ORIGINAL ARTICLE

Age-Dependent Sensitization to the 7S-Vicilin-Like Protein Cor a 11 From Hazelnut (*Corylus avellana*) in a Birch-Endemic Region

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■ **Abstract**

Background: Hazelnut (*Corylus avellana*) allergy exhibits age and geographically distinct sensitization patterns that have not yet been fully resolved.
Objective: To study sensitization to Cor a 11 in different age groups of hazelnut-allergic patients and infants with atopic dermatitis (AD) sensitized to hazelnut in a birch-endemic region.
Methods: Sera from 80 hazelnut-allergic patients, 33 infants under 1 year of age with AD (24 sensitized and 9 not sensitized to hazelnut), 32 healthy control individuals, and 29 birch pollen–allergic but hazelnut-tolerant individuals were tested for immunoglobulin (Ig) E reactivity to Cor a 11 by ImmunoCAP. IgE reactivity to Cor a 1.01, Cor a 1.04, Cor a 8, and Cor a 9 was studied by ISAC microarray.
Results: Forty patients (22 preschool children, 10 schoolchildren, and 8 adults) with systemic reactions on consumption of hazelnut were sensitized to Cor a 11 (respective rates of 36%, 40%, and 12.5%). Forty patients (6 preschool children, 10 schoolchildren, and 24 adults) reported oral allergy syndrome but only 2 of them (of preschool age) were sensitized to Cor a 11.
Two (8%) of the AD infants sensitized to hazelnut showed IgE reactivity to Cor a 11. This reactivity was not observed in any of the AD infants without sensitization to hazelnut, in any of the birch-pollen allergic patients without hazelnut allergy, or in any of the healthy control individuals.
Conclusion: Sensitization to Cor a 11 in a birch-endemic region is predominantly found in children with severe hazelnut allergy, a finding that is consistent with observations concerning sensitization to Cor a 9.

Key words: Basophil activation. Birch. Cor a 11. Component-resolved diagnosis. Hazelnut. IgE.

■ **Resumen**

Introducción: La alergia a avellana (Corylus avellana) exhibe unos patrones de sensibilización diferentes según la edad y área geográfica que no han sido totalmente elucidadas.
Objetivos: El objetivo de este estudio fue analizar la sensibilización frente a Cor a 11 en diferentes grupos de edad en pacientes adultos y en niños con dermatitis atópica (DA) sensibilizados a avellana procedentes de un área geográfica endémica
Métodos: Se cuantificó la IgE específica frente a Cor a 11 mediante ImmunoCAP en sueros procedentes de 80 pacientes alérgicos a avellana, 33 niños menores de 1 año con DA, (24 sensibilizados y 9 no sensibilizados a avellana), 32 controles sanos y 29 alérgicos a polen y tolerantes a avellana. Además se evaluó la IgE específica frente a Cor a 1.01, Cor a 1.04, Cor a 8, y Cor a 9 mediante microarray ISAC.
Resultados: Cuarenta pacientes (22 niños preescolares, 10 escolares y 8 adultos) con reacciones sistémicas tras la ingesta de avellana, estaban sensibilizados a Cor a 11 (36%,40%,12.5% respectivamente). Cuarenta pacientes (6 preescolares, 10 escolares y 24 adultos) tenían síndrome de alergia oral, pero únicamente dos de ellos (preescolares) estaban sensibilizados a Cor a 11. Dos (8%) de los niños con DA sensibilizados a avellana mostraban reactividad a Cor a 11. Esta reactividad no se observó en ningún niño con DA sin sensibilización a avellana, o en los pacientes alérgicos a polen de abedul sin alergia a avellana o en los controles sanos.
Conclusiones: En conclusión la sensibilización a Cor a 11 se encuentra de forma predominante en niños con alergia grave a avellana, en una población endémica de abedul y es consistente con las observaciones concernientes a la sensibilización a Cor a 9.

Palabras clave: Activación de basófilos. Abedul. Cor a 11. Diagnóstico por componentes. Avellana. IgE.

Introduction

Hazelnut constitutes an important food allergy in children and adults [1-3] with geographic and age-related variations in clinical severity according to the sensitization profile of the patient [4-6]. In birch-endemic regions, hazelnut allergy is predominantly observed in adults with oral allergy syndrome (OAS) due to cross-reactivity between Cor a 1.04 and Bet v 1, the major allergen from birch (*Betula verrucosa*) pollen [4-8]. The more severe, systemic reactions seen in adults from Mediterranean regions and children from birch-endemic regions are generally not associated with pollen allergy and are frequently related to sensitization to the hazelnut lipid transfer protein (LTP) Cor a 8 [6,8-10] or the 11S legumin-like seed-storage protein Cor a 9 [4,6,11]. Sensitization to Cor a 8 and Cor a 9 can also occur in young children [10,12].

However, 2 studies have shown that sensitization patterns remain to be elucidated in 40% of children with severe pollen-unrelated hazelnut allergy [4] and infants with atopic dermatitis (AD) sensitized to hazelnut [12]. Recently, the hazelnut 2S albumin Cor a 14 [8,13], the 7S vicilin-like protein Cor a 11 [6,14], and oleosin [15] were also identified as allergenic components. Data on the prevalence and clinical relevance of sensitization to Cor a 11 are divergent and restricted to adults with OAS [6,14].

The aim of this study was to establish sensitization rates to Cor a 11 in children and adults with hazelnut allergy and in infants with AD sensitized to hazelnut in birch-endemic regions.

Materials and Methods

Study Participants

A total of 174 individuals were studied. Eighty consecutive patients with a compelling history of immediate allergic symptoms upon consumption of raw or processed hazelnut were enrolled and stratified into 6 groups according to both age (0-6 years, 7-18 years, and >18 years) and clinical severity (OAS or systemic reactions) [4].

To assess possible differences in sensitization patterns between patients with genuine hazelnut allergy and those with mere sensitization to hazelnut, 29 patients with documented birch pollen allergy who did not develop symptoms on eating hazelnut were also studied, as was a control group of 27 nonallergic, nonsensitized age-matched individuals.

Sera from 33 infants under the age of 1 year with AD and sensitization to cow's milk, hen's egg, wheat, soy, potato, or peanut were selected as described elsewhere [12]. Twenty-four of the infants (17 males; median age, 9 months [range, 4-11 months]) showed specific immunoglobulin (sIg) E reactivity to hazelnut by Cor a 1.04-spiked ImmunoCAP. The remaining 9 infants (all male; median age 6.5 months [range, 5-11 months]) had negative IgE to hazelnut. Additionally, sera of 5 healthy control infants (4 males; median age, 8 months [range, 5-11 months]) without AD or sensitization to any of the above-mentioned food allergens were studied.

All the patients and controls, or their legal guardians, completed a standardized questionnaire about symptoms following the consumption of hazelnut or hazelnut-containing foods. Challenges were not conducted in patients with systemic reactions due to the severity of the reported symptoms and the consequential potential risk of eliciting serious reactions [6,11,16]. In OAS, challenges were deemed unnecessary as the clinical history in such cases is highly reliable and symptoms are easily recognized and described by the patient [17].

The local ethics committee approved the study and all the participants (or their legal representatives) gave their informed consent in accordance with the Declaration of Helsinki.

Purification of Cor a 11

Nine different brands of hazelnut were purchased in local supermarkets; eight brands were raw, non-roasted hazelnuts and one was roasted. A hazelnut mixture was made by taking equal amounts from each brand. The hazelnuts were frozen with liquid nitrogen and ground first with a blender (Moulinex) and then with an Ultra Turrax t25 (IKA). The hazelnut vicilin Cor a 11 was isolated using a modification [18] of the original protocol described by Rigby et al [19]. The purity of Cor a 11 was assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and gel filtration chromatography.

Total and Specific IgE

Singleplexed Assay

Total IgE and sIgE to the Cor a 1.04-supplemented hazelnut extract and bromelain were quantified according to the manufacturer's instructions (FEIA ImmunoCAP, Phadia). The ImmunoCAP bromelain was used as a marker for sensitization to MUXF cross-reactive carbohydrate determinants (CCDs).

Purified nCor a 11 was coupled to ImmunoCAPs by ThermoFisher Phadia. Allergen-specific IgE levels of 0.10 kU_A/L or higher were regarded as positive (Table 1).

Multiplexed Assay (CRD Microarray)

An allergen microarray immunoassay containing 103 components (ImmunoCAP ISAC, Phadia) to determine sensitization profiles was employed as described previously [4]. Results were expressed as ISAC Standardized Units (ISU/L) and values of greater than 0 were regarded as positive. Recombinant components are denoted by 'r' and natural purified proteins by 'n'.

Basophil Activation Test

The basophil activation test (BAT) is described elsewhere [4]. Briefly, blood samples were stimulated with 4 concentrations of purified Cor a 11 (0.001, 0.01, 0.1, and 1 μg/mL) and a positive control (anti-human IgE, 10 μg/mL, BD Biosciences) or buffer (Gibco, Invitrogen) to measure spontaneous CD63 expression. Analysis of basophil activation was performed using side scatter, CD123+ and HLA-DR- to gate out the basophils. Within this gate, the percentage of activated basophils, ie, those expressing CD63, was measured. Results were expressed as percentages of CD63+ basophils after subtraction of the negative control value.

Table 1. Characteristics of Children and Adults Stratified by Clinical Manifestations and Age.

		No.	Age, y			Total IgE, kU$_A$/L			IgE HZN, kU$_A$/L			IgE Cor a 11, kU$_A$/L		
			Median	Range	P[a]	Median	Range	P[a]	Median	Range	P[a]	Median	Range	P[a]
HZN+SR+	Preschool	22	2	(0-4)	.001[b]	341	(12-4347)	.02[b]	24.5	(<0.10->100)	<.001[b]	<0.10	(<0.10-0.67)	.01[b]
	School	10	10	(7-18)	–	559	(86-5054)	.009[b]	25.7	(1.72->100)	<.001[b]	<0.10	(<0.10-7.98)	.003[b]
	Adults	8	27.5	(24-44)	–	339	(64-1747)	<.001[b]	9.2	(<0.10-37.00)	<.001[b]	<0.10	(<0.10-0.15)	–
	Total	40												
HZN+OAS	Preschool	6	4.5	(2-6)	–	415	(25-4751)	.03[b]	40	(<0.10->100)	.004[b]	<0.10	(<0.10-0.22)	–
	+School	10	11.5	(7-16)	–	434	(43-2168)	.004[b]	54.8	(1.49->100)	<.001[b]	<0.10	(all<0.10)	–
	Adults	24	34	(19-63)	–	197	(9-679)	<.001[b]	11.75	(<0.10->100)	<.001[b]	<0.10	(all<0.10)	–
	Total	40												
HC	Preschool	5	5	(3-6)		16.1	(16-278)		<0.10	(all <0.10)		<0.10	(all<0.10)	
	School	13	10	(8-13)		143	(6-436)		<0.10	(all <0.10)		<0.10	(all<0.10)	
	Adults	9	35	(21-56)		32	(2-73)		<0.10	(all <0.10)		<0.10	(all<0.10)	
	Total	27												
HZN-BP+	Preschool	10	4	(2-6)	–	196	(16-1294)	–	12.27	(0.39->100)	.001[b]	<0.10	(all<0.10)	–
	School	9	12	(7-15)	–	333	(88-1758)	.02[b]	8.9	(0.34-86.70)	<.001[b]	<0.10	(all<0.10)	–
	Adults	10	33.5	(21-53)	–	199	(55-2408)	<.001[b]	4.7	(0.14-56.00)	<.001[b]	<0.10	(all<0.10)	–
	Total	29												
			Age, mo											
AD HZN+		24	9	(4-11)	–	223	(7-2135)	<.001[b]	5.65	(0.43->100)	<.001[b]	<0.10	(<0.10-0.42)	<.001[b]
AD HZN-		9	6.5	(5-11)	–	25	(15-396)	–	<0.10	(<0.10-0.17)	–	<0.10	(all<0.10)	–
HC		5	8	(5-11)		10	(5-17)		0.17	(0.17-0.23)		<0.10	(all<0.10)	
	Total	38												

Abbreviations: AD HZN+, infants under 1 year of age with atopic dermatitis and positive sIgE to hazelnut; AD HZN-, infants under 1 year of age with atopic dermatitis and negative sIgE to hazelnut; HC, healthy controls; HZN, hazelnut; HZN-BP+, patients allergic to birch pollen but tolerant of hazelnut; HZN+OAS+, hazelnut-allergic patients with oral allergy syndrome; HZN+SR+, hazelnut-allergic patients with systemic reactions; sIgE. Specific immunoglobulin E.
[a]P values denote statistical significance between patients and controls (Mann-Whitney U test).
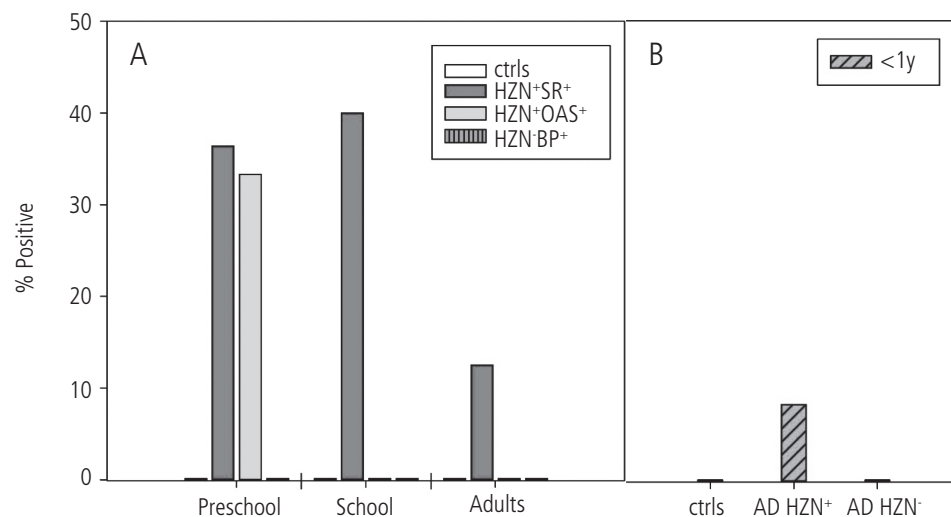[b]Statistically significant.

Figure 1. A, Sensitization (%) to Cor a 11 in healthy controls (ctrls), patients with a systemic reaction on consumption of hazelnut (HZN⁺SR⁺), hazelnut-allergic patients with OAS only (HZN⁺OAS⁺), and patients tolerant of hazelnut but allergic to birch pollen (HZN⁻BP⁺). These groups were stratified into 3 age categories (preschool 0-6 y, 7-18 y, and >18 y). B, Sensitization (%) to Cor a 11 in healthy control infants, infants with atopic dermatitis sensitized to hazelnut (AD HZN⁺), and not sensitized to hazelnut (AD HZN⁻). All these infants were under 1 year of age. For absolute numbers see Table 1. χ^2 P values <.05 for bars higher than 10% (controls vs patients).

Statistical Analysis

Results were expressed as median values (range). Nonparametric tests were used where appropriate and frequencies were compared using χ^2 analysis. IBM SPSS 17 (IBM) software was used for data analysis. A P value of less than .05 was regarded as statistically significant.

Results

Age and IgE Levels

Table 1 summarizes the age of the patients, total IgE levels, and singleplexed sIgE levels to the Cor a 1.04-spiked hazelnut and nCor a 11.

Sensitization to nCor a 11

Figure 1 displays the percentages of sensitization to nCor a 11. The most interesting observations were that 8 (36%) of 22 preschool children and 4 (40%) of 10 schoolchildren with systemic reactions after consumption of hazelnut were sensitized to nCor a 11. By contrast, sensitization to nCor a 11 was seen only in 1 of the 8 adults with systemic reactions to hazelnut and in 2 of the 6 preschool children, but not in the school-aged children or adults with OAS. None of the birch-pollen allergic patients without hazelnut allergy or the healthy controls showed IgE reactivity to nCor a 11 (Figure 1A).

Figure 1B shows that only 2 (8%) of the 24 infants with AD with a positive sIgE to hazelnut showed sIgE reactivity to nCor a 11. None of the infants with AD without sensitization to hazelnut or the healthy control infants displayed sensitization to nCor a 11.

Table 2 shows the demographic and clinical data and relevant sIgE results for the 17 patients sensitized to nCor a 11. It should be noted that 1 of the children with hazelnut allergy in whom diagnosis was confirmed by skin testing showed a negative sIgE result to the nCor a 1.04-spiked hazelnut ImmunoCAP. As can be seen in Table 2, this child had generalized urticaria and angioedema and was monosensitized to nCor a 11.
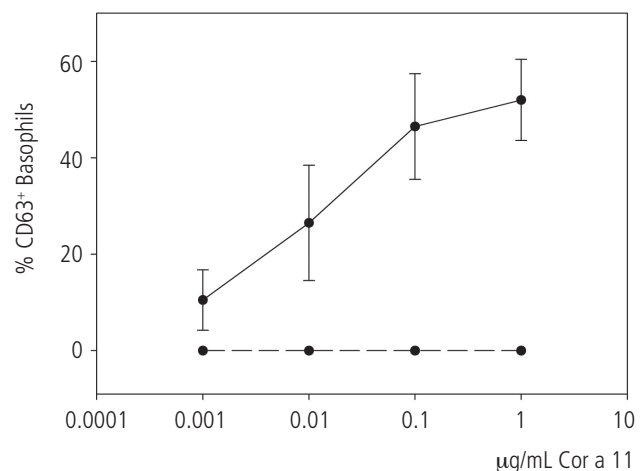

Figure 2. Percentages of CD63⁺ basophils after stimulation with 4 concentrations of Cor a 11 in 8 patients with hazelnut allergy sensitized to Cor a 11 (black line) and in 8 control individuals. Five of them were allergic to hazelnut but not sensitized to Cor a 11 and the other 3 were hazelnut tolerant (dashed line). Results are expressed as the percentage of CD63⁺ basophils (mean [SEM]).

Table 2. Characteristics of Patients Allergic to Hazelnut and Sensitized to Cor a 11.

Patient	Age, y[a]	Sex	Symptoms[b]	Grass Pollen Allergy[c]	ImmunoCAP, kU$_A$/L				ISAC, ISU/L			
					Total IgE	HZN	Cor a 11	Bromelain	Cor a 104	Cor a 1.0 1	Cor a 8	Cor a 9
1	7 (mo)	M	C	–	56	0.45	0.29	–	0	0	0	0
2	7 (mo)	M	AD	–	2135	100	0.19	–	0	0	0	22.85
3	9 (mo)	M	AD	–	1858	100	0.42	–	0	0	0	13.22
4	2	M	A, OAS	+	2007	100	0.13	–	0	0	1.01	0.86
5	2	F	C	–	12	<0.10	0.11	–	0	0	0	0
6	3	M	C, R	–	808	46.6	0.12	–	1.91	0	0	1.13
7	3	F	A, C	+	567	44.3	0.20	–	0	0	0	0.56
8	3	M	OAS	+	83	41.5	0.22	–	18.3	3.35	6.52	0
9	4	M	A, AD	+	4347	>100	0.67	–	13.39	0	0	3.38
10	4	M	GI, R	+	1715	>100	0.37	–	16.95	11.18	0	1.77
11	4	M	C, OAS	–	687	23.4	0.19	–	0	0	0	1.68
12	6	M	OAS	+	4751	97.7	0.12	0.67	10.07	16.05	0	0
13	7	M	A, C, R	+	3561	>100	7.98	–	0	0	8.98	13.75
14	10	M	GI	+	1370	25.7	0.12	–	0	49.88	0	0.64
15	13	M	R	+	5045	100	0.22	0.74	33.08	0	0	1.3
16	13	F	A	+	525	38.9	0.46	–	2.6	0	0	0.87
17	27	M	R	–	1747	37	0.15	–	0	0	0	2.15

Abbreviations: +, positive; –, negative; A, angioedema; AD, atopic dermatitis; C, immediate cutaneous symptoms; F, female; GI, gastrointestinal symptoms; Ig, immunoglobulin; M, male; OAS, oral allergy syndrome; R, respiratory symptoms.
[a]Age is displayed in years, except for patients 1-3, in whom it is shown in months.
[b]Clinical symptoms following consumption of hazelnut.
[c]Grass pollen allergy according to history and ISAC results for genuine grass pollen components.

Sensitization to Cross-Reactive Carbohydrate Determinants

Seroreactivity to the glycosylated purified Cor a 11 could result from sensitization to carbohydrate determinants (CCDs), particularly grass pollen CCD [20]. Based on the data shown in Table 2, 10 (59%) of the 17 patients sensitized to Cor a 11 had grass pollen allergy (according to clinical history and seroreactivity to the recombinant grass pollen specific markers Phl p (*Phleum pratensis*) 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6 and/or Phl p 11 spotted on the ISAC). Only 2 of these 17 patients had evidence of sensitization to CCDs [20].

BAT With Purified Cor a 11

Figure 2 shows a dose-dependent upregulation of CD63 upon stimulation of basophils with purified Cor a 11 in hazelnut-allergic children sensitized to nCor a 11 (n=8). No such upregulation was seen in 5 hazelnut-allergic patients without sensitization to nCor a 11 or in 3 healthy, hazelnut-tolerant controls.

Cor a 11 Compared With Cor a 9

Figure 3 shows the percentages of sensitization to nCor a 11 and nCor a 9 in the different patient groups. Sensitization to nCor a 11 and nCor a 9 was mainly seen in children with systemic reactions but not in those with OAS. Sensitization to nCor a 11, by contrast, was hardly present in infants with AD.

Discussion

The most interesting and novel observation of this study is that sensitization to Cor a 11 in our birch-endemic region was seen mainly in young children who developed more severe clinical symptoms following the consumption of hazelnut. Indeed, sensitization to Cor a 11 was 3 to 4 times more prevalent in preschool and schoolchildren than in adults, and was most common in patients with systemic reactions. Our findings in this respect clearly differ from those of Lauer et al [14], who described IgE-binding to Cor a 11 in almost half of the adult population with hazelnut-related OAS tested in a birch-endemic region. The exact reason or reasons for this discrepancy remain elusive but they might be related to the use of different IgE assays. By contrast, our data are consistent with observations by Hansen et al [6], who, using a similar ImmunoCAP technique, were unable to confirm sensitization to Cor a 11 in hazelnut-allergic adults in a birch-endemic region.
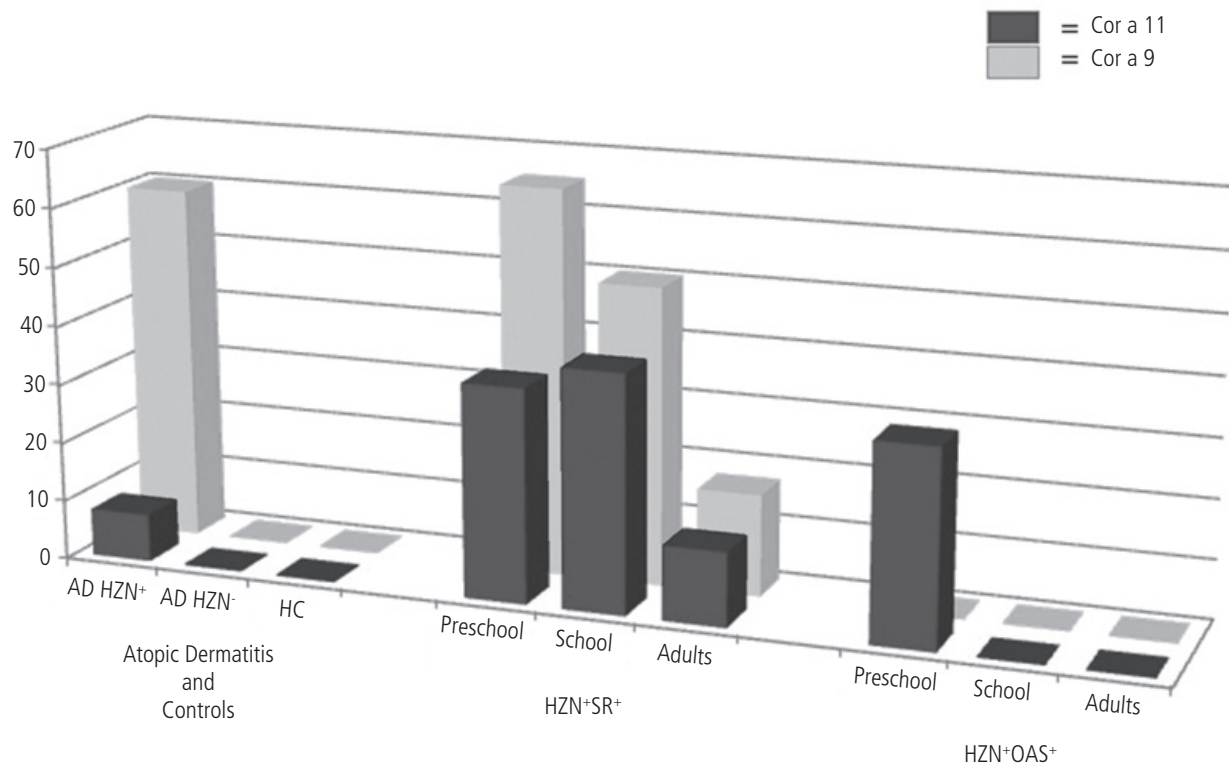
Figure 3. Comparison of sensitization (%) to Cor a 11 and Cor a 9 in a birch-endemic region in different groups stratified by age and severity of clinical reactions following consumption of hazelnut-containing foods: patients <1 year of age with atopic dermatitis sensitized to hazelnut (AD HZN+) and not sensitized to hazelnut (AD HZN-); healthy controls (HC); patients with systemic reactions following consumption of hazelnut-containing foods (HZN+SR+) or with mere OAS (HZN+OAS+) divided into 3 groups (preschool, schoolchildren, and adults). For absolute numbers see Table 1.

It is not clear when or through which route or routes children become sensitized to Cor a 11. We therefore extended our study by including infants under the age of 1 year with AD, since this condition is frequently associated with food sensitization. However, there were practically no cases of sensitization to Cor a 11 in this population. Therefore, it appears that sensitization to vicilins starts at a later age than sensitization to Cor a 9 and probably results from 1 or more routes of exposure other than in utero sensitization or sensitization through breastfeeding or inhalation (eg, oral consumption) [12].

Interestingly, sensitization to Cor a 11 in older patients with overt hazelnut allergy, seemed to be generally associated with sensitization to Cor a 9, as 13 of the 17 Cor a 11-positive patients in our series also had IgE reactivity to Cor a 9.

A potential criticism of our study, in the absence of double-blind, placebo-controlled challenges with hazelnut, could be that we did not directly assess the clinical relevance of the in vitro sIgE observations. However, a positive challenge with whole hazelnut does not offer absolute proof that Cor a 11 is the offending component. Therefore, in order to further and safely investigate the clinical relevance of anti-nCor a 11 sIgE antibodies, we performed flow-assisted analysis of nCor a 11–activated basophils. Our findings show that these antibodies,

even in low titers, can trigger significant activation of basophils in an assay that closely resembles the in vivo pathway that leads to the symptoms of an allergic reaction.

Another criticism of our results could be that our sensitization data for the purified Cor a 11 are the result of sensitization to CCD, as Cor a 11 is glycosylated. This would probably indicate clinically irrelevant sensitization. However, only 1 out of 10 patients in our series were sensitized to CCD.

The present study adds to the observation that hazelnut allergy exhibits age-related variations in the severity of symptoms according to the sensitization profile of the patient. We have shown that sensitization to Cor a 11 in a birch-endemic region predominantly occurs in children with severe hazelnut allergy and in preschool children with OAS, but is absent in adults with OAS related to their underlying birch pollen allergy. Moreover, our findings on sensitization to Cor a 11 are largely consistent with prior observations of sensitization to Cor a 9, another member of the cupin superfamily. In our study, however, sensitization to Cor a 9 was absent in patients with OAS and onset of sensitization seemed to start earlier in life, probably as a result of an alternative route or alternative routes of exposure.

Acknowledgements

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