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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

To the Editor:

Up to half of the adult population has allergen specific immunoglobulin E antibodies (sIgE)^{1,2}. Even though females and males are not equally affected relatively little is known regarding mechanisms behind differences in IgE sensitization between females and males. IgE poly-sensitization has been defined as IgE reactivity to several nonrelated (or not obviously related) allergenic source materials and has been shown to increase with age and correlate with disease expression and multimorbidity^{3,4}. Although females and males do not seem to differ regarding IgE poly-sensitization in adulthood,⁵ a recent study indicate that IgE poly-sensitization is more common among boys than girls in childhood⁵. Interestingly, findings from the longitudinal Isle of Wight study indicate that more females than males tend to outgrow their IgE sensitization⁶. Investigation of the nature history of IgE sensitization requires large longitudinal population-based studies including repeated measurements of sIgE. We therefore undertook a study to explore the dynamics of IgE sensitization from early childhood to young adulthood in relation to sex, including new and resolved IgE sensitization, poly-sensitization as well as IgE trajectories over time. In the Swedish population-based birth cohort BAMSE (N=4089) participants have been followed up to young adulthood including collection of blood at ages 4 years, 8 years, 16 years and 24 years for analysis of specific IgE to 14 common food (peanut, soy, wheat, milk, egg, cod) and airborne (timothy, birch, mugwort, cat, dog, horse, house dust mites, and *Cladosporium herbarum*) allergens⁷. Females (n=656) and males (n=570) with complete data on sIgE at all four follow ups were included for the current study. *Any IgE sensitization* was defined as sIgE ≥ 0.35 kU_A/l to one or more of the tested foods and/or airborne allergens. *Poly-sensitization* was defined as sIgE ≥ 0.35 kU_A/l to four or more specific allergens at the same follow-up, as in previous reports from BAMSE⁸. *New IgE sensitization*: any IgE sensitization at a follow up in an individual with no sIgE sensitization at the previous follow-up. *Resolved IgE sensitization*: no IgE sensitization to any of the specific allergens at a follow-up in an individual that displayed any IgE sensitization at the previous follow-up.

The study was approved by the regional ethics committee in Stockholm, ethics approval number; 2016/1380-31/2 and participants and/or caregivers provided written informed consent. Details on data collection and definitions are presented in the Methods section in this article's supporting information.

A similar proportion of females (22.6%) and males (23.7%) were IgE sensitized to any of the 14 allergens at 4 years, Figure 1. With increasing age, the prevalence of any IgE sensitization increased for both sexes, but a steeper increase was seen for males. Figure 1 also shows how many allergens females and males were sensitized to at the different ages. Fewer IgE sensitized females than males were poly-sensitized to four or more allergens at all ages with the most pronounced difference at age four years, 12.6% (20/148) vs 31.1% (42/135). The corresponding rates at 8 years were 30.7% (65/212) vs 42.9% (85/198). Differences diminished further with age and at 16 and 24 years differences were small. Very few poly-sensitized individuals outgrew their sensitization between follow-ups; zero individuals between 4 years and 8 years, one between 8 years and 16 years and five between 16 years and 24 years. Thus, in total four females and two males with poly-sensitization outgrew their IgE sensitization over time.

We further explored IgE-poly-sensitization in relation to sex over time using Generalized estimating equations and the same analysis was used to explore new and resolved IgE sensitization, Figure 2. Covariates included in Table S2 were tested as potential confounders using backward selection, but none of the factors impacted the associations between sex and any of the outcomes and therefore unadjusted analysis are presented. Male sex was significantly associated with IgE poly-sensitization at 4 years (OR: 2.75, 95% CI: 1.41-5.37) but not at other ages, Figure 2. Sex was not associated with new IgE sensitization at the ages 4 and 8 years. However, at ages 16 and 24 years more males than females developed new IgE sensitization. The overall OR for the impact of male sex for new IgE sensitization was 1.15, 95% CI: 0.97-1.33. In contrast, resolved IgE sensitization was less common among males than females at all ages, overall OR: 0.37, 95% CI: 0.25-0.53. Resolved IgE sensitization was seen both for food and airborne allergens with comparable sex-differences (data not shown).

Both new and resolved IgE sensitization was characterized by significantly lower levels of specific IgE at all ages while IgE poly-sensitization was associated with significantly higher levels of specific IgE at all ages (data not shown).

The current study includes only 30 % of the original cohort. To facilitate evaluation of generalizability we present baseline characteristics, allergic diseases and any IgE sensitization at ages 4 years, 8 years and 16 years for the 1226 individuals and for the original cohort, Table S1. As shown in Table S1 there were some differences in basic characteristics. Also, allergic diseases and IgE sensitization tended to be more prevalent, especially at higher ages, in the group that provided blood at all follow-ups, even though differences were small.

A higher proportion of females (656/2024, 32.4%) than males (570/2064, 27.6%) provided blood at all 4 follow-ups and were included in the current study. To evaluate whether selection bias might impact the sex differences found in our study we compared background factors between included females and males. Differences were small, Table S2. In accordance with the data in Table S1, any IgE sensitization tended to be more prevalent both among included females and males. However, differences were not more pronounced among males (data not shown).

In this longitudinal population-based study we show that a comparable number of females (22.6 %) and males (23.7 %) display any IgE sensitization at 4 years. With age, IgE sensitization became increasingly more common among both females and males. The increase was steeper among males with 52.6 % of males displaying any IgE sensitization at age 24 years compared to 38.7 % of females. We found that differences in trajectories of IgE sensitization from childhood to young adulthood between females and males can largely be explained by resolved IgE sensitization among females and early IgE poly-sensitization among males. The finding that females outgrow their IgE sensitization to a higher degree than males confirm previous results from the Isle of Wight study in which females tended to be more likely to outgrow their sensitization than males⁶.

Poly-sensitized individuals were unlikely to outgrow their sensitization. Thus, the accumulating sex differences in overall sensitization seen with increasing age (Figure 1) is probably partly explained by the high degree of early poly-sensitization among boys. Our results are in accordance with data from a cross-sectional population-based Polish study including 1409 individuals aged 6 to 44 years⁵. They found polyvalent sensitization (defined as sIgE to two or more allergens) to be significantly more prevalent among 6-7 year old boys compared to girls the same age while there were no significant differences in the older age groups⁵. Results from the German Multi-Centre Allergy Study birth cohort indicate that the earlier the sensitization onset, the stronger the

tendency for poly-sensitization⁹. Thus, even though a similar proportion of females and males displayed IgE sensitization at 4 years it is possible that males developed their sensitization at a younger age compared to females (i.e. below age 4). The genetic influence on poly-sensitization has only been evaluated in a limited number of studies and suggests associations with the *HLA* and *C11orf30-LRRC32* regions, as well as Th2 signaling genes³. Sex-specific genetic effects on sensitization and allergic diseases have been reported in the literature¹⁰, but no consistent picture or explanation of the underlying biology has emerged. Whether there are primarily genetic, hormonal or environmental factors associated with the observed sex differences in IgE sensitization trajectories in our study, resolved sensitization in females and poly-sensitization in males, remain to be further investigated.

Strengths of our study include the population-based design, long follow-up time and that blood was collected for analyses of sIgE at four time points. Adjustment for several potential confounders did not affect the results, although residual confounding effects can never be ruled out in an observational study. A limitation is that only 30% of the original cohort were included and rates of sensitization are probably higher compared to the general population, which may affect the generalizability of the findings. We explored three outcomes and if the Bonferroni method had been applied a p-value of 0.017 would be significant. The associations for sex and IgE poly-sensitization at 4 years as well as all associations regarding sex and resolved IgE sensitization are significant at that level. At the same level the association for sex and new IgE sensitization was significant at 16 years but not at 24 years. A weakness of our study is that IgE sensitization was not measured before age 4 years.

In summary, sex impacts IgE trajectories from childhood to young adulthood. We identified two factors that contribute to these sex differences: resolution of allergen specific sensitization in females and higher rates of allergen poly-sensitization in males. Further analyses of the underlying determinants for these immunological events are warranted.

Figure legends

Figure 1. IgE sensitization from 4 to 24 years among females (n=656) and males (n=570) in the BAMSE birth cohort. Bars represent the proportion of IgE-sensitized females and males with any IgE sensitization at the respective age. Color sections in bars show the proportions with IgE sensitization to one, two or three, four to seven and more than seven of the 14 tested specific allergens at each follow-up.

* p-value <0.05 regarding differences in any IgE sensitization between females and males

Figure 2. Impact of male sex on new IgE sensitization, resolved IgE sensitization and IgE-poly-sensitization up to age 24 years in the BAMSE cohort. Odds ratios were obtained by Generalized estimating equations including 570 males and 656 females (reference group)

Conflict of interest

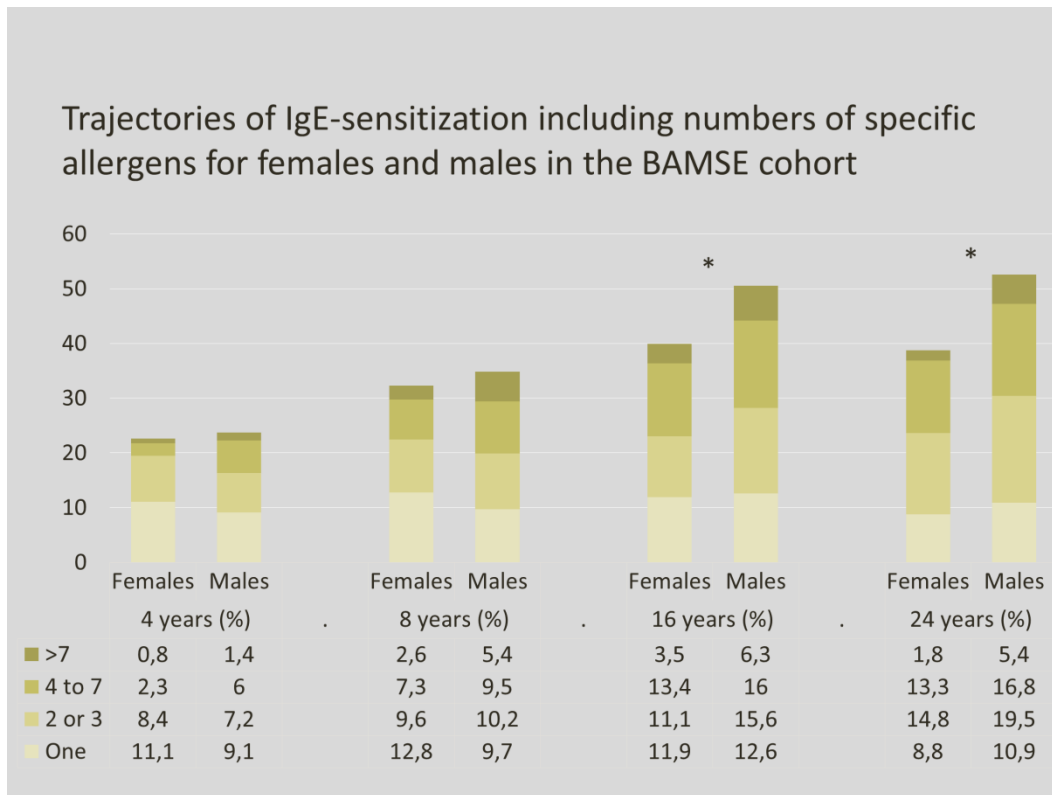
EM has received lecture fees from Novartis, Sanofi and Thermo Fisher Scientific outside the submitted work. NB has received consultancy fees from Pfizer and Sanofi outside the submitted work. MvH has received lecture fees from Thermo Fisher Scientific and ALK; and consultancy fees from Biomay AG, Vienna, Austria and Hycor Biomedical LLC, CA, US, outside the submitted work. Dr. Westman reports personal fees from ALK (consultancy fees), outside the submitted work. The other authors report no conflict of interest relevant to this article.

Authors contributions

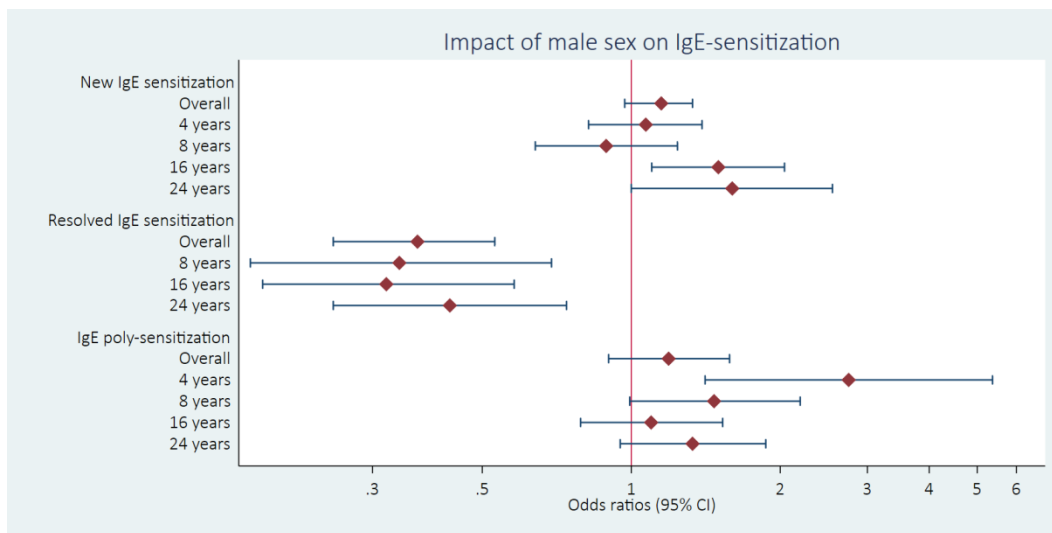
Data collection was managed by EM, IK and AB. Statistical analysis was conducted by NB and EM. Analysis and drafting of the manuscript were conducted by NB and EM. All authors participated in critical revision of the manuscript, provided important intellectual input and approved the final version.

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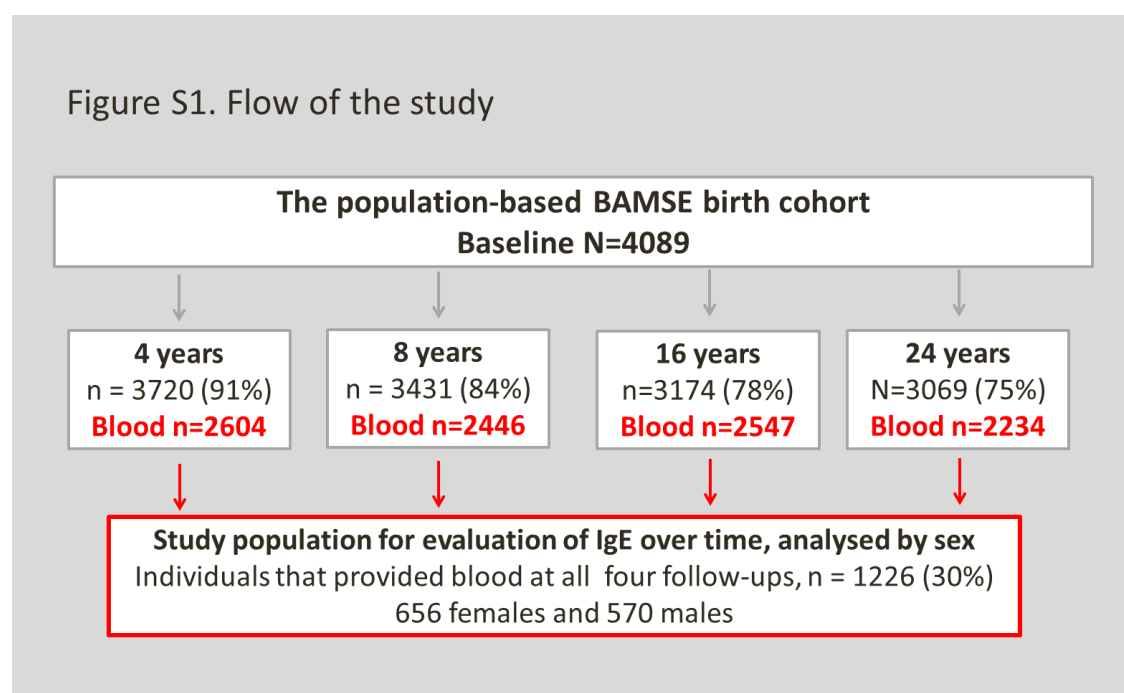
SUPPORTING INFORMATION

Resolved allergen specific IgE-sensitization among females and early poly-sensitization among males impact IgE-sensitization up to age 24 years

METHODS

Study design and study subjects

BAMSE is a population-based cohort from Stockholm, Sweden, in which newborn children were recruited from 1994 to 1996 and followed up to 24 years of age.¹ The BAMSE cohort comprised 4089 infants, corresponding to 75% of the eligible subjects.² When the children were 2 months (baseline) parents completed questionnaires on background factors and follow-up questionnaires including questions related to symptoms of eczema, asthma, and rhinitis were sent out and answered at 1, 2, 4, 8, 12, 16 and 24 years of age. Sera was collected at 4 years, 8 years, 16 years and 24 years. The study was approved by the regional ethics committee in Stockholm and participants and/or parents provided written informed consent. Individuals with complete data on IgE-sensitization from all four follow-ups were included, n=1226, (30%), Figure S1.



Definitions

Sensitization

Allergen-specific IgE antibodies were analyzed with the ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden) as recommended by the manufacturer. The results were expressed in kilounits of allergen per liter, and a positive test result was defined as ≥ 0.35 kU_A/l. Sera were analyzed for a mix of common food allergens with fx5[®] and a mix of common airborne allergens with Phadiatop[®].³ Sera that scored positive (IgE ≥ 0.35 kU_A/l) for a mix were further analyzed for the single allergens included in the food mix (fx5; peanut, soy, wheat, milk, egg and cod) and/or the mix of airborne allergens (Phadiatop; timothy, birch, mugwort, cat, dog, horse, house dust mites (*Dermatophagoides pteronyssinus* at ages four years and eight years and *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae* at ages 16 years and 24 years), and *Cladosporium herbarum*). All samples were analyzed at the Department of Clinical Immunology Karolinska University Hospital Solna, Stockholm, Sweden.

Any *IgE-sensitization* was defined as sIgE ≥ 0.35 kU_A/l to one or more of the tested food and/or airborne allergens (14 in total). *Poly-sensitization* was defined as sIgE ≥ 0.35 kU_A/l to four or more specific allergens. *New sensitization* was defined as any IgE-sensitization at a follow-up in an individual without sIgE-sensitization at the previous follow-up. *Resolved sensitization* was defined as no IgE-sensitization to any of the specific allergens at a follow-up in an individual that displayed any IgE-sensitization at the previous follow-up.

Statistical analysis

Chi-square tests were used for evaluation of differences between females and males for dichotomous outcomes. For evaluation of differences in levels of IgE to specific allergens for the groups with new IgE sensitization and resolved IgE sensitization compared to other

sensitized individuals, quantile regression was used. The median level of IgE was calculated by adding levels for all positive (≥ 0.35 kUA/l) sIgE:s divided by the number of positive tests among IgE-sensitized individuals. Generalized estimating equations (GEEs) with an unstructured correlation matrix were used to assess the impact of sex over time for IgE-poly-sensitization, new IgE-sensitization and resolved IgE-sensitization. Potential confounders were tested using exploratory backward stepwise logistic regression. None of the tested factors; parental allergy, low birthweight (< 2500 g), exclusive breast-feeding ≥ 4 months, maternal smoking during pregnancy and at enrollment, low parental socio-economy, young mother (< 26 years) and caesarian delivery confounded the association between sex and IgE-sensitization. All statistical analyses were performed with STATA Statistical Software (release 14.2; Stata-Corp, College Station, TX, USA).

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Table S1. Comparison of the original cohort and the study population that provided blood at all follow-ups used for analysis of sensitization turnover

	Original cohort (n=4089)		Study population (n=1226)		
	Obs	%	Obs	%	95 % CI
Males	2065	50.5	570	46.5	43.7 - 49.3
Atopic dermatitis 4 years	3721	20.7	1222	20.5	18.2 - 22.7
Atopic dermatitis 8 years	3330	16.3	1196	16.9	14.8 - 19.0
Atopic dermatitis 16 years	3164	8.2	1213	10.3	8.6 - 12.0
Asthma 4 years	3704	7.0	1219	6.2	4.8 - 7.5
Asthma 8 years	3397	6.3	1218	6.9	5.5 - 8.3
Asthma 16 years	3115	6.4	1200	7.1	5.6 - 8.5
Rhinitis 4 years	3682	10.5	1204	11.1	9.4 - 12.9
Rhinitis 8 years	3394	13.3	1221	14.8	12.8 - 16.8
Rhinitis 16 years	3109	25.4	1190	28.9	26.3 - 31.5
Any IgE-sens 4 years	2604	21.8	1226	23.1	20.2 - 25.4
Any IgE-sens 8 years	2446	32.4	1226	33.4	30.6 - 36.1
Any IgE-sens 16 years	2547	42.7	1226	44.9	40.8 - 47.6
Any IgE-sens 24 years	2234	43.4	1226	45.2	41.4 - 48.0
Parental allergy †	4045	43.2	1216	46.0	43.1 - 48.8
Low parental socio-economic status*	4018	17.3	1210	13.4	11.5 - 15.3
Environmental tobacco smoke‡	4086	13.8	1226	10.6	8.9 - 12.3
Maternal age ≤ 25 years	4088	7.8	1226	5.8	4.5 - 7.1
Exclusive breastfeeding ≥4 months	3919	79.5	1212	81.1	78.9 - 83.3
Birth weight <2500g	4044	3.6	1219	3.6	2.6 - 4.7
Caesarean delivery	4011	4.3	1204	3.7	2.6 - 4.7

†Doctor's diagnosis of asthma and/or hay fever in combination with allergy to furred pets and/or pollen and/or doctor's diagnosis of eczema at enrolment in one or both parents

*Parental blue collar worker status

‡Mother smoked at least one cigarette per day during pregnancy and/or at the time of enrolment

Table S2. Comparison of basic characteristics between included females and males

	Females n=656	Males n=570	p-value*
Parental allergy †	44.1 %	48.1 %	0.15
Low parental socio-economic status [∞]	12.9 %	14.0 %	0.56
Environmental tobacco smoke‡	10.8 %	10.5 %	0.79
Maternal age ≤ 25 years	5.3 %	6.3 %	0.46
Exclusive breastfeeding ≥4 months	79.2 %	83.3 %	0.07
Birth weight <2500g	4.3 %	2.8 %	0.17
Caesarean delivery	4.1	3.1	0.37

† Doctor's diagnosis of asthma and/or hay fever in combination with allergy to furred pets and/or pollen and/or doctor's diagnosis of eczema at enrolment in one or both parents

[∞] Parental blue collar worker status

‡ Mother smoked at least one cigarette per day during pregnancy and/or at the time of enrolment

* calculated by chi-two-test