

Sibling effect on atopy in children of patients with asthma

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Summary

Background Multiple population studies have shown the presence of a sibling effect on atopic disease. However, it is unclear if the sibling effect is also of importance in subjects who are genetically at high risk for the development of atopy.

Objective To study the presence of a sibling effect on markers of atopy (serum total IgE, specific IgE, skin tests) and asthma (bronchial hyper-responsiveness to histamine) in families ascertained through a parent with asthma.

Methods First-degree offspring in 200 asthma families were studied ($n = 541$). Mixed effects regression models were used to account for the dependence of the observations within a family, and to adjust for possible confounding variables.

Results Multiple regression analysis showed that having older siblings was inversely related to atopy, defined as ≥ 2 , ≥ 3 , ≥ 4 , or ≥ 5 skin tests ($P = 0.07$ – 0.009). In addition, family size (number of siblings) had a significant protective effect on the presence of specific IgE to common aeroallergens ($P = 0.03$). Exposure to cigarette smoke in the first 3 years of life significantly increased the risk of having specific IgE to common aeroallergens ($P = 0.04$). No sibling effect was detected for serum total IgE or bronchial hyper-responsiveness to histamine.

Conclusions This study shows a protective sibling effect on the presence and severity of atopy but not on bronchial hyper-responsiveness in children who are genetically at risk. The identification of the sibling effect in high-risk families stresses the need to understand the basis of this effect, in order to design future prevention programmes.

Keywords asthma, atopy, IgE, sibling effect, skin test, specific IgE

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Introduction

The worldwide increase in prevalence of atopic diseases has urged the scientific community to identify and prevent factors provoking this increase. These factors may be closely related to Western lifestyle, such as diet and the change in housing conditions leading to elevated levels of allergen exposure [1]. Identification of preventive factors of atopy development is of special interest, as this may lead to new strategies for disease prevention. Possible preventive factors include early childhood infections [2] or endotoxin exposure [3], early day care attendance [4, 5], living on a farm [6, 7] and a sibling effect [8].

The sibling effect includes a protective effect of sibling order on atopic phenotypes, i.e. the number of older siblings or an effect of family size, thus both older and younger siblings. Sibling effects on atopy have been identified in multiple population studies, in which atopy was defined by questionnaire [9–12], by allergy skin tests [13–16] and/or by measurements of specific IgE to aeroallergens [17–19]. Several studies have also

identified a sibling effect on asthma and wheeze [5, 20, 21], but not on bronchial hyper-responsiveness [13]. Most previous studies have been performed in general populations, although the sibling effect has been included in two family studies [9, 19]. Consequently, published data on the interaction between atopic disease of various family members and the presence of the sibling effect in high risk families is limited. In 1997, Strachan predicted that families with a genetic predisposition ‘... may demonstrate steeper risk gradients with increasing number of siblings than surveys of the general population’ [22]. This was partially confirmed by the study of Mattes et al. in which a sibling effect on atopy was seen in children of an atopic father, although not in children of an atopic mother [9]. In contrast, results from the European Community Respiratory Health Survey showed no sibling effect in children from atopic parents, suggesting that environmental factors are less important in children with a strong genetic predisposition [19]. Because prevention programmes are likely to be focussed on children with a family history of atopy, it is important to know if environmental factors play a role in children with a strong genetic predisposition. Therefore, the aim of the present investigation was to identify a sibling effect on markers of atopy and asthma in offspring of parents with predominantly atopic asthma.

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In addition, we assessed whether parental smoking in the first 3 years of life of the child may explain the sibling effect.

Methods

Study population

Between 1962 and 1975, patients with asthma from the northern part of the Netherlands were referred to Beatrixoord, a regional asthma centre in Haren, the Netherlands. Approximately 1800 newly diagnosed patients with symptomatic asthma who did not have an asthma exacerbation underwent a standardized, complete evaluation. For inclusion in the current study, at the time of initial testing all probands were younger than 45 years of age, had a doctor's diagnosis of asthma and showed bronchial hyper-responsiveness (BHR) to histamine ($PC_{20} \leq 32$ mg/mL, 30 s inhalation protocol [23]). All available charts of these asthma patients were screened and current addresses retrieved if possible, and patients and their families invited to participate in the study if the proband had at least two children. Between 1990 and 1999, 200 probands with asthma were restudied, together with their spouses, at least two children and available grandchildren aged 6 years or older. For the analysis presented in this paper, the first generation offspring of these families was selected. In this generation, every child had at least one parent with asthma (82% atopic as defined by having one or more positive skin tests), and data on family structure were available. Every child was assigned a sibling order according to the date of birth. Twins were assigned the same number equal to the number of the second twin. In addition, sibling size was recorded. Paternity status was verified by genotyping over 300 DNA markers for the genetic studies [24].

Clinical and laboratory evaluation

All individuals answered a modified version of the British Medical Council questionnaire on respiratory symptoms, housing conditions and smoking. Questions on medication use were added. Smoking history was recorded by asking each subject the number of cigarettes smoked per day for every year of their life. From this, smoking during pregnancy was determined as well as exposure to cigarette smoke of their children during the first 3 years of life.

All individuals underwent spirometry, bronchodilator reversibility to 800 µg of inhaled albuterol (salbutamol) and bronchial responsiveness testing to histamine using a 30-s inhalation protocol previously described [23]. Bronchial hyper-responsiveness was defined as a provocative concentration (PC_{20}) ≤ 32 mg/mL (30-s method).

Skin tests were performed in adults by intracutaneous tests, and in children with skin prick tests (SPTs), with a positive and negative control. For the current analysis, 10 allergens used both in adults and children were selected: mixed grasses, tree pollens and weeds, house dust mite, animal dander of dog, cat, horse, a mixture of guinea pig and rabbit, and the moulds *Aspergillus fumigatus* and *Alternaria alternata* (ALK-Abello, Nieuwegein, the Netherlands). The maximum diameter and the perpendicular diameter of the wheal size were recorded after 15 min. An intracutaneous skin test was considered to be positive if the mean wheal diameter was ≥ 5 mm; a SPT was considered positive if the mean wheal diameter was ≥ 3 mm.

The skin tests were not used for further analysis if the negative control gave a positive reaction.

Total IgE was measured by solid-phase immunoassay in the first 92 families. The mean of two duplicate tests of IgE was used, and measurements were repeated if the difference between duplicates was $> 5\%$. (Pharmacia, Uppsala, Sweden). In the second set of 108 families, serum IgE levels were measured by an enzyme-linked fluorescent assay (Mini Vidas, Biomerieux Inc., Marcy, France). Specific IgE was measured by an *in vitro* test system (Pharmacia CAP system, Phadiatop FEIA) according to the manufacturer's instructions (Pharmacia Diagnostics AB, Uppsala, Sweden). In this assay, IgE is measured against a mixture of inhalant-allergens and is used as a general assessment of allergic responsiveness. It determines the presence of specific IgE to the following antigens: house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), timothy grass (*Phleum pratense*), birch (*Betula verrucosa*) and olive pollen (*Olea europea*), two different weeds (*Artemisia vulgaris* and *Parietaria officinalis*), a mould (*Cladosporium herbarum*) and, finally, cat, dog and horse dander. The test was regarded positive if the fluorescence score of the subject's serum was higher than that of the reference as provided by the manufacturer. Finally, eosinophils were counted in a counting chamber. All participants were asked to stop asthma and allergy medication before the clinical testing if possible: specifically inhaled corticosteroids were stopped for 14 days, inhaled long acting beta-mimetics and oral antihistamines for 48 h, inhaled short acting beta-mimetics and anticholinergics for 8 h. Asthma patients did not have an asthma exacerbation or a course of oral prednisone in the 6 weeks prior to the study.

Informed consent

The study was approved by the Medical Ethics Committee of the University Hospital Groningen. In addition, the study was approved by the Institutional Review Board of the University of Maryland and Wake Forest University. Written informed consent and written parental consent were obtained from all participating adults and children, respectively.

Statistical methods

Serum total IgE was log transformed. Both parametric (*t*-test, ANOVA) and non-parametric analyses (Mann-Whitney, Kruskal-Wallis tests) were used to study differences in sibling order, depending on the normality of the distribution of the variables using SPSS 10.0. Sibling effects on atopy and BHR were assessed using mixed-effects regression models containing fixed effects in addition to random effects. These models adjust for the dependence of the observation within one family [25]. The sibling effect was adjusted for age, sex and smoking habits. In addition, parental atopy (the presence and the severity of atopy), passive smoking in the first 3 years of life (measured by smoking of the parents during that period), maternal age at birth and having pets or outdoor animals, were included in the model one by one. The latter variables only stayed in the model when they were significantly related to one of the markers of atopy or BHR in the child or when they influenced the effect of sibling order or family size. Models with sibling order (the total number of older siblings) and family size (the total number of siblings in the family) were run, and the best fitting models were

used for further analysis based on Akaike's information criterion. In addition to assessment of the sibling effect on the presence of positive skin tests and BHR, the sibling effect on severity of skin test positivity and bronchial responsiveness was assessed. This was done by using different cut-off values: ≥ 2 , ≥ 3 , ≥ 4 , and ≥ 5 positive skin tests and $PC_{20} \leq 16$, 8 and 4 mg/mL histamine.

Results

Study population

In the 200 families, 586 children were 6 years or older. Of these 586 children, 541 (92.3%) were investigated. Reasons for non-participation were living abroad ($n = 5$), deceased/disappeared ($n = 2$) and refusal ($n = 38$). In the first-degree offspring, one case of non-paternity was identified and paternal status was set to unknown. Baseline characteristics of the first-degree offspring, the probands and the spouses are shown in Table 1. The majority of the first-degree offspring was adult, the mean age was 24 years. Half of the children were atopic, as detected by one or more positive skin tests (50%) and 44% had specific IgE to common aeroallergens. In addition, 46% of the children had BHR to histamine.

Sibling effect – descriptive analysis

Mean ages were significantly different with sibling order 1, 2, 3 and ≥ 4 (Table 2). Specifically, first-born children were older than second- and third-born children ($P < 0.05$). First-born children had the highest prevalence of one or more positive skin tests (55%), specific IgE to common aeroallergens (46%) and BHR (50%). We observed lower prevalence rates for the consecutive siblings; the lowest rate was seen in the group of children born fourth or later: 43% had one or more positive skin tests, 34% had specific IgE to common aero-allergens and 38% had BHR. A similar trend was observed for geometric mean serum total IgE levels ranging from 67.3 kU/L in first-born children to 54.6 in children born fourth or later. Finally, the mean number of positive skin tests decreased significantly, with sibling order being 2.2 in the first-born to 1.2 in the last-born (≥ 4) children (Fig. 1).

Skin tests

In the multivariate models, sibling order fit the data better than did family size. Different cut-off levels for skin test positivity were assessed: ≥ 1 , ≥ 2 , ≥ 3 , ≥ 4 and ≥ 5 skin tests. When atopy was defined as at least one positive skin test, having one or more older sibling was not significantly related to atopy [odds ratio (OR) 0.92, 95% confidence interval (CI) 0.70–1.21]. However,

Table 1. Clinical characteristics of probands, spouses and their first degree offspring in 200 Dutch families

	First-degree offspring	Probands	Spouses
Number	541	200	201*
Age, mean (SD), years	24.2 (9.2)	52.1 (8.4)	51.0 (9.2)
Male, %	45.2	62.0	37.8
Total IgE, geometric mean, kU/L (range)	62.7 (0–3360)	93.0 (1.0–2880)	26.2 (0.5–1940)
≥ 1 positive skin test, %	50.1	81.9	31.0
Specific IgE, % positive (Phadiatop)	43.9	72.4	15.1
FEV ₁ , % predicted pre-medication (mean, SD)	93.8 (12.3)	69.6 (24.5)	98.4 (14.1)
FEV ₁ , % predicted post-medication (mean, SD)	100.3 (11.9)	82.4 (23.5)	103.9 (13.7)
BHR to histamine, % $PC_{20} \leq 32$ mg/mL	46.3†	88.2†	25.6

BHR, bronchial hyper-responsiveness. *One proband married twice. Missing data due to following reasons: not enough blood for serum total IgE measurement in two children, and for specific IgE measurements in 13 children; skin tests refused by four children. Spirometry before and after medication could not be performed reproducible in two children. †Thirty probands and eight children were not tested due to an FEV₁ that was too low to be tested safely; or FEV₁ measurements that were not reproducible.

Table 2. Markers of atopy and asthma in children of patients with asthma stratified on birth order

	Sibling order			
	1	2	3	≥ 4
Number	188	182	96	75
Age (mean, SD)*	25.8 (8.9)*	23.2 (9.3)*	23.0 (9.1)*	25.5 (9.7)
Male, %	39.3	47.3	51.0	48.0
Serum total IgE, kU/L geometric mean (range)	67.3 (1.0–3360)	61.1 (0–3040)	60.3 (1.0–1490)	54.6 (1.0–2000)
Skin tests				
≥ 1 positive, %	54.5	48.9	49.5	42.7
≥ 4 positive, %	29.7	22.2	18.9	12.0
Number of skin tests positive (median, IQR)	1 (0–4)†	0 (0–3)†	0 (0–2)†	0 (0–1)†
Specific IgE, % positive	46.2	46.0	43.0	34.2
BHR: $PC_{20} \leq 32$ mg/mL, %	50.2	46.9	44.2	37.8

IQR, interquartile range; BHR, bronchial hyper-responsiveness. * $P < 0.05$ sibling order 1 versus 2 and 3. †Significant differences between four sibling order groups, Kruskal–Wallis test ($P < 0.05$).

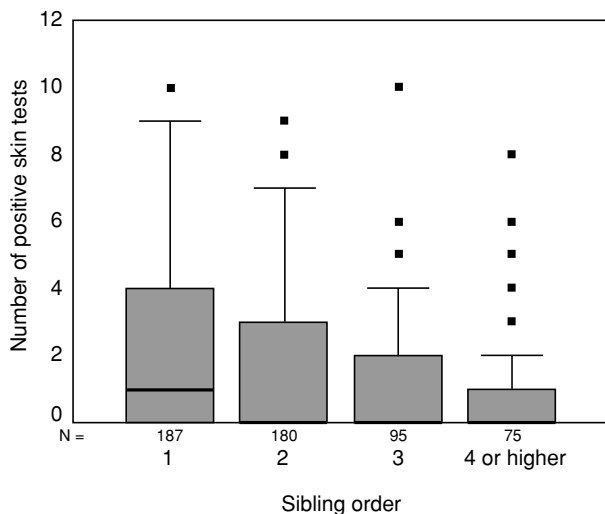


Fig. 1. Number of positive skin tests for every birth order group. Boxplot shows medians (bars) and interquartile ranges. Dots are outliers.

when more stringent definitions of atopy (≥ 2 , ≥ 3 , ≥ 4 and ≥ 5 positive skin tests) were used in the same model, sibling order was significantly and inversely associated with severe atopy. Table 3 shows the results for skin test positivity defined as two or more positive skin tests. The sibling effect on severe atopy was confirmed by analysis of higher cut-off values of skin test positivity, i.e. siblings with higher birth order are less likely to have ≥ 2 , ≥ 3 , ≥ 4 and ≥ 5 positive skin tests than first-born siblings. OR and 95% CI of sibling order for atopy defined as ≥ 3 positive skin tests was 0.70 (0.36–0.93), for ≥ 4 positive skin test 0.76 (0.55–1.03) and was 0.70 (0.50–0.97) for ≥ 5 positive skin tests. Possible confounding variables, such as the presence of indoor pets, the presence of outdoor cattle, smoking during pregnancy, current (passive) smoking, passive smoking during the first 3 years of life of the child and maternal age at birth of the child were not associated with skin test positivity, nor influenced the relationship of sibling order with skin test positivity.

Specific IgE to common aeroallergens

In the multivariate models, using family size described the data better than did sibling order. Family size was significantly and negatively related to the presence of specific IgE to common aeroallergens (Table 4). Thus, for larger families, the prevalence of specific IgE to common aeroallergens in the children is lower. In addition, exposure to cigarette smoke during the first 3 years of life was associated with having positive specific IgE to common aeroallergens.

BHR to histamine and serum total IgE levels

Sibling order and family size were not associated with the presence of BHR in a multivariate model after adjustment for age, sex, smoking and eosinophil counts (sibling order: OR 1.09, 95% CI 0.85–1.41; $P = 0.48$). Similar results were observed when more severe cut-off levels for BHR were taken ($PC_{20} \leq 16$, 8 or 4 mg/mL). In addition, sibling order and family size were not associated with serum total IgE levels (reference category first-born; second-born OR 0.97, 95% CI 0.88–1.07,

Table 3. Sibling order effect on skin test positivity (≥ 2 positive skin test)

Variable	OR	95% CI	P-value
Intercept	0.01	0.00–0.06	0.000
Male sex	1.38	0.74–2.58	0.311
Smoking habits			
Ex-smoker	1.01	0.39–2.59	0.98
Smoker	1.22	0.34–2.38	0.55
Age (quartiles)*			
> 17–24 years	3.42	1.47–7.95	0.004
> 24–30 years	6.49	2.34–17.98	0.000
> 30 years	1.52	0.57–4.06	0.392
Skin tests, parent			
Number of positive skin tests father	1.08	0.94–1.24	0.24
Number of positive skin tests mother	0.96	0.82–1.12	0.60
Sibling order	0.72	0.55–0.95	0.018

Presented are odds ratios (OR) with 95% confidence intervals (CI). Skin test positivity corrected for bronchial hyper-responsiveness, serum total IgE levels and eosinophil count. *Reference category: ≤ 17 years.

Table 4. Family size effect on the presence of specific IgE to common aeroallergens (Phadiatop)

Variable	OR	95% CI	P-value
Family size	0.73	0.56–0.97	0.03
Male sex	3.06	1.37–6.85	0.007
Age (tertiles)*			
19–28 years	2.80	0.99–7.92	0.05
> 28 years	1.45	0.53–3.93	0.47
Smoking habits			
Ex-smoker	1.28	0.50–3.29	0.63
Smoker	0.78	0.36–1.71	0.54
Passive smoking in first 3 years of life	2.83	1.02–7.84	0.04

Presented are odds ratios (OR) with 95% confidence intervals (CI). Corrected for bronchial hyper-responsiveness, serum total IgE levels and eosinophil count. *Reference category is ≤ 19 years.

$P = 0.62$; third-born OR 1.0, 95% CI 0.87–1.15, $P = 0.96$; $\geq$ fourth-born OR 1.0; 95% CI 0.85–1.17, $P = 0.97$).

Discussion

This study shows that in families with a parent with asthma, children with older siblings are less likely to have positive skin tests (defined as ≥ 2 skin tests), whereas children with more siblings (both older and younger) are less likely to have positive specific IgE to common aeroallergens. Furthermore, children who were exposed to passive smoking during the first 3 years of life were more likely to have elevated specific IgE levels. This effect, however, was not consistently identified for different atopic phenotypes. In contrast, we found no sibling effect on serum total IgE and BHR in children from patients with asthma. Environmental factors as detected by the sibling effect may modify the outcome of atopy but not of BHR in children who are at high risk due to genetic predisposition.

We observed a significant protective effect of a larger family size on the presence of specific IgE to common aeroallergens. This confirms several reports showing a similar effect in

population studies and in one study investigating 1440 families [9, 17–19]. In the latter study, a sibling effect was present in children of atopic fathers, but not of atopic mothers. Families were investigated with questionnaires, and paternal atopic status was assessed by asking one parent, generally the mother. Thus, bias could have been introduced in that study if only more severe atopic fathers were reported. In addition, recall bias may influence the results of questionnaire-based family studies. This has been recently shown in the MAS study, in which self-reported atopy by the parents was influenced by current atopy of the child [26]. We studied objective markers of atopy, such as skin tests and specific IgE to common aeroallergens, and found effects in families with atopic asthmatic fathers and/or mothers. Differences in results may also be explained by methods of family ascertainment. In the present study, ascertainment was based on asthmatic status of one of the parents, and a high percentage (>92%) of all children were studied. Therefore, it seems unlikely that selection bias may have confounded our results.

Our findings appear to be in contrast with the results from the European Community Respiratory Health Survey (ECHRS). In this survey, a sibling effect was present in children of non-atopic parents, but not in children of atopic parents. However, the severity of atopy was not taken into account, as atopy was defined as the presence of one or more positive skin tests [19]. In contrast to the ECHRS, we investigated offspring of predominantly atopic asthmatic probands and found a protective effect of sibling order on skin test positivity, yet only when we defined a more severe atopic phenotype. We speculate that although genetic predisposition in these children may lead to skin test positivity, the sibling effect appears to modify the severity of atopy (i.e. smaller number of positive skin tests). These conflicting results may indicate that the sibling effect is smaller in children who are genetically at risk. The sibling effect on severe atopy has also been indicated by an Italian study of recruits, which defined severe atopy by a higher cut-off point for specific IgE to common aeroallergens [18]. In addition, in a German population of children, the sibling effect on atopy was studied by assessment of skin test positivity. In this study, the sibling effect appeared to be present, with higher cut-off values for the mean diameter of the skin test (i.e. ≥ 4 or ≥ 5 mm) but not with lower values (≥ 2 mm) [14]. For skin test positivity and specific IgE to common aeroallergens, different models fit the data best for sibling order and family size. The trends in the data were similar (data not shown) and we have chosen the best fitting models in our multivariate analyses. The sample size of our study did not allow us to further disentangle effects of size and order, or effects of the gender of the older siblings. The ascertainment of families with at least two siblings further strengthens the association between family size and sibling order [8]. Moreover, as we only ascertained families with at least two siblings, the children at highest risk for atopy (one child per family) were not included in our study. Thus, we conclude that given the relatively small sample size the sibling effect is an important environmental factor acting in these high-risk families.

Our study did not find a sibling effect on serum total IgE levels and BHR. Similar results have been observed in other studies [13, 17, 19]. It appears that environmental factors are less able to modify serum total IgE levels than specific IgE levels or skin test positivity. This is consistent with genetic studies in

our families, showing high heritability estimates of serum total IgE levels of 55%, compared to 41% for specific IgE to common aeroallergens and 25% to skin tests [24]. No sibling effect was observed on BHR. Other studies have identified a sibling effect on doctor-diagnosed asthma [5] or self-reported asthma symptoms in children [20, 21, 27], although the number of studies supporting a role on hayfever and atopic sensitization appear to be larger [10, 11, 14–16, 18, 19, 28, 29]. However, there appeared to be a trend indicating a protective effect of the number of siblings on BHR in the univariate analysis. We cannot exclude that this study was not powerful enough to show a significant sibling effect on BHR. However, other explanations, such as differential environmental effects on asthma and atopy need to be considered.

Strachan hypothesized that the sibling effect could be explained as a protective effect of childhood infections on the development of atopy [12]. Children who grow up in larger families report more childhood infections, such as prolonged colds [30]. This is supported by immunological data, indicating that infections may have a skewing effect on the Th1–Th2 balance towards the Th1 pathway [31]. Alternative explanations for the sibling effect, such as changing smoking patterns in the parents, have also been put forward as an explanation. Parents could respond to the presence of allergy in the first child and change their smoking behaviour for the consecutive children, leading to lower prevalences of atopy in these children. This was not the case in our study. Although passive smoking in the first 3 years of life was associated with the presence of specific IgE to common aeroallergens, it did not appear to be associated with sibling order.

It has been suggested that the sibling effect is an interaction of genes with environment (infections) [32]. Further studies of genes that respond to these infections are needed. Interesting candidate genes are genes that encode for receptors of lipopolysaccharides or other bacterial wall components, such as CD14 [33, 34] and the Toll-like receptor 4 [35]. A precise understanding of protective effects of infections may lead to new preventative measures, which may possibly be applied in high-risk families. In summary, this study shows a sibling effect on the presence and severity of atopy, defined by specific IgE to common aeroallergens and skin test positivity in families at high risk for atopy and asthma. Furthermore, an independent negative effect of smoking during the first 3 years of life on atopy was observed. The identification of the sibling effect in high-risk families stresses the need to understand the sibling effect, in order to design better preventive programmes.

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