

Component-resolved *in vitro* diagnosis of hazelnut allergy in Europe

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Background: Food allergy to hazelnut occurs both with and without concomitant pollen allergy.

Objective: We sought to evaluate a panel of hazelnut allergens for diagnosis of hazelnut allergy in Spain, Switzerland, and Denmark.

Methods: Fifty-two patients with a positive double-blind, placebo-controlled food challenge result with hazelnuts; 5 patients with a history of anaphylaxis; 62 patients with pollen allergy but hazelnut tolerance; and 63 nonatopic control subjects were included. Serum IgE levels to hazelnut extract, recombinant hazelnut allergens (rCor a 1.04, rCor a 2, rCor a 8,

rCor a 11), and native allergens (nCor a 9, nCor a Bd8K, nCor a Bd11K) were analyzed by means of ImmunoCAP.

Results: Among patients with hazelnut allergy, 91% (Switzerland/Spain, 100%; Denmark, 75%) had IgE to hazelnut extract, 75% to rCor a 1.04, 42% to rCor a 2, 28% to rCor a 8, and 2% to rCor a 11. The highest rate of sensitization to Cor a 1.04 was found in the northern regions (Switzerland/Denmark, 100%; Spain, 18%), whereas IgE to the lipid transfer protein rCor a 8 prevailed in Spain (Spain, 71%; Switzerland, 15%; Denmark, 5%). IgE to profilin rCor a 2 was equally distributed (40% to 45%). Among control subjects with pollen allergy, 61% had IgE to hazelnut extract, 69% to rCor a 1.04, 34% to rCor a 2, 10% to rCor a 8, and 6% to rCor a 11.

Conclusion: Component-resolved *in vitro* analyses revealed substantial differences in IgE profiles of hazelnut allergic and hazelnut tolerant patients across Europe. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Birch pollen, Cor a 1, double-blind, placebo-controlled food challenge, food allergy, hazelnut, IgE, lipid transfer protein, recombinant allergens

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Tree nuts are common causes of food allergy in Europe and occasionally of severe systemic reactions.¹⁻⁵ Hazelnut allergy is seen both with and without relation to pollen allergy. There seems to be geographic variation regarding allergen recognition and severity of symptoms in patients with plant food allergy.⁵⁻⁸ The literature suggests different routes of sensitization between pollen-related plant food allergy and plant food allergy in patients without pollinosis.^{9,10} In patients with hazelnut allergy but no pollen allergy, the sensitization to the plant food is assumed to take place through ingestion of the food (ie, the oral route). In patients with pollen-related hazelnut allergy, the sensitization is more likely to occur through inhalation of pollen allergens and subsequent development of food-induced allergic symptoms caused by cross-reactivity between IgE raised against pollen allergens with homologous proteins in plant foods.

Evaluation of a carefully recorded case history and assessment of food sensitization are cornerstones in the diagnosis of food allergy for most patients. In the absence of detectable sensitization with a highly sensitive method, IgE-mediated allergy to the specific food investigated can be ruled out with a high level of certainty. However, the diagnostic set-up, including skin tests and determination of serum specific IgE is insufficient to distinguish between symptomatic food allergy and mere sensitization regarding most food allergen systems, especially in the case of pollen-related food allergy, and often fail to predict the severity of

Abbreviations used

CCD: Cross-reactive carbohydrate determinant
 DBPCFC: Double-blind, placebo-controlled food challenge
 LTP: Lipid transfer protein
 OAS: Oral allergy syndrome
 SPT: Skin prick test

symptoms.¹¹⁻¹⁶ Food challenge is considered the gold standard in the diagnosis of food allergy,¹⁷ but it is still of great interest to improve the performance of IgE measurements.

According to recent literature, component-resolved IgE analyses seem to improve the diagnostic efficacy for some plant food allergies, and further results suggest differences in IgE patterns related to clinical reactivity.^{5,10,18-20} A number of different IgE-binding components from hazelnut have been proposed as candidates for both sensitization and elicitation of allergic reactions. Some of the most intensively studied molecules during the last decade are the Bet v 1 homologue Cor a 1,²¹ the hazelnut profilin Cor a 2,²² and the lipid transfer protein (LTP) Cor a 8.^{5,23} Other molecules that have been investigated in connection with hazelnut allergy are the 11S globulin Cor a 9,²⁴ the vicilin Cor a 11,²⁵ hazelnut oleosin,²⁶ and the specific carbohydrate structures, so-called cross-reactive carbohydrate determinants (CCDs), for which bromelain has been used as source.^{25,27,28} The clinical importance of the latter structures is yet uncertain.

The aim of this study was to evaluate the use of a panel of native and recombinant hazelnut allergens for IgE analyses in 3 subsets of individuals: patients with hazelnut allergy, patients with birch or olive pollen allergy without allergy to hazelnut, and nonatopic control subjects. Furthermore, we wanted to compare the pattern of sensitization in patients with hazelnut allergy by means of specific IgE measurement among 3 different climatic regions of Europe represented by Spain, Switzerland, and Denmark, respectively.

METHODS**Design**

This was a multicenter study with participation of 3 clinical centers in Madrid, Spain; Zurich, Switzerland; and Copenhagen, Denmark. Further partners were the Division of Allergology at the Paul-Ehrlich-Institut in Langen, Germany, and the Department of Research and Development at Phadia in Uppsala, Sweden.

The clinical part of the study took place between 2002 and 2004. The study was approved by the local ethic committees, and all patients provided written informed consent before entering the study.

Results from patients with hazelnut allergy were compared with results from a group of pollen-sensitized but hazelnut-tolerant patients to determine the specificity and sensitivity of the tests and to assess the degree of cross-reactivity with homologous pollen allergens without a corresponding clinical response. Furthermore, nonallergic nonsensitized persons were included as control subjects.

Subjects

Patients and control subjects were recruited in Spain, Switzerland, and Denmark either by means of advertisement or from the participating clinics.

In total, 182 individuals were included in the study: 57 patients with hazelnut allergy, 62 patients with allergy to either birch or olive pollen but tolerance to hazelnut, and 63 nonatopic control subjects. Hazelnut allergy was confirmed by means of double-blind, placebo-controlled food challenge

(DBPCFC) for the patients with food allergy, except for 5 Spanish patients for whom the clinicians of the 3 centers unanimously accepted reliance on a medical history of severe systemic reaction on ingestion of hazelnut. All 62 control subjects with pollen allergy had a convincing history of pollinosis either to birch or olive, a positive skin prick test (SPT) response to the relevant pollen from each country, and a negative open food challenge with hazelnut. The nonatopic control subjects had no history of any allergic symptoms and a negative SPT response to a panel of inhalation and plant food allergens and were further submitted to open food challenge with raw hazelnuts to ensure tolerance.

Median age and sex were distributed as follows: patients with hazelnut allergy, 27 years (range, 14-60 years), 29 female/28 male patients; control subjects with pollen allergy, 28 years (range, 19-59 years), 30 female/32 male patient; and nonatopic control subjects, 25 years (range, 16-64 years), 39 female/24 male patients.

Food challenges

All food challenges took place out of the pollen season, and the blinded challenges were carried out as previously described.²⁹

SPTs

Birch and olive pollen extracts (Soluprick, ALK-Abelló, Hørsholm, Denmark; Stallerpoint, Stallergènes SA, Antony Cedex, France), hazelnut extract (ALK-Abelló), and raw hazelnut were used for SPTs. SPTs were carried out in accordance with European guidelines.³⁰ SPTs with raw nuts were performed by using the prick-prick technique.^{31,32} The skin wheal areas were determined by means of computer scanning.³³

Recombinant and native allergens

Recombinant hazelnut allergens were produced as hexahistidine-tagged proteins in *Escherichia coli* by using a pET vector and strain BL21, as previously described.²⁰ The proteins were purified by using immobilized metal ion affinity chromatography and size exclusion chromatography. Native hazelnut allergens were purified by means of ion exchange, hydrophobic interaction, and size exclusion chromatography; analyzed for purity by means of SDS-PAGE; identified by using N-terminal sequencing; and provisionally evaluated for IgE binding by using ImmunoCAP (Phadia, Uppsala, Sweden) and an in-house panel of sera. Details of Cor a Bd8K and Cor a Bd11K, which show similarity to vicilin-like proteins and 2S albumin, respectively, will be reported elsewhere.

Specific IgE measurements

For all patients, serum IgE levels to rBet v 1, CCDs, bromelain, and natural extracts of hazelnut, birch pollen, olive pollen, hazel pollen, timothy grass pollen, and plane pollen were measured by using regular ImmunoCAP tests. After the clinical part of the study was concluded, the original hazelnut assay (f17, Phadia) was replaced by an upgraded assay (hazelnut [f17], upgraded) spiked with rCor a 1.04,²⁰ and therefore data for both assays are included in the following.

IgE levels to recombinant and native hazelnut allergens were analyzed by using experimental ImmunoCAP tests carrying rCor a 1.04, rCor a 2, rCor a 8, rCor a 11, nCor a 9, nCor a Bd8K, and nCor a Bd11K. IgE values of 0.1 kU_A/L or greater were regarded as positive (subject sensitized), and values of less than 0.1 kU_A/L were regarded as negative (subject not sensitized). The absolute values obtained are shown in the figures. All IgE measurements were carried out with an ImmunoCAP 1000 instrument at Phadia, and 18% were performed in duplicate. Average and median coefficients of variation for replicates were 6.4% and 5.1%, respectively.

Statistics

Differences in specific IgE results between patients with positive and negative DBPCFC results were evaluated by using nonparametric methods

TABLE I. Symptoms of hazelnut allergy

		Symptoms, hazelnut allergy							
		History					DBPCFC		
	No.	Oral symptoms	Other than OAS	Respiratory	Severe	Cook/roast	Oral symptoms	Other	Severe
Denmark	20	18*	9	7	0	7	20	2	0
Switzerland	20	20	6	3	1	6	20	1	0
Spain	17	17	9	3	5†	17	12‡	3	0

*The history for 2 patients was unknown.

†No DBPCFCs were performed for 5 patients because of anaphylaxis.

TABLE II. IgE (≥ 0.1 kU_A/L), hazelnut allergens

Allergen/extract	Patients with hazelnut allergy (%)				Control subjects with pollen allergy (%)			
	All (n = 57)	Denmark (n = 20)	Switzerland (n = 20)	Spain (n = 17)	All (n = 62)	Denmark (n = 21)	Switzerland (n = 21)	Spain (n = 20)
Hazelnut extract (f17)	91*	75	100*,†	100*,‡	61	48	71	65
rCor a 1.04	75	100*,‡	100§	18	69	81	95†,§	30
rCor a 2	42	45*	40	41	34	14	43†	45‡
rCor a 8	28*	5	15	71*,‡,§	10	0	0	30‡,§
nCor a 9	30	20	25	47	27	19	14	50‡,§
rCor a 11	2	0	0	6	6	0	5	15
nCor a Bd8K	25	10	20	47‡	24	14	14	45‡,§
nCor a Bd11K	30	10	20	65‡,§	26	14	10	55‡,§
Bromelain	51	40	45	71	53	29	57	75‡
CCDs	25	15	35	24	35	19	33	55‡
Hazelnut extract (f17), upgraded	100*	100*	100	100*	81	81‡	100†,§	60
rCor a 1.04 + rCor a 8	96*	100*,‡	100§	88*	77	86‡	100†,§	55

The †, ‡, and § symbols mark significant differences between countries within patient groups:

*Shows significant differences (χ^2 test, $P < .05$) between patients with hazelnut allergy and control subjects with pollen allergy.

†Denmark versus Switzerland

‡Denmark versus Spain

§Switzerland versus Spain. The symbols/letters are placed at the highest value.

(Mann-Whitney rank-sum and χ^2 tests). The applied statistical program was the SigmaStat statistical software system (Systat Software, GmbH, Erkrath, Germany).

The sensitivity and specificity of selected IgE assays were evaluated. Sensitivity is defined as the fraction of patients with hazelnut allergy with a positive test result, and specificity is defined as the fraction of control subjects with pollen allergy with a negative test result.

RESULTS

Clinical history and food challenge

Table I shows the clinical reactivity evaluated based on history and during DBPCFCs. Five Spanish patients were not subjected to DBPCFCs because of previous severe systemic reactions on ingestion of hazelnuts. Moreover, all Spanish patients reported symptoms on ingestion of cooked or roasted hazelnuts.

Two Danish patients did not remember having any symptoms on ingestion of hazelnuts but had positive open challenge results with hazelnut and subsequently also on DBPCFCs.

All patients reported oral allergy syndrome (OAS) or other mild symptoms in combination with oral symptoms, and relatively few had more severe symptoms.

Among the 7 Spanish patients with either severe systemic symptoms or respiratory symptoms, 5 had IgE to LTP but only 1 at a high value (14.9 kU_A/L). All Spanish patients reported symptoms to roasted or cooked nuts compared with 14 (35%) of those

from Denmark and Switzerland. Fewer patients reported reactions to roasted than to raw nuts, oral symptoms were reported by all symptomatic patients, and anaphylaxis was most commonly caused by raw nuts. The level of IgE to LTP in patients with severe systemic reactions or respiratory symptoms did not differ significantly from those seen in the rest of the Spanish patients. None of the Swiss or Danish patients with severe symptoms based on history or symptoms other than OAS had IgE to LTP. Six control subjects with pollen allergy and 1 nonatopic control subject, all from Spain, had IgE to LTP.

Allergic symptoms during DBPCFCs were generally less severe than those reported based on history, and OAS was the most common symptom. One Danish patient had rhinoconjunctivitis, and another had conjunctivitis alone. One Swiss patient had conjunctivitis, and 2 Spanish patients had milder gastrointestinal discomfort. None of the patients in either country had asthmatic symptoms or anaphylaxis during food challenges.

Sensitization to hazelnut allergens

Table II summarizes the IgE results for both hazelnut allergic patients and control subjects with pollen allergy. In the total group of patients with hazelnut allergy versus control subjects with pollen allergy, significantly more patients had IgE against the hazelnut extract (f17), rCor a 8 and hazelnut extract (f17), upgraded. There were statistically significant differences between the

number of patients with hazelnut allergy and control subjects with pollen allergy reacting to rCor a 1.04 and rCor a 2 in Denmark and to the hazelnut extract in both Switzerland and Spain, and sensitization to rCor a 8 was clearly more frequent among patients with hazelnut allergy in Spain. Interestingly, 2 of the Spanish patients with more significant reactions to hazelnut displayed high-level sensitization to rCor a 1.04 while having negative test results for rCor a 8. Spanish patients with hazelnut allergy were more frequently sensitized to nCor a 9, nCor a Bd8K, and nCor a Bd11K, whereas sensitization against rCor a 1.04 was most common in Danish and Swiss patients with hazelnut allergy. All IgE responses to nCor a 9, rCor a 11, nCor a Bd8K, and nCor a Bd11K were of very low magnitude.

For the control subjects with pollen allergy, IgE against rCor a 2 was less common in Denmark than in the other countries, and IgE to nCor a 9, bromelain, and CCDs was most frequently seen in Spain. IgE to nCor a Bd8K and nCor a Bd11K was most frequent in Spanish patients.

Overall, there were no differences between patients with hazelnut allergy and control subjects with pollen allergy regarding the native allergens.

The combination of rCor a 1.04 and rCor a 8 is shown in the last row of Table II. In the total group of patients with hazelnut allergy, in patients from Denmark, and in patients from Spain, significantly more had IgE against 1 or 2 of these allergens compared with the control subjects with pollen allergy. Among patients with hazelnut allergy, significantly fewer in Spain versus Denmark and Switzerland had IgE. The same picture was found in the pollen control group, but here Denmark and Switzerland differed significantly, with most subjects reacting in Switzerland.

Fig 1 shows the IgE measurements for all hazelnut allergens tested. Data for patients with hazelnut allergy and control subjects with pollen allergy are shown both as geographically merged and separate groups.

In patients from both Denmark and Switzerland, the median level of IgE to hazelnut extract differed significantly between patients with hazelnut allergy and control subjects with pollen allergy (27-fold and 3.8-fold, respectively). For rCor a 1.04, the difference was 25-fold in patients from Denmark and 3.5-fold in those from Switzerland. In patients from Spain, the median level of IgE to hazelnut extract was 2.8 times higher in the group of patients with hazelnut allergy than among the control subjects with pollen allergy, and for rCor a 8, the difference was 24-fold. Interestingly, with respect to the magnitude of specific IgE, none of the other allergen components showed the same discrimination between patients with hazelnut allergy and control subjects with pollen allergy. In fact, rCor a 2 in particular and to some extent also bromelain and CCD showed a tendency in the opposite direction. The levels of specific IgE to the native hazelnut allergens tested were mostly very low, exceeding 1 kU_A/L in only 1 of the allergic subjects.

For the control subjects with pollen allergy, IgE against rCor a 2 was less common in patients from Denmark than in those from the other countries, and IgE to nCor a 9, bromelain, and CCDs was most frequently seen in patients from Spain. Six control subjects with pollen allergy and 1 nonatopic control subject, all from Spain, had detectable IgE to rCor a 8.

In Table E1 (available in this article's Online Repository at www.jacionline.org) the data for the nonatopic control subjects are shown. Only a few control subjects had IgE to the individual

hazelnut allergens and in a very low amount (maximum value for the hazelnut extract, 3.5 kU_A/L; for rCor a, 1.04; and for Cor a 8, 0.2 kU_A/L).

Pollen sensitization

For more information on pollen sensitization, see Table E2 and the Results section in this article's Online Repository at www.jacionline.org.

Sensitivity and specificity

The sensitivity and specificity are shown in Fig 2 as receiver operating characteristic curves of selected IgE assays at different cutoff levels (0.1, 0.35, 0.7, 3.5, and 17.5 kU_A/L). According to the comparisons shown in Table II, the allergens with significant geographic differences were chosen. Comparisons are made with both the worst-case control group, namely the pollen-positive, hazelnut-negative patients, and with the nonatopic control subjects. The upper left corner of the diagram represents the optimal performance of the assay. As expected, rCor a 1.04 shows the best sensitivity and specificity in Danish and Swiss patients, and rCor a 8 shows the best sensitivity and specificity in Spanish patients. For both the original hazelnut extract (f17) and the upgraded hazelnut extract (f17), the best performance was found in Swiss and Spanish patients but with a very high sensitivity in all 3 regions. As shown in the lower part of Table II, the spiked upgraded hazelnut extract (f17) had the highest sensitivity but a correspondingly lower specificity. The combination of rCor a 1.04 and rCor a 8 does not seem to improve either the sensitivity or specificity compared with the spiked extract.

DISCUSSION

To our knowledge, this study is the most comprehensive evaluation of IgE reactivity in hazelnut allergy published thus far. Several food allergen families have been taken into consideration: pathogenesis-related protein 10 (Cor a 1), profilin, pathogenesis-related protein 14 (LTP), 11 S globulin (Cor a 9), vicilin, and 2S albumin. Depending on the availability of material, either recombinant or purified native proteins were used as test antigens. All included patients and control subjects were extensively examined and characterized based on history, skin test results, and food challenge results. The patients with hazelnut allergy were submitted to DBPCFCs, and the control subjects had open food challenges to ensure tolerance. We included patients with pollen allergy as control subjects because discrimination between a relevant test result and plant food sensitization without clinical implications in patients with pollen allergy is a very common problem in daily allergology practice, and regarding performance of a diagnostic test, this setting represents the worst-case scenario.

By choosing centers with locations in the Mediterranean area, the central European region, and Scandinavia, respectively, diversity in climate and vegetation and thereby diversity in natural occurrence of pollen was ensured in the study. It is important to emphasize that this study is not an epidemiologic survey but a descriptive comparison of hazelnut allergy in patients recruited and evaluated by using the same principles from 3 different European regions. There is no doubt that local habits regarding selection of fruits, nuts, and vegetables and the amount

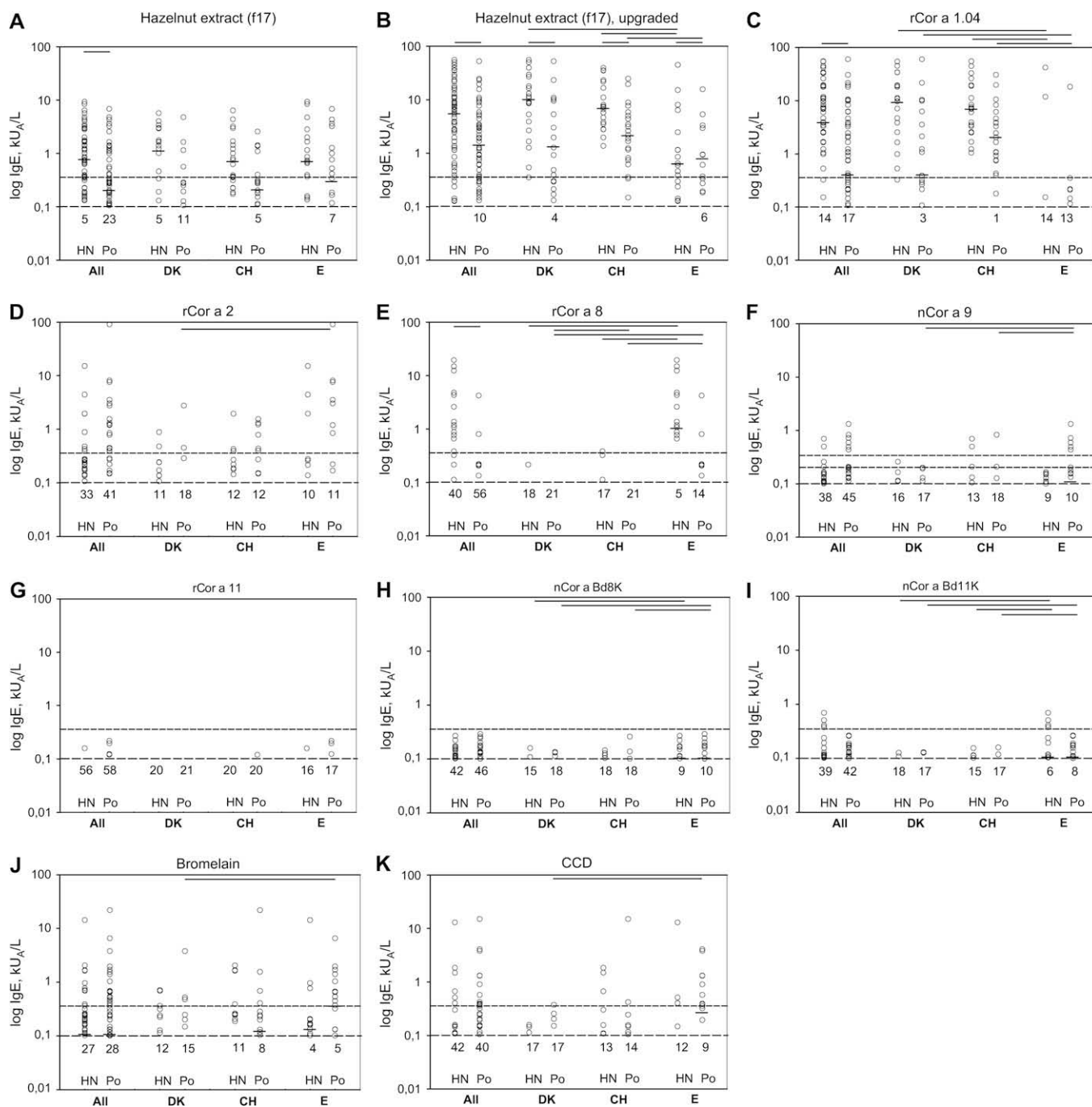


FIG 1. Level of IgE for all hazelnut allergens. The y axis represents the common log scale. Hatched lines indicate cutoff values at 0.1 and 0.35 kU_A/L . The number of values of less than 0.1 kU_A/L is indicated for each plot. The median value for each data set is marked by a short horizontal line. Lines at the top show significant differences ($P \leq .05$). HN, Patients with hazelnut allergy; Po, control subjects with pollen allergy; DK, Denmark; CH, Switzerland; E, Spain.

of plant food intake differ across Europe, but whether these differences can explain the differences in IgE reactivity is at present unknown.

It has been questioned whether the very high load of birch pollen allergens in the northern regions and thereby the high prevalence of sensitization to Bet v 1 and related plant food allergens in some way might inhibit or at least decrease the probability of primary sensitization to the other allergens in a

specific plant food (eg, LTP).³⁴ However, the immunologic mechanism behind such a phenomenon remains to be identified.

Five Spanish patients had a history of anaphylaxis, only 1 Swiss patient reported severe symptoms, and none of the included Danish patients reported severe symptoms to hazelnut. This fact, combined with the fact that all Spanish patients reported symptoms to processed hazelnuts, correlates to more common sensitization to the heat-stable LTP rCor a 8 in Spain found by other

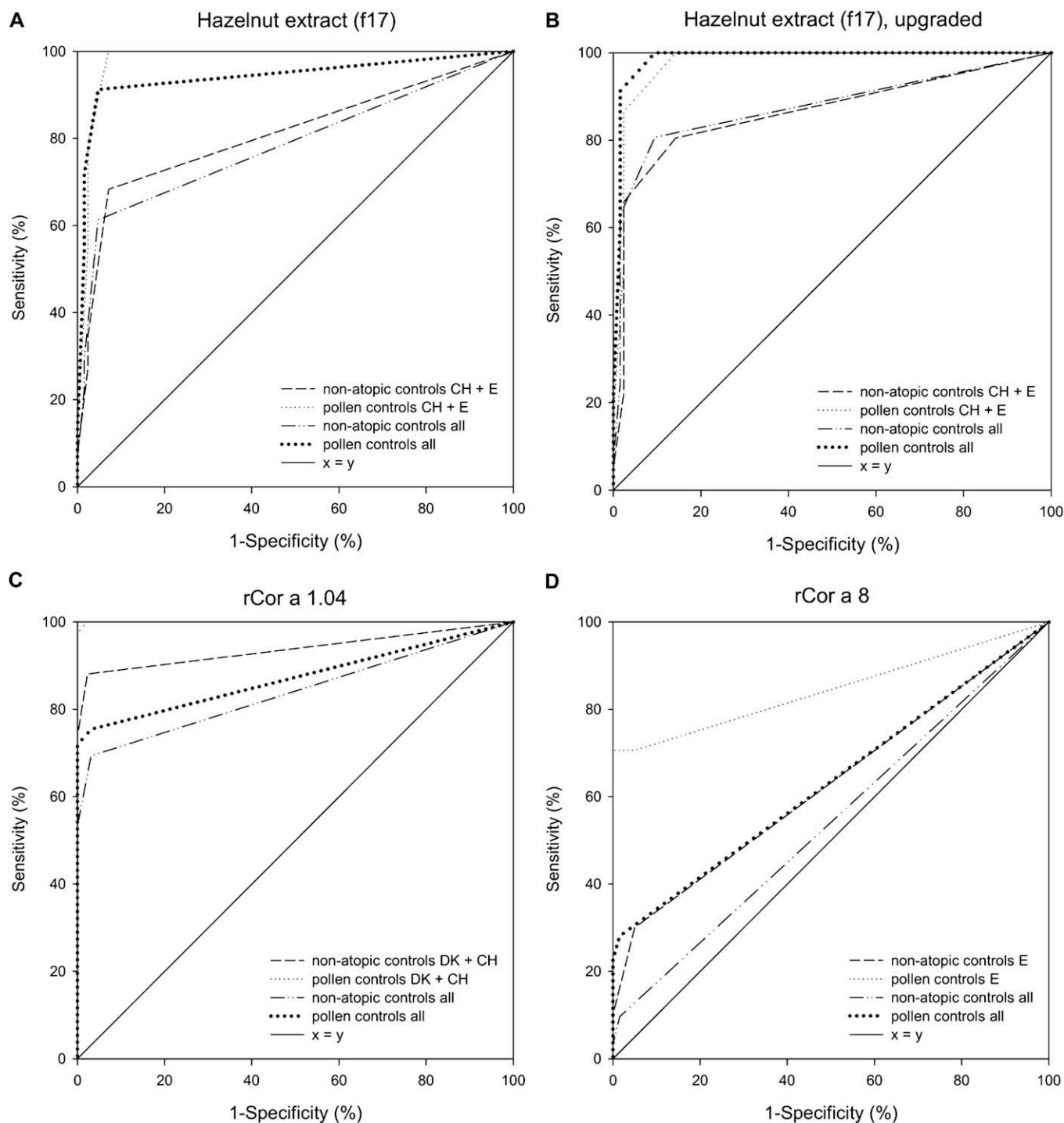


FIG 2. Receiver operating characteristic curves of sensitivity and specificity of IgE analyses at different cutoff levels: 0.1, 0.35, 0.7, 3.5, and 17.5 kU_A/L. Sensitivity is shown along the y-axis, and 1-specificity is shown along the x-axis. Dashed lines represent the nonatopic control group, and dotted lines represent the control subjects with pollen allergy. The solid line indicates $x = y$. CH, Switzerland; DK, Denmark; E, Spain.

authors. However, because one third of patients with hazelnut allergy from Denmark and Switzerland reacted to cooked or roasted hazelnuts, allergen components relevant to patients with hazelnut allergy in this region might be incompletely inactivated by heat treatment, as suggested by Hansen et al.²² Sensitization to

rCor a 1 dominated in the northern regions, thus confirming the results of previous investigations.

In accordance with previous studies, it seems that the Mediterranean area has more severe clinical cases of hazelnut allergy than the northern parts of Europe. This study demonstrates that

sensitization to hazelnut LTP definitely is more common in Spanish patients. In Switzerland and Denmark LTP sensitization was restricted to symptomatic patients with hazelnut allergy, but no indication for LTP causing severe symptoms in patients from Denmark or Switzerland was found. Neither control subjects with pollen allergy nor nonatopic control subjects from Denmark and Switzerland showed IgE to LTP, whereas 6 control subjects with pollen allergy and 1 nonatopic control subject from Spain showed IgE to LTP.

Among the patients with hazelnut allergy from all centers, severe symptoms to hazelnut were reported in 5 (31%) of 16 LTP-sensitized and in 2 (5%) of 41 non-LTP-sensitized subjects. Regarded the opposite way, LTP sensitization was present in 5 (71%) of 7 patients with severe symptoms to hazelnut and in 11 (21%) of 52 patients with milder reactions. Despite the fact that that some patients were LTP sensitized but only showed mild reactions and a few reacted severely in the absence of LTP sensitization, the results suggest an association between severe reactions to hazelnut and LTP sensitization, even if not quite as strong as that reported for certain other foods. However, given the fact that the frequency of Cor a 8 sensitization did not differ between Spanish patients with severe or mild reactions, it is possible that a separate or additional determinant of reaction severity exists, primarily occurring in the southern region.

Interestingly, a recent investigation suggested hazelnut LTP sensitization as a risk factor for objective symptoms in children from a birch-endemic area.³⁵ This is in contrast to our findings and could either be related to different patient selection or to impurities in the natural purified Cor a 8, which in our experience might be difficult to obtain entirely free from other IgE-binding proteins.

Because of the inherent regional differences in both the sensitization pattern and clinical reactivity, it proved necessary to recruit patients according to the predominant clinical manifestations in the 3 countries: pollen allergy with or without concomitant food allergy in Denmark and Switzerland and food allergy in Spain. This approach resulted in 19 of 20 patients with both hazelnut allergy and pollen allergy in Denmark and Switzerland and 15 of 17 in Spain. Clearly, there was a difference in severity of the hazelnut allergy according to history and also the level of pollen-specific IgE between the regions.

The clinical role of sensitization to profilin is probably limited, but in this material it seems that in Denmark sensitization to rCor a 2 is less likely among hazelnut-tolerant patients with pollen allergy than among patients with hazelnut allergy with positive challenge results (14%/45%, $P < .05$).

The use of rCor a 11, nCor a Bd8K, nCor a Bd11K, CCDs, and bromelain, the latter 2 being virtually identical with bromelain and more often producing values just above the cutoff level (Fig 1, J and K), did not increase the ability to discriminate between patients with hazelnut allergy and hazelnut-tolerant patients. There were, however, significant differences in the prevalence of sensitization and the level of IgE between the regions for most allergens.

Regarding sensitization to nCor a 9, of the 7 Spanish patients with more severe symptoms, 4 had IgE to nCor a 9 (data not shown). Among control subjects with pollen allergy, Cor a 9 sensitization was significantly more common in Spain than in Denmark and Switzerland. In this study there were no significant differences between the regions regarding the prevalence of sensitization to nCor a 9 among patients with hazelnut allergy and

no differences between patients with hazelnut allergy and control subjects with pollen allergy in any of the 3 countries. Therefore our results cannot with certainty confirm the link between this allergen and systemic allergic reactions, as suggested by the literature for pediatric patients.²⁴ It is still possible that Cor a 9 or other storage proteins are important to a relatively rare subgroup of patients with hazelnut allergy who are not well represented in this study. This question would need to be addressed in a study specifically designed to recruit patients with non-pollen-associated hazelnut allergy.

The use of component-resolved IgE analyses showed an excellent clinical sensitivity, but unfortunately, as an inherent problem, the ability to discriminate between patients with hazelnut allergy and hazelnut-tolerant control subjects with pollen allergy was poor. None of the investigated hazelnut allergens was exclusively correlated with severe allergic reactions. Neither did this study find a clear correlation between the level of sensitization to any of the investigated allergens and the severity of allergic reactions in any of the involved regions. The use of decision-point levels of IgE above which DBPCFC can be omitted, as published by Sampson and Ho³⁶ for selected food allergens, is currently not recommendable in pollen-sensitized subjects for the diagnosis of clinical hazelnut allergy. For safety reasons, we chose to avoid food challenges in 5 patients with a history of severe systemic reactions. However, it seems that the protocol used for blinded challenges in this study offers little risk of uncontrollable allergic reactions because none of the 52 patients who went through DBPCFCs experienced any severe symptoms during the challenges. All in all, DBPCFCs must still be considered the gold standard for the final diagnosis of hazelnut allergy.¹⁷

In conclusion, this study clearly showed differences in IgE reactivity between symptomatic patients with hazelnut allergy and hazelnut-tolerant control subjects with pollen allergy but also between different European regions. The diversity of IgE patterns between the involved regions could suggest the need for a geographically differentiated use of allergens both for diagnostic procedures and in the future also for therapeutic purposes in patients with plant food allergy.

Clinical implications: Diversity in IgE patterns suggests a need for a geographically differentiated use of allergens in the diagnosis of hazelnut allergy.

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RESULTS

Sensitization to hazelnut allergens

The data for the nonatopic control subjects are shown in Table E1.

Pollen sensitization

Table E2 shows the distribution of pollen sensitization between patient groups and countries. Data are shown as the number of positive, median, and maximum IgE values (in kilounits of allergen per liter). The data confirmed that sensitization to birch and rBet v 1 was more common in patients from Denmark and Switzerland than in those from Spain. The difference was most pronounced for rBet v 1.

Two Danish control subjects with birch pollen allergy with a convincing history of pollinosis in the birch pollen season and a positive SPT response to birch showed no specific IgE to either birch or Bet v 1. One of them had a low amount of IgE to olive and also a positive SPT response to olive. Both had Danish background.

As expected, IgE against olive pollen was less common in Denmark, but 13 of 20 patients with hazelnut allergy and 14 of 21

control subjects with pollen allergy had measurable IgE levels to olive, although in lower amounts than in seen in patients from Switzerland and Spain (data not shown).

Grass pollen sensitization (timothy) was equally distributed in percentage between countries and patient groups, but the level of IgE was significantly higher among the control subjects with pollen allergy. Plane sensitization was more common in Switzerland and Spain than in Denmark, but again it was surprising to find that of the Danish patients with hazelnut allergy and control subjects, 7 of 20 and 5 of 21 were sensitized to plane.

Sensitization to hazel pollen was equally distributed in percentage between countries and patient groups, but the level of IgE was significantly higher among patients with hazelnut allergy versus control subjects with pollen allergy in Denmark and Switzerland, and the level of IgE was higher among patients with hazelnut allergy in Denmark and Switzerland than in Spain (data not shown). The median level of IgE to hazel pollen in Spanish patients with hazelnut allergy was very low (0.5 kU_A/L) compared with approximately 20-fold higher levels in Denmark and Switzerland.

TABLE E1. IgE (≥ 0.1 kU_A/L), hazelnut allergens, nonatopic control subjects

Allergen/extract	Nonatopic control subjects				Maximum value IgE (kU _A /L)
	All (n = 63)	Denmark (n = 21)	Switzerland (n = 22)	Spain (n = 20)	
Hazelnut extract (f17)	3	0	1	2	3.5
rCor a 1.04	2	0	1	1	0.2
rCor a 2	1	0	1	0	0.9
rCor a 8	1	0	0	1	0.2
rCor a 9	2	0	1	1	1.8
rCor a 11	0	0	0	0	0
nCor a Bd8K	1	0	0	1	0.2
nCor a Bd11K	1	0	0	1	0.2
Bromelain	6	0	4	2	3.1
CCDs	2	0	0	2	3.1
Hazelnut extract (f17), upgraded	6	0	3	3	3.3

TABLE E2. IgE pollen allergens

		Patients with hazelnut allergy						Control subjects with pollen allergy					
		Birch	Bet v 1	Hazel	Olive	Grass	Plane	Birch	Bet v 1	Hazel	Olive	Grass	Plane
		n = 57						n = 62					
All	No. positive	51	43	54	47	51	35	55	41	44	54	54	37
	Median (kU _A /L)	10.7	8.6	7.6	1.7	5.0	0.2	3.3	0.7	1.4	2.0	18.7	0.43
	Maximum (kU _A /L)	97.2	87.5	60.0	37.4	248.7	24	119.2	106.6	86.8	112.5	138.1	128.5
		n = 20						n = 21					
Denmark	No. positive	20	20	19	13	16	7	19*	18*	17	14	16	5
	Median (kU _A /L)	19.8	15.9	9.6	0.1	1.5	0	2.1	1.9	0.8	0.3	5.7	0
	Maximum (kU _A /L)	97.2	83.5	31.1	2.9	43.4	2.1	119.2	106.6	27.5	4.5	75.5	1.9
		n = 20						n = 21					
Switzerland	No. positive	20	20	20	20	19	12	21	20	21	20	19	14
	Median (kU _A /L)	13.4	11.4	10.9	2.5	8.8	0.2	4.5	3.7	4.2	2.2	22.2	0.4
	Maximum (kU _A /L)	92.4	87.5	60.0	11.3	126.5	3.4	74.8	88.7	56.3	18.1	138.1	13.9
		n = 17						n = 20					
Spain	No. positive	11	3	15	15	16	16	15	3	16	20	19	18
	Median (kU _A /L)	0.3	0	0.5	3.1	4.0	3.9	0.7	0	0.6	12.4	41.7	2.5
	Maximum (kU _A /L)	37.4	47.9	28.1	37.4	248.7	24.0	99.8	17.9	86.8	112.5	120	128.5

*Denmark: 2 patients with negative results for Bet v 1 and birch pollen but 1 with a weak positive result to olive pollen.