

# Atopic wheezing and early life antibiotic exposure: a nested case–control study

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Several factors including early-life antibiotic usage have been implicated in the rising prevalence of allergic sensitization and asthma. A nested case–control study comparing antibiotic exposure of 37 sensitized children with recurrent wheeze (age 3–5 yr) and 37 non-sensitized children who had never wheezed was carried out within a population-based birth cohort (matching for age, sex, parental atopy, allergen exposure, and pet ownership). We collected data on antibiotic prescriptions during first 3 yr of life (timing, type, indication) from the primary care medical records. Significantly, more cases than controls received one or more antibiotic courses during the first year of life (92% vs. 70%,  $p = 0.04$ ). The median time to first antibiotic course was shorter for the cases than the controls (6 vs. 8 months,  $p = 0.03$ ). The total number of antibiotic receipts was greater amongst cases in each of the first 3 yr of life, but this reached significance only when the whole three-year period was considered (249 vs. 182 courses,  $p = 0.05$ ). The increased ratio of antibiotic receipt in cases over controls was highest in the first year of life (1.32, 95% CI 0.99–1.78). Significantly more cases than controls were prescribed antibiotics for lower respiratory tract infection during the first 3 yr ( $p = 0.007$ ), but not during the first year of life ( $p = 0.52$ ). Antibiotics use by class was similar in the two groups. Our data support the hypothesis that early life exposure to broad-spectrum antibiotics may have a causative role in sensitisation and the expression of wheeze.

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The causes of the recent increase in allergic sensitisation and asthma are incompletely understood (1). The hygiene hypothesis postulates that decreased early-life microbial exposure may increase the risk of allergic diseases (2). A recent extrapolation of the hypothesis examined the link between gut microbiota and the development of allergy (3). The neonate is born with a sterile gastrointestinal tract, acquires micro-organisms in early life and continues to develop gut microbiota over the first year of life. The gut acts as an interface between the developing immune system and microbial antigens, and intestinal microbiota may play a role in immune maturation. Animal models suggest that rendering the neonatal gut sterile by antibiotics can

result in altered function of the immune system (4).

The receipt of broad spectrum oral antibiotics affects gut microbiota in healthy subjects (5). Studies using parental recall of antibiotic use have reported associations between early-life antibiotic receipt and subsequent asthma and atopy (6–8) but may be confounded by recall bias. It is interesting to note that studies using health care records of antibiotic prescriptions have reported conflicting results (9–13). Whether children developed allergic disease because of antibiotic receipt, or whether they received antibiotics because of symptoms resulting from existent disease requires further clarification. In fact, some studies that have examined the

indication for antibiotic prescription have suggested a reverse causation (13). Ideally, the method of investigation of this hypothesis would be a prospective randomized controlled study, but this would be unethical. We have used a nested case-control design within the context of a prospective birth cohort to investigate the relationship between antibiotic receipt in early life and atopic wheeze.

## Methods

### Study population

The Manchester Asthma and Allergy Study is a population-based birth cohort described in detail elsewhere (14). Study was approved by the Local Research Ethics Committee and informed consent was obtained from all parents. The subjects were recruited prenatally by screening parents using skin testing and questionnaires. During the first 3 yr of life parents kept diary cards regarding their child's health. All participants attended a review at age 3 yr ( $\pm 4$  wk). A standard respiratory questionnaire was interviewer-administered. Children were skin prick tested (*D. pteronyssinus*, cat, dog, mixed grasses, egg, milk, positive, and negative control; sensitization defined as a weal at least 3 mm greater than the negative control). Following the three-year follow-up visit, parents were invited to enter their children into the case-control study. Cases were defined using the following criteria:

- Sensitized to at least one allergen.
- At least three parentally-reported wheezy episodes.

We matched cases with control children (skin test negative, no history of wheeze) according to parental atopy (history and skin prick test results of both parents), gender, month of birth, pet ownership, and allergen exposure.

### Antibiotic receipt information

The UK health care system ensures that a single medical record is held for all residents, which follows the patient as they change General Practitioners (GPs) and provides a full record of encounters with health professionals. GPs are legally required to maintain accurate records of all prescribing. We collected data from the GP-held medical records on antibiotic prescriptions (including timing, type, and indication) during the first 3 yr of life. Antibiotics prescriptions were classified according to category:

$\beta$ -lactam penicillins (ampicillin, amoxicillin and co-amoxycylav), phenoxymethylpenicillin (Penicillin V), macrolides, cephalosporins and others (principally trimethoprim and flucloxacillin). The indication was classified as 'lower respiratory' (LRTI; one or more of cough, wheeze, and breathlessness), 'upper respiratory' (one or more of sore throat, tonsillitis, earache, otitis media) and 'other' (principally skin infections, urinary tract infections and gastro-intestinal infections).

### Statistical analysis

Statistical analysis was carried out using SPSS Version 11.0 and STATA Version 6.0 (STATA Corp., TX, USA). Comparisons between cases and matched controls were carried out using paired *t*-tests and McNemar's test as appropriate. Antibiotic exposure data was log-normally distributed; geometric means (GM) and 95% Confidence Intervals (CI) are presented. We used Kaplan-Meier plot and a log-rank equality of survival test to compare the time to first antibiotic receipt.

## Results

Of 541 children reviewed at age 3 yr, 43 were skin test positive and had a history of recurrent wheeze. Of these, 37 parents agreed to take part in this study. All 37 (20 males) were matched with a non-sensitized, non-wheezy control subject. The demographic characteristics of the study population are presented in Table 1. There were no significant differences between the groups in terms of height, weight, mode of delivery, history of and duration of breast feeding, maternal smoking or position in sibship. Neither was there any difference in pet or dust mite allergen levels in homes (data not shown).

### Antibiotic receipt amongst cases and controls

In the first year of life, significantly more cases than controls received one or more antibiotic

Table 1. Demographics of the study population

|                                                 | Cases (n = 37)     | Controls (n = 37)  | p Value |
|-------------------------------------------------|--------------------|--------------------|---------|
| Age (yr) – mean, 95% CI                         | 4.36, 4.22–4.50    | 4.38, 4.24–4.52    | 0.62    |
| Height (cm) – mean, 95% CI                      | 104.0, 102.4–105.6 | 104.7, 103.2–106.2 | 0.51    |
| Weight (kg) – mean, 95% CI                      | 17.9, 17.0–18.8    | 18.2, 17.4–19.0    | 0.59    |
| Vaginal delivery                                | 26 (70.3%)         | 30 (81.1%)         | 0.28    |
| First-born                                      | 15 (40.5%)         | 18 (48.6%)         | 0.64    |
| Maternal smoking                                | 8 (21.6%)          | 4 (10.8%)          | 0.35    |
| Duration of breast feeding (wk: median (range)) | 8 (0–55)           | 18 (0–56)          | 0.20    |

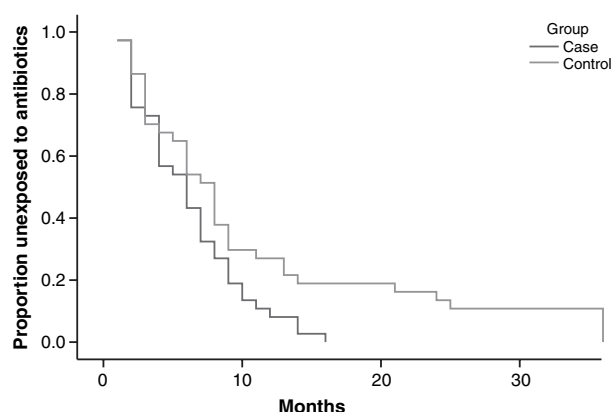


Fig. 1. Kaplan-Meier plot of time to first antibiotic receipt over the first 3 yr of life in cases and controls.

courses (92% vs. 70%,  $p = 0.04$ ). By age 3 yr, all the cases and 86% of the controls had received at least one course of antibiotics ( $p = 0.06$ ). Fig. 1 illustrates the time to first antibiotic receipt for cases and controls during the first 3 yr of life. The median (95% CI) time to first antibiotic course was 6 months (4–8) for the cases and 8 months (6–10) for the controls ( $p = 0.03$ ).

The total number of antibiotic receipts was greater amongst cases compared to controls in each of the first 3 yr of life, but this reached statistical significance only when the whole three-year period was considered (first 3 months: 17 vs. 7 courses,  $p = 0.14$ , first year: 97 vs. 69 courses  $p = 0.06$ , second year: 85 vs. 68 courses  $p = 0.35$ , third year 67 vs. 45 courses  $p = 0.15$ , total courses over 3 yr 249 vs. 182 courses,  $p = 0.05$ ). The increased ratio of antibiotic receipt in cases over controls was highest in the first year of life (1.32, 95% CI 0.99–1.78; Table 2), with a trend already apparent as early as the first three months of life (1.14, 95% CI 0.96–1.36,  $p = 0.14$ ).

The majority of antibiotic receipts in both groups were for broad-spectrum antibiotics. The receipt of antibiotics by class was similar in the two groups (proportion of receipt by class:  $\beta$ -lactam penicillins 60% vs. 58%, macrolides 15% vs. 15%, cephalosporins 7% vs. 9%, penicillin V

6% vs. 2%, others 12% vs. 16%; cases vs. controls, respectively).

#### Indication for antibiotic prescription

Significantly more cases ( $n = 32$ ) than controls ( $n = 21$ ) were prescribed antibiotics for LRTI during the first 3 yr of life ( $p = 0.007$ ). However, there was no difference in the numbers of children receiving antibiotics for LRTIs during the first year of life (cases vs. controls, 21 vs. 17,  $p = 0.52$ ). A trend emerged during the second and third years of life for more cases to receive antibiotics than controls for LRTI ( $p = 0.11$  and  $p = 0.08$ , respectively). Similar numbers of cases ( $n = 28$ ) received antibiotics for URTIs over the first 3 yr of life compared with controls ( $n = 26$ ;  $p = 0.75$ ), but there was a statistical trend for more cases ( $n = 19$ ) to receive antibiotics in the first year of life for URTI than controls ( $n = 11$ ;  $p = 0.08$ ).

Among children who were prescribed antibiotics, the first antibiotic prescription was most commonly for a LRTI, 19 (51%) of cases had a prescription for LRTI compared with 14 (42%) of controls). The next most common indication was for URTI (13 children in each group). Overall, there was no significant difference in the indication for the first antibiotic prescription between groups ( $p = 0.73$ ; chi-square test).

#### Discussion

In this nested case-control study, we found that sensitized children with recurrent wheezing during the first 3 yr of life had increased early life antibiotic exposure compared to matched children with neither wheezing nor sensitization. The consistent trends to increased antibiotic receipt were evident from the first 3 months of life, and were more apparent in the first than the second and third years. Although the proportion of antibiotic prescribing for LRTI was higher amongst the cases during the first 3 yr, this did not differ between the groups in the first year of

Table 2. Number of antibiotic courses received per child per time period

| Time period | Geometric mean (95% CI) [Range] |                       | Ratio for cases/controls (95% CI) | Significance (paired <i>t</i> -test) |
|-------------|---------------------------------|-----------------------|-----------------------------------|--------------------------------------|
|             | Cases                           | Controls              |                                   |                                      |
| Age 0–3 yr  | 5.4 (4.2, 6.9) [0–21]           | 3.4 (2.3, 4.7) [0–16] | 1.47 (1.01, 2.13)                 | 0.05                                 |
| Age 0–1 yr  | 2.1 (1.5, 2.8) [0–9]            | 1.3 (0.9, 1.9) [0–7]  | 1.32 (0.99, 1.78)                 | 0.06                                 |
| Age 1–2 yr  | 1.6 (1.1, 2.3) [0–9]            | 1.2 (0.8, 1.8) [0–7]  | 1.18 (0.83, 1.68)                 | 0.35                                 |
| Age 2–3 yr  | 1.2 (0.7, 1.8) [0–12]           | 0.7 (0.4, 1.1) [0–8]  | 1.27 (0.91, 1.77)                 | 0.15                                 |

life. In addition, there was a statistical trend towards increased antibiotic receipt for URTI in cases during the first year of life compared with controls.

These findings are suggestive of a causative association between antibiotic exposure in early life and the development of atopic wheeze. Antibiotic receipt was greater in the first year than in subsequent years and prescribing for LRTI was not significantly increased until the third year. It is in contrast to the results of the adult cohort study by Cullinan et al. who reported relationships between antibiotic use and asthma to be statistically significant and strengthening with increasing age of prescription (13), thus implying a tendency for antibiotics to given for the manifestations of pre-existing asthma.

The strength of our study is the careful definition of outcomes and matching according to a number of factors known to be associated with sensitisation and wheeze. This may be of particular importance as some studies have shown the effect of antibiotic exposure on atopy and asthma to be modified by parental atopy (6) and pet exposure (11). The main weakness is the small number of participants. However, it is worth emphasising that we had to screen more than 500 children to recruit cases and controls. Furthermore, they represented the extreme ends of a spectrum, i.e. those who by age 3 yr were skin test positive and had three or more wheezy episodes were compared with non-atopic children who have never wheezed.

Several studies support the relationship between antibiotic use and subsequent diagnosis of allergic disease (9, 7, 12). Farooqi and Hopkin reported a doubling of risk of diagnosed atopic disorders in association with antibiotic receipt in the first 2 yr of life, which remained significant whether antibiotics were given for respiratory or non-respiratory indications (9). In a retrospective survey in New Zealand, a four-fold increased of risk of asthma was observed in association with antibiotic receipt in the first year of life (7). In addition, they reported a dose response effect with increasing risk of asthma with increasing number of courses in the first year. McKeever et al also reported a dose response effect with increasing hazard ratios for asthma with increasing number of antibiotic courses (12). However, other studies results have raised the question of whether children developed asthma and sensitisation because of antibiotic receipt, or whether they received antibiotics because of symptoms resulting from already existent disease (10, 13, 15).

The importance of this issue lies in the potential to modify the risk exposure if a causal relationship is established. Much of the prescribing of antibiotics in young children occurs inappropriately in viral illnesses (16), and so is potentially modifiable. The high levels of antibiotic prescribing in the community we observed (with 92% of cases and 70% of controls receiving any antibiotics in the first year of life) may point to an increasing use in this age group over time (13).

Although our study cannot confirm or refute the causal relationship between antibiotic exposure and outcomes, the results support the hypothesis that early-life exposure to broad-spectrum antibiotics has a causative role in atopic wheeze and adds further evidence to this controversial subject. While we cannot exclude the possibility of reverse causation, the early trend to increased antibiotic exposure is much more consistent with a causal relationship. Indeed, if antibiotics were prescribed for wheeze, the clinical manifestation of the disease must have begun very early in life. In addition, the proportion of antibiotics prescribed for LRTI, although higher in the cases than controls overall, was very similar in the first year of life.

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