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ABSTRACT

The aim of this thesis is to explore the possible effects of exposure to cigarette smoking during foetal and early postnatal life on lower respiratory disease and sensitisation in children up to four years of age. A prospective birth cohort of 4,089 newborn infants (BAMSE) was followed during four years using parental questionnaires. When the infant was two months old the parents completed a questionnaire on various lifestyle factors, including maternal smoking during pregnancy and parental smoking after birth. At one, two and four years of age information was obtained by questionnaire on symptoms of allergic and respiratory diseases as well as on environmental exposures, particularly exposure to environmental tobacco smoke (ETS). At four years of age the response rate was 91% (3,619 children) and among these 73% participated in a clinical investigation including peak flow measurements and blood sampling for analyses of IgE antibodies to common inhalant and food allergens. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression with adjustments for confounders.

Maternal smoking during pregnancy, parental smoking after the baby was born and keeping of cat or dog were all associated with short education. The risk of respiratory illness in children increased with exposure to parental cigarette smoking. When the mother had smoked during pregnancy but not after that, there was an increased risk of recurrent wheezing up to two years´ age, OR_{adj}=2.2 (95% CI 1.3 – 3.6). The corresponding OR was 1.6 (95% CI 1.2 – 2.3) for reported exposure to ETS with or without maternal smoking during pregnancy. Follow up at four years´ age showed that exposure *in utero* to maternal smoking was associated with an increased risk of transient (OR=1.8, 95% CI 1.2 – 2.5), persistent (OR=2.1, 1.5 – 2.8) and late-onset wheezing (OR=1.5, 1.0 – 2.2). There was no strong association between exposure to parental smoking and any kind of wheezing. Neither prenatal nor postnatal exposure to tobacco smoking was associated with impairment of lung function (peak expiratory flow). An increased risk with ETS was found for sensitisation to inhalant and/or food allergens, OR_{adj}=1.3, (95% CI 1.0 – 1.6). Among single allergens the effect of ETS on sensitisation to cat was particularly strong, OR_{adj}=2.00 (95% CI 1.3 – 3.0). A dose-response effect was found for exposure to ETS from parental smoking during the first few months of life and IgE-sensitisation.

In conclusion, our data indicate that exposure to maternal cigarette smoking *in utero* is a risk factor for wheezing up to four years of age and exposure of infants to ETS increases the risk of IgE-sensitisation to indoor inhalant and food allergens.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Lannerö E, Kull I, Wickman M, Pershagen G, Nordvall SL. Environmental risk factors for allergy and socioeconomic status in a birth cohort (BAMSE). Pediatr Allergy Immunol 2002;13:1-8.

II. Lannero E, Wickman M, Pershagen G, Nordvall L. Maternal smoking during pregnancy increases the risk of recurrent wheezing during the first years of life (BAMSE). Respir Res. 2006 Jan 5;7:3.

III. Bergström A, Lannerö E, Nordvall L, Pershagen G, Hallberg J and Wickman M. Maternal smoking during pregnancy in relation to lung function and wheezing in children – results from the BAMSE cohort. *Submitted.*

IV. Lannerö E, Wickman M, van Hage M, Bergström A, Pershagen G and Nordvall L. Exposure to environmental tobacco smoke and risk of sensitisation in children. *Thorax. In press.*

CONTENTS

LIST OF ABBREVIATIONS

ANC	Antenatal clinics
BAMSE	Children, Allergy; Milieu, Stockholm, Epidemiological survey
BMHE 03	The Children´s Environmental Health Questionnaire Survey 2003
CHC	Child Health Centre
CI	Confidence interval
ETS	Environmental tobacco smoke
IgE	Immunglobulin E
LRI	Lower respiratory tract illness
OR	Odds ratio
PEF	Peak Expiratory Flow
SEI	Socioeconomic index measured by the Nordic standard occupational classification (NYK) and Swedish socioeconomic classification (SEI)
SES	Socioeconomic status

1 BACKGROUND

1.1 CIGARETTE SMOKING

History

Tobacco is made from dried leaves of the tobacco plant. The use of tobacco started among native Americans 5000 years BC. Columbus first brought tobacco seeds from the West Indies to Europe (Portugal and Spain) in 1492. Jean Nicot, a French ambassador, brought tobacco seeds to Paris and grew them in his garden. In Europe it was initially used for medical purposes and e.g. the queen of France, Catherine of Medici, was cured from abdominal disease by tobacco. As she got the tobacco from Jean Nicot she called it "Nicotiana". Later Linné named the plant Nicotiana tabacum.
The industrial production of cigarettes and cigars started in America during the late part of the 19th century and in Sweden some decades later. During the two world wars in the 20th century soldiers spread the use of tobacco, which was initially used as snuff, but also smoked in pipes. (Goodman J., 1995)

Cigarette smoking was initially a masculine habit. Women took up smoking during and after Wold War II why their children got in closer contact with environmental tobacco smoke (ETS). Cigarette smoking became a sign of independence among the well-educated and career-oriented women. Other groups in society subsequently acquired the habit, which is now most prevalent among women with short education. (Torell U, 2002)

The first version of the Swedish Tobacco Law was passed in 1993 and was tightened up in 1994, 1997, 2002 and 2005. The law regulates where smoking is prohibited and the use of warning labels on cigarette packets. Furthermore, cigarette advertising and the purchase of cigarettes by juveniles under 18 years of age are not permitted. In the EU the politicians now try to form a standardized legislation and set up goals for tobacco prevention. (WHO, 2002)

Epidemiology

Going back to the 1940´s half of the men in Sweden were regular smokers and less than 10 percent of the women while in the 1960´s 49 and 23 percent, respectively, were smokers. In the 1980´s among 16-84 year olds, 36% of men and 29 % of women smoked, which can be compared with a recent report in which the proportions of daily smokers in 2006 are 12 % among men and 17% among women and the number of current smokers is estimated to be 1.2 million. (The Swedish Council for Information on Alcohol and other Drugs, CAN, 2007)

In Sweden fewer parents smoke and according to "The Children´s Environmental Health Questionnaire Survey from 2003 (BMHE 03) about five percent of the children are exposed daily to tobacco smoke in their homes and ten percent of the mothers smoke during pregnancy. (Environmental health report, 2005)

The National Board of Health and Welfare started to collect information in 1983 on maternal smoking during pregnancy through the antenatal clinics (ANC) in Sweden.

The reported maternal smoking rates during the first trimester have decreased from 32% in 1983 to 6.5% in 2004. In a similar way data are collected from the child health centres (CHC) when the child is one and eight months old. Since 1999 the proportion of parents who smoke has been reduced from about 10 percent for maternal smoking and 14 percent for paternal smoking to seven and 12 percent, respectively. (Tobaksvanor hos spädbarnsföräldrar (Swedish), 2005) Even though there has been a decrease in parental smoking and new legislation restricts cigarette smoking in public places including restaurants, children are still exposed to ETS and mainly in their homes. For young children the major source of exposure to ETS is the home environment. (Jarvis *et al*., 2000) For the foetus and the newborn child maternal smoking is typically the largest source of exposure because of the cumulative effects of exposure *in utero* and the closeness with the mother during the breast-feeding period when the child is exposed to the mother´s active as well as passive smoking.

1.2 MATERNAL CIGARETTE SMOKING DURING PREGNANCY – HEALTH EFFECTS ON THE FOETUS

Foetal life

One of the most hazardous environmental exposures of children is that of maternal cigarette smoking during the foetal development. The foetus is affected in a number of ways resulting in impaired foetal growth (Horta *et al*., 1997) as well as an increased risk of late foetal death. (Cnattingius *et al*., 1988) The foetuses of smoking women are exposed to similar levels of nicotine as those of active smokers from the time of conception. (Foundas *et al*., 1997)

Animal studies show that exposure to cigarette smoke during pregnancy reduces the rate of foetal breathing movements, which may have a longlasting impact on the foetal airways by predisposing to limited airway growth. (Hofhuis *et al*., 2003) Moreover, maternal cigarette smoking during pregnancy exposes the foetus to carcinogens and other toxins. (Lackman *et al*., 1999) Prenatal maternal smoking also lowers maternal uterine blood flow by constricting the uterine vessels why transportation of oxygen from the uterus to the placenta is reduced. (Morrow *et al*., 1988) It is likely that this results in intrauterine growth retardation. (Horta *et al*., 1997) and low birth weight (Secker-Walker *et al*., 1997) Further, the risk of very preterm birth increases with exposure *in utero* to cigarette smoke (Kyrklund-Blomberg N.B. and Cnattingius S., 1998) and in a recent prospective study the risk was more than doubled among heavy smokers. (Kyrklund Blomberg *et al*., 2005) A small head circumference for gestational age is also associated with maternal smoking during pregnancy. (Källen K., 2000)

Neonatal period

In many earlier studies of the effects on children of exposure to ETS it has not been possible to separate the effect of exposure *in utero* from that of exposure postnatally. In the newborn child, however, all measures, including lung function, may only reflect the effects of exposures *in utero*. Several epidemiological studies on maternal smoking during pregnancy and lung function of the neonate have been performed. An increased risk of lung function impairment reflected in low spirometric flow due to impaired

growth and development of the foetal lung has been found. (Stick *et al*., 1996, Lødrup Carlsen *et al*., 1997, Elliot *et al*., 2003)
There is also evidence of an association between foetal exposure to cigarette smoking and sudden infant death syndrome. (Alm *et al*., 1998, Wisborg *et al*., 2000, Mitchell E.A. and Milerad J., 2006)

Childhood

The relation between parental smoking and asthma has mainly focused on postnatal studies of exposure, combining or separating maternal and paternal smoking. (Strachan D.P. and Cook D.G., 6. 1998, Jaakkola J.J. and Jaakkola M.S., 2002) However, some studies have addressed the role of smoking in pregnancy and there is now a growing body of evidence of a major effect from exposure to cigarette smoking *in utero* on wheezing and asthma. (Stein *et al*., 1999, Lødrup Carlsen K.C.and Carlsen K.H., 2001). This effect is independent of the effect of ETS in some (Lux *et al*., 2000), but not all studies. (Li *et al*., 2005) Exposure to cigarette smoking *in utero* seems to be as harmful as ETS and causes an increased risk for wheezing among children 18-30 months of age. (Lux *et al*., 2000) The effects also appear to be long lasting and data from a large prospective Finnish birth cohort indicate that prenatal exposure to cigarette smoking increases the risk of asthma up to seven years of age. (Jaakkola J.J. and Gissler M., 2004) In a large cross-sectional study of school-children there was a significant association between exposure *in utero* and doctor´s diagnosed asthma. It was calculated that an elimination of such exposure would prevent 5-15 % of the cases. (Gilliland *et al*., 2001)

In a large prospective cohort study recruited before birth it was noted that prenatal exposure to smoking was more important than postnatal for the risk of otitis media. (Stathis *et al*., 1999) An association has also been found between exposure to smoking *in utero* and neuro-behaviourial problems. (Rodriguez A. and Bohlin G., 2005)

1.3 ENVIRONMENTAL TOBACCO SMOKE

Several terms have been used to describe non-smokers´ inhalation of tobacco smoke produced by others e.g. passive, involuntary or second hand smoking. In this thesis the term ETS (environmental tobacco smoke) is used.

Composition

ETS is derived both from the mainstream smoke (MS) i.e. smoke from cigarettes, pipes or cigars that has been inhaled and then exhaled by the smoker. Side stream smoke (SS) is the smoke that is emitted from the smouldering tobacco that may be inhaled by the non smoker between the puffs. Thus, the side stream smoke contains the same harmful substances as mainstream smoke and in fact more of these substances because of the lower burning temperature between the puffs. More than 75% of the smoke that is produced from a cigarette is emitted as side stream smoke. (Benowitz N.L., 1996)

Thousands of substances have been identified in tobacco smoke including more than 50 mutagens. ETS is a mixture of gas and particle phase chemicals composed of tar, water, nicotine and other nicotine-like alkaloids and the composition changes through the

dilution and distribution in the environment. (Benowitz N.L., 1996) Many of the chemicals found in ETS pass through the placental barrier. (Jordanov J.S., 1990)

Assessment of exposure to ETS

In epidemiological studies it is crucial that information on exposure is accurate. To test whether self-reported active smoking information among pregnant women is valid, plasma levels of cotinine were measured and the conclusion was that information on active smoking could be trusted. (George *et al*., 2006) Evidence of exposure to ETS in non smokers can be found by measuring substances or metabolites originating from the smoke in body fluids and constitute a useful tool for the validation of questionnaire data on smoking. The most widely used method is to measure cotinine in the urine and it is by some considered to be the "gold standard". (Benowitz N.L., 1996, Johansson *et al*., 2005)

1.4 ETS – ADVERSE HEALTH EFFECTS ON CHILDREN

It appears that the adverse impact of adult tobacco use in the home on the health of children is significant and increases the incidence of media otitis, (Ståhlberg *et al*., 1986, Ilicali *et al*., 2001) pneumonia, cough, bronchitis and other lower respiratory tract illnesses (Fergusson *et al*., 1980) and bronchiolitis (Pullan C.R. and Hey E.N., 1982) as well as risk of sudden infant death syndrome. (Mitchell *et al*., 1993, Alm *et al*., 1998, Mitchell E.A. and Milerad J. 2006) Exposure to ETS also causes reduction in pulmonary function in small children. (Cook *et al*., 1998, Jones *et al*., 2000) However, it is likely that some of the adverse effects on lung function in the newborn child are attributable to cigarette exposure *in utero.* (Stick *et al*., 1996, Lødrup Carlsen *et al*., 1997)

Wheezing / asthma / allergy

Wheeze in early childhood seems to be induced by exposure to ETS. Daily postnatal exposure during the first six months was associated with an increased risk of wheezing and a positive dose-response effect was indicated. (Lux *et al*., 2000) ETS was also found to increase the occurrence of asthma among children below five years of age. (Gergen *et al*., 1998) Daily exposure to ETS was associated with an increased risk of recurrent episodes of wheezing and maternal smoking was related to the highest risk. (Halken *et al*., 1991) Among young children admitted to hospital because of severe wheezing one third could be attributed to parental smoking. (Rylander *et al*., 1993)

Effects of ETS due to parental smoking on respiratory health in preschool and school ages have been described in a number of epidemiological studies. In a systematic review of epidemiological studies both the occurrence and severity of asthma were increased by exposure to ETS and the risk of developing asthma was 1.37 if either parent smoked. (Strachan D.P. and Cook D.G.,.6. 1998) Maternal smoking was related to an increased incidence of wheezing. (Strachan D.P. and Cook D.G.,.6. 1998) ETS also increases the frequency of asthma attacks. (Strachan D.P. and Cook D.G., 1997) Thus, it seems that there is a causal relationship between ETS and asthma in children, but the exact mechanism has not been established. (DiFranza J.R. and Lew R.A., 1996, Cook D.G. and Strachan D.P., 1997) Gilliland and Lux have both hypothesized that

exposure to ETS works as a trigger factor for wheezing rather than a cause of asthma. (Gilliland *et al*., 2001, Lux *et al*., 2000) However, ETS exposure in combination with exposure to cat during the first years of life significantly increased the risk of sensitisation to cat and the development of more severe asthma up to four years of age. (Lindfors *et al.,* 1999, Melén *et al*., 2001) These findings rather indicate that ETS may be looked upon as a cause of allergic asthma.

1.5 EXPOSURE TO MATERNAL CIGARETTE SMOKING DURING PREGNANCY AND / OR ETS AND SENSITISATION OF THE CHILD

Already in 1981 Kjellman suggested that ETS increases the risk of allergic disease. (Kjellman N.I., 1981) A number of studies have investigated sensitisation in relation to ETS. Strachan and Cook concluded in a comprehensive review from 1998 that parental smoking, either before or immediately after birth, is unlikely to increase the risk of IgE-sensitisation in children. (Strachan D.P. and Cook D.G., 5. 1998) After that Murray as reported from "The National Asthma Campaign in Manchester" found little or no effect of ETS on the development of atopy. (Murray *et al*., 2004) Neither did Tariq find any significant association between passive smoking and allergic sensitisation by assessing skin-prick tests of children at one, two and four years of age and no effect was found of ETS on allergic sensitisation, neither to food- or aero allergen at four years of age. (Tariq *et al*., 2000)

On the other hand Kulig, in a birth cohort study, reported an association between pre- and postnatal exposure to tobacco smoke and sensitisation to food allergens but not to inhalant allergens. (Kulig *et al*., 1999) In a study of young asthmatic children atopic sensitisation to cat was found to be promoted by ETS. (Lindfors *et al*., 1999)

1.6 ASTHMA / WHEEZING

History

The word asthma originates from the Greek verb "aazein" ααζειν, and means "to breath with open mouth or to pant". It was first recognized in Homer´s "Iliad" *Ιλιαδ*. From around 400 BC it was used in the Corpus Hippocratum in a medical sense. It was later mentioned by Aratues Cappadocia, who described the clinical symptoms of asthma in the first century AD. Galen (130-200 AD) mentioned asthma many times and his descriptions were similar to those in the Hippocratic texts. (Marketos S.G. and Ballas C.N., 1982)

Definition

There is no uniform definition of asthma that can be applied to all cases. The "definitions" that are currently used are in fact descriptions of the characteristics of the disease and there are a number of reasons for that: there is no clinical test or single marker for asthma, the symptoms vary, asthma has multiple aetiological factors and the responses to treatment differ as well. Thus, asthma is a complex disease entity, encompassing many phenotypes such as allergic and exercise or cold induced asthma and early childhood virus-associated wheezing. (Sears M.R., 1997) Interestingly, it has lately been proposed that the name "asthma" is abolished and the reason is that asthma

is not a single disease entity. (Editorial, The Lancet, 2006) In the GINA guidelines asthma is described as a chronic inflammatory disorder of the airways in which different inflammatory cells are involved. This inflammation is strongly associated with airway hyper-responsiveness and asthma symptoms and may cause recurrent episodes of wheezing, breathlessness, chest tightness and cough. In clinical practice asthma is diagnosed on the basis of these symptoms. (Sears MR, 1997, GINA 2006)

Thus, there are no unanimous criteria for asthma neither among infants or preschool children nor school-children or adolescents. Consequently, in epidemiological studies the definitions and terminology of respiratory disease often differ between investigators and also the methods that are used. However, recurrent wheezing is considered to be associated with asthma and the diagnosis is confirmed by the relapsing feature of the disease entity and the number of episodes during the previous year. (Koopman *et al*., 2001) In epidemiological research, questionnaires are used to collect data on wheezing episodes, shortness of breath and recurrent cough.

Assessment of subtypes of asthma / wheezing in children

In preschool children three different expressions of asthma have been described: transient early wheezers, who exhibit at least one lower respiratory tract illness (LRI) with wheezing during the first three years of life but no wheezing at 6 years of age; late onset wheezers: no LRI with wheezing during the first three years of life but who had wheezing at six years of age; persistent wheezers: at least one LRI with wheezing during the first three years of life and wheezing at 6 years of age. (Martinez *et al*., 1995)

From a longitudinal study of children followed from birth to 6 years of age it was reported that almost 50% of 826 children, who were followed from birth, developed early, late or persistent wheezing and one fifth of this cohort developed early transient wheezing before the age of three. The children in the latter group exhibited reduced pulmonary function indicative of reduced airway calibre both before the age of one year and at the age of six years and were more likely than the other children to have smoking mothers. (Martinez *et al.,* 1995) Transient early wheezing is regarded as a benign condition usually associated with respiratory viral infections neither related to subsequent wheeze nor asthma. (Wright A.L., 2002) Late onset wheezers and persistent wheezers did not have reduced lung function in early life. Late and persistent wheezers are likely to exhibit atopy i.e. increased IgE antibody levels, skin test positivity and usually have a strong family history of asthma. (Martinez *et al*., 1995)

Atopy

The Greek word atopy (ατοπια), which means something "peculiar, out of place or strange", was in 1922 proposed to be used as a term to characterize diseases like hay fever and asthma, and the characteristic feature that they become sensitised to certain environmental proteins. (Coca A.F. and Cooke R.A. 1923) Later Pepys defined atopy as an immunologic reactivity characterised by the production of reaginic antibodies as a response to exposure to common allergens. (Pepys J, 1975) After that atopic has been used synonymously with "IgE mediated". A revised nomenclature for allergy was proposed in 2001, and was later revised. Atopy can be defined as a personal and/or familial tendency to produce IgE antibodies in response to low doses of allergens,

usually proteins. (Johansson S.G. and Haahtela T., 2004) According to this definition atopy means sensitisation with development of IgE antibodies, the consequence of which may be symptoms of asthma, rhino-conjunctivitis and/or eczema. During the first year of life the production of IgE antibodies is usually against food allergens and occurs among approximately 10 % of the children. The synthesis of IgE antibodies towards inhalant allergens dominates among children up to school age and approximately 25 % are sensitised. (Illi *et al*., 2001, Wickman M., 2004) Sensitisation is of importance for incident asthma among children. (Rönmark *et al*., 2001)

IgE and IgE sensitisation

Studies in adults of serum immunoglobulin E (IgE) levels have shown an increased level in smokers. (Burrows *et al*., 1981, Bahna *et al*., 1983,) In a study of occupational allergy smoking has also been reported to facilitate sensitisation against occupational allergens. (Zetterström *et al*., 1981) In 1985 Zetterström *et al*. also demonstrated an association between tobacco smoke, increased serum IgE levels and IgE sensitisation in rats. (Zetterström *et al*., 1985)

Prevalence and incidence

Allergic disorders are considered to be a major burden world-wide although the prevalence rates vary immensely. Epidemiological studies on prevalence have mostly been based on self reported questionnaires but measures of airway lability and signs of atopy, such as measurements of IgE antibodies have also been included.

The ISAAC (International Study of Asthma and Allergies in Childhood) Steering committee has performed systematic international comparisons of the prevalence of asthma. They report large world-wide variations of the occurrence of asthma among 12-13 year old teenagers. For example, in the UK, Australia and New Zealand the 12-month prevalences of self reported asthma was 25-30% as compared to prevalences below five percent in Latvia, China and India. (ISAAC, 1998) From epidemiological studies in the European countries the prevalence of childhood asthma defined as doctor´s diagnosis or any other more strict definition than wheezing alone has been calculated to be six to eight percent (Lau *et al*, 2000, Perzanowski *et al*., 2002, Kull *et al*., 2004) and an incidence rate of 0.9/100/year has been reported from Northern Sweden. (Rönmark *et al*., 2001)

Reports from several epidemiological studies indicate a rise in the prevalence of asthma during the last decades (Åberg N., 1989, Ninan T.K. and Russell G., 1992, Åberg *et al*., 1995), and even after 1988. (Burr *et al*., 2006) Some results, however, indicate that the upward trend has been broken and that there is a recent decline. (Andersson *et al.,* 2004, Braun-Fahrländer *et al.,* 2004, Zöllner I.K. 2005)

1.7 EPIDEMIOLOGY

It is often considered that the core science of public health is epidemiology (epi=among, demos=people, logos=doctrine.) The aim of epidemiological studies is to assess relations between exposure and occurrence of disease in human populations. (Rothman KJ, 2002)

Cohort studies

The term cohort was originally used in the Roman Army and consisted of a unit of 300 to 600 men and ten cohorts constituted a legion. A cohort study in our context consists of a group or groups of individuals, unexposed or exposed, who are followed over a period of time with regard to outcome. To test whether there is an association between exposure and outcome the incidence of the outcome among exposed and non-exposed is compared in the two groups. For common diseases cohort studies are useful to identify the incidence and to follow the natural history of a disease. This study design is also useful for analyses of multiple outcomes after a single exposure as well as in the study of rare exposures. Another advantage is that both the exposed and unexposed are often seen to be free of the outcome at the start of the study and consequently the data on exposure precede the identification of outcome. (Grimes D.A. and Schulz K.F., 2002, Rothman K.J. and Greenland S., 1998)

Although cohort studies have a number of appealing features there are limitations. For example, selection bias may be of importance in cohort studies if families with a history of atopic disease might be more willing to participate in studies on this topic. Another cause of non-respondent bias is that smoker may be less prone to answer questionnaires on smoking habits than non-smokers (Seltzer *et al*., 1974).
Information bias occurs when information on or from a study subject is incorrect and leads to misclassification of disease and exposure. Another disadvantage of cohort studies is loss to follow up especially in longitudinal studies that may continue for many years. Accounting for the biases resulting from non response is important in the analyses of longitudinal data. Another cause of bias is "reverse causation bias" which may occur when outcome precedes exposure or measurements of exposure and the occurrence of disease leads to modification of exposure. (Hernán M.A., 2004) Further, a confounder co-variates with the exposure and influences the risk of the studied disease. Confounding may be adjusted for in the statistical analyses. (Grimes D.A. and Schulz K.F., 2002)

In order to calculate risk associations it is essential that exposure and outcomes are clearly defined and specified at the beginning of the study to minimize disease related misclassification. (Rothman K.J., 1998) Cigarette smoke exposure for example can be estimated by reported smoking by the parents at home or by objective measures such as cotinine in urine, thus avoiding bias from misreporting. (Pershagen G., 1997)

Outcomes can be assessed through reported symptoms, diagnoses and medication. To measure association, such as odds ratio (OR), outcomes in terms of disease in exposed are compared to disease in unexposed. To measure the precision of study results confidence intervals (CIs) are used, usually the 95 % CI. The interval provides a set of possible values for a variable, such as the odds ratio (OR), that has a specified probability of containing the true value for the whole population from which the study sample emanates. By the use of OR and CI the direction, strength and plausible range of an effect as well as the likelihood of chance to occur are revealed. (Grimes D.A., 2002)

CONCLUSIONS FROM THE BACKROUND OF THIS THESIS

Cigarette smoke has severe effects on pulmonary health but also affects many other organs. There is little doubt that the use of tobacco must be in focus in any health preventive effort. There are, however, several questions that still need to be studied more in detail.

Although there are a number of studies on the effects of passive smoking on wheezing in children, it has been difficult to distinguish between the effects of maternal tobacco smoking during pregnancy and those when the child is exposed to parental tobacco smoke after birth. Indeed, in most studies this important distinction is not even made. The reason for the difficulty is obvious, most mothers who smoke during pregnancy continue after having given birth.

Another issue that remains unresolved is that of a possible risk of sensitisation with exposure to smoking. A systematic review on the topic from 1998 concludes that sensitisation assessed by skin prick tests or measurements of IgE antibodies is unlikely to be increased neither by pre- nor postnatal smoke exposure. (Strachan D.P. and Cook D.G, .5. 1998) After that two large epidemiological studies provide contradictory results. (Kulig *et al*., 1999, Murray *et al*. 2004) It is of interest in the context, however, that a considerably smaller case-control study from Stockholm indicated that smoke exposure in early life is associated with an increased risk of sensitisation. (Lindfors *et al*., 1999) Just as with the detrimental effects on wheezing, the role of pre- and postnatal tobacco smoke exposure needs to be sorted out.

Another issue of importance in the case of smoking is its well recognized association with the social status and educational level of the family. The possibility that educational level is associated with other exposures of importance remains a matter of concern for any bias. Further, considering the possible applicability of any of the results under study in prevention, it appeared particularly valuable to study more in detail associations between smoking, other exposures of interest in the context of allergic disease and educational level of the parents.

2 AIM OF THE STUDY

The overall objective of this thesis is to study the effect of exposure to maternal smoking during pregnancy and exposure to parental smoking early in life on allergic airway disease in children up to four years of age. Specific aims include:

- To elucidate the importance of various risk factors for allergic airway disease in early infancy in relation to parental educational level and the interrelationships between risk factors.
- To discriminate the effects of exposure to cigarette smoking during the foetal period from those of ETS in early life on lower respiratory disease.
- To study the effects of ETS in relation to the development of wheezing up to four years of age.
- To investigate the effects of pre and/or postnatal exposure to cigarette smoke on the development of sensitisation in children.

3 MATERIAL AND METHODS

All data presented in the four papers of this thesis originate from the longitudinal birth cohort study, BAMSE, with the main aim to study environmental factors and the development of allergic disease in children.

3.1 STUDY POPULATION

A cohort of 4,089 new-born infants identified in the Swedish Medical Birth Register and born in predefined areas of Stockholm, was recruited from Child Health Centres between February 1994 and November 1996 (The BAMSE study). During the recruitment period 7,221 children were born in the study area and 1,256 were excluded according to the study plan. (Figure 1) Another 477 families could not be reached because the address was unknown. Of 5,488 eligible newborn infants and their families 4,089 (75%) answered the inclusion questionnaire and thus constituted the final cohort. (Figure 1)

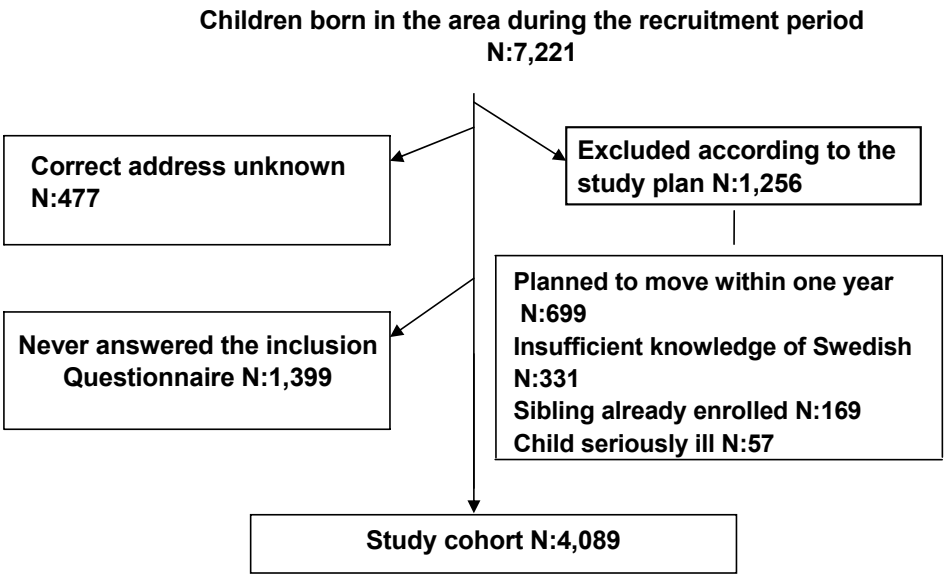

Figure 1. Description of the cohort

Access to a community population register ensured that the parents of all children born in the area during the period under study could be approached.

To study the possible biases introduced by non participation (non-responders or actively excluded children) in the BAMSE study a separate short questionnaire, with key questions (answered retrospectively) concerning environmental exposures and family history of allergic disease, was mailed in May 1996 to 1,418 children. The

questions were chosen from the first questionnaire in the BAMSE study. The age of the children targeted was 3-20 months and the response rate was 67%. The results from the non-responders and actively excluded families were compared with those of the study population. The proportion of children with any or double heredity and keeping of pets did not differ between the non-responders and actively excluded families compared with the whole BAMSE-cohort. However, a significant difference was recorded for parental smoking which was more prevalent among non-responders and actively excluded families than among the participating families (maternal smoking 18% vs. 9%, and paternal smoking 23% vs. 17%), respectively.

3.2 QUESTIONNAIRES

The families were informed about the study by the attending nurse at their first visit to the Child Health Centres or contacted directly by the study co-ordinating office. At the time of enrolment, at two months of age (median), the parents filled in the first questionnaire, which aimed to cover heredity, socioeconomic and environmental factors encountered by the newborn. (Q0) The follow-up questionnaires were distributed by mail to the parents, at one, two and four years of age of the child, and contained questions on symptoms of disease and environmental exposures. (Q1, Q2, Q4)

The response rates of the questionnaires when the children were one, two and four years of age were 96%, 94% and 91%, respectively. (Figure 2) Three thousand and six hundred nineteen families (88%) answered all four questionnaires.

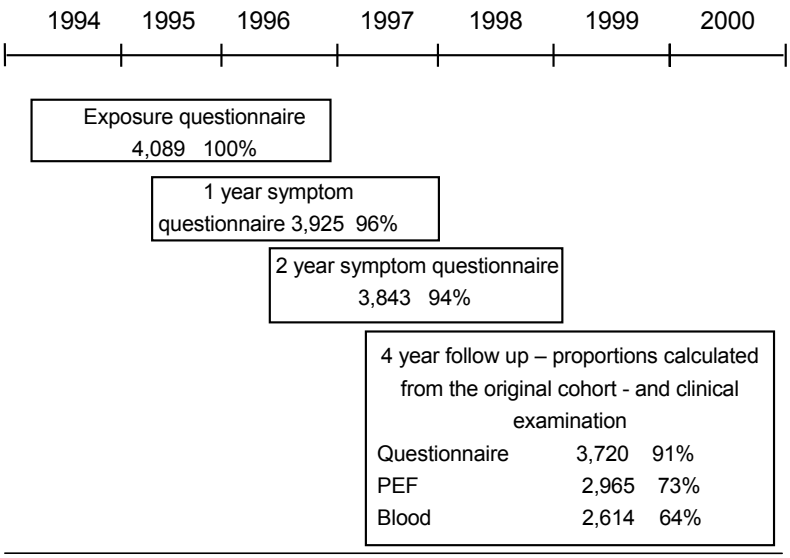

Figure 2. Flow chart of the BAMSE study

3.3 CLINICAL EXAMINATION

At four years of age all children (n=3,670) with questionnaire data from Q4 were invited to a clinical examination and 81% (2,965) agreed to participate. The clinical examination included blood sampling and peak flow measurements (PEF). Serum samples were obtained from 2,614 children, 64% of the original cohort and 88% of those who participated. Lung function was assessed by PEF measurements and were performed by 93% of the participating children.

3.4 DEFINITION OF SOCIOECONOMIC STATUS

Socioeconomic status was based on the educational level of the parents. (I) The cohort was divided into three strata according to the reported highest parental education. Level I comprised families with at least one parent holding a college or university degree. Level II included those with up to three years of upper secondary education (qualifying for college and university studies) and level III comprised the rest, most of which had completed compulsory school (equivalent to primary and lower secondary education) or two years of upper secondary education. The socioeconomic status was also assessed using questions about education, Socioeconomic Index (SEI). (SEI -Occupations in Population and Housing Census 1985 (FoB 85).

3.5 ASSESSMENT OF PRE- AND POSTNATAL EXPOSURE

Prenatal exposure to maternal cigarette smoking was reported in Q0 as daily number of smoked cigarettes for each trimester of pregnancy separately. Exposure *in utero* was defined as maternal daily smoking of one cigarette or more during any period of pregnancy. The number of cigarettes was quantified for each trimester separately. Information on paternal smoking during pregnancy was not collected.
Postnatal maternal and paternal smoking (ETS) was defined as cigarette smoking of one cigarette or more per day. Daily consumption of cigarettes by the parents was assessed in each of the questionnaires. In Q0 smoking parents also indicated if they smoked at home and when they did whether they smoked on the balcony / at an open window / under the fan, thus actively avoiding direct exposure of the child. In paper II ETS was defined as exposure to maternal smoking of one cigarette or more daily during the first months of life and/or maternal smoking at one year of age of the child. In III and IV ETS was defined as exposure to parental smoking of one cigarette or more daily during the first two months of life according to Q0.

3.6 ASSESSMENT OF HEALTH OUTCOME

In paper **II** three different definitions of lower respiratory disease are presented:
Recurrent wheezing was defined as three episodes of wheezing or more after three months of age in combination with the use of inhaled glucocorticoids and/or signs of bronchial hyperreactivity (wheezing or severe coughing when playing or being excited, or a disturbing cough at night not associated with common cold).
Doctor´s diagnosed asthma was defined among children who were reported to be diagnosed with asthma by a doctor at one and/or two years of age.
Any wheezing was defined as wheezing and/or disturbing cough at night, not associated with a common cold during the first and/or second year of life.

In paper **III** three definitions of outcome at four years of age were used:
Transient wheezing was defined as three or more episodes of wheezing between three months and two years of age, but none in the last 12 months.
Persistent wheezing was defined as at least one episode of wheezing between three months and two years of age and one episode or more in the last 12 months.
Late-onset wheezing was defined as no episode of wheezing between three months and two years of age, but at least one episode in the last 12 months.

3.7 ASSESSMENT OF LUNG FUNCTION

Lung function was assessed by peak flow measurements of expiratory flow (PEF) using the normal range Ferraris Peak Flow Meter®, (Ferraris Medical Limited, Hertford, UK). Each child performed several PEF readings according to the European Respiratory Society (ERS) guidelines (Quanjer PH *et al*., 1997) Exclusion criteria were: 1) coded as poor quality by the test leader, and 2) inability to perform two reproducible PEF values. The highest value of PEF was used for analysis.

3.8 MEASUREMENTS OF SENSITISATION

Blood samples were obtained from 2,614 of 4089 children (64%) and serum was kept frozen (-20°C). As a screening procedure serum IgE antibodies to a mixture of inhalant (Phadiatop®) and food (fx5®) allergens, respectively – (Immuno CAP Phadia AB, Uppsala, Sweden), were analysed according to the instructions of the manufacturer. Sera found to be positive ($\geq$0.35 kU_A/L) were further analysed for IgE antibodies to the respective individual allergen (birch, timothy, mugwort, cat, dog, horse, *Dermatophagoides pteronyssinus*, *Cladosporium herbarum* and cow´s milk, hen´s egg, peanut, soybean, wheat and codfish). Only sera positive to at least one single allergen were considered positive. Any inhalant allergen refers to a positive test ($\geq$0.35 kU_A/L) to birch, timothy and/or mugwort, *Dermatophagoides pteronyssinus* (house dust mite) and *Cladosporium herbarum* (mould), cat, dog and/or horse and any food allergen to a positive test to any of the foods tested. Separate statistic analyses were also performed using 3.5 kU_A/L as cut-off. (**IV**)

3.9 STATISTICAL ANALYSES

For all proportions exact binomial confidence intervals were calculated. The Mantel extension test (Mantel N., 1963) was used to test for trend of proportions across categories of parental education, stratifying by maternal age. The rank sum test developed by Cuzic was used to test for trend across ordered groups. (**I**)

The differences in the distribution of baseline characteristics between groups were tested by the chi-square test and the Fisher exact test. (**II**) Logistic regression was used to analyse the association between exposure and health outcome (**II, III**) / sensitisation (**IV**) and was presented as odds ratio (OR) and ninety-five percent confidence interval (95% CI). (**II-IV**) To identify potential confounders several models including various covariates were tested. Finally adjustments were made for parental allergy (defined as doctor´s-diagnosed asthma and asthma medication and/or allergic rhino-conjunctivitis diagnosed by a doctor in combination with reported allergy to furred pets and/or pollen in one or both parents), exclusive breastfeeding

during 4 months or more and maternal age ≥26 years (**II, III**) plus adjustments for traffic related air pollution, (NO_x) and municipality. (**III**) In analyses of ETS maternal smoking during pregnancy was also included and vice versa. (**III, IV**) In **paper IV** parental allergy and SEI (SEI -Occupations in Population and Housing Census 1985 (FoB 85) were included in the logistic regression model.

To test for interaction between smoking and other covariates an interaction term was used in the logistic regression model. (**II, III)** Interaction terms between maternal smoking during pregnancy or ETS at baseline, respectively and category of wheeze group were included in the linear regression model to compare differences in PEF. (**III**) A p-value of the interaction term of <0.05 was considered statistically significant.

Statistical analyses were made with the Stata Statistical Software: release 6.0 **(I),** release 8.0 (**II-IV)** (College Station, Texas, USA). SAS 9.1 software (SAS Institute, 2001) was also used in paper **III**.
The study was approved by the ethical committee at the Karolinska Institutet, Stockholm, Sweden.

4 RESULTS

4.1 SOCIOECONOMIC STATUS AND RISK FACTORS FOR ALLERGY

In paper **I** the associations between socioeconomic status, based on information on the educational level of the parents, and a number of risk factors for development of atopic disease were explored. The educational level influenced the proportion of children exposed to maternal cigarette smoking *in utero* and the highest rate of maternal smoking (22%) during pregnancy was associated with short maternal education (educational level III). Twenty-eight percent of the mothers who smoked in the beginning of the pregnancy reported to have given up smoking during the second and third trimester.

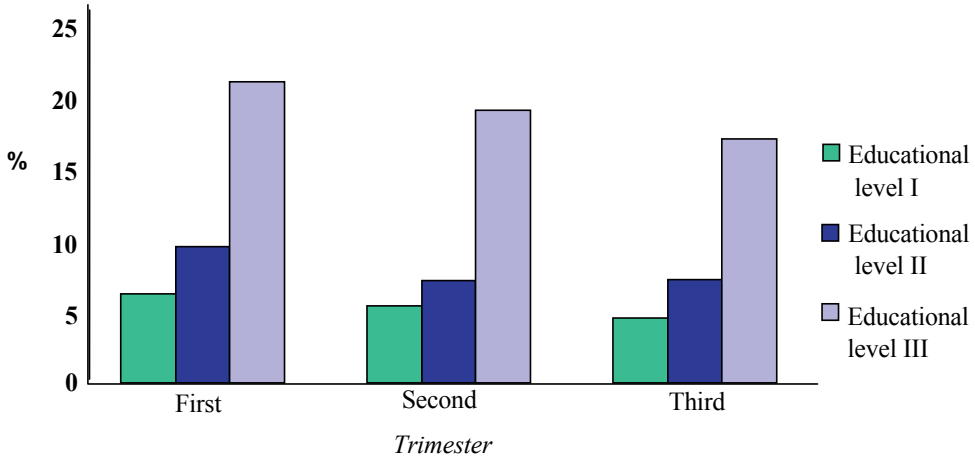

Figure 3. Smoking during pregnancy and maternal educational level

Smoking was almost five times more prevalent among those with the shortest education (educational level III) compared to those with the longest education (educational level I). This was true both for pregnancy and the first few months after birth. (Figure 3 and 4)

Almost one fifth of the cohort families kept a pet of some kind (cat, dog, rodent or bird). Cats and dogs were most common and the keeping of a pet was significantly associated with low parental educational level (Figure 4) and smoking. Indicators of dampness and poor ventilation were more common in the homes of those with the shortest education and were also related to parental smoking. Thus, strong interrelationships between risk factors were found.

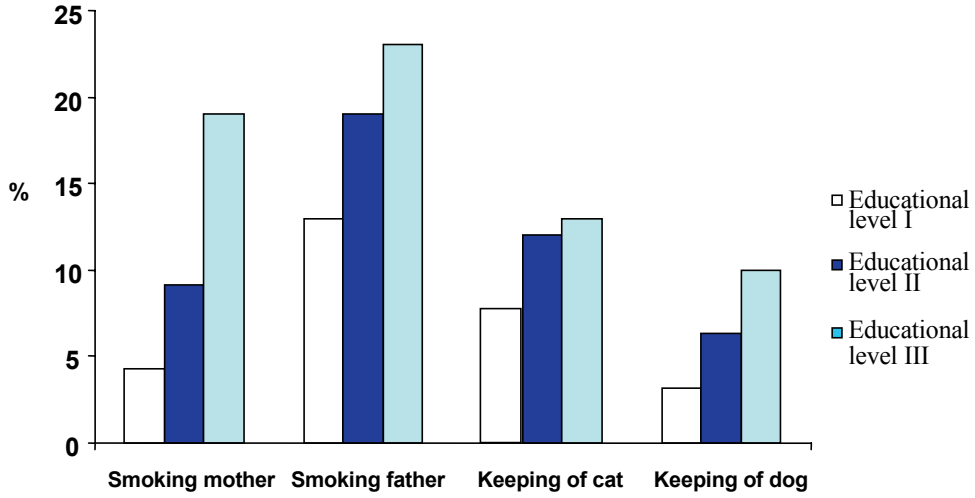

Figure 4. Parental smoking and keeping of furred pets in relation to parental educational level at two months of age of the child

4.2 EXPOSURE TO CIGARETTE SMOKING AND WHEEZING

The parents of 3791 (93 %) children answered the three questionnaires when the children were 0, 1 and 2 years of age. (**II**) Among them 8.5% fulfilled the criteria of recurrent wheezing up to two years of age.

Maternal smoking during any period of pregnancy, but not after giving birth was associated with an increased risk of recurrent wheezing at two years of age, (OR_{adj}=2.2, 95% CI 1.3-3.6). The effect appeared most pronounced when there was maternal smoking during the first and / or second trimester. (OR_{adj} =2.5, 95 % CI 1.5-4.0).

Exposure to ETS alone or in combination with exposure *in utero* was associated with an increased risk of recurrent wheezing (OR_{adj}=1.6, 95 % CI 1.2 – 2.3) and the risk estimates were similar in the different exposure groups for doctor´s diagnosed asthma and any wheezing up to two years of age, respectively.

Exposure to cigarette smoking during pregnancy and to maternal smoking during the child's first year of life increased the risk of recurrent wheezing as well as of doctor´s diagnosed asthma and any wheezing, respectively, at one year of age, in a similar way as reported for children at 2 years of age.

The results in paper **II** indicate a strong effect of exposure *in utero* on lower respiratory disease such as recurrent wheezing as well as asthma diagnosed by a doctor and wheezing at two years of age, even after adjusting for potential confounders. The effects associated with ETS were considerably weaker, possibly due to avoidance of direct exposure of the newborn child. Indeed, 94% of the parents reported to smoke in such a way that they avoided direct exposure of their children.

At four years´ age the parents of 3,619 children (88%) had answered all four questionnaires. (**III)** To be included in the analyses information on wheezing up to

the age four years as well as maternal smoking during pregnancy and parental smoking at baseline were also required. These criteria were fulfilled by 3,570 (87% of the original cohort) families. The proportion of smoking among parents with asthma or respiratory allergy (defined as doctor´s-diagnosed asthma and asthma medication and/or allergic rhino-conjunctivitis diagnosed by a doctor in combination with reported allergy to furred pets and/or pollen in one or both parents) compared to those without such symptoms differed significantly up to four years of age. (Figure 5)

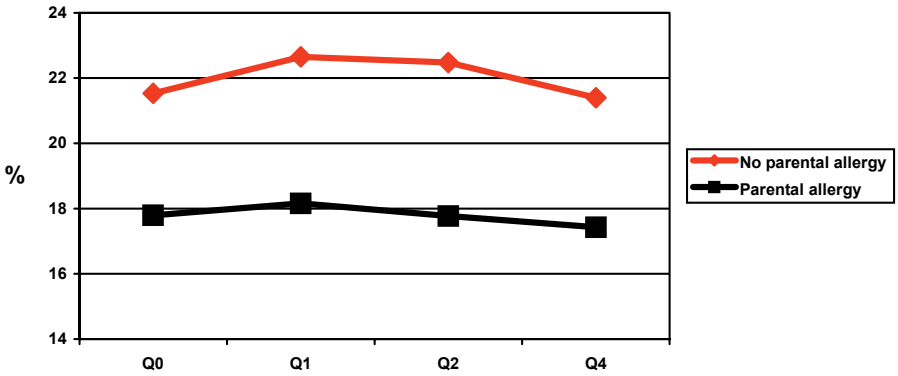

Figure 5. Proportion of parental smoking in allergic and non allergic families as indicated in the four questionnaires (Q0-Q4).

Three definitions of outcome at four years of age were used; transient, persistent and late-onset wheezing. Eight percent of the children were classified as having transient, 9% persistent and 5% as late-onset wheezing. (**III**) Exposure *in utero* irrespective of ETS was significantly associated with an increased risk of transient (OR=1.75, 95% CI 1.24-2.48), persistent (OR=2.08, 1.52-2.83), and late-onset wheezing (OR=1.47, 0.96-2.24). If parental smoking at about two months of age was included in the model the associations were attenuated only to a limited extent. The risk estimates of the three wheezing groups were comparable among children with or without parental allergy as well as among boys and girls. (**III**)

The risk estimates for transient and persistent wheezing were higher among children whose mothers only smoked during the first two trimesters compared to those children whose mothers smoked throughout pregnancy. There was a dose-response relation (p-value for trend <0.05) between the number of cigarettes smoked per day during the first and second trimester and risk of transient and persistent wheezing. (**III)** Thus, the effect seemed to be most pronounced when there was exposure in early pregnancy and not as much in the late phase of pregnancy.

When the analyses were stratified for foetal exposure to maternal smoking *in utero,* there was a strong tendency to an increased risk of persistent and late onset wheezing among those children who were not exposed during pregnancy. (**III)** The results indicate a strong effect of exposure *in utero* on lower respiratory disease such as persistent wheezing at 4 years´ age. (Figur 6)

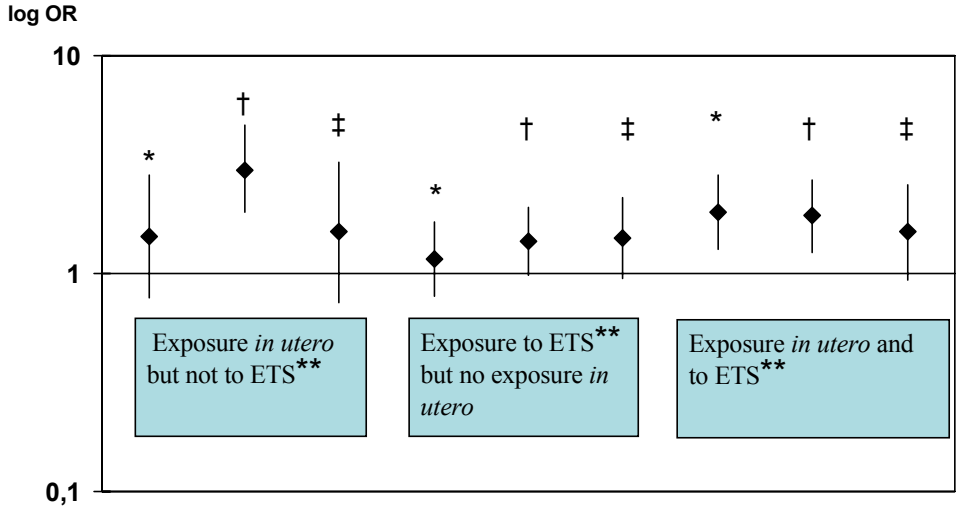

Figure 6. Pre-and postnatal cigarette smoke exposure and wheezing at four years of age. Transient wheezing Persistent wheezing † Late-onset wheezing‡ ETS (Parental smoking when the child was two months old) ***

4.3 EXPOSURE TO CIGARETTE SMOKING AND LUNG FUNCTION

Inclusion in the analyses of lung function required a valid PEF value (Hallberg *et al*., 2006), as well as information on age and height at the clinical examination, leaving 61% (2,487) of 4089 children. (**III**)

Significantly lower mean PEF were found in children with transient and persistent wheezing compared with those without wheezing but neither smoking during pregnancy nor ETS exposure in early life had any effect on pulmonary function (PEF) at age four. (**III)**

4.4 ETS AND SENSITISATION

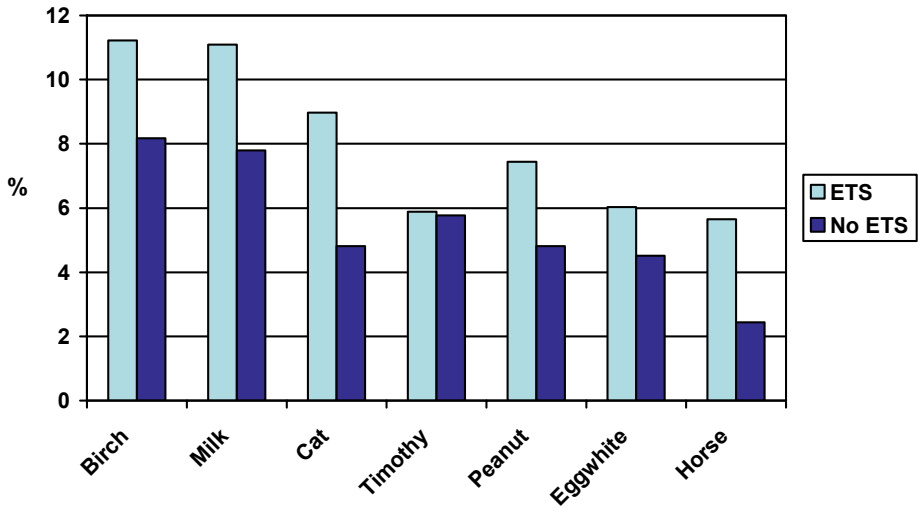

Figure 7. Exposure to ETS (yes/no) and the proportion of children sensitised (≥0.35 kU_A/L) at 4 years of age to the seven most common sensitising allergens within the BAMSE cohort (N=2534)

Only children whose parents answered all four questionnaires, and had complete sets of information on IgE sensitisation and ETS exposure at about two months of age were included in the final analyses. (**IV**) This was true for 2534 children i.e. 62% of the original cohort. Six hundred nine children (24%) were IgE sensitised at four years´ age.

The 2534 participants did not differ from the whole data set i.e. those who had answered all four questionnaires (3619) with respect to reported parental smoking at age two months and characteristics such as sex, parental allergy, duration of breast feeding and keeping of pets.

If the mother had smoked during any of the trimesters but not thereafter there was no increased risk of IgE sensitisation OR_{adj}=1.00 (95% CI 0.61-1.66). Similar results were found for single allergens. In contrast, exposure to ETS, without previous exposure to maternal smoking *in utero*, tended to be associated with an increased risk of sensitisation OR_{adj}=1.26 (95% CI 0.95-1.68).

For postnatal ETS exposure with or without exposure *in utero*, the adjusted OR:s for sensitisation to inhalant allergens were 1.12 (95% CI 0.84 to 1.48), for food allergens 1.46 (95% CI 1.11 to 1.93) and for any sensitisation 1.28 (95% CI 1.01 to 1.62). Among single allergens postnatal ETS exposure alone was associated with a doubled risk of sensitisation to cat (OR_{adj} =1.96 (95% CI 1.28 – 2.99) but there were no significant effects as regards sensitisation to pollens. (Figure 7)

A dose response effect was also found for the association between ETS and IgE-sensitisation and sensitisation to food allergens, p_{trend} =0.019 and 0.006, respectively

and the strongest trend was found for cat (p_{trend}=0.003). Further, this effect of exposure seemed stronger among children with non allergic parents (ORadj 1.95 (95% CI 1.19-3.20) than among those with allergic parents (ORadj 1.06 (95% CI 0.47-2.35).

Separate analyses of sensitisation using a cut off value of $\geq$3.5 kU_A/L of IgE antibody were also performed. Exposure to ETS at two months of age resulted in an adjusted OR_{adj} of 1.26 (95% CI 0.86-1.84). Among single allergen sensitisation to cat was significantly associated with ETS, OR_{adj}=2.59 (95% CI 1.40 - 4.78).

5 DISCUSSION

The foetus may be exposed to tobacco smoke *in utero* via a smoking mother or as a result of ETS exposure mainly from a smoking father. Thus, the unborn child is both exposed to the active smoking of the mother during pregnancy but also to the mother´s exposure to ETS. (Dejmek *et al*., 2002, Hanke *et al*., 2004, Hegaard *et al*., 2006) After birth ETS by parental smoking is a matter of concern and has been studied in great detail. The two types of exposure to cigarette smoking under study are biologically entirely different but intertwined in such a way, that it is very difficult to disentangle which effects are caused by the prenatal systemic exposure and which are the consequences of exposure in the airways after being born. The reason for this difficulty is obvious; mothers who smoke during pregnancy usually continue to smoke after giving birth.

Due to the large size of the present study, we have a sufficient number of mothers, who only reported to have smoked all through or during part of pregnancy, why the effect of exposure *in utero* could be estimated. This effect was evident and appeared to be considerably stronger than that of ETS as far as symptoms of wheezing are concerned. (II, III) The effect of prenatal exposure was present during the two first years of life, but was also obvious at four years´ age and then strongest in the subgroup of wheezers with an early and persistent pattern of disease. At that age there was even a dose-response effect of smoking during pregnancy on transient and persistent wheezing. (III) Our results are in good agreement with recent findings of others'. (Jaakkola J.J. and Gissler M., 2004, Håberg *et al*., 2007) Smoking during pregnancy is likely to affect pulmonary growth and the development of foetal airways, an effect that is reflected by lowered pulmonary function in the neonate. (Lødrup Carlsen *et al*., 1997, Young *et al*., 2000, Gilliland *et al*., 2003) These effects seem to persist for long. Thus, it appears that the effect on lung function in the neonate remains during childhood and even into adulthood. (Svanes C *et al.,* 2003) Smoke exposure during pregnancy has also been found to be associated with asthma among female teenagers (Alati *et al*., 2006), and smoking increased the risk of non-allergic asthma in adolescents, especially if they had also been exposed to maternal smoking *in utero*. (Gilliland *et al*., 2006) Exposure *in utero* to cigarette smoke has even been found to cause asthma in early adulthood. (Goksör *et al*., 2007)

It is noteworthy that ETS exposure of the pregnant mother has been found to be associated with an effect on birth weight, height, foetal mortality and rate of preterm delivery. (Hanke *et al*., 2004, Kharrazi *et al*., 2004) Thus, we may deal with a potent effect that appears even with a low degree of exposure.

Another finding is that the strongest effect of smoking during pregnancy on wheezing was related to exposure during the first two trimesters. (II, III) One may speculate that this is due to a high foetal vulnerability in early pregnancy. Some support of this interpretation comes from the study by Hoo, who studied smoking effects when there was premature delivery and found that the adverse effect was not limited to the last weeks of pregnancy. (Hoo *et al*., 1998) It is, however, difficult to explain that smoking all through pregnancy seemed to be associated with lower risk of wheezing than smoking exclusively during the first two trimesters. Possibly different subsets of mothers give up rather than continue smoking when becoming aware of pregnancy why this result at least in part may reflect residual confounding with heredity. An alternative

explanation is that mothers who smoke during the whole pregnancy, and often also after giving birth, are less likely to report respiratory symptoms than mothers who do not smoke or give up smoking. Thus, there may be a bias caused by differential reporting leading to misclassification of disease. This is in agreement with a recent study which reported that mothers who smoke underutilize health care for their children with mild respiratory symptoms compared with mothers who do not smoke. (Jacobs-van der Bruggen *et al*., 2007)

Our study also allowed for analyses of effects caused by ETS separately. A multitude of studies have addressed ETS and its effect on wheezing and it is notable that most of them have not taken the separate effect of smoke exposure during pregnancy into account. Our results indicate that the effect of ETS is weak. There is, however, an obvious explanation of this in our data in that parents reported not to smoke in the presence of their infants. (II) This could well be true and be a beneficial result of efforts to diminish smoking in our society. Another explanation may be that exposure, in spite of our prospective design, is not independent of very early symptoms - disease related modification of exposure. Results from studies in countries, where smoking is more prevalent and where the awareness of the detrimental effects of smoking is possibly not so well recognized, indicate that an effect of ETS does exist and may be significant. (Cunningham J., 1996, Strachan D.P. and Cook D.G., .6., 1998, Pattenden *et al*., 2006) Most probably, exposure to ETS in the airways of small children is an important cause of early wheezing. (Håberg *et al*., 2007) This effect appears to be reinforced by interactions with other risk factors such as heredity, upper respiratory tract infection, short duration of breastfeeding (Rylander *et al*., 1993), and exposure to other pollutants. (Pershagen *et al*., 1995)

Peak respiratory flow (PEF) was used to measure lung function among the four year old children in the cohort (Hallberg *et al*., 2006) but neither maternal smoking during pregnancy nor exposure to ETS influenced PEF. Maternal smoking *in utero* as well as exposure to ETS has, however, previously been found to be associated with impaired lung function in school children. (Cook *et al.*, 1998, Gilliland *et al*. 2003) The reason for the negative finding in our study may be that tobacco smoke exposure primarily affects the small airways, whereas PEF measurements primarily reflect airflow in large central airways.

Albeit, there was only a small effect on wheezing, exposure to ETS exhibited an effect on sensitisation, most evident to our most important indoor allergen, the cat. (IV) There were also significant effects on IgE-sensitisation to foods, but only tendencies as regards IgE-sensitisation to pollens. The fact that ETS, to which small children are exposed mainly indoors, increases sensitisation to indoor, but not to the same extent to outdoor allergens, adds to the trustworthiness of the results together with the finding of dose-response effects. This sensitising effect of smoke exposure may in the long run be very important since atopy (Rönmark *et al*., 2002) and particularly sensitisation to cat plays an important role for incident asthma. (Perzanowski *et al*., 2002) Since the review by Strachan and Cook on the subject of smoke exposure and sensitisation, it has been held, that there is little IgE-sensitising effect of ETS, and the results of studies after that have yielded inconsistent results. (Strachan D.P. and Cook D.G., .5., 1998. Kulig *et al*., 1999, Raherison *et al*., 2007)

What are then the important differences explaining the diverging results of different studies in the context of smoking and sensitisation? The lack of focus on a potentially important time window for sensitisation may have contributed to the negative findings

in previous studies on ETS exposure and sensitisation. Thus, it appears that exposure during the first months of life may be particularly detrimental for induction of sensitisation, as indicated for exposure to pollen (Björksten F. and Suoniemi I., 1976, Kihlström *et al*., 2002) but also for exposure to ambient air pollution. (Nordling *et al*., 2007) It may also be that study designs are not strictly prospective and allow for modification or misreporting of smoking habits as a consequence of symptoms in the child and lead to bias. Animal experimental data support the concept that exposure to ETS may cause sensitisation. (Zetterström *et al*., 1985, Moerloose *et al*., 2006)

If ETS increases the risk of sensitisation, it is tempting to speculate that the increased prevalence and incidence of asthma in the seventies and eighties may to some extent be explained by the high occurrence, especially of maternal smoking in the sixties and seventies. Recent reports indicate that the prevalence of asthma in children seems to level out in Sweden. (Asher *et al*., 2006) An explanation of this may be that smoking has diminished and that all effects on airway morbidity of exposure to tobacco smoking currently decrease in children. In this context decreasing risks of IgE sensitisation may be particularly important and possibly in the long run lead to lowered asthma incidence.

The mechanisms that cause the different adverse effects of pre- and postnatal exposure to smoking on the respiratory tract are not well understood. However, the effects of exposure to cigarette smoking *in utero* are possible consequences of the absorption of nicotine which diminishes placental blood flow and hampers foetal growth. (Morrow *et al*., 1988) Carbon monoxide may have similar effects by diminishing oxygenation. (Lambers D.S. and Clark K.E., 1996) Exposure to postnatal ETS by tobacco smoke products causes inflammation in the airways, oedema and a reduced airway diameter which in turn may cause wheezing and symptoms of asthma. Inflammation is likely to increase the risk of sensitisation and induce allergy according to "the mucosal theory of atopy". (Lescowitz *et al*., 1972) According to this hypothesis altered reactivity to allergens can be explained by the existence of a local defence barrier deficit that allows for increased absorption of antigens across mucosal surfaces and/or more efficient contact between antigen and immunologically competent cells. Tobacco smoke may act as an adjuvant by breaking immunologic tolerance to otherwise innocuous substances. (Moerloose *et al*., 2006) Further, with regard to IgE-sensitisation a number of studies indicate that there is a relationship between traffic related air pollution and allergic sensitisation. (Ishizaki *et al*., 1987, Krämer *et al*., 2000, Janssen *et al*., 2003, Penard-Morand *et al*., 2005, Nordling *et al*., 2007)

Living conditions and social factors have an impact on different risk factors for health and cigarette smoking is no exception. (Morales *et al*., 1997) Low educational groups are to a considerably higher extent smokers than groups with high education (Stronks *et al*., 1997) also in Sweden. (Lindqvist R. and Åberg H., 2001) Thus, socioeconomic status is of great importance in the context of smoke exposure of children. (I) A strong association between smoking habits and parental educational level was found. Those with the shortest education smoked almost five times more than those with the highest education. This was true both during pregnancy and after birth. (I) Recent data from "The Children´s Environmental Health Questionnaire Survey 2003" (BMHE 03) show that one third of Swedish women with short education smoke during pregnancy as compared to only three percent of pregnant women with a university or college degree. In families with high education only one to three percent of the eight months old children are exposed daily to ETS in their homes as compared to 10 % of the children in families with short education. (Barns hälsa och miljö i Stockholms län (Swedish), 2006) The impact of socioeconomic status on smoking habits has also been reported

from a large cross-sectional British study among young women and a strong association was found between socioeconomic disadvantage and tobacco use. (Harman *et al*., 2006) Thus, it appears important to create strategies in primary prevention that especially target those with short education. (Hovell *et al*., 2000)

The BAMSE study has a prospective and population based design with a large study size that allows for discrimination of prenatal from postnatal effects of tobacco smoke. Other strengths of the study are the detailed exposure assessments of maternal smoking during the different periods in pregnancy as well as of parental smoking during the child´s first years of life. Unfortunately, we lack information on maternal smoking before pregnancy and of paternal smoking during pregnancy. Since the latter may cause effects such as reduced birth weight (Dejmek *et al*., 2002, Hanke *et al*., 2004, Hegaard *et al*., 2006) it appears likely that such exposure is also of some importance in the context of asthma and wheezing.

Data on maternal smoking during pregnancy were collected when the child was about two months old. Thus, there could be a risk of recall bias. However, maternal recall of smoking during pregnancy has been found to be accurate as well as reproducible. (Murray *et al*., 2004, George *et al*., 2006) The assessment of exposure to cigarette smoke pre- and postnatally was done by the parents´ questionnaire-based reports and an obvious disadvantage in our design is the lack of objective smoke exposure assessments. However, a strong correlation has been found by others between assessment of ETS exposure by using urinary cotinine as well as measurements of air nicotine concentrations and self reported data. (Brunekreef *et al*., 2000, Willers *et al*., 2000, Gehring *et al*., 2006) The potential for selection bias has to be considered when the findings are interpreted. For example bias may be introduced by non-participation which is most likely to be affected by parental awareness of health hazards associated with cigarette smoking. Thus, smokers may to a higher extent than non-smokers have chosen not to join the study. A study of non- responders and actively excluded families of the BAMSE study showed that the latter parents smoked more than those included in the cohort. The study base may then be less representative of the population, but the risk estimates of smoking related health effects would most likely not be affected. Another possibility is that parents with allergic diseases would possibly more readily join the study than parents without such diseases but we found no evidence of such selection. Since exposure information was collected before outcome, misclassification of exposure would only be non-differential and lead to underestimation of the true association.

In analysing the significance of various risk factors both for wheezing in early childhood and atopic sensitisation potential confounders were considered. Logistic regression models were used and in the final models parental allergy, duration of breastfeeding, maternal age and / or socioeconomic status, municipality and traffic NO_x were used as confounding variables. Further, since any confounding associated with educational level might affect risk ratios, adjustment for such factors is particularly important. In this work we chose socioeconomic status of the parents as indicated by occupation (SEI) (Boström *et al*., 1993, Stronks *et al*., 1997) since this allowed for categorization of all families. Only in the first paper (I) was educational level used to label socioeconomic status.

The important implication of this study is that the foetus and the growing child after birth may be innocent victims of parental smoking which causes considerable and lasting harm. From the perspective of the little child the exposure is both involuntary

and unavoidable, why it is to a high extent the responsibility of health personnel to act on its behalf in the clinical situation as well as when it comes to preventive efforts in the society.

6 CONCLUSIONS

I Even in a society with comparatively large economic and social equality there were considerable differences between different socioeconomic strata with regard to exposures of relevance, i.e. exposure *in utero* to cigarette smoking and after birth to ETS. This is of great importance in the context of preventive work addressing tobacco smoking.

II Exposure to maternal smoking *in utero* is a risk factor for wheezing up to four years of age. This exposure appears to cause both early and persistent airway wheezing.

III An effect on asthma symptoms of ETS was difficult to establish. A possible explanation for this result may be that exposure of the newborn child was indeed avoided as indicated in the questionnaires - only six percent admitted to smoke in the presence of their child.

IV Exposure to ETS in early childhood appears to be associated with an increased risk of being sensitised to common allergens (indoor perennial allergens, cat in particular and foods) at four years of age. The finding of a dose-response relationship supports the interpretation of causality.

V Since childhood health is negatively influenced by maternal smoking during pregnancy and by ETS, smoking cessation both before and during pregnancy should be encouraged in public health programs also including recommendations on how to protect children from ETS.

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