

Lipid transfer protein–linked hazelnut allergy in children from a non-Mediterranean birch-endemic area

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Background: Hazelnut allergy in birch pollen–exposed areas is usually due to cross-reactivity (Cor a 1 and 2) and is usually mild in nature (oral allergy). In areas without birches, severe reactions are more prevalent and linked to sensitization to the lipid transfer protein (LTP) Cor a 8.

Objective: We sought to investigate whether sensitization to LTP plays a role in more severe (objective) hazelnut-induced symptoms in children from a birch-endemic area.

Methods: Sensitization to Cor a 8, Cor a 2, Cor a 1, and Bet v 1 was determined by means of RASTs and immunoblotting in hazelnut-sensitized children with (n = 8) and without (n = 18) objective reactions during double-blind, placebo-controlled food challenges. Additionally, samples from 191 hazelnut-sensitized nonchallenged children were analyzed.

Results: Children with objective reactions during double-blind, placebo-controlled food challenge had higher IgE titers to hazelnut ($P < .001$) and recognized more allergens on immunoblotting ($P = .001$) than those without such reactions. All children with objective symptoms were sensitized to Cor a 8 (0.51–23.3 IU/mL) compared with only 1 child without objective reactions (0.90 IU/mL). In a multivariate analysis only IgE against Cor a 8 remained as an independent risk factor (undefined odds ratio; $P < .0001$). In the group of nonchallenged children (n = 191), the prevalence of LTP sensitization was greater than 30%. Unexpectedly, sensitization to Cor a 1 was observed in children not sensitized to Bet v 1.

Conclusion: Sensitization to hazelnut LTP is a risk factor for objective symptoms in children from a birch-endemic area. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Hazelnut allergy, birch pollen, Cor a 1, pediatric allergy, Cor a 8, lipid transfer protein

Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
OAS: Oral allergy syndrome
ROC: Receiver operating characteristic

In areas in which the birch is endemic, hazelnut allergy has mainly been associated with cross-reactive IgE to Bet v 1 and Bet v 2 (profilin), usually causing mild symptoms known as the oral allergy syndrome (OAS).^{1–3} The cross-reactive structures in hazelnut implicated in this so-called parabirch syndrome are Cor a 1 and Cor a 2. In Mediterranean areas without exposure to birch pollen, reactions to hazelnut have been associated with sensitization to Cor a 8, a lipid transfer protein (LTP).⁴ Sensitization to LTP has convincingly been shown to be a risk factor for severe allergic reactions in these areas, not only to hazelnut⁴ but in particular also to apple and peach.^{5,6} A recent study among subjects with cherry and pollen allergy from Central Europe showed a prevalence of sensitization to LTP in 11%.⁷ In northern European countries, such as The Netherlands,⁵ Austria,⁵ and Germany,⁸ sensitization to LTP has only rarely been reported for patients with plant food allergies, and most of these studies were performed among adults. Although patients from birch pollen–endemic areas predominantly present with mild oral allergy to hazelnut, severe symptoms can occur, and even fatal reactions to hazelnut have been reported.^{9–11} Many of the more severe reactions were found in children. Recently, in a study among Dutch children with double-blind, placebo-controlled food challenge (DBPCFC)–proved hazelnut allergy, we reported that 8 of 12 had challenge-induced objective symptoms (generalized urticaria, angioedema, vomiting, and bronchoconstriction) in addition to mild oral symptoms reported by the whole group.¹² Remarkably, 3 of the 8 children with objective reactions had no or very low IgE levels to birch (or grass) pollen, suggesting that the origin of sensitization was not pollen cross-reactivity. Interestingly, these children were sensitized to multiple nuts and peanut. Such a pattern of fruit allergy, nut allergy, or both in the absence of, or at least not necessarily linked to, pollen allergy is typical for LTP-related allergy in Mediterranean countries, such as Spain¹³ and Greece.¹⁴ The route of sensitization is still largely a mystery but is likely to be different from pollen-induced cross-reactivity.

The aim of this study was to investigate whether sensitization to hazelnut LTP (Cor a 8) plays a role in children from birch-endemic areas presenting with more severe hazelnut allergy. This might challenge the dogma that has taken hold in the scientific community that sensitization to LTP is a Mediterranean problem.

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METHODS

Study population

Sera from 26 children with specific IgE to hazelnut (8 girls and 18 boys; median age, 7.0 years) were collected between January 2003 and June 2004. These children belonged to a group of children who underwent a DBPCFC with increasing doses of hazelnut. Sera used in the present study were taken before the challenge. The outcome of the DBPCFCs was reported elsewhere.¹¹ Of the 26 challenged children, 14 were shown to have negative results on challenge, 4 had only OAS, and 8 additionally demonstrated objective symptoms (angioedema, generalized urticaria, vomiting, diarrhea, rhinoconjunctivitis, and/or dyspnea).¹² To identify risk factors for objective symptoms, the subjects without symptoms ($n = 14$) and with only OAS ($n = 4$) were analyzed together ($n = 18$) and compared with those with objective symptoms ($n = 8$).

In the same period, all children (between 0.4 and 16.6 years of age) visiting the outpatient clinic for pediatric allergology ($n = 1290$) were routinely screened for sensitization to various food allergens, including hazelnut, by using the CAP-FEIA system (Phadia, Uppsala, Sweden). Of these, 310 (24%) children were sensitized. Remaining serum after determination of hazelnut-specific IgE was stored at -20°C . In total, 191 serum samples from children with sensitization to hazelnut were available for further analysis in this study. This group was not different from the total group ($n = 310$) with respect to sex (67 female and 124 male subjects vs 115 female and 195 male subjects), age (median age, 4.5 vs 4.1 years), and hazelnut-specific IgE levels (median, 2.4 vs 2.2 kU/L).

Determination of hazelnut-specific IgE levels by means of CAP

Levels of specific IgE to hazelnut were determined by using the CAP-FEIA system, according to manufacturer's instructions. IgE levels of greater than 0.35 kU/L were considered positive.

Determination of specific IgE levels by means of RASTs

RASTs were performed as described previously.¹⁵ In short, 100 μg of purified nBet v 1, nCor a 1, rCor a 1 (rCor a 1.0401; kindly provided by Stefan Vieths, Langen, Germany),¹⁶ nCor a 8, or rPru p 3¹⁷ was coupled to 100 mg of cyanogen bromide-activated Sepharose (GE Healthcare, Uppsala, Sweden). nCor a 1 and nBet v 1 were both purified by means of affinity purification with mAb 5H8, as described elsewhere.¹⁸ nCor a 8 was purified by means of a combination of ion-exchange and size-exclusion chromatography similar to that described for apple LTP.¹⁹ The purity of nCor a 1 and nCor a 8 was assessed by means of SDS-PAGE and silver staining (Fig 1). RAST analysis for profilin (Cor a 2) was performed by using Sepharose-coupled poly-L-proline as an affinity matrix to bind Cor a 2 from hazelnut extract.²⁰ After washing away unbound hazelnut protein, this solid phase was used for detecting Cor a 2-specific IgE. RASTs were carried out by means of overnight incubation of 50 μL of serum with 0.5 mg of Sepharose in a final volume of 300 μL of PBS/0.3% BSA/0.1% Tween 20. Negative controls consisted of a nonatopic control serum and secondary antibody without serum. After washing away unbound material with PBS/0.1% Tween 20, bound IgE was detected by means of overnight incubation with iodine 125-radiolabeled sheep anti-human IgE (SH25-1-p7; Sanquin, Amsterdam, The Netherlands). After washing, bound radioactivity was measured in a γ -counter. Results were expressed in international units of IgE per milliliter by using a standard curve of chimeric monoclonal IgE antibodies against Der p 2 and Sepharose-coupled rDer p 2.²¹ A result of greater than 0.35 IU/mL was considered positive.

SDS-PAGE and immunoblotting with hazelnut extract

Crude hazelnut extract (20 $\mu\text{g}/\text{cm}$ total protein) or purified nCor a 8 (15 $\mu\text{g}/\text{cm}$) was separated by means of SDS-PAGE under reducing conditions with NuPAGE-Bis-Tris gels (10%; Novagen, Groningen, The Netherlands) and transferred onto nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany), according to the manufacturer's instructions. A buffer containing

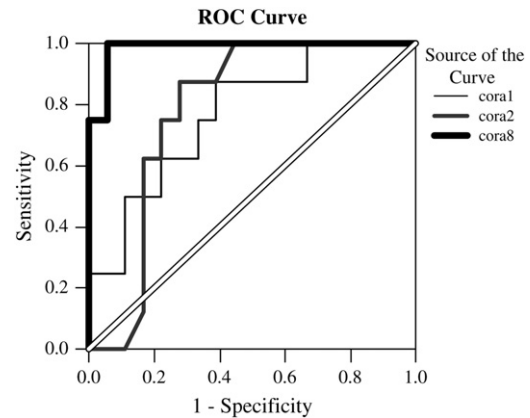


FIG 1. ROC curves for nCor a 8, nCor a 2, and nCor a 1 determined for the group that underwent DBPCFCs with hazelnut. The group with no reaction and OAS was regarded as negative, and the group with objective reactions was regarded as positive.

polyvinylpolypyrrolidone and diethyldithiocarbamate was used. For immunoblot analysis, serum samples were diluted 1:30 in PBS containing 0.05% Tween 20. Serum from a nonatopic subject was used as a negative control. For detection of bound IgE antibodies, the strips were incubated with iodine 125-radiolabeled sheep anti-human IgE (Sanquin) overnight. Radioactivity was measured by exposing the strips overnight to KODAK BioMax MS films (Rochester, NY).

Statistics

For statistical analyses, cut-off values for sensitization to individual allergens were established by means of receiver operating characteristic (ROC) analysis instead of using the standard assay cut-off of 0.35 IU/mL. For the ROC-curve analysis, objective symptoms during DBPCFCs were classified as positive, and OAS and negative symptoms were classified as negative. The resulting cut-off values for each allergen were used in univariate analysis. The Fisher exact test (2-way analysis) was used to establish associations between sensitization to individual allergens and objective symptoms during challenge. For age and challenge outcome, the Mann-Whitney U test was used. Significant associations ($P < .15$) from the univariate analyses were analyzed in a multivariate analysis by using a logistic regression model. Correlations between sensitization to different allergens were performed with the nonparametric Spearman rank or gamma test. All calculations were performed with SPSS software (version 12; SPSS, Inc, Chicago, Ill). P values of less than .05 were considered significant.

RESULTS

Sensitization to hazelnut LTP (Cor a 8) is a risk factor for objective symptoms

The hazelnut allergens Cor a 8 and Cor a 1 were purified from hazelnut extracts and shown to be 99% pure by means of SDS-PAGE and silver staining (see Fig E1 in the Online Repository at www.jacionline.org). Purified Cor a 8 consisted of several bands around 7 kd (see Fig E2 in the Online Repository at www.jacionline.org). Table I lists the RAST results for these allergens and for nBet v 1, a recombinant version of Cor a 1 (rCor a 1), nCor a 2, and peach LTP (rPru p 3) and the previously reported CAP data for specific IgE against hazelnut and birch pollen extract.¹² Levels of hazelnut-specific IgE were significantly higher ($P < .001$) for children with objective reactions (median, 32.5 kU/L) compared with those of children without objective reactions (median, 1.5 kU/L). All 8 children with objective symptoms were sensitized to nCor a 8 (median, 3.25 IU/mL; range, 0.51–23.3 IU/mL).

TABLE I. Sensitization to hazelnut, birch pollen, nBet v 1, nCor a 1, rCor a 1, nCor a 2, nCor a 8, and rPru p3 in children who were challenged with hazelnut

Children		DBPCFC		At DBPCFC							
Patient no.	Sex	Allergy*	Age (y)	Hazelnut†	Birch pollen†	Bet v 1†	Cor a 1†	rCor a 1†	Cor a 2†	Cor a 8†	Pru p 3†
1	M	None	8	8.5	>100	86.8	10.7	20.0	Negative	Negative	Negative
2	F	None	9	2.0	76.0	12.7	4.5	2.3	Negative	Negative	Negative
3	M	None	14	1.4	56.0	18.4	4.2	3.4	Negative	Negative	Negative
4	M	None	8	3.7	>100	39.7	8.0	26.0	Negative	Negative	Negative
5	M	None	16	1.0	Negative	Negative	Negative	Negative	Negative	Negative	Negative
6	M	None	14	26.1	39.0	13.8	2.4	6.3	Negative	Negative	Negative
7	M	None	7	1.1	26.7	5.7	2.6	2.2	Negative	Negative	Negative
9	M	None	4	1.3	53.0	15.4	3.0	4.0	Negative	Negative	Negative
11	M	None	7	3.5	>100	49.4	14.0	6.5	Negative	Negative	Negative
12	M	None	5	11.0	>100	87.5	4.6	28.0	2.0	Negative	1.3
13	M	None	4	0.7	Negative	Negative	Negative	Negative	0.7	Negative	Negative
14	F	None	7	1.3	1.6	Negative	0.7	Negative	12.7	Negative	Negative
15	M	None	6	4.2	>100	37.3	Negative	7.0	Negative	Negative	Negative
16	F	None	4	0.5	Negative	6.2	7.4	1.4	Negative	Negative	Negative
17	M	OAS	12	8.4	51.0	4.2	3.1	1.0	0.4	0.9	Negative
18	F	OAS	4	1.5	27.4	Negative	Negative	Negative	39.0	Negative	Negative
19	F	OAS	10	1.0	80.0	16.6	2.9	5.0	Negative	Negative	Negative
20	M	OAS	5	0.7	2.6	0.7	0.6	Negative	Negative	Negative	Negative
21	M	Objective	4	16.1	9.1	13.5	2.0	1.0	Negative	3.7	Negative
22	M	Objective	6	4.4	34.9	5.9	3.5	0.7	Negative	0.5	Negative
23	M	Objective	8	45.0	>100	56.6	9.2	21.0	1.0	2.8	9.1
24	M	Objective	13	20.1	62.0	19.4	7.0	2.0	0.5	2.3	0.6
25	F	Objective	4	38.0	Negative	Negative	8.6	Negative	0.7	23.3	Negative
26	F	Objective	8	48.0	>100	76.3	15.5	17.0	2.0	9.9	Negative
27	F	Objective	6	27.1	0.5	Negative	15.3	Negative	1.5	12.1	Negative
28	M	Objective	6	55.0	Negative	Negative	4.2	Negative	0.8	0.8	Negative
				$P < .001$	$P = .567$	$P = .567$	$P = .030$	$P = .567$	$P = .022$	$P < .001$	$P = .331$

Differences in levels of specific IgE and number of recognized allergens between children with and without objective symptoms are indicated with a P value on the bottom of each column.

M, Male; F, female.

*Allergy: *None*, no allergy; *objective*, objective reaction to hazelnut.

†Sensitization in international units per milliliter; negative: 0.35 IU/mL or less.

Two of them were also sensitized to peach LTP (rPru p 3). The ingestion of peach did not cause allergic symptoms in one child, and the other never ingested peach. Only one child in the group without objective reactions was sensitized to nCor a 8 (0.9 IU/mL). This child refused to complete the challenge after experiencing OAS on 3 subsequent portions. Three children with objective symptoms demonstrated significant levels of specific IgE against nCor a 1 but had negative results for rCor a 1 and nBet v 1. Sensitization to birch pollen, nBet v 1, and rCor a 1 was similar in both groups ($P > .5$).

By using ROC curves (Fig 1), cut-off values for the individual allergens were calculated: 0.65 IU/mL for nCor a 8, 0.45 IU/mL for nCor a 2, 0.65 IU/mL for nCor a 1, 0.35 IU/mL for rCor a 1, 0.35 IU/mL for nBet v 1, and 0.65 IU/mL for rPru p 3. In a univariate analysis only sensitization to nCor a 8 ($P < .001$) and nCor a 2 ($P = .026$) was associated with objective reactions to hazelnut during DBPCFCs. Combining sensitization to nCor a 8 and nCor a 2 in a multivariate analysis by using a logistic regression model revealed that only sensitization to nCor a 8 was independently associated with the appearance of objective reactions in the DBPCFCs (undefined odds ratio, $P < .001$). The probability of having an objective reaction during a DBPCFC with hazelnut was 87.5% when the IgE titers to Cor a 8 were 0.65 IU/mL or greater, decreasing to 5.5% for

IgE titers of less than 0.65 IU/mL. Therefore from all studied allergens, sensitization to nCor a 8 is the only significant and strong risk factor for objective reactions to hazelnut during a DBPCFC.

Complexity of IgE reactivity to hazelnut is particularly observed in objective reactions

By using crude hazelnut extract on immunoblots, IgE-binding proteins were identified between 5 and 90 kd (Fig 2, A). The IgE of children with objective reactions to hazelnut recognized significantly more hazelnut proteins than the IgE of children without objective reactions (median, 7 vs 2.5; $P = .001$). This might be in line with the fact that these children were also sensitized to several other nuts (walnut, pecan, Brazil nut, pistachio, and cashew) and peanut.¹² The children without objective reactions who were also sensitized to numerous other nuts, subjects 6, 12, and 17, also showed more IgE-binding bands on the blot.

Five of the 8 children with objective reactions recognized proteins at approximately 8 kd, corresponding to the expected size of the LTP. Immunoblots with purified nCor a 8 were positive for the same 5 children and showed similar bands at approximately 8 kd (Fig 2, B). Inhibition of the hazelnut extract immunoblots with nCor a 8 showed that these bands disappeared (Fig 3).

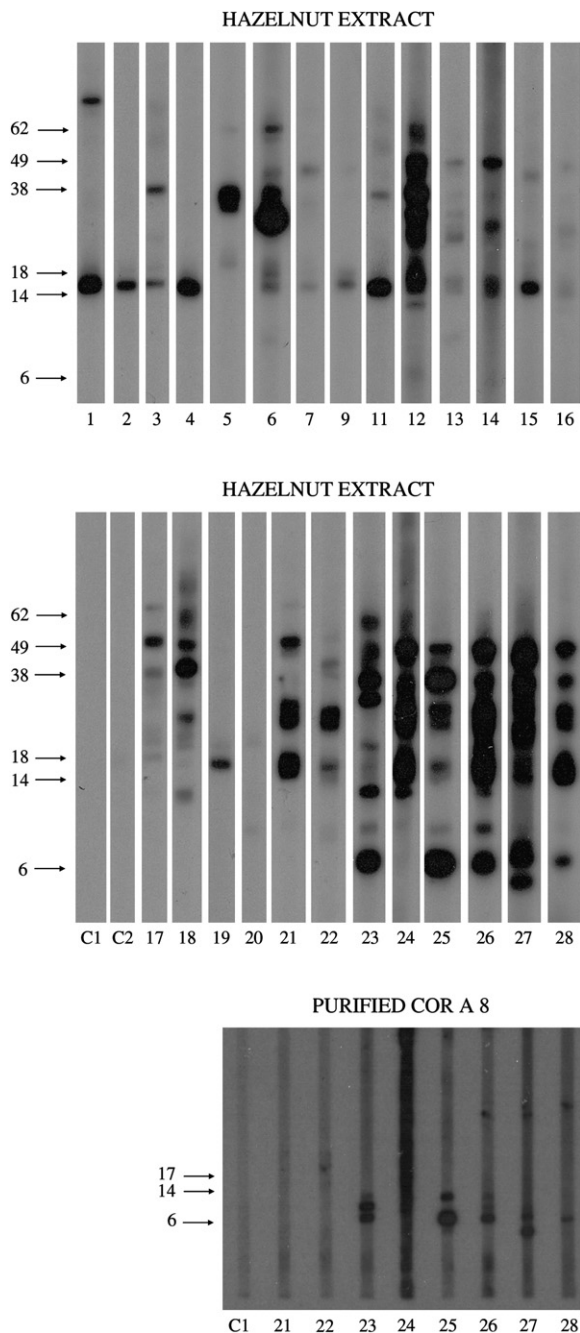


FIG 2. Immunoblots with hazelnut extract for all children who underwent DBPCFCs with hazelnut. The numbers under each lane correspond with the numbers of the children in Table I. The first lanes on the second row (C1 and C2) represent a nonatopic control and secondary antibody alone. The bottom row consists of immunoblots with nCor a 8 for all children with objective reactions to hazelnut.

Sensitization to hazelnut LTP is common for hazelnut-sensitized children in a birch-endemic area

One hundred ninety-one sera of children with a positive CAP result for hazelnut were evaluated for sensitization to nCor a 8 to assess whether sensitization to hazelnut LTP (Cor a 8) is common among hazelnut-sensitized children in The Netherlands. In addition, IgE levels against nCor a 1 and nBet v 1 were measured. Specific IgE against nCor a 8 was present in 30.9% of

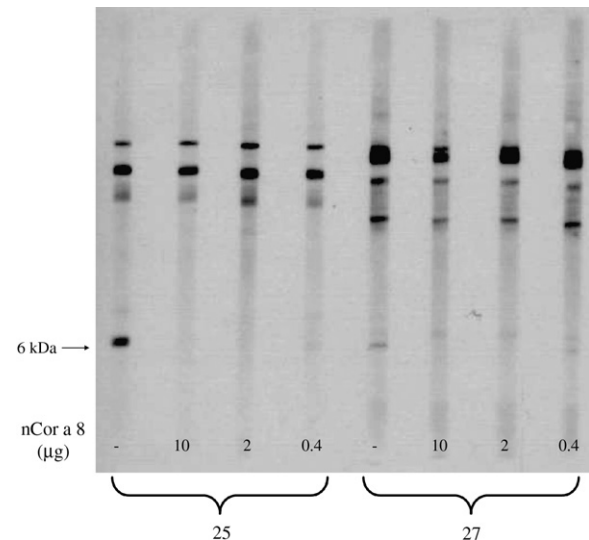


FIG 3. Inhibition of hazelnut immunoblots with nCor a 8 for 2 subjects with objective symptoms (25 and 27). The bands around 8 kDa are inhibited by nCor a 8.

the children (median, 1.4 IU/mL; range, 0.4–32.6 IU/mL). As expected for a birch-endemic area, the prevalence of sensitization to nCor a 1 and nBet v 1 was high: 59.7% had positive results for nCor a 1 (median, 3.1 IU/mL; range, 0.4–29.5 IU/mL), and 52.3% had positive results for nBet v 1 (median, 27.3 IU/mL; range, 0.5–>100 IU/mL). Fifty-seven (32%) of all children were sensitized to neither nBet v 1, nCor a 1, nor nCor a 8. These children were significantly younger (mean age, 2.9 vs 7.7 years; $P < .001$) and had lower antihazelnut IgE titers (1.6 vs 2.8 IU/mL; $P < .001$).

All challenged children were 3.6 years of age or older. In the group of sensitized children, more than half (105/191) were 3.6 years of age or older, with a median age not significantly different from that of the challenged children (8.9 vs 7.0 years). The median level of hazelnut-specific IgE of these older children (≥ 3.6 years of age) was not significantly different from that of the challenged children (2.35 vs 3.95 kU/L, $P = .137$). The prevalence of sensitization to Cor a 8, Cor a 1, and Bet v 1 was similar for these age-matched groups (Table II), thus supporting that the challenged group was representative of the larger group of hazelnut-sensitized children.

Sensitization patterns to Cor a 8, Cor a 1, and Bet v 1 at different ages

The prevalence of sensitization to nCor a 8 increased up to the age of 3 years to a level of approximately 30%, around which it fluctuated in older children (Fig 4). Nineteen (32%) of the 59 Cor a 8-sensitized children were not sensitized to Bet v 1. This group was significantly younger than the other nCor a 8-sensitized children ($P < .001$), although hazelnut- and nCor a 8-specific IgE levels were similar.

The prevalence of sensitization to nBet v 1 was 9% at 1 year of age and 67% at 6 years of age and also in older children (Fig 4). For nCor a 1, a similar pattern was observed of 16% and 67%, respectively. The levels of specific IgE to both nBet v 1 and nCor a 1 were positively correlated with age ($r = 0.598$ and $r = 0.630$,

TABLE II. Number and percentages of sensitization to nCor a 8, nCor a 1, and nBet v 1 for hazelnut-sensitized children older than 3.6 years compared with the challenged group (≥ 3.6 years)

	Unchallenged group (≥ 3.6 y)			Challenged group (≥ 3.6 y)		
	Cor a 8	Cor a 1	Bet v 1	Cor a 8	Cor a 1	Bet v 1
Tested samples, n	107	107	102	26	26	26
Positive samples, n	41	88	80	9	22	19
Percentage	38%	82%	78%	35%	85%	73%

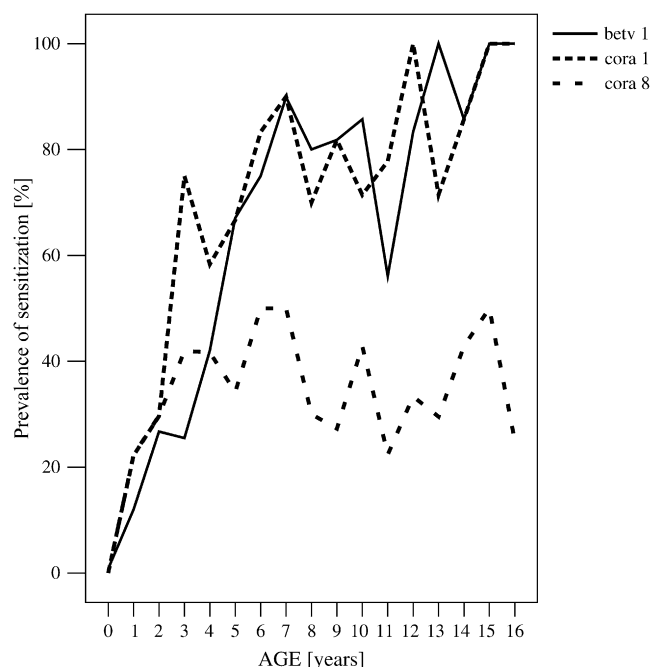
respectively; $P < .001$). Twenty-two children had specific IgE antibodies to nCor a 1, without detectable sensitization to nBet v 1. These children were significantly younger than those with sensitization to both nCor a 1 and nBet v 1 (median, 3.1 vs 8.6 years; $P < .001$). Fourteen children were sensitized to both nCor a 8 and nCor a 1, without detectable sensitization to nBet v 1.

DISCUSSION

Sensitization to hazelnut LTP (Cor a 8) was previously reported as a risk factor for more serious reactions for patients from the Mediterranean area.⁴ Similar findings were reported for apple LTP.⁵ In the present study it was shown that sensitization to hazelnut LTP is a significant risk factor in children from a birch-endemic area as well, where hazelnut allergy is usually associated with birch pollen sensitization and mild oral symptoms.¹⁻³ Of the entire hazelnut-sensitized pediatric population visiting our hospital over a period of 18 months, 30.9% were sensitized to Cor a 8.

Children with challenge-induced objective reactions were consistently sensitized to Cor a 8, whereas none of the children were sensitized who had completed the DBPCFC to the highest dose (open challenge with 10 hazelnuts) without having objective symptoms. One child sensitized to Cor a 8 reported only subjective symptoms but refused to finish the challenge up to the highest dose. Three RAST-positive sera did not bind to Cor a 8 on immunoblotting, both purified and in extract. The (partial) disturbance of the 3-dimensional structure of Cor a 8 during the immunoblotting procedure might explain this discrepancy. Purified Cor a 8 was able to inhibit binding to 6- to 8-kD proteins on the immunoblots with hazelnut extract. Our observation is in line with other studies that also showed an association between sensitization to LTP and systemic symptoms.⁴⁻⁷ However, a recent study from Central Europe reported sensitization to cherry LTP in children without clinical relevance.⁸ The fact that the children from our study were not sensitized to peach LTP and tolerated peach, apple, and cherry might indicate that there is poor cross-reactivity between hazelnut LTP and fruit LTP. Apparently, hazelnut LTP behaves differently than fruit LTP, which might explain the discrepancy in clinical relevance. From our results we can conclude that sensitization to nCor a 8, in particular greater than 0.65 IU/mL, is a risk factor for objective reactions.

In the statistical analyses children with OAS and those with a negative DBPCFC result were taken together and compared with those with objective symptoms. At first sight, this might not seem logical from a clinical perspective. Our hypothesis was that IgE against one or more individual allergens was a risk factor for objective symptoms. IgE recognition of individual allergens was lowest in the group with a negative challenge result (3/14 for Cor a 2 and 0/14 for Cor a 8), intermediate for those with OAS (2/4 and

**FIG 4.** Prevalence of sensitization to nBet v 1, nCor a 1, and nCor a 8 in different age groups (range, 0.5–16.6 years; median, 4.5). The horizontal axis represents the different age groups. Age group 0 consists of children aged 0.01 to 1.00 years and so on.

1/4, respectively), and highest for those with objective symptoms (6/8 and 8/8, respectively). Therefore pooling both groups is justified because it meant that statistical analysis was performed under unfavorable conditions with respect to the hypothesis that sensitization to certain individual allergens is a risk factor for the onset of objective symptoms during a DBPCFC.

In addition to sensitization to Cor a 8, children with challenge-induced objective reactions recognized more allergens on hazelnut immunoblotting and had significantly higher levels of specific IgE to hazelnut than those without objective symptoms. These included allergens of higher molecular mass, possibly Cor a 9, Cor a 11, or both, the 11S and 7S globulins of hazelnut, respectively.^{22,23} Interestingly, children from our study with objective reactions were sensitized to significantly more other nuts (≥ 5) and peanut.¹² Even in children without objective reactions to hazelnut, an association was observed between the number of allergens recognized on the blot and sensitization to other nuts. Possibly cross-reactive IgE responses against 7S and 11S globulins are at the basis of the observed polysensitization to nuts and peanut and might play a role in the development of more severe allergic symptoms. Purification of Cor a 9 and Cor a 11 is currently in progress to investigate this potential cross-reactivity.

Cross-reactivity between sensitization to hazelnut and birch pollen is commonly observed in individuals who live in an environment with birches.¹⁻³ As was expected, the prevalence of sensitization to birch pollen and its major allergen, Bet v 1, was lower at 1 year than at 6 years of age.^{24,25} This was associated with sensitization to the cross-reactive hazelnut allergen Cor a 1. However, particularly younger children with Cor a 8-specific IgE antibodies were not always sensitized to Bet v 1. This suggests early sensitization to LTP (before and) independent from pollen sensitization. Interestingly, our study has also

demonstrated that sensitization to Cor a 1 occurred independently from sensitization to Bet v 1. These children had negative results to rCor a 1, indicating that the relevant isoform in the affinity-purified nCor a 1 was a different isoform than Cor a 1.0401. Sensitization to hazelnut Cor a 1 is generally accepted to be a cross-reactive phenomenon after primary sensitization to Bet v 1.¹ Our data are contradictory to a role of birch pollen as a primary sensitizer, at least for some children, and possibly point toward primary sensitization to hazelnut Cor a 8 in conjunction with sensitization to Cor a 1. The majority of Cor a 8-sensitized children (14/19) without Bet v 1 sensitization were sensitized to Cor a 1. How this occurred remains an open question because for most children challenged with hazelnut, the DBPCFC was the first known exposure to hazelnut. Reactions on first exposure have been described earlier, and several explanations have been proposed, ranging from unknown oral exposure (chocolate products) and cross-reactivity to another food to sensitization *in utero*, through breast-feeding, or through skin exposure. For Mediterranean patients allergic to LTP, peach has been implicated as the primary sensitizer.²⁶ For our group of children, this is highly unlikely, because only 2 of 9 Cor a 8-sensitized children had IgE antibodies against peach LTP (Pru p 3). Moreover, most of these children had eaten peach without having allergic symptoms. Of course, alternative explanations can be proposed, such as primary sensitization to hazel pollen Cor a 1, resulting in cross-reactivity to nut Cor a 1. Another explanation might be primary sensitization to other nuts or peanut. Longitudinal cohort studies are needed to firmly establish this sequence of events.

In conclusion, sensitization to Cor a 8 in children is a risk factor for severe (objective) symptoms in response to ingestion of hazelnut not only in the Mediterranean area but also in birch-endemic areas. Sensitization to Cor a 8, but also to Cor a 1, was not always accompanied by birch pollen sensitization, especially in younger children.

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Clinical implications: Determination of sensitization to hazelnut allergens might differentiate between mild and more severe hazelnut allergy.

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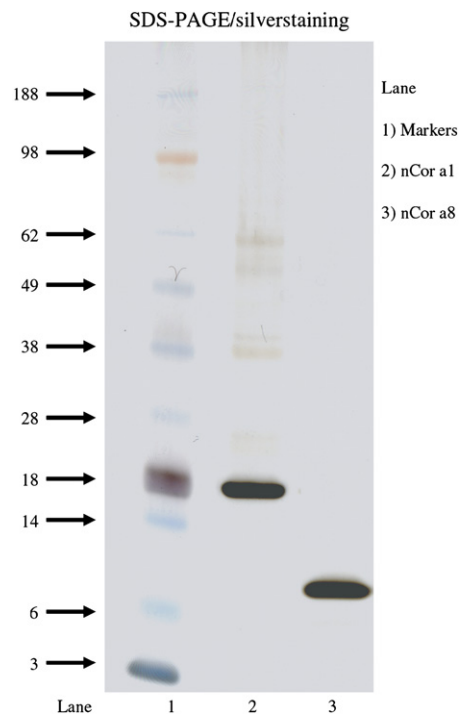


FIG E1. Silver staining of purified Cor a 1 and Cor a 8.

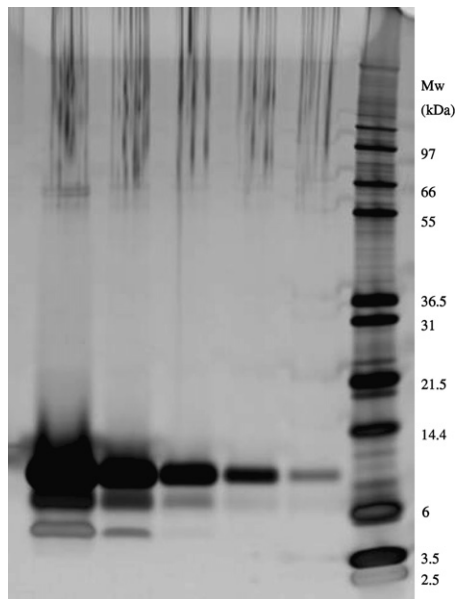


FIG E2. Silver staining of purified Cor a 8 at several concentrations. *Mw*, Molecular weight.