Childhood asthma and allergy:

the role of vaccinations and other early life events

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Childhood asthma and allergy:
the role of vaccinations and other early life events

Astma en allergie bij kinderen: de rol van vaccinaties
en andere omstandigheden in de vroege jeugd

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1

General Introduction

Atopic disorders

Asthma, allergy, hay fever and eczema are collectively known as "atopic disorders", diseases which are associated with dysfunction of the immune system. In the western world the prevalence of these disorders in children has increased dramatically over the last decades of the past century. However, it is difficult to present exact figures, as estimates of prevalences and increases differ considerably between studies, but figures from repeated cross-sectional surveys indicate about a two- to threefold increase upto 30% over the past 20 years (1-4). During the last decade this increase seems to level off (5). The fact that the increasing prevalence in the western world parallelled increasing prosperity and development and the fact that prevalences in the western world are about a tenfold of those in the non-western world (6), strongly suggest that aspects of modernisation are related to this increase. Support for this hypothesis came from research in West and East Germany (7, 8) which showed that five years after the unification (which rapidly changed living conditions) the prevalence of atopy in Eastern Germany had increased.

The hygiene hypothesis

Aspects of modern lifestyle are more hygienic living conditions and smaller families. Golding and Peters in 1986 were the first to describe the protective effect of family size or sibship size (the so-called "sibling effect") for eczema and hay fever (9). This motivated David Strachan in 1989 to put forward a hypothesis, stating that more hygienic living conditions, and consequently less exposure to pathogens at a young age, would increase the risk of atopy (10). This idea was prompted by the finding that adults coming from large families had less hay fever than those from small families. This finding, and also the finding that individuals with more older siblings have less atopy, has been confirmed by many studies since (11). The link between this finding and the hygiene hypothesis is, that children with many siblings are more exposed to pathogens at a young age.

Biological mechanism

The hygiene hypothesis was first explained by the paradigm of an equilibrium between Th1- and Th2-lymphocytes. The immune system of a newborn baby is Th2-skewed because during pregancy the immune system of the mother shifts towards the Th2 side. Without this shift, no pregnancy would be successful, as Th1-lymphocytes are harmful to the fetus. After birth the balance between Th1- and Th2-lymphocytes shifts towards the Th1 side as a result of exposure to

pathogens. If there is too little exposure, this shift would allegedly not take place completely and a Th2 environment, which promotes allergic inflammation, would be the result. However, several observations contradicted this paradigm, as for instance the prevalence of autoimmune diseases (which are Th1 related) has also increased. New research has led to the formulation of an interpretation in which early exposure to pathogens and allergens promotes the development of IL-10 producing T-regulatory cells, which downregulate both the Th1 and the Th2-lymphocytes (12, 13).

Evidence

A substantial amount of research has been carried out to test the hygiene hypothesis. As exposure to pathogens is difficult to measure, many studies investigated the relationship between infections and subsequent atopy. This may be the reason for the inconsistent evidence resulting from these studies. Considering infections as a proxy for exposure to pathogens harbours the possibility of reverse causation, for children predisposed to atopy have a different anti-viral immunity (14). Studies on the association between infections with orofecal pathogens (hepatitis A, Toxoplasma gondii) and parasites (helminths) showed an inverse relationship (15-17), which clearly support the hypothesis.

Day care attendance at a young age is obviously associated with more exposure to pathogens and, in terms of exposure to other children, more or less comparable with a large family size. A German study (18) found a (positive) dose-response relationship between age at entry and the risk of atopy in small families, but this relationship was absent in children from large families. Some cross-sectional Scandinavian studies, however, did not confirm this finding, which might be due to differences in study design, but could also be due to residual confounding.

Growing up on a traditional farm, especially one with cattle, was associated with a much smaller risk of atopy according to studies carried out in the Alps (19, 20). Early contact with bacterial components (such as endotoxines), which is common on farms, has been suggested to be protective for the development of atopy, although a “healthy worker effect” cannot be rules out.

Pet keeping at a young age has been shown to be protective for atopy at a later age (21), but confounding by (contra-)indication can never be excluded in these studies.

In summary, although the evidence is mostly based on observational studies and several forms of bias cannot be excluded, there is some evidence in favour of the hygiene hypothesis.

Vaccinations

The National Vaccination Programme in the Netherlands was started in 1957, when it was realized that vaccination of infants and young children against serious infectious diseases is among the most cost-effective intervention in preventive health care. The programme has been extended gradually over time. Currently it comprises vaccinations for diphtheria tetanus pertussis poliomyelitis (DTPPo), Haemophilus influenzae type b (Hib), measles, mumps, rubella (MMR) and meningococcal c (Neis Vax). However, potential adverse effects of these childhood vaccinations have often been the subject of heated debate, both in the lay press and in the scientific literature. Several organisations of opponents of vaccinations, notably in the UK and the Netherlands, have diffused biased interpretations of scientific results, thus stimulating an ongoing trend, where longing for a natural lifestyle became more important than preventing serious infectious diseases. Ignoring the fear of adverse effects by public authorities could lead to a dangerously decreasing vaccination rate.

A potential relationship between vaccinations and atopy could be thinkable in two ways in the context of the hygiene hypothesis: on the one hand vaccinations prevent infections, and thus contribute to the "hygiene effect"; on the other hand vaccinated children are at a very young age exposed to (attenuated) pathogens or components of pathogens, a favourable situation according to the hygiene hypothesis. Also the exposure to vaccine additives, such as aluminium containing adjuvants, might change the risk of subsequent atopy. It is impossible to validly predict the relationship on theoretical grounds.

Evidence

We aim at investigating the longterm relationship between vaccinations and atopy. Short term reactions have been extensively studied and are less controversial.

A randomized controlled trial with an unvaccinated control group is not feasible for ethical reasons. Therefore almost all available studies are observational, implying that groups were compared without being randomized. Therefore it is likely that vaccinated and unvaccinated groups differed in more aspects than vaccination alone. This may be an explanation for diverging study results.

For the MMR vaccination the available studies show no difference, or even a lower risk of atopy in vaccinated children (22-24). So regarding MMR we can conclude that there is no evidence for a higher risk of atopy in vaccinated children (25).

The Meningococcal group C vaccination has been introduced in the Netherlands in 2003. For this vaccination no evidence regarding the longterm relationship with atopy is available.

The relationship between the DTP(Po) vaccination, or the pertussis component alone, and atopy has been addressed in many studies (25-33), but the conclusions are inconsistent. Some studies found a higher risk of atopy in DTP vaccinated children (26-30), while other studies reported no difference or even a lower risk of atopy (22-24, 31-32). It is difficult to draw conclusions from the available evidence, because all studies are possibly subject to confounding by indication, as the reason for not vaccinating may be health related. Only one study was a randomized controlled trial (32). In this trial the additional effect of the pertussis component (in various forms) to the diptheria tetanus vaccinations was studied. No effect was found, but it was not possible to compare with an unvaccinated group.

For the Hib vaccination the available studies show no effect (23, 33) or a slightly higher risk of atopy for vaccinated children (24, 34), but the conclusion of these latter studies is that this weak relationship may be due to residual confounding.

Birth order and sibship size

The proportion of atopy cases attributable to the sibling effect has been estimated to be approximately 30% (35). But what is the underlying mechanism? Is it because children with many siblings are more exposed to "dirt" than children with no siblings or is it something else? Identification of the underlying mechanism is important for the design of a possible prevention strategy. However, different definitions of the sibling effect have been used, relating atopy to the number of older siblings, birth order, family size, the number of younger siblings and sibship size. In order to detect the underlying mechanism, we first need to know which definition of the sibling effect is the "real" risk factor. Several definitions are (almost) equivalent: the number of older siblings and birth order are equivalent, because birth order equals the number of older siblings plus one; sibship size parallels family size; and sibship size minus the number of younger siblings equals the number of older siblings plus one. Therefore it would be sufficient to disentangle the roles of birth order and sibship size as risk factors for atopic disease. The underlying mechanism could be either prenatal or postnatal or both.

Perinatal risk factors

It has been suggested that some important risk factors for atopy already operate during pregnancy and many studies have focussed on the relationship between perinatal data and atopy. The effect of prematurity on subsequent lung function and the positive relationship with wheezing is well known (36). On the other hand, there are also reports of high gestational age being a risk factor for atopic disease in adults (37). However, conclusions are not unequivocal, because many other studies did not find such relationships. Also head circumference at birth and subsequent atopy has been the subject of a number of studies. A positive association between neonatal head circumference and total IgE concentration was first reported in 1994 (38) and confirmed by others (39-41). This finding raised the hypothesis that this positive association might reflect the long-term effect of sustaining fetal brain growth at the expense of the trunk, in particular the thymus (38). More recently, this hypothesis was challenged by the finding that thymus size is not related to allergic disease (42) and that the relationship between the ratio of head circumference to birth weight and hay fever is negative (43).

Complications and interventions during labour, for instance caesarean section, were also found in some studies to be related to asthma in later life (42, 44). Understanding the mechanisms underlying these relationships could possibly contribute to the prevention of these disorders.

Objectives of the studies

The following objectives were formulated in order to address the questions introduced above:

1. To assess the relationship between the DTPPo vaccination and reported atopic disease at age 8-12.
2. To assess the relationship between the Hib vaccination and reported atopic disease at age 8-12.
3. To disentangle the effects of birth order and sibship size on reported atopic disease at the age of six years.
4. To assess the relationships of various perinatal characteristics and obstetric complications with reported atopic disease at age six.

The studies

Study population I

In the Netherlands, the Orthodox Reformed religion is a subdivision of the Protestant Church. About 6% of the Dutch population adheres to this religion (45). Children from this religious group attend Orthodox Reformed schools which are clustered in an area which stretches from the South-West of the Netherlands to the East. Many of these people refuse vaccinations for religious reasons. This group is very suitable to answer our first two questions, because within one, rather homogeneous, population there are exposed and unexposed individuals. Moreover, the reason for refusing vaccinations is essentially religious, and not, as in other studies on this subject, possibly health related.

Our method was to distribute questionnaires in classes 5-8, corresponding to age 8-12, of Orthodox Reformed primary schools. These questionnaires included, apart from a Dutch translation of the ISAAC-questionnaire (46) on symptoms of atopic disorders, questions on childhood vaccinations and other known risk factors for atopy. Altogether 4480 questionnaires were distributed and 1875 (42%) were returned. In a subgroup of 100 children blood samples were taken with two purposes: 1) to determine specific IgE for house dust mite, cat, dog, grass pollen and birch pollen to get an objective measurement of atopy and 2) to determine levels of tetanus and diphteria antibodies in order to get a validation of the reported vaccination status. As an additional validation we compared the vaccination status reported in the questionnaire with that officially registered in National Vaccination Registries in another subgroup of 120 children.

Study population II

To address the third question (birth order and sibship size) we selected a retrospective cohort of 700 families in the municipality of Zwijndrecht with index children born in 1988-1990 and, from files of the District Health Service of Zwijndrecht, extracted data concerning atopic disease at the age of six years and other relevant variables of all the index children and their brothers and sisters. Children in the Netherlands get a routine health check-up at the age of six and data concerning atopic disease were reported by a parent during these check-ups and recorded by the examining physician. The files also contain data collected at other points in time, like perinatal data and vaccination data. The perinatal data comprised data on special interventions before or during labour (such as caesarean section, vacuum or forceps extraction, induced labour), gestational age, anthropometric data of the newborn baby, and data on breastfeeding.

A part of these children was not vaccinated for religious reasons and we were therefore able to address the first question as well. However, the number of unvaccinated children was small (n=44), and we were not able to differentiate between the pertussis component alone, the cocktail of DTPPo, or the mumps measles rubella (MMR) vaccination.

The data, routinely collected during health check-ups from birth onward, enabled us to also address the fourth question regarding the relationship between perinatal characteristics and atopic disorders at age six.

Outline of the thesis

Chapter 1: gives an introduction and discusses the background of the thesis.
Chapter 2: addresses the question on the relationship between the DTTPo vaccination and reported atopic disease at age 8-12 years (study population I, objective 1).
Chapter 3: addresses the relationship between the pertussis component of the DTPPo vaccination and reported atopic disease at age six in study population II (objective 1).
Chapter 4: addresses the question on the relationship between the Hib vaccination and reported atopic disease at age 8-12 years (study population I, objective 2).
Chapter 5: addresses the question on disentangling the relationships of birth order and sibship size with reported atopic disease at age six (study population II, objective 3).
Chapter 6: addresses the question on the relationship between perinatal characteristics and reported atopic disease at age six (study population II, objective 4).
Chapter 7: gives a discussion of the results of this study, recommendations for future research and for communication of study results and possible prevention strategies.

References

1. Peat JK, van den Berg RH, Green WF, Mellis CM, Leeder SR, Woolcock AJ. Changing prevalence of asthma in Australian children. Br Med J 1994;308:1591-6.
2. Platts-Mills TA, Carter MC, Heymann PW. Specific and nonspecific obstructive lung disease in childhood: causes of changes in the prevalence of asthma. Environ Health Perspect 2000;108 Suppl 4:725-31.
3. Upton MN, McConnachie A, McSharry C, Hart CL, Smith GD, Gillis CR, Watt GC. Intergenerational 20 year trends in the prevalence of asthma and hay fever in adults: the Midspan family study surveys of parents and offspring. Br Med J 2000;321:88-92.
4. Ceuppens J. Western lifestyle, local defenses and the rising incidence of allergic rhinitis. Acta Otorhinolaryngol Belg 2000;54:391-5.
5. Toelle BG, Ng K, Belousova E, Salome CM, Peat JK, Marks GB. Prevalence of asthma and allergy in schoolchildren in Belmont, Australia: three cross sectional surveys over 20 years. Br Med J 2004;328:386-7.
6. Anonymous. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998;351:1225-32.
7. 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;149:358- 64.
8. Weiland SK, von Mutius E, Hirsch T, Duhme H, Fritzsch C, Werner B, Husing A, Stender M, Renz H, Leupold W, Keil U. Prevalence of respiratory and atopic disorders among children in the East and West of Germany five years after unification. Eur Respir J 1999;14:862-70.
9. Golding J, Peters T. Eczema and hay fever. In: Butler NR, Golding J, eds. From birth to five, a study of the health and behaviour of Britain's 5-year-olds. Oxford: Pergamon Press, 1986, pp 171-86.
10. Strachan DP. Hay fever, hygiene, and household size. Br Med J 1989;299:1259-60.
11. Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidemiol Community Health 2002;56:209-17.

12. McGuirk P, Mills KH. Pathogen-specific regulatory T cells provoke a shift in the Th1/Th2 paradigm in immunity to infectious diseases. Trends Immunol 2002;23:450-5.
13. Prescott SL. New concepts of cytokines in asthma: is the Th2/Th1 paradigm out the window? J Paediatr Child Health 2003;39:575-9.
14. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Hiemke HJ, Gentile K, Gangnon RE, Lemanske RF Jr. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 2004;170:175-80.
15. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. Br Med J 2000;320:412-7.
16. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.
17. McIntire JJ, Umetsu SE, Macaubas C, Hoyte EG, Cinnioglu C, Cavalli-Sforza LL, Barsh GS, Hallmayer JF, Underhill PA, Risch NJ, Freeman GJ, DeKruyff RH, Umetsu DT. Immunology: hepatitis A virus link to atopic disease. Nature 2003;425:576.
18. Kramer U, Heinrich J, Wjst M, Wichmann HE. Age of entry to day nursery and allergy in later childhood. Lancet 1999;353:450-4.
19. Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, Vuille JC, Wuthrich B. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. SCARPOL team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution. Clin Exp Allergy 1999;29:28-34.
20. Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy 2000;30:194-200.
21. Platts-Mills T, Vaughan J, Squillace J, Sporik R. Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study. Lancet 2001;357:752-6.
22. Anderson HR, Poloniecki JD, Strachan DP, Beasley R, Bjorksten B, Asher MI; ISAAC Phase 1 Study Group. Immunization and symptoms of atopic disease

in children: results from the International Study of Asthma and Allergies in Childhood. Am J Public Health 2001;91:1126-9.
23. Gruber C, Illi S, Lau S, Nickel R, Forster J, Kamin W, Bauer CP, Wahn V, Wahn U; MAS-90 Study Group. Transient suppression of atopy in early childhood is associated with high vaccination coverage. Pediatrics 2003;111:e282-8.
24. DeStefano F, Gu D, Kramarz P, Truman BI, Iademarco MF, Mullooly JP, Jackson LA, Davis RL, Black SB, Shinefield HR, Marcy SM, Ward JI, Chen RT; Vaccine Safety Datalink Research Group. Childhood vaccinations and risk of asthma. Pediatr Infect Dis J 2002;21:498-504.
25. Koppen S, de Groot R, Neijens HJ, Nagelkerke N, van Eden W, Rümke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. Vaccine 2004;22:3375-85.
26. Odent MR, Culpin EE, Kimmel T. Pertussis vaccination and asthma: is there a link? JAMA 1994;272:592-3.21.
27. Kemp T, Pearce N, Fitzharris P, Crane J, Fergusson D, St George I, Wickens K, Beasley R. Is infant immunization a risk factor for childhood asthma or allergy? Epidemiology 1997;8:678-80.
28. Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. Thorax 1998;53:927-32.
29. Hurwitz EL, Morgenstern H. Effects of diphtheria-tetanus-pertussis or tetanus vaccination on allergies and allergy-related respiratory symptoms among children and adolescents in the United States. J Manipulative Physiol Ther 2000;23:81-90.
30. Yoneyama H, Suzuki M, Fujii K, Odajima Y. The effect of DPT and BCG vaccinations on atopic disorders. Arerugi 2000;49:585-92.
31. Henderson J, North K, Griffiths M, Harvey I, Golding J. Pertussis vaccination and wheezing illnesses in young children: prospective cohort study. The Longitudinal Study of Pregnancy and Childhood Team. Br Med J 1999;318:1173-6.
32. Nilsson L, Kjellman NI, Bjorksten B. Allergic disease at the age of 7 years after pertussis vaccination in infancy: results from the follow-up of a randomized controlled trial of 3 vaccines. Arch Pediatr Adolesc Med 2003;157:1184-9.
33. Mommers M, Weishoff-Houben M, Swaen GM, Creemers H, Freund H, Dott W, van Schayck CP. Infant immunization and the occurrence of atopic disease in

Dutch and German children: a nested case-control study. Pediatr Pulmonol 2004;38:329-34.

34. Laubereau B, Grote V, Holscher G, Holscher B, Frye C, Wichmann HE, Heinrich J. Vaccination against Haemophilus influenzae type b and atopy in east German schoolchildren. Eur J Med Res 2002;7:387-92.
35. Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidemiol Community Health 2002;56:209-17.
36. von Mutius E, Nicolai T, Martinez FD. Prematurity as a risk factor for asthma in preadolescent children. J Pediatr 1993;123:223-9.
37. Pekkanen J, Xu B, Jarvelin MR. Gestational age and occurrence of atopy at age 31--a prospective birth cohort study in Finland. Clin Exp Allergy 2001;31:95-102.
38. Godfrey KM, Barker DJ, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994;24:641-8.
39. Leadbitter P, Pearce N, Cheng S, Sears MR, Holdaway MD, Flannery EM, Herbison GP, Beasley R. Relationship between fetal growth and the development of asthma and atopy in childhood. Thorax 1999;54:905-10.
40. Gregory A, Doull I, Pearce N, Cheng S, Leadbitter P, Holgate S, Beasley R. The relationship between anthropometric measurements at birth: asthma and atopy in childhood. Clin Exp Allergy 1999;29:330-3.
41. Oryszczyn MP, Annesi-Maesano I, Campagna D, Sahuquillo J, Huel G, Kauffmann F. Head circumference at birth and maternal factors related to cord blood total IgE. Clin Exp Allergy 1999;29:334-41.
42. Benn CS, Jeppesen DL, Hasselbalch H, Olesen AB, Nielsen J, Bjorksten B, Lisse I, Aaby P. Thymus size and head circumference at birth and the development of allergic diseases. Clin Exp Allergy 2001;31:1862-6.
43. Katz KA, Pocock SJ, Strachan DP. Neonatal head circumference, neonatal weight, and risk of hayfever, asthma and eczema in a large cohort of adolescents from Sheffield, England. Clin Exp Allergy 2003;33:737-45.
44. Xu B, Pekkanen J, Jarvelin MR. Obstetric complications and asthma in childhood. J Asthma 2000;37:589-94.
45. Kerncijfers 2003. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 2003.
46. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, Strachan D, Weiland SK, Williams HC. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.

2

The diphtheria tetanus pertussis poliomyelitis vaccination and reported atopic disorders in 8-12 year old children

Abstract

Background: Evidence for the relationship between the diphtheria tetanus pertussis (DTP) vaccination and atopic disorders is inconclusive, because the available studies that constitute the evidence are liable to confounding by indication.

Study objective: To assess the relationship between diphtheria-tetanus-pertussis-poliomyelitis (DTPPo) vaccination in the first year of life and reported atopic disorders at primary school age.

Method: 1875 children attending Orthodox Reformed (Protestant) primary schools in the Netherlands returned questionnaires with data on vaccination status, atopic symptoms and lifetime atopic disorders (asthma, hay fever, eczema and food allergy), and possible confounders.

Results: The adjusted odds ratio of any atopic disorder (vaccinated/unvaccinated) was 1.00 ($CI_{95\%}$: 0.80 - 1.24). For asthma, hay fever, eczema and food allergy the results were respectively: 1.04 ($CI_{95\%}$: 0.76 - 1.42), 0.79 ($CI_{95\%}$: 0.55 - 1.12), 0.87 ($CI_{95\%}$: 0.66 - 1.14) and 1.13 ($CI_{95\%}$: 0.71 - 1.81).

Conclusion: The DTPPo vaccination was not related to reported atopic disorders at primary school age.

Key words: DTP vaccination, atopy, asthma

Submitted

Introduction

The prevalence of allergic disorders has increased during the past decades and much effort has been put into identifying risk factors for these disorders. These include elements of western lifestyle such as increased hygiene and subsequent decreased exposure to pathogens and number of infectious diseases. The potential influence of childhood vaccinations is often the subject of heated debate, both in the lay press and in the scientific literature. Organisations of opponents of vaccinations (especially in the UK and the Netherlands) have spread biased interpretations of scientific results, thus stimulating an ongoing trend whereby the longing for a natural lifestyle takes precedence over the fear of serious infectious diseases.

Many studies have investigated the relationship between childhood vaccinations and allergic disorders. A recent systematic review concluded that these vaccinations do not cause allergic diseases (1). However, concerning the diphtheria tetanus pertussis (DTP) vaccination, this conclusion is controversial (2), mainly because of the possibility of bias in these studies. For in all these (observational) studies the reason for not vaccinating may well have been health-related and thus outcome-related, which is a classic example of confounding by indication. A randomised controlled trial would avoid this bias, but may not be feasible for ethical reasons.

In the Netherlands about 6% of the population adhere to the Orthodox Reformed (Protestant) religion and many in this group refrain from vaccinations for religious reasons. We conducted an observational cross-sectional study within this religious group to study the relationship between the DTP-Poliomyelitis (DTPPo) vaccination and atopic disorders, at the same time minimizing the problem of confounding by indication.

Aim of the study

To assess the relationship between receiving the DTPPo vaccination in the first year of life and reported atopic disorders at primary school age.

Methods

Childhood vaccinations in the Netherlands

According to the National Vaccination Programme, infants get four vaccinations of DTPPo and Haemophilus influenzae type B (HiB) in their first year of life. At 14 months children are immunized with the measles mumps rubella vaccine (MMR) and meningococcal c vaccine (Neis Vax). At four years children receive a

vaccination for diphtheria tetanus poliomyelitis (DTPo) and acellular pertussis (aP), and at nine years DTPo and MMR. Because the Hib vaccination was introduced in 1993 and the Neis Vax in 2003, part of the population under study did not regularly receive the HiB vaccination and the whole population received the Neis Vax (if accepted) at age 7 years or older.

Study area, population and design

In the Netherlands the Orthodox Reformed religion is a subdivision of the Protestant Church. Children from this religious group attend Orthodox Reformed schools which are clustered in an area which streches from the south-west of the Netherlands to the east. Many of these people refuse vaccinations for religious reasons.

In a cross-sectional design we assessed by means of questionnaires the relationship between receipt of the DTPPo vaccination in the first year of life and atopic symptoms or lifetime disease at age 8-12 years.

Permission was requested from the Boards of the Orthodox Reformed primary schools to distribute letters of invitation, information and questionnaires directed to the parents of children aged 8-12 years. In most cases these letters were also signed by a staff member of the coordinating District Health Service in the region at issue.

The study was approved by the Ethics Committee of Erasmus MC and all participants (parents) gave informed consent for participation in the study.

The study was financed by the Department of General Practice of Erasmus MC. AstraZeneca, who provided the anaesthetic patches, had no role in the design, collection, analysis and interpretation of the data, writing of the report or the decision to submit the paper for publication.

Sample size calculation

We assumed a significance level of 5%, a power of 80%, and a prevalence of allergic disorders of 10% in the lowest risk group. Our aim was to detect an absolute risk difference of at least 5%. It was unknown beforehand what the distribution of the risk factor under study would be in our population. However, we assumed a distribution that would not be skewed more than 3:7, i.e. 30% vaccinated children and 70% unvaccinated children (or vice versa). In the statistically most unfavourable situation, 10% atopic disorders in the smallest group (30%) and 15% atopic disorders in the other group (70%), we would need a total of 1769 children to detect this difference (corresponding to an odds ratio of 1.6) with a two-sided test.

Participating schools and children

Relevant schools were approached by mail (and subsequently by telephone) requesting permission to distribute packages containing a letter of invitation, information about the study, informed consent form and a questionnaire. We continued approaching schools until we judged that the required number of questionnaires would be returned. Of the 55 schools that were asked for permission 17 (31%) refused, mainly because the Boards expected the topic of vaccinations to be too sensitive. In total 4480 packages were distributed in the period autumn 2003 - spring 2004 in classes 5-8 (corresponding to age 8-12 years) of the 38 participating schools. In total 1875 questionnaires (42%) were returned of which 1872 were suitable for analysis.

Data collection

One of the parents, preferably the mother, was asked to fill out the questionnaire. Informed consent contained questions on consent to participate in the study, consent for the investigator to obtain officially registered vaccination data, blood sampling (for validation purposes in a subset of the participants), and storage of the data. The questionnaire asked for symptoms of (and physician diagnosis of) asthma, hay fever and eczema (Dutch translation of the ISAAC questionnaire (3, 4)), physician-diagnosed food allergy ever, childhood vaccinations, BCG vaccinations and influenza vaccinations, gender, passive smoking (prenatally, during the first year of life and currently), date of birth, perinatal data, birth order, ZIP code, year of birth of the mother, any atopic disorders of parents or siblings, family composition, education, income, living conditions (current and during the first year of life), day care attendance, breast feeding, nutrition during the past month, frequency of exercise, body length and weight, and having had common infectious diseases preventable by vaccinations (measles, mumps, rubella and pertussis).

Statistical analysis

The relationship between the DTPPo vaccination in the first year of life and (current symptoms or "ever had") asthma, hay fever, eczema, food allergy, and "any atopic disorder" (at least one of these diseases) was evaluated by means of logistic regression. In the main analyses we ignored the questions on physician diagnosis because the more conservative religious adherents are possibly less inclined to visit their general practitioner thus introducing bias. However, we repeated all analyses with physician-diagnosed disorders as outcomes. Analyses were performed both in univariate and multivariate models. Potential confounders

are shown in Table 1. A variable was included in the multivariate model if it changed the univariate point estimate by at least 10% (5).

The question on income is often a sensitive one which people tend to skip. Therefore, for those participants who did not answer this question we ranked them according to income data linked to their ZIP code (6) and imputed a code for income such that the total distribution of income remained the same as it was for the participants who did answer this question.

The main analyses were repeated taking the multilevel structure (level 1: children/level 2: families/level 3: schools) into account.

For the imputation procedure (see "Objective measurement of allergy") we used a multiple imputation technique with five imputed data sets (7). Analyses were carried out on each of these five sets and results were pooled (7). Imputation was done with IVEware version 2.0.

SPSS version 11.0 and ML-win version 1.1 were used for all other computations. A two-sided p-value of 0.05 or less was considered significant in all tests.

Objective measurement of allergy

In total 72% of the participants gave informed consent for a blood sample (73.6% of the vaccinated, and 70.3% of the unvaccinated children). From these children we selected a random stratified sample of 100 children. Stratification was performed such that there was a 1:4 distribution of DTPPo vaccination (yes/no) and an equal distribution of atopic symptoms (yes/no) and primary school class (5/6/7/8). Two research assistants visited the participating schools to take the blood samples. Emla® anaesthetic patches (AstraZeneca, Zoetermeer, The Netherlands) were used to minimize needle pain. We performed RAST tests using the Pharmacia® RIA method to determine specific IgE to five of the most common aero allergens in the Netherlands (house dust mite (d1), cocksfoot pollen (g3), common silver birch pollen (t3), cat epithelium and dander (e1) and dog dander (e5)) in the sera of these 100 children to obtain an objective measurement of allergy.

Allergy was defined as at least one RAST class 2 or higher (i.e. IgE >=0.7 IU/ml); we linked this objective variable to reported asthma or hay fever (current symptoms or "ever had"). Moreover, on the basis of these objective allergy data of 100 children and other relevant variables, we imputed the objective allergy for the remaining 1773 children.

Table 1 Potential confounders in the relationship between the DTPPo vaccination and atopic disorders

Season of birth
Birth order
Gender
Gestational age
Birth weight
Age of the mother at the time of delivery
Exposure to smoking (prenatally, during the first year of life and currently)
Breast feeding for four months or more (yes/no)
Housing in the first year of life (rural and living on a farm with livestock/
rural other / city)
Pet keeping (furry pets or birds yes/no) during the first year of life and
currently
Day care starting at age 6 months or less (yes/no)
Current age
Asthma and/or allergy of the parents and/or siblings
Highest educational level of the parents
Family income
Current level of urbanization (five levels)
Living on a farm with livestock (yes/no)
Sibship size
Mould in the child's bedroom in the past year
Frequent (more than five days/week) consumption of
fruit (yes/no)
(raw or cooked) vegetables (yes/no)
anti-oxidants (yes/no)
unskimmed dairy products (yes/no)
wholemeal bread (yes/no)
Frequent (at least one day/week) consumption of fish
Frequent exercise (school gym at least once a week and playing games with
physical activity for at least half an hour a day and either being a
member of a sporting club or walking or cycling from home to school
vice versa for at least one hour a day)
Body mass index
HiB vaccination

Validation of the risk factor

To validate our question on the DTPPo vaccination we performed tests for tetanus toxoid IgG and diphtheria IgG antibodies in the sera of the 80 children (from the sample of 100 mentioned under "Validation of the outcome") that according to their parents were DTPPo unvaccinated. All sera were stored at minus 20 degree celcius until being tested in one run. The following EIA kits were used to test for tetanus toxoid IgG and diphtheria IgG antibodies: RIDASCREEN® Tetanus test and RIDASCREEN® Diphtheria test (both R-Biopharm, Germany). Children with titres of at least 0.6 IU/ml were considered as being vaccinated for the pathogen concerned.

In addition we randomly selected a group of 120 children [of those 1761 (94.1%, 99.0% of the vaccinated and 85.2% of the unvaccinated children) who gave informed consent to obtain officially registered vaccination data] and compared the official registrations of the vaccinations in the first year of life with those reported by the parents.

Assessment of selection bias

In case of a low response rate the possibility of selection bias had to be evaluated. If selection bias would be present, at least one of the four cells (no atopy/noDTPPo, atopy/noDTPPo, no atopy/DTPPo, atopy/DTPPo) would be over- or underrepresented, thus causing a spurious relationship. Selection bias is unlikely if no selection on DTPPo vaccination has occurred. In order to verify this we asked the District Health Services covering the participating schools to estimate from their records the vaccination coverage per school. Thereafter, we compared the vaccination coverage of the responders for each school separately, and the total in all schools, with the vaccination coverage reported by the District Health Services.

Finally, we assessed the odds ratios (ORs) pertaining to the relationship under study and the response rate for each school separately and checked by means of linear regression, weighted with the inverse of the squared standard error of the log(OR), whether there was a relationship between the log(OR) and the response rate. In case of selection bias one would expect a (negative or positive) relationship between ORs and response rate.

Results

Relationship between DTPPo and atopy

A total of 1875 children returned the questionnaire (a response rate of 42%), of which three questionnaires were excluded because of missing vaccination status. Of the remaining 1872 children, 671 (35.8%) were not vaccinated for DTPPo in the first year of life. Of the 1201 vaccinated children, 21 (1.7%) were incompletely vaccinated for DTPPo (i.e. they did not receive all four vaccinations scheduled in the first year of life).

Of all children, 873 (46.6%) reported any form of lifetime atopic disorder. Characteristics of the children, by DTPPo vaccination status, are presented in Table 2.

Table 3 shows prevalences and both univariate and multivariate ORs with 95% confidence intervals ($CI_{95\%}$) for the relationships between DTPPo vaccinations and atopic disorders. Atopy appeared to be slightly more prevalent in vaccinated children [univariate OR=1.27, $CI_{95\%}$=(1.05-1.59)], but this difference disappeared when we adjusted for confounders [OR=1.00, $CI_{95\%}$=(0.80-1.24)]. The differences for the separate disorders were small and not significant (Table 3). In all models family size, education and atopic history of parents or siblings proved to be confounders. When we analysed the models with physician-diagnosed diseases as outcome variables the adjusted ORs tended to be somewhat larger, although they were never statistically significant: for any atopic disorder the adjusted OR was 1.04 [$CI_{95\%}$=(0.82-1.31)] (Table 4).

Multilevel analysis of the multivariate models presented in Table 2 yielded similar results (data not shown).

Objective measurement of allergy

Of the 100 children who donated blood, 46 (46.0%) reported symptoms of (or ever had) asthma or hay fever. Of these, 26 (56.5%) were allergic according to the RAST results. Of the remaining 54, who reported neither asthma nor hay fever, 40 (74.1%) were not allergic according to the RAST results. Of the remaining 14 (who had at least one RAST class 2 or higher and did not report asthma or hay fever), 7 (50%) reported symptoms of eczema. Table 5 shows these data, also broken down by vaccination status. It seems that, although the numbers are small, vaccinated children who reported asthma or hay fever, have less allergy according to our objective measurements than unvaccinated children (27% and 66%, respectively).

Table 2 Characteristics of the participating children by DTPPo vaccination status

		Unvaccinated N=671 *	%	Vaccinated N=1201 *	%
Gender	Male		52.5		50.3
	Female		47.5		49.7
Age (years)	< 10		44.0		42.2
	>= 10		55.7		57.4
	Unknown		0.3		0.4
Atopic disorders in parents and/or siblings	Yes		61.3		65.5
	No		38.6		34.0
	Unknown		0.1		0.5
Highest education of parents	Primary or less		0.4		0.4
	Lower professional & lower secondary		34.1		23.8
	Medium professional		28.5		29.2
	Higher secondary		10.1		15.0
	High professional & university		26.5		31.1
	Unknown		0.3		0.4
Number of children in the family	1		0.7		1.2
	2		1.8		15.2
	3		9.2		30.1
	4		22.5		22.6
	5+		65.6		30.8
	Unknown		0.1		0.2
Pertussis infection**	Yes		42.2		8.9
Mumps infection**	Yes		7.3		2.9
Measles infection**	Yes		86.1		18.4
Rubella infection**	Yes		22.2		6.5
HiB vaccination 1st year	Yes		0.0		54.3
	No		99.6		34.8
	Unknown		0.4		10.9
Pertussis part of DTPPo 1st year (vaccinated only)	Yes				90.1
	No				3.8
	Unknown				6.1
MMR at 14 months	Yes		0.1		89.7
	No		99.6		8.4
	Unknown		0.3		1.9

* Unvaccinated means: no DTPPo vaccination in the first year of life; vaccinated means: at least one DTPPo vaccination in the first year of life. For 3 children this characteristic was unknown.

** Reported infection

Table 3 Atopic disorders for (diphtheria tetanus pertussis poliomyelitis) vaccinated and unvaccinated children: prevalences (%) and crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

		%	OR	Adj OR	$CI_{95\%}$
Asthma*	Vaccinated	15.2	1.28	1.04	0.76-1.42
	Unvaccinated	12.4			
Hay fever*	Vaccinated	17.9	1.14	0.79	0.55-1.12
	Unvaccinated	16.2			
Eczema*	Vaccinated	34.2	1.14	0.87	0.66-1.14
	Unvaccinated	31.1			
Food allergy*	Vaccinated	7.9	1.29	1.13	0.71-1.81
	Unvaccinated	6.3			
Any atopic disorder*	Vaccinated	48.7	1.27	1.00	0.80-1.24
	Unvaccinated	42.9			

*Ever had the disease or having symptoms at present

Table 4 Physician diagnosed atopic disorders for (diphtheria tetanus pertussis poliomyelitis) vaccinated and unvaccinated children: prevalences (%) and crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

		%	OR	Adj OR	$CI_{95\%}$
Asthma	Vaccinated	11.4	1.23	1.03	0.72-1.46
	Unvaccinated	9.5			
Hay fever	Vaccinated	5.5	1.30	1.06	0.59-1.90
	Unvaccinated	4.3			
Eczema	Vaccinated	22.6	1.19	0.96	0.73-1.25
	Unvaccinated	19.7			
Food allergy	Vaccinated	7.9	1.29	1.13	0.71-1.81
	Unvaccinated	6.3			
Any atopic disorder	Vaccinated	33.2	1.27	1.04	0.82-1.31
	Unvaccinated	28.3			

Table 5 Reported asthma and/or hay fever versus objective allergy in a subset of participants (n=100)

		Objective allergy**		Total
Reported symptoms*		No	yes	
No	DTPPo no	33	12	45
		73%	27%	100%
	yes	7	2	9
		78%	22%	100%
	Total	40	14	54
		74%	26%	100%
Yes	DTPPo no	12	23	35
		34%	66%	100%
	yes	8	3	11
		73%	27%	100%
	Total	20	26	46
		44%	56%	100%

*Symptoms or ever had asthma and/or hay fever

** at least one RAST class 2 or higher (i.e. IgE ≥ 0.7 IU/ml) (out of five RASTs for: house dust mite, grass pollen, birch pollen, cat dander and dog dander)

Analysis of the imputed datasets yielded an adjusted OR for objective allergy (DTPPo+/DTPPo-) of 0.38 [$CI_{95\%}$: (0.18-0.82)], suggesting a protective effect of vaccinations.

Validation of the risk factor

Of the 80 allegedly DTPPo unvaccinated (in the first year of life) children tested for tetanus toxoid IgG and diphtheria IgG antibodies, six proved to have titres of tetanus toxoid IgG of at least 0.6 IU/ml. However, four of these indicated in the questionnaire that they had a tetanus vaccination after an accident. Two of the remaining children also had titres of diphtheria IgG of at least 0.6 IU/ml. A third child had a diphtheria IgG titre over 0.6 IU/ml, but its tetanus titre was only 0.32 IU/ml. These results raise doubt about the vaccination status of three out of 80 allegedly unvaccinated children (3.8%).

We requested official vaccination registration data for 120 children, of whom 3 turned out to be untraceable. Comparison of reported infant vaccinations with the official registration data in the remaining 117 participants yielded a perfect match in 107 children (91.5%): in four cases there was disagreement about the pertussis component and in five cases about the HiB vaccination. On the DTPPo vaccination there was disagreement in only one case.

Selection bias

For each school we compared the vaccination coverage according to the records of the District Health Service with the vaccination coverage in our responders. Results for all 38 schools are shown in Figure 1. The mean difference is 0.1% (sd 6.4%). The vaccination coverage for all schools, estimated with the help of the records of the District Health Service, was 63.8%, whereas the vaccination coverage of the responders was 63.1%.

Figure 2 shows for 37 schools (for one school which had 100% vaccination coverage, no OR was computed) the relationship between the ln(OR) of atopy (DTPPo+/DTPPo-) and the response rate. The weighted linear regression line seems to be descending slightly, indicating a possible small decrease of the OR with increasing response rate.

Figure 1 Vaccination coverage in the 38 schools

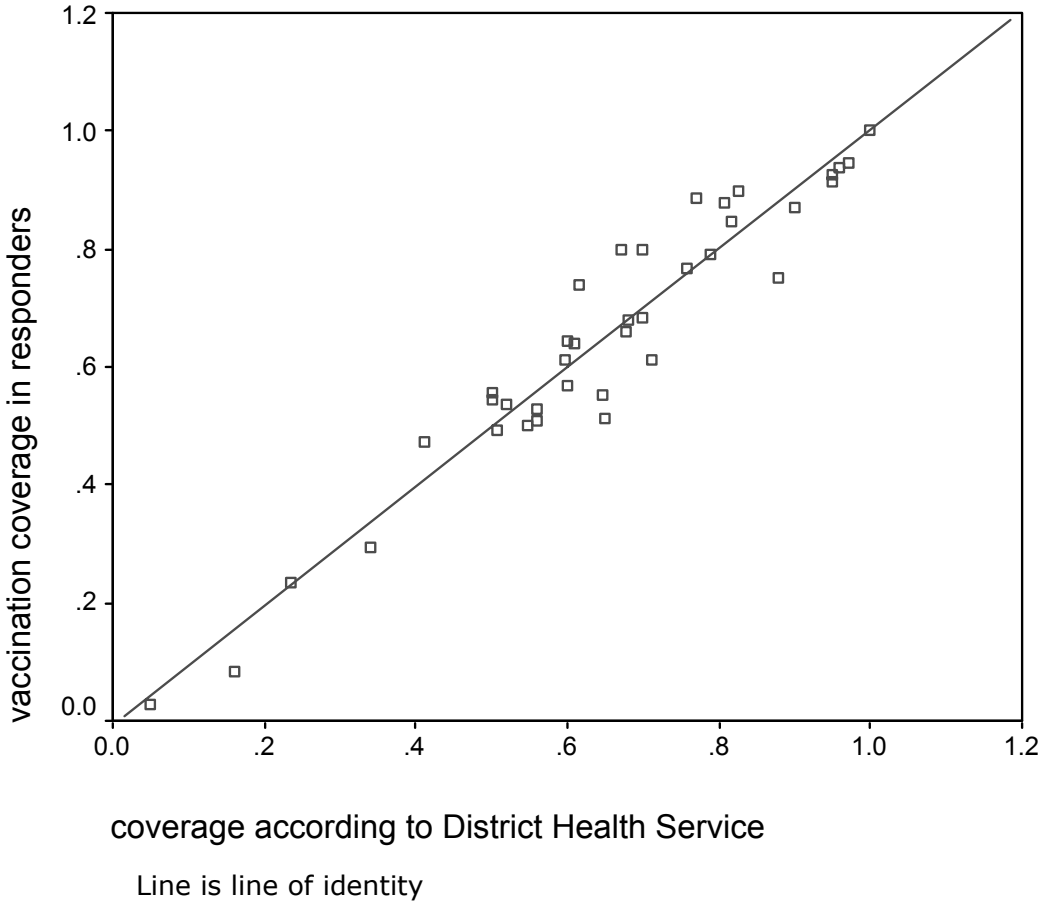
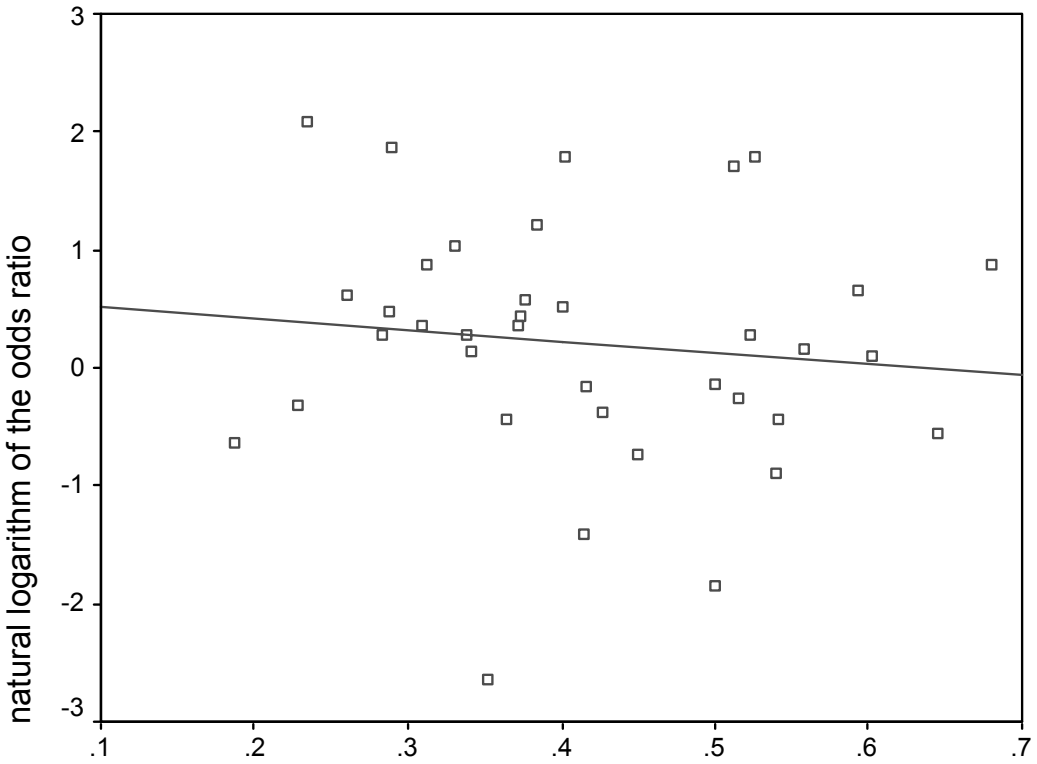


Figure 2 Relationship between the logarithm of the odds ratio (any atopy) (DTPPo+/DTPPo-) and response rate to the questionnaire in 37 schools*



Values > 0 on the y-axis correspond to a higher risk of atopy for vaccinated children

Linear regression line: beta = -1.06 $CI_{95\%}$ = (-3.15,1.04)

* In one school no odds ratio could be estimated because all children were vaccinated

Discussion

The present study showed no relationship between the DTPPo vaccination in the first year of life and reported atopic disorders in primary school children. This is the first study to compare vaccinated and unvaccinated children in a population where refraining from vaccinations is unlikely to be health related. Moreover, the two groups had the same religious background, lived in the same area and attended the same schools. Possible differences were extensively surveyed and controlled for in the analyses. This situation is second best after a randomized controlled trial, which would not be feasible for ethical reasons.

Koppen et al. concluded in their review (1), that the DTP vaccination does not cause allergic disease. This conclusion was not entirely justified, because confounding by indication could have caused biased results in all studies included in their review and, moreover, the conclusions of these studies were not unequivocal (2). However, the results of the present study support the conclusion that the DTPPo vaccination indeed is not associated with allergic disease.

Atopic disorders of family members could be viewed as intermediate variables in the relationship between vaccinations and atopy, because the vaccination status of family members is likely to be highly correlated. However, we considered this predictor to be too important to omit from the models.

Vaccinations other than the DTPPo were not included in the model, because having had other vaccinations was highly correlated to acceptation of the DTPPo vaccination. An exception was the HiB vaccination; this was introduced in 1993 and, consequently, a major part of the otherwise vaccinated children did not get the HiB vaccination.

Another consequence of the fact that almost all DTPPo unvaccinated children were completely unvaccinated is that our conclusion, that the two groups do not differ in prevalence of atopic disorders, applies to childhood vaccinations in general. In this respect also concerns about additives (such as the aluminium-containing adjuvans), are not supported by the present study.

We did not include histories of infections of pertussis, mumps, measles and rubella in the multivariate model, as these are obviously intermediate variables in the relationship between vaccinations and atopy. Moreover, also reversed causation could play a role because children predisposed to allergic disease have a different antiviral immunity (8). Finally, it is plausible that these diseases were more often diagnosed in the unvaccinated group, also because they probably result in more symptoms. Children with a reported history of

pertussis, mumps and measles reported more atopy, but for children who allegedly had had rubella less atopy was reported.

Physician-diagnosed atopy showed similar results as self-reported atopy, but indicating slightly more atopy in the vaccinated group. This difference may be explained by the possibility that unvaccinated (probably more traditional) people tend to be less inclined to visit their general practitioner. Possible health care utilization bias was the reason why we took reported symptoms and disease as the main outcome variable.

In a previous study (not included in the review mentioned above) we concluded that pertussis vaccinated children had a lower risk of atopic disorders (9). However, this conclusion was based on only 44 possibly genetically related, unvaccinated children who attended the same school. Also, our analysis of objective allergy with imputed data showed an almost identical result, but these estimates were based on a subsample of 100 children (with only 20 vaccinated children) and should therefore be viewed with caution. Maybe parents' reports of their child's symptoms are influenced by the knowledge that they are participants in a study, whereas objective measurements are not.

Limitations of the study

Design: The design was cross-sectional, implying that some variables were assessed retrospectively. This applies mainly to confounders concerning pregnancy and the first year of life, but not to vaccinations, as these were registered at the time they were administered. Parents receive a list with their child's vaccinations.

Reliability of the outcome: The outcomes analysed in the present study are symptoms of atopic disease reported by the parents. Total agreement with our objective definition of allergy was 66%: just over half of the children reporting symptoms showed allergic sensitization and about one quarter of the children without symptoms were sensitized. Similar figures are also found in the literature (10).

Reliability of the risk factor: In sera of a random stratified sample of 80 children who were not DTPPo vaccinated in the first year, we checked whether tetanus toxoid IgG or diphtheria IgG antibodies above a relevant level could be detected. Moreover, in a random sample of 100 children, we compared the reported DTPPo vaccination with the officially registered vaccination data. Based on the results of these checks, we conclude that the main risk factor was fairly reliably reported, although some misclassification was detected. Moreover, only 85% of the unvaccinated children (vs 99% of the vaccinated children) gave

informed consent to obtain official registration data; for blood samples this was 73% and 75%, respectively. These figures could raise some concern about the reliability of the reported risk factor, because parents may have reasons to conceal the true vaccination status of their children. On the other hand, it would be more likely that these people would refuse to participate in the study at all.

Response rate: Because only 42% of the approached children responded, the possibility of selection bias had to be evaluated. We checked this in two ways: first we compared the vaccination coverage of the responders with the vaccination coverage of the target group. The difference was less than 2%, implying that no selection on vaccination status had occurred; this made selection bias unlikely. Secondly, for the 38 participating schools we investigated the relationship between the response rate and the relationship between DTPPo and atopic disorder. There appeared to be a slightly negative trend, i.e. if there would be selection bias, the "true" OR would be smaller (i.e. a relatively smaller risk of atopy in vaccinated children) than the OR in our results.

External validity: The study was conducted in a relatively homogeneous group of people, all from a common cultural background. Although this is a strong aspect of the study design, it could also raise questions about the generalizability of the conclusions. However, there is no reason to assume that the group of people under study differ biologically from the general Dutch population such that childhood vaccinations would have a different impact on atopy.

Conclusion

In the Dutch population the DTPPo vaccination is not associated with a higher risk of reported atopic disorders at primary school age.

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References

1. Koppen S, De Groot R, Neijens HJ, Nagelkerke N, Van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. Vaccine 2004;22:3375-85.
2. Bernsen RM, van de Wouden JC. Re: No epidemiological evidence for infant vaccinations to cause allergic disease. Letter to the Editor. Vaccine 2005;23:1427.
3. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
4. Anyo G, Brunekreef B, de Meer G, Aarts F, Janssen NA, van Vliet P. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. Clin Exp Allergy 2002;32:361-6.

5. Rothman K. Modern Epidemiology. Philadelphia: Lippincott-Raven 1998: 256-7.
6. Kerncijfers vierpositie postcodegebieden. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 1995.
7. Rubin DB, Schenker N. Multiple imputation in health care databases: an overview and some applications. Stat Med 1991;10:585-98.
8. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Hiemke HJ, Gentile K, Gangnon RE, Lemanske RF Jr. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 2004;170:175-80.
9. Bernsen RM, de Jongste JC, van der Wouden JC. Lower risk of atopic disorders in whole cell pertussis-vaccinated children. Eur Respir J 2003;22:962-4.
10. Kurukulaaratchy RJ, Fenn M, Matthews S, Arshad SH. Characterisation of atopic and non-atopic wheeze in 10 year old children. Thorax 2004;59:563-8.

3

Lower risk of atopic disorders in whole cell pertussis vaccinated children

Abstract

Study question: Do whole cell pertussis-vaccinated children have a different risk of atopic disorders compared to children who did not receive this vaccination?

Methods: Data on vaccination status, atopic disorders and child and family characteristics of children of 700 families were collected in a retrospective study. A minority of these 700 families refused vaccinations for religious reasons. The relation between pertussis-vaccination status and atopic disorders was analysed by means of adjusted logistic regression for repeated measurements in order to account for the correlation between sibship members.

Results: The 700 families comprised 1961 children. Data on vaccination status and atopic disorders were available for 1724 children. Vaccinated children had a reduced risk of atopic disorders (adjusted OR: 0.37, $CI_{95\%}$: 0.16-0.87).

Answer to the question: Whole cell pertussis vaccination is associated with a lower risk of atopic disorders, but we cannot exclude that other vaccine components (diphtheria, tetanus, poliomyelitis) or other vaccinations are also involved.

Keywords: Whole cell pertussis vaccination, atopy, asthma, allergy, eczema

Eur Respir J 2003;22:962-4.

Introduction

An increased risk of atopic disorders in pertussis or diphtheria tetanus pertussis (DTP) vaccinated children has been reported (1, 2, 3, 4), but this was not confirmed by more recent observational studies (5, 6, 7), a randomized placebo-controlled trial (8), and an ecological study (9). Reports of adverse effects of vaccinations usually cause a lot of commotion and debate between advocates and opponents of vaccination programs. Within a study of family size and birth order as risk factors for asthma, allergy and eczema in a population of 700 families in the Netherlands we evaluated the role of the pertussis vaccination as risk factor for atopic disorders.

Material and Methods

Study subjects: In a study in the Netherlands designed to determine the role of sibship size and birth order as risk factors for asthma, allergy and eczema 700 families with index children born in 1988, 1989 or 1990 were sampled from files administered by the Municipal Health Service covering the municipality of Zwijndrecht, a town with over 40,000 inhabitants. A subgroup of these families adhere to the orthodox reformed religion; their children mostly attend orthodox reformed schools. Some of the parents of this group refrain from vaccinations for religious reasons. According to the official schedule in the Netherlands children receive four vaccinations of DTP (whole cell)-Poliomyelitis and Haemophilus influenzae type B under the age of one year (before 1998 at the age of 3, 4, 5 and 11 months), mumps-measles-rubella (MMR) at the age of 14 months, a booster of DT-Poliomyelitis at the age of 4 and 9 years and a booster of MMR at the age of 9 years. Since 2002 a booster of acellular pertussis is administered at the age of 4 years.

Study design: Retrospective study using datafiles from routine health check-ups from birth till adolescence.

Methods: Data on preventive health check-ups of index children performed by the Municipal Helath Service at the age of six years were collected from the files. Data from files of their siblings were also extracted. The following data were used: presence and type of allergy, asthma, eczema, current medication, birth order, sibship size, date of birth, gender, date of check-up, year of birth of the mother, postal code, country of birth of the parents, occupation of the bread-winner, atopic disorders of the parents, vaccination status, and duration of breast-feeding. A child was considered having asthma, allergy or eczema, if this

was mentioned in the file by the physician who did the check-up, in the questionnaire filled out by the parents or in a letter from a paediatrician/pulmonologist.

Analysis: The independent relation of pertussis-vaccination status with allergy, eczema, asthma and any atopic disorder (allergy, eczema or asthma) was evaluated within families by means of logistic regression for repeated measurements (Generalized Estimating Equations) (10), families being the units of analysis, which takes correlation between family members into account (SAS PROC GENMOD). Analyses were performed both in univariate and multivariate models with adjustments for the subset of the following variables which changed the univariate point estimate by at least 10% (11): sibship size, birth order, year of birth, season of birth, gender, breast feeding for more than one month (yes/no), age at the time of check-up, allergy or asthma of the parents, level of occupation of the bread-winner (five levels), age of the mother at the time of delivery, level of urbanization (two levels) and country of origin (both parents born in the Netherlands yes/no). A two-sided p-value of 0.05 was considered significant.

Results

The 700 families comprised 1961 children. Both vaccination status and atopic status was available for 1724 children; of these 44 (2.6%) were not vaccinated for pertussis, mostly for religious reasons. Of these 39 had received no vaccinations at all, four received only the polio-vaccination, and one received DT-polio (without pertussis). The 1724 children had their health check-up at a mean age of 5.9 years (sd: 0.6 years). The types of allergy mentioned were: house-dust mite, pollen, furry pets, food allergy, hay fever and allergic rhinitis. The prevalence of atopic disorders in both groups is shown in the table. Crude odds ratios for atopic disorders (vaccinated/unvaccinated) varied between 0.33 and 0.69. Adjusted odds ratios ranged from 0.23 to 0.40, consistent with a substantially reduced risk of atopy in pertussis-vaccinated children. The same analysis in the subgroup of children attending orthodox reformed schools (39 unvaccinated and 128 vaccinated children) yielded similar point estimates. For "any atopic disorder" the adjusted odds ratio (vaccinated /unvaccinated) was 0.16 ($CI_{95\%}$ 0.04 to 0.67, data not shown in table).

Table Prevalence of atopic disorders among unvaccinated and vaccinated children and crude and adjusted odds ratios for atopic disorders (vaccinated/unvaccinated) with 95% confidence intervals ($CI_{95\%}$)

		Allergy	Eczema	Asthma	Any atopic disorder
	N	%	%	%	%
Unvaccinated	44	11.4	13.6	11.4	22.7
Vaccinated	1680	5.2	4.9	8.7	13.8
		OR	OR	OR	OR
Crude		0.40	0.33	0.69	0.51
Adjusted*		0.23	0.26	0.40	0.37
$CI_{95\%}$		0.07 – 0.79	0.11 – 0.63	0.10 – 1.70	0.16 – 0.87

*Variables included in the multivariate model were for

allergy: birth order, family size, year of birth, atopy of the father, age of mother at birth

eczema: birth order, atopy of the father, age of mother at birth

asthma: birth order, family size, year of birth, atopy of the father, age of mother at birth

any atopy: birth order, family size, atopy of the father, age of mother at birth

Discussion

In the present study we found that pertussis-vaccinated children were at a considerably lower risk of atopic disorders than unvaccinated children. Most vaccinated children received the combined DTP-polio vaccine. Hence, the comparison of children with and without pertussis vaccination was almost equivalent to the comparison of DTP-polio vaccination and no vaccination at all. Data used for this study were not collected to answer the present research question, and consequently no sample sizes for vaccinated and unvaccinated groups were computed. We found 44 unvaccinated children and the relations studied were significant. Adjustment for confounders could have been a problem with so few children at risk, with resulting empty cells and huge confidence intervals. However this was not the case and the results of the adjusted analyses showed similar relations. All data were collected retrospectively from files and possibly misreporting, most likely underreporting, of atopic diseases may have occurred. It could be that this misclassification is differential, i.e. related to the risk factor under study. This would for instance mean that parents of vaccinated children report less atopy than unvaccinated children. However, the relations found persisted, even became somewhat stronger when we performed the analyses in the more homogenous group of children attending orthodox reformed schools. Even if within this more homogenous group underreporting would be differential, it would be more likely that parents of unvaccinated children (more orthodox religious families) would report less than parents of vaccinated children. However, this bias would lead to an attenuation of the odds ratio and the unbiased odds ratio would even be more extreme.

In the Netherlands pertussis is endemic in childhood with four-yearly peaks (12). The highest incidence is reported in infants younger than one year (35 per 100,000 per year in the period 1989-1993). Pertussis cases are reported both in vaccinated and in unvaccinated children, the incidence in the latter group being roughly tenfold the incidence in the vaccinated group. The relatively low incidence in the unvaccinated group (compared to completely unvaccinated groups in Germany) can be explained by herd immunity.

As only 0.7% of our study population was born after 1997, the role of acellular pertussis vaccine is negligible in this study.

Given the hygiene hypothesis we would expect the immune system of the unvaccinated children to have shifted more towards the Th1-side, inconsistent with a raised risk of atopy. On the other hand, the immune system of vaccinated children is triggered with the (albeit killed) micro-organisms at a very young age

and from this point of view our findings do fit in the hygiene hypothesis and are biologically plausible.

Our findings are consistent with a recent study in Germany by Grüber et al (7). Several studies found an increased risk of asthma and allergy in DTP- or pertussis-vaccinated children (1,2,3,4), but this may be due to residual confounding or confounding by indication as the reasons for not vaccinating are not described and may be related to the outcome. A number of studies found no relation between the DTP- or the pertussis-vaccination and atopy (5, 6, 8, 9). One of these is a randomized controlled trial (8) which compared three groups which received different cocktails of DTP with a control group which received DT only. However the age of evaluation was rather young: 2½ years, and this may account for the lack of an effect. Henderson et al (5) found no difference, but in this study the outcome was wheezing at the age of not more than 42 months. In all (but one) studies we are dealing with observational studies, and residual confounding cannot be excluded, especially when the reason for refraining from vaccination is not known. It is important for future studies to include groups which are as homogenous as possible and describe well the differences between vaccinated and unvaccinated groups.

Conclusion

Our results suggest that whole cell pertussis-vaccinated children have a lower risk of atopic disorders. However we cannot exclude that other vaccine components (diphtheria, tetanus, poliomyelitis) or other vaccinations played a role as well.

References

1. Odent MR, Culpin EE, Kimmel T. Pertussis vaccination and asthma: is there a link? JAMA 1994; 272:592-3.
2. Kemp T, Pearce N, Fitzharris P, Crane J, Fergusson D, St George I, Wickens K, Beasley R. Is infant immunization a riskfactor for childhood asthma or allergy? Epidemiology 1997; 8(6):678-80.
3. Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. Thorax 1998; 53:927-32.
4. Hurwitz EL, Morgenstern H. Effects of diphtheria-tetanus-pertussis or tetanus vaccination on allergies and allergy-related respiratory symptoms among

children and adolescents in the United States. J Manipulative Physiol Ther 2000; 23: 81-90.

5. Henderson J, North K, Griffiths M, Harvey I, Golding J. Pertussis vaccination and wheezing illnesses in young children: prospective cohort study. Br Med J 1999; 318:1173-6.
6. DeStefano F, Gu D, Kramarz P, Truman BI, Iademarco MF, Mullooly JP, Jackson LA, Davis RL, Black SB, Shinefield HR, Marcy SM, Ward JI, Chen RT. Vaccine Safety Datalink Research Group Childhood vaccinations and risk of asthma. Pediatr Infect Dis J 2002; 21: 498-504.
7. Gruber C, Illi S, Lau S, Nickel R, Forster J, Kamin W, Bauer CP, Wahn V, Wahn U. MAS-90 Study Group. Transient suppression of atopy in early childhood is associated with high vaccination coverage. Pediatrics 2003; 111:e282-8.
8. Nilsson L, Kjellman M, Bjorksten B. A randomized controlled trial of the effect of pertussis vaccines on atopic disease. Arch Pediatr Adolesc 1998; 152:734-8.
9. Anderson HR, Poloniecki JD, Strachan DP, Beasley R, Bjorksten B, Asher MI; ISAAC Phase 1 Study Group. Immunization and symptoms of atopic disease in children: results from the International Study of Asthma and Allergies in Childhood. Am J Public Health 2001; 91: 1126-9.
10. Diggle PJ, Liang KY, Zeger SL. Analysis of Longitudinal Data. Oxford: Clarendon Press 1994.
11. Rothman K. Modern Epidemiology. Philadelphia: Lippincott-Raven 1998: 256-7.
12. de Melker HE, Conyn-van Spaendonck MA, Rumke HC, Sprenger MJ, Schellekens JF. Whooping cough in The Netherlands, 1989-1994 (Article in Dutch). Ned Tijdschr Geneeskd 1995;139(25):1280-6.

4

The Haemophilus influenzae type b vaccination and reported atopic disorders in 8-12 years old children

Abstract

Background: Because the Haemophilus influenzae type b (Hib) vaccination was added recently to most existing vaccination programmes, no conclusive data on the relationship with atopic disorders are yet available.

Study objective: To assess the relationship between Hib vaccination in the first year of life and reported physician-diagnosed atopic disorders at age 8-12 years.

Method: Parents of 1201 children attending Orthodox Reformed (Protestant) primary schools in the Netherlands returned questionnaires reporting data on vaccination status, atopic symptoms and physician-diagnosed lifetime atopic disorders (asthma, hay fever, eczema and food allergy), and possible confounders. This study was conducted within the framework of a larger study on the relationship between the diphtheria-tetanus-pertussis-poliomyelitis (DTPPo) vaccination and reported atopic disorders.

Results: The adjusted odds ratio of any atopic disorder (HiB vaccinated/unvaccinated) was 1.09 ($CI_{95\%}$ 0.79 - 1.50). For asthma, hay fever, eczema and food allergy the results were respectively: 0.89 ($CI_{95\%}$ 0.55 - 1.43), 0.94 ($CI_{95\%}$ 0.47 - 1.90), 1.09 ($CI_{95\%}$ 0.75 - 1.58) and 0.68 ($CI_{95\%}$ 0.38 - 1.19).

Conclusion: In the Dutch population there is no indication for a higher risk of reported physician-diagnosed atopic disorders at primary school age after the Hib vaccination in the first year of life.

Submitted

Introduction

The prevalence of atopic disorders has increased during the second half of the 20th century and explanations for this are still being sougth. Because the highest prevalence is found in modern societies and is increasing in those societies changing to a more modern lifestyle, elements of western lifestyle have been investigated, including childhood vaccination programmes. Such studies are also important because some countries a growing resistance to the childhood vaccination programmes, resulting in a declining vaccination coverage. Because serious infectious diseases have almost been eradicated, for part of the population possible adverse effects of vaccinations may take precedence over their preventive action.

The possible effects of the measles-mumps-rubella vaccination (MMR) and the diphtheria-tetanus-pertussis vaccination on atopic disease have been well studied (1-5). However, less is known about the relationship between the Haemophilus influenzae type b (Hib) vaccination and atopic disorders. The few studies published so far (4, 6, 7) found no evidence of more atopy in Hib vaccinated children. In these (observational) studies, information bias, residual confounding and confounding by indication could not be excluded. Therefore we need more evidence concerning the Hib vaccination as a potential risk factor for atopic disorders. We analysed data of a study conducted to investigate the relationship between the diphtheria-tetanus-pertussis-poliomyelitis (DTPPo) vaccination and atopy in the Orthodox Reformed (Protestant) population in the Netherlands, which also collected data on the HiB vaccination. Some of the study population was born before, and some after the introduction of the Hib into the Dutch National Vaccination Programme (i.e. April 1 1993).

Aim of the study

To assess the relationship between Hib vaccination in the first year of life and reported atopic disorders at age 8-12 years.

Methods

Childhood vaccinations in the Netherlands

According to the National Vaccination Programme, infants get four vaccinations of DTPPo and Hib in their first year of life. At 14 months children are immunized with the MMR and the meningococcal c vaccine (Neis Vax). At 4 years children receive a vaccination for diphtheria-tetanus-poliomyelitis (DTPo) and acellular pertussis (aP), and at 9 years DTPo and MMR. The Hib vaccination (administered at 2, 3 and 4 months) was introduced in April 1993 and the Neis Vax in 2003.

Consequently, part of the population under study did not regularly receive the Hib vaccination. The whole study population was offered the Neis Vax at age 7 years or older.

Study area, population and design

The present study was conducted in the framework of an earlier study designed to assess the relationship between the DTPPo vaccination and reported atopic disease (5). Briefly, in 2003 and 2004, we sent questionnaires to 4480 children (aged 8-12 years) of 38 Orthodox Reformed (Protestant) primary schools in the Netherlands. Many parents of children attending these schools refuse vaccinations for religious reasons. A total of 1875 questionnaires (42%), of which 1872 were suitable for analysis, were returned. The present study population comprised the 1201 children who all received the DTPPo vaccination; 41% of these children were born before April 1 1993 and therefore did not regularly receive the Hib vaccination in their first year of life. However, in the year before April 1993 it was possible to receive the Hib on special request.

Data collection

The questionnaire asked for symptoms and physician diagnosis of asthma, hay fever and eczema (ever) (Dutch translation of the ISAAC questionnaire (8, 9)), physician-diagnosed food allergy ever, childhood vaccinations, BCG vaccinations and influenza vaccinations, gender, passive smoking (prenatally, during the first year of life and currently), date of birth, perinatal data, birth order, ZIP code, year of birth of the mother, any atopic disorders of parents or siblings, family composition, education, income, living conditions (current and during the first year of life), day care attendance, breast feeding, nutrition during the past month, frequency of exercise, body height and weight, and having had common infectious diseases preventable by vaccinations (measles, mumps, rubella or pertussis).

Statistical analysis

The relationship between receipt of the Hib vaccination in the first year of life and asthma, hay fever, eczema, food allergy, and "any atopic disorder" (ever diagnosed by a physician, at least one of these diseases) was evaluated by means of logistic regression. Analyses were performed both in univariate and multivariate models. Potential confounders are shown in Table 1. A variable was included in the model if it changed the univariate point estimate by at least 10% (10).

Table 1 Potential confounders in the relationship between the Hib vaccination and atopic disorders

Season of birth
Birth order
Gender
Gestational age
Birth weight
Age of the mother at the time of delivery
Exposure to smoking (prenatally, during the first year of life and currently)
Breast feeding for four months or more (yes/no)
Housing in the first year of life (rural, living on a farm with livestock/rural other / city)
Pet keeping (furry pets or birds yes/no) during the first year of life and currently
Day care starting at age 6 months or less (yes/no)
Current age
Asthma and/or allergy of the parents and/or siblings
Highest educational level of the parents
Family income
Current level of urbanization (five levels)
Living on a farm with livestock (yes/no)
Sibship size
Mould in the child's bedroom in the past year
Frequent (more than five days/week) consumption of
    fruit (yes/no)
    (raw or cooked) vegetables (yes/no)
    anti-oxidants (yes/no)
    unskimmed dairy products (yes/no)
    wholemeal bread (yes/no)
Frequent (at least one day/week) consumption of fish
Frequent exercise (school gym at least once a week and playing games with physical activity for at least half an hour a day and either being a member of a sporting club or walking or cycling from home to school vice versa for at least one hour a day)
Body mass index
MMR vaccination
Pertussis vaccination

Because the question on income is a sensitive one which is often skipped, participants who did not answer this question were ranked according to income data linked to their ZIP code (11), and we imputed a code for income such that the total distribution of income remained the same as it was for the participants who did answer this question.

The Hib vaccination was first introduced in the Dutch National Vaccination Programme for the cohort born on April 1 1993; during one year before the official introduction it was available on special request. We assessed the risk factor in three different ways: 1) as reported, i.e. children whose parents indicated they did not know whether or not their child received the HiB vaccination in the first year of life were omitted from the analysis (main analysis), 2) all children with missing Hib vaccination status were categorized as "no" (i.e. did not receive the Hib vaccination in their first year of life), 3) all children with missing vaccination status and born before April 1 1992 were categorized as "no", all other children of this group were categorized as "yes" (i.e. received at least one Hib vaccination in their first year of life).

The main analyses were repeated taking the multi-level structure into account (level 1: children; level 2: families; level 3: schools).

SPSS version 11.0 and ML-win version 1.1 were used for all computations. A two-sided p-value of 0.05 or less was considered significant in all tests.

Assessment of selection bias

The method of evaluation of selection bias has been described elsewhere; we concluded that no selection on DTPPo vaccination status had occurred (5).

Results

Relationship between Hib and atopy

Of the1201 children, 1070 (89.1%) reported their Hib vaccination status. Of these 1070 children, 652 (60.9%) reported that they had at least one Hib vaccination in the first year of life. Of these 652 Hib vaccinated children, 45 (6.9%) indicated that they were incompletely vaccinated for Hib. Characteristics of the children, by Hib vaccination status, are presented in Table 2.

Table 3 shows prevalences, and univariate and multivariate ORs with 95% confidence intervals ($CI_{95\%}$) for the relationships between the Hib vaccination and atopic disorders. Atopy had a similar prevalence in Hib vaccinated and unvaccinated children (adjusted OR: 1.09 ($CI_{95\%}$=(0.79-1.50)). The separate disorders also showed (clinically and statistically) no significant differences. The

Table 2 Characteristics of study population (N=1201) by Hib vaccination status

		Unvaccinated group * N=418	%	Vaccinated group * N=652	%	Vaccination status unknown N=131	%
Gender	Male		49.3		50.3		53.4
Year of birth	1990/1991		16.8		2.0		14.5
	1992		46.4		7.4		35.9
	1993		22.7		26.9		20.6
	1994		10.5		40.4		21.4
	1995/1996		3.6		23.2		7.6
	Unknown		0.0		0.1		0.0
Atopic disorders in parents and/or siblings	Yes		69.6		62.5		64.9
	No		30.1		36.8		35.1
	Unknown		0.2		0.7		0.0
Highest education of parents	Primary or less		0.5		0.4		0.0
	Lower professional & lower secondary		24.6		22.2		28.2
	Medium professional		27.5		31.1		23.7
	Higher secondary		15.1		14.9		16.0
	High professional & university		32.1		31.0		31.3
	Unknown		0.2		0.4		0.8
Number of children in the family	1		0.2		1.8		1.5
	2		8.9		18.5		14.5
	3		25.8		33.8		23.7
	4		24.2		21.6		26.0
	5+		40.7		24.1		34.4
	Unknown		0.2		0.1		0.0
Pertussis part of DTPPo first year	Yes		89.2		91.9		84.0
	No		6.0		2.5		3.8
	Unknown		4.8		5.7		12.2
MMR at 14 months	Yes		80.6		95.1		88.5
	No		17.7		3.2		5.3
	Unknown		1.7		1.7		6.1
Reported infections:							
Pertussis	Yes		8.4		10.1		4.6
Mumps	Yes		5.0		1.8		1.5
Measles	Yes		26.6		12.7		20.6
Rubella	Yes		7.7		5.2		9.2

*Unvaccinated means no Hib vaccination in the first year of life; vaccinated means at least one Hib vaccination in the first year of life

Table 3 Physician diagnosed atopic disorders for (Haemophylus influenzae type b) vaccinated children and unvaccinated children (children who reported Hib vaccination status, N=1070): prevalences (%) and crude and adjusted (adj) odds ratios (OR) (HiB+/HiB-) with 95% confidence intervals ($CI_{95\%}$)

		%	OR	AdjOR	$CI_{95\%}$
Asthma	Vaccinated	11.0	0.79	0.89	0.55-1.43
	Unvaccinated	13.6			
	Unknown	7.8			
Hay fever	Vaccinated	5.1	0.81	0.94	0.47-1.90
	Unvaccinated	6.3			
	Unknown	5.4			
Eczema	Vaccinated	23.5	1.04	1.09	0.75-1.58
	Unvaccinated	22.8			
	Unknown	18.6			
Food allergy	Vaccinated	7.3	0.72	0.68	0.38-1.19
	Unvaccinated	9.8			
	Unknown	5.4			
Any atopic disorder	Vaccinated	33.9	0.93	1.09	0.79-1.50
	Unvaccinated	35.7			
	Unknown	28.6			

results of the analyses with the two alternative assessments of the risk factor show similar nonsignificant relationships (data not shown).
Multilevel analysis of the multivariate models presented in Table 3 yielded similar results (data not shown).

Validation of the risk factor

We compared the reported Hib vaccination of 74 children with the officially registered vaccination data; disagreement emerged in five of these cases (6.8%).

Discussion

In the present study no significant relationship was found between Hib vaccination in the first year of life and physician-diagnosed atopic diseases in primary school children. Our study population was a homogeneous group of primary school children (all adhering to the same religious group), all of whom were DTPPo vaccinated. In this study, the main reason for not being vaccinated for Hib is that this vaccination had not yet been introduced in the National Vaccination Programme until 1993. This implies that probably only age was a confounder (for which we adjusted in the analyses). However, data on the MMR vaccination coverage in the two groups (Hib vaccinated and Hib unvaccinated) indicate that some of the Hib unvaccinated group may be those with fundamental objections to vaccinations, but nevertheless accepted the DTPPo for fear of serious diseases. The relationship studied pertains to a possible additional risk to other childhood vaccinations.

The relationships found in our study are similar to, or even weaker than those reported earlier (4, 6, 7), thus confirming the conclusion that there is no indication for a higher risk of atopic disorders in Hib vaccinated children.

Histories of infections of pertussis, mumps, measles and rubella were not included in the main multivariate model because these are obviously intermediate variables in the relationship between the respective vaccinations and atopy. Moreover, reverse causation could also play a role because children predisposed to allergic disease may have a different antiviral immunity (13).

Limitations of the study

Reliability of the risk factor: 6.8% (5 of 74 children) of the answers on the question about the Hib vaccination did not agree with the official registrations. This uncertainty may be because the Hib was only introduced in 1993. Therefore some misclassification cannot be excluded and this (non-differential) misclassification may have diluted the effect estimates. Moreover, a relatively large number of parents (10.9%) did not answer the question on Hib vaccination.

However, we used different approaches to allocate these children to one of the two groups (vaccinated or unvaccinated), and all approaches led to similar conclusions. Thus, we argue that the findings are relatively robust.
Allergic rhinitis: Because our questionnaire did not include questions on allergic rhinitis, a number of allergic cases may have been missed in both groups.
External validity: This study was conducted in a relatively homogeneous group of people, all from a common cultural background. Although this is a strong aspect of the study design, it could at the same time raise questions about the generalizability of the conclusions. However, there is no reason to assume that the study population differs biologically from the general Dutch population such that childhood vaccinations would have a different impact on atopy.
In conclusion, we found that there is no indication for a higher risk of reported physician-diagnosed atopic disorders due to the Hib vaccination in addition to other childhood vaccinations in the Dutch population.

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References

1. Koppen S, De Groot R, Neijens HJ, Nagelkerke N, Van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. Vaccine 2004;22:3375-85.
2. Bernsen RM, van de Wouden JC. Re: No epidemiological evidence for infant vaccinations to cause allergic disease. Letter to the Editor. Vaccine 2005;23:1427.
3. Bernsen RM, de Jongste JC, van der Wouden JC. Lower risk of atopic disorders in whole cell pertussis-vaccinated children. Eur Respir J 2003;22:962-4.
4. Mommers M, Weishoff-Houben M, Swaen GM, Creemers H, Freund H, Dott W, van Schayck CP. Infant immunization and the occurrence of atopic disease in Dutch and German children: a nested case-control study. Pediatr Pulmonol 2004;38:329-34.
5. Bernsen RM, de Jongste JC, Koes BW, Aardoom HA, van der Wouden JC. The diphtheria tetanus pertussis poliomyelitis vaccination and reported atopic disorders in primary school children. Submitted.
6. Laubereau B, Grote V, Holscher G, Holscher B, Frye C, Wichmann HE, Heinrich J. Vaccination against Haemophilus influenzae type B and atopy in east German schoolchildren. Eur J Med Res 2002;7:387-92.
7. DeStefano F, Gu D, Kramarz P, Truman BI, Iademarco MF, Mullooly JP, Jackson LA, Davis RL, Black SB, Shinefield HR, Marcy SM, Ward JI, Chen RT; Vaccine Safety Datalink Research Group. Childhood vaccinations and risk of asthma. Pediatr Infect Dis J 2002;21:498-504.
8. Asher MI, Keil U, Anderson HR et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
9. Anyo G, Brunekreef B, de Meer G, Aarts F, Janssen NA, van Vliet P. Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children. Clin Exp Allergy 2002;32:361-6.
10. Rothman K. Modern Epidemiology. Philadelphia: Lippincott-Raven 1998: 256-7.
11. Kerncijfers vierpositie postcodegebieden. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 1995.
12. Rubin DB, Schenker N. Multiple imputation in health care databases: an overview and some applications. Stat Med 1991;10:585-98.

13. Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, DaSilva DF, Hiemke HJ, Gentile K, Gangnon RE, Lemanske RF Jr. Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. Am J Respir Crit Care Med 2004;170:175-80.

5

Birth order and sibship size as independent risk factors for asthma, allergy, and eczema

Abstract

Study objective: To disentangle the independent relations of birth order and sibship size with the presence of asthma, allergy and eczema.

Method: In a retrospective study, 700 families in the Netherlands were selected with index children born in 1988-1990. Data were extracted from reports of health examinations at the age of 6 years of these children and their siblings.

Results: Birth order, and not sibship size, appeared to be a strong risk factor for allergy (excluding eczema). Children with higher birth order had a lower risk of allergy compared to first-borns (adjusted odds ratios: 0.43, 0.26 and 0.05 for second-, third- and fourth- or higher borns, respectively; $p<0.0001$). Allergy including eczema also had a significant relation with birth order ($p=0.001$). For asthma there appeared no clear relation with birth order. For asthma a non-significant relationship with sibship size (adjusted for birth order) was found ($p=0.06$): first-born children in small sibships were more at risk than those in larger sibships. For allergy and eczema no such trend was observed.

Conclusion: Birth order is inversely related to the risk of allergy, independent of the size of the sibship.

Keywords: asthma, allergy, eczema, sibship size, birth order.

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Introduction

The prevalence of asthma and allergy has increased during the past decades and much effort has been put into identifying risk factors for these disorders, including elements of western lifestyle. Golding and Peters were the first to describe the protective effect of sibship size for eczema and hay fever (1). Since 1986 many studies have demonstrated a lower prevalence of atopic disease in large families (2). There has been much speculation on the mechanism underlying this "sibling effect". According to the hygiene hypothesis, a reduced opportunity of cross-infection in small families could increase the risk of hay fever (3, 4). Also, prenatal mother-child interactions might be different after multiple births (5, 6).

The proportion of allergy cases attributable to the sibling effect is approximately 30% (2). Hence, identification of the underlying mechanism is potentially important. However, different definitions of the sibling effect have been used, relating allergy to the number of older siblings, birth order, family size, the number of younger siblings and sibship size. Several definitions are (almost) equivalent: the number of older siblings and birth order are equivalent, because birth order equals the number of older siblings plus one; sibship size parallels family size; and sibship size minus the number of younger siblings equals the number of older siblings plus one. Therefore it suffices to study the role of birth order and sibship size as risk factors for atopic disease. However, it is difficult to disentangle the effects of these two factors (7). In any random sample of individuals the two variables are strongly correlated, and including them both into a regression model would not yield a definite answer, unless both effects are assessed within families. To examine all children in a family at a fixed age in a prospective study would be difficult because maturation of the increasing sibship may take a long time. Therefore, we performed a retrospective study investigating within families the independent role of birth order and sibship size as determinants for asthma, allergy and eczema at the age of 6 years.

Methods

Study area and population

In the Netherlands all children fall under the auspices of a Municipal Health Service, which invites them for health examinations by a physician at the age of 6 years. Parents are requested to fill out a questionnaire with details on their child's health. Reports of these examinations are stored in a file together with the

questionnaire and data from birth up to the last examination in secondary school, including letters from specialists after referrals. Files are kept at the Service for about 25 years. We focused on the municipality of Zwijndrecht, a town south of Rotterdam with over 40,000 inhabitants. From the files of the Municipal Health Service of Zwijndrecht, 700 families with at least two children with a written report of a check-up at the age of 6 years were randomly selected. At least one of the children in the family had to be born in 1988, 1989 or 1990.

Sample size calculation

We assumed a prevalence of allergy of 10% in first-born children and 5% in their siblings. In order to detect this difference with a power of 0.80 and alpha=0.05 a sample size of 700 families was required.

Data extraction

From the files the following data were extracted: current asthma, allergy, eczema (as recorded by the physician who did the check-up; in the questionnaire or mentioned in letter(s) from a paediatrician/pulmonologist), current medication, birth order, sibship size, date of birth, gender, date of check-up, year of birth of the mother, postal code, country of birth of the parents, occupation of the bread-winner, atopic disorders of the parents, diphtheria tetanus pertussis poliomyelitis (DTPP) vaccination status and duration of breast-feeding.

Blinding

To avoid possible bias the researcher (RMDB) classified children as being asthmatic, allergic or having eczema only if this was explicitly mentioned in the file by the physician who did the check-up or by a paediatrician or other specialist in a letter preserved in the file. In case of doubt the researcher consulted one of the Municipal Health Service's physicians who was blind for the risk factors under study.

Derived variables

The following variables were derived from data extracted from the files:

- age of the mother at the time of delivery.
- level of urbanization derived from the zip code (8). Because of the homogeneity of urbanization in the study area, two levels were recognized: heavily urbanized and moderately urbanized.
- level of occupation using the classification system of the Statistics Netherlands (9). Occupation was categorized into 5 levels: elementary, low, average, high, academic. In case of missing occupation, we estimated the level of occupation as follows: first (for all patients with known occupation) the mean income per bread-winner, derived from the ZIP code,(8) was used

as predictor in a linear regression model with level of occupation as outcome variable (after checks of assumptions); for cases with "occupation" missing the mean income per bread-winner was imputed from this linear equation.

- country of origin; a dichotomy was used: both parents born in the Netherlands (yes/no).
- season of birth divided in three categories: June-September, October-January and February-May (10-12).
- Breast feeding for one month or more. This cut-off point was chosen because it has been observed by Sears et al (13) that children who were breastfed for less than one month have a risk of asthma and allergy similar to those who are not breastfed at all.

Statistical analysis

The independent relation of birth order and sibship size with asthma, allergy (including house-dust mite, pollen, furry pets, hay fever, rhinitis, excluding food allergy and eczema) eczema (including food allergy), allergy (including eczema/food allergy), and any atopic disorder (asthma, allergy, and/or eczema) was evaluated within families by means of logistic regression for repeated measurements (Generalized Estimating Equations) (14), families being the units of analysis, which takes correlation between family members into account (SAS PROC GENMOD). Analyses were performed both in univariate and multivariate models with adjustments for sibship size or birth order (depending on which model was applicable) and the subset of the following variables which changed the univariate point estimate by at least 10% (15): year of birth, season of birth, gender, age at the time of check-up, asthma, allergy and/or eczema of the parents, level of occupation of the bread-winner, age of the mother at the time of delivery, level of urbanization, country of origin and DTPP vaccination status. A two-sided p-value of 0.05 was considered significant. For the two main risk factors (birth order and sibship size) also tests for linear trend were performed. We performed the multivariate analyses both with and without "breast-feeding for one month or more" in the model, as the causal pathway between breast-feeding and atopy is unclear, and may be in two directions: breast-feeding may have implications for the development of atopic disorder (13, 16), but on the other hand children who are at risk of developing an atopic disorder are more likely to have been breast fed.

The SPSS (version 10) and SAS (version 6.12) programs were used for all analyses.

Results

Data were obtained of 1961 children from 700 families. For 1727 of these children (88%) a health check-up file was available. The average age at which the health check-up was performed was 5.9 years (sd 0.6 years). 246 children (14%) reported at least one of the atopic disorders under study. Allergy has a strong relation with birth order: adjusted odds ratios 0.43, 0.26 and 0.05 for second-, third- and fourth- or higher borns, respectively (first-borns reference category); p-value for trend < 0.0001. Only sibship size was included in the multivariate model. Also allergy according to the more comprehensive definition (including eczema and food allergy) showed a clear relation with birth order (adjusted odds ratios 0.47, 0.35 and 0.39, p-value for trend=0.001). The odds ratio for birth order four and higher is somewhat raised due to more eczema in this category). For asthma there appeared no clear inverse relation with birth order. For none of the five outcome variables did addition of sibship size to the model substantially change the odds ratios for birth order. Table 1 lists the characteristics of the 700 families, table 2 gives data on their 1,961 children, and table 3 presents data on the health examinations. Table 4 shows prevalences, odds ratios and adjusted odds ratios for asthma, allergy (without food allergy and eczema), eczema (including food allergy), allergy (including eczema/food allergy), and any atopic disorder, by birth order.

Sibship size was not strongly related to the outcome variables. Only asthma seems to be less prevalent in larger families (after adjustment for birth order, age of the mother and age at the time of the check-up), although the relationship is not significant (p-value for trend: 0.06). For first-borns the prevalences of asthma were 10%, 7%, 6%, and 3% in families with 2, 3, 4, and 5 or more children, respectively (data not in table). Table 5 presents results by sibship size.

Inclusion of breast-feeding in the multivariate model made no difference to these outcomes.

Discussion

It is generally assumed that birth order is a risk factor for allergy but as yet no within-family study has demonstrated its effect independent of sibship size. We found that birth order, but not sibship size, is a risk factor for allergy. Our data show no relationship between birth order and asthma; however, there seems to be a lower prevalence of asthma in larger families, especially after adjustment for

Table 1 Data on 700 families with two or more children, one of which born in 1988-1990

		N	%
Sibship size (no. of children)	2	368	53
	3	210	30
	4	73	10
	5-9	49	7
Country of origin	The Netherlands	606	87
	Other	94	13
Mother atopic/asthmatic	Yes	103	15
	No	590	84
	Unknown	7	1
Father atopic/asthmatic	Yes	68	10
	No	625	89
	Unknown	7	1
Occupation level	Elementary	23	3
	Low	104	15
	Average	467	67
	High	73	10
	Academic	33	5
Urbanization	Heavily urbanized	339	49
	Moderately urbanized	358	51
	Unknown	3	0

Table 2 Data on the 1,961 children in the study

		N	%
Birth order	1	708	36
	2	711	36
	3	330	17
	4	128	7
	5-9	71	4
Year of birth	1969-75	17	1
	1976-80	53	3
	1981-85	270	14
	1986-90	1085	55
	1991-95	455	23
	1996-2000	77	4
	Unknown	4	0
Season of birth	June-September	619	32
	October-January	553	28
	February-May	633	32
	Unknown	156	8
Gender	Male	1011	52
	Female	944	48
	Unknown	6	0
Age (years) of mother at delivery	<26	400	20
	26-30	809	41
	31-35	567	29
	>35	149	8
	Unknown	36	2
Breast-feeding $\geq$1 month	Yes	896	46
	No	753	38
	Unknown	312	16
DTPP vaccination	Yes	1730	88
	No	46	2
	Unknown	185	9

Table 3 Data on health check-ups at age 6 years in 1,727 of the 1,961 children included in the study

		N	%
Health check-up file	Available	1727	88
	Not available	234	12
Data on 1727 children with available health check-up records:			
Asthma	Yes	153	9
	No	1574	91
Allergy*	Yes	77	4
	No	1650	96
Eczema and food allergy	Yes	106	6
	No	1623	94
Allergy, incl. food allergy and eczema	Yes	160	9
	No	1567	91
Asthma and/or any allergy and/or	Yes	246	14
Eczema	No	1481	86
			Mean (sd)
Age (years) at check-up		1727	5.9 (0.6)

* including: hay fever, rhinitis, house dust mite allergy, pet allergy, excluding food allergy/eczema

Table 4 Atopic disorders by birth order: prevalences (%), crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

	Asthma				Allergy (excl food allergy and eczema)				Allergy (incl. food allergy and eczema)				Asthma or allergy or eczema			
	%	OR	OR adj$	$CI_{95\%}$	%	OR	OR adj#	$CI_{95\%}$	%	OR	OR adj$$	$CI_{95\%}$	%	OR	OR adj##	$CI_{95\%}$
Birth order																
1 *	8.7	1.00	1.00	--	7.4	1.00	1.00	--	13.4	1.00	1.00	--	16.3	1.00	1.00	--
2	8.9	1.02	0.82	0.52-1.29	3.4	0.43	0.43	0.26-0.70	6.9	0.47	0.47	0.31-0.71	12.8	0.74	0.71	0.49-1.02
3	9.0	1.14	0.85	0.46-1.61	2.5	0.29	0.26	0.11-0.59	6.5	0.41	0.35	0.18-0.68	12.6	0.74	0.66	0.38-1.15
4+	9.0	1.35	1.26	0.51-3.11	0.6	0.06	0.05	0.01-0.37	7.2	0.45	0.39	0.16-0.96	14.5	0.92	0.93	0.45-1.95
p-value for trend			0.99				<0.0001				0.001				0.36	

* reference category

$ adjusted for sibship size, year of birth, age at check-up, age mother at delivery

adjusted for sibship size

$$ adjusted for sibship size, year of birth, season of birth, DTPP vaccination, age at check-up, atopy mother, age mother at delivery, country of origin, urbanization

adjusted for sibship size, year of birth, season of birth, DTPP vaccination, age at check-up, atopy father, age mother at delivery, country of origin, urbanization

Table 5 Atopic disorders by sibship size: prevalences (%), crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

	Asthma				Allergy (excl food allergy and eczema)				Allergy (incl. food allergy and eczema0				Asthma or allergy or eczema			
	%	OR	OR adj$	$CI_{95\%}$	%	OR	OR adj#	$CI_{95\%}$	%	OR	OR adj$$	$CI_{95\%}$	%	OR	OR adj##	$CI_{95\%}$
Sibship size																
2 *	10.2	1.00	1.00	--	4.8	1.00	1.00	--	9.4	1.00	1.00	–	15.5	1.00	1.00	--
3	7.8	0.73	0.68	0.41-1.11	4.2	0.89	0.99	0.55-1.80	8.5	0.89	0.92	0.59-1.42	12.2	0.75	0.71	0.48-1.06
4	8.4	0.81	0.63	0.32-1.23	4.4	0.92	1.59	0.70-3.60	10.2	1.10	1.34	0.74-2.42	15.6	1.01	0.95	0.57-1.59
5+	7.4	0.71	0.40	0.12-1.31	3.9	0.85	1.37	0.49-3.81	9.8	1.05	0.93	0.42-2.06	13.7	0.86	0.54	0.29-1.07
p-value for trend			0.06				0.34				0.77				0.11	

* reference category

$ adjusted for birth order, year of birth, DTPP vaccination, age at check-up, atopy mother, atopy father, age mother at delivery

\# adjusted for birth order, season of birth, DTPP vaccination, age at check-up, atopy mother, atopy father, age mother at delivery,country of origin, urbanization

$$ adjusted for birth order, year of birth, season of birth, atopy mother, atopy father, age mother at delivery, country of origin, level occupation, urbanization

adjusted for birth order, season of birth, DTPP vaccination, age at check-up, atopy mother, atopy father, age mother at delivery, country of origin, level occupation, urbanization

birth order, implying that first-borns in small families have a higher prevalence of asthma than first borns in large families. An explanation for this phenomenon could be that parents decide not to plan for more children if a serious disease in their first child becomes manifest.

We found a risk of eczema and food allergy for children with birth order four and higher which was higher than the risk for birth order two and three. We wondered whether this might be due to a different awareness over time (of doctor or parents). We therefore checked whether there was a raised risk of (diagnosis of) eczema over time by considering the fraction of eczema reporteded for each year of check-up for first borns and for second borns separately. This did not reveal any time dependent pattern. We can not explain our finding.

Three other studies have investigated the relationship between birth order and atopy within families (17,18,19). Two of these (17,18) selected families with at least one atopic child and did not consider the relationship between sibship size and atopy. The third study (19) evaluated the relationship between atopic disease and number of older siblings in a cross-sectional design, but evaluated children at different ages. After adjustment for other family characteristics (including "number of younger siblings" which is almost equivalent to adjustment for sibship size), they found a protective effect of high birth order only in children with atopic fathers. Our data do not confirm this finding: the relationship in children of non-atopic fathers was even somewhat stronger than in children of atopic fathers.

In the Netherlands the proportion of first borns has risen from 27% in 1950 to 45% in 1990, whereas the proportion of fourth (and higher) borns was 31% in 1950 and 7% in 1990 (20). If we assume that the prevalences and odds ratios for allergy (excl. eczema/food allergy) found in this study apply to the period from 1950 to 1990 and that children without siblings (a category we did not consider) have the same risk as first-borns in larger sibships, the increase of allergy during these years due to this reduced family size would be 41%.

We considered it unlikely that breast-feeding is just a confounder. The causal path between atopy and breast-feeding is probably in two directions: breast-feeding may have implications for atopy (13, 16), but on the other hand mothers of children at risk may be recommended to start or continue breast-feeding. Therefore, we analysed our data with and without "breast-feeding for at least one month" in the multivariate model. Adjustment for breast-feeding resulted in similar odds ratios. Breast-feeding appeared protective for asthma (6% vs 12%) and for any atopic disorder (12% vs 17%). Thus, if we could

eliminate this "confounding by indication", the protective effect might be even larger.

Misclassification

The data of this study are extracted from routinely collected records, and inaccurate reporting or underreporting of the disorders under study may have occurred. The prevalences of 9%, 4%, 6% and 14% for asthma, allergy, eczema and any atopic disease, respectively, suggest that there may be limited underreporting as higher prevalences have been published for the period most of the children in our study had their health examination (21, 22). This does not necessarily invalidate our findings, as misclassification is unlikely to be differential, i.e. related to birth order or sibship size. An increased underreporting of disease with increasing birth order (the mother "getting used" to diseases of her children) would explain the lower prevalence in children with higher birth order. However, results of an unpublished study conducted in 1998/1999 by physicians of the Municipal Health Service, where we extracted the data, suggest that there is no need for this concern (personal communication H. Aardoom, May 2002). They asked the accompanying parent of 400 children during the health check-up to fill out the ISAAC questionnaire for asthma symptoms (23). Self-reporting slightly increased with increasing birth order and increasing sibship size.

These routinely collected records inevitably have limitations. They did not provide information on relevant conditions in the child's early life, apart from breast feeding. One can argue that these conditions usually don't change dramatically within families. On the other hand, a change in smoking habits or disposal of pets in case of an allergic first born can not be excluded.

In conclusion, we demonstrated that birth order is inversely related to the risk of allergy, independent of the size of the sibship. However, it is still a question whether (within families) the reduced risk of later born children is due to an increased exposure to pathogens or to a with increasing parity changing prenatal mother-child interaction. Further research is needed to detect which antenatal and/or environmental factors explain this relationship. The answer to this question could help us in the prevention of atopic disorders.

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References

1. Golding J, Peters T. Eczema and hay fever. In: Butler NR, Golding J, eds. From birth to five, a study of the health and behaviour of Britain's 5-year-olds. Oxford: Pergamon Press, 1986:171-86.
2. Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidemiol Community Health 2002;56:209-17.
3. Strachan DP. Hay fever, hygiene, and household size. Br Med J 1989: 299: 1259-60.
4. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". Thorax 2000;55 Suppl 1:S2-10.
5. Karmaus W, Arshad H, Mattes J. Does the sibling effect have its origin in utero? Investigating birth order, cord blood immunoglobulin E concentration, and allergic sensitization at age 4 years. Am J Epidemiol 2001;154:909-15.
6. Sunyer J, Anto JM, Harris J, Torrent M, Vall O, Cullinan P, Newman-Taylor A. Maternal atopy and parity. Asthma Multi-centre Infants Cohort Study. Clin Exp Allergy 2001;31:1352-5.
7. Ponsonby AL, Couper D, Dwyer T, Carmichael A. Cross-sectional study of the relation between sibling number and asthma, hay fever and eczema. Arch Dis Child 1998;79:328-33.
8. Kerncijfers vierpositie postcodegebieden. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 1995.
9. Standaard Beroepenclassificatie. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 1992.
10. Smith JM, Springett VH. Atopic disease and month of birth. Clin Allergy 1979;9:153-7.
11. Pedersen PA, Weeke ER. Month of birth in asthma and allergic rhinitis. Scand J Prim Health Care 1983;1:97-101.
12. Korsgaard J, Dahl R. Sensitivity to house dust mite and grass pollen in adults: influence of the month of birth. Clin Allergy 1983;13:529-35.

13. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 2002;360:901-7.
14. Diggle PJ, Liang KY, Zeger SL. Analysis of Longitudinal Data. Oxford: Clarendon Press 1994.
15. Rothman K. Modern Epidemiology. Philadelphia: Lippincott-Raven 1998:256-7.
16. Schoetzau A, Filipiak-Pittroff B, Franke K, Koletzko S, Von Berg A, Gruebl A, Bauer CP, Berdel D, Reinhardt D, Wichmann HE; German Infant Nutritional Intervention Study Group. Effect of exclusive breast-feeding and early solid food avoidance on the incidence of atopic dermatitis in high-risk infants at 1 year of age. Pediatr Allergy Immunol 2002;13:234-42.
17. Olesen AB, Ellingsen AR, Larsen FS, Larsen PO, Veien NK, Thestrup-Pedersen K. Atopic dermatitis may be linked to whether a child is first- or second-born and/or the age of the mother. Acta Derm Venereol 1996;76:457-60.
18. Bradley M, Kockum I, Soderhall C, et al. Characterization by phenotype of families with atopic dermatitis. Acta Derm Venereol 2000;80:106-10.
19. Mattes J, Karmaus W, Moseler M, Frischer T, Kuehr J. Accumulation of atopic disorders within families: a sibling effect only in the offspring of atopic fathers. Clin Exp Allergy 1998;28:1480-6.
20. Kerncijfers bevolking. Voorburg/Heerlen: Centraal Bureau voor de Statistiek 2002.
21. Anonymous. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998;12:315-35.
22. Duhme H, Weiland SK, Rudolph P, Wienke A, Kramer A, Keil U. Asthma and allergies among children in West and East Germany: a comparison between Munster and Greifswald using the ISAAC phase I protocol. International Study of Asthma and Allergies in Childhood. Eur Respir J 1998;11:840-7.
23. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.

6

Perinatal characteristics and obstetric complications as risk factors for asthma, allergy and eczema at the age of six years

Abstract

Background: Considerable effort has been put into identifying early determinants for atopic disorders. Many studies have evaluated the role of foetal development and obstetric complications. However, the results are not unequivocal.

Study objective: To assess the relationship between perinatal characteristics and obstetric complications, and the presence of reported current asthma, allergy and eczema at the age of six years in the framework of a previously conducted study.

Method: 700 families in the Netherlands with index children born in 1988-1990 were retrospectively selected. Data were extracted from the Municipal Health Service's records of health examinations of these children and their siblings. These examinations were carried out at the age of six years. The records contained data on reported atopic disorders and perinatal characteristics.

Results: Gestational age was inversely related to the risk of asthma (p for trend: 0.03). Children with low birth weight tended to have a lower risk of any allergy, albeit not significant (p=0.07). However, no link was found between neonatal head circumference and atopic disorders. The ratio of neonatal head circumference to birth weight was positively associated with the risk of atopic disorders, especially with the risk of asthma (OR=1.87 $CI_{95\%}$ = [1.11, 3.15]). Vacuum extraction was a risk factor for allergy (OR=1.84, $CI_{95\%}$ = [1.03, 3.28]), but not for asthma. Induced labour was positively associated with the risk of inhalant allergy (OR=2.22, $CI_{95\%}$ = [1.09, 4.51]) and, to a lesser extent, asthma (OR=1.72, $CI_{95\%}$ = [0.95, 3.10]). For caesarean section and forcipal extraction there were no such relationships.

Conclusions: Prematurity is a risk factor for asthma reported at six years. A high ratio of head circumference to birth weight is a risk factor for any atopic disorder. Vacuum extraction was associated with a higher risk of allergy, and induced labour is a risk factor for inhalant allergy. All results should be viewed with the possibility of residual confounding.

Keywords: Gestational age, birth weight, neonatal head circumference, asthma, allergy, eczema.

In press, Clin Exp Allergy

Introduction

The prevalence of asthma and allergic disorders has increased over the past decades and much effort has gone into identifying risk factors for these disorders. The relationship between fetal development and obstetric complications with childhood asthma and atopic disorders has been studied extensively (1-27). Results are not unequivocal: although several studies have reported that low gestational age (or low birth weight) is a risk factor for the development of subsequent asthma (3, 4, 6, 11, 20, 21), others could not confirm this relationship (8, 10, 13, 16, 27). Similarly, although some studies have found a link between high gestational age (or high birth weight) and either a high risk of asthma, atopic disease or a high IgE concentration (5, 9, 16, 21), other studies have not (7, 8, 12, 13). A positive association between neonatal head circumference and total IgE concentration was first reported in 1994 (5) and confirmed by others (12-14). This finding raised the hypothesis that this positive association might reflect the long-term effect of sustaining fetal brain growth at the expense of the trunk, in particular the thymus (5). More recently, this hypothesis was challenged by the finding that thymus size is not related to allergic disease (18) and that the relationship between the ratio of head circumference to birth weight and hay fever is negative (24).

Several studies found that caesarean section is a risk factor for subsequent development of asthma (15, 19, 20, 23, 26) and allergic disease (18, 23, 24), although this last result was not confirmed by Xu et al (19). Other complications during labour (vacuum extraction, forcipal extraction and induced labour) were also found to be positively related to the risk of asthma (15, 20).

Within the framework of a retrospective study of birth order and sibship size as risk factors for reported asthma, allergy, and eczema in children (28) we recorded various perinatal characteristics (gestational age, birth weight and neonatal head circumference) and obstetric complications. Because of the lack of consensus mentioned above, we aimed to contribute a piece of evidence to the bulk of results that already exists. The current study focused on the relationships between perinatal characteristics and obstetric complications on the one hand and asthma, allergy, and eczema as reported during health examinations at age six years on the other.

Methods

Study area and population

Population, sample size calculation and blinding have been described in detail elsewhere (28). Briefly, data on health check-ups in children of 700 families were extracted from the files of a Municipal Health Service in the Netherlands. Only families with at least two children were included and index children were born in 1988-1990. The files contained health data from birth up to the examination at the age of six years. These examinations are approximately monthly during the first year of life, but primary school children get only one routine comprehensive examination at six years.

Data extraction

All data, including those on current atopic disorders, were extracted from the records as reported by the physician who did the check-up, or as reported in a questionnaire filled out by the parents, or as reported in letter(s) from a paediatrician/pulmonologist that was included in the file. The following items were extracted: gestational age, birth weight, neonatal head circumference, obstetric complications (such as caesarean section, vacuum extraction, forcipal extraction and induced labour), birth order, sibship size, date of birth, gender, year of birth of the mother, ZIP code, parents' country of birth, breadwinner's occupation, atopic disorders in the parents, diphtheria tetanus pertussis poliomyelitis (DTPP) vaccination status and duration of breast-feeding, diagnosis of current asthma, allergy, eczema and specific medication for these disorders at the age of six years and date of check-up. Length at birth was also recorded in the files, but we did not use this characteristic, as it is an unreliable measure (29).

Statistical analysis

We evaluated the relationship between perinatal characteristics (gestational age, birth weight, neonatal head circumference and the ratio of neonatal head circumference to birth weight) and obstetric complications (caesarean section, vacuum extraction, use of forceps, induced labour) on the one hand and, on the other, reported asthma, inhalant allergy (including house-dust mite, pollen, furry pets, hay fever, rhinitis), eczema (including food allergy), allergy (inhalant allergy and/or eczema), and any atopic disorder (asthma and/or allergy). Gestational age was categorized as follows: <36 weeks, 36-<41 weeks and ≥41 weeks. Birth weight was categorized in <2500 grams, 2500-<4500 grams and ≥4500 grams, head circumference in <37 cm and ≥37 cm, and the ratio of head circumference and birth weight in below the median and greater than or equal to

the median. The analysis was carried out within families by means of logistic regression for correlated measurements (Generalized Estimating Equations) (30). With this technique correlation between family members is taken into account. Analyses were performed both in univariate and multivariate models with adjustments for the subset of the following possible confounders which changed the univariate point estimate by at least 10% (31): sibship size, birth order, year of birth, season of birth, gender, age at the time of check-up, atopic disease of the parents, level of occupation of the bread-winner, age of the mother at the time of delivery, level of urbanization, country of origin and DTPP vaccination status. For the relationships with obstetric complications we also considered gestational age, birth weight, neonatal head circumference and the ratio of neonatal head circumference to birth weight as possible confounders. However, because obstetric complications are likely to be intermediate variables in the relationship between perinatal data and atopic disorders, these complications were not considered as confounders. Similarly, in the relationships between perinatal data and atopic disorders, we did not consider the remaining perinatal data as confounders, because these variables are very likely to be strongly correlated and including them in the model could obscure possible relationships. A two-sided p-value of 0.05 was considered significant. For the risk factors with more than two categories (gestational age and birth weight) we also performed tests for linear trend. Breast-feeding was excluded from the model for two reasons: Firstly, the causal pathway between breast feeding and atopy is unclear, and may be in two directions: breast-feeding may have implications for the development of atopic disorders (32, 33), but on the other hand mothers of children who are at risk of developing an atopic disorder are commonly advised to breastfeed (34); secondly, breast-feeding may act as an intermediate variable in the relationship between perinatal factors and allergic disorders: adjustment for this variable could conceal any of the relations studied.

For many children one or more of the perinatal characteristics was missing and consequently sometimes an adjusted analysis became problematic. In case no adjusted analysis was feasible we used a multiple imputation technique with five imputed data sets (35). Analyses were carried out for these five sets and results were pooled. Imputation was done with IVEware version 2.0. For all other analyses SPSS (version 11) and SAS (version 8.2) were used.

Results

Data were obtained on 1,961 children from 700 families. At least one atopic parent was present in 150 families (21%); of 606 families (87%) both parents were born in the Netherlands. Health check-up data at the age of six years were available for 1,727 of the children (88%). The average age at which the health check-up was performed was 5.9 years (sd 0.6 years). Gestational age was recorded for 1,666 children (85%), birth weight for 1,710 children (87%) and neonatal head circumference for 861 children (44%). For 246 children (14%) at least one of the atopic disorders under study was reported.
For further details of the 700 families and their 1,961 children we refer to a previous publication on this study (28). Perinatal characteristics and obstetric complications are presented in table 1, data on the health examinations at age six in table 2.

Relations of perinatal data and atopy

Table 3 shows the relationships (prevalences, ORs and adjusted ORs) between the perinatal data and asthma, inhalant allergy, eczema, any allergy, or any atopic disorder.

Gestational age was inversely related to the risk of asthma (p for trend:0.03): children born after a gestational period of less than 36 weeks had a higher risk of asthma (OR=2.03, $CI_{95\%}$ = [1.03, 4.01]), whereas children born after a gestational period of 41 weeks or more had a lower risk (OR=0.71, $CI_{95\%}$ = [0.44, 1.14]) .

Children with low birth weight tended to have a lower risk of allergy compared to children with normal birth weight, although the relationship was not significant (p=0.07). This relationship was of similar magnitude in the subgroup of children of normal gestational age (data not shown).

Neonatal head circumference showed no significant relationships with the outcome variables. However the ratio of neonatal head circumference to birth weight showed a significant relationship with atopic disorders: children born with a large head circumference relative to their weight had a higher risk of asthma and "any atopic disorder".

Obstetric complications

After adjustment for confounders neither caesarean section nor forcipal extraction were related to the outcome variables (table 4). However, vacuum extraction was related to a higher risk of allergy, whereas induced labour was associated with a higher risk of inhalant allergy. There was some evidence of a

Table 1 Perinatal data and obstetric complications

		N	%
Perinatal data			
Gestational age	< 36weeks	62	3
	36-< 41weeks	1250	64
	≥ 41weeks	354	18
	Unknown	295	15
Birth weight	< 2500gr	81	4
	2500-< 4500gr	1601	82
	≥ 4500gr	28	1
	Unknown	251	13
Neonatal head circumference	< 37cm	788	40
	≥ 37cm	73	4
	Unknown	1100	56
Ratio head circumference to	< median	429	22
birth weight (cm/gr)	≥ median	432	22
(median=0.01015)	Unknown	1100	56
Obstetric complications			
Caesarean section	Yes	85	4
	No	1627	83
	Unknown	249	13
Vacuum extraction	Yes	92	5
	No	1620	83
	Unknown	249	13
Forcipal extraction	Yes	26	1
	No	1686	86
	Unknown	249	13
Induced labour	Yes	129	7
	No	1583	81
	Unknown	249	13

Table 2 Data on health check-ups at age 6 years in 1,727 of the 1,961 children included in the study

		N	%
Health check-up file	Available	1727	88
	Not available	234	12
Data on 1,727 children from available health check-up records:			
Asthma	Yes	153	9
	No	1574	91
Inhalant allergy	Yes	77	4
	No	1650	96
Eczema	Yes	106	6
	No	1621	94
Allergy*	Yes	160	9
	No	1567	91
Atopic disorder (any)	Yes	246	14
	No	1481	86
			Mean (sd)
Age (years) at check-up		1727	5.9 (0.6)

*inhalant allergy and/or eczema

Table 3 Atopic disorders by perinatal data: prevalences (%), crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

	Asthma				Inhalant allergy				Eczema				Allergy (inh.allergy and/or eczema)				Any atopic disorder			
	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$
Gestational age (weeks)																				
< 36	18.3	2.28	2.03	1.03-4.01	3.3	0.67	0.58	0.17-1.96	1.7	0.27	0.30	0.14-1.97	3.3	0.32	0.32	0.09-1.17	18.3	1.29	1.13	0.55-2.32
36 -< 41*	9.2	1.00	1.00	-	5.1	1.00	1.00	-	6.1	1.00	1.00	-	9.8	1.00	1.00	-	14.8	1.00	1.00	-
≥ 41	6.0	0.73	0.71	0.44-1.14	3.2	0.63	0.60	0.31-1.16	6.3	1.04	1.06	0.64-1.74	8.0	0.81	0.78	0.50-1.20	11.2	0.77	0.77	0.53-1.12
p-value for trend		0.03	0.03			0.24	0.22			0.37	0.39			0.92	0.77			0.12	0.16	
Birth weight (grams)																				
< 2500	12.3	1.32	1.35	0.60-3.00	3.7	0.75	0.67	0.20-2.25	2.5	0.40	0.38	0.09-1.57	3.7	0.37	0.34	0.11-1.09	12.3	0.81	0.79	0.37-1.69
2500-< 4500*	8.6	1.00	1.00	-	4.7	1.00	1.00	-	6.2	1.00	1.00	-	9.5	1.00	1.00	-	14.1	1.00	1.00	-
≥ 4500	3.7	0.55	0.41	0.07-2.44	0.0	#0.37	-	-	7.4	1.21	1.54	0.35-6.76	7.4	0.77	0.95	0.22-4.10	7.4	0.52	0.52	0.13-2.05
p-value for trend		0.33	0.24			0.75				0.12	0.09			0.09	0.05			0.98	0.93	
Head circumfer. (cm)																				
< 37cm *	8.7	1.00	1.00	-	4.7	1.00	1.00	-	6.3	1.00	1.00	-	9.7	1.00	1.00	-	14.3	1.00	1.00	
≥ 37cm	9.1	1.11	1.02	0.34-2.80	4.5	0.92	0.72	0.20-2.55	4.5	0.75	0.72	0.22-2.32	9.1	0.94	0.82	0.33-1.99	12.1	0.87	0.80	0.34-1.84
p-value		0.81	0.96			0.89	0.61			0.63	0.58			0.89	0.66			0.73	0.59	
Ratio headc./ birth weight																				
< median *	6.3	1.00	1.00	-	3.4	1.00	1.00		5.1	1.00	1.00	-	7.5	1.00	1.00		11.1	1.00	1.00	-
≥ median	11.1	1.80	1.87	1.11-3.15	6.0	2.01	1.61	0.81-3.19	7.2	1.46	1.25	0.20-2.24	11.8	1.68	1.50	0.93-2.44	17.1	1.68	1.68	1.11-2.54
p-value		0.02	0.02			0.03	0.17			0.20	0.45			0.03	0.10			0.01	0.01	

*reference category

Due to the absence of children allergic for airborn allergens in the group with high birth weight (both in the original and in the imputed data) no OR could be estimated by the usual method. We omitted the multivariate analysis for this relation and only estimated a univariate OR and $CI_{95\%}$ (0.02-6.11) by adding 0.5 to all cells, also ignoring the correlation between family members.

Table 4 Atopic disorders by obstetric complications: prevalences (%), crude and adjusted (adj) odds ratios (OR) with 95% confidence intervals ($CI_{95\%}$)

	Asthma				Inhalant allergy				Eczema				Allergy (inh.allergy and/or eczema)				Any atopic disorder			
	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$	%	OR	OR adj	$CI_{95\%}$
Caesarean section																				
No*	8.5	1.00	1.00	-	4.7	1.00	1.00	-	6.1	1.00	1.00	-	9.2	1.00	1.00	-	13.8	1.00	1.00	-
Yes	12.3	1.31	#1.03	0.51-2.08	2.5	0.41	0.57	0.12-2.76	4.9	0.80	0.94	0.26-3.33	7.4	0.73	0.86	0.31-2.40	17.3	1.14	#1.12	0.56-2.24
p-value		0.50	0.93			0.28	0.48			0.73	0.40			0.53	0.77			0.71	0.74	
Vacuum extraction																				
No*	8.7	1.00	1.00	-	4.3	1.00	1.00	-	5.8	1.00	1.00	-	8.7	1.00	1.00	-	13.7	1.00	1.00	-
Yes	8.7	1.03	#0.92	0.43-1.98	8.7	2.20	1.53	0.68-3.44	9.8	1.83	1.67	0.83-3.35	17.4	2.29	1.84	1.03-3.28	19.6	1.61	1.68	1.01-2.79
p-value		0.94	0.83			0.04	0.31			0.08	0.15			0.003	0.04			0.05	0.04	
Forcipal extraction																				
No*	8.6	1.00	1.00	-	4.6	1.00	1.00	-	6.0	1.00	1.00	-	9.2	1.00	1.00		13.9	1.00	1.00	-
Yes	15.4	1.14	#1.44	0.48-4.28	3.8	0.88	#0.77	0.10-5.93	3.8	0.74	0.59	0.09-3.94	7.7	0.91	#0.62	0.14-2.65	19.2	1.22	0.98	0.30-3.14
p-value		0.84	0.52			0.90	0.80			0.74	0.59			0.89	0.52			0.71	0.97	
Induced labour																				
No*	8.1	1.00	1.00	-	4.2	1.00	1.00	-	6.1	1.00	1.00	-	9.1	1.00	1.00	-	13.5	1.00	1.00	-
Yes	17.2	1.78	1.72	0.95-3.10	8.6	1.93	2.22	1.09-4.51	5.2	0.86	1.47	0.50-4.36	10.3	1.07	1.25	0.66-2.37	20.7	1.42	1.49	0.91-2.44
p-value		0.04	0.07			0.07	0.03			0.72	0.49			0.83	0.50			0.15	0.12	

* reference category

Due to lack of data no estimations were possible in the adjusted model; we used the five imputed data sets in order to estimate the ORs and $CI_{95\%}$

higher risk of asthma in children delivered after induced labour, although this was not significant in the adjusted model (p=0.07).

Discussion

The present study showed that gestational age is inversely related to the risk of asthma and that a high ratio of neonatal head circumference to birth weight is a risk factor for atopic disorders, especially asthma. Both vacuum extraction and induced labour appeared to be related to a higher risk of allergic disease.

Perinatal data

Gestational age was in the present study shown to be related to a higher risk of asthma at six years. This is a confirmation of earlier findings (4, 6). One study relating gestational age to asthma in adulthood reported a similar result (11), but two other studies did not find this relation (10, 16). Kuehr et al reported an increased sensitization to aeroallergens in children born with a low gestational age (2). High gestational age was found to be associated with the risk of atopic dermatitis (9) and atopy (16), low gestational age was reported to be protective for rhinitis (11). Even though the observed relationships were not significant, our data tend to support these findings. A lack of association was reported earlier: between gestational age and the risk of sensitization to aeroallergens (7) and the risk of any atopy (8).

Low birth weight has been shown to be associated with a higher risk of asthma (1,3,4,6,20,21), whereas high birth weight has been shown to be positively related to total IgE (5), the risk of atopic dermatitis (9) and the risk of asthma (12,21). The result of the present study, showing an inverse relationship between birth weight and the risk of asthma, although not statistically significant, is in concordance with the literature. Although a higher risk of asthma or a higher risk of atopic dermatitis in children with high birth weight could not be confirmed in this study, we observed a lower risk of eczema in low birth weight children. This was also reported by Siltanen et al (17). An explanation for the higher risk of asthma in prematurely born children and low birth weight children might be that these conditions are characterized by disturbed lung growth and result in reduced lung function. Small airway size gives rise to wheezing symptoms during viral infections, especially in the first year of life, and this is often labelled as asthma (36). The inconsistency of study results concerning the relationship between birth weight and asthma might be explained by the different phenotypes of asthma: a higher risk of non-atopic asthma in low birth weight groups and, seeing the

positive relationship between birth weight and IgE, a higher risk of atopic asthma in high birth weight groups could be conceivable.

Neonatal head circumference was not found to be related to any of the outcome variables in the present study. Several studies have reported positive relationships between neonatal head circumference and the risk of asthma or increased total IgE (5,8,12,13,14), although the findings are not unequivocal. Some studies reported the lack of a relationship with asthma (10,12,13) or atopic disorders (8,12,13). The positive relationship between neonatal head circumference and total IgE concentration has raised the hypothesis, put forward by Godfrey et al (5), that a sustaining brain growth at the expense of the trunk, in particular the thymus, promotes the development of atopic disease. However, this hypothesis has been falsified by Benn et al (18) who found a positive relationship between neonatal head circumference and thymus size in the first week of life. He also showed that neonatal thymus size is not associated with allergic disease at the age of five years. Katz et al (24) examined the relationship between hayfever and the ratio of neonatal head circumference to birth weight, a measurement which better reflects the relative growth of the brain than absolute neonatal head circumference. They found that children with a high ratio had a lower risk of hay fever. Our own results suggest an increased risk of atopy in newborns with a relatively large ratio of neonatal head circumference to birth weight and hence support the hypothesis put forward by Godfrey et al (5) .

Obstetrical complications

Children delivered by caesarean section have a higher risk of asthma (15, 19, 23, 26), and allergy (18, 23). Other obstetric complications are also reported to be associated with a higher risk of asthma (15, 20). Although, in line with other studies (15, 19, 23, 26), we found a 50% higher prevalence of asthma in children delivered by caesarean section, the relationship disappeared after adjustment for confounding factors. One of these confounding factors was the ratio of neonatal head circumference to birth weight in the multivariate model. However, neither this measure nor neonatal head circumference alone was considered as a confounder in the four studies mentioned. We therefore suggest that the excess risk found in other studies may be due to residual confounding.

Like Xu et al (19) we did not observe a relationship between caesarean section and allergy. Unlike them (15), however, we did not find an excess risk of asthma in children delivered with vacuum extraction, although we did find an increased risk of allergy in such children. Children delivered after induced labour had a higher risk of inhalant allergy and (to a lesser extent) asthma; Annesi-

Maesano et al (20) also found a weak relationship with asthma. The mechanisms underlying the relationships between obstetric complications and atopic disorders are unclear. It has been suggested that incubator care, being more probable after a delivery with complications, might delay the contact of the newborn with microbes and thus affect the Th2→ Th1 shift (15).

Limitations of this study

As stated before (28) the data used in this study were obtained from routinely collected records and we cannot exclude inaccuracy of data, including under- or overreporting of the diseases under study. Therefore it is possible that some of the relationships found in this study are due to residual confounding.

Selection bias cannot be excluded, because, due to the study design, families with only one child were not included. Consequently possibly atopic children with perinatal problems are underrepresented in this study. This may have attenuated the resulting relationships.

Neonatal head circumference was often not recorded, because this measurement is generally not performed in home deliveries. Firstly this affected the power of the statistical tests. Secondly, while analyses with this variable were probably restricted to a selected population (i.e. hospital deliveries), this could have yielded spurious results. However, missing data on neonatal head circumference was not related to any of the outcome measures, and hence selection bias can be excluded. Provided a report of the delivery was available, the other perinatal variables were complete. The lack of a delivery report is unlikely to be related to the health status of the newborn or the child at the age of six years: files with missing delivery reports were either very old or contained data of children born in a different region or country.

No data were available on smoking by the mother during pregnancy. Intrauterine exposure to tobacco components is related to both birth weight and childhood respiratory symptoms (37) and should therefore be considered as an important confounder in the relationship between asthma and birth weight. Therefore the weak relationship observed between birth weight and asthma could be due to residual confounding. However, other studies which did adjust for smoking during pregnancy reported the same relationship.

Because no distinction could be made between the different phenotypes of asthma at the age of six years and observations at earlier ages were lacking, we could not examine associations pertaining to a specific phenotype.

Conclusion

Prematurity is a risk factor for asthma at six years, and special procedures during delivery, such as vacuum extraction and induced labour, are associated with a higher risk of atopic disorders. We also demonstrated that a high ratio of neonatal head circumference to birth weight is related to a higher risk of atopic disorders. Although some of these results confirm earlier findings, all results should be viewed with the possibility of residual confounding. Therefore further research into these relationships and their underlying mechanisms is needed. A better understanding could contribute to the prevention of atopic diseases.

References

1. Schwartz J, Gold D, Dockery DW, Weiss ST, Speizer FE. Predictors of asthma and persistent wheeze in a national sample of children in the United States. Association with social class, perinatal events, and race. Am Rev Respir Dis 1990;142:555-62.
2. Kuehr J, Frischer T, Karmaus W, Meinert R, Barth R, Herrmann-Kunz E, Forster J, Urbanek R. Early childhood risk factors for sensitization at school age. J Allergy Clin Immunol 1992;90:358-63.
3. Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. Clin Exp Allergy 1993;23:504-11.
4. von Mutius E, Nicolai T, Martinez FD. Prematurity as a risk factor for asthma in preadolescent children. J Pediatr 1993;123:223-9.
5. Godfrey KM, Barker DJ, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994;24:641-8.
6. Schaubel D, Johansen H, Dutta M, Desmeules M, Becker A, Mao Y. Neonatal characteristics as risk factors for preschool asthma. J Asthma 1996;33:255-64.
7. Strachan DP, Harkins LS, Johnston ID, Anderson HR. Childhood antecedents of allergic sensitization in young British adults. J Allergy Clin Immunol 1997;99:6-12.
8. Fergusson DM, Crane J, Beasley R, Horwood LJ. Perinatal factors and atopic disease in childhood. Clin Exp Allergy 1997;27:1394-401.
9. Olesen AB, Ellingsen AR, Olesen H, Juul S, Thestrup-Pedersen K. Atopic dermatitis and birth factors: historical follow up by record linkage. Br Med J 1997;314:1003-8.

10. Hagstrom B, Nyberg P, Nilsson PM. Asthma in adult life--is there an association with birth weight? Scand J Prim Health Care 1998;16:117-20.
11. Braback L, Hedberg A. Perinatal risk factors for atopic disease in conscripts. Clin Exp Allergy 1998;28:936-42.
12. Leadbitter P, Pearce N, Cheng S, Sears MR, Holdaway MD, Flannery EM, Herbison GP, Beasley R. Relationship between fetal growth and the development of asthma and atopy in childhood. Thorax 1999;54:905-10.
13. Gregory A, Doull I, Pearce N, Cheng S, Leadbitter P, Holgate S, Beasley R. The relationship between anthropometric measurements at birth: asthma and atopy in childhood. Clin Exp Allergy 1999;29:330-3.
14. Oryszczyn MP, Annesi-Maesano I, Campagna D, Sahuquillo J, Huel G, Kauffmann F. Head circumference at birth and maternal factors related to cord blood total IgE. Clin Exp Allergy 1999;29:334-41.
15. Xu B, Pekkanen J, Jarvelin MR. Obstetric complications and asthma in childhood. J Asthma 2000;37:589-94.
16. Pekkanen J, Xu B, Jarvelin MR. Gestational age and occurrence of atopy at age 31 - a prospective birth cohort study in Finland. Clin Exp Allergy 2001;31:95-102.
17. Siltanen M, Kajosaari M, Pohjavuori M, Savilahti E. Prematurity at birth reduces the long-term risk of atopy. J Allergy Clin Immunol 2001;107:229-34.
18. Benn CS, Jeppesen DL, Hasselbalch H, Olesen AB, Nielsen J, Bjorksten B, Lisse I, Aaby P. Thymus size and head circumference at birth and the development of allergic diseases. Clin Exp Allergy 2001;31:1862-6.
19. Xu B, Pekkanen J, Hartikainen AL, Jarvelin MR. Caesarean section and risk of asthma and allergy in adulthood. J Allergy Clin Immunol 2001;107:732-3.
20. Annesi-Maesano I, Moreau D, Strachan D. In utero and perinatal complications preceding asthma. Allergy 2001;56:491-7.
21. Xu B, Pekkanen J, Laitinen J, Jarvelin MR. Body build from birth to adulthood and risk of asthma. Eur J Public Health 2002;12:166-70.
22. Hesselmar B, Dahlgren J, Wennergren G, Aberg N, Albertsson-Wiklan K. Born small for gestational age: relation to future allergy and asthma. Acta Paediatr 2002;91:992-4.
23. Kero J, Gissler M, Gronlund MM, Kero P, Koskinen P, Hemminki E, Isolauri E. Mode of delivery and asthma - is there a connection? Pediatr Res 2002;52:6-11.

24. Katz KA, Pocock SJ, Strachan DP. Neonatal head circumference, neonatal weight, and risk of hayfever, asthma and eczema in a large cohort of adolescents from Sheffield, England. Clin Exp Allergy 2003;33:737-45.
25. Edwards CA, Osman LM, Godden DJ, Campbell DM, Douglas JG. Relationship between birth weight and adult lung function: controlling for maternal factors. Thorax 2003;58:1061-5.
26. Hakansson S, Kallen K. Caesarean section increases the risk of hospital care in childhood for asthma and gastroenteritis. Clin Exp Allergy 2003;33:757-64.
27. Sin DD, Spier S, Svenson LW, Schopflocher DP, Senthilselvan A, Cowie RL, Man SF. The relationship between birth weight and childhood asthma: a population-based cohort study. Arch Pediatr Adolesc Med 2004;158:60-4.
28. Bernsen RM, de Jongste JC, van der Wouden JC. Birth order and sibship size as independent risk factors for asthma, allergy, and eczema. Pediatr Allergy Immunol 2003;14:464-9.
29. Johnson TS, Engstrom JL, Haney SL, Mulcrone SL. Reliability of three length measurement techniques in term infants. Pediatr Nurs 1999;25:13-7.
30. Diggle PJ, Liang KY, Zeger SL. Analysis of Longitudinal Data. Oxford: Clarendon Press 1994.
31. Rothman K. Modern Epidemiology. Philadelphia: Lippincott-Raven 1998: 256-7.
32. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. Lancet 2002;360:901-7.
33. Schoetzau A, Filipiak-Pittroff B, Franke K, Koletzko S, Von Berg A, Gruebl A, Bauer CP, Berdel D, Reinhardt D, Wichmann HE; German Infant Nutritional Intervention Study Group. Effect of exclusive breast-feeding and early solid food avoidance on the incidence of atopic dermatitis in high-risk infants at 1 year of age. Pediatr Allergy Immunol 2002;13:234-42.
34. NHG-standaard "Voedselovergevoeligheid bij zuigelingen". Huisarts Wet 1995;38:178-84.
35. Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat Med 1991;10:585-98.
36. Merkus PJ, ten Have-Opbroek AA, Quanjer PH. Human lung growth: a review. Pediatr Pulmonol 1996;21:383-97.

37. Kurz H, Frischer T, Huber WD, Gotz M. Adverse health effects in children caused by passive smoking. Review in German. Wien Med Wochenschr 1994;144:531-4.

7

General Discussion

Vaccinations

DTPPo

In chapter 2 we concluded that, at primary school age, DTPPo vaccinated children were not more often atopic than unvaccinated children. As this study was conducted in a religious group in The Netherlands, where part of the population refuses vaccinations for religious reasons, the problem of confounding by indication, which may have biased other studies, was no reason for concern (1,2). Moreover, the majority of the group that was not vaccinated for DTPPo did not receive other childhood vaccinations either, so our findings also appear to exonerate additives used in these vaccines, such as aluminium containing adjuvants. This conclusion is very reassuring for parents who fear possible long term adverse effects of vaccinations for their children and, if communicated well, our finding may help reverse the declining vaccination coverage.

In an unvaccinated subgroup of study population II (chapter 3) and a subgroup of study population I (chapter 2) we even found that at primary school age vaccinated children were less atopic than their unvaccinated counterparts. Although the basis of this conclusion is not very robust because of the small numbers in these subgroups, similar results were obtained in the MAS-cohort (3) and in a worldwide ecological study (4). This result would fit well in the hygiene hypothesis as vaccinations expose children at a very young age to (attenuated) pathogens, thus stimulating their immune system to the development of IL-10 producing T-regulatory cells. If this is a "true" difference between vaccinated and unvaccinated children, we may speculate that the reason why we did not find this effect in the reported symptoms in chapter 2 is that reports obtained from parents may be biased towards a "desirable" study conclusion. It may be worthwhile to conduct another study with objective measurements of asthma and atopy as primary outcome.

Hib

In chapter 4 we concluded that there was no additional risk of atopy due to the Hib vaccination in primary school children who were DTPPo vaccinated. As these findings agree with those from the (few) other available studies (3-7), we are clearly justified to conclude that Hib vaccination carries no additional risk of atopy in childhood .

Birth order and sibship size

In previous studies the "sibling effect" was explored by relating atopy to the number of older siblings, birth order, family size, the number of younger siblings and sibship size (8). As several of these variables are linearly dependent on each other, as explained in chapter I (General Introduction), it sufficed to study the role of birth order and sibship size as risk factors for atopic disease. Identifying which of the two drives the development of atopy could be an essential step in unraveling the pathogenesis of atopy. However, it is difficult to disentangle the effects of these two factors (9). In a random sample of individuals sibship size and birth order are strongly correlated, and including them both into a regression model would not yield a definite answer, unless both effects are assessed within families. We demonstrated in a within-family study, that birth order, and not family size, was a risk factor for inhalant allergy. This confirms Strachan's conclusion that older siblings have a stronger impact on allergy than younger siblings (10).

Timing of birth order effect

Knowledge of the age the birth order effect develops would also help to understand its underlying mechanisms. A negative relationship between maternal atopy and parity was found by Sunyer et al (11). Murine experiments have demonstrated that the status of the placenta changes after multiple births (12), and IgE measurements in cord blood have shown that the birth order effect is already present at birth (13, 14). Surely, this prenatal birth order effect does not preclude a possible, additional effect of more exposure to pathogens from other siblings in the family. While this prenatal effect could be due to more exposure to pathogens of the mother during pregnancy if she already has more children, a more plausible explanation would be that the gene/maternal-fetal interaction changes with increasing parity (14). Genetic research and research on a molecular level has to reveal the exact mechanism. Recent research has indeed shown that the maternal-fetal interaction (and thus prenatal development) is influenced by parity and genetic make-up (15) and even by the gender of older siblings (16). During childbirth, fetal cells may enter the maternal circulation and during this process the mother's immune system may develop tolerance to fetal antigens. During successive pregnancies the fetuses may take advantage of this tolerance, especially if they have the same biological father as the first child.

Genetic susceptibility

Another aspect of the birth order effect, is its genetic susceptibiliy, i.e. are there genes, of the mother or the child, that are predictors of the birth order effect? In other words: do some people benefit from the birth order effect while others do not? Knowledge on this area would both provide insight into the underlying mechanism of the birth order effect and outline which groups of people would benefit from preventive actions.

Perinatal data

One of our findings in chapter 6 (study population 2) was, that children with a gestational age of less than 36 weeks had a higher risk of asthma at six years of age. This was a confirmation of findings in other studies. Preterm infants often have a surfactant deficiency in their lungs, leading to respiratory distress syndrome, lower lung function and a higher risk of wheezing (17).

Our finding that a high ratio of head circumference and birth weight was associated with a higher risk of atopic disorders needs confirmation in other studies. The association should not be seen as support for the hypothesis that an excessive fetal growth of the brain at the expense of the thymus (reflected by a high head circumference to birth weight ratio) leads to atopy at a later age (18), because no relationship between thymus size and atopy was found (19). The association rather appears to be based on a complicated interplay between genes, mother-fetal interaction and probably environment, as was suggested by the recent demonstration of a positive association between the INS VNTR III/III genotype and head circumference; a relationship that increased with parity (15). Also, head circumference and birth weight are both associated with birth order, first borns being lighter and having smaller heads than second and higher borns (20).

A positive relationship between caesarean section and atopy was not confirmed by our study. However, we found a higher risk of allergy in children delivered by vacuum extraction and after induced labour. The mechanism underlying these associations is unclear. It could be that incubator care is a causal factor, but confounding factors in the intrauterine environment would seem more plausible.

Limitations of the studies

The design of the study with study population I (answering the questions on vaccinations) was cross-sectional. The main disadvantage of this type of design is that several variables are assessed retrospectively. The risk factor under study, however, was validated in a random subgroup by comparing reported data with officially registered data, a comparison that yielded an almost 100% agreement. Validation of reported data by means of determination of IgG antibodies in sera identified some misclassification, but this is unlikely to have influenced our conclusions.

The study with population II (answering the questions on sibling effect and perinatal factors) was a retrospective cohort study. The main disadvantage of this design is that data were collected during routine health check-ups which may have introduced some amount of misclassification (likely non-differential and thus effect diluting). Moreover, as some possibly important confounders, such as exposure to smoking, were not available, residual confounding is possible. However, probably most of these confounders were family characteristics and since comparisons were made within families, this omission is unlikely to be consequential.

Recommendations for daily practice

It is important to communicate the results of vaccination studies to the general public in order to allay the fear of long term side-effects. These results can help preventive health care workers to advise parents in the process of considering the pro's and contra's of childhood vaccinations. Therefore these results should not only be published in scientific journals, but also communicated in public newspapers and magazines.

Recommendations for future research

We recommend to monitor the development of atopy when new vaccinations are added to National Vaccination Programmes.

We also recommend to study the mechanism underlying the in utero birth order effect. Epidemiological research, comparing sibships with one biological father with those with more biological fathers (not uncommon in Africa), could reveal whether mothers develop tolerance to fetal antigens after multiple births, an issue that may also be addressed by genotyping mother, child, and older siblings. If the hypothesis proves to be true, this could be a starting point for

preventive measures, for instance by artificially inducing tolerance to paternal tissues before preganacy. Genotyping research is also recommended to identify polymorphisms associated with susceptibility to the birth order effect.

The mechanism(s) underlying the relationships of perinatal data and interventions during labour with atopy in childhood could be elucidated by prospective studies which measure IgE in cord blood to assess whether a higher risk of atopy in childhood was already present in utero.

References

1. Koppen S, De Groot R, Neijens HJ, Nagelkerke N, Van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. Vaccine 2004;22:3375-85.
2. Bernsen RM, van der Wouden JC. Re: No epidemiological evidence for infant vaccinations to cause allergic disease. Letter to the Editor. Vaccine 2005;23:1427.
3. Gruber C, Illi S, Lau S, Nickel R, Forster J, Kamin W, Bauer CP, Wahn V, Wahn U; MAS-90 Study Group. Transient suppression of atopy in early childhood is associated with high vaccination coverage. Pediatrics 2003 Mar;111:e282-8.
4. Anderson HR, Poloniecki JD, Strachan DP, Beasley R, Bjorksten B, Asher MI; ISAAC Phase 1 Study Group. Immunization and symptoms of atopic disease in children: results from the International Study of Asthma and Allergies in Childhood. Am J Public Health 2001;91:1126-9.
5. Mommers M, Weishoff-Houben M, Swaen GM, Creemers H, Freund H, Dott W, van Schayck CP. Infant immunization and the occurrence of atopic disease in Dutch and German children: a nested case-control study. Pediatr Pulmonol 2004;38:329-34.
6. Laubereau B, Grote V, Holscher G, Holscher B, Frye C, Wichmann HE, Heinrich J. Vaccination against Haemophilus influenzae type B and atopy in east German schoolchildren. Eur J Med Res 2002;7:387-92.
7. DeStefano F, Gu D, Kramarz P, Truman BI, Iademarco MF, Mullooly JP, Jackson LA, Davis RL, Black SB, Shinefield HR, Marcy SM, Ward JI, Chen RT; Vaccine Safety Datalink Research Group. Childhood vaccinations and risk of asthma. Pediatr Infect Dis J 2002;21:498-504.
8. Karmaus W, Botezan C. Does a higher number of siblings protect against the development of allergy and asthma? A review. J Epidemiol Community Health 2002;56:209-17.

9. Ponsonby AL, Couper D, Dwyer T, Carmichael A. Cross-sectional study of the relation between sibling number and asthma, hay fever and eczema. Arch Dis Child 1998;79:328-33.
10. Strachan DP. Hay fever, hygiene, and household size. Br Med J 1989;299:1259-60.
11. Sunyer J, Anto JM, Harris J, Torrent M, Vall O, Cullinan P, Newman-Taylor A; AMICS study. Asthma Multi-centre Infants Cohort Study. Maternal atopy and parity. Clin Exp Allergy 2001;31:1352-5.
12. Lagadari M, Blois S, Margni R, Miranda S. Analysis of macrophage presence in murine placenta: influence of age and parity status. Am J Reprod Immunol 2004;51:49-55.
13. Karmaus W, Arshad H, Mattes J. Does the sibling effect have its origin in utero? Investigating birth order, cord blood immunoglobulin E concentration, and allergic sensitization at age 4 years. Am J Epidemiol 2001;154:909-15.
14. Devereux G, Barker RN, Seaton A. Antenatal determinants of neonatal immune responses to allergens. Clin Exp Allergy 2002;32:43-50.
15. Ong KK, Petry CJ, Barratt BJ, Ring S, Cordell HJ, Wingate DL, Pembrey ME, Todd JA, Dunger DB; Avon Longitudinal Study of Pregnancy and Childhood Study Team. Maternal-fetal interactions and birth order influence insulin variable number of tandem repeats allele class associations with head size at birth and childhood weight gain. Diabetes 2004;53:1128-33.
16. Cote K, Blanchard R, Lalumiere ML. The influence of birth order on birth weight: does the sex of preceding siblings matter? J Biosoc Sci 2003;35:455-62.
17. Speer CP, Silverman M. Issues relating to children born prematurely. Eur Respir J 1998;27 (Suppl):13s-16s.
18. Godfrey KM, Barker DJ, Osmond C. Disproportionate fetal growth and raised IgE concentration in adult life. Clin Exp Allergy 1994;24:641-8.
19. Benn CS, Jeppesen DL, Hasselbalch H, Olesen AB, Nielsen J, Bjorksten B, Lisse I, Aaby P. Thymus size and head circumference at birth and the development of allergic diseases. Clin Exp Allergy 2001;31:1862-6.
20. Ong KK, Preece MA, Emmett PM, Ahmed ML, Dunger DB; ALSPAC Study Team. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: longitudinal birth cohort study and analysis. Pediatric Research 2002;52:863-867.

8

Summary & samenvatting

Summary

Atopic disorders have become increasingly prevalent in the western world during the last decades of the 20th century. Research concerning the cause(s) of this increase has so far suggested a complex interplay between genetic and environmental factors. These studies have also indicated that priming starts at a very young age, even before birth. Therefore most studies on this subject have been in children. No clear causes of the increase of these disorders have come up so far, although there are indications that the increased hygienic living-conditions could play a role. Knowledge of causal factors would open opportunities for prevention. The aim of this thesis was to contribute a small piece to the immense endeavour of building foundations for prevention strategies. It also aimed at yielding objective information on implications of childhood vaccinations as a tool for workers in the field of preventive health care and for the general public.

The study population described in the chapters 2 and 4 were 8-12 year old children of the Orthodox Reformed (Protestant) population, living in an area in the Netherlands which stretches from the south-west to the east (study population I). Chapters 3, 5 and 6 apply to retrospectively collected health report data of a cohort of 700 families with index children born in 1988-1990 (study population II). These files contained reports of health check-ups of children living in Zwijndrecht, a town south of Rotterdam, at the age of 6 years.

Chapter 1 gives an introduction and discusses the background of the thesis: the prevalence of atopic disorders has increased dramatically in the western world over the last decades of the past century and much effort has been put into finding the cause(s) of this increase. The hygiene hypothesis assumes that this increase is caused by a too hygienic environment where children are not enough exposed to pathogens. This exposure is said to be necessary for a healthy development of their immune system. Extensive research has been conducted to test the hygiene hypothesis, and, although much of the evidence is conflicting, there is some evidence that some aspects of hygiene are related to the development of atopic disorders. In this thesis we focused on two aspects of "hygiene": vaccinations and family size.

Chapter 2 presents a study of the relationship between the diphtheria-tetanus-pertussis-poliomyelitis vaccination (DTPPo) in the first year of life and reported atopic disorders at age 8-12 years. We conducted this study in children of Orthodox Reformed (Protestant) families in the Netherlands (study population I). A part of these families refuse vaccinations for religious reasons, and therefore

we could compare vaccinated and unvaccinated children in a rather homogenous population. We included children via Orthodox Reformed schools, sent in total almost 4500 questionnaires of which 1875 (42%) were returned. The conclusion of this study is, that vaccinated and unvaccinated children have an equal risk of atopy.

In **Chapter 3** we addressed the same research question as in chapter 2. However, this study was conducted in children of 700 families with index children born in 1988-1990 (study population II) and the outcome of interest was atopy at age 6 years. Data were collected from reports of routinely conducted health examinations at the age of 6 years. Although we focussed on the pertussis vaccination at first, it became evident that there was almost 100% overlap with the DTPPo vaccination. The conclusion of this study is, that unvaccinated children had a higher risk of atopy at age 6 years. However, the number of unvaccinated children in this study was small and therefore the result is not reliable on its own.

Chapter 4 describes a study on the relationship between the Haemophilus influenzae type b vaccination (Hib) and reported atopic disorders at age 8-12 years. This study was conducted in the DTPPo vaccinated part of study population I. The Hib vaccination was introduced in the Netherlands in 1993, implying that a part of these DTPPo vaccinated children did not regularly get the Hib vaccination in the first year of life. This study concluded that there was no indication for a different risk of atopy due to the Hib vaccination.

In **Chapter 5** we investigated the so-called "sibling effect" (the phenomenon first described in 1986 that children from larger families have a smaller risk of atopic disorders than children from small families). This study was conducted in study population II. Because we studied the sibling effect within families, we were able to disentangle the independent effects of sibship size and birth order. In an ordinary cohort of children these variables are usually strongly correlated and it is consequently not possible to disentangle their effects From this study it became evident, that a higher birth order (or a higher number of older siblings) is associated with a lower risk of allergy, independent of the size of the sibship.

In **chapter 6** we studied the associations of atopy at age 6 years with perinatal characteristics (gestational age, birth weight and neonatal head circumference) and obstetric complications. These associations were studied in population II. We concluded, confirming earlier findings, that premature children have a higher risk of asthma at age 6 years. Other findings were that children with a high ratio of head circumference to birth weight, children delivered with vacuum extraction, and children delivered after induced labour had a higher risk of allergy.

Chapter 7 discusses the findings in this thesis, also in relation to what was already known on the etiology of atopic disorders.

The finding that the DTPPo vaccination and the Hib vaccination are not related to atopic disorders is reassuring for those parents who are hesitating about the health effects of vaccinations and could, if communicated well, contribute to stop the, in some countries (like the UK and the Netherlands), steady decrease of the vaccination coverage.

The findings on birth order and perinatal risk factors are mere links in a chain of hypotheses on the mechanisms underlying atopic disorders and could thus contribute to preventive strategies in the future.

We finally speculate on possible mechanisms on the basis of our findings and results of studies conducted on other topics and make recommendations for daily practice and future research.

Samenvatting

Atopische aandoeningen komen steeds vaker voor. Deze stijging heeft in de laatste decennia van de vorige eeuw plaatsgevonden. Onderzoek naar de oorzaken van deze stijging heeft een ingewikkeld samenspel tussen aanleg en omgevingsfactoren laten zien. Uit dit onderzoek is ook gebleken, dat de aandoening al op heel vroege leeftijd begint, zelfs al voor de geboorte. Daarom wordt er veel onderzoek in kinderen gedaan. Tot nu toe heeft men niet echt duidelijke oorzaken voor het meer vóórkomen kunnen vinden. Er zijn wel aanwijzingen dat misschien de toegenomen hygiëne een rol speelt. Als we precies zouden weten wat de oorzaken zijn, zouden we een preventieprogramma kunnen opzetten. Het doel van deze studies was een klein stukje bij te dragen aan de grote klus om een goede fundering voor zo'n preventieprogramma te leggen. Een ander doel was objectieve informatie te vergaren over de impact van kindervaccinaties op het krijgen van atopie. Deze informatie is bedoeld voor mensen die werken in de preventieve gezondheidszorg en voor de hele bevolking.

De studiepopulatie die wordt beschreven in hoofdstuk 2 en 4 (studie-populatie I) waren 8-12 jaar oude kinderen uit de Reformatorische groep die woont in een strook die zich uitstrekt van het zuid-westen naar het oosten an Nederland (ook wel de "Bible Belt" genoemd). Hoofdstuk 3, 5 en 6 hebben betrekking op gegevens uit rapporten van schoolartsbezoeken van een cohort van 700 gezinnen met index-kinderen, die tussen begin 1988 en eind 1990 zijn geboren (studie-populatie II). Deze kinderen woonden ten tijde van hun bezoek aan de schoolarts (op 6-jarige leeftijd) in Zwijndrecht.

Hoofdstuk 1 is een inleiding en bespreekt de achtergronden van de studies in dit proefschrift: atopische aandoeningen komen steeds vaker voor in de westerse wereld. De echte toename heeft gedurende de laatste decennia van de vorige eeuw plaatsgevonden. Er is veel onderzoek gedaan naar de oorzaken van deze toename. Volgens de hygiënehypothese is de oorzaak een te hygiënische leefomgeving, waarin kinderen niet genoeg aan ziekteverwekkers worden blootgesteld. Men neemt aan, dat deze blootstelling nodig is voor een gezonde ontwikkeling van het immuunsysteem. Er zijn veel studies gedaan met als doel de hygiënehypothese te testen. Hoewel veel resultaten elkaar tegenspreken, lijkt het er toch op, dat sommige aspecten van hygiëne gerelateerd zijn aan het risico op atopische aandoeningen. In dit proefschrift worden twee aspecten van hygiëne bestudeerd: vaccinaties en gezinsgrootte.

Hoofdstuk 2 beschrijft een studie naar de relatie tussen de difterie-tetanus-kinkhoest-polio vaccinatie (DKTP) in het eerste levensjaar en gerapporteerde atopische aandoeningen op de leeftijd van 8-12 jaar. Deze studie werd uitgevoerd in een groep reformatorische kinderen in Nederland (studiepopulatie I). Een deel van de ouders van deze kinderen wijst vaccinaties af uit godsdienstige overwegingen. Daarom was het mogelijk binnen een redelijk homogene groep gavccineerde en ongevaccineerde kinderen met elkaar te vergelijken. We includeerden kinderen via reformatorische scholen en verzonden bijna 4500 vragenlijsten, waarvan er 1875 (42%) ingevuld werden teruggestuurd. De conclusie van deze studie is, dat gevaccineerde en ongevaccineerde kinderen een even grote kans hebben op een atopische aandoening.

In **hoofdstuk 3** onderzochten wij dezelfde relatie als in hoofdstuk 2, maar nu in de kinderen van 700 gezinnen in Zwijndrecht met index-kinderen geboren in 1988-1990 (studie-populatie II). In deze studie werd gekeken naar atopische aandoeningen op de leeftijd van 6 jaar. Data werd verzameld uit verslagen van schoolartsonderzoeken op de leeftijd van 6 jaar. Onze hypothese betrof eigenlijk de kinkhoest vaccinatie, maar het bleek dat als een kind niet gevaccineerd was tegen kinkhoest, hij/zij bijna altijd ook niet gevaccineerd was met de DKTP-cocktail. De conclusie van deze studie is, dat ongevaccineerde kinderen een grotere kans hebben op een atopische aandoening op de leeftijd van 6 jaar. Echter, het aantal ongevaccineerde kinderen in deze studie was klein en daarom is dit resultaat alleen niet echt een sterk bewijs.

In **hoofdstuk 4** beschrijven wij een studie naar de relatie tussen de Haemophilus influenzae type b vaccinatie (Hib) en gerapporteerde atopische aandoeningen op de leeftijd van 8-12 jaar. De onderzoeksvraag werd onderzocht in het DKTP gevaccineerde deel van studiepopulatie I. De Hib vaccinatie werd in Nederland in 1993 aan het Rijks Vaccinatie Programma toegevoegd. Studiepopulatie I werd deels vóór, deels na 1993 geboren. Daardoor heeft een deel van deze DKTP gevaccineerde kinderen de Hib in het eerste levensjaar niet ontvangen. De conclusie is, dat er geen aanwijzing is voor een veranderd risico op atopische aandoeningen ten gevolge van de Hib vaccinatie.

In **hoofdstuk 5** onderzochten wij het zogenoemde "sibling effect". Deze term staat voor het verschijnsel (voor het eerst beschreven in 1986), dat kinderen met veel broers en zussen minder kans op een atopische aandoening hebben dan kinderen met minder broers en zussen. We voerden deze studie uit in studiepopulatie II. Omdat de onderzoekseenheden in deze studiepopulatie

gezinnen zijn, waren we in staat de variabele “gezinsgrootte” te onderscheiden van de variabele “plaats in de kinderrij”. In een “gewoon” cohort van kinderen zijn deze twee variabelen n.l. sterk gecorreleerd, en zijn de effecten niet goed te onderscheiden. Uit deze studie bleek, dat hoe hoger de plaats in de kinderrij binnen een gezin (of: hoe meer oudere broers en zussen) des te kleiner de kans op een atopische aandoening, onafhankelijk van de gezinsgrootte.

Hoofdstuk 6 beschrijft de relaties van atopische aandoeningen op de leeftijd van 6 jaar met geboortegegevens (zwangerschapsduur, geboortegewicht, schedelomtrek en complicaties bij de geboorte, zoals een keizersnede, vacuumverlossing, tangverlossing of een ingeleide bevalling). Deze studie werd gedaan in studiepopulatie II. We vonden, dat te vroeg geboren kinderen een grotere kans hebben op astma op zesjarige leeftijd dan kinderen die à terme geboren zijn. Een andere bevinding was, dat kinderen met een relatief grote schedelomtrek (ten opzichte van hun gewicht), kinderen die met vacuum extractie waren geboren en kinderen bij wie de weeën waren opgewekt een grotere kans hadden om allergisch te zijn op zesjarige leeftijd.

Hoofdstuk 7 bespreekt de bevindingen van dit proefschrift, ook in het licht van wat al bekend was over de etiologie van atopische aandoeningen.

De conclusie dat vaccineren niet uitmaakt voor het krijgen van atopische aandoeningen is geruststellend voor ouders die twijfels hebben over de effecten van vaccinaties op de gezondheid van hun kinderen. Als dit resultaat bredere bekendheid krijgt, kan het ertoe bijdragen dat de (in sommige landen zoals Engeland en Nederland) dalende vaccinatiegraad wordt omgebogen.

De resultaten over plaats in de kinderrij en perinatale risicofactoren zijn bijdragen aan de vorming van hypothesen over de mechanismen in het ontstaan van atopische aandoeningen. Zij kunnen aldus bijdragen aan de ontwikkeling van toekomstige preventieprogramma’s.

Verder speculeren we op grond van onze bevindingen en andere onderzoeksresultaten over mogelijke mechanismen en we doen aanbevelingen voor de dagelijkse praktijk en toekomstig onderzoek.

Dankwoord

Het proefschrift is bijna klaar en wat nog rest is het dankwoord. Veel mensen hebben een bijdrage aan het totstandkomen geleverd en deze mensen wil ik hier bedanken.

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Over de auteur

Roos Bernsen werd op 13 april 1953 geboren in 's-Hertogenbosch. Zij behaalde in 1971 het diploma gymnasium-beta in Voorburg en studeerde aansluitend wiskunde aan de Universiteit Leiden, alwaar zij in februari 1976 het doctoraal diploma behaalde. Bijvakken waren mathematische statistiek en medische sociologie voor ontwikkelingslanden. Tijdens haar studie werkte zij als student-assistent bij het Economisch Instituut van de Universiteit Leiden en later tevens als assistant hoofddocent wiskunde aan de Leidse Onderwijsinstellingen.
Na het behalen van het doctoraal examen werkte zij gedurende een jaar als adviserend statisticus in dienst van de Universiteit van Amsterdam. In 1977 deed zij drie jaar lang onderwijservaring op als docent wiskunde aan een avondschool voor HAVO en VWO in Amsterdam. In 1980 trad zij in dienst als statisticus bij Boehringer Ingelheim in Alkmaar. Deze baan werd in 1987 vaarwel gezegd wegens vertrek naar Kenia, waar haar echtgenoot naar werd uitgezonden. In Kenia werkte zij nog enige tijd op afstand voor Boehringer Ingelheim, maakte zij een database voor een epilepsiekliniek en gaf zij wiskundeles aan een internationale school.
Na terugkeer in Nederland in 1990 werkte zij op free-lance basis voor diverse farmaceutische bedrijven, tot zij met haar gezin in 1993 opnieuw voor een periode van 3 jaar naar Kenia vertrok. Gedurende deze 3 jaar werkte zij ten behoeve van een Canadees-Keniaans AIDS-project. Hier beheerde zij o.m. een database van verticale transmissie-gegevens, ontwierp zij databases en begeleidde zij lokale onderzoekers in het beheren en verwerken van hun onderzoeksgegevens.
Na terugkeer in Nederland kwam zij begin 1997 in dienst van de afdeling Huisartsgeneeskunde van de Erasmus Universiteit in Rotterdam, waar zij tot op heden werkt. Zij volgde het Nihes-programma Epidemiologie en sloot dit in 1998 af met het behalen van het Master of Science-diploma Epidemiologie.

Author's publications related to the subject of this thesis

Bernsen RM, de Jongste JC, Koes BW, Aardoom HA, van der Wouden JC. Perinatal characteristics and obstetric complications as risk factors for asthma, allergy and eczema at the age of 6 years. In press, Clin Exp Allergy.

Bernsen RM, van de Wouden JC. Re: No epidemiological evidence for infant vaccinations to cause allergic disease. Letter to the Editor. Vaccine 2005;23:1427.

Bernsen RM. Discussion "Consumption of milk fat and reduced asthma risk in pre-school children". Thorax 2005; 60 (electronic letter).

Bueving HJ, van der Wouden JC, Raat H, Bernsen RM, de Jongste JC, van Suijlekom-Smit LW, Osterhaus AD, Rimmelzwaan GF, Molken MR, Thomas S. Influenza vaccination in asthmatic children: effects on quality of life and symptoms. Eur Respir J 2004;24:925-31.

Bueving HJ, Bernsen RM, de Jongste JC, van Suijlekom-Smit LW, Rimmelzwaan GF, Osterhaus AD, Rutten-van Molken MP, Thomas S, van der Wouden JC. Does influenza vaccination exacerbate asthma in children? Vaccine 2004;23:91-6.

Bueving J, Bernsen RM, de Jongste JC, van Suijlekom-Smit LW, Rimmelzwaan GF, Osterhaus AD, Rutten-van Molken MP, Thomas S, van der Wouden JC. Influenza vaccination in children with asthma: randomized double-blind placebo-controlled trial. Am J Respir Crit Care Med 2004;169:488-493.

Bernsen RM, de Jongste JC, van der Wouden JC. Birth order and sibship size as independent risk factors for asthma, allergy, and eczema. Pediatr Allergy Immunol 2003;14:464-9.

Bernsen RM, de Jongste JC, van der Wouden JC. Lower risk of atopic disorders in whole cell pertussis-vaccinated children. Eur Respir J 2003;22:962-4.

van der Wouden JC, Tasche MJ, Bernsen RM, Uijen JH, de Jongste JC, Ducharme FM. Inhaled sodium cromoglycate for asthma in children. Cochrane Database Syst Rev 2003;(3):CD002173.

van der Wouden JC, Bernsen RM. Exposure to pets in childhood and risk of atopic disorders. JAMA 2003;289:841.

Bernsen RM, Van Der Wouden JC. Re:Does the sibling effect have its origin in utero? investigating birth order, cord blood ImmunoglobulinE concentration, and allergic sensitization at age 4 years (letter to the editor). Am J Epidemiol 2002;156:882.

Tasche MJA, Uijen JHJM, Bernsen RMD, de Jongste JC, van der Wouden JC. Inhaled sodium cromoglycate in children with asthma (Authors' reply). Thorax 2002;57:751-2.

van der Wouden JC, Bueving HJ, Bernsen RM. Influenza vaccine and asthma. J Pediatr 2002;140:278-9.

Tasche MJA, Uijen JHJM, Bernsen RMD, van der Wouden JC. Sodium cromoglycate in childhood asthma (Authors' reply). Thorax 2001;56:331-332.

Tasche MJA, Uijen JHJM, Bernsen RMD, de Jongste JC, van der Wouden JC. Time to consign cromoglycate to history? (Authors' reply). Thorax 2001;56:503.

Tasche MJA, Uijen JHJM, Bernsen RMD, de Jongste JC, van der Wouden JC. Inhaled sodium cromoglycate (Authors' reply). Thorax 2001;56:585.

Tasche MJ, Uijen JH, Bernsen RM, de Jongste JC, van der Wouden JC. Inhaled sodium cromoglycate. Thorax 2001;56:585.

Bernsen RM, van der Wouden JC. Re:Declining incidence of episodes of asthma. Letter to the editor. Thorax 2001;56:245-6.

Tasche MJ, Uijen JH, Bernsen RM, de Jongste JC, van Der Wouden JC. Inhaled disodium cromoglycate (DSCG) as maintenance therapy in children with asthma: a systematic review. Thorax. 2000;55:913-920.

Tasche MJA, van der Wouden JC, Uijen JHJM, Ponsioen BP, Bernsen RMD, van Suijlekom-Smit LWA, de Jongste JC. Inhalatie therapie met cromoglicaat by kleine kinderen met matig ernstig astma. Huisarts Wet 1998;5:229-35.

Tasche MJA, van der Wouden JC, Uijen JHJM, Bernsen RMD, van Suijlekom-Smit LWA, de Jongste JC. Randomised placebo-controlled trial of inhaled sodium cromoglycate in 1-4 year old children with moderate asthma. Lancet 1997;350:1060-4.

Molkenboer JFWM, Cornelissen PJG, Bernsen R. Berodual versus ipratropium and fenoterol. Tijdschrift voor therapie geneesmiddel en onderzoek 1992;supp 2:31-5.

Maesen FPV, Smeets JJ, Bernsen R, Cornelissen PJG. Clinical experiences with powder inhalations in asthma patients. In: W. Hofmann (ed.) Deposition and clearance of aerosols in the human respiratory tract. Arbeitsgemeinschaft fuer Aerosole d. Oesterreichischen Gesellschaft fuer Lungenerkrankungen u. Tuberkulose, 1987.

Maesen FPV, Smeets JJ, Bernsen R, Cornelissen PJG. Ipatropium Bromide (Atrovent) as inhalation powder. A double blind study of comparison with Ipratropium as a pressure aerosol in patients with reversible airways obstruction. Allergy 1986;41:37-42.

Maesen FPV, Smeets JJ, Bernsen R,Cornelissen PJG. Comparative study of the bronchospasmolytic effect of fenoterol (0.2 mg) and salbutamol (0.4 mg) as powder inhalations in 20 patients with reversible bronchial obstruction. Respiration 1984;45:265-70.