



# Season of the first trimester of pregnancy predicts sensitisation to food allergens in childhood: a population-based cohort study from Finland

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## ABSTRACT

**Objective** To examine whether the season of birth or season of the early phase of gestation is associated with sensitisation to food allergens in children, with special reference to mothers' pollen exposure in spring.

**Design** A population-based cohort study linking information from a questionnaire survey to allergy tests performed on the target population and regional pollen counts.

**Population** Children born in 2001–6 who were resident in the province of South Karelia, Finland, at the time of the survey (N=5920).

**Main Outcome Measures** A positive result in any food allergy test or food-specific immunoglobulin E test (sIgE).

**Results** The cumulative incidence of a positive food allergy test up to the age of 4 years was highest among children born in October–November (10%) and lowest among those born in June–July (5%), and correspondingly highest among children who were in their 11th gestational week in April–May (11%), the season of high concentrations of birch and alder pollen, and lowest among those reaching that stage in December–January (6%). The amplitude of seasonal variation in any test, estimated as the relative ratio between the peak and trough of the smoothed incidence curve over the year, was 2.03 (95% CI 1.52 to 2.76). The amplitudes of positive sIgE were especially pronounced for milk (3.07; 95% CI 1.81 to 5.50) and egg (3.03; 95% CI 1.86 to 5.18).

**Conclusions** Children having their early gestational period in the pollen season for broad-leaved trees are more prone to sensitisation to food allergens than other children.

Children born in autumn or winter have a higher occurrence of atopic dermatitis and bronchial asthma and higher concentrations of food-specific immunoglobulin E antibodies than children born in spring or summer.<sup>1–4</sup> The reasons for this are not known but there may be some seasonally varying environmental factors that influence the production of immunoglobulins. The fetus begins to produce immunoglobulin E antibodies at approximately the 11th gestational week<sup>5–6</sup> and allergen-specific immunoglobulin E antibodies at the end of the second trimester of pregnancy.<sup>6</sup> An allergic-type immunological response is necessary for the pregnancy to continue, but in some cases this type of reaction persists throughout the neonatal period.<sup>5–6</sup> While seasonal environmental factors could interfere with this process, the timing of the development of sensitisation, a prerequisite for clinical

immunoglobulin E-mediated allergic disease, has remained a controversial issue.<sup>7–9</sup>

In the context of the South Karelian Allergy Research Project (SKARP),<sup>10</sup> (K Pyrhönen *et al*, manuscript in preparation), an epidemiological study of the occurrence of food allergies and hypersensitivities and risk factors for these, we examined whether the season of birth or the season of the early fetal period was related to the incidence of positive results in food allergy tests during childhood. We also compared the seasonal variation in the incidence with concentrations of pollen grains in the environment and with the relevant climatological data for the study area concerned.

## METHODS

### Study population

The study population comprised all 5973 children born between 1 April 2001 and 31 March 2006, who were resident in the province of South Karelia in south-east Finland at the time when the questionnaire survey was conducted in 2005–6. The subjects were identified and their demographic details obtained from the Finnish Population Register Centre<sup>11</sup> (K Pyrhönen *et al*, manuscript in preparation). We estimate that 99% of the children were born in Finland.

### Data collection

Details of the study design and data collection have been reported elsewhere (K Pyrhönen *et al*, manuscript in preparation).<sup>10</sup> Briefly, we conducted a questionnaire survey in close cooperation with local child health clinics<sup>12</sup> during the period April 2005 to September 2006, when the children were 0–4 years old. Altogether the parents of 3899 children returned the questionnaire, the participation rate being 66%, varying from 59% to 72% by month of birth. The questionnaires are available at <http://kelo.oulu.fi/tutkimus/EKAT/>.

The results of food allergy tests carried out by order of a clinician from August 2001 to September 2006 were collected from patient and laboratory records at all hospitals, health centres, private clinics and laboratories in the area independently of and concurrently with the questionnaire survey. The test data were intended to cover the whole population, including those who failed to return the questionnaire. The data include the dates and results of the tests and the child's unique personal identity code.<sup>11</sup> Based on parental reports in the questionnaire we estimated that our test data

covered at least 90% of all children tested for suspected allergy, and 0.6% of the participants had been tested elsewhere only (K Pyrhönen *et al*, manuscript in preparation).

As part of the Finnish pollen information network,<sup>13</sup> daily regional pollen counts were measured throughout the pollen season, from 1 March to the end of August in 2002–5, with a Burkard 7-day volumetric sampler<sup>14</sup> located 119 m above sea level on the roof of a 19-m high building in the town of Joutseno (61° 18' N, 28° 41' E) in the middle of the area concerned. Since the location of the sampler was moved from a higher to a lower roof after the pollen season in 2001, the pollen concentrations from the period before 2002 were not comparable to those during the years 2002–5. The sampling, slide preparation and data interpretation took place according to the standard methodology adopted by the Finnish pollen information network and following the principles of the European Aeroallergen Network (<http://www.polleninfo.org/>). The pollen concentrations were expressed as daily mean counts of pollen grains per cubic metre of air.

The mean monthly temperature in Jyväskylä as well as the length of the day (hours) and monthly averages of sunshine hours per day in Helsinki were measured by the Finnish Meteorological Institute over the years 1973–2000 (<http://www.fmi.fi>).

### Ethics and permissions

The protocol was reviewed by the Ethical Committee of the Northern Ostrobothnia Hospital District, and a notification of the collection of the data was sent to the Data Protection Ombudsman. The allergy test data were collected from the patient records with the permission of the Finnish Ministry of Social Affairs and Health. All 11 healthcare centres in the region consented to cooperate. We excluded from further analysis 53 children in the questionnaire survey whose parents declined data linkage. None of the authors provided medical care in the area.

### Outcomes

The following outcomes were considered: the first positive result in (1) specific immunoglobulin E antibodies to a food item (sIgE); (2) skin prick tests (SPT) for a food item; (3) open food challenges (OFC); and (4) any food allergy test. We also report the occurrence of the first food allergy test (by any food allergy test method) as well as positive test results separately for milk and egg allergens.

The mean of two orthogonal diameters of the urticarial wheal equal to or above 3 mm, with positive and negative controls, was taken as the cut point for a positive SPT. The cut point for sIgE was equal to or above 0.35 kU/l in 98% of the tested subjects and in 99% of all positive tests, and 1.43 SU/ml among the rest. The results of the OFC were transcribed as they were in the patient record, that is, negative, positive or not known.

### Explanatory variables

The month of birth is indicated in the personal identity code.<sup>11</sup> Parents recorded the duration of pregnancy from the maternity card on the questionnaire. In Finland, for over 90% of pregnancies gestational age is ascertained with ultrasound scan during the 11th to 22nd gestational weeks,<sup>15</sup> considered to have an accuracy of 7 days. We took as another explanatory variable the calendar month of the 11th week considered critical for the developing immune system.<sup>5,6</sup>

Gender, birth order, year of the 11th gestational week, history of hay fever or pollen allergy in the biological mother and maternal smoking during pregnancy were included in some analyses as covariates (figure 1). Birth order was classified into

'firstborn' (including both of firstborn twins), and 'not firstborn'. Year of the 11th gestational week was used as a crude indicator for annual pollen exposure, and it was categorised based on the comparability of the pollen measurements across the years (see above) and on the measured annual cumulative pollen counts during 2002–5. Pollen allergy in the biological mother was classified as follows: 'No pollen allergy' and 'Yes, either self-perceived or physician-diagnosed pollen allergy'. Mother's smoking during pregnancy was categorised as 'No', 'Yes, occasionally', and 'Yes, regularly'.

### Statistical methods

The duration of pregnancy was missing for 2325 subjects (39%) of the population (figure 1). This lack of data was compensated by multiple imputation,<sup>16</sup> in which 10 simulated values for the duration of pregnancy based on the observed distribution of this variable among those providing this information were drawn at random for each subject with missing data. Subtracting each of those values from the date of birth (known for all) and adding 77 days produced 10 imputed values for the timing of the 11th week of gestation. Any subsequent modelling involving the 11th week of gestation as an explanatory variable was then repeated 10 times, each including one of the imputed versions of this key variable. The final point estimates were obtained by averaging or pooling the results of the separate analyses, and in the assessment of the precision of the pooled estimates for the regression coefficients both the within-imputation and between-imputation variability was taken into account as appropriate.<sup>16</sup> For the technical details of the imputation procedure, see the statistical appendix.

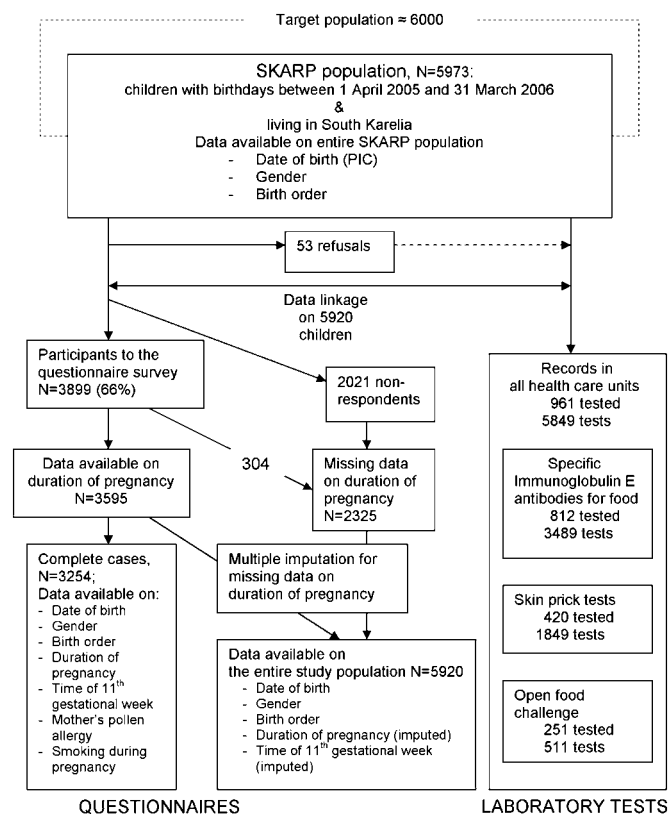


**Figure 1** Flow diagram showing subsets of the study population for which different items of data were available and for which certain items were imputed. PIC, personal identity code; SKARP, South Karelian Allergy Research Project.

Kaplan–Meier estimates of the cumulative incidences of each outcome studied by age were computed in groups defined by bimonthly periods of birth and the 11th gestational week, respectively. The event in these analyses was the first occurrence observed for a given outcome, and the event age was the child's age on the date of the event. The risk time began at birth and ended at the event age if an event occurred. Among the children in whom no such event was observed, the risk time ended with censoring on the closing date of data collection (30 September 2006). These descriptive computations according to the 11th week of pregnancy were based on conditional mean imputation of the missing data.

Seasonal variations in the incidences of the different outcomes both by month of birth and by that of the 11th gestational week were further analysed using the Cox proportional hazards model including two periodic terms of length one year and half a year, as well as gender, birth order, the year of the 11th gestational week, maternal smoking during pregnancy and maternal history of pollen allergy as covariates.<sup>17</sup> The point estimates of the regression coefficients and their estimated covariance matrix in the final model were obtained by pooling the results from replications of the analysis with the different imputed datasets. The smoothed predictions of daily incidence rates were based on the final pooled models. The ratio between the highest and lowest predicted incidence rate was taken as the estimated relative amplitude of the seasonal variation. The 95% confidence

limits for the amplitude were approximated by a parametric bootstrap simulation based on the pooled results of the fitted model.<sup>18–19</sup> For further details, see the statistical appendix. Circular correlation coefficients<sup>20</sup> were computed to describe seasonal associations of the time of the first food allergy test with the time of the 11th gestational week and the date of birth.

The pollen counts for alder, birch, grasses and mugwort were converted to 7-day mean concentrations and averaged over the years 2002–5.

The R environment (release 2.9.2) was used for checking and correcting the data and transforming the variables, and also for performing and documenting the analyses.<sup>21</sup>

## RESULTS

Out of all 5920 children, 961 had been tested for food allergies, 812 had at least one test result for sIgE, 420 for SPT and 251 for OFC (figure 1 table 1). The proportion of children tested for food allergies by 4 years of age was overall 18%, varying by the season of birth from 15% (February–March) to 21% (August–September), and by that of the 11th gestational week from 20% (April–May) to 16% (August–September) (table 1).

The cumulative incidence of positive test results increased steeply during the first year of life and typically levelled off thereafter (figure 2). By 4 years the incidence of a positive result in any test according to season of birth varied from 5% for

**Table 1** Numbers of food allergy-tested subjects and those with a positive result in these tests and their cumulative incidences (%) up to 4 years of age by month of birth and month in which the 11th gestational week occurred

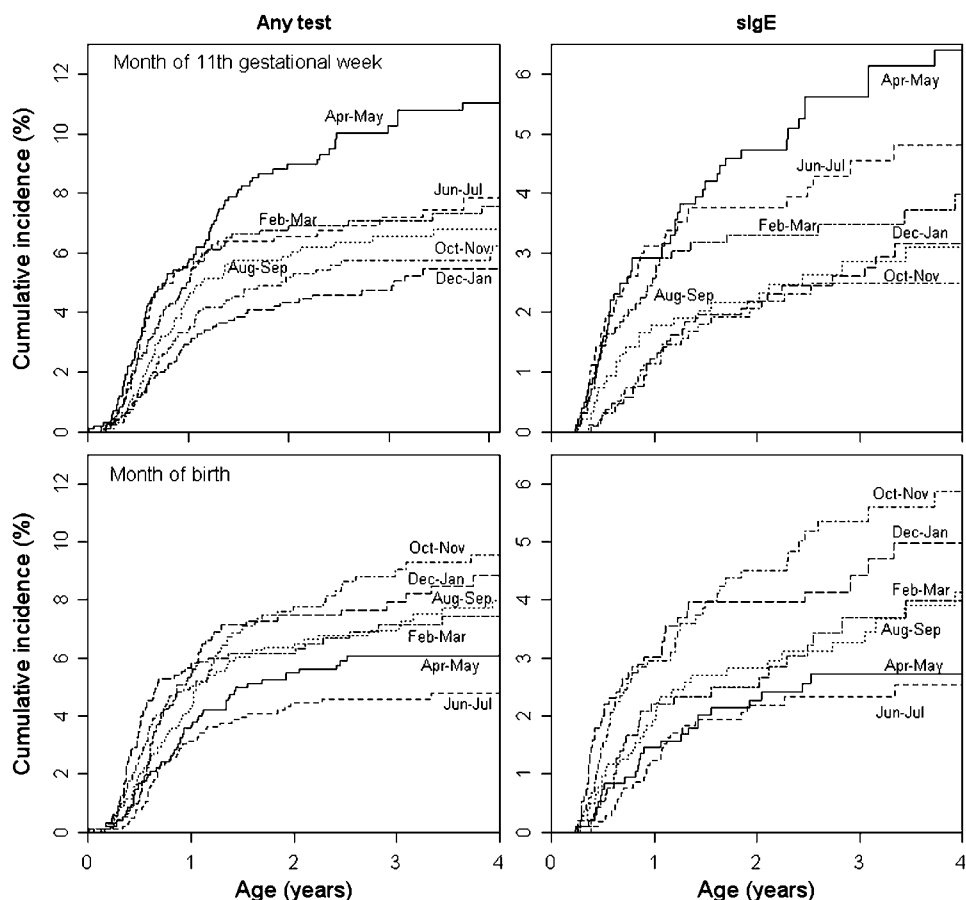
| Test method               | Month     | By month of birth* |                      | By month of the 11th gestational week† |                      |
|---------------------------|-----------|--------------------|----------------------|----------------------------------------|----------------------|
|                           |           | Testing % (N)      | First positive % (N) | Testing % (N)                          | First positive % (N) |
| Specific immunoglobulin E | Apr–May   | 16 (144)           | 2.7 (26)             | 17 (147)                               | 6.4 (51)             |
|                           | June–July | 15 (149)           | 2.5 (26)             | 15 (123)                               | 4.8 (39)             |
|                           | Aug–Sept  | 17 (157)           | 4.1 (36)             | 13 (112)                               | 3.1 (25)             |
|                           | Oct–Nov   | 15 (131)           | 5.9 (49)             | 16 (146)                               | 2.5 (25)             |
|                           | Dec–Jan   | 17 (134)           | 5.0 (39)             | 15 (150)                               | 3.2 (29)             |
|                           | Feb–March | 13 (97)            | 4.0 (28)             | 16 (134)                               | 4.0 (35)             |
|                           | Overall   | 15 (812)           | 4.0 (204)            | 15 (812)                               | 4.0 (204)            |
| Skin prick test           | Apr–May   | 8 (76)             | 2.3 (23)             | 8 (73)                                 | 2.9 (25)             |
|                           | June–July | 6 (60)             | 1.5 (16)             | 8 (68)                                 | 2.7 (23)             |
|                           | Aug–Sept  | 9 (86)             | 3.0 (28)             | 9 (71)                                 | 3.1 (26)             |
|                           | Oct–Nov   | 8 (69)             | 2.7 (24)             | 7 (61)                                 | 2.1 (20)             |
|                           | Dec–Jan   | 9 (74)             | 3.3 (27)             | 7 (72)                                 | 1.4 (15)             |
|                           | Feb–March | 8 (55)             | 2.6 (20)             | 9 (75)                                 | 3.1 (29)             |
|                           | Overall   | 8 (420)            | 2.5 (138)            | 8 (420)                                | 2.5 (138)            |
| Open food challenge       | Apr–May   | 5 (42)             | 3.4 (31)             | 7 (59)                                 | 5.0 (42)             |
|                           | June–July | 3 (30)             | 1.8 (19)             | 5 (40)                                 | 3.7 (31)             |
|                           | Aug–Sept  | 5 (48)             | 3.2 (33)             | 5 (39)                                 | 3.8 (31)             |
|                           | Oct–Nov   | 6 (52)             | 4.3 (36)             | 4 (37)                                 | 3.0 (27)             |
|                           | Dec–Jan   | 5 (41)             | 3.2 (29)             | 3 (33)                                 | 1.9 (20)             |
|                           | Feb–March | 5 (38)             | 4.6 (33)             | 5 (43)                                 | 3.1 (30)             |
|                           | Overall   | 5 (251)            | 3.4 (181)            | 5 (251)                                | 3.4 (181)            |
| Any FA tests              | Apr–May   | 18 (167)           | 6.1 (59)             | 20 (171)                               | 11.1 (91)            |
|                           | June–July | 17 (166)           | 4.8 (49)             | 18 (149)                               | 7.9 (66)             |
|                           | Aug–Sept  | 21 (194)           | 8.0 (73)             | 16 (137)                               | 6.8 (56)             |
|                           | Oct–Nov   | 18 (157)           | 9.5 (81)             | 18 (159)                               | 5.8 (56)             |
|                           | Dec–Jan   | 20 (163)           | 8.9 (72)             | 18 (174)                               | 5.5 (52)             |
|                           | Feb–March | 15 (114)           | 7.4 (56)             | 19 (171)                               | 7.6 (69)             |
|                           | Overall   | 18 (961)           | 7.4 (390)            | 18 (961)                               | 7.4 (390)            |

\*The bimonthly periods from April–May to February–March included 951, 1046, 1030, 1018, 950 and 925 children, respectively.

†The bimonthly periods from April–May to February–March included 999, 973, 958, 957, 1057 and 976 children, respectively. Missing data on the duration of pregnancy were imputed employing the mean duration in the answers given in the questionnaire survey, 278 days.

FA, food allergy.

**Figure 2** Kaplan–Meier curves showing the cumulative incidences of positive results in food allergy tests during the first 4 years of life, classified by the calendar month in which the 11th gestational week occurred (the upper two panels) and the month of birth (the lower two panels) from April–May to February–March. The food allergy tests (on the left) included food-specific immunoglobulin E antibodies (sIgE), skin prick tests and open food challenges, of which the cumulative incidences of positive sIgE are shown separately (on the right).



June–July to 9.5% in October–November. The 4-year cumulative incidence was 11% among the children whose 11th gestational week had been in April–May, but it remained lower (5.5–8%) among the others (table 1); the April–May group had an exceptionally steep increase even after the first year of life (figure 2). These patterns were largely similar, too, for the sIgE and OFC separately, but differed for the SPT in that no particular season stood out clearly from the rest (table 1).

The smoothed incidence from the fitted harmonic model for any positive test, and also for a positive sIgE test alone, was highest in the children whose 11th gestational week had occurred in April–May and lowest in those for whom it fell in October–December (figure 3A table 2). Regarding any food allergy test, the incidence was doubled from the date of the seasonal trough to that of the seasonal peak, the amplitude being particularly high for milk and egg-specific sIgE (table 2). Similar curves and amplitudes were observed according to the month of birth, too, but the peaks were in November–December and the troughs in May–June; that is approximately 7 months later than those by the time of the 11th gestational week (table 2). The seasonal variation in the relative incidence of food allergy testing was very modest compared with that of the positive test results (figure 3A). Among the food allergy tested children the season when the first test was performed was only weakly associated with the season of birth and of the 11th gestational week (circular correlations 0.18 and 0.17, respectively).

The concentrations of alder and birch pollen in the area were highest in April and May and were followed by smaller increases in grass and mugwort pollen 2–3 months later (figure 3B). In the years 2002–5, during which the measurements of pollen

counts were comparable, the annual cumulative sums of pollen grains (including birch, alder, mugwort and grass) per cubic metre were 61 000, 30 000, 29 000 and 27 000, respectively. When the year of the 11th gestational week was added to the harmonic model, the estimated relative rate of a positive result from any food allergy test was 1.16 (95% CI 0.90 to 1.49) and from a sIgE test it was 1.69 (95% CI 1.19 to 2.40) for children whose 11th gestational week fell in 2002 rather than in 2003–5. The seasonal peaks in mean temperature, day length and sunshine hours are located in May–July (figure 3C).

Information on the duration of pregnancy was provided by the parents of 3595 children (92% of the survey participants and 61% of the whole population, figure 1). The proportion of ‘complete cases’ (children for whom this information was available) varied by month of birth, from 57% (December–March) to 64% (April–July). The mean duration of pregnancy among the complete cases was 278 days. Almost 90% of the reported gestational ages were at least 38 weeks but less than 43 weeks, that is, within an interval of 5 weeks. The incidence of positive results was somewhat higher among the complete cases (4-year overall incidence 9.0%) than in the whole population (7.4%, table 1). Yet, the seasonal variation was not essentially greater for the complete cases (estimated relative amplitude by the 11th gestational week being 2.17 for any positive food allergy test) than for all children (2.03, table 2), and the overall patterns of the harmonic curves remained similar, too. Inclusion of mother’s pollen allergy and smoking during the pregnancy in the harmonic models fitted for the complete cases ( $N=3254$ , figure 1) produced only a negligible change to the estimates of the relative amplitudes of interest. Maternal pollen allergy was associated with the overall incidence of positive test

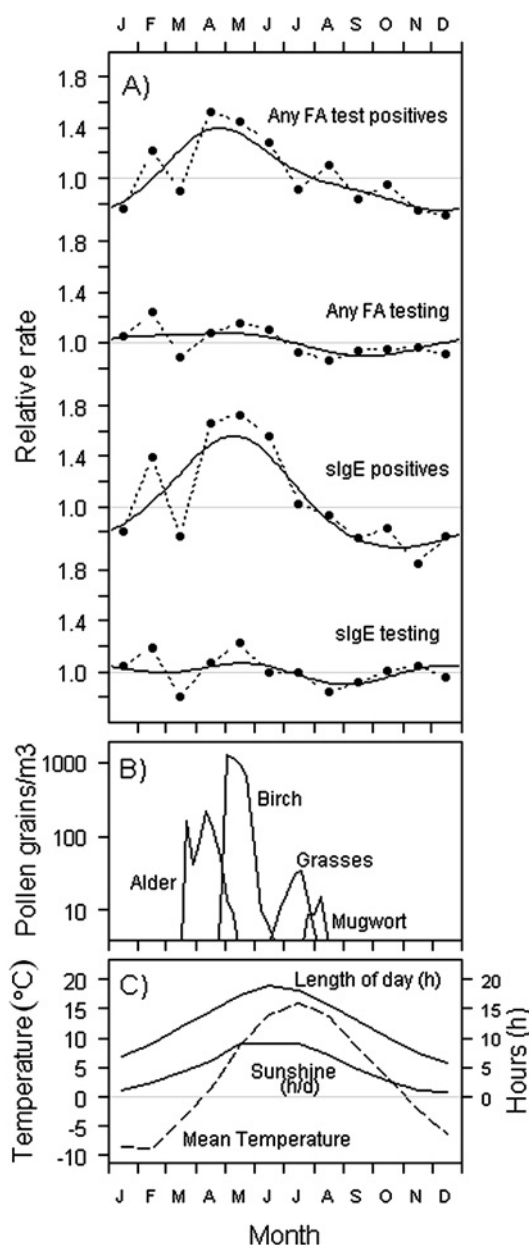


**Figure 3** Incidence of any positive food allergy (FA) test, FA testing, a positive test result in specific immunoglobulin E tests (sIgE) and sIgE testing by the month in which the 11th gestational week occurred (A), mean weekly concentrations of environmental pollen (averaged over the years 2002–5) (B) and the climatic pattern (C). The points in panel A indicate monthly estimates of relative rates (from the Cox model, adjusted for gender, birth order and year of 11th gestational week) and the continuous lines values smoothed with a second-order harmonic model.

results (rate ratio 1.67, 95% CI 1.29 to 2.16). However, the results from a model including the pertinent interaction terms contained no sufficient evidence for the seasonal variation in the outcome to be modified by whether the mother had pollen allergy or not.

## DISCUSSION

### Principal findings

We found a higher incidence of positive results in food allergy tests among children born in October or November than among those born in other months in an unselected population. The

incidence of such results was particularly high and especially pronounced for milk and egg among children who had their 11th gestational week in April or May, the season during which the concentrations of pollen from birch and alder are highest in the area concerned. In addition, the occurrence of positive sIgE tests was clearly elevated in children who had their 11th gestational week in 2002 when the pollen concentrations were exceptionally high compared with average years.

### Strengths and weaknesses

Our design, albeit observational, can actually be viewed as a natural experiment with respect to the key explanatory factors, because the timing of pregnancy most plausibly is distributed quite independently of any other biological and psychosocial risk factors of food allergies. Therefore, the observed seasonal patterns and amplitudes should be practically free from confounding. This assertion was supported by our finding that the location of the fitted harmonic curves and the estimated relative amplitudes were unaffected by the inclusion of gender, birth order, year of the 11th gestational week, pollen allergy in biological mother and mother's smoking during the pregnancy.

Another major strength lies in the carefully compiled population-based data on laboratory tests linked with a questionnaire survey on an individual basis using the children's personal identity codes. As the dispersion of the duration of pregnancy is quite small compared with the time span of a whole year, and as the lack of this item can reasonably be considered missing at random,<sup>16</sup> we were able to increase the validity and precision of our analyses as to the 11th gestational week by means of a fairly simple, yet sound and efficient, imputation procedure. The results obtained were compared with those for the complete cases as recommended in the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines,<sup>22</sup> and the small differences can be explained by a slight overrepresentation of food allergy tested children among the respondents.

Measurement of outcomes was based on existing patient records with all their real-life imperfections. Diverse food allergy tests were performed on children at different ages by several laboratories with varying practices and procedures. Despite all this heterogeneity, we expect the resulting misclassification to be basically non-differential with respect to the timing of pregnancy. This does not affect the form of the observed seasonal curves otherwise, but the estimated amplitudes are more likely to be attenuated rather than exaggerated. Even though records on food allergy tests were identified for less than 20% of the population, we have good grounds to believe that we have not missed any substantial number of potentially positive food allergy test results among those children on whom no test data were found. In Finland almost all children visit child health clinics several times during their early years.<sup>12–23</sup> Based on the experience of public health nurses and physicians in the child health clinics practically all children with significant food allergy symptoms are identified and referred to food allergy tests. In any case, the possible under-coverage of positive food allergy test results should not be selective as to the seasons of different phases of pregnancy. As neither SPT nor sIgE are painless tests and both SPT and food challenges may cause systemic reactions,<sup>24–26</sup> conducting these tests for research purposes on such a large population of children having no food allergy symptoms and some of whom had previously had a severe reaction to food items would be both unethical and unfeasible.

**Table 2** Peak (max), trough (min) and amplitude (min/max ratio) of the incidence of the first positive food allergy test by month of birth and of the 11th gestational week in the total series (N=5920)

| Test method | Food item | By month of birth |         |                     | By month of the 11th gestational week‡ |        |                     |
|-------------|-----------|-------------------|---------|---------------------|----------------------------------------|--------|---------------------|
|             |           | Max               | Min     | Amplitude (95% CI)  | Max                                    | Min    | Amplitude (95% CI)  |
| slgE*       | Milk      | 26 Nov            | 23 June | 3.07 (1.81 to 5.52) | 2 May                                  | 6 Dec  | 3.07 (1.81 to 5.50) |
|             | Egg       | 22 Nov            | 4 May   | 3.07 (1.87 to 5.23) | 23 Apr                                 | 2 Nov  | 3.03 (1.86 to 5.18) |
|             | Any food  | 27 Nov            | 19 May  | 2.47 (1.71 to 3.67) | 9 May                                  | 31 Oct | 2.47 (1.68 to 3.71) |
| Any†        | Milk      | 4 Dec             | 16 June | 2.09 (1.48 to 3.04) | 6 May                                  | 27 Nov | 2.06 (1.43 to 3.05) |
|             | Egg       | 22 Nov            | 16 May  | 2.98 (1.93 to 4.74) | 27 Apr                                 | 19 Nov | 3.01 (1.94 to 4.83) |
|             | Any food  | 12 Nov            | 26 June | 2.01 (1.50 to 2.74) | 23 Apr                                 | 8 Dec  | 2.03 (1.52 to 2.76) |

Based on incidence rates smoothed with a second-order periodic regression and adjusted for gender and birth order.

\*Food-specific immunoglobulin E (slgE) antibodies.

†Includes positive results in slgE for food items, in skin prick tests for food items or open food challenges.

‡Missing data on the duration of pregnancy (2325 children) were replaced with imputed values.

The wide seasonal variations in exposure to pollen and sunlight in Finland (figure 3) enabled us to observe their associations with the outcomes. Sampling of pollen at only one location is an obvious limitation. However, this sampler has been regarded to measure pollen counts adequately in an urban area of 250 km<sup>2</sup>.<sup>27</sup> Because mixed forests with both leafy trees (birch and alder) grow relatively homogeneously all over the study region, one local sampler in the middle of the area at a height of 20 m reflects reasonably well the regional concentrations of pollen grains. Stationary sampling at house roof level measures regional pollen concentrations, which have been observed to agree well enough with the actual (local) pollen exposure at breathing height.<sup>28–30</sup> Concentrations of alder and birch pollen in April–May were clearly higher than those of grass and mugwort pollen grains in July–August (figure 3). Grass and mugwort are low growing and their pollen grains do not spread as widely in the environment as do alder and birch pollen. This explains the higher concentrations of tree pollen than grass and mugwort pollen in various months. Although we did not have individual data on sunlight exposure either, the seasonal variation in sunlight is known to be related to the synthesis of vitamin D and its concentration in the blood.<sup>31 32</sup> In winter the newborn infants are more exposed to viral infections than those born in summer, but unfortunately we had no relevant data on infections.

### Comparison with other observations

Our study is the first to examine the association between environmental pollen concentrations during the first trimester of pregnancy and the incidence of a positive result in food allergy tests. A Swedish study (n=209) found a higher prevalence of slgE antibodies to egg, milk and wheat in children born in autumn or winter than in those born in summer.<sup>2</sup> A study of 369 Japanese children with atopic dermatitis showed high egg-specific immunoglobulin E (IgE) among those born in winter.<sup>1</sup> In a Dutch survey of 90 000 patients under 4 years of age, the highest prevalence of a strongly positive egg or cow milk slgE test was associated with autumn or winter birth.<sup>3</sup> Our findings agree with the above,<sup>1–3</sup> and add SPT and OFC results, the timing of the 11th gestational week and data on concomitant variations in environmental pollen concentrations.

In our study, the observed seasonal variations in the incidence rates are equally well concordant with the underlying reasons being linked with either the season of birth or the season of the 11th gestational week, or even both. Children whose 11th gestational week falls in April–May might also be more heavily exposed than other children to viral infections and deficiency of vitamin D in their neonatal period occurring in mid-winter,

which may also have an impact on their immunological development.

A low concentration of vitamin D has previously been found to be associated with a low level of cytokine interleukin-10,<sup>33</sup> considered a central regulator in the development of allergy.<sup>34</sup> A low intake of vitamin D during pregnancy has been associated with asthma and allergic rhinitis in the child.<sup>35</sup> Vitamin D deficiency in children born in winter, or delayed effects of winter darkness during early pregnancy, may interfere with the development of sensitisation. Cytokine responses of cord blood have previously been found to be generally lower in the offspring of allergic than non-allergic parents and higher in those who were born in spring or summer than in autumn or winter.<sup>36 37</sup> Seasonal variation in cytokines of cord blood has been discussed to be a possible result from maternal immune response to viral or bacterial pathogens that occur in the autumn and winter.<sup>36</sup> On the other hand, the risk of allergies, also for food allergies, has previously been found to be higher in the firstborn than in the others,<sup>10 38</sup> even though the latter are probably more exposed to viral infections at home during their prenatal and neonatal period. In addition, a higher risk, particularly of a slgE-positive result in food allergy tests in the offspring of mothers who were exposed to twofold higher concentrations of pollen in 2002 than those in 2003–5, strengthens the evidence for maternal pollen exposure during the first trimester affecting the development of sensitisation in the fetus.

### What is already known on this subject

Prevalences of milk and egg allergy and specific IgE antibodies to these food items are higher in children born in winter than in those born in other seasons.

### What this study adds

- The 4-year cumulative incidence of positive results in food allergy tests, particularly in slgE tests, was highest among children born in October–November.
- The seasonal effect was particularly pronounced in the incidences of positive slgE for milk and egg among children who had their 11th gestational week in April–May, the pollen season.

## Implications of the findings

Even if an association between the season of the 11th gestational week and the incidence of positive food allergy tests existed, due caution should be exercised as to causality between pollen exposure in early pregnancy and subsequent sensitisation.

It is plausible, though, that gestational exposure to potent allergens such as alder, birch, grass and mugwort pollen could induce an IgE response and inflammation. This might affect the immunological development by an unknown mechanism during the crucial first trimester of pregnancy.

Positive test results are considered 'sensitisation' rather than 'clinical food allergy'. A positive OFC is the basis of a physician's diagnosis of food allergy.<sup>26 39 40</sup> The small number of positive OFCs did not allow any precise conclusions to be drawn, but seasonal variation was similar.

## Unanswered questions and future research

The aetiological role of the mother's pollen exposure and a possible IgE-mediated inflammation process during early pregnancy for the development of sensitisation in the child should be elucidated further. The natural history of food allergies and potential comorbidity in relation to the season of birth and early environmental exposure should be examined in cohorts with follow-up times extending to adulthood.

## CONCLUSIONS

Children having the 11th gestational week in April–May are more likely to have a food allergy test with a positive result. One possible explanation would be exposure to pollen from leafy trees that shows similar seasonal variation.

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## STATISTICAL APPENDIX

The imputations with regard to the timing of the 11th week of pregnancy were performed in phases 1 to 4 as follows:

1. The distribution of the completed gestational weeks among those with missing data was assumed to follow a multinomial probability distribution in which the week-specific probabilities were estimated from the corresponding observed proportions in the participants for whom data on the duration of pregnancy were provided.
2. For each child  $i$  with a missing duration of pregnancy the following steps (i) and (ii) were repeated 10 times ( $j=1, \dots, 10$ ): (i) a random value  $w_{ij}$  was drawn from the multinomial distribution as specified above using the `rmultinom()` function in R,<sup>21</sup> and another random value  $d_{ij}$  from a discrete uniform distribution with support  $\{0, 1, \dots, 6\}$  using the same function, (ii) the  $j$ th imputed value for the duration of pregnancy  $GA_{ij}$  in days was computed as  $GA_{ij}=7 \cdot w_{ij}+d_{ij}$ .
3. 10 imputed datasets  $D_j$ ;  $j=1, \dots, 10$ , were created as follows. For the presumably completely observed variables (date of birth, gender, types and times of outcome events) the values were the same in each set as recorded in the original database. For those subjects  $i$  for whom questionnaire data on gestational age were available, the recorded value  $GA_i$  was also repeated as such in all datasets. For those with missing gestational age, one of the simulated values  $GA_{ij}$  was substituted for it in each version  $D_j$ .
4. The value representing the timing of the 11th week of pregnancy in each imputed dataset  $D_j$  was computed from the known date of birth and either the recorded duration of pregnancy  $GA_i$  or the imputed value  $GA_{ij}$ .

Descriptive Kaplan–Meier estimates were produced in groups, the division being based on conditional mean imputation such that the mean value of 278 days observed among those providing this item of information was substituted for the missing durations of pregnancy. The function `survfit()` in the survival package of R<sup>41</sup> was used for the computation and graphics.

The seasonal variations associated with the month of birth and the 11th week of pregnancy were analysed by fitting Cox proportional hazards regression models to the observed numbers of outcome events and the accumulated risk times since birth. The linear predictor in these models was specified as a second-order harmonic function of time<sup>17</sup> plus terms for gender and birth order:

$$\alpha_1 \cos(2\pi m) + \beta_1 \sin(2\pi m) + \alpha_2 \cos(4\pi m) + \beta_2 \sin(4\pi m) + \gamma G + \delta B$$

where  $m$  is the midpoint of the month in fractions of a year computed as exact midpoints between 1 January, 1 February etc, divided by 365;  $G$  indicates gender (1=boy, 0=girl) and  $B$  birth order (1=firstborn, 0=not firstborn). Additional main effect terms were included in some models for year of the 11th gestational week, maternal smoking during pregnancy and maternal history of pollen allergy. The models were fitted using the `coxph()` function in the survival package of R.<sup>41</sup> From among the results of fitting the pertinent model to each imputed dataset  $D_j$  the vector  $C_j$  containing the estimated regression coefficients and their estimated covariance matrix  $V_j$  was stored. The results of fitting the various imputed datasets were pooled by averaging the point estimates and computing the estimated covariance matrix as the sum of the within-imputation and between-imputation components<sup>16</sup> using the mitools package in R.<sup>42</sup> A seasonally smoothed curve of predicted incidence rates was thus obtained based on the pooled point estimates of the harmonic model. The locations of the highest and lowest predicted rate in the curve were obtained to an accuracy of 1 day, and the ratio between them was calculated to describe the relative amplitude of the estimated seasonal variation. The precision of the estimated amplitude was assessed through a simulation of 10 000 samples from an assumed multivariate Gaussian distribution for the parameter estimates of the harmonic model drawn by `mvrnorm()` function in the R package MASS.<sup>43</sup> The mean and covariance matrix of that distribution were plugged in from the vector  $C$  of averaged point estimates and the pooled covariance matrix  $V$  obtained above. Finally, the 2.5% and 97.5% quantiles among the simulated estimates of relative amplitude were taken as confidence limits.<sup>18</sup> Models including higher-order periodic terms and interactions between periodic terms and some of the covariates were also considered, but the statistical signal for such effects was very weak or non-existent, so they were not pursued any further.