ASTHMA

Association between *Chlamydia pneumoniae* antibodies and wheezing in young children and the influence of sex

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Background: The proposed association between *Chlamydia pneumoniae* (Cpn) infection and wheezing needs further clarification.
Methods: Serum samples obtained from 1581 children aged 4 years in a population based cohort were tested for antibodies to Cpn and IgE antibodies to common allergens. Data on environmental factors and disease were collected prospectively from birth.
Results: The occurrence of IgG antibodies to Cpn at 4 years of age was associated with reported wheezing at different ages; however, these findings were most often not significant. In girls, the occurrence of anti-Cpn IgG was associated with wheezing at the ages of 1, 2, and 4 years (odds ratios (ORs) 3.41 (95% confidence interval (CI) 1.46 to 7.96), 2.13 (95% CI 1.02 to 4.44), and 2.01 (95% CI 1.14 to 3.54), respectively), and even higher ORs were observed for each age category when only high level antibody responses to Cpn were analysed. At the time of blood sampling the association between anti-Cpn IgG and wheezing was restricted to girls without atopic sensitisation (OR 2.39 (95% CI 1.25 to 4.57). No associations with wheezing were detected in boys, in whom IgE sensitisation was inversely associated with the presence of anti-Cpn IgG (OR 0.49 (95% CI 0.26 to 0.90).
Conclusions: This study suggests an association between evidence of earlier Cpn infection and a history of wheezing in young girls. Infection with Cpn may be an important risk factor for wheezing and possibly for non-atopic asthma, predominantly in girls.

Respiratory tract infections are well recognised as triggers of wheezing in asthma. It is suggested that infections may also contribute to the pathogenesis during an induction phase and promote the development of asthma. Asthma is an inflammatory disease and the inflammation could be a consequence of a preceding or ongoing infection.[1 2]

Chlamydia pneumoniae (Cpn) is a respiratory tract pathogen associated both with acute and chronic disorders. It has been associated with pharyngitis, acute bronchitis, pneumonia, and chronic obstructive pulmonary disease.[3 4] This bacterium is frequently found in young children when using polymerase chain reaction (PCR) on throat swabs.[5] Cpn has been linked to exacerbations of asthma and it has been suggested that it could contribute to the development of the disease.[6–8] In 1991 Hahn was the first to describe an association between Cpn and asthma in adults, and he later reviewed subsequent reports on the topic.[8 9] In 1994 Emre *et al*[10] described an association between Cpn and reactive airway disease in children. However, Mills *et al*[11] were unable to show an association between asthma and Cpn serological status in a retrospective study of subjects aged 21 years who reported ever having asthma compared with healthy controls. Recently, Korppi *et al*[12] did not find an association between antibodies to Cpn and asthma in a case-control study of children aged 1–6 years with newly diagnosed asthma.

All previous studies have been performed on patient samples originating from different clinical settings. In his review, Hahn argued for large, prospectively designed, population based studies.[8] We have tried to accomplish this by examining the relationship between asthma, allergy, and antibodies to Cpn using a large birth cohort of children.

METHODS

Population

The investigated population was collected from an ongoing prospective birth cohort study (BAMSE: Barn = Children, Allergy, Milieu, Stockholm, Epidemiological survey). The study design is described in detail elsewhere.[13] In summary, data were collected by means of questionnaires at the age of 2 months and 1, 2, and 4 years. These data included detailed information about the child's health with an emphasis on asthma and other allergic diseases. The original study population comprised 4089 children, of whom 91.5% had completed questionnaires at 4 years of age. At this age, blood was collected from 2561 of the participating children. Information on longitudinal changes in the population has been published elsewhere.[14] Important risk factors for wheezing, educational level, and disease outcomes did not differ significantly between children from whom blood was collected and those from whom a specimen was not taken. Serum was analysed for IgE antibodies to common allergens and, when enough sample remained, it was used for the analyses of Cpn antibodies. A random quantity of blood was originally obtained and some samples had been used up during other projects.[14 15] The present study included 1581 children of mean age 4.3 years (median age 4.3 years, range 4.0–5.4). The ethical committee of Karolinska Institutet, Stockholm, Sweden approved the study.

Definitions of disease

Wheezing at 1 year of age: More than two episodes of wheezing after the age of 3 months including at least one episode without apparent infection, or the use of inhaled steroids.

Wheezing at 2 years of age: As above but with more than two episodes of wheezing after the age of 1 year.

Wheezing at 4 years of age: At least one exacerbation of wheezing during the last year.

At 4 years of age:

Abbreviations: Cpn, *Chlamydia pneumoniae*; MIF, microimmunofluorescence

Asthma: More than three episodes of wheezing over the last year or more than one episode over the last year if the child had been given inhaled steroids.

Eczema: Dry skin with itchy rashes in characteristic areas during the last year or a doctor's diagnosis of atopic dermatitis after the age of 2 years.

Suspected allergic rhinitis: Non-infectious rhinitis during the last year or rhinitis combined with itchy red eyes.

IgE sensitised: Serum IgE antibody levels ⩾0.35 kU_A/l to any of the following allergens: house dust mite, cat, horse, dog, pollen from birch, timothy or mugwort, mould, hen's eggs, wheat, fish, cow's milk, soy, or peanuts. For sensitisation to inhalants positive Phadiatop tests were used and for food sensitisation positive fx5 tests were used (Phadia CAP System, Uppsala, Sweden).

Serology

IgG, IgA, and IgM antibodies to Cpn, *Chlamydia trachomatis*, and *Chlamydia psittaci* were analysed by the microimmunofluorescence (MIF) technique as described in detail elsewhere.[16] One experienced investigator (JG), who interpreted all tests, was blinded to all other study data. Standard high and low positive serum samples were included to ensure consistency and quality control of MIF tests. Antibody titres were expressed as reciprocal titres. IgG antibodies were removed by immunoprecipitation with GullSORB (Meridian Bioscience Inc, OH, USA) before testing for IgA antibodies. Serum samples negative for IgG were not analysed for IgA, and all children negative for IgG were also considered as negative for IgA. A positive MIF titre was defined as ⩾16 (inverted titre).

Statistics

Data were collected and analysed with STATA/SE 8.2 for Windows (StataCorp, College Station, TX, USA). The confidence level was set at 95%.

Odds ratios (ORs) for 2×2 tables were calculated using the maximum likelihood estimate, also when including an interaction variable. The homogeneity of ORs between strata was analysed by χ^2 test. Mantel-Haenszel estimate was used to calculate ORs when adjusting for IgE sensitisation. The p values were calculated by χ^2 except for analyses of the group of children with high titres of anti-Cpn IgG when the Fisher's exact test was used to ensure statistical accuracy for small numbers. When the study population was stratified by sex or by different serological characteristics, and when several different outcomes were analysed simultaneously, the Simes procedure was used to adjust for multiple statistical comparisons.[17]

RESULTS

With the exception of eczema, higher prevalences of study diagnoses were reported for boys. One hundred and fifty nine children (10.1%) had detectable anti-Cpn IgG levels with no significant difference between the sexes. The results for all study variables are shown in table 1.

The occurrence of anti-Cpn IgG was associated with a history of wheezing, most evident when reported at 2 years of age (table 2).

Only 42 children had anti-Cpn IgA, which made possible associations between serology and clinical diagnoses difficult to study. Few children with anti-Cpn IgA had any of the study diagnoses reported at 4 years of age. Children with IgA antibodies to Cpn had a reduced risk of reactive airway disease and of being IgE sensitised at the time of blood sampling. None of the associations in table 2 remained statistically significant after adjusting for multiple statistical comparisons.

When the sexes were examined separately, an observed association between anti-Cpn IgG and wheezing was restricted to girls (table 3). The respective ORs calculated for boys and girls regarding IgE sensitisation were also different in that the occurrence of IgG antibodies to Cpn was associated with a lowered risk of IgE sensitisation in boys and an increased risk of IgE sensitisation in girls. When the statistics in table 3 were adjusted for multiple comparisons, including stratification by sex and the nine different outcomes, the lowest ordered p value had to be <0.0028 to be considered as significant; this requirement was true only for the association between anti-Cpn IgG and wheezing in girls at 1 year of age.

Post hoc analyses were performed to further scrutinise indicated associations. The associations found between anti-Cpn IgG and wheezing in girls were unchanged after adjusting for IgE sensitisation (data not shown). When stratifying for IgE sensitisation, anti-Cpn IgG was associated with an increased risk of wheezing in non-IgE sensitised girls at 4 years of age (OR 2.39 (95% CI 1.25 to 4.57), p = 0.0064) but not in IgE sensitised girls (OR 1.13 (95% CI 0.34 to 3.70)). No such association was found for boys, with or without sensitisation. IgE sensitisation was a risk factor for wheezing in 4 year old boys and for asthma in both sexes (data not shown).

To test whether high levels of anti-Cpn IgG would affect the results, another cut off level was chosen (IgG >1/512), yielding a group which comprised 10% of Ig positive children with raised titres. Sixteen children had anti-Cpn IgG above this level, 10 of which were girls. In girls a high level of anti-Cpn IgG was associated with an even higher risk for airway disease: wheezing at 1 year (OR 10.8 (95% CI 2.60 to 44.7)),

Table 1 Prevalence of study variables in children at the age of 4 years according to sex

Variables (no of children)	Boys (n = 827)	Girls (n = 754)	OR (95% CI)*
Diagnoses			
Wheeze at 1 year (n = 88)	57/820 (7.0%)	31/743 (4.2%)	1.72 (1.09 to 2.69)
Wheeze at 2 years (n = 139)	82/813 (10.1%)	57/740 (7.7%)	1.34 (0.94 to 1.92)
Wheeze at 4 years (n = 285)	167/824 (20.3%)	118/749 (15.8%)	1.36 (1.05 to 1.76)
Asthma (n = 148)	94/821 (11.4%)	54//749 (7.2%)	1.66 (1.17 to 2.37)
Suspected allergic rhinitis (n = 69)	49/818 (6.0%)	20/737 (2.7%)	2.28 (1.34 to 3.89)
Eczema (n = 313)	155/825 (18.8%)	158/753 (21.0%)	0.87 (0.68 to 1.12)
Sensitisation to inhalants (n = 239)†	154/827 (18.6%)	85/754 (11.3%)	1.80 (1.35 to 2.40)
Sensitisation to foods (n = 234)†	128/827 (15.5%)	106/754 (14.1%)	1.12 (0.85 to 1.48)
Any IgE sensitisation (n = 371)	214/827 (25.9%)	157/754 (20.8%)	1.33 (1.05 to 1.68)
Anti-Cpn Ig			
Anti-Cpn IgG (n = 159)	85/827 (10.3%)	74/754 (9.8%)	1.05 (0.76 to 1.46)
Anti-Cpn IgA (n = 42)	16/827 (1.9%)	26/754 (3.4%)	0.55 (0.29 to 1.04)

*Odds ratios are given using girls as the reference.
†Sensitisation to inhalants = Phadiatop >0.35 kU_A/l; sensitisation to foods = fx5 >0.35 kU_A/l.

Table 2 Association between study diagnoses and anti-Cpn IgG and IgA in children at 4 years of age

	Ig−	IgG+ and IgA− OR (95% CI)*	IgG+ and IgA+ OR (95% CI)	Ig+ OR (95% CI)
Wheeze at 1 year	74/1404 (5.3)†	10/117 (8.6) 1.68 (0.84 to 3.35)	4/42 (9.5) 1.89 (0.66 to 5.45)	14/159 (8.8) 1.74 (0.96 to 3.15)
Wheeze at 2 years	117/1397 (8.4)	16/116 (13.8) 1.75 (1.00 to 3.07)	6/40 (15.0) 1.93 (0.79 to 4.70)	22/156 (14.1) 1.80 (1.10 to 2.93)
Wheeze at 4 years	249/1414 (17.6)	30/117 (25.6) 1.61 (1.04 to 2.50)	6/42 (14.3) 0.78 (0.32 to 1.87)	36/159 (22.6) 1.37 (0.92 to 2.04)
Asthma	129/1411 (9.1)	16/117 (13.7) 1.57 (0.90 to 2.75)	3/42 (7.14) 0.76 (0.23 to 2.51)	19/159 (11.9) 1.35 (0.81 to 2.25)
Suspected allergic rhinitis	63/1399 (4.5)	6/116 (5.2) 1.16 (0.49 to 2.73)	0/40	6/156 (3.8) 0.85 (0.36 to 1.94)
Eczema	285/1419 (20.1)	21/117 (18.0) 0.87 (0.53 to 1.42)	7/42 (16.7) 0.80 (0.35 to 1.81)	28/159 (17.6) 0.85 (0.55 to 1.31)
Sensitisation to inhalants‡	217/1422 (15.3)	19/117 (16.2) 1.08 (0.65 to 1.80)	3/42 (7.1) 0.42 (0.13 to 1.40)	22/159 (13.8) 0.89 (0.56 to 1.43)
Sensitisation to foods‡	210/1422 (14.8)	20/117 (17.1) 1.19 (0.72 to 1.97)	4/42 (9.5) 0.61 (0.21 to 1.72)	24/159 (15.1) 1.03 (0.65 to 1.62)
Any IgE sensitisation	339/1422 (23.8)	28/117 (23.9) 1.01 (0.65 to 1.56)	4/42 (9.5) 0.34 (0.12 to 0.95)	32/159 (20.1) 0.80 (0.54 to 1.21)

*Odds ratios are given using seronegative values (Ig–) for each diagnosis as the reference.
†Number of children with diagnosis/total number of children within category (%).
‡Sensitisation to inhalants = Phadiatop >0.35 kU_A/l; sensitisation to foods = fx5 >0.35 kU_A/l.

wheezing at 2 years (OR 3.51 (95% CI 0.71 to 17.4)), wheezing at 4 years (OR 5.54 (95% CI 1.56 to 19.6)), and asthma (OR 5.78 (95% CI 1.44 to 23.2)). The association in girls between high levels of anti-Cpn IgG and wheezing at 1 and 4 years remained statistically significant after adjustment for analysing four different outcomes.

There were no detectable antibodies to *C psittaci* or *C trachomatis* in children, nor were there any IgM antibodies to Cpn.

DISCUSSION

The results of this study suggest an association between evidence of earlier Cpn infection and wheezing in girls at 1, 2 and 4 years of age, especially in girls who were not IgE sensitised. This association was even stronger when the antibody response was pronounced, indicating a dose-response relationship. It is tempting to speculate whether one or several Cpn infections trigger both a strong antibody response and wheezing in some individuals or, alternatively, whether some wheezing children are prone to exhibit strong antibody responses to Cpn. No corresponding association was found for boys. Instead, boys exhibited an inverse relationship between anti-Cpn IgG and IgE sensitisation to common allergens.

Pre-school children are frequently infected by Cpn, and there is no reason to believe that exposure to the agent would differ between the sexes.[5] In the present study almost the same proportion of boys and girls had antibodies to Cpn, indicating equal exposure. Most exacerbations of wheezing in young children are probably triggered by respiratory tract infections, some of which may be Cpn infections.[6 18] The timing of Cpn exposure cannot be determined in this study, nor related to any specific episode of wheezing. The objective of the study was to examine the association between earlier exposure to Cpn and a susceptibility to reactive airway diseases. Surprisingly, such an association was indicated only for girls.

A difference between the sexes has not been addressed in other studies of Cpn and reactive airway disease in children.[8] However, von Hertzen *et al* reported an association between

Table 3 Association between study diagnoses and anti-Cpn IgG in children at 4 years of age according to sex

	Boys			Girls			
Diagnoses	IgG+	IgG−	OR (95% CI)	IgG+	IgG−	OR (95% CI)	p value†
Wheeze at 1 year	6/85 (7.1)*	51/735 (6.9)	1.02 (0.42 to 2.45)	8/74 (10.8)	23/669 (3.4)	3.40 (1.46 to 7.96)‡	0.046
Wheeze at 2 years	12/84 (14.3)	70/729 (9.6)	1.57 (0.81 to 3.04)	10/72 (13.9)	47/668 (7.0)	2.13 (1.02 to 4.44)	0.542
Wheeze at 4 years	17/85 (20.0)	150/739 (20.3)	0.98 (0.56 to 1.72)	19/74 (25.7)	99/675 (14.7)	2.01 (1.14 to 3.54)	0.075
Asthma	10/85 (11.8)	84/736 (11.4)	1.03 (0.51 to 2.08)	9/74 (12.2)	45/675 (6.7)	1.94 (0.90 to 4.15)	0.230
Suspected allergic rhinitis	4/84 (4.8)	45/734 (6.1)	0.77 (0.27 to 2.19)	2/72 (2.8)	18/665 (2.7)	1.03 (0.23 to 4.52)	0.751
Eczema	12/85 (14.1)	143/740 (19.3)	0.69 (0.36 to 1.30)	16/74 (21.6)	142/679 (20.9)	1.04 (0.58 to 1.87)	0.341
Sensitisation to inhalants§	12/85 (14.1)	142/742 (19.1)	0.69 (0.37 to 1.31)	10/74 (13.5)	75/680 (11.0)	1.26 (0.62 to 2.56)	0.217
Sensitisation to foods§	8/85 (9.4)	120/742 (16.2)	0.54 (0.25 to 1.15)	16/74 (21.6)	90/680 (13.2)	1.81 (0.99 to 3.29)	0.011
Any IgE sensitisation	13/85 (15.3)	201/742 (27.1)	0.49 (0.26 to 0.90)	19/74 (25.7)	138/680 (20.3)	1.36 (0.78 to 2.36)	0.013

*Number of children with diagnosis/total number of children within category (%).
†Test of homogeneity of ORs between sexes.
‡Statistically significant also when adjusting for multiple testing (p = 0.0026).
§Sensitisation to inhalants = Phadiatop >0.35 kU_A/l; sensitisation to foods = fx5 >0.35 kU_A/l.

anti-Cpn IgG and longstanding asthma in adults.[19] As in the present study, the association between wheezing and an immune response to Cpn was significant only for women and was most apparent for those without IgE sensitisation. The scientific literature is limited with regard to data on differences between the sexes with respect to immune response to various microbes in relation to wheezing. von Hertzen *et al* detected a difference between sexes when studying *Mycobacterium tuberculosis* where previous infection was associated with a reduced risk for subsequent allergic disease and asthma in females.[20] The present study cohort has previously been investigated with serology to Epstein-Barr virus and cytomegalovirus, but no differences between the sexes were found (M Wickman, personal communication).[14 15]

There may be several different explanations for the observed differences between the sexes. In spite of the large study population, the power may have been insufficient to detect an existing association in boys. A contributing factor could be that the association was masked in boys due to the Carter effect.[21] This theory is based on the observation that some factors involved in diseases with multiple risk factors may be more easily detected in subjects lacking one dominating risk factor. Consequently, it may be easier to confirm a risk factor common for both sexes in the sex with the lowest prevalence of disease (in this case girls) because they are less influenced by another more important risk factor.[22] Of the possible factors of importance in this context, atopic allergy appears particularly plausible since boys were more often IgE sensitised than girls. A possible association between Cpn infection and reactive airway disease in boys might therefore have needed a larger study population to be detected.

Given that there is a true sex difference, this might be explained by differences in respiratory tract reactivity upon Cpn infection as well as in differences in immune responses to Cpn. It has been suggested that girls are more susceptible to selected non-allergen effects in their airways, and are thus possibly more susceptible to reactive airway disease when infected with Cpn.[23–25] Another explanation could be that some individuals, most often girls, might have an impaired ability to eliminate Cpn when infected.[26] If persistently infected over an extended period of time, the immune system might respond with higher levels of antibodies to Cpn which may not be protective. Such persistent Cpn infection might induce a chronic inflammatory response and thus wheezing in some individuals.

The prevalence of non-atopic asthma is increasing, especially in girls.[27] Chronic or repetitive infections with, for example, Cpn and increased susceptibility to airway irritants in females could possibly cause a higher prevalence of asthma in teenage girls than in boys.[23] The results obtained from this and other studies suggest that the sexes develop their airway disease based on different prerequisites and/or on different time scales.[28 29]

In the present study there were some indications of an inverse association between anti-Cpn Ig and IgE sensitisation to common allergens. These findings are in agreement with two recent follow up studies reporting a lower prevalence of allergy in children previously infected with Cpn.[30 31] The findings fit with the ''hygiene hypothesis'', which suggests that infections in early life may protect against atopic disease.[32] However, others have reported a positive association between atopy and Cpn infection.[33 34] The latter studies differ from the present in that they correlated evidence of allergy with detection of bacteria, thus indicating an ongoing infection. Those studies did not include observations derived from earlier infections and this might possibly explain the disparate results compared with those of the present study.

This study cannot establish whether the observed associations between wheezing and positive serology to Cpn are due to acute or chronic infection with this bacterium. There is little information available on how to interpret serological reactions to Cpn infection in children. The detection of anti-Cpn IgG reflects earlier exposure to Cpn, but some children do not develop levels of antibodies detectable by MIF, especially when they are very young.[35 36] In this study, children without anti-Cpn IgG were not tested for IgA antibodies because we have never found a child with anti-Cpn IgA who did not also have IgM or IgG antibodies to Cpn.[5 35] In adults, anti-Cpn IgA is suggested to be associated with persistent infection but there is no information regarding children, even though the results of this study may indicate some interesting findings in children with anti-Cpn IgA. No method for bacterial detection was included in the present study since host reactivity to the agent was the major parameter of interest.

A cohort study such as this has important limitations. Children who continue to participate in a longitudinally designed investigation may be different from those who drop out—that is, in this study continuing children possibly had more respiratory tract diseases. This creates a problem for the ability to formulate generalisations but may improve the efficiency of analyses of associations between asthma and environmental risk factors including infections. However, it cannot be excluded that such a selection bias introduced unknown confounding factors into this study. Importantly, those who donated blood did not differ with respect to parental educational level and important risk factors, but there was a tendency for children with wheeze to remain in the study.[14] In addition, findings in subgroups may be misleading by selection, but it appears unlikely that such biases would be associated with the sex of the children. It is also important to acknowledge the increased risk of getting significant results obtained by chance when performing multiple statistical tests. For that reason, the Simes procedure was carried out on the different groupings of analyses and only a few associations between girls and wheezing remained significant after adjusting for multiple comparisons. Furthermore, even though the size of the study population was large, subgrouping was associated with an important loss of power. Some important differences may have been too small to be detected, particularly when the low sensitivity of the serological method is considered. MIF is, however, considered as the gold standard for serological testing to this organism and it is commonly applied in studies of seroprevalence.[37] The study is also limited by the fact that only one blood sample was obtained from each child and that the serological response reflects a previous event, making this information retrospective. Since a low prevalence of self-produced antibodies to Cpn can be expected in even younger children when using MIF, an earlier blood sampling in this study cohort was not justified. Korppi *et al*[12] studied younger individuals, which may be the reason why they could not detect an association between Cpn and asthma.

In summary, this study of young children suggests an association between evidence of earlier Cpn infection and wheezing, but only in girls. This result implies that, even when subjects are very young, the influence of sex should be included in studies on reactive airway diseases.[38] This cohort of children will be followed longitudinally to further determine the role of Cpn infection on asthma and allergy.

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