in food sensitization rates were first observed at age 3 years, these differences between the 2 folate groups were not statistically significant until age 6 years. Because of the modest sample size, we were unable to determine whether the risk of food sensitization was also borne out as an increased risk in likely food allergy.

The mechanisms underlying our observations are unclear. Although folate has a myriad of biologic effects, one current hypothesis posits that it may promote DNA methylation since it serves as a methyl donor, thereby suppressing the expression of key immune regulatory genes. Although these findings point to the potential for folate to act via an epigenetic mechanism in early childhood, folate has many roles in cellular function so that it may act on the pathogenesis of allergic sensitization by other mechanisms.

Other potential confounding factors may include breastfeeding, consumption of high-folate-containing (or folate-fortified) foods. In this study, we found no significant difference between breast-feeding in both clusters (data not shown). Although we cannot ascertain exactly what role diet may have contributed to variability in our findings, in this study dietary folate acid intake would not be considered a true confounder as it is present in the causal pathway; since dietary intake is the major driver of plasma folate levels, our use of plasma folate levels already captures the dietary intake of folic acid. However, it is possible that plasma folate levels are simply a marker in general of a healthy diet and that some other component of a healthy diet—such as another micronutrient—could be the true driver of allergic sensitization risk rather than folate. The observational study design does not allow for the evaluation of this question, and so this limitation could not be controlled for in a natural history study such as Childhood Origins of Asthma.

In summary, we found in a high-risk birth cohort that higher folate levels in early childhood were significantly associated with the increased incidence of both food and aeroallergen sensitization, suggesting that folate may confer the risk of allergy not only in utero but also in the first few years of life. These findings suggest that modification of folate intake in early childhood could reduce the risk of allergic sensitization and support the conduct of larger prospective studies to determine whether these findings are reproducible and whether folate affects the risk of allergic disease as well as the risk of allergic sensitization.

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Anaphylaxis caused by hidden soybean allergens in pillows

To the Editor:

Pillow stuffing can contain polyester, feathers, down, or more recently, soy-based materials. Component-resolved diagnosis and microarray technology might be useful in patients with food-sensitized allergies or anaphylaxis caused by hidden allergens.

We report 4 patients with repeated anaphylaxis, which was defined as a decrease in blood pressure and possibly life-threatening adverse reactions during sleep at home but not in other places. Patient 1 was a 64-year-old woman with previous nocturnal rhinitis who began to experience anaphylaxis during sleep. Patient 2 was a 58-year-old man with progressive rhinitis, asthma, and anaphylaxis after changing his bedroom furniture and bed linen. Patient 3 was a 64-year-old woman with previous sensitization to Anisakis simplex and nocturnal pharyngeal angioedema unrelated to eating fish or other sources of A simplex who experienced severe anaphylaxis during sleep. Patient 4 was a 69-year-old woman with nocturnal anaphylaxis for 1 year before diagnosis.

All patients had food-related rhinitis and asthma, although the sources were not clear. Patient 4 also had severe anaphylaxis after eating soy sauce in a Japanese restaurant, which led to our initial suspicion. All patients had negative skin prick test (SPT) responses and IgE determination (ImmunoCAP Assay; Phadia, Uppsala, Sweden) to conventional aeroallergens and foods, including soybean.

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The only common finding among these patients was the purchase of a viscoelastic pillow before symptom onset. Information was obtained on the soy oil composition of the pillow matrices, and an allergy study focusing on the proteins contained within the oil bodies of soybean seeds was performed. Symptoms disappeared after the pillows were removed.

After informed consent, new SPTs with soybean nut, hull, and oil were performed. Specific IgE levels were analyzed by using IgE microarrays (ISAC) and immunoblots with proteins extracted from plant lipid storage bodies (most allergens are obtained through hydrosoluble methods, which remove the lipid derivatives from the allergenic extract) and proteins extracted from the pillow core.

All SPT responses to different nut extracts were negative. Only SPT responses to soybean oil were positive, with a mean wheal diameter for soy oil of $8 \times 8 \pm 3$ mm. The mean wheal diameter for histamine was $5 \times 5$ mm.

Microarray-based IgE detection assay (ISAC) revealed hypersensitivity to $\beta$-conglycinin (nGly m 5) in all patients.

Western blot experiments detected serum-specific IgE to several proteins, with molecular masses compatible with the allergens Gly m 5, Gly m 6, and oleosins, and dot blots confirmed these IgE bindings. Sera from all 4 patients showed a positive response to the aqueous fraction and lipoprotein fraction extracts of soybean seeds and the core extract of the pillows. Sera from nonatopic control subjects did not react to any product tested (Fig 1).

Sensitization to soy and soy oil have been described. Exposure to soy antigens has been associated with asthma in community and workplace outbreaks. Recent evaluation of 135 Tennessee soy flake–processing workers for immune reactivity to soy showed that allergic sensitization to soy was common and 5 times more prevalent than in control health care workers with no known soy exposure. Nano high-performance liquid chromatography mass spectrometry analysis showed the high-molecular-weight soybean storage proteins $\beta$-conglycinin (Gly m 5) and glycinin (Gly m 6) were the proteins that bound soy flake–processing worker IgE.

Gly m 5 and Gly m 6 might be respiratory sensitizers in occupationally and nonoccupationally exposed workers. Soy protein has shown great potential for use in biobased adhesives, $\beta$-Conglycinin, a major component of soy protein, accounts for 30% of the total storage protein in soybean seeds. $\beta$-Conglycinin was isolated and purified, and the physicochemical and adhesive properties of its subunits ($\beta$ and $\alpha'$) were characterized. Soy is also used in pharmaceutical products as soymorphins or drug excipients.

The diagnostic workup for suspected soy allergy includes food allergy screening tests, responses to an elimination diet, and an oral food challenge. No screening test, alone or in combination, can definitively diagnose or exclude soy allergy. A wider panel of proteins extracted from plant lipid storage bodies called oil bodies in combination with diagnostic microarray methods, including those that focus on immune responses to specific food proteins or protein epitopes, are being studied.

Oral allergy syndrome to soy milk is classified as a phenotype of pollen-food allergy syndrome (PFAS). Gly m 4 (Bet v 1 homolog, 17 kDa) and oleosin (23 kDa) are reported as causative antigens. Recently, 2 cases of PFAS to soy milk have been reported, with positive reactions to soy milk in SPTs and to Gly m 4 in specific serum IgE antibody assays. Microarray analysis of specific serum IgE antibodies of soy-related proteins showed a positive reaction to Bet v 1 in both cases, and this was diagnosed as PFAS to Gly m 4.

In our patients protein microarray analysis was useful as a screening test for immediate allergy, such as PFAS. The reason for the negative SPT responses and IgE assay results to soy in our patients might be due, in part, to the localization of oleosins (and other as yet uncharacterized proteins) in oil bodies because they are underrepresented or denatured in most diagnostic extracts of nuts and seeds that are usually extensively defatted. The absence or presence of denatured (during defatting) oleosins might provoke a false-negative in vitro diagnosis of some nut and seed allergies. Acidic subunits of glycinin and $\beta$-conglycinin, major soybean storage proteins, might be absent or present in much reduced amounts in these techniques. Immunoblotting with sera from patients with soy allergy indicates alteration, reduction, or loss of IgE binding in the commercial extracts compared with extracts of soy flour. Preparation methods appear to be partially responsible for the variable allergen content in commercial soybean SPT extract.

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**FIG 1.** IgE immunoblotting after exposure to soybean seed extracts. A, Responses of sera from patients 1 to 4 and a negative control subject (C-). Lane 1, Aqueous fraction of soybean seeds; lane 2, lipid fraction of soybean seeds; lane 3, protein extract from the pillow core. B, Responses of sera from patients 1 to 4 to the aqueous fraction (A), lipoprotein fraction (B), and pillow core (C).
The major limitation of this study was the lack of specific oral challenge with soybean. However, the severity of the anaphylaxis precluded these tests.

In summary, soy is recognized as a common food allergen. IgE antibodies from soybean-sensitive patients recognize more than 15 soybean proteins,9 some of which can be found as hidden allergens in pillows. Sensitization to the soybean allergens Gly m 5, Gly m 6, and oleosins is potentially indicative of severe allergic reactions to soy, as in our patients.10

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Validation of the Cork-Southampton Food Challenge Outcome Calculator in a Canadian sample

To the Editor:

We have previously demonstrated the clinical usefulness and diagnostic value of a calculator (the Cork-Southampton calculator) using routinely collected data in predicting oral food challenge (OFC) outcomes in a diverse sample of children in an Irish pediatric allergy clinic.1 The calculator consists of 6 clinical factors: skin prick test responses, serum specific IgE levels, total IgE levels minus serum-specific IgE levels, symptoms, sex, and age. The probability scores generated by the calculator range from 0 (no allergy) to 1.0 (allergy), corresponding to a 0% to 100% chance of a positive or negative outcome. Our model showed an advantage in clinical prediction compared with serum specific IgE levels only, skin prick test responses only, and serum specific IgE levels plus skin prick test responses (92% accuracy vs 57% to 81%, respectively).1

We have examined the wider utility of the calculator in a different clinical setting, with different criteria for selection of children for challenge, different challenge protocols, and different food preparations. In addition, we now report likelihood ratios (LRs), and in response to correspondence on our previous article, we have developed a prechallenge probability level at, above, or below which a clinician can manage a patient’s ongoing food allergy care without recourse to OFCs.

The retrospectively collected dataset consisted of children in Montreal, Quebec, Canada, who had undergone food challenges for peanut, milk, or egg in a tertiary pediatric allergy clinic (Table I). The mean age of the sample (n = 46) was 7.4 years (Table I). The cases were chosen consecutively from existing clinic datasets. Analyses were carried out for all 6 clinical factors and for skin prick test responses and serum specific IgE measurements only (separately and together). Analysis was performed (by A.D.) blind to OFC outcomes.

Seventeen patients underwent food challenge for peanut, 9 for egg, and 20 for milk. There were 8 positive and 9 negative results for peanut, 1 positive and 8 negative results for egg, and 6 positive and 14 negative results for milk. Egg OFCs included raw, baked, and meringue egg.

In total, 42 (91%) of 46 OFC outcomes were correctly predicted based on prechallenge data. In the case of peanut OFCs, the calculator correctly predicted 15 of 17 outcomes, with all 8 positive and 7 of 9 negative results correctly predicted. The single positive OFC result for egg was correctly predicted, as were 7 (88%) of 8 negative outcomes. Milk OFC outcomes were predicted correctly in 19 (95%) of 20 cases (all 6 positive and 13 of 14 negative milk OFC results). The only incorrect prediction for milk OFC outcomes had an intermediate probability of 0.6 (and the result was negative).

| TABLE I. Clinical characteristics of the sample (n = 46) |
|-------------|-----------|-----------|-----------|
| Male/female sex | 34/12     | 74/26     | 24         | 52     |
| Skin or oral or gastrointestinal or upper respiratory tract symptoms only* | | | |
| Upper respiratory tract and gastrointestinal or 2 systems* | 15 | 33 |
| Lower respiratory tract or 3 systems* | 6 | 13 |
| Cardiovascular or 4 systems* | 1 | 2 |

*There were no significant differences (P > .05) in the number and severity of symptoms experienced among the 3 foods.