
1 year	7 years	3.54 (2.10-5.97)	<0.0001
	23 years	1.73 (0.71-4.23)	0.16
7 years	23 years	48.38 (6.32-370.66)	<0.0001

† OR cannot be calculated as all subjects who were atopic at age 6 months were also atopic at age 23 years

4.7b) Serum total IgE results

We will now present changes in total IgE over the years in this cohort. The distribution of IgE is well known to be highly skewed and therefore we have taken log values to undertake analysis and report the geometric mean. Table 4.3 summarises the distribution of IgE at different ages using the geometric mean, median, maximum and minimum. The maximum levels are found at age 7 years and 23 years.

Table 4.3 The value of IgE in maternal blood, cord blood and subjects at age 3 months, 7 years and 23 years.

	Number tested	Minimum	Maximum	Median	Geometric Mean
Maternal IgE	433	0.2	10240.0	42.0	42.0
Cord Blood IgE	386	0.1	447.0	0.3	0.3
3 months IgE	450	0.1	151.00	1.5	1.5
7 year IgE	338	0.1	4137.00	44.0	47.6
23 years	99	0.3	2287.00	44.5	47.4

Table 4.2 showed that there were strong associations between atopy (defined as positive skin prick test) at different ages. Although atopy has not yet been defined using IgE, Table 4.4 shows associations between IgE at different ages. Linear regression analysis has been used to analyse total IgE at different ages and the results are summarised using the values of R^2; this measures the square of a multivariate correlation coefficient but here it has been adjusted to show the percentage of variation explained only by the earlier IgE value. As with atopy defined using positive skin prick tests (table 4.2), there are substantial associations between IgE levels at different ages. There is also an increase in median IgE up to age 7 years (table 4.3) but the behaviour from age 7 to age 23 years is very different to skin prick test results which continue to rise especially for aeroallergens as shown in figure 4.1.

Table 4.4 The relationships between log serum IgE at different ages

Earlier age	Later age	R^2	P value
Newborn (cord)	3 months	0.057	<0.0001
	7 years	0.019	0.076
	23 years	0.004	0.589
3 months	7 years	0.101	<0.0001
	23 years	0.095	0.002
7 years	23 years	0.284	<0.0001

†adjusted for gender and randomisation group and the R^2 values are derived from the variation that are not explained by these two variables.

4.8 Definition of atopy as determined by total IgE- Establishing a cutpoint of serum total IgE to define atopy in the MAP study cohort

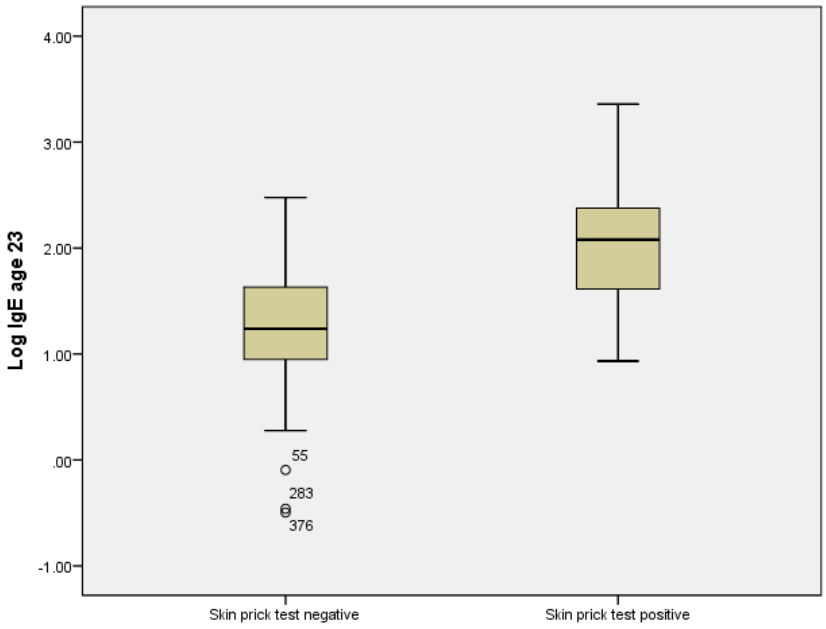
Although we know that high values of total IgE are associated with atopy and asthma, it is difficult to demarcate the upper limit of normal in a population. However not all populations are the same and it may be that the population of the MAP study may have a natural cutpoint. With this in mind we will attempt to establish a cutpoint of total IgE that may define atopy in this cohort.

One approach to establishing a cutpoint for total IgE is to take a fixed threshold based on previous work. Another approach is to use the skin prick

test results, as a ‘Gold standard’ and to choose the threshold for IgE in relation to this standard. Having a positive skin prick test is a binary outcome which we can use to define atopy. We need to find a threshold above which atopy is indicated, i.e. one that can define an increased risk of atopic disease. However, we must first prove there is an association between positive skin prick test and log total IgE.

Figure 4.2 shows there is a relationship between total IgE at age 23 years and skin prick testing at age 23. Using the student t test the mean log total IgE at age 23 in those who are skin prick test positive is significantly higher than those who are skin prick test negative $p<0.0001$. Similar is true of log IgE at age 7 years and skin prick test result at age 7 years ($p<0.0001$) and for log IgE at 3 months and skin prick test result at 6 months ($p<0.0001$). This shows there is a relationship between total IgE and skin prick test results in our cohort but does not necessarily mean that we can define atopy reliably using total IgE.

Figure 4.2 Distributions of log IgE at age 23 years for subjects who are skin prick positive and negative at age 23 years



Using our own data to establish a cutpoint there are two possible methods: one is to find the threshold for total IgE which maximises the sum of the sensitivity and specificity against a gold standard such as a positive skin prick test, while another is to choose a cutpoint which gives approximately the same prevalence as the gold standard (point prevalence).

Figure 4.3 shows the ROC curve for the analysis at age 23, plotting sensitivity against 1-specificity over all possible cutpoints with atopy defined by skin prick testing as the 'Gold Standard'. The ROC curves for the other ages are not shown. The area under the ROC curve (AUC) is 82% here. At 3 months, relating total IgE to positive skin prick test at age 6 months, the AUC was 0.59

which is only just above 50%. At age 7 the AUC was 0.79, this suggests that at age 3 months the total IgE can only just discriminate between atopic and non-atopic subjects as defined by skin prick test at age 6 months, while at ages 7 and 23 the total IgE is a much better discriminator. This may be at least partly because there was no time lag between the two tests being undertaken, as there was when the subject was an infant.

A perfect test would have sensitivity and specificity both equal to 1. If a cut-off value existed to produce such a test then the sensitivity would be 1 for any non-zero values of 1- specificity. The ROC curve would start at the origin (0,0), go vertically up the y-axis to (0,1) and then horizontally across to (1,1). A good test would be somewhere close to this ideal[215]. The ROC curve in figure 4.3 approximates this idealised curve but is not perfect.

Figure 4.3 ROC of log IgE age 23 years compared to positive skin prick test age 23 years

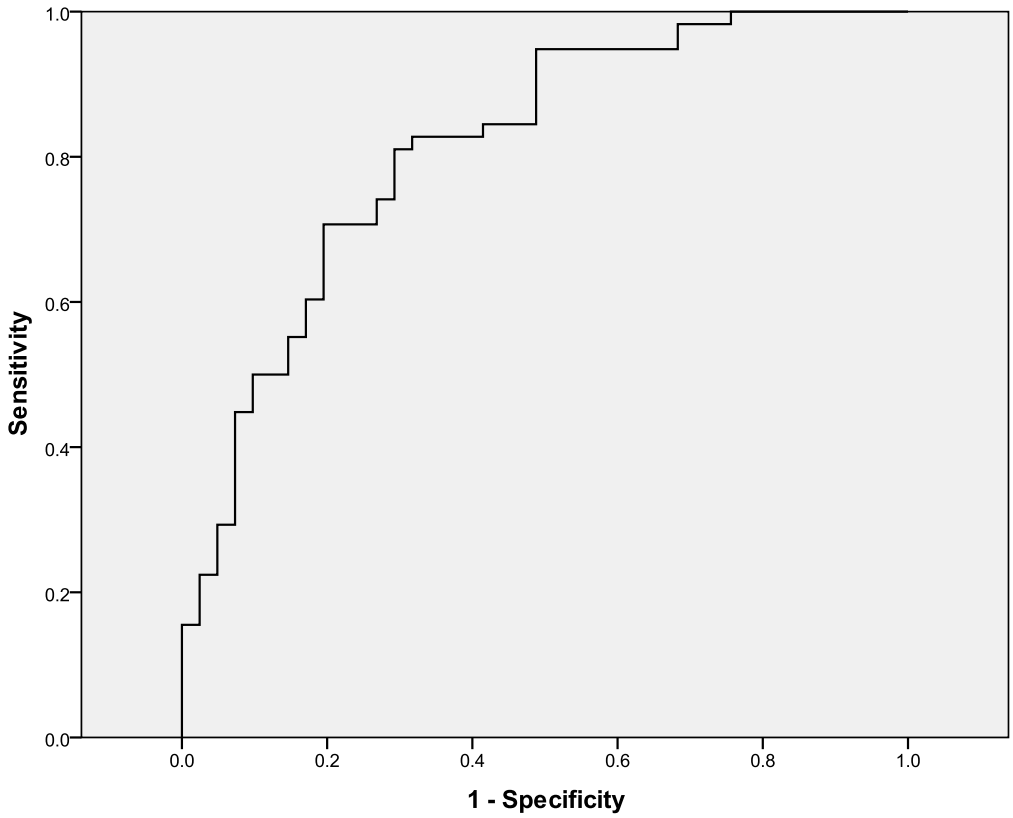


Table 4.5 shows cutpoints for total IgE at ages 3 months, 7 years and 23 years, based on maximizing the sum of the sensitivity and specificity, as well as the sensitivity and specificity and the prevalence for that cutpoint, that is the percentage of subjects whose IgE value exceeds the cutpoint. At age 3 months, the cutpoint is only 1.45 iu/ml and the prevalence of atopy, based on this cut point, is 52% compared to 9.4% using skin prick testing. This suggests this is a poor cutpoint for defining atopy at age 3 months. At age 7 years the cutpoint is higher at 45.5 iu/ml, with a consequent prevalence of atopy of 48%, compared to 25.3% using a skin prick test. Again the cutpoint

could be considered too low as too many subjects have total IgE above the cutpoint. At age 23 the cutpoint of IgE is a little higher than at age 7 and the prevalence of atopy is 48% based on this, compared to 58% using skin prick tests.

Table 4.5 IgE cut off, equivalent to a positive skin prick test, calculated using sensitivity and specificity.

Age of Serum IgE level	**Total IgE cut off based on maximised sum of Sensitivity and Specificity (iu/ml)**	**Age of SPT undertaken**	**% SPT positive (positive/total tested)**	**Sensitivity**	**Specificity**	**% of subjects with total IgE above cutpoint value**	**PPV**
3 months	1.45	6 months	9.4%	0.79	0.51	52%	0.14
7 years	45.5	7 years	25.3%	0.77	0.61	48%	0.4
23 years	49.2	23 years	58.8%	0.70	0.83	48%	0.85

PPV positive predictive value of this cut off point for IgE compared to positive skin prick testing

SPT skin prick test

These results are rather poor and so using the criterion of ensuring similar prevalences may be more useful. Therefore cutpoints are chosen to approximate the same proportions of positive results we would obtain with our 'gold standard', i.e. the proportion above the cutpoint at age 3 months would be 9.4% and 25.3% at age 7 years and 58.8% at age 23 years. The resulting

cutpoints, and the corresponding values of sensitivity and specificity, are shown in Table 4.6.

Table 4.6 IgE cut off equivalent to positive skin prick test calculated using point prevalence

Age of Serum IgE level	**IgE cut off based on point prevalence (iu/ml)**	**Age SPT undertaken.**	**% SPT positive (positive/total tested)**	**Sensitivity**	**Specificity**	**% of subjects with total IgE above cut off value**	**PPV**
3 months	7.7	6 months	9.4%	0.33	0.93	9.6%	0.34
7 years	131.0	7 years	25.3%	0.56	0.85	25.1%	0.55
23 years	35.0	23 years	58.8%	0.80	0.73	58.6%	0.80

PPV positive predictive value of this cut off point for IgE compared to positive skin prick testing

SPT skin prick test

Using point prevalence to find the cutpoint, there is an improvement in the positive predictive value at ages 3 months and 7 years, but no change at age 23 years although the positive predictive value was already relatively high at age 23 years. The important issue is whether or not this cutpoint for total IgE can define the clinical syndrome of atopy and we will therefore investigate if there is an association between atopy, defined using these cutpoints, and asthma and wheeze at age 23 years.

4.9 Association between markers of allergy and wheeze and asthma at age 23 years

In this section we will investigate associations between atopy, defined using total IgE levels, and outcomes at 23 years and compare these to associations between atopy defined as one or more positive skin prick tests and outcomes at age 23 years. These results, including odds ratios and corresponding confidence intervals, are given in table 4.7. Neither version of atopy at age 3 months or 6 months is associated with outcomes at age 23 years. However, a positive skin prick test at both 1 year and also at 7 years does have a significant association with both wheeze and asthma at age 23 years. A high total IgE using point prevalence to determine the cutpoint at age 7 years is associated with asthma, but not wheeze, at age 23 years. At age 23 years the odds ratios are high but the small sample sizes mean the results are mostly not significant, as can be seen from the wide confidence intervals. Atopy, based on IgE and a cutpoint ascertained by using the ROC, i.e. maximising the sum of sensitivity and specificity, gives larger effect sizes, as shown in table 4.7, at age 23 years than when using a cutpoint based on matching the prevalence.

Table 4.7 Associations between three different definitions of atopy* and wheeze and asthma at age 23 years.

Age of total IgE or positive skin prick test	Wheeze age 23			Asthma age 23		
	IgE using sensitivity vs 1-specificity	**IgE using point prevalence**	**Skin prick test**	**IgE using sensitivity vs 1-specificity**	**IgE using point prevalence**	**Skin prick test**
3 months	1.03 (0.63-1.70) 0.50	1.17 (0.5-2.77) 0.44		1.16 (0.62-2.16) 0.38	1.27 (0.45-3.60) 0.41	
6 months			1.52 (0.73-3.16) 0.18			1.87 (0.81-4.29) 0.11
1 year			2.35 (1.30-4.24) 0.004*			1.96 (0.98-3.90) 0.04*
7 years	1.09 (0.63-1.89) 0.44	1.32 (0.71-2.46) 0.24	1.92 (1.11-3.32) 0.019*	2.00 (0.97-4.12) 0.07	2.78 (1.33-5.82) 0.006*	4.26 (2.23-8.11) < 0.0001*
23 years	2.15 (0.95-4.85) 0.07	1.41 (0.62-3.20) 0.27	2.88 (1.33-6.25) 0.005*	3.01 (1.10-8.23) 0.02*	2.22 (0.79-6.29) 0.10	2.30 (0.93-5.71) 0.052

Unadjusted results

*Atopy defined using three different definitions, 1 IgE with cutpoint using ROC, 2 IgE with cutpoint using point prevalence of skin prick test and 3 positive skin prick tests

4.10 Relationship between measures of allergy and asthma and wheeze at age 23 years

This cohort has a high prevalence of wheeze (32.8%) and asthma (17.6%) at age 23 years. The rate of positive skin prick test in each was 73.1% and 73.3% respectively. To determine the relationship with IgE we can model the relationship between wheeze and asthma and Log IgE by using binary logistic regression. Before undertaking binary regression to model the relationship between log IgE and asthma at age 23 years we must check for a linear relationship between logit (p (asthma)) and log IgE. We need to undertake a similar procedure for wheeze. To do this we have used the Hosmer Lemeshow test which is available on SPSS. This test did not give a significant result for either asthma or wheeze, suggesting we do not have to reject the model. Log IgE at age 23 is significantly associated with asthma age 23 years OR 2.82 (95% CI 1.22-6.52) p=0.015. When this is adjusted for skin prick test result, the analysis is no longer statistically significant but the OR remains high OR 2.25 (95% CI 0.88-5.79) p=0.09. The OR for log IgE and wheeze is 1.72 (0.93-3.19) p=0.086 and when adjusted for positive skin prick test the OR is 1.27 (0.6-2.67) p=0.52. The results of the association with log IgE suggest that the relationship of IgE with asthma is different to the relationship with wheeze.

4.11 Prevalence of atopy (defined by positive skin prick test) amongst subjects who wheeze

Childhood atopy in this cohort appears to have a significant but low effect on wheeze. Table 4.8 illustrates that among those who wheeze, from age 6 months to age 23 years, atopy increases from 13.3% at age 6 months to 72.5% at age 23 years. Of 28.8% who wheeze at age 7 years, only 50% are atopic. A total of 21.1% of those subjects who had never had a positive skin prick test result had a wheeze at age 23 years. Atopy becomes more common the older the subjects become, and was relatively uncommon in childhood. This cohort appears to suffer, commonly, from non-atopic wheeze in childhood and atopic wheeze and asthma in adulthood.

Table 4.8 Prevalence of atopy in those who wheeze at ages 1, 7 and 23, in 118 subjects available for skin prick testing at 1, 7 and 23 years.

Age	Number who wheeze	Number who are atopic	Atopic subjects with wheeze /total no. with wheeze
6 months†	30 (25.4%)	16 (13.6%)	4/30 (13.3%)
1 year††	36 (30.5%)	30 (25.4%)	11/36 (30.6%)
7 year	34 (28.8%)	35 (29.7%)	17/34 (50.0%)
15 year	37 (33.9%)	NA	18/31 (58.1%)†††
23 years	51 (43.2%)	70 (59.3%)	37/51 (72.5%)

† Subjects asked on 2 occasions if wheeze in past 3 months at age 3 months and age 6 months
†† Subjects asked on 3 occasions if wheeze in past 3 months at age 3 months and 6 months and if wheeze in past 6 months at age 1 year. Wheeze at 6 months is subset of wheeze at 1 year.
††† Atopy at age 7 used for this calculation since skin prick test not undertaken at age 15 years.

Of the 118 subjects who were tested for atopy on all four occasions, 29 (24.6%) had asthma at age 23. These subjects with asthma had a rate of atopy of 72% which was about the same as those who had a wheeze at age 23 years.

Figure 4.4 shows the rate of wheeze by age in subjects who were atopic at age 7 years and those who were not atopic at age 7 years. Table 4.9 gives OR for the association between atopy at age 7 and wheeze at the stated age.

Figure 4.4 Wheeze prevalence in those who are and are not atopic at age 7 years

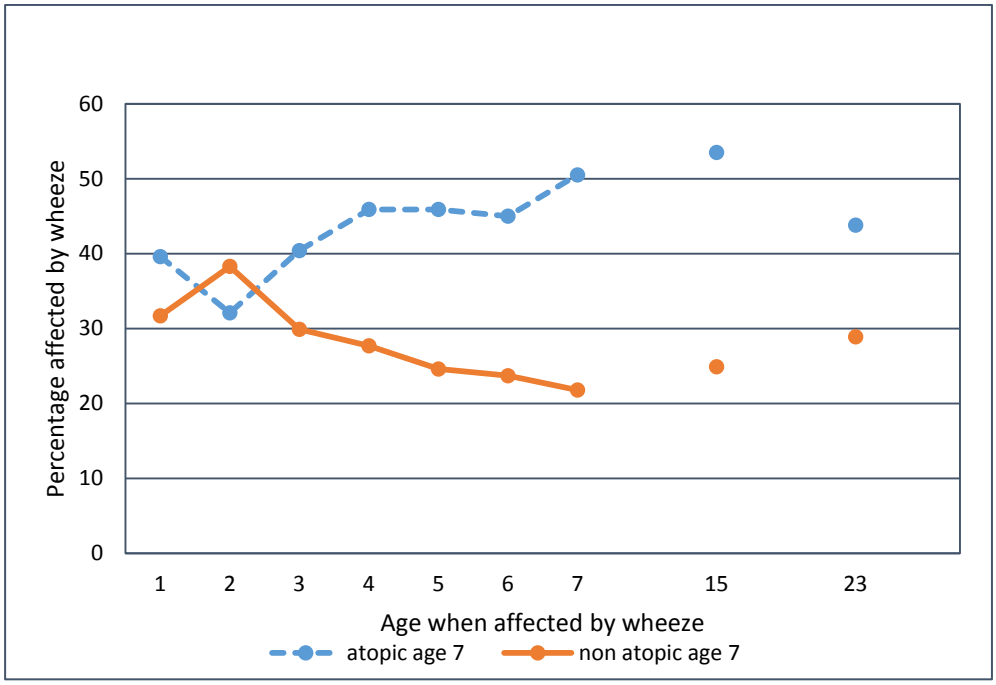


Table 4.9 Year of wheeze and association with atopy at age 7 years.

Age of wheeze	OR (95% CI) p=
1 year	1.41 (0.90-2.23) p=0.14
2 years	0.76 (0.48-1.21) p=0.25
3 years	1.59 (1.01-2.49) p=0.045*
4 years	2.21 (1.41-3.46) p=0.001*
5 years	2.60 (1.65-4.09) p<0.0001*
6 years	2.63 (1.67-4.16) p<0.0001*
7 years	3.65 (2.31-5.78) p<0.0001*
15 years	3.47 (2.08-5.77) p<0.0001*
23 years	1.92 (1.11-3.32) p=0.019*

* Significant results

Even though absolute numbers of atopy are low, in this cohort, figure 4.4 shows us that childhood atopy (at age 7 for this cohort) is associated with an increased risk of wheeze up to adulthood.

4.12 Discussion

We found that the sensitivity to aeroallergens increased from age 6 months to age 23 years. These findings have been shown in a number of other studies. The longitudinal study from the Isle of Wight reported an increase in allergic sensitisation to at least one allergen from 19.7% at age 4 years, to 26.9% at age 10 years and 41.3% at age 18 years[216]. The TCRS found an increase in prevalence of sensitisation from age 6 years (38.6%) to age 11 years (58.1%). The Swedish (BAMSE) longitudinal study also found an increase in atopy up to age 8 years, especially sensitisation to aeroallergens which increased from 15% to 25% between ages 4 and 8 years[194].

There was a rise in sensitivity to aeroallergens over time, while that to food allergens decreased to near zero by early childhood. The reason for the transitory nature of food allergen sensitisation, and the more permanent nature of aeroallergen sensitisation, is not clear but may be related to the large amount of food allergen that children are exposed to early in life compared to the small amounts of aeroallergen. It has been hypothesised by Holt that the infant is exposed to many food allergens and therefore we must have a highly effective mechanism for selective down-regulation of the immune response to ubiquitous environmental allergens found in the gastrointestinal tract. Holt suggests that this mechanism forms the basis for long-term 'protection' of the majority of individuals against allergic sensitisation to many food and inhalant allergens, and that the occasional failure of this process is responsible for food

allergies and leads to lgE production against these allergens[217]. In short advanced mechanisms of tolerance induction probably occur when allergen levels are high. It is likely that such tolerance induction can only occur early in life. One example of tolerance induction in individuals exposed to large amounts of allergen was shown by Nilsson et al, who found that children born during the tree pollen season were less likely to develop allergic rhinoconjunctivitis or IgE antibodies to pollen, than children born during the rest of the year[218].

We have reviewed the relationship between two immunological measurements, and found that there is a significant relationship between positive skin prick test at age 23 years, 7 years and 6 months and log IgE concurrently at 23 and 7 years and log IgE at 3 months respectively. This close relationship between total IgE and positive specific sensitivity has been shown before. Oryszczyn et al found a strong association between total IgE and skin prick test result, on two occasions 5 years apart[219]. They also found a higher total IgE level at the initial assessment in those subjects who converted to a positive skin prick test compared to those who were consistently negative and a lower total IgE level at initial assessment in those who reverted compared to those who remained positive.

The German MAS study undertook a longitudinal study of total IgE beginning at the age of 5 years and found that total serum IgE levels in children evolved in parallel with overall specific IgE levels. They found that variations in total IgE levels at school age were closely reflected by variations in overall specific

IgE levels, in their cohort[220]. However they also found there were a number of different longitudinal patterns of total IgE: declining, flat or increasing with different profiles. These studies suggest that although there is a relationship between total IgE and specific sensitivity, they are probably measuring different aspects of allergic disease and therefore they are not equivalent.

To define atopy using the total IgE values, we determined a cutpoint at each age; values above this indicate atopy. We have chosen cutpoints by using the criterion of maximising the sum of the sensitivity and specificity as well as seeking to have a similar prevalence to that using a skin prick test. The results show that at age 3 months the total IgE can only just discriminate between atopic and non-atopic subjects as defined by a skin prick test at age 6 months, but at ages 7 and 23 years it can discriminate reasonably well. It is not clear why the strength of the association between skin prick tests and IgE changes with age in this way.

We found that the rate of wheeze at each age was high but despite the fact the subjects were at high risk of atopy, only 50% of those who wheezed at age 7 years were atopic. The Isle of Wight longitudinal study also found that 50% of 10 year olds who had wheeze were atopic and 50% non-atopic, however their study was a population study with only 10% of subjects having wheeze[221]. A Spanish cross-sectional population study of 9-12 year olds found that the prevalence of current wheeze was 13.1% and 62.4% of children with current wheezing were atopic[222]. In our cohort there was a much higher rate of wheeze, at almost 30% at age 7 years but a low prevalence of atopy (50%) among those who had a wheeze. However atopy was more prevalent at

age 23 years (59.3%) and therefore prevalence of atopy in subjects who had wheeze was also more common (73%). The increased prevalence of atopy as subjects increase in age, cannot be due to self-selection of subjects at age 23 since table 4.8 only considers subjects who attended on all four occasions for skin prick testing. It may be that those subjects who attended on all four occasions were more likely to be from higher social class since we know that loss to follow-up was greater in lower social class. It is known that subjects from higher social class are more likely to develop sensitisation, as shown in the NHANES II study where white subjects with education more than 13 years had prevalence of positive skin prick test of 25.0% (SD 1.11) while those with education of 0-8 years had prevalence of positive skin prick test of 12.2% (SD 1.22)[223]. Alternatively it may be that this increase in the atopic nature of subjects longitudinally measured and especially those who wheeze has not been shown previously and is something specific to our population. For some reason our subjects are not too atopic in childhood but it appears that those who wheeze are more likely to be atopic as they get older.

However, a positive skin prick test or raised specific IgE, even in an asthmatic, may only indicate that a subject is sensitised against an allergen, and does not constitute proof that the symptoms of asthma are caused by an allergic response to that particular allergen[224]. Burrows et al investigated parental and child IgE levels and found that in subjects with childhood asthma, there was a significantly higher level of IgE than would be expected on the basis of parental IgE levels. They speculated that the inflammatory changes in the airways of asthmatic children may release mediators that directly or indirectly

tend to elevate serum IgE levels above those expected on the basis of their parents' IgE values[225]. This could explain a previous finding from Burrows et al that there was a very close relationship of asthma prevalence to age and sex-standardized IgE levels regardless of skin-test reactivity[41].

Our subjects displayed an association of total IgE with wheeze and asthma. However this association was not statistically significant once adjusted for positive skin prick test at age 23 years. This was different to the findings of Sunyer et al who showed that asthma was associated with increased levels of total IgE, even in subjects negative for specific IgE to common allergens[207]. This study suggested, like others before [41], that the association of total IgE with asthma was present even in non-atopic asthma sufferers. This is supported by the findings of other studies that have shown that mechanisms of inflammation in severe asthma are the same regardless of the atopic or non-atopic nature of the disease[226]. However the recent use of anti IgE therapy in the treatment of severe asthma[227] has nonetheless established the causal link between IgE and at least the symptoms of atopic asthma, although inception of asthma cannot be confirmed.

Thus we are faced with a conundrum: asthma is associated with atopy, atopy is more common in higher social class but asthma is commoner in lower social class. Despite the significant association with atopy there are likely to be other, important aetiological factors in the development of asthma and these factors may also impact on atopy. We can venture that atopy may be an aetiological factor for asthma in some subjects but in other subjects it may be a

sequela of asthma. As Custovic expressed in a recent review, ' It is increasingly clear that childhood asthma and atopy are not single phenotypes, and it is likely that allergen exposure has different effects on distinct subgroups under the umbrella terms of 'sensitization' and 'asthma'[228]. This view is supported by the worldwide epidemiological studies that have shown that the effect of atopy on the prevalence of asthma varies widely between worldwide centres both in adult disease[229] and paediatric disease[230].

In summary we have characterised atopy over the years of follow up in our cohort. We have defined atopy on the basis of skin prick test positivity and sought to define atopy by total IgE. Although IgE probably has a relationship with asthma which is not dependent on specific sensitivity we have been unable to show this due to the small population tested at age 23 years. These measures of allergy are an important part of the disease process that is asthma but it is not clear if allergy causes asthma, is a bystander in the asthma process, is caused by asthma or maybe a combination of all these.

Chapter 5

The association of infant diet and asthma in adults

5.0 Introduction-Dietary factors in the development of asthma

One environmental factor that the neonate encounters within the first weeks of life is diet. Early tolerance to food is the usual outcome in the development of the human infant. Sensitisation to food allergens is often only a transient state, but the inability to tolerate food allergens in early life is thought to be important in the aetiology of atopic disease such as eczema[231] and therefore early tolerance induction may be a factor in reducing the risk of developing asthma later in life. Holt et al have reviewed the evidence regarding primary sensitisation to aeroallergens in infancy and have argued that the combination of exposure to environmental "risk factors", along with the immaturity of the mucosal component of the infant's immune system, may provide a basis for the increased risk of allergic sensitisation in early childhood[35].

Cows' milk protein has been reported to be a potent allergen in children and cows' milk sensitivity occurs in 2-3% of infants in developed countries[232]. Early use of cows' milk based formula compared to banked breast milk in the preterm neonate has been reported to be associated with an increased risk of atopic disease in infants of atopic parents[233].

Høst et al reported that solely breast-fed children were exposed to small amounts of cows' milk protein in breast milk, but if solely breast-fed they were unlikely to develop cows' milk allergy[234]. Despite the fact that cows' milk protein has been considered allergenic, most alternatives to breast milk

are often based on cows' milk, and recent studies suggest that partially or extensively hydrolysed cow's milk formulae may protect against the development of allergy in high risk children[235].

5.1 Breast feeding and asthma prevalence

Breastfeeding is thought to protect against allergic disease, either by removing allergenic foods or through immunological factors from the mother that suppress the exaggerated immune responses that may lead to asthma. There have been a number of studies investigating the effect of breast feeding on wheeze and asthma at varying ages up to adulthood. However these have not given a clear perspective on breast feeding and protection against atopic disease. Each study has different definitions of outcomes and of breast feeding and this may be the main reason for no clear consensus on whether breast feeding protects or not. Many have not gathered prospective breast feeding history and those that have taken a prospective history have not recorded data weekly. All these studies can therefore introduce recall bias. Kramer et al in 1988 attempted to make sense of the conflicting results with regard to breast feeding and atopy. He developed 12 points to standardise the study of breast feeding with regard to atopic disease[236]. One of these standards is 'non-reliance on prolonged maternal recall' of breast feeding. He stated that because of recent publicity concerning infant feeding and atopic disease, some women who have babies with first degree relatives with atopy may preferentially choose to breast feed in an attempt to "protect" their child. This tends to bias the results against a protective effect of breast feeding,

because the breast-fed infant would be at a greater genetic risk for developing atopic disease[236]. Here we will review the studies in detail, dividing them into cross-sectional and longitudinal studies. The longitudinal studies are further divided into those that do not give a prospective breast feeding history, those that do give some prospective history, but not on a weekly or monthly basis, and those that give a weekly or monthly prospective history.

5.2 Cross-sectional studies on breast feeding and asthma prevalence

Cross-sectional studies depend on recall for a history of breast feeding and although this is not as accurate as a prospective history of breast feeding, it is a way of gathering a large amount of data on a large population.

The ISAAC study on breast feeding was an international cross-sectional study that asked parents to give a breast feeding history when the child was 8-12 years. The researchers investigated the association between breast feeding and wheeze. They divided countries into non affluent (Gross National Income per capita (GNI) less than US$9200) and affluent (GNI greater than US$9200). There was a reduced risk of wheeze with breast feeding in both affluent (adjusted OR 0.87 95% CI 0.78-0.97) and non- affluent countries (adjusted OR 0.80 95% CI 0.68-0.94). There was, however, a stronger protective effect of breast feeding against non-atopic wheeze in non-affluent countries (adjusted OR 0.69 95% CI 0.53–0.90)[20]. Breast feeding was associated with a reduced prevalence of wheeze, especially in non-atopic subjects from non-affluent countries.

5.3 Longitudinal studies with retrospective history of breast feeding

5.3a) The Tucson Children's Respiratory Study (TCRS)

The Tucson birth cohort of 1246 infants was recruited from a Hospital Maintenance Organisation (HMO), a managed care organisation that provides general health care coverage in the United States and the TCRS would therefore be considered a population based study. However it must be remembered that all subjects had to have medical insurance to be part of the HMO[81]. The primary outcome at age 2 years was wheeze; beyond this age the outcome was asthma, defined as physician diagnosed asthma in the child and wheeze or asthma symptoms reported on two or more questionnaires from age 6 to 13 years. Breast feeding history was taken at each health surveillance interview; this appears to be yearly, but full details are not given. In addition a full breast feeding history was taken when the subject was age two years. There was a 90% concordance between the different methods of extracting a history on breast feeding. The authors reported that breast feeding for more than 4 months was associated with significantly lower rates of recurrent wheeze up to 2 years of age (OR 0.45, 95% CI 0.2 to 0.9). Breast feeding was not associated with a lower rate of wheeze or asthma after the age of 2. In fact, there was no association between duration of exclusive breast feeding and asthma by age 13 years in the total group. They did, however, find that where there was a maternal history of asthma there was an increased risk of asthma if the mother breast fed (OR 8.7 95% CI 3.4-22.2)[81]. With this finding the team hypothesised that there might be a maternal asthma factor that is passed through breast milk from mother to baby. However, women who had asthma

were significantly more likely to breast feed exclusively than women who did not have asthma[81] (i.e. reverse causality is a possible explanation of the finding). Asthmatic mothers may be more likely to remember if they had breast fed asthmatic children than mothers of children who are not asthmatic, which is why a prospective history is of such importance.

5.3b) The Dunedin birth cohort

Sears et al studied an unselected birth cohort of 1037 subjects from Dunedin in New Zealand. They were born between 1972 and 1973, recruited at the age of 3 years, and followed up to the age of 26 years[75]. The investigators found that the risk of current asthma (defined as a positive response to the question "Do you (does your child) have asthma?" together with symptoms reported within the previous 12 months) was significantly higher at age 9 years [OR 2·54 (1·45–4·44) 0·0008] and 26 years [1·74 (1·26–2·40) 0·0008] in those that were breast fed. The breast feeding history was taken when the subjects were three years old, and the investigators reviewed notes from a community based nurse programme, which kept weekly records on breast feeding and formula feeding, to check for consistency.

The normal postnatal practice in Dunedin, at the time, was to give a formula feed for the first few nights of the new-born's life. Most women stayed in hospital for 3 to 4 nights and although the authors state that bottle feeding was therefore minimal, others have suggested that one 4 ounce feed of formula milk exposes the baby to as much cows' milk protein as would be present in breast milk after 17 years of breast feeding[237]. However there is evidence that

a one off dose of cows' milk during the neonatal period does not increase asthma symptoms up to the age of 5 years compared to placebo[238]. In summary this study found asthma risk was significantly positively associated with breast feeding but although the breast feeding history was viewed from prospective notes, breast feeding mothers were encouraged to feed their new born babies cows' milk based formula for the first post-natal days. For this reason this study was flawed and may not conclusively show that breast feeding increases the risk of asthma.

5.3c) The Tasmanian asthma study (TAS)

The Tasmanian cohort consisted of 8,000 subjects born in 1961 who were recruited when they were 7 years old. Current asthma was defined as self-reported ever asthma followed by answering ''in the last 12 months'' to the question, ''How long since the last attack?''. Breast-feeding was defined by the question, ''How was he/she fed in the first three months of life?''. The parent could answer (1) breast-fed only, (2) bottle-fed only, or (3) breast-fed and bottle-fed. Exclusively breast-fed was defined as the breast-fed only group. Exclusively breast-fed babies with a maternal history of atopy were less likely to develop asthma before the age of 7 years, (odds ratio[OR] 0.8; 95% CI, 0.6-1.0). After age 7 years, the risk reversed, and exclusively breast-fed children had an increased risk of current asthma at age 14 (OR 1.46; 95% CI 1.02-2.07), age 32(OR 1.84; 95% CI 1.06-3.3), and age 44 (OR 1.57; 95% CI 1.15-2.14) years [239].

5.3d) The Isle of Wight study

Tariq et al investigated 1218 children born in the Isle of Wight[101]. The breast feeding history was taken from parents at age 1 year and 2 years, but not prospectively. A diagnosis of asthma was defined as three or more separate episodes of wheeze, each lasting 3 days or more. There was a statistically significant association between breast feeding and asthma at the age of 4 years. . The OR, comparing infants whose mothers refrained from using feeding formula before the age of 3 months to those who did refrain, was 0.56 (95% CI 0.38-0.83) $p<0.01$.

5.3e) The Stockholm (BAMSE) birth cohort

Kull et al recruited over 75% of all the births in Stockholm from 1994-1996. Asthma age 4 years was defined as 4 or more episodes of wheezing during the past year or just one episode of wheezing in subjects who had a prescription for inhaled corticosteroids. Breast feeding data was collected retrospectively at age 1 year. Exclusive breast feeding was defined as consuming breast milk and no other nutrient. They found that infants who had 4 months or more of exclusive breast feeding had a reduced risk of asthma at age 4 compared to those who had less than 4 months exclusive breast feeding (adjusted Odds Ratio was 0.73, 95% CI 0.53-0.97)[240].

5.4 Longitudinal studies with some prospective breast feeding history but not weekly or monthly

5.4a) The Prevention and Incidence of Asthma and Mite Allergy (PIAMA)

The PIAMA (Prevention and Incidence of Asthma and Mite Allergy) birth cohort study of Dutch children born in 1996/1997 recruited mothers from the general population during pregnancy. Asthma was defined as an episode of wheeze and or a prescription of inhaled corticosteroids. They found breast feeding for more than 16 weeks compared to no breast feeding was protective up to age 8 (OR 0.57 [95% CI0.41 to 0.80])[90]. Breast feeding history was gathered prospectively, but not weekly, as parents gave a breast feeding history at age 3 months and then age 1 year[90].

5.4b) The Western Australian Pregnancy Cohort Study (WAPCS)

WAPCS was established between 1989-92 and recruited 2979 subjects, before birth, by 18 weeks gestation[241]. Asthma was defined as 'ever diagnosed by a physician and wheeze in the last year'. At discharge parents were given a diary to record episodes of illness, feeding history, and other key events in the first year of the child's life. Children were further assessed at 6 years of age. Exclusive breast feeding was defined as using no formula or other milk. After adjustment for sex, gestational age, infection, smoking exposure and maternal asthma, exclusive breast feeding for 4 months was associated with a reduced risk of current asthma in the children at age 6 (OR 0.74 95% CI0.55-1.00 p=0.049)[241]. However, information on the introduction of solid food was not collected and therefore those who the researchers judged as having had

exclusive breast feeding, may have been weaned early, thereby negating exclusive breast feeding. This was a flaw in the study design.

5.4c) The Childhood Asthma Prevention Study (CAPS)

This Australian study recruited 601 babies from high risk families. They defined current asthma as any wheeze in the preceding 12 months in subjects who either had asthma diagnosed by a doctor or at a hospital at any time during the first 5 years or had a positive bronchodilator test at the 5 year assessment. Breast feeding history was taken when subjects were reviewed at 1, 3, 6, 9 and 12 months. They defined 'ever breastfed' as having ever consumed breast milk, even once, and 'fully breast fed' as babies whose mothers reported at the 3 month and the 6 month visit that the subject had not been fed food or breast milk substitute. However, they did not gather information on fruit juice and water consumption, therefore exclusive breast feeding for the time stated cannot be confirmed. A total of 85% were ever breast fed and the OR for asthma was: 0.59 (0.30–1.16). Only 2.3% were exclusively breastfed for at least 6 months, the OR was 0.90 (0.58–1.40). This study found that prolonged breast feeding and late weaning did not reduce the risk of current asthma at age 5 years[242].

5.5 Longitudinal studies with prospective history of breast feeding on weekly or monthly basis

5.5a) Helsinki Birth cohort

Saarinen et al investigated 236 healthy babies born in the first 3 months of 1975 in Helsinki[243]. The breast feeding history was taken prospectively at 2 weeks, then at, 1, 2, 4, 6, 9, and 12 months of age. The outcome they investigated was 'respiratory allergy' which was defined as one of the following: allergic asthma diagnosed at hospital, three or more separate episodes of respiratory distress with wheezing" (at 3-10 years only); history of wheezing in association with three or more respiratory infections (at 3-5 years only); seasonal rhinoconjunctivitis, usually associated with positive prick or RAST tests; or a history of wheezing and/or rhinoconjunctival symptoms associated with animal contact. A total of 150 subjects were followed up until age 17 years. There were three groups of breast feeding history depending on the length of exclusive breast feeding: prolonged (>6 months) 36/150, 24%; intermediate (1-6 months) 66/150, 44%; and short or no (<1 month) breastfeeding 48/150, 32%. Those with short or no breast feeding were more likely to suffer with respiratory allergy (p=0.01, no OR given)[243].

5.5b) The Melbourne Atopy Cohort Study (MACS)

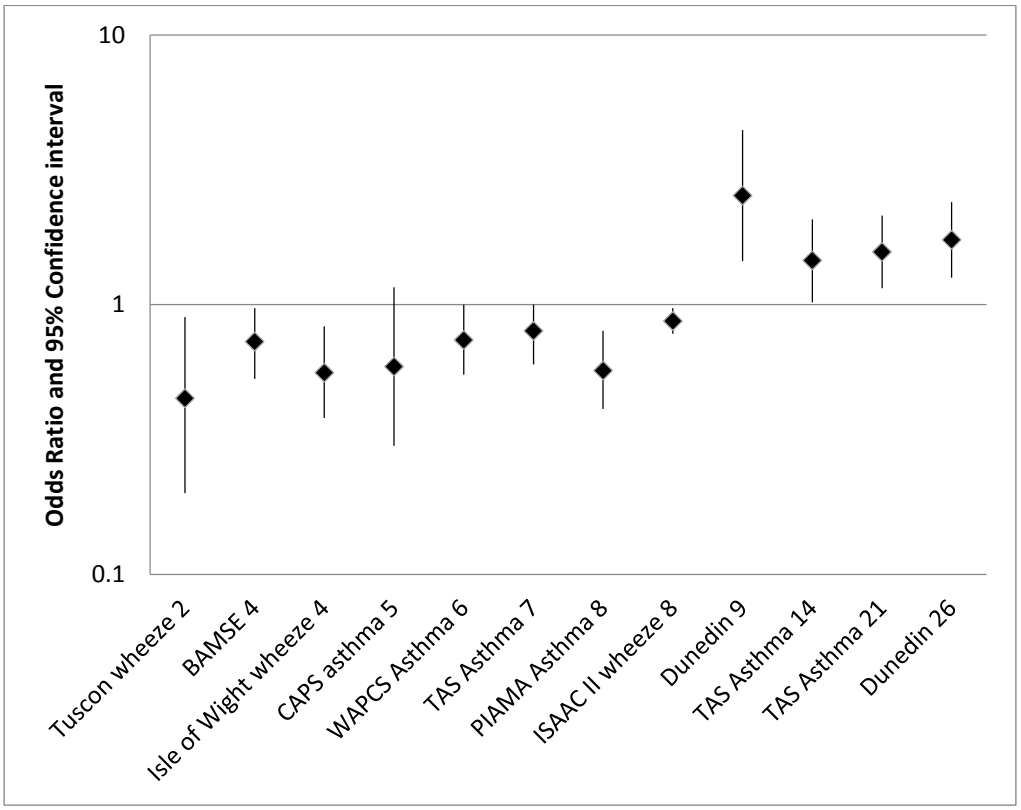
The MACS cohort study was a longitudinal study in the 1990s which investigated 620 children with a family history of atopic disease[77]. Breast feeding history was ascertained by an allergy-trained nurse phoning the nursing mother every four weeks until the subject was age 64 weeks. During

the telephone interview the nurse asked questions related to signs of asthma, eczema, food reaction and use of asthma medications. Skin prick testing was undertaken at 6 months and 12 months. There was a significant relationship between signs of early atopic disease and the mother being less likely to stop breast feeding. This was expressed as a lower rate of cessation of breast feeding if the baby had any early atopic disease, (HR 0.69 [0.52-0.93] p=0.015). This phenomenon, known as 'reverse causality', is a possible explanation for the fact that that longer breast feeding was associated with a greater risk of risk of atopic disease.

5.6 Comparison of results of longitudinal Studies

Figure 5.1 shows odds ratios from the major studies on asthma and breast feeding; the majority of studies show a protective effect with breast feeding in childhood. In adulthood there are only two studies and only one is represented on the graph. The study by Saarinen with outcomes at age 17 years is not included in the figure as no odds ratios were quoted, although that study suggested a protective effect from breast feeding on symptoms in 17 year olds. The MACS study is also excluded as this gives hazard ratios for cessation and atopic features.

Figure 5.1 Association of breast feeding and asthma (log scale)



BAMSE Stockholm age 4 asthma[240], Tucson[81], The Childhood Asthma Prevention Study-CAPS[242], WAPCS[241], PIAMA[90], Dunedin[75], ISAAC II[20], The Tasmanian Asthma Study-TAS[239]

Some studies define breastfeeding as any amount of breastfeeding, not necessarily exclusive, while other studies only consider exclusive breast feeding for 4 or 6 months. This makes it difficult to compare studies. If we choose only the highest quality population studies with prospective data on breast feeding, (CAPS, BAMSE Stockholm and WAPCS studies), these give a favourable outcome in early childhood for breast feeding and asthma. On the whole the evidence suggests that breast feeding may be protective during childhood, but that this protective effect potentially reverses in adolescence and adulthood.

Table 5.1 Longitudinal studies that report on Breast Feeding and their definition of breast feeding

Study group (year initially recruited/birth year if different)	Age of subject at time of study	Outcome measured	Prospective history of breast feeding	Definition of breast feeding
MACs (1990-94)	2	Cessation of breast feeding	Prospective	Exclusive after first few days.
BAMSE Stockholm (1994-96)	4	Wheeze or asthma	Prospective	Exclusive for 4 months
Tucson Children's Respiratory Study (1980-84)	2, 6, 13	Wheeze age 2, asthma age 6-13	Retrospective age 2 but also some cases taken prospectively	Exclusive for 4 months
Isle of Wight (1989-90)	4	Asthma	Retrospective at age 1 and 2	Exclusive for 3 months
CAPS (1997-99)	5	Asthma	Prospective history at 1, 3, 6, 9,12 months	Ever Breast Fed, even once
WAPCS (1989-92)	6	Asthma	Prospective	No account taken of when weaned onto solids
Dunedin (1972-73)	7, 9-26	Asthma	Retrospective at age 3	Exclusive after first few days
PIAMA (1996-7)	8	Asthma	Prospective at 3 months and 1 year	No history on weaning onto food therefore not exclusive
TAS (1968/1961)	7, 14, 21	Asthma	Retrospective age 7 years	Exclusive for first 3 months

5.7 Infant diet and outcomes in adults

Lucas et al showed that early exposure to cows' milk in the preterm neonate was associated with an increased risk of atopic disease in infants of atopic parents[233]. Few longitudinal studies have investigated asthma and early consumption of foods. Zutavern et al investigated preschool wheeze and infant weaning on to foods. They found no evidence that late weaning affected the risk of preschool wheeze. Late introduction of egg (late introduction was not defined but the median of introduction of egg into diet was 8 months) was associated with a non-significant increased risk of preschool wheezing (OR 1.5, 95% CI 0.92 to 2.4)[244]. The Childhood Asthma Prevention Study (CAPS) also found no reduced risk of asthma in 5 year olds with late weaning. The OR of asthma at age 5, comparing children weaned by age 3 months to those not weaned at age 3 months, was 0.65 (0.36–1.15). They did, however, find that solid food introduction, by 3 months of age, was associated with a reduced risk of atopy OR 0.54 (95% CI 0.33–0.87)[242].

Scott et al reviewed the Isle of Wight birth cohort at age 18 years[212]. A total of 120 subjects were randomised from birth to have low exposure to allergens, for the first year of life or to normal diet and environment. Those in the intervention arm were given a strict anti-house dust mite regime and a low allergenic load in their diet including extensively hydrolysed formula in those not breast fed, and avoidance of dairy products, eggs, soya, fish, shellfish, peanut and tree nuts in the first year of life. Asthma was defined as a positive response to 'Has a doctor diagnosed you with asthma?' and, either a wheeze in the last 12 months or use of inhaled corticosteroids. Atopic asthma was defined by the combination of asthma plus atopy, defined as at least one

positive skin prick test. The intervention was associated with a lower rate of asthma at age 18 years (adjusted OR: 0.23, 95% CI 0.08 to 0.70, p=0.01). They statistically tested the hypothesis that the mechanism through which the intervention reduced the risk of asthma was by a reduction in atopy, using a binary logistic regression model which was run with the interaction between atopy and the group as the sole explanatory variable. The interaction term did not have a significant association with asthma at 18 years, which was unsurprising given the small numbers. So although the hypothesis is a plausible one, a larger study would be required to test it properly. In this chapter we will examine the association of diet in early childhood with asthma in early adulthood.

5.8 Aims and objectives

The aim of this chapter is to examine the effect of diet in early life and its association with asthma, wheeze and atopy in adulthood.

Objectives

- To investigate the association between the original randomised groups, of cows' milk exclusion and normal diet, and asthma, wheeze and atopy in early adulthood.
- To investigate the association of breast feeding with outcomes in early adulthood.
- As a subgroup analysis we will investigate associations in subjects who have asthmatic mothers.

- To investigate the association between the age at weaning onto food types and outcomes in early adulthood

5.9a) Methods

Full methods regarding the original randomisation, breastfeeding and outcomes up to age 7 are given in chapter 2 and have been published previously[107]. A total of 487 live births were randomised to the intervention group, in which mothers were asked to exclude all cows' milk protein from the infants' diet for the first 4 months of life, or the control group, which was a normal diet. In order to exclude cows' milk protein, those subjects randomised to the intervention arm of the study were offered free soya protein based infant formula.

Mothers were asked to keep a diary and were visited by a dietitian weekly for the first 26 weeks of the subject's life, in which period data were gathered on the type of milk and food the subject consumed. Details of different feeding routines are given in the questionnaire appendix 2b. The feeding regime of each subject was categorised into one of: Breast milk only, breast and cows' milk, breast and soya supplement, cows' milk only and soya supplement only. Ever having been breast fed was defined as having had breast feeding for at least 1 week. Information was gathered on the age in weeks when the baby first consumed the following foods: beef, cereal, egg, fruit, fish and meat other than beef.

5.9b) Statistical Methods

The original randomisation was analysed on an intention to treat (ITT) basis. This means that after randomisation even those who did not adhere to the proposed diet were analysed in the group to which they were randomised.

Breast feeding was encoded into a binary variable: ever breast fed or never breast fed and was analysed using chi-square tests and logistic regression. The time until weaning on a particular food is a continuous variable, which may have been censored in that the time until a child first ate a food may only be known to be greater than 26 weeks. The distribution of the time until weaning can be estimated using Kaplan Meier methods and different groups compared using the log rank test. Control for confounders was achieved using Cox's Proportional Hazards model. We will use Cox's model to analyse the first exposure to cows' milk, producing an estimate of the hazard ratio, assumed constant, essentially comparing the risk of wheezing in different groups while adjusting for confounders.

5.10a) Results

5.10b) Breast feeding rates and association with outcomes

A total of 180 out of 477 (37.7%) subjects were ever breastfed for at least one week. There was an association between the randomisation group and the prevalence of breast feeding: 78/238 (32.8%) ever breast fed in the intervention group and 104/249 (41.8%) ever breast fed in the control group

(OR 1.47 (95%1.02-2.13) p= 0.041). This statistically significant association may have occurred because soya milk was provided free of charge to the intervention group. Social class was also significantly associated with breast feeding choice: A total of 53 out of 119 (56%) subjects from families in social class I and II were breast fed, while 135 out of 358 (32%) subjects from families in social class III, IV, V or unemployed were breast fed: OR 2.67 (95% CI 1.74-4.07) p <0.0001. Those with parental allergy were more likely to be breast fed: OR 2.15 -95% CI 1.29-3.58 p= 0.003. In addition those who were not breast fed were significantly more likely to be lost to follow up at age 23 years (1.59 95%CI 1.08-2.34 p=0.019).

Table 5.2 shows the results of associations of ever being breast fed or not and outcomes in childhood and at age 23 years. Wheeze at age 1 year was significantly less likely in those who were breast fed. No other associations were significant, though in the majority of cases the odds ratio was less than 1, suggesting breast feeding may protect against wheeze at other ages and asthma at 23 years. The statistically significant association at age 1 would fit with our understanding of the role of breast feeding since it is known that the mother gives immune support through the passage of IgA antibodies in breast milk which protect the infant against upper respiratory tract infections[245]. We will investigate the effects of breast feeding in different wheeze phenotypes in chapter 7 but using the established variables there is no evidence of a significant effect after the age of 1 year.

Table 5.2 Association of breast feeding with allergic manifestations in childhood and at age 23 years

	Ever breast fed†	**Never breast fed**	**Crude OR (95%CI) p**	**Adjusted†† OR (95%CI) p**
Wheeze age 23 years	39/124 (31.5%)	59/175 (33.7%)	0.90 (0.55-1.48) 0.68	0.81 (0.47-1.39) 0.44
Wheeze age 15	45/142 (31.7%)	70/214 (32.7%)	0.95 (0.61-1.50) 0.84	1.03 (0.64-1.68) 0.89
Wheeze age 7	42/170 (24.7%)	86/276 (31.2%)	0.73 (0.47-1.11) 0.14	0.71 (0.45-1.13) 0.15
Wheeze age 1	39/177 (22.0%)	119/284 (41.9%)	0.39 (0.26-0.60) <0.0001*	0.48 (0.30-0.75) 0.001*
Asthma age 23	19/122 (15.6%)	33/174 (19.0%)	0.79 (0.42-1.46) 0.45	0.71 (0.36-1.41) 0.33
Atopy age 23	28/49 (57.1%)	42/70 (60.0%)	0.89 (0.42-1.87) 0.76	0.82 (0.35-1.91) 0.64
Atopy age 7	46/161 (28.6%)	63/269 (23.4%)	1.31 (0.84-2.04) 0.24	1.25 (0.78-2.00) 0.36
Atopy age 1	37/178 (20.8%)	48/291 (16.5%)	1.33 (0.83-2.14) 0.24	1.22 (0.74-2.03) 0.43

* Significant result † Breast fed for at least one week.

††Adjusted for social class age 1, parental allergy, gender and original randomisation.

In the introduction we presented data from the Tucson study that suggested that subjects with asthmatic mothers had an increased risk of asthma if they were breast fed. We have therefore investigated the association of breast feeding in the 94 subjects who had asthmatic mothers. As shown in table 5.3 there was no statistically significant association between breast feeding and wheeze, asthma or atopy at age 23. The ORs for wheeze and asthma at age 23 were less than one, suggesting that there may be a protective effect from breast feeding, but the small sample size led to wide confidence intervals.

Table 5.3 Association of breast feeding with allergic manifestations in childhood and at age 23 years only in subjects with asthmatic mothers

	Ever Breastfed	Never Breast fed	OR (95% CI) p	adjusted§ OR (95% CI) p
Wheeze 1	9/31 (29.0%)	24/63 (38.1%)	0.67 (0.26-1.68) 0.39	0.67 (0.24-1.84) 0.43
Wheeze 7	12/28 (42.9%)	22/59 (37.3%)	1.26 (0.51-3.15) 0.62	1.36 (0.51-3.59) 0.54
Wheeze 15	10/26 (38.5%)	18/45 (40.0%)	0.94 (0.35-2.52) 0.90	1.24 (0.44-3.53) 0.69
Wheeze 23	5/17 (29.4%)	13/34 (38.2%)	0.67 (0.19-2.35) 0.54	0.71 (0.19-2.63) 0.61
Asthma 23	2/17 (11.8%)	6/34 (17.6%)	0.62 (0.11-3.47) 0.59	0.90 (0.13-6.19) 0.92
Atopy 23	3/6 (50.0%)	5/11 (45.5%)	1.20 (0.16-8.80) 0.86	1.22 (0.15-9.98) 0.85

§Adjusted for social class age 1, parental allergy, gender and original randomisation.

There is no evidence that breast feeding is detrimental in our cohort.

5.10c) Cows' milk exclusion and soya formula supplementation up to 4 months- the results of the RCT

Of the 487 subjects, 249 were randomised to normal diet and 238 to soya substitute and cows' milk avoidance. Of those assigned to cows' milk exclusion 41 (17.2%) used cows' milk before 16 weeks of life and of those who were randomised to usual diet, a total of 32 (13%) of subjects had soya in the first 16 weeks of life. At age 23 a total of 304 subjects completed questionnaires but only 123 were available for skin prick testing.

The associations between the randomisation groups and wheeze, asthma and atopy at age 23 years and atopy and wheeze in childhood were analysed on an ITT basis and results are shown in Table 5.4. There was no evidence that exclusion of cows' milk protein was associated with outcomes in childhood, but asthma and atopy at age 23 years were both significantly associated with cows' milk exclusion. The effect was in the opposite direction to that expected, i.e. the intervention was associated with a significantly increased risk of atopy and asthma at age 23 years. It was not possible to elucidate if this result was because the subjects had avoided cows' milk or because they were given soya milk supplement. All we can say is that the substitution of soya based formula instead of cows' milk protein in the first 4 months of life led to statistically significantly increased rates of asthma and atopy at age 23 years compared to the control group. Later we will use survival techniques to analyse time to first use of cows' milk and outcomes at age 23 years.

Table 5.4 Associations between the randomisation groups and allergic manifestations

Allergic disease	intervention group	control group	OR (95% CI) p	Adjusted§ OR (95% CI) p
Wheeze age 23	54/146 (37.0%)	44/153 (28.8%)	1.45 (0.90-2.36) 0.13	1.43 (0.87-2.37) 0.16
Wheeze age 15	61/180 (33.8%)	54/176 (30.7%)	1.16 (0.74-1.81) 0.52	1.11(0.71-1.76) 0.65
Wheeze age 7	58/215 (27.0%)	70/231 (30.3%)	0.85 (0.56-1.28) 0.44	0.89 (0.58-1.36) 0.59
Wheeze age 1	80/227 (35.2%)	78/234 (33.3%)	1.09 (0.74-1.60) 0.67	1.04 (0.69-1.57) 0.85
Asthma age 23	33/145 (22.8%)	19/151 (12.6%)	2.05 (1.0-3.80) 0.023*	1.95 (1.03-3.70) 0.04*
Atopy age 23	41/58 (70.7%)	29/61 (47.5%)	2.66 (1.25-5.67) 0.011*	2.97 (1.30-6.80) 0.01*
Atopy age 7	58/208 (27.9%)	51/222 (23.0%)	1.30 (0.84-2.00) 0.24	1.41 (0.90-2.21) 0.13
Atopy age 1	46/232 (19.8%)	39/237 (16.5%)	1.26 (0.78-2.0) 0.34	1.27 (0.79-2.05) 0.33

§Adjusted for social class age 1, parental allergy, gender and breast feeding.

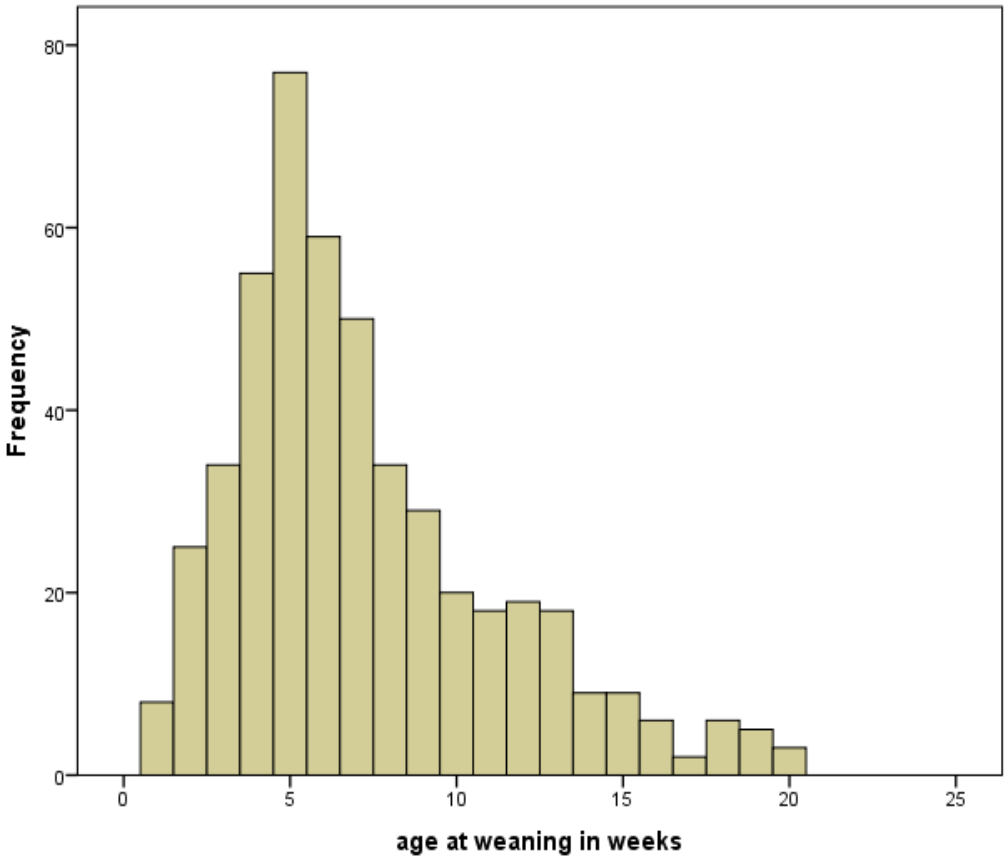
*Significant result

5.11a) Patterns of weaning

All subjects apart from one (486/487) had eaten solid food by 26 weeks. A total of 478 (98.2%) had eaten fruit by 26 weeks, 466 (95.7%) had eaten cereal, beef was eaten by 436 (89.5%) and other meats by 446 (91.6%). Egg was eaten by 357 (73.3%) and fish by only 86 (17.7%) subjects.

Figure 5.2 gives a histogram of the age in weeks at weaning with any food type. The one subject who did not wean by 26 weeks of age has been excluded from the histogram for ease of illustration.

Figure 5.2 Age in weeks when first weaned onto solids



For some subjects and certain foods the age of weaning is known only to be greater than 26 weeks. We have therefore presented the median age at weaning, rather than the mean in Table 5.5. This table also shows that 75% of subjects had eaten some solid food by 9 weeks. Despite the fact that the WHO current recommendation is for sole breast feeding until the child is 26 weeks, in the 1980s the practice, in the MAPS cohort, was for the majority of neonates to be weaned by just over 6 weeks.

Table 5.5 Age in weeks when weaned onto different solid foods

	Minimum	25th Percentile	Median	75th Percentile
Any Food	1	4	6	9
Fruit	1	5	7	12
Cereal	2	9	12	15
Meat other than Beef	3	14	16	20
Beef	3	14	16	20
Egg	3	15	20	>26
Fish	3	>26	>26	>26

By age 12 weeks 75% of subjects had been weaned onto fruit and weaning started as early as 1 week for some subjects. Those who avoided cows' milk were more likely to avoid egg; this was because there are many foods that contain both egg and milk, as has been described in a previous publication regarding this cohort[100].

5.11b) Time to weaning

Using the Student's t test to analyse subjects who were weaned before 26 weeks (all but one subject) it was possible to show that there was a significant difference between age at weaning for subjects who were from non-manual families (mean age at weaning 8.27 weeks) at age 1 year and subjects who were from manual families (mean age at weaning 7.0 weeks) at age 1 year ($p=0.003$). However we can use survival techniques to analyse the data we

have regarding weaning and wheeze. In this case the event of interest is the time to wean the subject. Figure 5.3 shows the Kaplan Meier estimates of the time to weaning, allowing for censoring, subdivided into those who wheezed and those who did not wheeze at age 23. This shows the proportion of subjects who have not been weaned onto solid food by age. At most ages the proportion not weaned was lower in those who wheezed at age 23, suggesting that wheezing was more common in those weaned at an early age. Comparing the distributions using the log rank test showed there was a significant difference between groups (p=0.039).

Figure 5.3 Kaplan Meier graph of time to weaning and wheeze status at age 23 years

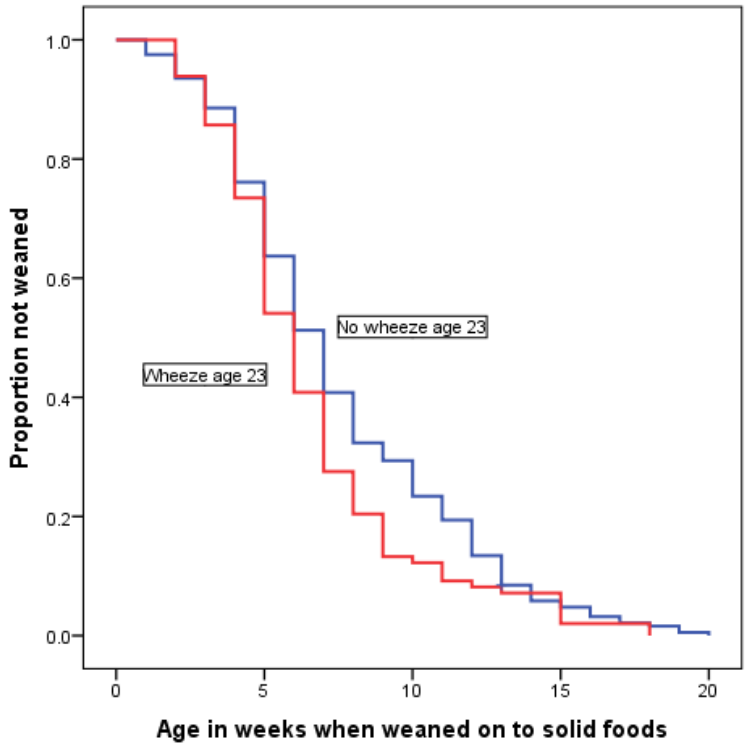
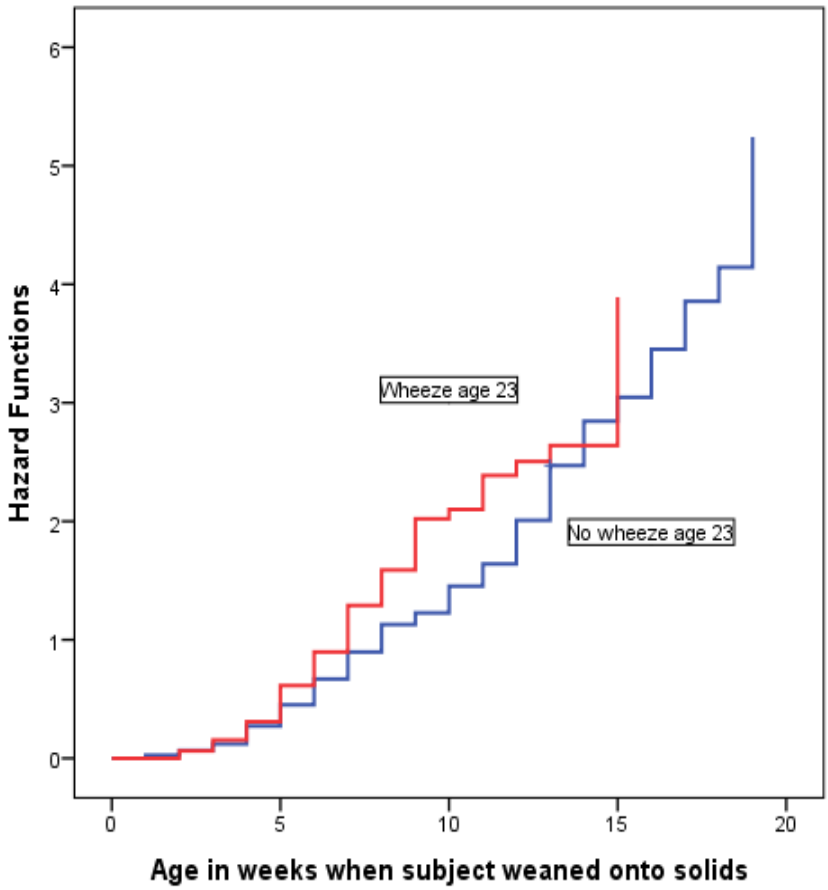


Figure 5.4 also compares the distributions but this time we have a hazard plot comparing the hazard of weaning between those who wheeze and those who do not. The hazard function gives the instantaneous event rate i.e. the probability that an individual is weaned at time t, conditional on them not having been weaned up to that point. The hazard function tends to be higher in the wheeze group, consistent with the survival function plot in Figure 5.3.

Figure 5.4 Hazard function showing age at weaning onto solids by wheeze status at age 23 years.



A Hazard Ratio (HR) greater than 1, comparing those who wheeze with those who do not, corresponds to the weaning distribution being shifted earlier in the wheeze group, i.e. earlier wean would make wheeze more likely. Adjustment for confounders is achieved using Cox's Proportional Hazards model. This assumes that the hazard functions for the two groups have a constant ratio over time; the plot of the survival curves in Figure 5.3 and the hazard plot in figure 5.4 suggests that is not unreasonable.

The results of this analysis are shown in Table 5.7. For wheeze the unadjusted hazard ratio was 1.26. As this is greater than 1 it suggests that the risk of wheeze is greater in those weaned early, but it was marginally non-significant. Adjustment for confounders had little effect as can be seen in Table 5.7.

Table 5.7 Results from Cox's Proportional Hazards Model of age at weaning onto solid food and asthma, wheeze and atopy at age 23 years.

Outcomes at age 23	Asthma	Wheeze	Atopy
Number (censored)	296 (1)	299 (1)	119 (1)
Unadjusted hazard ratio	1.23 (0.91-1.67) 0.17	1.26 (0.99-1.61) 0.06	0.81 (0.56-1.17) 0.26
Adjusted Hazard Ratio†	1.27 (0.93-1.74) 0.13	1.28 (1.00-1.65) 0.052	0.84 (0.56-1.27) 0.41

*Significant result

†Adjusted for social class age 1, randomisation, parental allergy, gender and ever breast fed.

The above analysis refers to weaning on to any food; we now consider each main type of food. There was a significant association between time to weaning on to beef and wheeze at age 23 years, as shown in table 5.8. Associations with weaning on to other foods were not significant, though the relatively small samples sizes led to wide confidence intervals. The result on beef suggests that wheeze was less likely with early weaning onto beef; however, in the absence of an obvious mechanism, this may be a chance result arising from multiple testing.

Table 5.8 Time to wean on foods in those who wheeze and those who do not at age 23 years.

Number	Number/ censored (%)	Hazard Ratio no adjustment	Adjusted Hazard Ratio (95% CI)
Meat not Beef	298/23 (7.7%)	0.90 (0.70-1.16) 0.43	1.01 (0.94-1.09) 0.62
Fruit	299/6 (2.0%)	1.20 (0.94-1.53) 0.14	1.23 (0.96-1.57) 0.11
Fish	299/255 (85.3%)	1.18 (0.64-2.19) 0.59	1.13 (0.61-2.10) 0.71
Egg	298/91 (30.5%)	1.00 (0.75-1.34) 1.00	1.00 (0.74-1.35) 1.00
Cereal	298/13 (4.4%)	1.12 (0.87-1.43) 0.37	1.05 (0.81-1.35) 0.73
Beef	299/24 (8.0%)	0.75 (0.58-0.98) 0.03*	0.76 (0.59-0.99) 0.05*

*Significant result

†Adjusted for parental allergy, gender, social class age 1, original randomisation and breast feeding

5.12a) Time to event analysis for first exposure to cows' milk

In an earlier section we investigated the results from the two randomisation groups. That analysis was on an intention to treat basis, ignoring the fact that some in the intervention group were actually exposed to cows' milk at an early age. Now we will analyse the data regarding first exposure to cows' milk using Cox's Proportional Hazards model. The results are shown in table 5.9, and suggest that early exposure to cows' milk protects against asthma and wheeze; the hazard ratio for atopy was comparable to those for wheeze and

asthma but the smaller sample size has led to a much wider confidence interval.

Figure 5.5 Age in weeks when cows milk first consumed by randomisation group (log scale)

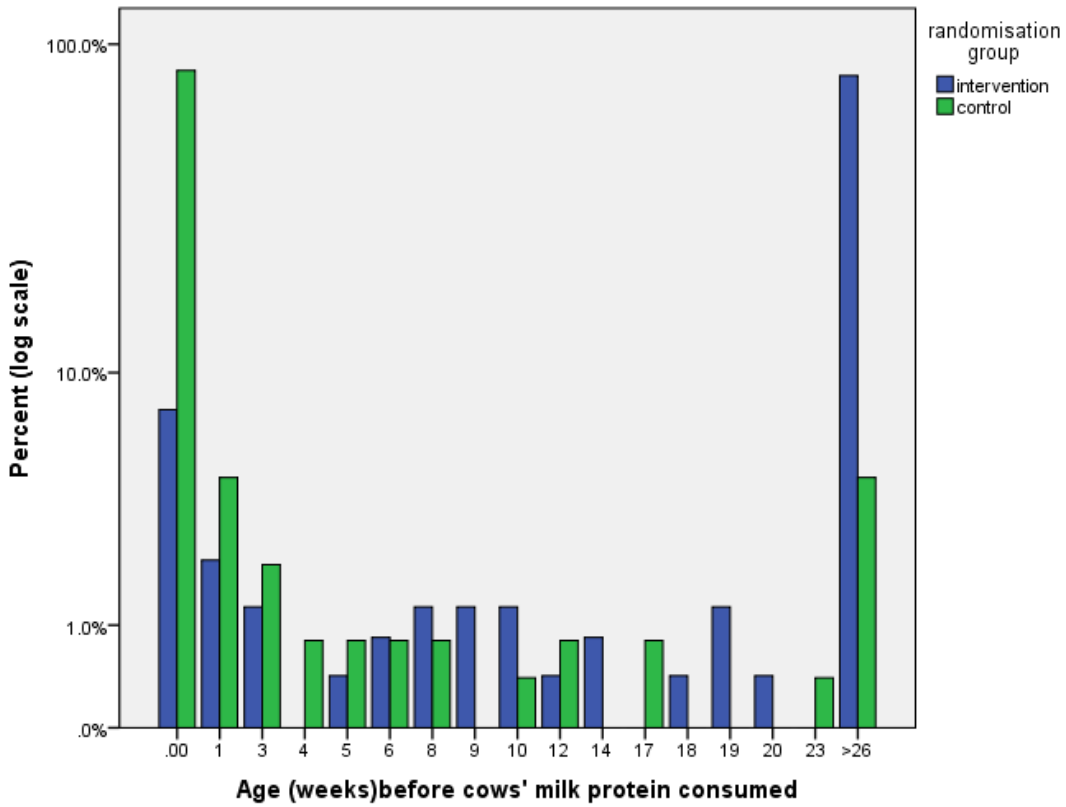


Table 5.9 Age in weeks of cows' milk protein exposure and association of outcomes at age 23

Outcomes at age 23	Asthma	Wheeze	Atopy
Number (censored)	296 (128)	299 (128)	119 (52)
Unadjusted hazard ratio	0.51 (0.31-0.84) 0.008*	0.67 (0.47-0.94) 0.02*	0.67 (0.41-1.08) 0.10
Adjusted Hazard Ratio†	0.51 (0.31-0.85) 0.009*	0.66 (0.47-0.94) 0.022*	0.63 (0.38-1.04) 0.71

*Significant result

†Adjusted for social class age 1, parental allergy, gender and ever breast fed.

5.13 Discussion

Neither breast feeding nor late infant weaning onto solid food was significantly associated with outcomes at age 23 years. However, few subjects were breast fed for more than 4 weeks and the majority of subjects were weaned onto solids within 4 months of birth. This poor exposure to being solely breast fed may explain why the results from the MAPS cohort were different to many other studies, in which a much higher percentage of subjects had sole breast feeding for much longer . However we were able to show a significant association between breast feeding and wheeze age 1. The protective effect of breast feeding with respect to wheeze at age 1 year may be exhibited, fairly quickly, through the passage of maternal IgA to protect against infections. Hence we found an association in this study. However, to protect against antigenic foods, or indeed to induce a poor tolerance reaction

due to lack of antigenic food, one could speculate that sole breast feeding would have to continue for longer and thereby not allow the subject to be exposed to antigenic foods. Prescott et al have demonstrated that the Th2-skewed responses to common environmental allergens, are present in virtually all new-born infants and that it is immune modulation toward a Th1 response in the early post natal period that determines the normal (non-atopic) state of the individual's immune system in most cases[246]. Since our subjects were not breast fed for long enough the results of our study cannot add to this evidence. One limitation in our RCT was the fact that a high percentage of those who were in the intervention arm did actually have exposure to cows' milk protein.

There are few longitudinal studies that have outcomes on breast feeding in adulthood. Matheson et al found that exclusively breast fed babies in the TAS cohort with a maternal history of either asthma or allergic rhinitis in the initial survey in 1968, were less likely to develop asthma before the age of 7(odds ratio [OR], 0.8; 95% CI, 0.6-1.0). However, after age 7 years, the risk reversed, and exclusively breast-fed children had an increased risk of current asthma at age 14 years (OR, 1.46; 95% CI, 1.02-2.07) and 32 years (OR, 1.84; 95% CI, 1.06-3.3) years[239]. However this study used a retrospective history of breast feeding taken when the subject was 7 years of age, relying on parental recall of breast feeding which may be inaccurate. Other studies have found much higher rates of breast feeding in their population but no other study to our knowledge has taken a weekly prospective diet history for the first 6 months of the subjects' life. We would therefore speculate that other studies may not be fully aware of their true prevalence of breast feeding.

We have shown that excluding cows' milk protein for the first four months of life did not reduce the risk of wheeze, asthma or atopy at age 23 years. Our results showed a significant increase in atopy and asthma at age 23 years in subjects who were allocated to the intervention arm. No other study has excluded cows' milk protein in an RCT and followed up subjects into adult life. When comparing soya based milk formulae to cows' milk based formulae, two early studies suggested that soya milk was not as atopy inducing as cows' milk[247, 248]. Lowe et al investigated a 3 group RCT of high risk infants fed a conventional cows' milk formula, extensively hydrolysed cows' milk formula or a soy formula when breast feeding had ceased. At age 6-7 there was no evidence of soya or extensive hydrolysed cows' milk formulas providing any protection from allergic disease or asthma specifically compared to the usual milk formula[249]. However the GINI plus study followed up children till age 6 and investigated eczema (not asthma) as an outcome. The intervention arm used extensively hydrolysed cows' milk (eHF-C) based formula. Researchers found that predisposed children without nutritional intervention had a 2.1 times higher risk for eczema [95% confidence interval (CI) 1.6 – 2.7] than children without a familial predisposition. The risk was smaller with nutritional intervention even levelling out to 1.3 (95% CI 0.9–1.9) in children fed eHF-C formula[250]. However the association between our intervention arm and asthma and atopy was not apparent until the MAPS cohort were adults and neither the Lowe et al study or the GINI plus study has reported on adult results. The only other study, to our knowledge, that has comparable results is the Isle of Wight study,

which reported on their subjects at age 18. Subjects were enrolled in a single blinded, randomised controlled trial. Infants in the intervention arm were either breast fed with the mother on a low allergen diet or given an extensively hydrolysed formula. Exposure to house dust mite allergen was reduced. The control group followed standard advice. Children were assessed at ages 1, 2, 4, 8 and 18 years for the presence of asthma and atopy. Children were assessed at 18 years for the presence of asthma and atopy. They showed that reducing allergenic exposure in the first year of life reduced the risk of asthma at age 18 years the OR being 0.23 (95% CI 0.08 - 0.70), p=0.01[212]. However this study, used a multifaceted approach and did not just remove one allergen; in addition, they removed exposure to soya in the treatment arm of their study because of its allergenic properties, so they are not completely comparable. The reason our study showed that cows' milk had a reduced allergenic effect may be because the control group was given soya based milk formula, which could have had an increased allergenic effect.

Our results may not be due to early use of soya in our intervention arm, rather it may be that in the MAPS cohort early exposure to cows' milk protein created tolerance in the subjects' immune systems. In this case our findings would be in keeping with the PIAMA cohort study where researchers found that the daily consumption of full fat milk and butter at the age of 2 reduced the risk of asthma and wheeze at age 3 when compared with children who did not have daily consumption[91]. Sudo et al investigated the role of intestinal bacterial flora in oral tolerance induction in germfree (GF) mice. When they tested mice without appropriate bowel flora for tolerance induction, they

found Th1 cytokines remained low and Th2 remained high. However when they were inoculated with Bifidobacferium infantis, one of the predominant bacteria in the intestinal flora, tolerance induction was maintained. However this only occurred in neonatal mice. Older mice were unable to develop tolerance[251].

Studies in mice regarding tolerance induction suggest that the neonatal period is critical for appropriate immune-modulation to allow adult Th1 type responses to develop in the infant[35, 252]. It may be that our cohort received cows' milk allergen at that critical period and had the correct bowel flora to develop tolerance in their neonatal immune systems. This is not the only example of exposure to an allergen in early life promoting tolerance, as already discussed in chapter 4, evidence suggests that babies born in the pollen season are less likely to have rhinitis or pollen sensitivity up to age 15 years[218]. The mechanism of this must also be through early tolerance induction.

Weaning onto solid food has not been extensively studied but two reports suggest that late weaning does not protect against wheezing[244] or asthma[242]. In the MAPS cohort there was no evidence that weaning was associated with asthma, wheeze or atopy. There was one significant result using Cox's proportional hazard modelling which showed a protective effect from early weaning and wheeze at age 23 years. However it is not clear if this is a true effect or just present due to multiple testing.

The investigators did not expect, when the study was initiated, that offering free soya formula to mothers of new born subjects might have an influence on their breast feeding activities. However our findings show that breast feeding rates were lower in the intervention group and it is possible that this arose because of the offer of free formula. Mothers from this deprived community may have decided to use formula rather than breast feed as a good source of nutrition or purely for convenience. If such a study was to be planned in the future then such possible effects would have to be considered by an Ethics panel.

Bias may have arisen from the fact that not all mothers kept rigidly to the randomisation group to which they were assigned. Some mothers not in the intervention arm did use soya milk for feeding the subjects, presumably because they believed it to be beneficial, and many in the intervention group were exposed to cows' milk at an early age. 17.2% of mothers in the intervention group admitted to using to using cows' milk products while 37% tested positive to IgG4 for cows' milk antigen at age 3 months, suggesting some exposure to cows' milk. The combined effects of these departures from protocol would be to lessen any effect of the intervention.

In summary we have found that the intervention (exclusion of cows' milk protein with replacement of soya supplementation in neonatal life) appeared to increase the risk of adult asthma and atopy. Further areas of research need to be undertaken to investigate the mechanism of this increased risk of asthma

and atopy with the intervention arm. This has not been shown in previous studies and may be specific to the population under study.

Chapter 6

Environmental factors in the aetiology of asthma.

6.0 Introduction

A wide variety of environmental influences has been implicated in the rise in prevalence of asthma. These include smaller family sizes[253]; less exposure to domestic animals in childhood[254]; and changes in diet[255]. However, no one environmental factor has been shown to be unfailingly protective against, or consistently a risk for, the development of asthma. Often it is difficult to determine which factor in the environment influences outcomes and when the factor has its effect. It is likely that both environmental and host influences interplay to produce an outcome, which may lead to the development of asthma. A risk factor can be either primary (i.e. increases the incidence of asthma) or secondary (triggers symptoms, exacerbates disease, or increases its severity)[256]. In this study we are investigating primary risk factors in early life that may have an effect later in life.

It is likely that there is a close interplay between environmental factors and genetic make-up which will become better informed with the research into epigenetics. However a full discussion of the epigenetics of asthma is beyond the scope of this work and is reviewed by Miller and Ho[257]. In this chapter we will investigate environmental factors in the prevalence of asthma and asthma symptoms in adults.

6.1 Evidence from migration studies

Smith et al investigated a group of school children in Birmingham who were first reviewed in 1956 and again in 1968. They showed an increasing prevalence of asthma over the 12 year period. They also showed that children from Asian and African backgrounds born overseas had a lower prevalence of asthma than children born in the UK (whether British or African and Asian parents)[258]. In the 1980s Gregg investigated the effect of migration of diverse people, such as Tokelauan citizens migrating to New Zealand and Xhosa children migrating to Cape Town in South Africa, on the prevalence of asthma, and suggested that as subjects moved from less developed to more developed communities, their prevalence of asthma increased[259].

Kuehni et al undertook a random postal questionnaire study of 2380 South Asian women and 5796 young white mothers residing in Leicester, UK. The survey showed a lower rate of asthma in the South Asian women (10.9%) compared to white women (21.8%) and an even lower rate (6.5%) in the South Asian women who had not migrated from India until they were over 5 years old[260]. Those who had migrated to the United Kingdom after the age of 5 years were less than half as likely to suffer with asthma as those who had been born in the United Kingdom or had migrated before the age of 5 years. This study suggests that exposure to Western environmental influences is particularly important at a young age.

The reunification of Germany in 1989 gave the ideal setting to compare the effects of genetic factors and environmental factors in the development of asthma and atopy. Despite the political separation of the two populations, their genetic make-up remained comparable. When the Cold War ended and the Berlin Wall came down, there was a lower prevalence of asthma among children age 9-11 years in the East compared to the West, despite the fact that the East was notorious for high levels of pollution[261]. Frye et al investigated the prevalence of bronchial hyper-reactivity in 8-14 year olds from schools in what was previously East Germany firstly in 1992 and again in 1995. They found an increase in bronchial hyper reactivity (BHR) from 6.4% in 1992-93 to 11.6% in 1995-96 (OR 1.9 95% CI 1.3-2.9) which they suggested was the first sign of an increase in asthma that was likely to occur[262].

A number of environmental factors had changed for children in the two German surveys over the 3 years including a significant change in heating from coal to central heating, less sharing of bedrooms, a greater percentage of children starting day care at age 6 months (7.3% vs 3.9%) and at age 1 year (12.5% vs 5.3%) and there was greater contact with pets. There is no single environmental influence that can be pin-pointed as the reason why these children have a greater prevalence of BHR compared with subjects of a similar age only three years before but it is suggested that a number of factors, increase the risk of BHR and asthma.

6.2 Endotoxin and asthma risk

Endotoxin is a lipopolysaccharide that is present within the cell wall of gram negative bacteria and can be measured in house dust. The level of endotoxin in house dust increases with greater contact with farm animals[263] and in the presence of domestic cats and dogs in the house[264] and where livestock are kept[265]. Reidler et al found that those children who had regular exposure to a farming environment and who drank unpasteurised milk before the age of 5, had a significantly lower risk of developing asthma at age 9-10 years than those who did not have this type of exposure[266]. A cross-sectional ISAAC study found a significantly lower prevalence of ever having been diagnosed with asthma or of having wheezed in the past year with a greater concentration of endotoxin in living room dust[267].

6.3 Domestic pets and asthma prevalence

Having a domestic pet is associated with increased levels of aeroallergens specific to the pet that is kept (i.e. fel d1 for cat and can f1 for dog)[268]. A Swedish study found that living with a cat was inversely related to the incidence of physician-diagnosed asthma[269]. De Marco et al in an ECRHS cross-sectional study in adults found that pet ownership in childhood resulted in protection against childhood asthma[270]. The ECRHS study found that the effects of pet-keeping in childhood varied according to the type of pet, the allergic sensitisation of the individual, and the wider environmental exposure to allergen. Cats owned in childhood were associated with more asthma in sensitised adults who grew up in areas with a low community prevalence of cat ownership while dogs owned in childhood seemed to protect against adult allergic disease but promote non-allergic asthma[271]. However one systematic

review showed that exposure to pets increased the risk of asthma and wheezing in older children[272], which suggests that studies, so far, have not answered the question on whether pet ownership is protective or not. What we may need is longitudinal studies to report on this issue. ALSPAC has reported on pet keeping only in childhood and shown a small but significant increase in wheeze up to age 42 months when cats and dogs are kept[93]. The PIAMA study found that although keeping pets at the age of 3 months reduced the incidence of sensitisation to aeroallergens, at age 8, there was no evidence that there was a reduction in the risk of asthma at this age[73]. Evidence from the Dunedin cohort suggested that owning both a cat and a dog in childhood, was associated with a lower risk of atopy at age 13 and further pet keeping protected against atopy in adulthood, but they have not reported on asthma or wheeze[273]. The Tucson Children's Respiratory Health study (TCRS) showed that early pet ownership was associated with a lower prevalence of wheeze up to age 11 years among children without parental asthma, but they have not given results on adult subjects[254]. However, a more recent study showed that subjects with asthma selectively avoid keeping a cat if they are allergic, and concluded that at least in part any apparent protective effect of cat keeping may be due to avoidance of cat keeping in those with asthma[23].

6.4 Indoor Environment

People living in urban areas spend most of their time (85–90%) in indoor environments[274], which have changed enormously over the past few decades with the introduction of soft furnishings and fitted carpets. There has been a

consequent increase in damp and reduction in ventilation with a subsequent increase in indoor allergen levels[268, 275]. Indoor air has been shown to contain increasing levels of indoor aeroallergens due to modern living, i.e. central heating and insulated windows[275]. The other factor in indoor air pollution is chemical pollutants from cooking and heating; these have been shown to increase asthma symptoms[276]. Aromatic compounds and carbonyls, in homes, offices, shopping malls and schools often have concentrations 2–5 times those found outdoors, and can occasionally reach 100 times outdoor levels[277]. In addition the rate at which indoor air is changed for fresh air is now lower than it was 30 years ago, leading to an increase in humidity, levels of indoor pollutants and air-borne allergens[277].

6.4a) Damp and mould in housing

One German study examined bedrooms both before and 7 months after double glazing and central heating were installed and found a significant decrease in air exchange rate and an increase in temperature and absolute humidity (water content of the air), although relative humidity (the percentage of water vapour in the air compared to the maximum for that temperature) did not significantly rise[275]. There was also an increase in house dust mite allergen and fungal spores in dust from carpets and mattresses in bedrooms[275]. The levels of cat (fel d1) and dog (can f1) allergens increase with higher humidity and lower ventilation[268]. Fungal spore levels[278] and house dust mite levels correlate with the presence of dampness[279]. House dust mite levels have been shown to correlate with the risk of incident asthma[180], and so it is no surprise that

measured dampness was significantly more frequent in asthma sufferers' homes than in those of age and sex matched controls[280]. In addition a randomised controlled trial of an intervention to remove mould from households of asthma sufferers compared to no intervention found a significant difference in asthma symptoms between the two groups[281]. These studies suggest that increased levels of allergens are present in damp poorly ventilated environments and that reducing dampness and mould may reduce asthma symptoms in those who suffer with asthma. However, it is not clear if removing damp from the indoor environment reduces the incidence of asthma.

6.4b) Heating and cooking- domestic fuels

The two most prevalent oxides of nitrogen are nitrogen dioxide (NO_2) and nitric oxide (NO). These are both indoor air pollutants although NO quickly oxidises to NO_2 when mixed with oxygen (O_2). Both are toxic gases with NO_2 being a highly reactive oxidant and corrosive. The primary sources indoors are combustion processes, such as unvented combustion appliances, e.g. gas stoves, vented appliances with defective installations, welding, and tobacco smoke[282]. A meta-analysis of studies investigating the respiratory effects of indoor NO_2 showed that, in children, gas cooking was associated with an increased risk of asthma and indoor NO_2 was associated with an increased risk of current wheeze[283]. A British study found that gas cooking was associated with current wheeze in 14-16 year old children when they were investigated using a questionnaire administered to parents[284]. An association between gas stove use and respiratory symptoms (cough and tight chest) was found in Australian 7-14 year olds, but there was no association of such

symptoms with nitrogen dioxide exposure in this study[285]. This last study suggests that the association of asthma symptoms with gas stove use may not be due to an association with raised indoor levels of nitrogen dioxide, but rather due to some other factor specific to the indoor use of gas.

The longitudinal Tasmanian Infant Health Survey (TIHS) reported that in 8 year old children there was an increased relative risk of sensitisation to indoor aeroallergens with the use of home gas appliances[286]. Exposure to fume emitting heaters in a cross-sectional study of school children age 8-11 in Belmont in NSW Australia showed no association with asthma, if exposure was current at the time of questioning. However, if exposure was in the first year of life, there was an association with increased airway hyper responsiveness in 8-11 year olds[287].

6.5a) Infection and related factors

The hygiene hypothesis suggests that increased cleanliness should be associated with an increased risk of atopic disease. The ALSPAC study found that high levels of hygiene at 15 months of age were independently associated with wheeze and atopic eczema reported between 30 and 42 months[288]. Korhonen et al investigated children with an atopic disease (defined as having sensitivity to a common allergen and one of eczema, asthma or hay-fever) and matched controls aged 5-11. They tested their serum for 12 different enterovirus serotypes and found that the controls were more likely to have the presence of recurrent enterovirus infections than those with atopic disease[289]. The Tasmanian longitudinal study which investigated asthma risk from early

life infections, showed that, overall childhood infections, including pneumonia reduced the risk of asthma persisting into later life[290]. However other studies have not found that early life infections are associated with a reduced risk of atopic disease. A cross-sectional ECRHS study found that a serious respiratory infection before the age of five years was associated with a significantly increased risk of asthma in adulthood[270]. An ISAAC study of 5-7 year olds and 9-11 year olds showed an association of increased risk of asthma, especially non-atopic, with repeated episodes of fever and antibiotic treatment in early life[291]. An investigation of maternal infections during pregnancy found an association of increased risk of asthma in the child up to age 6 if the mother had had a urinary tract infection during pregnancy[292]. The evidence regarding infection in early life and protection against allergic disease is therefore conflicting.

6.5b) Number of siblings

The association of older siblings and protection from hay fever at the age of 11 years and 23 years was the basis of Strachan's original paper that later became the foundation for the hygiene hypothesis. Other researchers have investigated the association of the presence of siblings and the acquisition of asthma or wheeze. The best known studies are presented in table 6.1. Bodner et al undertook a large cross-sectional study of 10-14 year olds in Aberdeen and showed that having 3 or more younger siblings was associated with a lower risk of asthma[293]. Similarly a GP database study demonstrated that children with increased numbers of older siblings had a lower risk of asthma than those with fewer siblings[294]. Ball et al studied the TCRS longitudinally

and showed that the presence of one or more older sibling at home protected against the development of physician diagnosed asthma at age 6-13 years[82]. However the cross-sectional ECRHS showed that a greater number of siblings was associated with a reduced risk of rhinitis in adults but associated with an *increased* risk of asthma[22]. The SIDIRA (Italian Study of Respiratory diseases) longitudinal study group found that having siblings was a risk factor for transient early wheezing (phenotype of early wheeze not linked to asthma) in 5-6 year olds but protective against late onset and persistent wheeze, (considered to be precursors to asthma)[99]. In a study of male conscripts born in Sweden between 1973-75, the presence of older siblings was associated with a reduced risk of rhinitis, though there was no such protective association with asthma[295]. Pekkanen, however, showed a positive but non-significant association with doctor diagnosed asthma and the presence of 3 or more siblings in the home. The relationship between family size and prevalence of asthma is not clear and remains controversial. Why there are differing outcomes with the SIDRIA and TCRS showing a protective effect for persistent wheezing, in children, while the ECRHS shows no protective effect in adults, is not clear, but it may be due to the age when the outcome is measured or due to the longitudinal nature of the Tucson and SIDRIA studies, which means these studies may be more able to record accurately a history of asthma. Although the results of the studies are not noticeably showing a benefit of having siblings, overall there does appear to be a lower risk of asthma and wheeze in childhood and adulthood in those with more siblings.

Table 6.1 studies investigating the association of the presence of siblings and asthma prevalence

Study	**OR (95%CI)**	**Outcome variable**	**Variable defined**	**Study design**
Braback[295]	1.31 (0.96-1.77) with rhinitis 0.92 (0.74-1.16) without rhinitis	Asthma age 17 years	4 or more siblings compared to none	Cross-sectional
Bodner[293]	0.4 (0.1 - 0.9)	Asthma age 10-14 years	>=3 siblings vs 0 sibs	Cross-sectional
ECRHS[296]	0.31 (0.11-0.83)	Asthma age 20-44 years	>2 sib vs 0 sibs	Cross-sectional
National Study of Health and Growth (NSHG)[133]	0.87 (0.76-0.98)	Asthma attacks or wheeze 5-11 years	>=1 sib vs 0 sibs	Cross-sectional
Pekkanen [297]	1.26 (0.85–1.88)	Doctor diagnosed asthma ever13-14 year old children	0 sibs vs 3 or more	Cross-sectional
SIDRIA[99]	1.41 (1.21–1.64)	Transient early wheeze 6-7 year olds	>=1 sib vs 0 sibs	longitudinal
SIDRIA[99]	0.83 (0.70–0.97)	Late onset wheeze 6-7 year olds	>=1 sib vs 0 sibs	Longitudinal
Tasmanian Infant Health Survey[298]	0.36 (0.17-0.74)	Current asthma (self- reported ever asthma and wheeze in past year) 7 year old	>=1 sib vs 0 sibs	Longitudinal
TCRS[82]	0.8 (0.7–1.0)	Asthma physician diagnosis ever and symptoms past year 6-13 year old	>=1 sib vs 0 sibs	Longitudinal

TCRS=Tucson Children's Respiratory Health study

6.6 Changing response to infection-alteration in immune competence

A number of factors have been proposed as modulating the immune response to infection and therefore these factors may have an effect on the incidence of asthma. Two of these modulating factors are immunisation, which causes a

naïve immune system to recognise a harmful microorganism, and caesarean section birth, since it has been hypothesised that delivery through the vaginal canal arms the new born with healthy maternal bacteria that colonise the gut and thereby reduce the risk of atopic disease[299].

6.6a) Immunisation

A study of 13-14 year olds who had participated in the French section of the ISAAC study and had vaccination records were found to have a reduced risk of asthma and atopy if they had been vaccinated when compared to those who had not been vaccinated. After adjustment for the usual confounders the odds ratio was 0.30; 95% CI: 0.10, 0.92. The relationship did not depend on the disease against which the vaccine was used as prophylaxis, adherence to the observance of the vaccination schedule or the number of inoculations. A higher protection was observed in the case of live attenuated vaccines (oral poliomyelitis and bacilli Camille-Guerin; OR = 0.26; 95% CI: 0.08, 0.83)[300]. However out of a group of 718 only 24 did not have immunisation and they are likely to be different in other ways to the children that did have immunisation.

Others have found that polio immunisation protects against asthma to age 6 years, while diphtheria and tetanus immunisation together gave an increased risk for asthma at age 6[301]. A large retrospective Canadian study, in which children received at least 4 doses of diphtheria, pertussis and tetanus (DPT), reported on the association between delay in first dose of DPT and association with asthma at age 7. If the first immunisation was delayed from before 2

months by at least 1 month there was a statistically significant association with asthma: OR 0.84 (0.75-0.95). They found that the risk of asthma was reduced to half in children whose first dose of DPT was delayed by more than 2 months. The likelihood of asthma in children age 7 with delays in all 3 doses was 0.39 (95% CI, 0.18-0.86)[302].

The Tasmanian Longitudinal Health Study (TLHS) investigated the association between asthma and childhood vaccination in a population in which immunisation against common infections was undertaken by 80-85% of the population. Researchers found no evidence of an association between asthma in adulthood and immunisation in childhood[303]. The Melbourne Atopy Cohort Study (MACS) found that polio immunisation before the age of 2 reduced the risk of doctor diagnosed asthma at age 6 in a high risk birth cohort[304]. Overall, it seems that there may be a protective effect from immunisation with live attenuated bacteria with in childhood asthma, but this is likely to be small. The effect on adult asthma prevalence is less clear as there are few longitudinal studies that have reported on outcomes in adults. In summary there is no clear picture regarding the association of immunisation with asthma and wheeze.

6.6b) Caesarean section and risk of asthma

The rate of caesarean section has risen from 9% of deliveries in England in 1980 to 24.8% in 2009[305]. A meta-analysis of studies between 1996-2006 investigating the association between asthma and caesarean section found a 20% increase in the odds of asthma in those born by caesarean section (OR

1.20 95% CI 1.14-1.26)[306]. A further meta-analysis investigating studies from 1966-2007 found a similar association between caesarean section and asthma (OR 1.18 95% CI 1.05–1.32) in all subjects up to adult ages[307]. The Epidemiology of Home Allergens and Asthma longitudinal study found an association between caesarean section and rhinitis at age 9 years (OR 1.9 95% CI 1.1-3.2 p=0.02) but not with asthma at the same age (OR 1.1 95% CI 0.6-2.3 p=0.7)[308]. The PIAMA study found that children born through caesarean section were significantly more likely to have asthma at the age of 8 (OR 1.79 95% CI 1.27-2.51) than those born with simple vaginal delivery, especially if they had allergic parents (OR 2.91 95% CI 1.20-7.05)[309]. The ALSPAC longitudinal study found no association of mode of delivery with asthma at 69–81 months (OR 1.16 95% CI 0.9-1.5); wheeze 69–81 months (OR 0.95 95% CI 0.7-1.3); physician diagnosed asthma (OR 1.14 95% CI 0.9-1.4); atopy (OR 1.04 95% CI 0.8-1.3)[88]. Breton et al in a data base study from Quebec investigated a cohort of 13 100 and 28 042 single pregnancies in women with and without asthma, respectively. The crude OR of perinatal mortality was 1.35 (95% CI 1.08 to 1.67), which decreased to 0.93 (95% CI 0.75 to 1.17) after adjustment for birth weight and gestational age at birth. Women with asthma had a higher rate of low birth weight babies and preterm delivery than those without asthma[310]. The authors concluded that the increased risk of low birth weight babies and premature delivery in women with asthma may partly explain the association between maternal asthma and the increased risk of perinatal mortality, i.e. reverse causality.

6.7 Environmental tobacco smoke

A large cross-sectional study of school students in Southern California in 4th grade (age 9-10 years), 7th grade (age 12-13 years) and 10th grade (age 15-16 years) was undertaken and based on a questionnaire administered to parents The questionnaire asked parents to recall their smoking history during the time when the subjects were still intrauterine so there may have been some recall bias. Despite this, the study showed that smoking during pregnancy was associated with asthma[311] and reduced lung function[312] in the child, however they could not show conclusively that environmental tobacco smoke was associated with asthma, although it was associated with wheeze[311]. A systematic quantitative review of 25 studies regarding passive smoking in school age children (5–16 years) and asthma, wheeze or cough in the child, was undertaken in 1997 by Cook et al and revealed a significant association for all these symptoms if either parent was a smoker compared to children not exposed to environmental tobacco smoke. The pooled odds ratios for either parent smoking and association of the following symptoms in the subject were 1.21 (95% CI 1.10-1.34) for asthma, 1.24 (95% CI 1.17 to 1.31) for wheeze, 1.40 (95% CI 1.27 to 1.53) for cough[313]. The association between passive smoking and childhood asthma was also shown in a meta-analysis by Tinuoye et al, where twenty relevant studies were identified. The pooled odds ratio was 1.32 (95% CI: 1.23 - 1.42, $p < 0.001$)[314]. A further meta-analysis of 29 studies showed that passive smoking increases the risk of asthma by at least 20% in exposed children[315]. The SIDRIA group studied two random samples of subjects ages 6-7 and 13-14 years in ten areas of northern and central Italy

and showed that exposure to smoke of at least one parent was associated with an increased risk of current asthma among children [odds ratio (OR) =1.34; 95% confidence interval (CI) = 1.11-1.62] and of current wheezing among adolescents OR = 1.24; 95% CI = 1.07-1.44)[316]. No study has reported on childhood exposure to passive smoke and adult risk, although one study showed that in utero exposure to tobacco was associated with a higher prevalence of smoking in adulthood[317]. Adult onset asthma has also been found to be associated with passive smoking in adults in one study from Finland. The risk of asthma was related to both workplace exposure (adjusted odds ratio [OR] = 2.16: 95% CI 1.26-3,72) and home exposure (OR=4.77; 95% Cl 1.29-17.7) in the past year[318].

6.8 Aims and objectives

The aim of this chapter is to investigate associations between the early life environment and asthma and wheeze in early adult life.

Objectives

1 To investigate the association of outcome variables at age 23 with

- The possession of household pets in early childhood
- Housing attributes such as the presence of damp and mould
- Domestic fuel use for heating and cooking
- Infection (chest infection in the first year of life)
- The presence of one or more sibling
- Immunisation and caesarean section
- Exposure to environmental tobacco smoke in early

6.9 Methods

In this section the methods were as outlined in chapter 2. All sections in this chapter are based on answers to the questionnaires at different ages. The questions asked with regard to damp housing, mould in housing, older siblings and early life chest infections are given in the questionnaires at age 3 months and 7 years, reproduced in the appendix (appendix 3b and 7b).

6.10 Results

6.10a) Domestic pet keeping and outcomes at age 23 years

A total of 15% of subjects from the MAP study had a domestic cat in the family at the age of 1 year and 22% at the age of 7 years. A total of 30% of the cohort had at least one dog in the family at the age of 1 and 42% at the age of 7 years.

Table 6.2 shows no significant associations between pet keeping at age 1 or 7 and any outcomes in adulthood. However, there is a low OR for wheeze and asthma at age 1 and an OR greater than 1 for wheeze and asthma at age 7. This suggests that pet keeping at age 1 year may be protective while pet keeping at age 7 may increase the risk of asthma and wheeze. The OR for atopy and pet keeping at age 1 and at age 7, are both low suggesting there may be some tolerance to sensitisation from regular pet keeping.

Table 6.2 Associations between pet ownership at ages 1 and 7 and outcomes at age 23

	Cat or dog or mammal age 1	No cat or dog or mammal age 1	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	38/124 (30.6%)	57/169 (33.7%)	0.87 (0.53-1.43) 0.58	0.89 (0.53-1.49) 0.46
Asthma age 23	16/126 (12.7%)	37/170 (21.8%)	0.52 (0.28-0.99) 0.047*	0.54 (0.27-1.05) 0.07
Atopy age 23	24/49 (49%)	46/70 (65.7%)	0.50 (0.24-1.06) 0.069	0.59 (0.26-1.34) 0.21
	Cat or dog or mammal age 7	No cat or dog or mammal age 7	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	61/170 (35.9%)	31/125 (28%)	1.44 (0.87-2.37) 0.15	1.64 (0.97-2.77) 0.065
Asthma age 23	33/172 (19.2%)	20/126 (15.9%)	1.26 (0.68-2.32) 0.46	1.59 (0.83-3.05) 0.16
Atopy age 23	35/68 (51.5%)	35/52 (67.3%)	0.52 (0.24-1.09) 0.08	0.54 (0.24-1.23) 0.14

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.11 Home environment

6.11a) Damp in housing.

Table 6.3 shows associations between outcomes at 23 years and damp and mould at age 3 months and age 7 years. There is only one significant association, between wheeze at age 23 years and damp and mould at age 7. However there are no other significant associations, and this single significant result may be by chance.

Table 6.3 Damp or mould at age 1 and age 7 years and association with outcomes at age 23 years

	Damp and or mould age 3 months	Damp and or mould age 3 months	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	40/111 (36.0%)	55/183 (30.1%)	1.31 (0.80-2.16) 0.29	1.36 (0.81-2.28) 0.24
Asthma age 23	23/112 (20.5%)	29/185 (15.7%)	1.39 (0.76-2.55) 0.29	1.50 (0.80-2.82) 0.21
Atopy age 23	25/42 (59.5%)	45/78 (57.7%)	1.08 (0.50-2.31) 0.85	1.23 (0.53-2.85) 0.63
	Damp and or mould age 7 years	No damp and or mould age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	33/71 (46.5%)	63/224 (28.1%)	2.22 (1.28-3.85) 0.004*	2.39 (1.34-4.26) 0.003*
Asthma age 23	14/71 (19.7%)	39/227 (17.2%)	1.18 (0.60-2.33) 0.63	1.34 (0.66-2.72) 0.42
Atopy age 23	20/33 (60.6%)	50/87 (57.5%)	1.14 (0.50-2.58) 0.76	1.04 (0.41-2.61) 0.94

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.11b) Domestic Fuels for heating and cooking

Details of the use of domestic fuel at the age of 1 showed that a total of 71 (14.6%) of subjects used coal at age 1. No further information was available on the use of fuels at that age. At age 7 a number of household fuels were used, including gas for cooking (52.9%), electric fire (8.1%), central heating (90.9%), coal fire (4.5%), electric fire (8.1%), gas fire (63.7%) and solid fuel heating (18.2%), while 8.1% used some other form of heating. Table 6.4 displays the association between outcomes at age 23 years and the presence of a coal fire in the home at age 1 or 7 years. There are no significant associations.

Table 6.4 Coal fire age 1 and age 7 years and association with outcomes at age 23 years

	Coal fire age 1 years	No coal fire age 1 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	18/41(43.9%)	77/252 (30.6%)	1.78 (0.91-3.49) 0.093	1.95 (0.94-4.04) 0.07
Asthma age 23	9/41 (22.0%)	44/255 (17.3%)	1.35 (0.60-3.03) 0.47	1.60 (0.66-3.85) 0.30
Atopy age 23	9/19 (47.4%)	61/100 (61.0%)	0.58 (0.22-1.54) 0.27	0.65 (0.21-1.95) 0.44
	Coal fire age 7 years	No coal fire heating age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	6/15(40.0%)	90/280 (32.1%)	1.41 (0.49-4.08) 0.53	1.48 (0.49-4.41) 0.49
Asthma age 23	5/16 (31.2%)	48/282(17.0%)	2.22 (0.74-6.67) 0.16	2. 48 (0.79-7.83) 0.12
Atopy age 23	3/8 (37.5%)	67/112 (59.8%)	0.40 (0.09-1.77) 0.23	0.57 (0.11-3.06) 0.51

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.5 shows associations between the use of gas for cooking in the family home at the age of 7 and outcomes at age 23 years. There is one significant association between gas as cooking fuel at age 7, and asthma 23 when adjusted for confounders. There is no other association.

Table 6.5 The use of gas for domestic cooking age 7 years and association with outcomes at age 23 years

	Gas for cooking age 7 years	No gas for cooking age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	50/154(32.5%)	46/141 (32.6%)	0.99 (0.61-1.62) 0.98	1.11 (0.69-1.84) 0.69
Asthma age 23	33/155 (21.3%)	20/143(14.0%)	1.66 (0.91-3.06) 0.10	2.02 (1.06-3.86) 0.033*
Atopy age 23	41/66 (62.1%)	29/54 (53.7%)	1.41 (0.68-2.94) 0.35	1.59 (0.70-3.60) 0.27

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.6 shows associations between the use of an electric fire in the home at age 7 years and outcomes at age 23 years. There are no significant associations.

Table 6.6 The use of an electric fire for heating age 7 and association with outcomes at age 23

	Electric fire age 7 years	No electric fire age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	10/26(38.5%)	86/269 (32.0%)	1.33 (0.58-3.05) 0.50	1.51 (0.64-3.55) 0.35
Asthma age 23	6/26 (23.1%)	47/272 (17.3%)	1.44 (0.55-3.77) 0.46	1.66 (0.61-4.50) 0.32
Atopy age 23	7/11 (63.6%)	63/109 (57.8%)	1.28 (0.35-4.62) 0.71	2.14 (0.54-8.49) 0.28

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.7 shows associations between the use of central heating in the home at age 7 and outcomes at age 23 years. There are no significant associations.

Table 6.7 The use of central heating for heating age 7 and association with outcomes at age 23 years

	Central heating age 7 years	No Central heating age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	90/267 (33.7%)	6/25 (24.0%)	1.61 (0.62-4.17) 0.33	1.24 (0.46-3.34) 0.68
Asthma age 23	49/270 (18.1%)	4/25(16.0%)	1.16 (0.38-3.54) 0.79	0.83 (0.26-2.67) 0.75
Atopy age 23	62/103 (60.2%)	8/15 (53.3%)	1.32 (0.45-3.93) 0.61	0.91 (0.25-3.37) 0.89

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.8 shows associations between gas fire as a heating source in the home at age 7 and outcomes at age 23 years. There are no significant associations.

Table 6.8 The use of a gas fire for heating age 7 and association with outcomes at age 23

	Gas fire age 7 years	No gas fire age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	56/189(29.6%)	40/106 (37.7%)	0.70 (0.42-1.15) 0.16	1.15 (0.62-2.14) 0.66
Asthma age 23	32/190 (16.8%)	21/108(19.4%)	0.84 (0.46-1.54) 0.57	0.79 (0.42-1.48) 0.45
Atopy age 23	48/79 (60.8%)	22/41 (53.7%)	1.34 (0.62-2.87) 0.46	1.28 (0.54-3.06) 0.58

†Adjusted for Social class 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.9 shows associations between the use of solid fuel heating (not including coal heating) in the home age 7 years and outcomes at age 23 years. There is one significant result with a large effect size. That is for atopy after adjustment for confounders. This result is similar to the result with coal except for coal it was not statistically significant. There may be a suggestion that the use of solid fuel for heating in the home during childhood years is associated with a reduction in atopy in the adult subject.

Table 6.9 Outcomes at age 23 years and association with the use of a solid fuel for heating age 7 years

	Solid fuel heating age 7 years	No Solid fuel heating age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	19/50(38.0%)	77/245 (31.4%)	1.34 (0.71-2.52) 0.33	1.30 (0.68-2.50) 0.43
Asthma age 23	10/50 (20.0%)	43/248(17.3%)	1.19 (0.55-2.57) 0.79	1.09 (0.49-2.40) 0.84
Atopy age 23	8/20 (40.0%)	62/100 (62.0%)	0.41 (0.15-1.10) 0.61	0.27 (0.09-0.82) 0.02*

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.10 shows associations between the use of other fuels for heating in the home at age 7 years and outcomes at age 23 years. The fuel is likely to be oil and may signify other environmental differences since this type of heating is often used in farms. However there is insufficient information for more detailed analysis. There are no significant results for these associations.

Table 6.10 Outcomes at age 23 years and association with the use of 'other' fuel for heating age 7

	'Other' fuel heating age 7 years	No 'other' fuel heating age 7 years	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	5/27(18.5%)	91/268 (34.0%)	0.44 (0.16-1.21) 0.11	0.45 (0.16-1.25) 0.13
Asthma age 23	4/27 (14.8%)	49/271(18.1%)	0.79 (0.26-2.38) 0.67	0.80 (0.26-2.46) 0.69
Atopy age 23	7/13 (53.8%)	63/107 (58.9%)	0.82 (0.26-2.59) 0.73	0.75 (0.21-2.72) 0.66

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.12 Infection and related factors

6.12a) Older siblings in first year of life

Figure 6.1 shows the number of older siblings for each child. For 40% of families the subject was the first born.

Figure 6.1 Frequency of one or more older siblings in MAPS cohort

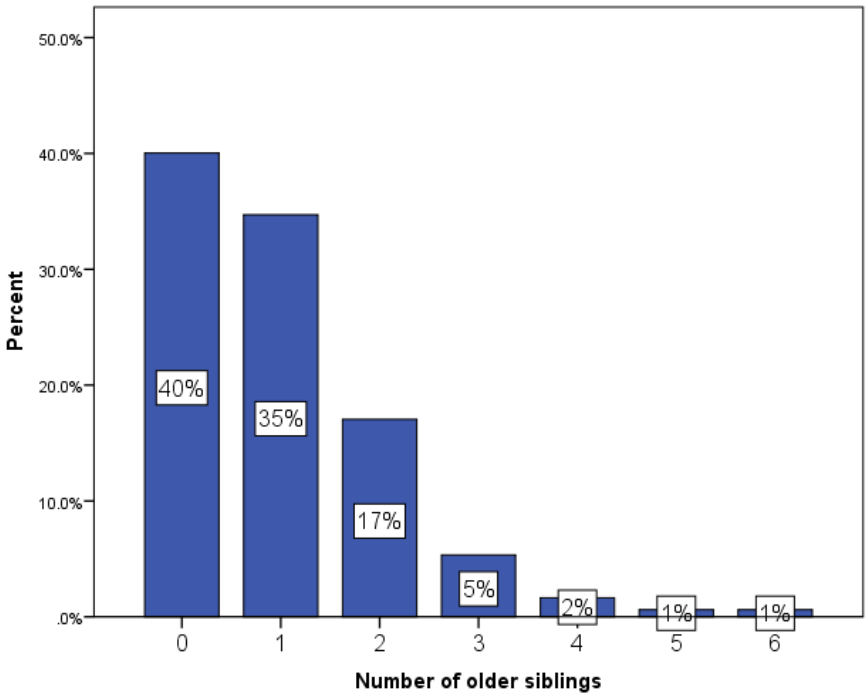


Table 6.11 shows associations of the presence of siblings when the subject was born and outcomes at age 23 years. There are no significant associations and so no strong evidence for an association between the presence of siblings and the risk of wheeze, asthma or atopy. It is interesting that all odds ratios are substantially less than one which suggests that the presence of older siblings in the house may have an association with a reduced prevalence of wheeze, asthma and atopy.

Table 6.11 Outcomes at age 23 years and association with presence of older siblings in the first year of life

	1 or more siblings at birth	No siblings at birth	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	51/180 (28.3%)	45/116 (38.8%)	0.62 (0.38-1.02) 0.06	0.78 (0.45-1.33) 0.36
Asthma age 23	28/180 (15.6%)	25/119 (21.0%)	0.69 (0.38-1.26) 0.23	0.83 (0.43-1.60) 0.58
Atopy age 23	40/72 (55.6%)	30/48 (62.5%)	0.75 (0.36-2.58) 0.45	0.80 (0.33-1.96) 0.63

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.12b) Association of chest infection in first year of life

Table 6.12 shows the association between having a chest infection before the age of 1 year and outcomes at age 23 years. There was no significant association.

Table 6.12 Outcomes at age 23 years and association with a chest infection in the first year of life

	Chest infection†† age 1	No chest infection age 1	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	21/58 (36.2%)	73/230 (31.7%)	1.22 (0.67-2.23) 0.52	1.32 (0.69-2.51) 0.41
Asthma age 23	15/58 (25.9%)	38/232 (16.4%)	1.78 (0.90-3.53) 0.10	1.92 (0.91-4.04) 0.086
Atopy age 23	15/26 (57.7%)	53/91 (58.2%)	0.98 (0.41-2.36) 0.96	1.17 (0.41-3.38) 0.77

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

††Chest infection definition at age 1 includes those who wheeze.

6.13 Changing immune response

6.13a) Immunisation

Table 6.13 shows the frequencies of the different immunisations that were given in the first 26 weeks of life. The rate of diphtheria, tetanus and polio immunisation was 78% at 13 weeks but for pertussis immunisation the rate was only 46%. This was because during the 1980s there was some difficulty in obtaining the pertussis vaccination in Wales. We have used the numbers for early immunisation (before 13 weeks) as this best approximates the current immunisation regime.

Table 6.13 Frequency of immunisation to Diphtheria, Pertussis and Tetanus and Polio

Age in weeks	<=13	14--26	Total immunised before 26 weeks
Number of subjects with Diphtheria and Tetanus immunisation at specified age	351 (78%)	82 (18%)	433 (96%)
Number of subjects with pertussis immunisation at specified age	205 (46%)	42 (9%)	249 (55%)
Number of subjects with polio immunisation at specified age	348 (78%)	82 (18%)	430 (96%)

Table 6.14 shows the association of immunisation against Diphtheria and Tetanus by 13 weeks and outcomes at age 23 years. There are no significant associations.

Table 6.14 Associations of outcomes at age 23 years with diphtheria and tetanus immunisation before 13 weeks of age

	Diphtheria and tetanus immunisation before 13 weeks	No Diphtheria and tetanus immunisation before 13 weeks	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	65/217 (30.0%)	31/79 (39.2%)	0.66 (0.39-1.13) 0.13	0.65 (0.37-1.13) 0.12
Asthma age 23	37/291(16.9%)	16/20 (20.0%)	0.81 (0.42-1.56) 0.53	0.84 (0.43-1.67) 0.63
Atopy age 23	55/88 (62.5%)	15/32 (46.9%)	1.89 (0.83-4.28) 0.13	1.80 (0.73-4.44) 0.20

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.15 shows the associations of immunisation with pertussis and outcomes at age 23 years. There are no significant associations.

Table 6.15 Outcomes at age 23 years and association with pertussis immunisation before 13 weeks of age

	Pertussis immunisation before 13 weeks	No Pertussis immunisation before 13 weeks	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	39/125 (31.2%)	57/171 (33.3%)	0.91 (0.55-1.49) 0.70	0.91 (0.54-1.52) 0.72
Asthma age 23	24/126 (19.0%)	29/173 (16.8%)	1.17 (0.64-2.12) 0.61	1.21 (0.65-2.27) 0.55
Atopy age 23	34/52 (65.4%)	36/68 (52.9%)	1.70 (0.80-3.53) 0.17	1.77 (0.77-4.09) 0.18

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.16 shows associations for polio immunisation with outcomes at age 23 years. There are no significant associations.

Table 6.16 Outcomes at age 23 years and association with polio immunisation before 13 weeks of age

	Polio immunisation before 13 weeks	No Polio immunisation before 13 weeks	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	64/216 (29.6%)	31/79 (39.2%)	0.65 (0.38-1.12) 0.12	0.64 (0.36-1.11) 0.11
Asthma age 23	36/218 (16.5%)	16/80 (20.0%)	0.79 (0.41-1.52) 0.48	0.82 (0.41-1.63) 0.58
Atopy age 23	54/87 (62.1%)	15/32 (46.9%)	1.86 (0.82-4.20) 0.14	1.78 (0.72-4.39) 0.21

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.13b) Caesarean section

Of the 485 subjects for whom we have information on mode of delivery 376 (78%) had a simple vaginal delivery, 53 (11%) had a caesarean section and 56 (12%) had a mode of delivery that was not caesarean section and would therefore have been through the vaginal canal. Since our hypothesis is that caesarean section does not allow the new born to acquire microorganisms through its usual route through the vaginal canal, we will compare caesarean section with all other modes, since all others would be through the vaginal canal.

Table 6.17 shows associations between caesarean section delivery and outcomes at age 23 years. There are no significant results however there is a large effect size for atopy. The number of people who had skin prick testing at age 23 was small and therefore the confidence intervals are wide. Since our sample is small we may not have found an association that was present, i.e. we may have failed to detect an effect that is present.

Table 6.17 Caesarean section and outcomes at age 23 years

	Caesarean Section	No Caesarean Section	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	10/37 (27%)	86/257 (34%)	0.74 (0.34-1.59) 0.28	0.80 (0.36-1.78) 0.58
Asthma age 23	6/38 (16%)	47/259 (18%)	0.85 (0.34-2.14) 0.5	0.88 (0.34-2.31) 0.80
Atopy age 23	12/15 (80%)	57/104 (55%)	3.30 (0.88-12.38) 0.06	2.57(0.63-10.35) 0.19

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.14a) Environmental tobacco smoke exposure

The environmental smoke exposure rate for our cohort was high. The mothers of the group under study had a smoking rate of 34% while pregnant with the subject which was higher than the national average for women in the UK in

1984. The national rate of smoking was 35% (men 38% and women 33%) in 1982[319]. The majority of children had a smoker in the home and only 41% of children had no smokers in the house at either 1 year or 7 years of age. However if one of the parents had allergy, subjects were much less likely to have a smoker in the house (i.e. environmental tobacco smoke ETS). The OR for parental smoking and either parent having allergy was 0.59 (95% CI 0.37-0.94) p=0.03. Of those with no ETS 50.3% had parents with allergy while 37.4% had parents with no allergy.

At age 1, wheeze was present in 26% of those with no smoker in the house, 33% with a smoker that was not the mother and 47% in those whose mother smoked. This suggests that the greater the exposure to environmental tobacco smoke the more likely the subject was to wheeze. Unsurprisingly there was a significant association between environmental exposure to smoke at age 1 and wheeze at age 1 year: OR 2.14 (1.44-3.18) p<0.0001. There was no significant association between exposure to environmental tobacco smoke and wheeze at age 7: OR 1.33 (0.88-2.01) p=0.18.

Table 6.18 shows there was no significant association between maternal smoking during pregnancy and outcomes in adults. Evidence suggests that maternal smoking during pregnancy is associated with an increased risk of smoking in the offspring when older[317]. This fact could bias the results of our analysis, since atopic mothers are less likely to smoke. Therefore the children of atopic mothers are less likely to smoke and this could reduce the measured effect of maternal smoking on the development of asthma. Subjects smoking

themselves would make wheezing disorder more likely and if this active smoking was not taken into account, the effect of maternal smoking on wheeze in offspring may be exaggerated. In the final column of table 6.18 we therefore give results of associations controlled for current smoking age 23 years.

Table 6.18 Outcomes at age 23 years and association with maternal smoking during pregnancy

	Maternal smoking during pregnancy	No Maternal smoking during pregnancy	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value	Adjusted†† OR (95%CI) p-value
Wheeze age 23	34/89 (38.2%)	62/207 (30.0%)	1.45 (0.86-2.43) 0.17	1.52 (0.87-2.65) 0.14	1.34 (0.75-2.39) 0.32
Asthma age 23	14/89 (15.7%)	39/210 (18.6%)	0.82 (0.42-1.60) 0.56	0.75 (0.37-1.56) 0.45	0.71 (0.33-1.51) 0.37
Atopy age 23	23/39 (59.0%)	47/81 (58.0%)	1.04 (0.48-2.26) 0.92	1.30 (0.53-3.18) 0.57	1.18 (0.48-2.91) 0.72

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

†† Additional adjustment for current smoking at age 23 years

Table 6.19 shows the association of passive smoking at age 1 and outcomes at age 23. There are no significant results, even when adjusted for current smoking at age 23 years.

Table 6.19 Association of outcomes at age 23 years and exposure to passive smoke age 1 year

	Passive smoke age 1	No Passive smoke age 1	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value	Adjusted†† OR (95%CI) p-value
Wheeze age 23	48/135 (35.6%)	47/158 (29.7%)	1.30 (0.80-2.13) 0.29	1.35 (0.80-2.28) 0.26	1.25 (0.73-2.12) 0.41
Asthma age 23	26/137 (19.0%)	27/159 (17.0%)	1.15 (0.63-2.08) 0.66	1.18 (0.62-2.23) 0.61	1.21 (0.63-2.30) 0.57
Atopy age 23	34/56 (60.7%)	36/63 (57.1%)	1.16 (0.56-2.41) 0.69	1.59 (0.68-3.71) 0.29	1.59 (0.68-3.72) 0.29

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

†† Additional adjustment for current smoking at age 23 years

Table 6.20 shows the association of passive smoking at age 1 or 7 and outcomes at age 23 years. There are no significant results although atopy gives a large effect size.

Table 6.20 Outcomes at age 23 years and association with Passive smoking age 1 or 7 years

	Passive smoking age 1 or 7	No Passive smoking age 1 or 7	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23	56/157 (35.7%)	39/135 (28.9%)	1.37 (0.83-2.24) 0.22	1.41 (0.84-2.37) 0.20
Asthma age 23	29/159 (18.2%)	24/136 (17.6%)	1.04 (0.57-1.89) 0.90	1.05 (0.56-1.97) 0.88
Atopy age 23	39/62 (62.9%)	31/57 (54.4%)	1.42 (0.68-2.96) 0.35	1.94 (0.83-4.53) 0.13

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

Table 6.21 shows that ever having smoked was associated with having a wheeze at age 23. This significant association was present after adjusting for class and family history, suggesting that there is a true association of smoking with a wheezing disorder. Although asthma does not appear to be associated with a history of smoking at age 23, wheeze does. The association of active smoking at age 23 years with wheeze at age 23, after adjustment is OR 2.19 (1.25-3.83) p=0.006.

Table 6.21 Association of smoking among subjects and outcomes at age 23 years.

	Ever smoker	Never smoker	Unadjusted OR (95%CI) p-value	Adjusted† OR (95%CI) p-value
Wheeze age 23 years	54/140 (39%)	42/155 (27%)	1.69 (1.03-2.76) 0.02*	1.79 (1.07-3.01) 0.03*
Asthma age 23 years	27/140 (19%)	25/155 (16%)	1.24 (0.68-2.26) 0.29	1.29 (0.68-2.44) 0.43
Atopy age 23 years	28/53 (52.8%)	42/67 (62.7%)	0.67 (0.32-1.39) 0.28	0.74 (0.32-1.71) 0.48

†Adjusted for social class age 1, gender, breast feeding, original randomisation, parental allergy.

6.15 Discussion

In this chapter we have found some positive sporadic findings on early environmental factors and outcomes at age 23 years but on the whole the results do not show a consistent association between early environmental exposure and outcomes at age 23 years. Pet ownership, however, may have a protective effect at least with respect to atopy. An ECRHS study showed that dogs owned in childhood seemed to protect against adult allergic disease but promote non-allergic asthma[271]. The MAP cohort results suggest some evidence of an association of wheeze with environmental features which are more commonly present in subjects of low prosperity. One example is damp

and mouldy housing at age 7, which was associated with wheeze at age 23 years but not with asthma at age 23 years in the MAP study.

Solid fuel use was associated with a reduction in atopy at age 23 years. This may be because houses with solid fuel heating were less likely to have high levels of damp and the usual indoor aeroallergens since they would have open fires and be more likely to draw air though the house. However this is contradicted by our data which shows that rather than a lower prevalence of damp with solid fuel use in the home at the age of 7, there was a greater prevalence of damp in homes using solid fuel compared to other forms of heating (30.9% vs 23.8% respectively). Unfortunately we do not have data on aeroallergen level and solid fuel use, but evidence suggests that high indoor humidity, presence of wall-to-wall carpets, and poor ventilation all increase the risk for high allergen exposure[268].

There was no association between chest infection in the first year of life and outcomes at 23 years, although there is an association with wheeze at age 7. This contradicts other published work such as De Marco et al which was a large ECRHS study on subjects age 0-44 years. They found that respiratory infections at an early age are associated with an increased prevalence of asthma[270]. Viral infections are associated with asthma as shown in the TCRS[69]. RSV has long been thought to promote the development of asthma in association with genetic factors but this remains controversial and is reviewed along with other viral infections by Busse et al[320]. A review of the effects of infectious exposure suggests that the prenatal period and early childhood are

likely to be critical for the establishment and maintenance of a normal Th-1/Th-2 balance, and that viral and/or microbial infections and/or their products may have bidirectional effects on the development of allergy and asthma[321].

Increasing allergen exposure may be the source of the rise in asthma when subjects move from low prevalence (eg Tokelauan) to high prevalence (eg New Zealand) areas, since recent evidence has shown that Tokelauan residents are exposed to lower levels of indoor aeroallergens compared to residents in New Zealand[322]. However, there is no evidence regarding allergen levels or sensitisation in any other population study and therefore it cannot be assumed that increased rates of asthma in Westernised communities are related to increased allergen exposure.

The relationship between allergen exposure and the incidence of asthma shown by Sporik et al[180] is further complicated by the fact that bacterial endotoxins may induce the Th1 immune pathway. A study examining the endotoxin in the house dust of 61 infants with at least 3 physician documented episodes of wheeze, reported that there was significantly less endotoxin in the homes of those infants who were sensitised to at least one aeroallergen (HDM, Cat, Dog and Mouse)[323], suggesting that endotoxin may be associated with a reduction in sensitisation. However, there is also evidence that a high endotoxin level can increase the risk of wheeze and asthma. The "Epidemiology of Home Allergens and Asthma" study found that there was an increased risk of wheeze in the first year of life in those with a family history of asthma or allergy and a high measured endotoxin level in living room

dust[324]. The Reidler group found a trend to an increased prevalence of non-atopic asthma and wheeze with increased levels of endotoxin, although this was not significant[325]. These studies suggest that the same environmental factor can have different effects in different settings and at different concentrations. Since our subjects were more likely to be from a deprived population than other populations in most longitudinal studies they may have had high levels of endotoxin which reduced their risk of atopy but also increased their risk of asthma and wheeze.

Immunisation may predispose to allergy since it may cause a reduction in exposure to infection and therefore encourage T-helper type 2 (Th2) immune responses. Alternatively, vaccination may expose the infant to attenuated pathogens and therefore promote a T-helper type 1 (Th1) pathway[326]. It is therefore unclear what outcome we might anticipate when investigating immunisation and prevalence of asthma. The MAP cohort found no conclusive results with immunisation and outcomes at age 23 years. Caesarean section was also found to have no association in the MAP cohort unlike published research as discussed in the introduction.

The main reason that MAPS showed no definite associations with environmental factors is because the sample size was too small to study the large number of potential environmental factors that might be responsible for incident asthma. However other factors are likely to be confounders, such as the high rate of environmental tobacco smoke (ETS) in this community since over 50% of children were exposed to passive smoke before the age of 7.

Smoking among pregnant women in this community was higher than women in the UK as a whole for the equivalent period, suggesting that this was a community with a very high prevalence of smoking. It is therefore possible that smoking was so common in this community that even those children who did not have smokers at home may have been exposed to tobacco smoke at the homes of friends and family, this could have reduced the effect size of any association we might have found.

In addition smoking is known to have effects on the immune system, some of which may be suppressive such as the results of a study undertaken by Nymand who investigated pregnant women and found that that 10.4% of non-smokers developed antibodies during the first pregnancy, rising to 31% after the fourth. The corresponding figures in smokers were 7.8% rising to 17.4%, which indicates significantly reduced immune recognition of foreign histocompatibility antigens among the smoking population[327]. Other studies have found that smoking can increase the risk of allergic disease as expounded in a recent meta-analysis where an association between ETS exposure in early childhood and the increased risk of allergic sensitisation was found. In addition, subgroup meta-analyses demonstrated that younger children suffer the most from detrimental immunomodulating effects of ETS exposure[328]. The evidence that tobacco smoke has profound effects on t cell activity and other humoral and cellular immune responses in experimental animals has long suggested that it also has significant effects on the human immune system, as reviewed by Holt et al[329]. Smoke can cause inhibition of the immune system in pregnant women, and enhancement of the immune system in children. It

may also have different effects determined by co factors in the environment or genetic factors in the child. This makes it difficult to elucidate a clear picture of environmental effects. Therefore the main finding in this section is that no association with early life environment has been proven. Our study can neither refute nor support the work presented in the introduction.

Chapter 7

The natural history of asthma and wheeze

7.0 The course of asthma from birth to adulthood

The course of asthma from birth to death is variable. Some asthma sufferers appear to recover completely. Others have long remissions with occasional mild relapses. Most seem to continue unchanged for many years, but some become more symptomatic and may develop irreversible airway obstruction. Rarely some die of acute asthma. Treatment of patients could be improved if we were able to understand the reasons for this variability[330]. Longitudinal population studies are an important way of increasing our understanding of the natural history of asthma. However despite the number of longitudinal studies already in place, our understanding of asthma aetiology, progression and complication rate is still poor. There are a number of reasons for this, including differing definitions of asthma or other end point used and multiple environmental factors that cannot all be controlled for. It is also debateable whether asthma is a disease with a single aetiology and many varying outcomes or a syndrome with many different factors that cause a variable phenotype[330]. However we know that asthma begins most often in infants as wheezing with respiratory infections. If these episodes are mild and infrequent, asthma does not usually continue into the school years. If wheezing episodes are more frequent and severe, then asthma is likely to persist. If atopy develops before the age of 3 years subjects are more likely to develop asthma and have impaired lung function by school age[49]. Patients

with frequent exacerbations have a significantly larger annual decline in forced expiratory volume in one second (FEV1)[331].

Asthma may remit at any age, especially during adolescent years, as shown in the Tucson Children's Respiratory Study (TCRS) where 42% of subjects had remission from asthma around the time of puberty[332]. However remission was based on symptoms of asthma in the TCRS study, rather than objective measurements. Vonk et al defined complete remission of asthma in 5-14 year old Dutch asthmatic children in a specialist clinic as having no current wheeze, no asthma attacks in the previous 3 years, no use of inhaled corticosteroids, normal lung function (FEV1 90% predicted) and absence of bronchial hyper-reactivity (BHR). They found that 22% had complete remission after a median of 30 years follow up and a further 30% (total 52%) of the group had no asthma symptoms and did not use steroid inhalers; this was termed clinical remission[333]. Panyhuysen et al found a remission rate in Swedish asthmatics of only 11% after 25 years follow up and found remission was associated with a younger age and less severe airway obstruction at first testing[334]. The Dutch team also found that more severe disease and continued allergen exposure cause the disease to persist and that a persistent decline in lung function was retarded, but not completely prevented, by aerosol glucocorticoids[335]. They also found that in subjects with established asthma, aged 13-44 years from secondary care clinics, there was evidence of irreversible airway obstruction and a low transfer coefficient, even though both these features are usually thought of as characteristics of COPD, they represented distinct entities in adult asthma [336].

7.1 Tracking of lung function from childhood into adulthood and risk of adult chronic obstructive pulmonary disease (COPD)

For decades asthma was considered to be a completely reversible obstructive airways disease but after a number of pathological investigations it became apparent that asthma is not always reversible and the term remodelling was used to describe the process of progression of disease[123]. Remodelling was first defined, clinically as asthma that loses its reversible aspect by Rasmussen et al studying the Dunedin birth cohort. In this study they measured lung function in their subjects at age 9, 11, 13 and 15 during childhood years and then again at age 18 and 26 years in adulthood. Those subjects who showed low post-bronchodilator ratios in childhood also showed a greater decline from ages 9 to 26 in the pre-bronchodilator ratio of forced expiratory volume divided by vital capacity (FEV_1/VC ratio) compared with those with normal post-bronchodilator FEV_1/VC ratios at both ages. Asthma, male sex, airway hyper-responsiveness, and low lung function in childhood were each independently associated with a low post-bronchodilator FEV_1/VC ratio, which in turn was associated with an accelerated decline in lung function and decreased reversibility. They postulated that low lung function tracks from childhood into adulthood, with greater loss of function in male than female asthmatics at age 26 years[80]. Their findings of irreversible obstruction would fit with a diagnosis of COPD. The TCRS also showed that poor lung function, measured using maximal expiratory flows at functional residual capacity in 169 infants by the chest compression technique at a mean age of 2·3 months (SD 1·9), was associated with low lung function at age 16 and 22 years. They postulated that poor airway function shortly after birth should be recognised as

a risk factor for airflow obstruction in young adults and that prevention of COPD might need to start in foetal life[337].

Thus far we have presented evidence regarding the natural history of asthma from inception to chronic non reversible lung disease. However it is the inception of asthma that is of interest in the understanding of the natural history of asthma and now we will present the evidence regarding patterns of early childhood wheeze and asthma in late childhood and adulthood.

7.2a) The natural history of early life wheezing disorder and wheeze phenotypes

In 1991 Sporik, Holgate and Cogswell investigated a high risk birth cohort and showed a bimodal distribution of wheeze with a peak before the age of 2 years and a gradual increase thereafter. Of the 21 children who wheezed before their second birthday, most never wheezed again and did not show bronchial hyper reactivity (BHR) at 11 years[338]. This early study was the first to suggest that not all early life wheezing was the same and that wheeze after infanthood was a significant sign of later childhood asthma, while early childhood wheeze might have different aetiology. In some children wheeze does continue and develop into asthma. Many longitudinal studies have investigated early life patterns of wheeze in order to understand which phenotypes predispose to the development of asthma.

7.2b) Phenotype studies using four phenotypes

A number of longitudinal studies have investigated the early childhood wheeze phenotypes by using variations of the TCRS phenotypes on their own cohort. The modifications from the TCRS phenotype definitions have been determined by when subjects were followed up in the particular longitudinal studies. All the phenotype studies are slightly different but generally they investigate subjects in early childhood (between 0-3 years old), and later, early childhood years (between 3 years old and 6 or 7 years old). The four phenotypes for TCRS are:

- Never wheeze (NW): no wheeze before 3 years of age and no wheeze up to 6 years of age.
- Transient early wheeze (TEW): wheeze before the age of 3 but no wheeze in the year of the 6th birthday.
- Late onset wheeze (LOW): no wheeze before age 3 years but wheeze in the year of the 6th birthday
- Persistent wheeze (PW): wheeze before age 3 years and wheeze in year of 6th birthday.

The TCRS participants had lung function measured in the first 2-4 months of life and those who had infant Vmax FRC in the lowest quartile also had lower values for the FEV1/FVC ratio up to age 22, after adjustment for height, weight, age, and sex, than those in the upper three quartiles combined. They also found that lung function during infanthood was significantly lower with the TEW phenotype, when compared to those who never wheeze ($p<0.01$) and when compared to LOW and PW ($p<0.05$)[92]. At ages 11 and 16 years

subjects who had been defined as having TEW phenotype, had the same risk of wheeze in later childhood as those who had never wheezed before the age of six. They also found that both TEW and PW had significantly lower FEV_1 and FEV_1/FVC ratio at 11 years and 16 years compared to the other two phenotypes[185]. The TCRS study found an association between LOW and PW phenotype at age 6 and newly diagnosed asthma at age 22 years[117].

The German Multicentre allergy study (MAS) followed 1314 children from 5 centres from birth. Subjects were reviewed at ages 1, 3, 6, 12 and 18 months, then yearly, within 3 months of their birthday, up to 7 years of age. They described four phenotypes, similar to those in the TCRS, based on early wheeze before the age of 3 and wheeze in the year of their 7th birthday. They examined lung function tests at age 7 and the results were expressed as a percentage of the predicted value according to internal reference values which were determined using statistical methods. Results of FEV_1 for the PW phenotype (96.5%+-9.3% predicted) and the LOW phenotype (94.1%+-12.2% predicted) were significantly different (p=0.01, p=0.001 respectively) from the NW phenotype (100.9% +-11.9% predicted). The TEW phenotype (99.3%+-12.3% predicted) was not significantly different from NW. The lower lung function tests in LOW and PW were therefore already apparent by age 7 years and these were more likely if subjects were atopic or had an atopic family history[96].

MAAS is a longitudinal population-based birth cohort also classified into the 4 phenotypes, based on the fact the parents were interviewed when the subject

was age 3 and also at age 5[98]. They found poor lung function at age 3 years was associated with the subsequent persistence of symptoms in children who had wheezed within the first 3 years (i.e. PW phenotype), but was not associated with the onset of wheeze after age 3 years in the children who had not wheezed previously (i.e. LOW phenotype).

All these three studies have used different measurements for lung function and at different ages so they are not necessarily comparable. Lung function measurements in young children are difficult to undertake and the difference between the studies may also be explained by this, although finding early changes in the TCRS study is due to the very early testing undertaken in this study. In summary, reduction in measures of lung function was present in the TCRS cohort in the TEW and PW phenotypes at age 11 and 16 and only in TEW in infanthood. In the German MAS the TEW phenotype did not show reduced lung function unless they had been exposed to a lower respiratory tract infection or the mother was a smoker; only LOW and PW had lower lung function. The MAAS group just found a reduced lung function at age 3, in those who went on to be defined as PW subjects at age 5 years. From these studies we can see that the PW phenotype has universally reduced lung function in all the studies. TEW may only have reduction in lung function during infanthood.

7.3 Exercise testing

Mochizuki et al undertook metacholine inhalations tests on subjects age 6 months and then again 18 months later. A positive test was defined by a 10% drop in transcutaneous oxygen tension. A positive test was only found in those who had established asthma, not in those who went on to develop asthma[339]. Porsbjerg et al showed that both exercise challenge (with 10% drop in FEV1) and chemical challenge (20% drop) may be positive in children who do not have asthma at the time of a positive result although they may subsequently develop it, up to 12 years later[340]. Jones showed that a positive exercise test, defined as a 15% or greater fall in peak expiratory flow rate after 6 minutes of exercise, in early childhood can predict asthma in later childhood[341], and Rasmussen et al undertook exercise testing in asymptomatic children at a mean age of 9.7 years. They found a percentage fall in FEV1 of 8.6% or greater was associated with wheeze in adulthood[342]. This suggests that positive exercise testing and chemical testing are associated with asthma later in life, although chemical challenge is also associated with established asthma.

7.4 Aims and objectives

The aim of this chapter is to investigate the natural history of the MAP cohort with regard to wheezing from birth to adulthood. We will use phenotypes based on established classifications in order to investigate if there is an association between these phenotypes and outcomes in later life. We will

investigate chronic lung disease in young adults and what factors are associated with low lung function measurements at age 23 years.

Objectives

- To categorise subjects using modified classifications for childhood wheezing phenotypes
- To investigate the association of a positive exercise test at age 7 with outcomes in adult subjects
- To investigate associations of these childhood wheezing phenotypes with the patterns of chronic lung disease commonly seen in adulthood.

7.5 Methods

Full methods are given in chapter 2.

7.5a Birthday cards

The Merthyr Allergy Prevention Study (MAPS) gathered information on wheeze and respiratory infections at pre-set interviews with predetermined questionnaires, with subjects at 3, 6 and 12 months of life. Each year between the ages of 2 and 6 years, subjects were sent a birthday card with a short parental questionnaire attached. This included a question regarding wheezing: 'Have you heard a wheeze coming from his/her chest'. The questionnaire is shown in appendix 6. Phenotypes in MAPS were coded using only complete

data, i.e. only using the 430 subjects on whom there was data regarding all time points up to age 7. Subjects with missing data were therefore not included.

Phenotypes were based on those in the TCRS but modified to take account of the data collection protocol:

- NW was no wheeze at any time point.
- TEW was wheeze between birth and age 3 years and not between 4 and 7 years.
- LOW was wheeze at some age between 4 and 7 but not before this.
- PW was wheeze at least once before 3 years and at least once between 4 and7 years.

The differences between definitions in MAPS, TCRS and German MAS are detailed in Table 7.1 below.

Table 7.1 Definitions of different wheeze phenotypes in TCRS[92], MAS and MAPS

	Tucson Children's Respiratory Study[92] (TCRS)	German MAS[96]	Merthyr Allergy Prevention Study (MAPS)
Never Wheeze (NW)	No Wheeze age 0-3† or at age 6	Never wheeze up to age 7	No Wheeze age 0-3 or age 4-7
Transient Early Wheeze (TEW)	Wheeze age 0-3 but no wheeze in previous 12 months at age 6	Wheeze at least once before 3 and not in past year at age 7	Wheeze age 0-3 but no wheeze age 4-7
Late onset Wheeze (LOW)	No wheeze age 0-3 but wheeze in previous 12 months at age 6	No wheeze before 3 but wheeze in the past year at age 7	No wheeze age 0-3 but at least one positive record of wheezing age 4-7
Persistent Wheeze (PW)	Wheeze age 0-3 and wheeze in previous 12 months at age 6	Wheeze at least once before age 3 and in past year at age 7	Wheeze age 0-3 and at least one positive record of wheezing age 4-7

† At least one physician-diagnosed wheezing lower respiratory illness (LRI) in the first 3 years of life.

7.5b) Exercise test age 7 years

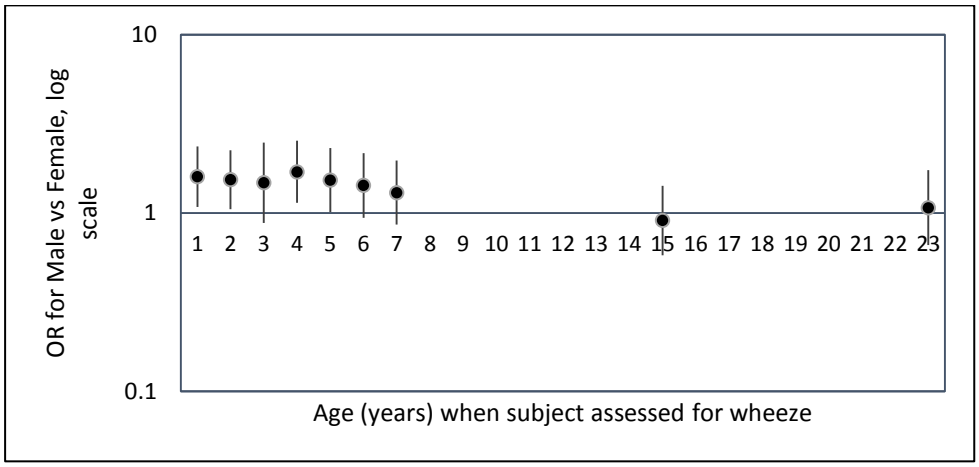
At age 7 years, each child was invited to undertake an exercise challenge test. Peak expiratory flow rate (PEFR) was measured 5 times before exercise began. The children then ran round a room for 6 minutes, and 5 further PEFR measurements were taken after 5 minutes' rest. On each occasion the mean of the 3 highest readings was taken as the estimated value. The results of the exercise testing have been published and a significant difference was found between the mean change in PEFR in those who wheeze and the mean in those who do not at age 7 years[107]. We have taken a drop in PEFR of 15% or greater as a positive exercise test.

7.6 Results

Up to age 7 years, 430 subjects out of a total of 487 (88.3%) gave complete information on the presence of wheeze at ages 0-7 years. There was complete data to age 15 on 345 (70.8%) subjects and to age 23 years on 255 (52.2%) subjects.

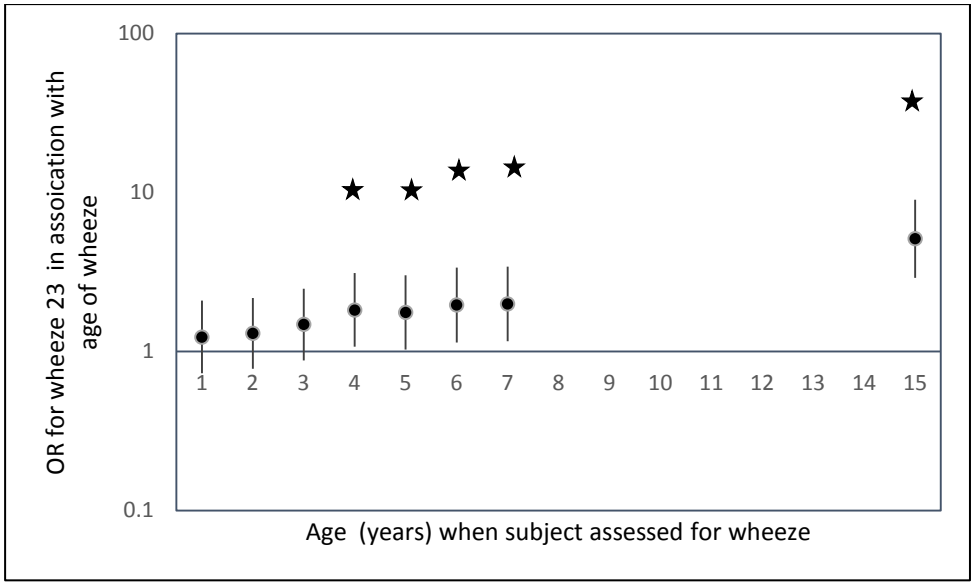
As shown in Figure 7.1, wheeze is more common in males than in females up to age 7. The OR for the association between wheeze and gender is more than 1 in favour of males at each wheeze age. While these results are not always significant, there is an obvious pattern towards more wheezing in males. However at ages 15 years and 23 years the OR is close to 1 suggesting no significant gender difference.

Figure 7.1 The Association of gender with age of wheeze (log scale)



The association of wheeze at age 23 with wheeze at different ages in childhood is displayed in figure 7.2 by plotting the OR measuring the association between wheeze at earlier ages with wheeze at age 23. A significant OR is indicated by a star. The figure demonstrates that in our cohort, wheeze from the age of 4 is significantly associated with wheeze at age 23 years. This suggests that early wheeze, which occurs before the age of 4, may be different in origin to adult wheeze, while late childhood wheeze may be a precursor of adult disease.

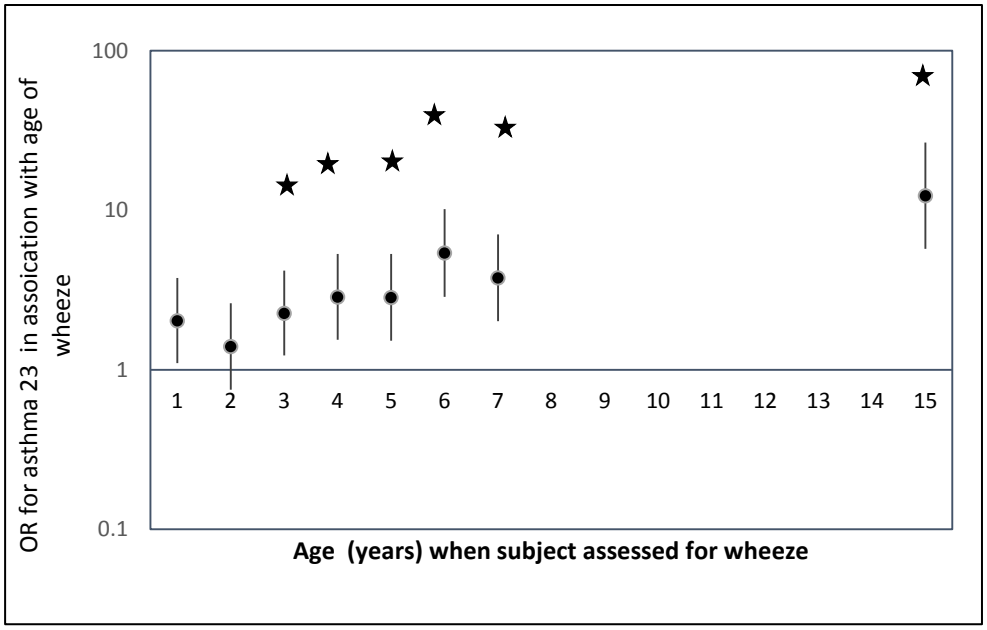
Figure 7.2 The association of wheeze age 23 years with wheeze in childhood (log scale)



A star signifies a statistically significant result

Figure 7.3 plots the OR for the association of asthma at age 23 years with wheeze at different ages in childhood. This suggests that wheeze before age 3 years is probably not a prequel to asthma but wheeze in late childhood is.

Figure 7.3 The association of asthma age 23 with wheeze in childhood (log scale)



A star signifies a statistically significant result

7.7 Exercise test results age 7 years

A total of 202 boys and 188 girls undertook an exercise test, in which 67 (33%) boys and 55 (29%) girls were positive. A positive exercise test was not associated with wheeze at age 7 (OR1.35, 95% CI 0.85-2.16 p=0.12), wheeze at 15 (OR 1.06, 95% CI 0.64-1.76, p=0.46) or wheeze at 23 years (OR 0.97, 95% CI 0.56-1.68, p=0.52), but was associated with asthma at age 23 years 2.15 95% CI 1.13-4.08, p=0.02. These findings suggest that wheeze at 23 years is different to asthma at 23 years, as has been shown on a number of occasions in this thesis.

7.8 Childhood wheeze and remission, relapse and late onset

To determine remission and relapse rates we used only those 255 subjects on whom we have outcome data at ages 1-7, 15 and 23 years. A total of 165/255 (64.7%) subjects had a wheeze at least once in the first 7 years. Remission of this childhood wheeze was found in just under 50 percent of subjects; 77 of the 165 who had a wheeze before the age of 7 (i.e. 30.2% of the original 255), did not wheeze again at age 15 or 23- early wheeze with remission. Of the 90 who did not wheeze before 7, 29 (11.4% of the original 255) went on to have a wheeze at age 15 or 23 years- later onset wheeze. Adult onset was found in 14 (5.5% of the original 255) out of the 90 who did not wheeze before age 7 years and who had a wheeze for the first time at age 23 years- adult onset wheeze. No information was gathered in the intervening years so it is possible that subjects did wheeze more frequently but we do not have that information. The early Cogswell group found that 75% of those who had wheeze onset before age 2 did not have wheeze at age 11[338]. We do not have data at age 11 but do have data on wheeze at age 15 and 23 years and asthma at age 23 years. Of those subjects in the MAPS cohort who had wheeze at or before the age of 2 years, 59.8% were wheeze free at age 15 years and 63.5% were wheeze free at age 23 years. These rates are lower than the Cogswell group at age 11 years. However when we investigate subjects with asthma at age 23 years, 77.2% of those who had a wheeze at age 2 years or younger were free from asthma at age 23 years.

7.9a) Childhood phenotypes in MAPS and comparison with other longitudinal studies

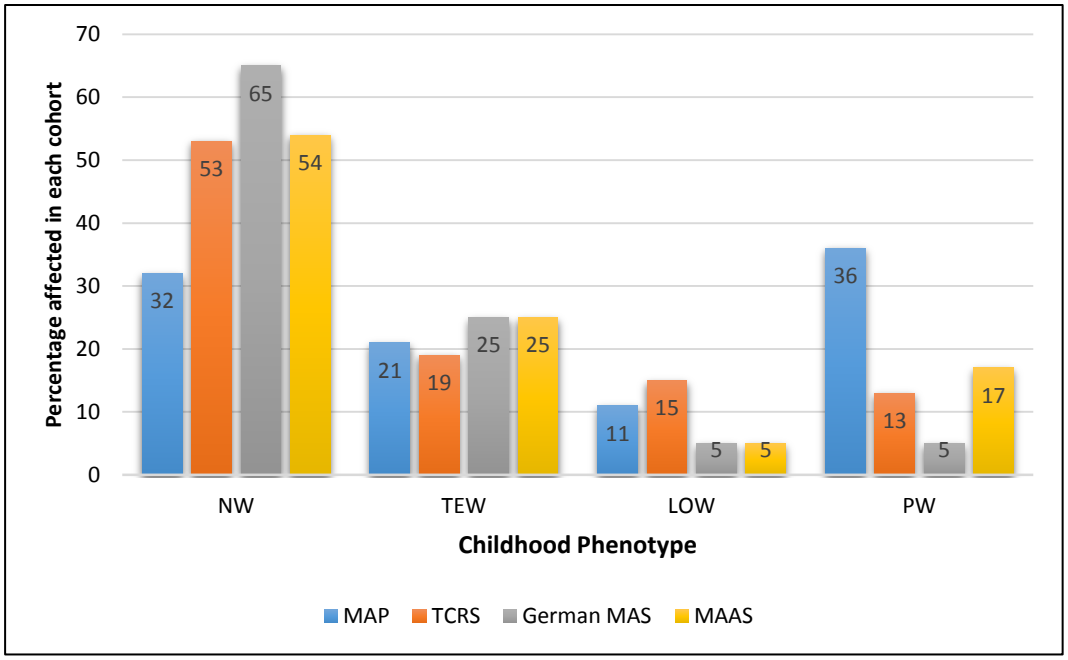
The MAP study reported on a greater number of possible observations than the other studies. This means there is a greater chance of someone being classed as having a wheeze. The TCRS, German MAS and MAAS have used only the ages stated and considered only wheeze in the year before the later observation year stated. This means that the definitions are not strictly comparable. In fact if we use TCRS definitions to analyse the MAP study data i.e. NW: no wheeze age up to 3 or at age 6, TEW: wheeze only in first 3 years, LOW: wheeze only at age 6 and PW: wheeze in first 3 years and again at age 6 years, the percentages of phenotypes are given in table 7.2. The directions of the differences in prevalence are all as expected, given the definitions.

Table 7.2 The prevalence of different phenotypes in the MAPS cohort using MAPS definitions and also TCRS definitions

	MAPS Definitions	TCRS Definitions
NW	32.1%	38.4%
TEW	20.7%	33.2%
LOW	10.9%	4.6 %
PW	36.3%	23.8%

Figure 7.4 shows the differences in phenotype frequencies between a number of longitudinal study cohorts. Some of the differences are due to varying definitions, as illustrated above, while others may arise from differences in the populations.

Figure 7.4 Rate of childhood phenotypes for MAPS compared to TCRS, German MAS and MAAS.



NW= Never Wheeze, TEW=Transient Early Wheeze, LOW=Late Onset Wheeze, PW=Persistent Wheeze

Table 7.3 displays a comparison of the phenotypes for the cohorts from MAPS, TCRS, German MAS and MAAS. The prevalence of atopy in childhood in each group for TCRS and German MAS (age 6 years for TCRS, age 7 years (at age 6 if age 7 not available) for MAS and age 7 years MAPS) is shown. It was surprising that the MAPS cohort was generally less atopic than the TCRS subjects, despite the fact that the MAPS group was recruited from a high risk population and therefore might be expected to have a greater rate of atopy. The prevalence of atopy was 51% in the TCRS, 41% in the German MAS group and only 34% in the MAPS cohort. However within each

cohort LOW was the most atopic phenotype and PW was the second most atopic phenotype. The prevalence of atopy was not given for MAAS.

Table 7.3 Childhood wheeze phenotype in MAP cohort compared to TCRS and MAAS

Childhood Wheeze Phenotype	**MAP study (430) High risk population**		**TCRS[92] (762) Unselected population**		**German MAS[96] 'Nested' population study†**		**MAAS[98] (463) Unselected population**
	Prevalence	% Atopic	Prevalence	% Atopic	Prevalence	% Atopic	Prevalence
Never wheeze	32%	17.3%	53%	33.8%	65%	35.4%	54%
Transient early wheeze	21%	13.3%	19%	38.4%	25%	42.6%	25%
Late onset wheeze	11%	43.5%	15%	55.7%	5%	75.7%	5%
Persistent Wheeze	36%	34.0%	13%	51.1%	5%	51.1%	17%

†1314=total population -499 high risk subjects 815 controls.

Gender differences in four childhood wheeze phenotypes are shown in table 7.4. Boys are known to wheeze more in childhood than girls and using NW as the reference variable, there is an association between male gender and the two phenotypes, TEW and PW. There is no definite association of LOW with gender, possibly due to the low frequency of this phenotype. These results

confirm that wheezing before three is more common in boys than girls, and therefore the prevalence of TEW and PW is higher in boys.

Table 7.4 Gender difference in the 4 early wheeze phenotypes

All subjects n=430	Male subjects	Female subjects	OR (95% CI) p-value with NW as reference variable	Adjusted† OR (95% CI) p-value with NW as reference variable
NW	59/111 (53.2%)	79/116 (68.1%)	1	1
TEW	52/111 (46.8%)	37/116 (31.9%)	1.88 (1.10-3.23) 0.02*	2.11 (1.19-3.75) 0.01*
LOW	23/82 (28.0%)	24/103 (23.3%)	1.28 (0.66-2.49) 0.46	1.31 (0.66-2.58) 0.43
PW	93/152 (61.2%)	63/142 (44.4%)	1.98 (1.24-3.14) 0.004*	1.91 (1.18-3.12) 0.009*

†Adjusted for social class age 1 year, breast feeding, original randomisation, parental allergy.

7.9b) Childhood wheeze phenotypes and outcomes at age 23 years in MAPS.

The definition of childhood phenotypes can help us to understand asthma in early years. However, it is clear that not all childhood wheeze occurs in children with asthma. It is therefore important to investigate which outcomes

are associated with different childhood phenotypes in later childhood and adult life in the MAP cohort and compare this to other established cohorts.

These childhood phenotypes are helpful only in as much as they can inform our understanding of the natural history of asthma. To examine the association with adult outcomes we have investigated associations between phenotypes and wheeze and asthma at age 23 years. These are shown in tables 7.5 and 7.6. TEW does not appear to be associated with either wheeze or asthma age 23 years. Wheeze at age 23 is only associated with PW and not with LOW while asthma age 23 is highly significantly associated with PW as expected and also there is a non-significant association with LOW.

Table 7.5 Childhood phenotypes and association with wheeze at age 23 years

All subjects n=285	Wheeze age 23 (93)	No wheeze age 23 (192)	OR (95% CI) p-value with NW as reference variable	Adjusted† OR (95% CI) p-value with NW as reference variable
NW (100)	25	75	1	1
TEW (59)	18	41	1.32 (0.64-2.69) 0.45	1.61 (0.73-3.55) 0.24
LOW (28)	10	18	1.67 (0.68-4.08) 0.26	1.56 (0.62-3.89) 0.34
PW (98)	40	58	2.07 (1.13-3.79) 0.02*	2.09 (1.09-4.00) 0.03*

†Adjusted for social class age 1 year, gender, breast feeding, original randomisation, parental allergy.

Table 7.6 Childhood phenotypes and association with asthma at age 23 years

All subjects n=285	Asthma age 23 (51)	No asthma age 23 (234)	OR (95% CI) p-value with NW as reference variable	Adjusted† OR (95% CI) p-value with NW as reference variable
NW (100)	9	91	1	1
TEW (59)	7	52	1.36 (0.48-3.87) 0.56	1.64 (0.52-5.11) 0.40
LOW (28)	5	23	2.20 (0.67-7.19) 0.19	2.09 (0.59-7.38) 0.25
PW (98)	30	68	4.46 (1.99-10.01) <0.0001*	4.99 (2.09-11.93) <0.0001*

†Adjusted for social class age 1 year, gender, breast feeding, original randomisation, parental allergy.

7.9c) Aetiological factors and childhood wheeze phenotypes in MAP

Table 7.7 shows a significant protective association between ever being breast fed and TEW and PW and no evidence of an association with LOW. These results reflect the earlier findings of a significant association of breast feeding with wheeze at age 1 but not with wheeze at age 7.

Table 7.7 Childhood phenotypes and association with breast feeding age 1 year

All subjects n=430	Ever breast fed	Never breast fed	OR (95% CI) p-value	Adjusted† OR (95% CI) p-value
NW (138)	66	72	1	1
TEW (89)	28	61	0.50 (0.29-0.88) 0.02*	0.54 (0.30-0.98) 0.04*
LOW (47)	21	26	0.88 (0.45-1.71) 0.71	0.87 (0.44-1.72) 0.68
PW (156)	50	106	0.52 (0.32-0.83) 0.006*	0.55 (0.33-0.92) 0.02*

†Adjusted for social class age 1year, gender, original randomisation, parental allergy.

Table 7.8 shows environmental tobacco smoke (ETS) exposure gives a statistically significant association with both TEW and PW, probably because smaller airways are more likely to be affected by the injurious properties of tobacco smoke. Both these phenotypes, TEW and PW, by definition have wheeze before the age of 3 years and when airways are small.

Table 7.8 Childhood phenotypes and association with environmental tobacco smoke (ETS) age 1 year, defined as mother or other person living with subject age 1 year being a regular smoker.

All subjects n=429	ETS age 1	No ETS age 1	OR (95% CI) p	Adjusted† OR (95% CI) p
NW (138)	52	86	1	1
TEW (89)	53	36	2.44 (1.41-4.20) 0.001*	2.17 (1.23-3.84) 0.008*
LOW (46)	22	24	1.52 (0.77-2.98) 0.23	1.57 (0.79-3.12) 0.2
PW (156)	89	67	2.20 (1.38-3.51) 0.001*	1.79 (1.09-2.94) 0.02*

ETS = Environmental tobacco smoke

†Adjusted for social class age 1 year, gender, original randomisation, parental allergy and ever breast fed.

Either parent having allergy is significantly inversely associated with TEW, possibly due to the reduced prevalence of smoking among atopic parents as discussed in chapter 6 section 6.14a. However when the association of TEW and either parent having allergy is adjusted for exposure to environmental

smoke age 1 year as well as the other confounders, the OR remains significant: 0.44 (95% CI 0.23-0.87) p=0.02, suggesting that there is a true inverse association between the TEW phenotype and having atopic parents in the MAPS cohort. Table 7.9 also shows a non-significant association of parental allergy with LOW. In our cohort there appears to be no association between parental allergy and PW, the phenotype most closely linked to asthma. This suggests environmental attributes may contribute more to the development of asthma than genetic characteristics in our cohort.

Table 7.9 Childhood phenotypes and association with parental allergy

n=430	Either parent allergy	Neither parent allergy	Unadjusted OR (95% CI) p-value	Adjusted† OR (95% CI) p-value
NW (138)	115	23	1	1
TEW (89)	61	28	0.44 (0.23-0.82) 0.01*	0.42 (0.22-0.83) 0.01*
LOW (47)	43	4	2.15 (0.70-6.58) 0.18	2.10 (0.68-6.49) 0.20
PW (156)	131	25	1.05 (0.56-1.95) 0.88	1.07 (0.56-2.06) 0.83

†Adjusted for social class age 1 year, gender, ever breast fed, original randomisation

In table 7.10 we show the association between atopy at age 7 years and these childhood wheeze phenotypes. It is to be noted that the wheeze phenotypes

are determined before the age of atopy and therefore it is not surprising that there is no association with TEW since this early wheeze predates the age of atopy. There is however an association of PW with atopy age 7 years and an even stronger association with LOW and atopy at age 7 despite the small numbers in the LOW group.

Table 7.10 Childhood phenotypes and association with atopy age 7 years

N=415	Atopic age 7	Not atopic age 7	Unadjusted OR (95% CI) p	Adjusted† OR (95% CI) p
NW (133)	23	110	1	1
TEW (83)	11	72	0.73 (0.34-1.59) 0.43	0.76 (0.33-1.74) 0.52
LOW (46)	20	26	3.68 (1.76-7.68) 0.001*	3.67 (1.73-7.78) 0.001*
PW (153)	52	101	2.46 (1.41-4.31) 0.002*	2.50 (1.39-4.50) 0.002*

†Adjusted for social class age 1 year, gender, ever breast fed, original randomisation, parental allergy.

7.10 Chronic changes age 23 years and association with childhood variables

A total of 14 subjects out of 120 who had spirometry, i.e. 11.7% of subjects had an FEV_1<80% of the predicted value. Out of 119 subjects for whom we have a valid post bronchodilator FEV_1, 8 (6.7%) had a low FEV_1 post bronchodilator. One subject was excluded because their FEV_1 dropped from above 80% to below after bronchodilators and therefore only seven subjects are included in the assessment of low FEV_1 post bronchodilator in table 7.11. Those with post bronchodilator FEV_1 <80% would fulfil the criteria for lung obstruction. Of these seven subjects only four had wheeze at age 23 years, and three did not suffer with wheeze. These seven were defined as having irreversible obstructive airways disease or remodelling, using the definition expounded by Rasmussen et al[80]. The other six subjects with low pre bronchodilator FEV_1 did reverse and therefore were consistent with poorly controlled asthma. These findings suggest a high prevalence of chronic lung disease at the early age of 23. Table 7.11 shows the gender differences between males and females for chronic lung disease in our cohort. Males appear to have a higher rate of chronic lung disease than females, even when results are adjusted for smoking. The reason for the higher level of chronic lung disease in males is not clear, but may be related to early wheeze in males.

Table 7.11 Gender differences in chronic lung disease

Chronic Phenotype	Male	Female	OR (95% CI) p-value	OR (95% CI) p-value Adjusted for ever smoking
Low FEV1 pre β agonist (Low FEV1)†	9/50 (18.0%)	4/70 (5.7%)	3.62 (1.05-12.52) p=0.04*	4.02 (1.14-14.20) p=0.003*
Low FEV1 post β agonist (irreversible obstruction phenotype)	6/50 (12.0%)	1/70 (1.4%)	9.41 (1.10-80.82) p=0.04*	12.42 (1.22-98.03) p=0.02*

† Group is made up of both the COPD and those with reversible obstructive airways

Table 7.12 shows the association between wheeze at different ages and the irreversible obstruction phenotype in adults as we have defined it (post bronchodilator FEV_1 less than 80% predicted). There were no statistically significant results but there was a non-significant association of the irreversible obstruction phenotype with wheeze at age 1 year or before. We might speculate that those subjects who wheeze before the age of 1 were similar to those subjects in the TCRS study who were in the lowest quartile for lung functions at 3 months of age. There was no other significant association between childhood wheeze and the adult irreversible obstruction phenotype although some ORs were moderately large.

Table 7.12 Association of irreversible obstruction phenotype with wheeze in childhood and adulthood

Age at wheeze	**Low FEV1 post β agonist - irreversible obstruction phenotype**	**No Low FEV1 post β agonist**	**OR (95% CI) p-value**	**OR (95% CI) p-value Adjusted for ever smoking**
1 year	4/7 (71.4%)	32/111 (28.8%)	3.29 (0.70-15.54) p=0.13	4.13 (0.82-20.78) p=0.085
7 years	2/7 (28.6%)	31/113 (27.1%)	1.06 (0.20-5.74) p=0.95	1.05 (0.19-5.90) p=0.96
15 years	2/7 (28.6%)	36/104 (34.6%)	0.76 (0.14-4.09) p=0.75	0.69 (0.12-3.91) p=0.68
23 years	4/7 (57.1%)	49/113 (43.4%)	1.74 (0.37-8.14) p=0.48	1.63 (0.32-8.29) 0.56

7.12 Discussion

We investigated the natural history of wheeze and asthma in MAPS. We found that before the age of 7 years, males were more likely to wheeze than females and that wheeze between ages 3 and 7 was especially associated with asthma and wheeze at the age of 23 years. We found that more than 50% of childhood wheeze sufferers went on to wheeze at the age of 15 or 23 years. A positive exercise test at age 7 was also associated with asthma at age 23 but not with wheeze at age 23 or younger. Both wheeze and asthma at age 23 years were significantly associated with the PW phenotype suggesting that this phenotype may be the precursor for chronic lung disease in this cohort. The four phenotypes could be considered to be really just two phenotypes; those who wheeze early (TEW and PW) and those who do not (NW and LOW). This would explain some of the findings such as the increased male tendency to have either TEW or PW phenotypes. However for some outcomes we could divide the group into two phenotypes in a different way. Since PW and LOW are both more likely to be associated with asthma and wheeze at age 23 years and TEW and NW are not significantly different in many of our outcome measures, as was found in the TCRS study[185], we might consider the two phenotypes to be NW and TEW and alternatively LOW and PW together.

Of interest was the comparison between the phenotypes and atopy in MAPS, TCRS and German MAS, since MAPS was generally less atopic in all the 4 phenotypes. This is probably because MAPS has a low general level of atopy. The significance of this is that atopy in childhood has been associated with

chronicity of lung disease in the German MAS cohort[49] but not with remodelling in the Dunedin cohort[80] nor low lung function in the TCRS[337]. In the MAPS study there was no evidence of an association of atopy with the low FEV_1 phenotype. In fact there was a non-significant negative association between atopy age 7 and low FEV_1 age 23 years (OR 0.38 -95% CI 0.04-3.26) and also no significant association with atopy at age 23 years and low FEV_1 (OR 0.96 -95% CI 0.21-4.49). We have found no evidence that atopy is associated with chronicity of lung disease in the MAPS cohort. However similar to the Dunedin study[80] we did find that low FEV_1 was more common in males than females. The cause for this worse outcome in some males in the MAPS cohort is not clear and may be due to wheeze occurring early in life in male subjects. Our study was not powered to investigate this issue but it does require further investigation.

We found that early wheeze was significantly associated with male gender, up to the age of 7. Early wheeze in boys is thought to be related to the relative size of airways in male lungs during childhood, which are relatively smaller than airways in female lungs[2]. This relative uncoupling of airway size and lung size is known as 'dysanapsis'[343]. In adult humans, the male has relatively larger airways than females and the propensity of males to wheeze is lost when they reach adulthood. At age 15 and 23 years there was no association with gender in the MAP cohort although other studies have found an increased rate of asthma in females in adulthood[296]. Abnormal spirometry at age 23 years in the MAPS cohort was significantly associated with gender. Boys were much more likely to have abnormal spirometry.

Subjects in the MAPS cohort were less likely to be wheeze free at age 15 if they had wheezed at or before the age of 2 years (59.8% wheeze free) than the Cogswell group at age 11 (75% wheeze free)[338]. However the results are comparable. Cogswell's cohort was also a high risk cohort from the UK selected before birth, and although those who had wheeze at 2 or younger and were wheeze free at age 15 in the MAPS cohort was lower than the Cogswells' group, it was not especially different.

Breast feeding was associated with an OR less than 1 in the three phenotype categories compared with NW in table 7.7. This suggests some protection from breast feeding in the MAPS cohort. Both TEW and PW were statistically significantly associated with breast feeding, which may at least in part, be related to the association of breast feeding with wheeze in the first year of life, since both TEW and PW are defined by wheeze before the age of 3 years. Passive smoking at age 1 was significantly associated with TEW and PW as has been shown in the TCRS longitudinal cohort where TEW was associated with parental smoking[92]. Both these phenotypes are defined by wheeze before the age of 3 years and it may be that environmental tobacco smoke exposure, which is known to increase the risk of wheeze at age 1 in this cohort, is impacting on our phenotype definitions. However this would not explain the differences between TEW and PW with respect to asthma and wheeze at 23 years. The TEW phenotype is closer to NW than PW when we consider asthma at age 23 years.

The TCRS study showed an association of asthma at age 22 years with LOW and PW phenotype at age 6[117], while MAPS showed an association between PW and probably LOW and asthma. The association between LOW and asthma was not significant but the effect size was large suggesting there may be an association. We have found that ETS was significantly associated with TEW and PW (table 7.8), as has been shown in TCRS study. We found TEW was statistically significantly inversely associated with parental allergy. This has not been described in other longitudinal studies.

Male gender in the MAPS cohort was more likely to be associated with a low $FEV_{1,}$ compared to female gender. Rasmussen et al[80] also found lower lung function had a propensity for males in the Dunedin cohort. They found that the mean FEV_1/ VC ratio was consistently higher among females than males and decreased significantly from late adolescence into adult life among males. The cause for this is not clear and may be due to wheeze occurring early in males more than females. Our study was not powered to investigate this issue but it does require further investigation.

Atopy was associated with LOW and PW in all the cohorts even though atopy was much less common in the MAPS cohort than in others. The rate of atopy was lower in every phenotype in the MAPS cohort than in the corresponding phenotypes in other cohorts ; despite this the rate of symptoms was high and chronic lung disease was also relatively common with 11% of subjects at the

age of 23 having a low FEV_1, less than 80% of predicted. Although low FEV_1 was not significantly associated with wheeze at age 1 there was a substantial effect size and a larger study may have given more definitive results. The TCRS study found that both the TEW group and the PW group were significantly more likely to be in the lower quartile for lung function than the other two phenotypes at age 16. The TCRS paper suggests that the relative differences in lung function were present at age 6 years and that there was no significant change in any phenotype subset relative to peers from age 6 years to 16 years[185]. This suggests that changes that occur before the age of 6 years are carried through to adult life. In the Dunedin study, Rasmussen et al showed there was tracking of poor lung function from age 9 through to age 26 years of age, suggesting this was due to airways remodelling as seen in pathological specimens of subjects with longstanding asthma. They suggested that reduced lung function tracks from childhood to adulthood[80] and further work has recently shown in pathological specimens that remodelling does occur early in subjects, sometimes even before the symptoms of asthma have begun[344]. The Dunedin study only recruited children at age 7 so they do not have early data like TCRS but their data does support TCRS in that they too show that any changes in lung function that are present at the age of 7 track through to adult like. The MAAS study measured lung function at age 3 years and they found that subjects who had continued wheezing after the age of 3 (subjects with PW) did have a reduction in lung function at age 6. We have not undertaken spirometry on our subjects until age 23 years so we are unable to comment on tracking of spirometry. However what the MAPS cohort does add to the discussion is that our non-reversible obstruction group at age 23

years, although very small, does show a non-significant association with subjects who wheeze at 1 year or younger. One might speculate that these subjects with low FEV_1 at age 23 years in MAPS were similar to the participants in the TCRS study who had infant Vmax FRC in the lowest quartile and then went on to have lower values for the FEV_1/FVC ratio up to age 22 years. It was suggested that these subjects were likely to be at higher risk of developing COPD than other subjects in the TCRS cohort. This chapter suggests, as shown by Stern et al in the TCRS cohort study that paediatric lung function is an important predictor in the development of adult lung disease[337].

The paediatric wheeze phenotypes have allowed us to categorise our subjects into groups from which we can further analyse them. Further research into these 4 wheeze phenotypes will help us to understand what childhood environmental features may be responsible for the rise in asthma and if there is any treatment we can undertake to change the course of one of these phenotypes as we currently understand them.

As yet, there is no preventive course of action that can alter the natural history of asthma and other paediatric wheeze disorders. However the MAPS cohort has shown that like other longitudinal studies, early life wheeze, is associated with low lung function in adulthood.

Chapter 8

General discussion and conclusion

8.0 Discussion

The aim of this thesis was to investigate the association of factors in early life with wheeze, asthma and atopy in adult years in our subjects. This included a randomised controlled trial of exclusion of cows' milk protein for the first four months of life with a soya preparation when breast feeding was not possible. A high risk birth cohort was recruited from a deprived area of the South Wales valleys. The themes considered were: social class, parental allergy and asthma, BMI, immunological factors, diet, the original randomisation, environmental factors and natural history.

8.1 Principal findings

There was a 62% response rate of the cohort as a whole at age 23 years, although there was a higher loss to follow up of subjects with wheeze at age 1, subjects from unemployed families at age 1 and male subjects. There was no evidence of an association with social class and any outcome at age 23 years. However at age 1 and age 7 there was an increase in wheeze with lower social class. There was an association between paternal allergy and paternal asthma and asthma and wheeze at age 23 years. The risk of wheeze at age 23 years increased with BMI at age 1, 7 and 23 years but only in girls.

Atopy, defined as a positive skin prick test, in early life was associated with atopy later in life. Total IgE in early life was also associated with the level of

total IgE in later life, up to adulthood. The results showed that childhood atopy, defined as a positive skin prick test at age 7 years, was associated with a significantly increased risk of wheeze from age 3 up to age 23 years. This was despite the fact that atopy had a prevalence of approximately 50% in those subjects who had wheeze at age 7, and suggests that although the prevalence of atopy was low in the MAPS cohort, atopy still had a strong association with asthma symptoms in childhood and adulthood.

We were able to use two methods to investigate an appropriate cutpoint for total IgE level that would define atopy. Using these cutpoints we found an association between total IgE and wheeze (non-significant) and asthma (significant and large effect size). There was evidence of an association between asthma and IgE even after adjustment for atopy based on positive skin prick test result, although the association was no longer significant after adjustment for skin prick test result. This relationship between total IgE and asthma even in the absence of specific sensitisation, was first reported by Burrows et al who suggested that these findings challenged the concept that there are basic differences between allergic and non-allergic asthma[41]. However we were unable to develop this hypothesis any further as we had a small sample size who attended at age 23 years for skin prick test investigations and this may explain why our results were not significant.

The low prevalence of atopy at age 7 years in the MAPS cohort was probably related to environmental factors that we were unable to measure. By the age of 23 years there was a higher prevalence of atopy especially among those

who were asthmatic at age 23 years with only 27% (8 subjects) non-atopic asthma sufferers. One may speculate that studying only the non-allergic subjects in the MAPS cohort may suggest other possible aetiological factors for asthma, but the MAPS cohort was not powered to investigate only those subjects without atopy, and therefore we have no evidence on this matter.

There was also a very low level of breast feeding in the group as a whole, and this was significantly lower in the intervention arm than the control arm. Since the intervention arm was associated with an increased risk of atopy, and asthma, we could argue that the two were related but it is not possible to demonstrate causality.

Rates of both asthma and atopy were significantly higher in the intervention arm of the RCT. This is likely to be due to specific characteristics in our cohort as other studies have found benefit from reduced exposure to allergens in the first year of life. Although there is some evidence of soya supplementation being inert, other studies have excluded soya when they have undertaken an allergen free diet[212]. So the results may be due to the fact that we used soya in our intervention arm. Alternatively it may be that early exposure to cows' milk in the MAPS cohort was associated with a reduction in atopy in later life.

The MAPS cohort showed no pattern of environmental factors affecting outcomes at age 23 years. Many environmental factors were investigated but combinations of them could not be investigated easily since the number of

subjects was too small. Few significant associations were found other than an association of a lower prevalence of atopy with solid fuel burning.

Early life wheeze was associated with adult wheeze and asthma in the MAPS cohort. Phenotyping of early childhood wheeze revealed that a higher proportion of subjects in the MAPS cohort had persistent wheeze (PW) than in other comparable cohorts. The MAPS cohort was a high risk one and the definitions for each phenotype varied between cohorts. In the MAPS cohort the phenotypes of late onset wheeze (LOW) and PW were both associated with asthma aged 23 years.

There was a strong but non-significant association between wheezing in the first year and having a non-reversible obstruction at age 23. In spite of the small sample size, this may contribute to the body of evidence that suggests that COPD is determined early in life, probably in infancy.

8.1 Strengths and limitations of study

The main strength of this work is that it was a large cohort in a deprived community in the South Wales valleys followed up for over 20 years. A large amount of information was gathered at or near birth and we have investigated associations of these early factors with outcomes in adults. Follow up to the age of 7 years was high, and annual follow up until the age of 7 years meant we have strong data to inform our childhood wheeze phenotypes. We know that early life events have an impact on outcomes in adulthood and so it was important to follow up subjects into adult life. Unlike some other longitudinal

studies, such as those in Tasmania and Dunedin, our study began before birth so the associations with early life events were prospectively gathered. This makes recall bias much less likely. This has been especially important in our evidence on breast feeding. There are only a handful of longitudinal studies that have gathered information on breast feeding and diet prospectively on a weekly basis and then continued to report outcomes in adults. Rates of breast feeding in this study were very low and it is perhaps not surprising that associations were not found with outcomes beyond the age of 1 year. We cannot speculate how the lack of rigour in collecting data in other studies may have affected their results.

The main weakness of this study was the loss to follow-up. Only 62% were seen at age 23 years, and only 25% were present for spirometry and skin prick testing at age 23 years. A larger cohort study, with higher attendance at clinics for testing, would have given us greater power in our calculations of associations. A large birth cohort such as the ALSPAC study in a similar area to that of the MAPS cohort would have been an excellent design for our study[345]. With a larger sample size we may have been able to find more and stronger associations with the variables we were reviewing.

We might have considered using extensively hydrolysed cows' milk formula for the intervention arm, if we were undertaking a similar study today, although, as Lowe et al showed in 2011, there is no evidence of an increased risk of asthma up to age 6 or 7 years with early life soya milk supplements[249]. However Berg et al have shown a reduction in eczema with the use of

extensively hydrolysed cows' milk formula in high risk children in the large GINI plus study[250].

In addition we may have considered undertaking serial specific IgE results as well as serial skin prick test results. Julge et al proposed that there may be a down regulation of skin prick test positive results compared to specific IgE response among children from countries without a market economy (i.e. non westernised)[346]. In a large international ISAAC study Weinmayr et al[347] found a pattern of discordance between positive skin prick test and positive specific IgE results which was greater in children from countries which were considered less affluent determined by gross national income of the country. With specific IgE levels we may have been able to obtain a better understanding of patterns of discordance in our non-affluent population in South Wales.

A useful further addition to the study would have been more consistent lung function testing. With childhood spirometry, and regular spirometry in the teenage years, we may have been able to have a clearer view of when the chronic changes of low FEV_1 may have been initiated and if there were any environmental factors that made these early changes more likely.

8.2 Interpretation of findings

The original premise of this study was that the exclusion of allergens early in life may protect children at high risk of asthma from developing the disease. The underlying hypothesis was that allergens are causally related to the

incidence of asthma and therefore the RCT of normal diet against cows' milk exclusion diet was initiated. In fact those subjects who had cows' milk early in life, had a reduced risk of asthma and atopy. This result may have been due to subjects in the control arm of the RCT receiving soya milk, which may have had a higher allergenic effect. Soya antigen has been shown to be associated with epidemics of asthma when soya beans are unloaded in port in Barcelona[348], but there is no convincing evidence thus far that soya is more allergenic when taken orally than cows' milk.

There have been a number of studies both longitudinal and cross-sectional that have investigated the natural history of asthma and wheezing disorder. However few longitudinal studies have followed subjects from early infanthood to adulthood and therefore there are few to compare with MAPS. The Melbourne[349] and Dunedin[116] studies have both investigated subjects into adulthood but neither have investigated subjects from infancy and therefore early life histories are based on recall and cannot be relied upon. TCRS is the only other large cohort that has reported on adult outcomes and taken a prospective history in infants[337].

The subjects in this cohort did have a lower rate of atopy in childhood than in other comparable cohorts. Atopy is known to have a higher prevalence in subjects of higher social class. Strachan et al found a prevalence of sensitisation to house dust mite of 25% in children from social class I and 16.5% in children from social class V[128]. Heinrich et al in Germany investigated the effect of social class, based on three groups determined by

parental education level. Those in the highest social class and with the most years of education had a sensitisation level of 22.1% (95% CI 19.5%-25.0%) N=904 and those from the lowest social group with the lowest level of education had a sensitisation level of 11.5% (95% CI 7.1-17.4 %) N=165[350]. The reason for the high prevalence of aotpy in association with high social class is not clear but may be related to reduced rates of infection at a young age causing changes in the subject's immune system, as proposed by Strachan[50]. However, an alternative interpretation of this hypothesis is that deviations in the composition of gastrointestinal microbiota as a result of westernised lifestyles (antibiotic use, diet) have disrupted the mechanisms involved in the development of immunological tolerance. Sudo et al have shown that tolerance induction in a murine model is related to adequate diversity of gut flora[251]. Abrahamsson et al showed that low diversity in gut microbiata in early infancy precedes the development of asthma at school age[351]. Sjogren et al investigated faecal samples collected up to age 2 months. Those subjects who develop allergy at age 5 years were significantly less often colonised with specific gut bacteria up to 2 months[339]. These studies support a view that a more diverse gut microbiota early in life might prevent allergy development. Our cohort may have had a low level of atopy because they were from a relatively deprived community and may therefore have been exposed to more bacteria early in life giving them greater diversity in gut bacteria. However despite the low level of atopy at age 7 years, there is no evidence that asthma was less frequent in this cohort.

Breast feeding was associated with a reduction in wheeze at age 1 year but not associated with outcomes at age 23 years. The rate of breast feeding was very low in MAPS, limiting the likely effect at population level, in spite of obtaining a weekly prospective history of feeding of the infant. The dietitian actually went to visit the mother in her home and therefore was able to see, first hand, what was being given to the infant. To our knowledge, no other study has been as thorough as this in obtaining a history of early infant feeding and therefore it is hard to compare with other studies. The World Health Organisation (WHO) has advised increasing exclusive breast feeding in all new-borns from 4-6 months, which was previously advised, to 6 months based on the fact that there is no evidence to suggest this will have a detrimental effect on the baby[352]. However, fewer than 1% of mothers exclusively breastfed for the recommended time in a 2005 survey[353]. We would propose that mothers are often under pressure to breast feed and therefore are more likely to suggest that they have breast fed for longer. Unless there is prospective gathering of breast feeding information it is not possible to confirm the accuracy of any study.

8.3 Implications for research and practice

Unfortunately as so many questions remain unanswered regarding dietary and environmental factors and the development of asthma, as yet, the implications for clinical practice are difficult to determine. However from the work undertaken we can identify that the early wheeze phenotyping is an effective way of categorising children who wheeze early in life and from these phenotypes we may be able to suggest prognosis. There is also the suggestion

that adult wheeze associated with abnormal irreversible lung function testing may not be only related to smoking and more work is required in this area to fully understand the different phenotypes of early onset COPD, which may help to deliver new therapies for our chronically disabled respiratory patients.

Further longitudinal studies of birth cohorts from socially deprived areas are required in order to investigate environmental factors that might reduce the prevalence of asthma. One of these factors would be the reduced rate of atopy. We may learn more about the aetiology of asthma investigating a large population based birth cohort from a less affluent area, where similar to MAPS we might expect a higher prevalence of non-atopic children who wheeze. More work is required to elucidate the environmental factors that are linked to incidence of asthma in both children and adults and this can best be achieved by large longitudinal population based studies. The key is understanding the natural history of wheezing disorder and which infants who wheeze will go on to develop adult wheezing disorders, both asthma and COPD, and which subjects who do not wheeze in childhood, do go on to develop asthma later in life.

As well as the epidemiological studies that have been summarised in each chapter of this thesis, we require a better understanding of the immunological factors on which the environment impacts. This means we need to investigate more models of early life immunity in the laboratory. The murine models worked on by Prescott et al and by Adkins have suggested that early environment can change the way the immune system develops during

childhood and therefore create an allergic or tolerant individual but our understanding of critical times points and antigen dose for both atopic and tolerant status are not clear. Once they are more fully understood, this will help us to look for appropriate environmental triggers in our epidemiological studies and will also assist us in developing asthma prevention studies. Cross-sectional studies have helped us to develop hypothesis for longitudinal studies but these types of studies are always dependent on recall bias, and therefore cannot give the detail of those early months of life that are so critical. These murine models may hold the key for hypothesis setting in the future for the next generation of longitudinal studies.

Despite this initial progress, fundamental questions remain that need to be addressed by well-designed research studies. Cohort-driven epigenetic research has the potential to address key questions, such as those concerning the influence of timing of exposure and dose of exposure required to increase susceptibility to asthma development. The presumption is that the development of asthma is not a foregone conclusion based on genetic makeup, and hence modifiable[354]. In addition asthma is a disease that exhibits a notoriously variable phenotype[355], again suggesting that environmental factors may determine phenotype. Epigenetic mechanisms such as-DNA methylation, histone modifications, and production of non-coding RNAs are epigenetic molecular changes that can alter gene transcription, without changing the DNA coding sequence, that occur prenatally or during other susceptible time periods and may modify the clinical manifestations and variable nature of this complex disease[354]. Large GP database studies may be another useful method;

large numbers of subjects can be included but the approach has limitations. Data contained in these databases are routine data and depend on a subject attending a GP. Data are recorded using Read codes and their use varies between GPs, some concentrating on diagnoses while others on symptoms, though medication use should be recorded in similar ways by all GPs. Despite these limitations, this may be a way forward if undertaken carefully.

References

1. Weiss ST. Epidemiology and heterogeneity of asthma. *Ann Allergy Asthma Immunol* 2001; **87**(1 Suppl 1): 5-8.
2. Weiss ST. Environmental risk factors in childhood asthma. *Clin Exp Allergy* 1998; **28 Suppl 5**: 29-34; discussion 50-1.
3. UK A. Asthma Facts and FAQs. 2013. http://www.asthma.org.uk/asthma-facts-and-statistics (accessed 11th August 2013 2013).
4. (ONS) OoNS. Avoidable mortality in England and Wales, 2012 - Reference table 1 (Excel sheet 165Kb) may 2014, 2012. http://www.ons.gov.uk/ons/datasets-and-tables/index.html?pageSize=50&sortBy=none&sortDirection=none&newquery=asthma&content-type=Reference+table&content-type=Dataset (accessed june 2014).
5. Svanes C, Sunyer J, Plana E, et al. Early life origins of chronic obstructive pulmonary disease. *Thorax* 2010; **65**(1): 14-20.
6. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006; **355**(21): 2226-35.
7. Pearce N, Ait-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2007; **62**(9): 758-66.
8. SIGN/BTS. British guideline on the management of asthma. Asthma priorities: influencing the agenda In: Scotland Hi, editor.; 2013.
9. GINA. Global initiative for asthma2014. http://www.ginasthma.org/local/uploads/files/GINA_Report_2015_May19.pdf (accessed 29th May 2015).
10. Van Wonderen KE, Van Der Mark LB, Mohrs J, Bindels PJ, Van Aalderen WM, Ter Riet G. Different definitions in childhood asthma: how dependable is the dependent variable? *Eur Respir J* 2010; **36**(1): 48-56.
11. Asher MI, Weiland SK. The International Study of Asthma and Allergies in Childhood (ISAAC). ISAAC Steering Committee. *Clin Exp Allergy* 1998; **28 Suppl 5**: 52-66; discussion 90-1.
12. European Community Respiratory Health S. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* 1996; **9**(4): 687-95.
13. Burney PG, Laitinen LA, Perdrizet S, et al. Validity and repeatability of the IUATLD (1984) Bronchial Symptoms Questionnaire: an international comparison. *Eur Respir J* 1989; **2**(10): 940-5.
14. Peat JK, Toelle BG, Marks GB, Mellis CM. Continuing the debate about measuring asthma in population studies. *Thorax* 2001; **56**(5): 406-11.
15. Jenkins MA, Clarke JR, Carlin JB, et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. *Int J Epidemiol* 1996; **25**(3): 609-16.
16. ISAAC. The ISAAC story2012, 2012. http://isaac.auckland.ac.nz/story/background/toolbox.php#writtenT (accessed 31st May 2015).
17. Chinn S, Jarvis D, Burney P, et al. Increase in diagnosed asthma but not in symptoms in the European Community Respiratory Health Survey. *Thorax* 2004; **59**(8): 646-51.

18. Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax* 2009; **64**(6): 476-83.
19. Anto JM, Sunyer J, Basagana X, et al. Risk factors of new-onset asthma in adults: a population-based international cohort study. *Allergy* 2010; **65**(8): 1021-30.
20. Nagel G, Buchele G, Weinmayr G, et al. Effect of breastfeeding on asthma, lung function and bronchial hyperreactivity in ISAAC Phase II. *Eur Respir J* 2009; **33**(5): 993-1002.
21. Beasley R, Clayton T, Crane J, et al. Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6-7 years: analysis from Phase Three of the ISAAC programme. *Lancet* 2008; **372**(9643): 1039-48.
22. Svanes C, Jarvis D, Chinn S, Omenaas E, Gulsvik A, Burney P. Early exposure to children in family and day care as related to adult asthma and hay fever: results from the European Community Respiratory Health Survey. *Thorax* 2002; **57**(11): 945-50.
23. Svanes C, Zock JP, Anto J, et al. Do asthma and allergy influence subsequent pet keeping? An analysis of childhood and adulthood. *J Allergy Clin Immunol* 2006; **118**(3): 691-8.
24. Beasley R. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *The Lancet* 1998; **351**(9111): 1225-32.
25. (GNA) GAN. Global Asthma Report. http://www.globalasthmareport.org/burden/burden.php; 2014.
26. Woolcock AaPAJ. Evidence for the increase in asthma worldwide. In: Chadwick DJaC, G, ed. the rising trends in asthma: wiley; 1997: 111-34.
27. Woods RK, Walters EH, Wharton C, Watson N, Abramson M. The rising prevalence of asthma in young Melbourne adults is associated with improvement in treatment. *Ann Allergy Asthma Immunol* 2001; **87**(2): 117-23.
28. Wickens K, Barry D, Friezema A, et al. Obesity and asthma in 11-12 year old New Zealand children in 1989 and 2000. *Thorax* 2005; **60**(1): 7-12.
29. Haahtela T, Lindholm H, Bjorksten F, Koskenvuo K, Laitinen LA. Prevalence of asthma in Finnish young men. *BMJ* 1990; **301**(6746): 266-8.
30. Brogger J, Bakke P, Eide GE, Johansen B, Andersen A, Gulsvik A. Long-term changes in adult asthma prevalence. *Eur Respir J* 2003; **21**(3): 468-72.
31. Anderson HR, Gupta R, Strachan DP, Limb ES. 50 years of asthma: UK trends from 1955 to 2004. *Thorax* 2007; **62**(1): 85-90.
32. Burr ML, Wat D, Evans C, Dunstan FD, Doull IJ. Asthma prevalence in 1973, 1988 and 2003. *Thorax* 2006; **61**(4): 296-9.
33. Johansson SG, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967; **13**(4): 381-94.
34. Arshad SH, Tariq SM, Matthews S, Hakim E. Sensitization to common allergens and its association with allergic disorders at age 4 years: a whole population birth cohort study. *Pediatrics* 2001; **108**(2): E33.
35. Holt PG, McMenamin C, Nelson D. Primary sensitisation to inhalant allergens during infancy. *Pediatric Allergy and Immunology* 1990; **1**(1): 3-13.
36. Cullinan P, MacNeill SJ, Harris JM, et al. Early allergen exposure, skin prick responses, and atopic wheeze at age 5 in English children: a cohort study. *Thorax* 2004; **59**(10): 855-61.
37. Von Linstow ML, Porsbjerg C, Ulrik CS, Nepper-Christensen S, Backer V. Prevalence and predictors of atopy among young Danish adults. *Clin Exp Allergy* 2002; **32**(4): 520-5.

38. Court CS, Cook DG, Strachan DP. Comparative epidemiology of atopic and non-atopic wheeze and diagnosed asthma in a national sample of English adults. *Thorax* 2002; **57**(11): 951-7.
39. Lau S, Illi S, Platts-Mills TA, et al. Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood--report of the German Multicentre Allergy Study (MAS 90). *Allergy* 2005; **60**(6): 766-73.
40. Warner JA, Miles EA, Jones AC, Quint DJ, Colwell BM, Warner JO. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; **24**(5): 423-30.
41. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989; **320**(5): 271-7.
42. Plaschke PP, Janson C, Norrman EVA, BjÖRnsson E, EllbjÄR S, JÄRvholm B. Onset and Remission of Allergic Rhinitis and Asthma and the Relationship with Atopic Sensitization and Smoking. *American Journal of Respiratory and Critical Care Medicine* 2000; **162**(3): 920-4.
43. WAO. World Allergy Organization white book on allergy 2011-12. 2011.
44. Host A, Halken S. The role of allergy in childhood asthma. *Allergy* 2000; **55**(7): 600-8.
45. Hoppin JA, Jaramillo R, Salo P, Sandler DP, London SJ, Zeldin DC. Questionnaire predictors of atopy in a US population sample: findings from the National Health and Nutrition Examination Survey, 2005-2006. *Am J Epidemiol* 2011; **173**(5): 544-52.
46. Sears MR, Burrows B, Flannery EM, Herbison GP, Holdaway MD. Atopy in childhood. I. Gender and allergen related risks for development of hay fever and asthma. *Clin Exp Allergy* 1993; **23**(11): 941-8.
47. Sears MR, Burrows B, Herbison GP, Holdaway MD, Flannery EM. Atopy in childhood. II. Relationship to airway responsiveness, hay fever and asthma. *Clin Exp Allergy* 1993; **23**(11): 949-56.
48. Simpson BM, Custovic A, Simpson A, et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001; **31**(3): 391-9.
49. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006; **368**(9537): 763-70.
50. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**(6710): 1259-60.
51. Martinez FD. Role of viral infections in the inception of asthma and allergies during childhood: could they be protective? *Thorax* 1994; **49**(12): 1189-91.
52. Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy Immunol* 1995; **6**(1): 1-10.
53. Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000; **320**(7232): 412-7.
54. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997; **275**(5296): 77-9.
55. Castro-Rodriguez JA, Garcia-Marcos L, Alfonseda Rojas JD, Valverde-Molina J, Sanchez-Solis M. Mediterranean diet as a protective factor for wheezing in preschool children. *J Pediatr* 2008; **152**(6): 823-8, 8 e1-2.
56. Wickens K, Barry D, Friezema A, et al. Fast foods - are they a risk factor for asthma? *Allergy* 2005; **60**(12): 1537-41.

57. Ellwood P, Asher MI, Garcia-Marcos L, et al. Do fast foods cause asthma, rhinoconjunctivitis and eczema? Global findings from the International Study of Asthma and Allergies in Childhood (ISAAC) phase three. *Thorax* 2013; **68**(4): 351-60.
58. Gotshall RW, Mickleborough TD, Cordain L. Dietary salt restriction improves pulmonary function in exercise-induced asthma. *Med Sci Sports Exerc* 2000; **32**(11): 1815-9.
59. Kocyigit A, Armutcu F, Gurel A, Ermis B. Alterations in plasma essential trace elements selenium, manganese, zinc, copper, and iron concentrations and the possible role of these elements on oxidative status in patients with childhood asthma. *Biological trace element research* 2004; **97**(1): 31-41.
60. Allen S, Britton JR, Leonardi-Bee JA. Association between antioxidant vitamins and asthma outcome measures: systematic review and meta-analysis. *Thorax* 2009; **64**(7): 610-9.
61. Troisi RJ, Willett WC, Weiss ST, Trichopoulos D, Rosner B, Speizer FE. A prospective study of diet and adult-onset asthma. *Am J Respir Crit Care Med* 1995; **151**(5): 1401-8.
62. Sin DD, Sutherland ER. Obesity and the lung: 4. Obesity and asthma. *Thorax* 2008; **63**(11): 1018-23.
63. Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JQ. Particulate air pollution and hospital emergency room visits for asthma in Seattle. *Am Rev Respir Dis* 1993; **147**(4): 826-31.
64. MAGAZINE BETE. EarthTalk: Is U.S. air quality getting better or worse? 2013. http://www.lung.org/associations/states/florida/news/top-story-in-the-news/earthtalk-is-us-air.html2014).
65. Lomborg b. Take a deep breath... air quality is getting better. 2001. http://www.theguardian.com/environment/2001/aug/15/physicalsciences.globalwarming2014).
66. Plumer b. Air pollution now kills more people than high cholesterol. 2012. http://www.washingtonpost.com/blogs/wonkblog/wp/2012/12/20/air-pollution-now-kills-more-people-than-high-cholesterol/2014).
67. Law BJ, Carbonell-Estrany X, Simoes EAF. An update on respiratory syncytial virus epidemiology: a developed country perspective. *Respiratory Medicine* 2002; **96, Supplement 2**(0): S1-S7.
68. Henderson J, Hilliard TN, Sherriff A, Stalker D, Al Shammari N, Thomas HM. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol* 2005; **16**(5): 386-92.
69. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; **354**(9178): 541-5.
70. Sigurs N, Aljassim F, Kjellman B, et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* 2010; **65**(12): 1045-52.
71. Stensballe LG, Simonsen JB, Thomsen SF, et al. The causal direction in the association between respiratory syncytial virus hospitalization and asthma. *J Allergy Clin Immunol* 2009; **123**(1): 131-7 e1.
72. Blanken MO, Rovers MM, Molenaar JM, et al. Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N Engl J Med* 2013; **368**(19): 1791-9.
73. Kerkhof M, Wijga AH, Brunekreef B, et al. Effects of pets on asthma development up to 8 years of age: the PIAMA study. *Allergy* 2009; **64**(8): 1202-8.
74. Taussig LM, Wright AL, Morgan WJ, Harrison HR, Ray CG. The Tucson Children's Respiratory Study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. *Am J Epidemiol* 1989; **129**(6): 1219-31.

75. Sears MR, Greene JM, Willan AR, et al. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002; **360**(9337): 901-7.
76. Henderson J, Granell R, Heron J, et al. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. *Thorax* 2008; **63**(11): 974-80.
77. Lowe AJ, Carlin JB, Bennett CM, et al. Atopic disease and breast-feeding--cause or consequence? *J Allergy Clin Immunol* 2006; **117**(3): 682-7.
78. Henderson AJ. What have we learned from prospective cohort studies of asthma in children? *Chronic respiratory disease* 2008; **5**(4): 225-31.
79. Burgess JA, Dharmage SC, Byrnes GB, et al. Childhood eczema and asthma incidence and persistence: a cohort study from childhood to middle age. *J Allergy Clin Immunol* 2008; **122**(2): 280-5.
80. Rasmussen F, Taylor DR, Flannery EM, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. *Am J Respir Crit Care Med* 2002; **165**(11): 1480-8.
81. Wright AL, Holberg CJ, Taussig LM, Martinez FD. Factors influencing the relation of infant feeding to asthma and recurrent wheeze in childhood. *Thorax* 2001; **56**(3): 192-7.
82. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000; **343**(8): 538-43.
83. Lau S, Illi S, Sommerfeld C, et al. Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet* 2000; **356**(9239): 1392-7.
84. Illi S, von Mutius E, Lau S, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 2004; **113**(5): 925-31.
85. Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001; **322**(7283): 390-5.
86. Shaheen SO, Newson RB, Sherriff A, et al. Paracetamol use in pregnancy and wheezing in early childhood. *Thorax* 2002; **57**(11): 958-63.
87. Elliott L, Henderson J, Northstone K, Chiu GY, Dunson D, London SJ. Prospective study of breast-feeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). *J Allergy Clin Immunol* 2008; **122**(1): 49-54, e1-3.
88. Maitra A, Sherriff A, Strachan D, Henderson J. Mode of delivery is not associated with asthma or atopy in childhood. *Clin Exp Allergy* 2004; **34**(9): 1349-55.
89. Simpson A, Tan VY, Winn J, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. *Am J Respir Crit Care Med* 2010; **181**(11): 1200-6.
90. Scholtens S, Wijga AH, Brunekreef B, et al. Breast feeding, parental allergy and asthma in children followed for 8 years. The PIAMA birth cohort study. *Thorax* 2009; **64**(7): 604-9.
91. Wijga AH, Smit HA, Kerkhof M, et al. Association of consumption of products containing milk fat with reduced asthma risk in pre-school children: the PIAMA birth cohort study. *Thorax* 2003; **58**(7): 567-72.
92. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995; **332**(3): 133-8.

93. Sherriff A, Peters TJ, Henderson J, Strachan D, Parents ASTALSo, Children. Risk factor associations with wheezing patterns in children followed longitudinally from birth to 3(1/2) years. *Int J Epidemiol* 2001; **30**(6): 1473-84.
94. Edwards CA, Osman LM, Godden DJ, Douglas JG. Wheezy bronchitis in childhood: a distinct clinical entity with lifelong significance? *Chest* 2003; **124**(1): 18-24.
95. Mai XM, Almqvist C, Nilsson L, Wickman M. Birth anthropometric measures, body mass index and allergic diseases in a birth cohort study (BAMSE). *Arch Dis Child* 2007; **92**(10): 881-6.
96. Lau S, Illi S, Sommerfeld C, et al. Transient early wheeze is not associated with impaired lung function in 7-yr-old children. *Eur Respir J* 2003; **21**(5): 834-41.
97. Sandin A, Bjorksten B, Braback L. Development of atopy and wheezing symptoms in relation to heredity and early pet keeping in a Swedish birth cohort. *Pediatr Allergy Immunol* 2004; **15**(4): 316-22.
98. Lowe LA, Simpson A, Woodcock A, et al. Wheeze phenotypes and lung function in preschool children. *Am J Respir Crit Care Med* 2005; **171**(3): 231-7.
99. Rusconi F, Galassi C, Corbo GM, et al. Risk factors for early, persistent, and late-onset wheezing in young children. SIDRIA Collaborative Group. *Am J Respir Crit Care Med* 1999; **160**(5 Pt 1): 1617-22.
100. Merrett TG, Burr ML, Butland BK, Merrett J, Miskelly FG, Vaughan-Williams E. Infant feeding and allergy: 12-month prospective study of 500 babies born into allergic families. *Ann Allergy* 1988; **61**(6 Pt 2): 13-20.
101. Tariq SM, Matthews SM, Hakim EA, Stevens M, Arshad SH, Hide DW. The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study. *J Allergy Clin Immunol* 1998; **101**(5): 587-93.
102. Basagana X, Sunyer J, Kogevinas M, et al. Socioeconomic status and asthma prevalence in young adults: the European Community Respiratory Health Survey. *Am J Epidemiol* 2004; **160**(2): 178-88.
103. Poyser MA, Nelson H, Ehrlich RI, et al. Socioeconomic deprivation and asthma prevalence and severity in young adolescents. *Eur Respir J* 2002; **19**(5): 892-8.
104. Miskelly FG, Burr ML, Vaughan-Williams E, Fehily AM, Butland BK, Merrett TG. Infant feeding and allergy. *Arch Dis Child* 1988; **63**(4): 388-93.
105. Burr ML, Miskelly FG, Butland BK, Merrett TG, Vaughan-Williams E. Environmental factors and symptoms in infants at high risk of allergy. *J Epidemiol Community Health* 1989; **43**(2): 125-32.
106. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur Respir J Suppl* 1993; **16**: 5-40.
107. Burr ML, Limb ES, Maguire MJ, et al. Infant feeding, wheezing, and allergy: a prospective study. *Arch Dis Child* 1993; **68**(6): 724-8.
108. Lowe GL, Burr M. Undiagnosed and untreated wheezing in a cohort of adolescents with a family history of allergic disease. *Br J Gen Pract* 2001; **51**(469): 664-5.
109. Asher MI, Keil U, Anderson HR, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; **8**(3): 483-91.
110. ISAAC. International study of asthma and allergy in childhood. Phase one manual. 1993
111. Berger A. Skin prick testing. *BMJ* 2002; **325**(7361): 414.

112. McGowan EM, Saad S, Bendall LJ, Bradstock KF, Clarke CL. Effect of progesterone receptor a predominance on breast cancer cell migration into bone marrow fibroblasts. *Breast Cancer Res Treat* 2004; **83**(3): 211-20.
113. Lundh A, Gotzsche PC. Recommendations by Cochrane Review Groups for assessment of the risk of bias in studies. *BMC Med Res Methodol* 2008; **8**: 22.
114. Altman DG. Statistics in medical journals: some recent trends. *Stat Med* 2000; **19**(23): 3275-89.
115. Johnston ID, Strachan DP, Anderson HR. Effect of pneumonia and whooping cough in childhood on adult lung function. *N Engl J Med* 1998; **338**(9): 581-7.
116. Sears MR, Greene JM, Willan AR, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med* 2003; **349**(15): 1414-22.
117. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. *Lancet* 2008; **372**(9643): 1058-64.
118. Fewtrell MS, Kennedy K, Singhal A, et al. How much loss to follow-up is acceptable in long-term randomised trials and prospective studies? *Arch Dis Child* 2008; **93**(6): 458-61.
119. Drivsholm T, Eplov LF, Davidsen M, et al. Representativeness in population-based studies: a detailed description of non-response in a Danish cohort study. *Scand J Public Health* 2006; **34**(6): 623-31.
120. Knudsen AK, Hotopf M, Skogen JC, Overland S, Mykletun A. The health status of nonparticipants in a population-based health study: the Hordaland Health Study. *Am J Epidemiol* 2010; **172**(11): 1306-14.
121. Harald K, Salomaa V, Jousilahti P, Koskinen S, Vartiainen E. Non-participation and mortality in different socioeconomic groups: the FINRISK population surveys in 1972-92. *J Epidemiol Community Health* 2007; **61**(5): 449-54.
122. Bergmann RL, Edenharter G, Bergmann KE, Lau S, Wahn U. Socioeconomic status is a risk factor for allergy in parents but not in their children. *Clin Exp Allergy* 2000; **30**(12): 1740-5.
123. Bousquet J, Chanez P, Lacoste JY, et al. Asthma: a disease remodeling the airways. *Allergy* 1992; **47**(1): 3-11.
124. Security. DoHaS. Inequalities in health: report of a research working group. . *London: DHSS, 1980* 1980.
125. Shohaimi S, Welch A, Bingham S, et al. Area deprivation predicts lung function independently of education and social class. *Eur Respir J* 2004; **24**(1): 157-61.
126. Williams HC, Strachan DP, Hay RJ. Childhood eczema: disease of the advantaged? *BMJ* 1994; **308**(6937): 1132-5.
127. Bugiani M, Carosso A, Migliore E, et al. Allergic rhinitis and asthma comorbidity in a survey of young adults in Italy. *Allergy* 2005; **60**(2): 165-70.
128. Strachan DP, Harkins LS, Johnston ID, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. *J Allergy Clin Immunol* 1997; **99**(1 Pt 1): 6-12.
129. Strachan DP, Anderson HR, Limb ES, O'Neill A, Wells N. A national survey of asthma prevalence, severity, and treatment in Great Britain. *Arch Dis Child* 1994; **70**(3): 174-8.
130. Baker D, Henderson J. Differences between infants and adults in the social aetiology of wheeze. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *J Epidemiol Community Health* 1999; **53**(10): 636-42.

131. Chung KF, Wenzel SE, Brozek JL, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; **43**(2): 343-73.
132. Braback L, Hjern A, Rasmussen F. Social class in asthma and allergic rhinitis: a national cohort study over three decades. *Eur Respir J* 2005; **26**(6): 1064-8.
133. Rona RJ, Hughes JM, Chinn S. Association between asthma and family size between 1977 and 1994. *J Epidemiol Community Health* 1999; **53**(1): 15-9.
134. Mitchell EA, Beasley R, Bjorksten B, et al. The association between BMI, vigorous physical activity and television viewing and the risk of symptoms of asthma, rhinoconjunctivitis and eczema in children and adolescents: ISAAC Phase Three. *Clin Exp Allergy* 2013; **43**(1): 73-84.
135. Portsmouth GHGUo. Liverpool through time | Historical Statistics on Social Structure for the District/Unitary Authority | Rate: Percentage of Working-Age Males in Class 1 & 2, A Vision of Britain through Time. 2014. http://www.visionofbritain.org.uk/unit/10105821/rate/RGSOC_12 (accessed Date accessed: 08th April 2014.
136. tradingeconomics. british unemployment rates. 2013. http://www.tradingeconomics.com/united-kingdom/unemployment-rate
137. Holloway JW, Beghe B, Holgate ST. The genetic basis of atopic asthma. *Clin Exp Allergy* 1999; **29**(8): 1023-32.
138. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008; **8**(3): 169-82.
139. Kumar A, Ghosh B. Genetics of asthma: a molecular biologist perspective. *Clin Mol Allergy* 2009; **7**: 7.
140. Burke W, Fesinmeyer M, Reed K, Hampson L, Carlsten C. Family history as a predictor of asthma risk. *Am J Prev Med* 2003; **24**(2): 160-9.
141. Sarafino EP. Connections among parent and child atopic illnesses. *Pediatr Allergy Immunol* 2000; **11**(2): 80-6.
142. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 1990; **142**(6 Pt 1): 1351-8.
143. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res* 2001; **4**(2): 81-93.
144. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Parental history and the risk for childhood asthma. Does mother confer more risk than father? *Am J Respir Crit Care Med* 1998; **158**(1): 176-81.
145. Alford SH, Zoratti E, Peterson EL, Maliarik M, Ownby DR, Johnson CC. Parental history of atopic disease: disease pattern and risk of pediatric atopy in offspring. *J Allergy Clin Immunol* 2004; **114**(5): 1046-50.
146. Jenkins MA, Hopper JL, Bowes G, Carlin JB, Flander LB, Giles GG. Factors in childhood as predictors of asthma in adult life. *BMJ* 1994; **309**(6947): 90-3.
147. Dold S, Wjst M, von Mutius E, Reitmeir P, Stiepel E. Genetic risk for asthma, allergic rhinitis, and atopic dermatitis. *Arch Dis Child* 1992; **67**(8): 1018-22.
148. Patino CM, Martinez FD. Interactions between genes and environment in the development of asthma. *Allergy* 2001; **56**(4): 279-86.
149. Somerville SM, Rona RJ, Chinn S. Obesity and respiratory symptoms in primary school. *Arch Dis Child* 1984; **59**(10): 940-4.
150. Ford ES. The epidemiology of obesity and asthma. *J Allergy Clin Immunol* 2005; **115**(5): 897-909; quiz 10.
151. Rodriguez MA, Winkleby MA, Ahn D, Sundquist J, Kraemer HC. Identification of population subgroups of children and adolescents with high asthma prevalence: findings from the Third National Health and Nutrition Examination Survey. *Arch Pediatr Adolesc Med* 2002; **156**(3): 269-75.

152. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; **320**(7244): 1240-3.
153. Chinn S, Jarvis D, Burney P, European Community Respiratory Health S. Relation of bronchial responsiveness to body mass index in the ECRHS. European Community Respiratory Health Survey. *Thorax* 2002; **57**(12): 1028-33.
154. Shaheen SO, Sterne JA, Montgomery SM, Azima H. Birth weight, body mass index and asthma in young adults. *Thorax* 1999; **54**(5): 396-402.
155. Murray CS, Canoy D, Buchan I, Woodcock A, Simpson A, Custovic A. Body mass index in young children and allergic disease: gender differences in a longitudinal study. *Clin Exp Allergy* 2011; **41**(1): 78-85.
156. Litonjua AA, Sparrow D, Celedon JC, DeMolles D, Weiss ST. Association of body mass index with the development of methacholine airway hyperresponsiveness in men: the Normative Aging Study. *Thorax* 2002; **57**(7): 581-5.
157. Celedon JC, Palmer LJ, Litonjua AA, et al. Body mass index and asthma in adults in families of subjects with asthma in Anqing, China. *Am J Respir Crit Care Med* 2001; **164**(10 Pt 1): 1835-40.
158. Delgado J, Barranco P, Quirce S. Obesity and asthma. *J Investig Allergol Clin Immunol* 2008; **18**(6): 420-5.
159. Group TES. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *European Respiratory Journal* 2003; **22**(3): 470-7.
160. Mosen DM, Schatz M, Magid DJ, Camargo CA, Jr. The relationship between obesity and asthma severity and control in adults. *J Allergy Clin Immunol* 2008; **122**(3): 507-11 e6.
161. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; **181**(4): 315-23.
162. Dixon JB, Chapman L, O'Brien P. Marked improvement in asthma after Lap-Band surgery for morbid obesity. *Obes Surg* 1999; **9**(4): 385-9.
163. Schmidt ME, Steindorf K. Statistical methods for the validation of questionnaires--discrepancy between theory and practice. *Methods Inf Med* 2006; **45**(4): 409-13.
164. Korkeila K, Suominen S, Ahvenainen J, et al. Non-response and related factors in a nation-wide health survey. *Eur J Epidemiol* 2001; **17**(11): 991-9.
165. Martin PE, Matheson MC, Gurrin L, et al. Childhood eczema and rhinitis predict atopic but not nonatopic adult asthma: a prospective cohort study over 4 decades. *J Allergy Clin Immunol* 2011; **127**(6): 1473-9 e1.
166. Punekar YS, Sheikh A. Establishing the sequential progression of multiple allergic diagnoses in a UK birth cohort using the General Practice Research Database. *Clin Exp Allergy* 2009; **39**(12): 1889-95.
167. Davies H, Joshi, H, Clarke, L. Is it cash that the deprived are short of? *Journal of the Royal Statistical Society* 1997; **160 (1)**: 107-26.
168. Chen Y, Dales R, Krewski D, Breithaupt K. Increased effects of smoking and obesity on asthma among female Canadians: the National Population Health Survey, 1994-1995. *Am J Epidemiol* 1999; **150**(3): 255-62.
169. Castro-Rodriguez JA, Holberg CJ, Morgan WJ, Wright AL, Martinez FD. Increased incidence of asthmalike symptoms in girls who become overweight or obese during the school years. *Am J Respir Crit Care Med* 2001; **163**(6): 1344-9.
170. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006; **444**(7121): 860-7.
171. Boulet LP. Obesity and atopy. *Clinical & Experimental Allergy* 2015; **45**(1): 75-86.

172. Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. Central obesity is associated with nonatopic but not atopic asthma in a representative population sample. *Journal of Allergy and Clinical Immunology*; **118**(6): 1284-91.
173. Mead J. Dysanapsis in normal lungs assessed by the relationship between maximal flow, static recoil, and vital capacity. *Am Rev Respir Dis* 1980; **121**(2): 339-42.
174. Wheeldon NM, Newnham DM, Coutie WJ, Peters JA, McDevitt DG, Lipworth BJ. Influence of sex-steroid hormones on the regulation of lymphocyte beta 2-adrenoceptors during the menstrual cycle. *British journal of clinical pharmacology* 1994; **37**(6): 583-8.
175. PASQUALI R, ANTENUCCI D, CASIMIRRI F, et al. Clinical and Hormonal Characteristics of Obese Amenorrheic Hyperandrogenic Women Before and After Weight Loss. *The Journal of Clinical Endocrinology & Metabolism* 1989; **68**(1): 173-9.
176. Delacourt C, Labbe D, Vassault A, Brunet-Langot D, de Blic J, Scheinmann P. Sensitization to inhalant allergens in wheezing infants is predictive of the development of infantile asthma. *Allergy* 1994; **49**(10): 843-7.
177. Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. *J Allergy Clin Immunol* 2006; **118**(2): 331-6.
178. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol* 2008; **8**(3): 183-92.
179. Deamer WC. Asthma and mites: An early theory. *Journal of Allergy and Clinical Immunology* 1971; **48**(4): 251-2.
180. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990; **323**(8): 502-7.
181. Sherrill D, Stein R, Kurzius-Spencer M, Martinez F. On early sensitization to allergens and development of respiratory symptoms. *Clin Exp Allergy* 1999; **29**(7): 905-11.
182. Illi S, von Mutius E, Lau S, et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. *J Allergy Clin Immunol* 2001; **108**(5): 709-14.
183. Kulig M, Bergmann R, Tacke U, Wahn U, Guggenmoos-Holzmann I. Long-lasting sensitization to food during the first two years precedes allergic airway disease. The MAS Study Group, Germany. *Pediatr Allergy Immunol* 1998; **9**(2): 61-7.
184. Lazic N, Roberts G, Custovic A, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy* 2013; **68**(6): 764-70.
185. Morgan WJ, Stern DA, Sherrill DL, et al. Outcome of asthma and wheezing in the first 6 years of life: follow-up through adolescence. *Am J Respir Crit Care Med* 2005; **172**(10): 1253-8.
186. Haahtela T, Bjorksten F, Heiskala M, Suoniemi I. Skin prick test reactivity to common allergens in Finnish adolescents. *Allergy* 1980; **35**(5): 425-31.
187. de Jong AB, Dikkeschei LD, Brand PL. Sensitization patterns to food and inhalant allergens in childhood: a comparison of non-sensitized, monosensitized, and polysensitized children. *Pediatr Allergy Immunol* 2011; **22**(2): 166-71.
188. Gergen PJT, Turkeltaub PC. P. C. Prevalence of Percutaneous Immediate Hypersensitivity and Histamine Reactivity in the U. S. Population: Data from the Second National Health and Nutrition Examination Survey. . In: Marsh DG B, M. N., editor. , ed. Genetic and environmental factors in clinical allergy: minnesota; 1990.
189. Gergen PJT, P. C. Prevalence of Percutaneous Immediate Hypersensitivity and Histamine Reactivity in the U. S. Population: Data from the Second National

Health and Nutrition Examination Survey. In: Marsh DG, Blumenthal, M. N., ed. Genetic and environmental factors in clinical allergy minnesota; 1990.
190. Arbes SJ, Jr., Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005; **116**(2): 377-83.
191. Barbee RA, Kaltenborn W, Lebowitz MD, Burrows B. Longitudinal changes in allergen skin test reactivity in a community population sample. *J Allergy Clin Immunol* 1987; **79**(1): 16-24.
192. Broadfield E, McKeever TM, Scrivener S, Venn A, Lewis SA, Britton J. Increase in the prevalence of allergen skin sensitization in successive birth cohorts. *J Allergy Clin Immunol* 2002; **109**(6): 969-74.
193. Stern DA, Lohman IC, Wright AL, Taussig LM, Martinez FD, Halonen M. Dynamic changes in sensitization to specific aeroallergens in children raised in a desert environment. *Clin Exp Allergy* 2004; **34**(10): 1563-669.
194. Asarnoj A, Ostblom E, Kull I, et al. Sensitization to inhalant allergens between 4 and 8 years of age is a dynamic process: results from the BAMSE birth cohort. *Clin Exp Allergy* 2008; **38**(9): 1507-13.
195. Burney P, Malmberg E, Chinn S, Jarvis D, Luczynska C, Lai E. The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1997; **99**(3): 314-22.
196. Janson C, Anto J, Burney P, et al. The European Community Respiratory Health Survey: what are the main results so far? European Community Respiratory Health Survey II. *Eur Respir J* 2001; **18**(3): 598-611.
197. Leynaert B, Neukirch C, Jarvis D, et al. Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood? *Am J Respir Crit Care Med* 2001; **164**(10 Pt 1): 1829-34.
198. Chan-Yeung M, Anthonisen NR, Becklake MR, et al. Geographical variations in the prevalence of atopic sensitization in six study sites across Canada. *Allergy* 2010; **65**(11): 1404-13.
199. Aslund N, Thomsen SF, Molgaard E, Nolte H, Backer V. Changes in skin test reactivity among adults with atopic disease: a 3-year prospective study. *Ann Allergy Asthma Immunol* 2008; **101**(5): 524-8.
200. Bodtger U, Linneberg A. Remission of allergic rhinitis: An 8-year observational study. *J Allergy Clin Immunol* 2004; **114**(6): 1384-8.
201. Matricardi PM, Bockelbrink A, Keil T, et al. Dynamic evolution of serum immunoglobulin E to airborne allergens throughout childhood: results from the Multi-Centre Allergy Study birth cohort. *Clin Exp Allergy* 2009; **39**(10): 1551-7.
202. Wahn U, Lau S, Bergmann R, et al. Indoor allergen exposure is a risk factor for sensitization during the first three years of life. *J Allergy Clin Immunol* 1997; **99**(6 Pt 1): 763-9.
203. Woodcock A, Lowe LA, Murray CS, et al. Early life environmental control: effect on symptoms, sensitization, and lung function at age 3 years. *Am J Respir Crit Care Med* 2004; **170**(4): 433-9.
204. Brown WG, Halonen MJ, Kaltenborn WT, Barbee RA. The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 1979; **63**(5): 328-35.
205. Klink M, Cline MG, Halonen M, Burrows B. Problems in defining normal limits for serum IgE. *J Allergy Clin Immunol* 1990; **85**(2): 440-4.
206. Sears MR, Burrows B, Flannery EM, Herbison GP, Hewitt CJ, Holdaway MD. Relation between airway responsiveness and serum IgE in children with asthma and in apparently normal children. *N Engl J Med* 1991; **325**(15): 1067-71.

207. Sunyer J, Anto JM, Castellsague J, Soriano JB, Roca J. Total serum IgE is associated with asthma independently of specific IgE levels. The Spanish Group of the European Study of Asthma. *Eur Respir J* 1996; **9**(9): 1880-4.
208. Sunyer J, Antó JM, Sabrià J, et al. Relationship between serum IgE and airway responsiveness in adults with asthma. *Journal of Allergy and Clinical Immunology* 1995; **95**(3): 699-706.
209. Brunekreef B, Smit J, de Jongste J, et al. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. *Pediatr Allergy Immunol* 2002; **13 Suppl 15**: 55-60.
210. Custovic A, Simpson BM, Murray CS, Lowe L, Woodcock A. The National Asthma Campaign Manchester Asthma and Allergy Study. *Pediatr Allergy Immunol* 2002; **13 Suppl 15**: 32-7.
211. Corver K, Kerkhof M, Brussee JE, et al. House dust mite allergen reduction and allergy at 4 yr: follow up of the PIAMA-study. *Pediatr Allergy Immunol* 2006; **17**(5): 329-36.
212. Scott M, Roberts G, Kurukulaaratchy RJ, Matthews S, Nove A, Arshad SH. Multifaceted allergen avoidance during infancy reduces asthma during childhood with the effect persisting until age 18 years. *Thorax* 2012; **67**(12): 1046-51.
213. Cline MG, Burrows B. Distribution of allergy in a population sample residing in Tucson, Arizona. *Thorax* 1989; **44**(5): 425-31.
214. Perkins NJ, Schisterman EF, Vexler A. Receiver operating characteristic curve inference from a sample with a limit of detection. *Am J Epidemiol* 2007; **165**(3): 325-33.
215. Bewick V, Cheek L, Ball J. Statistics review 13: receiver operating characteristic curves. *Crit Care* 2004; **8**(6): 508-12.
216. Roberts G, Zhang H, Karmaus W, et al. Trends in cutaneous sensitization in the first 18 years of life: results from the 1989 Isle of Wight birth cohort study. *Clin Exp Allergy* 2012; **42**(10): 1501-9.
217. Holt PG. Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol Today* 1994; **15**(10): 484-9.
218. Nilsson L, Bjorksten B, Hattevig G, Kjellman B, Sigurs N, Kjellman NI. Season of birth as predictor of atopic manifestations. *Arch Dis Child* 1997; **76**(4): 341-4.
219. Oryszczyn MP, Annesi I, Neukirch F, Doré MF, Kauffmann F. Longitudinal observations of serum IgE and skin prick test response. *American Journal of Respiratory and Critical Care Medicine* 1995; **151**(3): 663-8.
220. Matricardi PM, Bockelbrink A, Gruber C, et al. Longitudinal trends of total and allergen-specific IgE throughout childhood. *Allergy* 2009; **64**(7): 1093-8.
221. Kurukulaaratchy RJ, Fenn M, Matthews S, Arshad SH. Characterisation of atopic and non-atopic wheeze in 10 year old children. *Thorax* 2004; **59**(7): 563-8.
222. Garcia-Marcos L, Castro-Rodriguez JA, Suarez-Varela MM, et al. A different pattern of risk factors for atopic and non-atopic wheezing in 9-12-year-old children. *Pediatr Allergy Immunol* 2005; **16**(6): 471-7.
223. Gergen PJ, Turkeltaub PC, Kovar MG. The prevalence of allergic skin test reactivity to eight common aeroallergens in the U.S. population: results from the second National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 1987; **80**(5): 669-79.
224. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002; **57**(7): 643-8.
225. Burrows B, Martinez FD, Cline MG, Lebowitz MD. The relationship between parental and children's serum IgE and asthma. *Am J Respir Crit Care Med* 1995; **152**(5 Pt 1): 1497-500.

226. Morahan G, Huang D, Wu M, et al. Association of IL12B promoter polymorphism with severity of atopic and non-atopic asthma in children. *Lancet* 2002; **360**(9331): 455-9.
227. Holgate ST, Djukanovic R, Casale T, Bousquet J. Anti-immunoglobulin E treatment with omalizumab in allergic diseases: an update on anti-inflammatory activity and clinical efficacy. *Clin Exp Allergy* 2005; **35**(4): 408-16.
228. Custovic A. To what extent is allergen exposure a risk factor for the development of allergic disease? *Clin Exp Allergy* 2015; **45**(1): 54-62.
229. Sunyer J, Jarvis D, Pekkanen J, et al. Geographic variations in the effect of atopy on asthma in the European Community Respiratory Health Study. *J Allergy Clin Immunol* 2004; **114**(5): 1033-9.
230. Weinmayr G, Weiland SK, Bjorksten B, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. *Am J Respir Crit Care Med* 2007; **176**(6): 565-74.
231. Ring J, Brockow K, Abeck D. The therapeutic concept of "patient management" in atopic eczema. *Allergy* 1996; **51**(4): 206-15.
232. Host A. Frequency of cow's milk allergy in childhood. *Ann Allergy Asthma Immunol* 2002; **89**(6 Suppl 1): 33-7.
233. Lucas A, Brooke OG, Morley R, Cole TJ, Bamford MF. Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. *BMJ* 1990; **300**(6728): 837-40.
234. Host A, Husby S, Osterballe O. A prospective study of cow's milk allergy in exclusively breast-fed infants. Incidence, pathogenetic role of early inadvertent exposure to cow's milk formula, and characterization of bovine milk protein in human milk. *Acta Paediatr Scand* 1988; **77**(5): 663-70.
235. Sinn J, Osborn DA. Primary prevention with hydrolysed formula: does it change natural onset of allergic disease? *Clin Exp Allergy* 2010; **40**(4): 534-5.
236. Kramer MS. Does breast feeding help protect against atopic disease? Biology, methodology, and a golden jubilee of controversy. *J Pediatr* 1988; **112**(2): 181-90.
237. Zeiger RS. Dietary manipulations in infants and their mothers and the natural course of atopic disease. *Pediatr Allergy Immunol* 1994; **5**(6 Suppl): 33-43.
238. de Jong MH, Scharp-Van Der Linden VT, Aalberse R, Heymans HS, Brunekreef B. The effect of brief neonatal exposure to cows' milk on atopic symptoms up to age 5. *Arch Dis Child* 2002; **86**(5): 365-9.
239. Matheson MC, Erbas B, Balasuriya A, et al. Breast-feeding and atopic disease: a cohort study from childhood to middle age. *J Allergy Clin Immunol* 2007; **120**(5): 1051-7.
240. Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. *J Allergy Clin Immunol* 2004; **114**(4): 755-60.
241. Oddy WH, Peat JK, de Klerk NH. Maternal asthma, infant feeding, and the risk of asthma in childhood. *J Allergy Clin Immunol* 2002; **110**(1): 65-7.
242. Mihrshahi S, Ampon R, Webb K, et al. The association between infant feeding practices and subsequent atopy among children with a family history of asthma. *Clin Exp Allergy* 2007; **37**(5): 671-9.
243. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995; **346**(8982): 1065-9.
244. Zutavern A, von Mutius E, Harris J, et al. The introduction of solids in relation to asthma and eczema. *Arch Dis Child* 2004; **89**(4): 303-8.
245. Hanson LA, Soderstrom T. Human milk: Defense against infection. *Prog Clin Biol Res* 1981; **61**: 147-59.

246. Prescott SL, Macaubas C, Holt BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; **160**(10): 4730-7.
247. Halpern SR, Sellars WA, Johnson RB, Anderson DW, Saperstein S, Reisch JS. Development of childhood allergy in infants fed breast, soy, or cow milk. *J Allergy Clin Immunol* 1973; **51**(3): 139-51.
248. Johnstone DE, Dutton AM. Dietary prophylaxis of allergic disease in children. *N Engl J Med* 1966; **274**(13): 715-9.
249. Lowe AJ, Hosking CS, Bennett CM, et al. Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol* 2011; **128**(2): 360-5 e4.
250. Berg A, Kramer U, Link E, et al. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. *Clin Exp Allergy* 2010; **40**(4): 627-36.
251. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997; **159**(4): 1739-45.
252. Adkins B, Bu Y, Cepero E, Perez R. Exclusive Th2 primary effector function in spleens but mixed Th1/Th2 function in lymph nodes of murine neonates. *J Immunol* 2000; **164**(5): 2347-53.
253. Wickens KL, Crane J, Kemp TJ, et al. Family size, infections, and asthma prevalence in New Zealand children. *Epidemiology* 1999; **10**(6): 699-705.
254. Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. *J Allergy Clin Immunol* 2001; **108**(4): 509-15.
255. Kim JH, Ellwood PE, Asher MI. Diet and asthma: looking back, moving forward. *Respir Res* 2009; **10**: 49.
256. Becklake MR, Ernst P. Environmental factors. *Lancet* 1997; **350 Suppl 2**: SII10-3.
257. Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. *Am J Respir Crit Care Med* 2008; **177**(6): 567-73.
258. Smith JM, Harding LK, Cumming G. The changing prevalence of asthma in school children. *Clinical & Experimental Allergy* 1971; **1**(1): 57-61.
259. Gregg I, editor. Epidemiological aspects

Second ed: Chapman and Hall;; 1983.
260. Kuehni CE, Strippoli MP, Low N, Silverman M. Asthma in young south Asian women living in the United Kingdom: the importance of early life. *Clin Exp Allergy* 2007; **37**(1): 47-53.
261. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; **149**(2 Pt 1): 358-64.
262. Frye C, Heinrich J, Wjst M, Wichmann HE, Bitterfeld Study G. Increasing prevalence of bronchial hyperresponsiveness in three selected areas in East Germany. Bitterfeld Study Group. *Eur Respir J* 2001; **18**(3): 451-8.
263. Waser M, Schierl R, von Mutius E, et al. Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. *Clin Exp Allergy* 2004; **34**(3): 389-97.
264. Heinrich J, Gehring U, Douwes J, et al. Pets and vermin are associated with high endotoxin levels in house dust. *Clin Exp Allergy* 2001; **31**(12): 1839-45.
265. Doreswamy V, Peden DB. Modulation of asthma by endotoxin. *Clin Exp Allergy* 2011; **41**(1): 9-19.

266. Riedler J, Braun-Fahrlander C, Eder W, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**(9288): 1129-33.
267. Gehring U, Strikwold M, Schram-Bijkerk D, et al. Asthma and allergic symptoms in relation to house dust endotoxin: Phase Two of the International Study on Asthma and Allergies in Childhood (ISAAC II). *Clin Exp Allergy* 2008; **38**(12): 1911-20.
268. Munir AK, Bjorksten B, Einarsson R, et al. Cat (Fel d I), dog (Can f I), and cockroach allergens in homes of asthmatic children from three climatic zones in Sweden. *Allergy* 1994; **49**(7): 508-16.
269. Perzanowski MS, Ronmark E, Platts-Mills TA, Lundback B. Effect of cat and dog ownership on sensitization and development of asthma among preteenage children. *Am J Respir Crit Care Med* 2002; **166**(5): 696-702.
270. de Marco R, Pattaro C, Locatelli F, Svanes C, Group ES. Influence of early life exposures on incidence and remission of asthma throughout life. *J Allergy Clin Immunol* 2004; **113**(5): 845-52.
271. Svanes C, Heinrich J, Jarvis D, et al. Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey. *J Allergy Clin Immunol* 2003; **112**(2): 289-300.
272. Apelberg BJ, Aoki Y, Jaakkola JJ. Systematic review: Exposure to pets and risk of asthma and asthma-like symptoms. *J Allergy Clin Immunol* 2001; **107**(3): 455-60.
273. Mandhane PJ, Sears MR, Poulton R, et al. Cats and dogs and the risk of atopy in childhood and adulthood. *J Allergy Clin Immunol* 2009; **124**(4): 745-50 e4.
274. Carrer P, Maroni M, Alcini D, et al. Assessment through environmental and biological measurements of total daily exposure to volatile organic compounds of office workers in Milan, Italy. *Indoor Air* 2000; **10**(4): 258-68.
275. Hirsch T, Hering M, Burkner K, et al. House-dust-mite allergen concentrations (Der f 1) and mold spores in apartment bedrooms before and after installation of insulated windows and central heating systems. *Allergy* 2000; **55**(1): 79-83.
276. Blanc PD, Yen IH, Chen H, et al. Area-level socio-economic status and health status among adults with asthma and rhinitis. *European Respiratory Journal* 2006; **27**(1): 85-94.
277. Franchi M, Carrer P, Kotzias D, et al. Working towards healthy air in dwellings in Europe. *Allergy* 2006; **61**(7): 864-8.
278. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006; **27**(3): 615-26.
279. Nicolai T, Illi S, von Mutius E. Effect of dampness at home in childhood on bronchial hyperreactivity in adolescence. *Thorax* 1998; **53**(12): 1035-40.
280. Williamson IJ, Martin CJ, McGill G, Monie RD, Fennerty AG. Damp housing and asthma: a case-control study. *Thorax* 1997; **52**(3): 229-34.
281. Burr ML, Matthews IP, Arthur RA, et al. Effects on patients with asthma of eradicating visible indoor mould: a randomised controlled trial. *Thorax* 2007; **62**(9): 767-72.
282. USA EpaE. An introduction to indoor air quality (IAQ). 2012. http://www.epa.gov/iaq/no2.html#Sources (accessed 4th January 2014.
283. Lin W, Brunekreef B, Gehring U. Meta-analysis of the effects of indoor nitrogen dioxide and gas cooking on asthma and wheeze in children. *Int J Epidemiol* 2013.
284. Withers NJ, Low L, Holgate ST, Clough JB. The natural history of respiratory symptoms in a cohort of adolescents. *Am J Respir Crit Care Med* 1998; **158**(2): 352-7.

285. Garrett MH, Hooper MA, Hooper BM, Abramson MJ. Respiratory symptoms in children and indoor exposure to nitrogen dioxide and gas stoves. *Am J Respir Crit Care Med* 1998; **158**(3): 891-5.
286. Ponsonby AL, Dwyer T, Kemp A, Couper D, Cochrane J, Carmichael A. A prospective study of the association between home gas appliance use during infancy and subsequent dust mite sensitization and lung function in childhood. *Clin Exp Allergy* 2001; **31**(10): 1544-52.
287. Phoa LL, Toelle BG, Ng K, Marks GB. Effects of gas and other fume emitting heaters on the development of asthma during childhood. *Thorax* 2004; **59**(9): 741-5.
288. Sherriff A, Golding J, Alspac Study T. Hygiene levels in a contemporary population cohort are associated with wheezing and atopic eczema in preschool infants. *Arch Dis Child* 2002; **87**(1): 26-9.
289. Korhonen L, Kondrashova A, Tauriainen S, et al. Enterovirus infections in early childhood and the risk of atopic disease--a nested case-control study. *Clin Exp Allergy* 2013; **43**(6): 625-32.
290. Burgess JA, Abramson MJ, Gurrin LC, et al. Childhood infections and the risk of asthma: a longitudinal study over 37 years. *Chest* 2012; **142**(3): 647-54.
291. von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. *Eur Respir J* 1999; **14**(1): 4-11.
292. Collier CH, Risnes K, Norwitz ER, Bracken MB, Illuzzi JL. Maternal Infection in Pregnancy and Risk of Asthma in Offspring. *Maternal & Child Health Journal* 2013; **17**(10): 1940-50.
293. Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. The Aberdeen WHEASE Group. *Thorax* 1998; **53**(1): 28-32.
294. McKeever TM, Lewis SA, Smith C, et al. Siblings, multiple births, and the incidence of allergic disease: a birth cohort study using the West Midlands general practice research database. *Thorax* 2001; **56**(10): 758-62.
295. Braback L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. *Clin Exp Allergy* 1998; **28**(8): 936-42.
296. Sunyer J, Anto JM, Kogevinas M, et al. Risk factors for asthma in young adults. Spanish Group of the European Community Respiratory Health Survey. *Eur Respir J* 1997; **10**(11): 2490-4.
297. Pekkanen J, Remes S, Kajosaari M, Husman T, Soininen L. Infections in early childhood and risk of atopic disease. *Acta Paediatr* 1999; **88**(7): 710-4.
298. Ponsonby AL, Couper D, Dwyer T, Carmichael A, Kemp A. Relationship between early life respiratory illness, family size over time, and the development of asthma and hay fever: a seven year follow up study. *Thorax* 1999; **54**(8): 664-9.
299. Adams M, Doull I. Birth by caesarean section and asthma. *Clin Exp Allergy* 2008; **38**(4): 554-6.
300. Martignon G, Oryszczyn MP, Annesi-Maesano I. Does childhood immunization against infectious diseases protect from the development of atopic disease? *Pediatr Allergy Immunol* 2005; **16**(3): 193-200.
301. Thomson JA, Widjaja C, Darmaputra AAP, et al. Early childhood infections and immunisation and the development of allergic disease in particular asthma in a high-risk cohort: A prospective study of allergy-prone children from birth to six years. *Pediatric Allergy and Immunology* 2010; **21**(7): 1076-85.
302. McDonald KL, Huq SI, Lix LM, Becker AB, Kozyrskyj AL. Delay in diphtheria, pertussis, tetanus vaccination is associated with a reduced risk of childhood asthma. *J Allergy Clin Immunol* 2008; **121**(3): 626-31.
303. Nakajima K, Dharmage SC, Carlin JB, et al. Is childhood immunisation associated with atopic disease from age 7 to 32 years? *Thorax* 2007; **62**(3): 270-5.

304. Thomson JA, Widjaja C, Darmaputra AA, et al. Early childhood infections and immunisation and the development of allergic disease in particular asthma in a high-risk cohort: A prospective study of allergy-prone children from birth to six years. *Pediatr Allergy Immunol* 2010; **21**(7): 1076-85.
305. NICE. NICE guidance on Caesarean section. *http://wwwniceorguk/nicemedia/live/13620/57162/57162pdf*, 2011. (accessed 12th May 2013).
306. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy* 2008; **38**(4): 629-33.
307. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disease: meta-analyses. *Clin Exp Allergy* 2008; **38**(4): 634-42.
308. Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedon JC. Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy. *J Allergy Clin Immunol* 2008; **122**(2): 274-9.
309. Roduit C, Scholtens S, de Jongste JC, et al. Asthma at 8 years of age in children born by caesarean section. *Thorax* 2009; **64**(2): 107-13.
310. Breton MC, Beauchesne MF, Lemiere C, Rey E, Forget A, Blais L. Risk of perinatal mortality associated with asthma during pregnancy. *Thorax* 2009; **64**(2): 101-6.
311. Gilliland FD, Li YF, Peters JM. Effects of maternal smoking during pregnancy and environmental tobacco smoke on asthma and wheezing in children. *Am J Respir Crit Care Med* 2001; **163**(2): 429-36.
312. Gilliland FD, Berhane K, McConnell R, et al. Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. *Thorax* 2000; **55**(4): 271-6.
313. Cook DG, Strachan DP. Health effects of passive smoking. 3. Parental smoking and prevalence of respiratory symptoms and asthma in school age children. *Thorax* 1997; **52**(12): 1081-94.
314. Tinuoye O, Pell JP, Mackay DF. Meta-analysis of the association between secondhand smoke exposure and physician-diagnosed childhood asthma. *Nicotine Tob Res* 2013; **15**(9): 1475-83.
315. Burke H, Leonardi-Bee J, Hashim A, et al. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics* 2012; **129**(4): 735-44.
316. Agabiti N, Mallone S, Forastiere F, et al. The impact of parental smoking on asthma and wheezing. SIDRIA Collaborative Group. Studi Italiani sui Disturbi Respiratori nell'Infanzia e l'Ambiente. *Epidemiology* 1999; **10**(6): 692-8.
317. Al Mamun A, O'Callaghan FV, Alati R, et al. Does maternal smoking during pregnancy predict the smoking patterns of young adult offspring? A birth cohort study. *Tob Control* 2006; **15**(6): 452-7.
318. Jaakkola MS, Piipari R, Jaakkola N, Jaakkola JJ. Environmental tobacco smoke and adult-onset asthma: a population-based incident case-control study. *Am J Public Health* 2003; **93**(12): 2055-60.
319. (ASH) Aosah. Smoking statistics who smokes and how much. 2013. http://ash.org.uk/files/documents/ASH_106.pdf2014).
320. Busse WW, Lemanske RF, Jr., Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet*; **376**(9743): 826-34.
321. Renz H, Herz U. The bidirectional capacity of bacterial antigens to modulate allergy and asthma. *Eur Respir J* 2002; **19**(1): 158-71.
322. Lane J, Siebers R, Pene G, Howden-Chapman P, Crane J. Tokelau: a unique low allergen environment at sea level. *Clin Exp Allergy* 2005; **35**(4): 479-82.

323. Gereda JE, Leung DY, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; **355**(9216): 1680-3.
324. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; **163**(2): 322-8.
325. Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; **347**(12): 869-77.
326. Bernsen RM, van der Wouden JC, Nagelkerke NJ, de Jongste JC. Early life circumstances and atopic disorders in childhood. *Clin Exp Allergy* 2006; **36**(7): 858-65.
327. Nymand G. Letter: Maternal smoking and immunity. *Lancet* 1974; **2**(7893): 1379-80.
328. Feleszko W, Ruszczynski M, Jaworska J, Strzelak A, Zalewski BM, Kulus M. Environmental tobacco smoke exposure and risk of allergic sensitisation in children: a systematic review and meta-analysis. *Arch Dis Child* 2014.
329. Holt PG. Immune and inflammatory function in cigarette smokers. *Thorax* 1987; **42**(4): 241-9.
330. Reed CE. The natural history of asthma. *J Allergy Clin Immunol* 2006; **118**(3): 543-8; quiz 9-50.
331. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. *Eur Respir J* 2007; **30**(3): 452-6.
332. Guerra S, Wright AL, Morgan WJ, Sherrill DL, Holberg CJ, Martinez FD. Persistence of asthma symptoms during adolescence: role of obesity and age at the onset of puberty. *Am J Respir Crit Care Med* 2004; **170**(1): 78-85.
333. Vonk JM, Postma DS, Boezen HM, et al. Childhood factors associated with asthma remission after 30 year follow up. *Thorax* 2004; **59**(11): 925-9.
334. Panhuysen CI, Vonk JM, Koeter GH, et al. Adult patients may outgrow their asthma: a 25-year follow-up study. *Am J Respir Crit Care Med* 1997; **155**(4): 1267-72.
335. Dijkstra A, Vonk JM, Jongepier H, et al. Lung function decline in asthma: association with inhaled corticosteroids, smoking and sex. *Thorax* 2006; **61**(2): 105-10.
336. Vonk JM, Jongepier H, Panhuysen CI, Schouten JP, Bleecker ER, Postma DS. Risk factors associated with the presence of irreversible airflow limitation and reduced transfer coefficient in patients with asthma after 26 years of follow up. *Thorax* 2003; **58**(4): 322-7.
337. Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 2007; **370**(9589): 758-64.
338. Sporik R, Holgate ST, Cogswell JJ. Natural history of asthma in childhood--a birth cohort study. *Arch Dis Child* 1991; **66**(9): 1050-3.
339. Mochizuki H, Shigeta M, Arakawa H, Kato M, Tokuyama K, Morikawa A. Bronchial hyperresponsiveness before and after the diagnosis of bronchial asthma in children. *Pediatrics* 2000; **106**(6): 1442-6.
340. Porsbjerg C, von Linstow ML, Ulrik CS, Nepper-Christensen SC, Backer V. Outcome in adulthood of asymptomatic airway hyperresponsiveness to histamine and exercise-induced bronchospasm in childhood. *Ann Allergy Asthma Immunol* 2005; **95**(2): 137-42.
341. Jones A. Asymptomatic bronchial hyperreactivity and the development of asthma and other respiratory tract illnesses in children. *Thorax* 1994; **49**(8): 757-61.
342. Rasmussen F, Lambrechtsen J, Siersted HC, Hansen HS, Hansen NC. Asymptomatic bronchial hyperresponsiveness to exercise in childhood and the

development of asthma related symptoms in young adulthood: the Odense Schoolchild Study. *Thorax* 1999; **54**(7): 587-9.
343. Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 1999; **54**(12): 1119-38.
344. Pohunek P, Warner JO, Turzikova J, Kudrmann J, Roche WR. Markers of eosinophilic inflammation and tissue re-modelling in children before clinically diagnosed bronchial asthma. *Pediatr Allergy Immunol* 2005; **16**(1): 43-51.
345. Shaheen SO, Northstone K, Newson RB, Emmett PM, Sherriff A, Henderson AJ. Dietary patterns in pregnancy and respiratory and atopic outcomes in childhood. *Thorax* 2009; **64**(5): 411-7.
346. Julge K, Vasar M, Bjorksten B. Development of allergy and IgE antibodies during the first five years of life in Estonian children. *Clin Exp Allergy* 2001; **31**(12): 1854-61.
347. Weinmayr G, Genuneit J, Nagel G, et al. International variations in associations of allergic markers and diseases in children: ISAAC Phase Two. *Allergy* 2010; **65**(6): 766-75.
348. Sunyer J, Anto JM, Rodrigo MJ, Morell F. Case-control study of serum immunoglobulin-E antibodies reactive with soybean in epidemic asthma. *Lancet* 1989; **1**(8631): 179-82.
349. Phelan PD, Robertson CF, Olinsky A. The Melbourne Asthma Study: 1964-1999. *J Allergy Clin Immunol* 2002; **109**(2): 189-94.
350. Heinrich J, Popescu MA, Wjst M, Goldstein IF, Wichmann HE. Atopy in children and parental social class. *Am J Public Health* 1998; **88**(9): 1319-24.
351. Abrahamsson TR, Jakobsson HE, Andersson AF, Bjorksten B, Engstrand L, Jenmalm MC. Low gut microbiota diversity in early infancy precedes asthma at school age. *Clin Exp Allergy* 2014; **44**(6): 842-50.
352. Jones KD, Berkley JA, Warner JO. Perinatal nutrition and immunity to infection. *Pediatr Allergy Immunol*; **21**(4 Pt 1): 564-76.
353. Bolling K GC, Hamlyn B, Thornton A. Infant Feeding Survey 2005. . *The Information Centre, 2007* 2007.
http://www.ic.nhs.uk/webfiles/publications/ifs06/2005%20Infant%20Feeding%20Survey%20(final%20version).pdf (accessed.
354. L. MR. chapter 16 Asthma Epigenetics: Emergence of a New Paradigm? . In: Michels KB, editor. Epigenetic Epidemiology; 2012. p. 307-19.
355. Mandhane PJ, Greene JM, Cowan JO, Taylor DR, Sears MR. Sex differences in factors associated with childhood- and adolescent-onset wheeze. *Am J Respir Crit Care Med* 2005; **172**(1): 45-54.