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Does maternal immunoglobulin E decrease with increasing order of live offspring? Investigation into maternal immune tolerance

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Summary

Background Identifying the protective effect of a higher number of siblings is a significant finding in understanding the aetiology of allergic sensitization, asthma, eczema, and hayfever. Knowledge about causes behind the sibling effect may allow us to prevent atopic manifestations.

Objective We tested the hypothesis that rising order of live offspring increases maternal immune tolerance (immune non-reactivity) against allergens. To this end, we investigated whether maternal IgE levels are associated with the number of live offspring.

Methods In a cohort of 1456 newborns recruited between January 1989 and February 1990 on the Isle of Wight, UK, we determined maternal and cord serum IgE, and the order of live offspring. The data were analysed by means of linear and path analysis.

Results Maternal and cord serum IgE were available in 820 mother–infant pairs with birth order information. We found that the number of live offspring significantly reduces maternal IgE. The decline was more prominent in mothers with atopy ($n = 268$). The geometric means of IgE after the first, second, and third or higher delivery were 74.4, 66.6, and 43.0 kU/L, respectively. Findings of path analysis suggest a significant direct effect of birth order on maternal IgE, but no direct effect of birth order on cord serum IgE.

Conclusion The findings support that maternal immune tolerance against allergens may increase with increasing order of live offspring and thus pass on a lower risk of developing atopy in children of higher birth order.

Keywords atopy, birth order, cord serum, IgE, maternal, tolerance
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Introduction

The identification of the protective effect of a higher number of siblings on the risk of atopic manifestations is a major challenge for etiologic research on allergic sensitization, asthma, eczema, and hayfever [1, 2]. If we were able to identify the cause behind the sibling effect, we might be able to prevent 30–50% of all atopic manifestations [3].

One school of thought emphasizes exposure to infections during the post-natal period that may cause a switch from a Th2 to a Th1 pattern of immune response [4–6]. A new approach focuses on pre-natal factors that may program post-natal immune responses in children. This concept is supported by findings that cord serum IgE levels and responses of cord serum leucocytes to allergens are decreased with increasing birth order [7, 8]. Additionally, providing further evidence for a pregnancy-related aetiology, Sunyer et al. [9] showed that the number of pregnancies is lower in mothers with atopy. One explanation for these findings is that each successive pregnancy decreases the atopic response of the mother by induction of immune tolerance (de-sensitization or immune non-reactivity), and thus decreases the risk in subsequent offspring of developing atopy [9, 10]. These intriguing findings lead to the following two hypotheses:

1. Maternal IgE levels are decreased with birth order (*tolerance induction hypothesis*). Provided that maternal immune tolerance (indicated by lower IgE levels) is critical for atopic responses of the child, findings of a reduction in cord serum IgE with increasing number of live siblings [7] may not be an effect on its own, but might be explained by the reduction in maternal IgE. Thus we need to know if there is a direct link between birth order and cord serum IgE or an indirect link with maternal IgE acting as an intervening variable. We also hypothesize:
2. The reduction of maternal IgE with increasing order of live offspring is transmitted to subsequent offspring and thus explains a reduced cord serum IgE in offspring with a higher-order offspring (*tolerance transmission hypothesis*).

To test these hypotheses, we investigated maternal and cord serum IgE in mother–infant pairs from the Isle of Wight, UK. At the birth of the index child, the mothers had a varying

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number of live births, correspondingly the index child had a varying number of older siblings. For the offspring cohort, it has been shown that cord serum IgE is a risk factor for allergic sensitization at age 4 years [11, 12].

Population and methods

Study population

After approval by the Local Research Ethics Committee, a birth cohort of 1456 children born between January 1989 and February 1990 on the Isle of Wight, UK, was established. Informed written parental consent was obtained from all participants. Blood samples were obtained from 1056 mothers within a week of delivery to measure total maternal IgE levels. For reasons unrelated to the study, sampling for maternal IgE had to be discontinued before completion of the study. No pre-conceptional maternal IgE were collected. Cord serum IgE levels were available in 1358 children; the position of the child in the family was available for 1212 offspring.

Questionnaire

In the first survey completed at birth, parents were asked about their age at birth of the child, paternal and maternal smoking before and during pregnancy, and maternal and paternal history of atopy (asthma, hayfever, and atopic eczema). The order of live offspring was ascertained in the 48-month follow-up, and this restricted the sample used to analyse the effect of an increasing number of pregnancies on maternal IgE.

IgE determination

The method of cord IgE estimation was described in a previous report [13]. Briefly, total IgE in the cord serum was measured using Pharmacia IgE EIA® (Pharmacia Diagnostics AB, Uppsala, Sweden), which is designed to measure IgE between 0.2 and 50 kU/L on 0.1 mL of serum or plasma [11, 14]. Maternal IgE was determined using PRIST® (Pharmacia Diagnostics AB) designed to measure IgE between 2.0 and 1000 kU/L. Cord IgA was used to exclude contamination by maternal blood. In 13 infants, the sample was insufficient for IgA assay, and in 73, the serum IgA was more than 10 mg/L (mean+2 standard deviation), indicating contamination with maternal blood. Hence, 86 children were excluded from further analyses [13].

Number of offspring

Different terms are used to describe the protective 'sibling effect'. From the progeny's perspective, it may be defined as the 'number of siblings' or as 'birth order' (number of older siblings − 1). From the mother's perspective, it is the 'number of still living children' (excluding deceased children and pregnancies that did not end in live birth), which is parity minus the number of deceased children. In this study, we focus on mother–child pairs. To define this process from the perspective of mother–infant pairs, we use the term *order of live offspring*.

Statistical analysis

All statistical analyses were performed using SAS/STAT® program [15]. Since the distribution of IgE was not normal, we log-transformed maternal and cord serum IgE and presented the geometric means along with their 5th and 95th percentiles. Regarding order of live offspring, we considered three groups: first, second, and third or higher position. We categorized birth months of the index child into four indicator variables for seasons (Spring: March–May; Summer: June–August; Fall: September–November; Winter: December–April). Other potential confounders were maternal age (categorized: 16–23, 24–33, 34 years and older), maternal and paternal atopy (asthma, hayfever, and atopic eczema) and smoking as well as maternal smoking during pregnancy. To test the effect of order of live offspring on maternal IgE concentrations (tolerance induction hypothesis), we used linear regression adjusting for the potentially confounding factors (GLM procedure). To contrast the different levels of the predictors (order of live offspring and confounders), we estimated adjusted geometric means for the different levels of the predictors.

The tolerance transmission hypothesis proposes that the protective sibling effect on cord serum IgE is indirect, through maternal IgE. This hypothesis cannot be tested with ordinary linear regression models, as intervening variables do not qualify as confounders [16]. Controlling for an intervening variable will reduce the initial association between the risk factor and the outcome, as the one initial causal chain is split into two associations. Thus, we explored the relationship between order of live offspring, and maternal IgE and cord serum IgE by path analysis [17], using the CALIS procedure [18]. We used a two-stage estimation method, by first computing unweighted least-squares estimates of the model parameters and their residuals. Afterwards, these estimates were used as initial values for the optimization process to compute maximum likelihood parameter estimates. Regression parameters (path coefficients), their statistical importance, the explained variance and goodness of fit of the explanatory model, as well as the statistical significance of the deviation of the assumed covariance structure from the empirical covariance are provided in Fig. 1.

In order to estimate a parsimonious model for the path analysis, we only controlled for confounders that were of importance in our previous report on cord serum IgE (maternal atopy and gender) [7] and also those which were significant when testing the predictors for maternal IgE.

Results

Maternal IgE was determined in 1056 maternal blood samples (Table 1). Data on order of live offspring was ascertained at age 4 years for 1212 offspring, data on cord serum IgE was determined in 1358 infants. Information on order of live offspring, cord serum, and maternal IgE was complete for 820 pregnancies (Table 1), which was used in the statistical analyses of association with maternal IgE. There was no significant difference in the distribution of predictor variables (Table 1); however, fewer smoking mothers participated in the follow-up at 48 months (24.3% compared

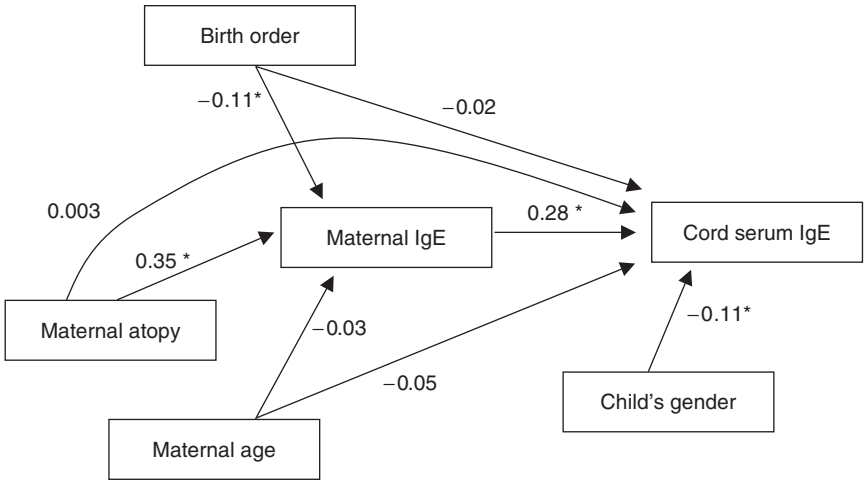

Fig. 1. Path-analytical model exploring the links between birth order, maternal and cord serum IgE, controlled for season (Spring, Summer, Fall, Winter) and maternal smoking ($n = 816$). The path coefficient represents the relative importance between -1 and $+1$. *$P \leqslant 0.05$. Goodness of fit adjusted for degrees of freedom: 0.99. Root mean residuals: 0.001. Deviance between the covariance structure and the empirical covariance: $\chi^2 = 0.065$, (df = 1), $P = 0.80$.

with 20.7%, $P = 0.065$). The geometric means for maternal and cord serum IgE were 32.7 and 0.13 kU/L, respectively. Both were correlated ($r_{Spearman} = 0.33$, $P = 0.0001$) and show a negative correlation with order of live offspring (maternal IgE: $r_{Spearman} = -0.09$, $P = 0.01$, cord serum IgE: $r_{Spearman} = -0.092$, $P = 0.008$).

Since we collected no pre-conceptional maternal IgE, all maternal IgE levels are responses to the order of the full-term pregnancy. Table 2 indicates that maternal IgE decreases with increasing number of preceding offspring. The reduction seems to be stronger for mothers with a history of atopy (74.14, 64.37, 39.03 kU/L). Maternal IgE also declined with age (Table 3). Within each age group, maternal IgE after delivery of the first offspring was higher than maternal IgE after having two or more live offspring.

Controlling for confounders, maternal IgE is significantly reduced with increasing number of preceding offspring: 40.37 kU/L after the first; 29.57 kU/L after the second; and 26.82 kU/L after third or higher order of live offspring (Table 4). Two other significant risk factors were maternal atopy and maternal age. IgE levels were lower in older mothers. Gestational age did not affect maternal IgE.

For women who had their first live birth ($n = 361$), we compared age at first delivery. Women with a history of asthma, hayfever, or atopic eczema ($n = 128$) were found to be 1.15 years older than those without (25.65 vs. 24.5 years, Wilcoxon's test; $P = 0.02$).

Results of the path analysis, including order of live offspring, maternal atopy and age, child's gender, maternal and cord serum IgE, while controlling for maternal smoking during pregnancy and season of delivery, are presented in Fig. 1. The model fits the empirical structure. The findings suggest a significant direct effect of birth order on maternal IgE (path coefficient = -0.11 per child, hence for two offspring: -0.22, for three+offspring: -0.33), but no direct effect of birth order on cord serum IgE (path coefficient = -0.02). The association between maternal and cord serum IgE (path coefficient = 0.28) implies an indirect effect of birth order on cord serum IgE with maternal IgE acting as an intervening variable. For maternal atopy, maternal IgE, and cord serum IgE, we found a similar indirect pattern of association. Maternal atopy did not have a direct effect on cord serum IgE (path coefficient = -0.003), but had an indirect effect via maternal IgE.

Discussion

In support of our tolerance induction hypothesis, maternal IgE concentrations are statistically significantly decreased with order of live offspring. Additionally, maternal IgE concentration, reduced by preceding births, explains the association between order of live offspring and reduced cord serum IgE of the offspring, thus supporting the tolerance transmission hypothesis.

The analysis of maternal and cord serum IgE was based on 820 complete mother–infant pairs from a population of 1456 infants recruited on the Isle of Wight between January 1989 and February 1990. In this subsample used in the analyses, the order of live offspring was available. Maternal blood samples were available for 1056 subjects. A comparison of the initial maternal sample ($n = 1056$) and the sample used in the analyses (Table 1) does not indicate a selection bias. Contamination of the cord serum with maternal blood was assessed by cord IgA. This led to the exclusion of 86 infants from further analysis [13]. Hence, a potential misclassification of cord serum IgE can be excluded.

Whereas one school of thought explains the sibling effect with post-natal exposures and consecutive adaptation of the newborn [4, 6, 19], new research suggested that this pattern of response (cord serum IgE levels or response of cord serum mononuclear cells) is determined *in utero* [7, 8]. For this cohort of children, we showed that cord serum IgE decreased with birth order, and that decreased cord serum IgE in turn decreases the susceptibility for allergic sensitization at age 4 years [7, 12]. Investigating maternal responses during pregnancy represents a further step 'back' in the etiologic web of atopic susceptibility. Our results suggest that the origin of the sibling effect may be in the mother, not the offspring.

These findings raise two questions: Do preceding pregnancies initiate maternal immune tolerance that benefits the next child? Or does atopy decrease fertility? [9, 10]. Our study was

Table 1. Composition of the original sample and the sample with maternal IgE used in analysis

Variable	Initial sample ($n = 1056$) *n* (%)	Sample used in analysis ($n = 820$) *n* (%)
Birth order		
First	363 (34.4)	345 (42.1)
Second	294 (27.8)	287 (35.0)
Third and more	193 (18.3)	188 (22.9)
Missing	206 (19.5)	
Season of birth		
Spring	292 (27.7)	223 (27.2)
Summer	284 (26.9)	225 (27.4)
Fall	227 (21.5)	190 (23.2)
Winter	253 (24.0)	182 (22.2)
Maternal atopy		
Yes	354 (33.5)	268 (32.7)
No	698 (66.1)	550 (67.1)
Missing	4 (0.4)	1 (0.1)
Paternal atopy		
Yes	259 (24.5)	612 (74.6)
No	787 (74.5)	203 (24.8)
Missing	10 (1.0)	5 (0.6)
Age of mother at delivery (years)		
Up to 23	302 (28.6)	211 (25.7)
24–33	634 (60.0)	515 (62.8)
34 and older	112 (10.6)	93 (11.3)
Missing	8 (0.8)	2 (0.2)
Gestational age (weeks)		
30–36	49 (3.2)	17 (2.1)
37–39	306 (19.9)	154 (18.8)
40	724 (47.1)	399 (48.7)
41–45	418 (27.2)	239 (29.2)
Missing	39 (2.5)	11 (1.3)
Gender of the offspring		
Girl	527 (50.2)	407 (49.8)
Boy	523 (49.8)	411 (50.2)
Maternal smoking during pregnancy		
Yes	257 (24.3)	170 (20.7)
No	795 (75.3)	648 (79.0)
Missing	4 (0.4)	2 (0.2)
Paternal smoking (cigarettes/day)		
Yes	400 (37.9)	283 (34.5)
No	628 (59.5)	517 (63.1)
Missing	28 (2.7)	20 (2.4)

not designed to test the reduced fertility hypothesis, however, we found that women who had a history of atopic disorders and gave birth to their first child, were significantly older than their counterparts without atopy (1.15 years). Since Sunyer et al. [9] suggested that mother's age when the first child is born is an indirect measure of fertility, our findings do not rule out that atopy may decrease fertility. Given that lower fertility may result in lower number of siblings [20, 21] and that maternal atopy is linked to higher probability of atopic offspring [22, 23], it is possible that a reduced number of siblings of atopic children may partially reflect a spurious correlation associated with the above-mentioned external factors. However, the results of our study also imply that the atopic response of the mother and the offspring diminished with increasing number of live births.

Our findings support the assumption that the newborn pattern of immune response may be primed *in utero* [24–26] and that birth order may influence this association [7–10]. Furthermore, Devereux et al. [26] suggested that it is not changes in the prevalence of *in utero* allergen sensitization, but the conditions under which this occurs that are responsible for the current epidemic of childhood allergy.

Additionally, we considered that mothers whose older siblings suffered from atopic manifestations (atopic eczema, asthma, or hayfever) might have adjusted their living conditions. They may have stopped having a pet, reduced wet spots in their homes, or stopped smoking during the next pregnancy. This might explain lower IgE levels during their next pregnancy. Hence, we investigated whether atopic manifestations in older children, both in atopic and non-atopic mothers, had influenced maternal smoking, moulds in the house, and having a pet. There was no such indication. Approximately 15% reported damp conditions, 54% had a pet, and 21% of the mothers were smokers, independent of parental or sibling history of atopy.

Previous studies have reported a moderate correlation between maternal and cord serum IgE that is comparable with our findings [27–30]. The correlation suggests that the maternal IgE may influence fetal IgE. One approach to explain the correlation focuses on the genes, which are shared between mother and child, and assumes that cord serum IgE levels are genetically determined [31, 32]. However, our findings reveal a substantial co-variation of cord serum IgE with birth order and maternal age (Tables 3 and 4) that cannot be explained by genes.

If not genetically determined, two questions arise: (1) How is the maternal immune response communicated to the child? (2) What diminishes the atopic response of the mother (reduced maternal IgE) with increasing number of births?

How is the maternal immune response communicated to the child?

The placenta provides the interface between the mother and fetus and is therefore involved in the transfer of information.

Table 2. Maternal IgE values (kU/L), determined after delivery, for order of live offspring and stratified by history of maternal atopy

	Without maternal atopy			With maternal atopy			Total		
Order of live offspring	*n*	Geometric mean	5–95% value	*n*	Geometric mean	5–95% value	*n*	Geometric mean	5–95% value
First	226	30.29	2–542	118	74.40	3–1000	345	41.19	3–825
Second	202	20.03	0.1–236	84	66.63	3–708	287	28.55	2–536
Third or higher	122	20.34	0.1–307	66	43.01	0.6–493	188	26.46	0.6–449

Table 3. Maternal IgE values, determined after delivery, for different order of live offspring and stratified by age of mothers

	Up to 23 years of age			24–33 years of age			34 years of age and more		
Order of live offspring	*n*	Geometric mean	5–95% value	*n*	Geometric mean	5–95% value	*n*	Geometric mean	5–95% value
First	150	41.54	2–876	179	41.87	3–825	15	31.27	3–526
Second	51	33.84	2–469	205	28.75	2–536	31	20.63	0.1–270
Third or higher	10	29.40	10–78	131	32.13	0.1–532	47	14.00	0.1–178

Table 4. Adjusted maternal IgE levels for different predictors ($n = 787$)

Variable	Adjusted geometric mean	*P*-value (*F*-test)[a]
Order of live offspring		
1	40.37	0.031
2	29.57	
3 or more	26.82	
Season of birth		
Spring	34.80	0.74
Summer	31.10	
Fall	28.83	
Winter	32.58	
Age of mother at birth (years)		
Up to 23	39.03	0.026
24–33	38.28	
34 and more	21.99	
Gestational age (weeks)		
30–36	27.70	0.97
37–39	34.01	
40	32.53	
41–45	33.17	
Maternal atopy		
No	20.03	<0.0001
Yes	50.32	
Maternal smoking during pregnancy		
No	30.14	0.53
Yes	33.45	
Paternal smoking (cigarettes/day)		
No	30.78	0.66
Yes	32.76	

[a]The *F*-test is based on log-transformed IgE values.

Transplacental priming of the T cell system against environmental allergens is assumed to result in a skewed Th2 response [33]. It has been determined that maternal IgE does not cross the placenta [30]. However, a recent report emphasized that trans-amniotic transfer of maternal IgE into the amniotic fluid results in higher amniotic IgE concentration when maternal levels are increased [34]. Since the fetus swallows amniotic fluid, the fetal immune system is exposed to information about the maternal immune reactivity. A number of other concepts also attempt to explain the immune communication. Szépfalusi et al. [35, 36] suggest that allergens are actively and selectively transferred across the placenta. Others speculate that maternal conditions may operate by influencing IFN-γ production by T cells in the offspring [37]. Feto-maternal cell trafficking has also been proposed as a means of communication [10]. Herzenberg et al. [38] reported that fetal cells can be identified in the blood of pregnant women as early as 15 weeks of gestation, and may persist for decades [39]. Additionally, maternal DNA was detected in cord plasma [40]. It is assumed that the feto-maternal interface allows leucocyte trafficking to regulate immune responses during pregnancy by secreting a variety of cytokines that modulate the Th2 bias [41].

IgG crosses the placenta, and animal studies have shown that maternal IgG antibodies can suppress the development of IgE in the offspring [36, 42–45]. In a small sample of children, the prevalence of positive skin prick test (SPT) against grass pollen allergens were lower (two of 11), when mothers underwent grass pollen immunotherapy during pregnancy compared with children without maternal immunotherapy (nine of 13). The author suggested that specific IgG blocking antibodies may freely cross the placenta [46]. Jenmalm and Bjorksten [47] reported in 96 children that high levels of cord serum IgG antibodies to cat and birch were associated with fewer atopic symptoms in the children during the first 8 years of life. Additionally, children with IgE antibodies to cat had lower levels of IgG antibodies to that specific allergen at birth. Hence, there is evidence to assume that maternal IgG may transfer information, affect the cytokine production of the child, and bias the immune response of the offspring.

The mechanisms outlined above represent epigenetic links between maternal and childhood atopy. The DNA sequence information provides the blueprint for the manufacture of the cytokine proteins and IgE. Pre-natal priming is based on an epigenetic model, which includes additional instructions on how, where, and when the blueprint should be used. Hence, as a result of the *in utero* environment, the child's genes may be expressed or silenced [48], depending on the immunologic information that the mother transfers to the child [49]. Following this model, changes in maternal atopy may explain changing probabilities of allergic manifestation in childhood.

What diminishes the atopic response of the mother with increasing order of live offspring?

To our knowledge, this is the first study to investigate the association between order of live offspring and maternal IgE. Sunyer et al. [9] showed, in accordance with our findings, that the prevalence of positive SPT, another marker of atopy, decreased with increasing birth order.

Based on findings of Jarrett and Hall [42] and Glovsky et al. [46] that immunotherapy during pregnancy may have an inhibitory effect on atopic responses in offspring, we speculate that each pregnancy may result in de-sensitization (tolerance induction hypothesis) and thereby reduce the maternal IgE level and consequently the risk of the next

offspring to become atopic. These assumptions are capable of explaining why the risk of atopy diminishes with increasing birth order.

The question, however, remains whether this model contributes to the explanation of an increased incidence of childhood allergy. Based on an epigenetic transfer of maternal atopy to their offspring during pregnancy, we can speculate that an increased prevalence of acquired maternal atopy may explain an increasing proportion of childhood asthma. Data from adults suggests that the prevalence of asthma has increased in the last 20 years [50, 51].

However, Devereux et al. [26] assume that it is not changes in the prevalence of *in utero* allergen sensitization, but the conditions under which this occurs that are responsible for the current epidemic of childhood allergy. Hence, the question is, which conditions during pregnancy have changed in the last decades?

In pregnant women, the effect of cytokines produced by Th2 cells predominate over those produced by Th1 cells and maintain the pregnancy [52]. In early pregnancy, Th2-derived cytokines may induce the release of human gonadotropin (HCG) from trophoblasts. HCG in turn seems to stimulate progesterone production from corpus luteum in pregnancy; and progesterone triggers the secretion of Th2 cytokines and reduces the secretion of Th1 cytokines [53–56]. Thus, Th2-type cytokines appear to contribute to the maintenance of pregnancy by controlling the immune [57] and endocrine systems. Another possible explanation for our findings related to the first hypothesis is that a minor endocrine disruption during pregnancy may change the Th2 response.

A limitation of our research is that no pre-conceptional comparisons were made. Maternal IgE was collected within a week of delivery, most likely representing IgE levels during pregnancy. For future research, we suggest assessing potential changes in atopic responses comparing pre-conceptional, pregnancy, and post-pregnancy IgE levels. Our study attempts to establish risk factors for offspring development of atopy. This is the reason why mother–child pairs, but no other women, were enrolled at delivery. Hence, comparisons with non-pregnant controls were not made.

Also, our study did not include follow-up of individual subjects over the course of several pregnancies, only comparisons between different mothers or mother–child pairs. Future research should thus investigate the extent of IgE changes within one subject.

Finally, we only have information on number of live offspring and not pregnancies. Devereux et al. [8] found a stronger association between the mother's total number of pregnancies (e.g. including miscarriages, stillbirths) and the atopic response of cord serum mononuclear cells. Hence, there is a need to investigate whether birth or pregnancy order is a better predictor.

In conclusion, our findings suggest that the protective effect of a higher number of siblings has its origin in the mother. The maternal immune status may be communicated to the child, possibly by maternal IgG that restricts the Th2 response of the offspring. The reduced level of maternal IgE with increasing order of live offspring may result from desensitization of the mother produced by fetal antigens of preceding pregnancies. A non-genetic transmission of the maternal immune status to the child is suggested as an explanation of the birth-order effect. Hence, the increase of atopy in children is likely the result of a preceding increase in the prevalence of maternal atopy in the last decades. Our study emphasizes the need to modify the focus of allergy research and to investigate the *in utero* origin of childhood allergy.

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