

Apple allergy across Europe: How allergen sensitization profiles determine the clinical expression of allergies to plant foods

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Background: Allergy to a plant food can either result from direct sensitization to that food or from primary sensitization to pollen, latex, or another food.

Objective: We sought to investigate the primary sensitizers in apple allergy across Europe, the individual allergens involved, and whether these differences determine the clinical presentation.

Methods: Patients (n = 389) with positive case histories and skin prick test responses to fresh apple were selected in the Netherlands, Austria, Italy, and Spain. Skin prick tests and RASTs to a panel of pollens and plant foods were performed, as well as RASTs to Bet v 1 and the apple allergens Mal d 1, 2, 3, and 4.

Results: In the Netherlands, Austria, and Italy apple allergy is mild (>90% isolated oral symptoms) and related to birch pollinosis and sensitization to Bet v 1 and its apple homologue, Mal d 1, which has an odds ratio of local reactions of 2.85 (95% CI, 1.47-5.55). In Spain apple allergy is severe (>35% systemic reactions) and related to peach allergy and sensitization to Mal d 3 (nonspecific lipid transfer protein), which has an odds ratio of systemic reactions of 7.76 (95% CI, 3.87-15.56).

Conclusion: The analysis of individual apple allergens in a clinical context has provided insight into the sensitization pathway and into the intrinsic risk an allergen bears to induce mild or severe food allergy.

Clinical implications: Information on the sensitization pathway is essential to develop preventive strategies in food allergy. The application of individual food allergens with a known intrinsic risk will improve the prognostic value of diagnostic tests.

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Key words: Apple, Bet v 1, birch pollen, food allergy, lipid transfer protein, Mal d 1, Mal d 2, Mal d 3, Mal d 4, peach

Allergy to plant foods is the most common food allergy among older children and adults. Fruits of the Rosaceae family are the plant foods most frequently involved in allergic reactions.¹⁻⁴ The first clinical descriptions came from northern and central Europe, where Rosaceae fruit allergy, mainly apple allergy, is associated with birch pollinosis and patients present with mild oropharyngeal symptoms known as oral allergy syndrome (OAS).^{1,2} However, in areas free of birch pollen (BP), such as Spain, peach is the Rosaceae fruit most frequently inducing allergic reactions, followed by apple. The clinical presentation includes mild OAS, but severe systemic manifestations are frequently found.^{3,5}

Food allergy can result from a primary sensitization to food allergens or from primary sensitization to inhalant allergens (pollens or latex in allergies to plant foods). It has been postulated that direct sensitization by food allergens through the oral route is only possible when they have high resistance to proteolysis in the digestive tract. This same property is thought to be decisive for the potential of food allergens to induce severe systemic reactions.⁶⁻⁹ Two recently identified classes of plant allergens possess such extreme stability, the nonspecific lipid transfer proteins

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Abbreviations used

BP:	Birch pollen
nsLTP:	Nonspecific lipid transfer protein
OAS:	Oral allergy syndrome
OR:	Odds ratio
SPT:	Skin prick test
TLP:	Thaumatococcus-like protein

(nsLTPs) and the thaumatococcus-like proteins (TLPs). Both have a very compact structure stabilized by 4 and 8 disulfide bridges, respectively.¹⁰⁻¹² Patients with IgE antibodies against nsLTPs have indeed been reported to have severe food anaphylaxis.¹³ The clinical relevance of TLPs has not yet been established. Thus far, pollen-food cross-reactive IgE antibodies have mainly been implicated in mild OAS. The main sources of cross-reactivity, the major BP allergen Bet v 1 and the pollen profilins, are extremely sensitive to pepsin digestion, explaining the restriction of symptoms to the oral cavity.^{6-8,14-17} Specific IgE to profilin has been reported to lack biologic activity,¹⁶ whereas other reports have linked profilin to mild food allergy.¹⁷

In this work apple was chosen as a model to study the relation between sensitization profiles and the clinical expression of food allergies. Apple is one of the most commonly eaten fruits in Europe, the prevalence of apple allergy is significant, and its most important allergens are available as purified natural or recombinant reagents (ie, Mal d 1 [Bet v 1 homologue], Mal d 2 [TLP], Mal d 3 [nsLTP], and Mal d 4 [profilin]). The IgE reactivity to these 4 apple allergens was analyzed in 389 patients from 4 distinct regions in Europe: the Netherlands, Austria, northern Italy, and central Spain. The aim of this study was to investigate the primary sensitizers responsible for apple allergy across Europe, to establish the individual allergens involved in sensitization, and to establish whether these differences have an effect on the clinical presentation.

METHODS**Patients**

Patients (n = 389) were selected in the Netherlands, Austria, Italy, and Spain from 2001 through 2003. Dutch patients (n = 99) were recruited at the Dermatology/Allergy Department of University Medical Centre Utrecht. Austrian patients (n = 94) were selected in Vienna at the Allergy Clinic Reumannplatz and at the Medical University. Italian patients (n = 97) were selected at the Allergy Unit of Ospedale Caduti Bollatesi in Bollate (Milan) and Spanish patients (n = 99) at the Allergy Unit of Fundación Hospital Alcorcón in Madrid. To enter the study, patients had to report immediate adverse reactions to apple ingestion together with a positive skin prick test (SPT) response to fresh Golden Delicious apple. Subjects were selected prospectively from the population of new patients referred for allergy study. All the patients who fulfilled the inclusion criteria were invited to participate. The study was performed with the approval of the local ethics committees and with consent of the patients (or their representatives).

Clinical evaluation

The clinical evaluation comprised a medical history, SPTs, and blood sampling. A thorough medical history was collected by using a standardized questionnaire. SPTs with fresh Golden Delicious apple and peach were performed according to the prick-prick method.¹⁸ Peel and pulp were tested separately because of their different allergenicities.^{19,20} Patients underwent SPTs with extracts of hazelnut, peanut, walnut, celery, and the pollens of *Betula verrucosa* (birch), *Phleum pratense* (timothy grass), *Olea europea* (olive), *Artemisia vulgaris* (mugwort), *Ambrosia elatior* (ragweed), and *Parietaria judaica* (pellitory; ALK-Abelló, Hørsholm, Denmark). Histamine hydrochloride (10 mg/mL) and saline served as positive and negative controls, respectively. At each clinical center, SPTs were carried out by the same investigator according to the European Academy of Allergy and Clinical Immunology recommendations.²¹ An SPT response was considered positive if the wheal area was 7 mm² (3 mm diameter) greater than that induced by the negative control.

The clinical reactivity to apple was confirmed in a subset of patients from the Netherlands (n = 26) and Spain (n = 77) by means of double-blind, placebo-controlled food challenges with apple, according to a procedure previously described.^{13,22}

Serum-specific IgE determinations by means of RAST

RAST analysis was performed at Sanquin Research, Amsterdam, the Netherlands, as described previously.¹⁴ For the solid phase, pollens were extracted in water,¹⁴ and food extracts were prepared according to the method of Björkstén et al.²³ Natural (n) Bet v 1 and nMal d 1 were affinity purified with mAb 5H8, as described elsewhere.²⁴ Natural Mal d 3 was purified by means of cation-exchange and size-exclusion chromatography.²⁵ The recombinant (r) apple TLP rMal d 2 was produced in tobacco plants,²⁶ rMal d 4 was produced in *Escherichia coli*,²⁷ and rPru p 3 (peach nsLTP) was produced in the yeast *Pichia pastoris*.²⁸

Food extracts (apple, hazelnut, peach, peanut, walnut, celery, and carrot) were coupled at 40 mg/g cyanogen bromide-activated Sepharose (Amersham-Pharmacia Biotech, Uppsala, Sweden), pollen extracts (*Betula verrucosa*, *Phleum pratense*, *Olea europea*, *Artemisia vulgaris*, *Ambrosia elatior*, *Parietaria judaica*, *Platanus acerifolia*, and *Chenopodium album*) at 25 mg/g, and purified allergens (nBet v 1, nMal d 1, rMal d 2, nMal d 3, rMal d 4, and rPru p 3) at 1 mg/g. Fifty microliters of serum and 0.5 mg of Sepharose (1.5 mg for food extracts) were used per test.

Statistics

Statistical analysis was performed with SPSS (SPSS Inc, Chicago, Ill) and Epi Info software (Centers for Disease Control and Prevention, Atlanta, Ga). Descriptive statistics included frequency of positive results with the 95% CI for qualitative variables. For quantitative variables, such as age, means and SDs were calculated, whereas for SPT and RAST results, medians and 25th and 75th percentiles were given. A χ^2 test was used for comparisons of frequencies. Differences in quantitative variables between countries were compared by means of ANOVA (age at study and age at onset) and median tests (SPTs and RASTs). A Wilcoxon test for paired data was used to compare the ages at onset of pollen and food allergies within the same individual. Paired correlations were calculated by using the Pearson test. The association of the clinical presentation (classified as local or systemic) with the variable of RAST results to Mal d 1, 2, 3, and 4 was analyzed with logistic regression, adjusting for age and sex. A logistic regression analysis was used to study the factors associated with the IgE response to Mal d 3 (categorized by its median). The variables included in the model were age, sex, and the RAST results to nBet v 1 and to the pollens of birch, *Phleum*,

Olea, *Artemisia*, *Ambrosia*, *Parietaria*, *Platanus*, and *Chenopodium* species. For the logistic regression analysis, age and RAST values were categorized by their median by using the values below the median as the reference category. The adjusted odds ratio (OR) with its 95% CI was calculated. Values were considered significant at a *P* value of less than .05.

RESULTS

Demographics

The 389 patients included in this cross-sectional study comprised 152 male (39.07%; 95% CI, 34.19% to 44.11%) and 237 female (60.93%; 95% CI, 55.88% to 65.80%) patients with a mean age of 33.91 years (SD, 12.93). Sex distribution was significantly different for the whole group and for the Netherlands (70.70% female), Austria (61.70% female), and Spain (59.60% F) separately ($P < .01$) but not for Italy (51.55% female). The mean age of patients selected in the Netherlands, Austria, Italy, and Spain was 36.91 (SD, 10.18), 35.33 (SD, 11.26), 40.05 (SD, 14.22), and 23.64 (SD, 9.23) years, respectively. In the Netherlands and Austria only, patients with an age of 18 years or older were included, whereas 4 Italian and 25 Spanish patients were younger than 18 years. Even excluding those younger than 18 years, Spanish subjects were younger (27.23 years [SD, 7.77]) than in the other 3 countries ($P < .001$). No differences in age were found between Dutch and Austrian patients ($P > .05$), and the mean age of the 93 adult Italian patients (40.85 years [SD, 13.72 years]) was higher than that of the patients from the Netherlands and Austria ($P < .05$).

Clinical presentation

Symptoms restricted to the skin or mucosal sites of contact with apple during consumption were designated as local symptoms, whereas urticaria, anaphylaxis, and anaphylactic shock were classified as systemic symptoms. Oral symptoms (OAS) were the most frequent local symptoms and were reported by 100%, 96.8%, 89.7%, and 79.8% of patients from the Netherlands, Austria, Italy, and Spain, respectively, with the frequencies in Italy and Spain being significantly lower. Urticaria was found in 4.2%, 2.1%, and 16.2% of Austrian, Italian, and Spanish patients ($P < .001$) and not described in patients from the Netherlands. Anaphylaxis was reported by 14.1% of Spanish patients ($P < .001$), and a single case of anaphylactic shock was recorded in the Netherlands. Apple allergy in Spain was more severe, with a frequency of systemic reactions of 35.4%, which is significantly higher than in Italy (8.2%), Austria (4.2%), and the Netherlands (1.0%; $P < .001$). There was an association ($P < .0001$) between systemic reactions induced by apple and living in Spain (OR, 11.65; 95% CI, 5.83-23.28).

Associations with pollen and food allergies

Pollen allergy was present in 97%, 96%, and 96% of patients from the Netherlands, Austria, and Italy,

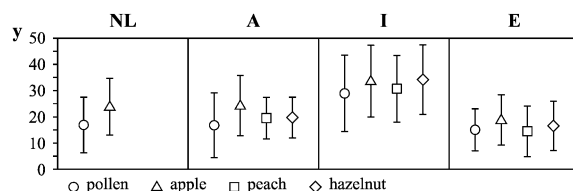


FIG 1. Age at onset of pollen, apple, peach, and hazelnut allergies. The age at onset of pollen and food allergies was calculated, excluding the patients with an age at the time of the study of less than 18 years. Mean age and SD are given. y, Years; NL, The Netherlands; A, Austria; I, Italy; E, Spain.

respectively but only in 88% of the Spanish subjects ($P < .05$). The clinically relevant pollen species differed among countries: BP dominated in the Netherlands, Austria, and Italy (85% to 93%), whereas grass pollen was the most prevalent in Spain (93%; $P < .05$).

Peach allergy was the food allergy most frequently associated with apple allergy in Spain (89.9%). Patients in the Netherlands, Austria, and Italy reported significantly less adverse reactions to peach ($P < .001$; ie, in 70.7%, 69.9%, and 66.0% of the cases, respectively). In the Netherlands and Austria hazelnut was the food most frequently associated with apple allergy (73.7% and 73.3%, respectively) and the second one after peach in Italy (57.7%). Hazelnut allergy was less frequently reported in Spain (21.2%, $P < .001$).

Age at onset of apple allergy

The age at onset of apple allergy was different across Europe (Fig 1): Spanish patients were the youngest ($P < .01$), Italian patients were the oldest ($P < .001$), and Dutch and Austrian patients started at a similar age ($P > .05$). Similar results were found for peach and hazelnut allergies. The age at onset of pollen allergies in the Netherlands, Austria, and Spain was comparable (15-17 years) and significantly lower than in Italy (29 years, $P < .001$). In all 4 countries pollen allergy started before apple allergy ($P < .05$). Peach and hazelnut allergies also started later than pollinosis in Austria and Italy ($P < .01$). However, peach allergy started earlier than apple and hazelnut allergies in Spain ($P < .05$), and no difference was found between peach and pollen allergy onsets in the Spanish patients.

Specific IgE assessed by means of SPTs and RASTs

SPT responses for apple were higher in Italy and were higher for peach in Spain. In Spain and, to a lesser extent, in Italy, skin reactivity to peel was higher than to pulp ($P < .001$, Fig 2). As expected, no significant skin reactivity to birch was observed in Spain. In contrast, grass pollen induced significantly higher SPT responses in Spain. Skin reactivity to hazelnut was significantly lower in Spain (Table I).

When analyzed by means of RASTs, IgE titers to apple were also higher in Italy, although this difference only

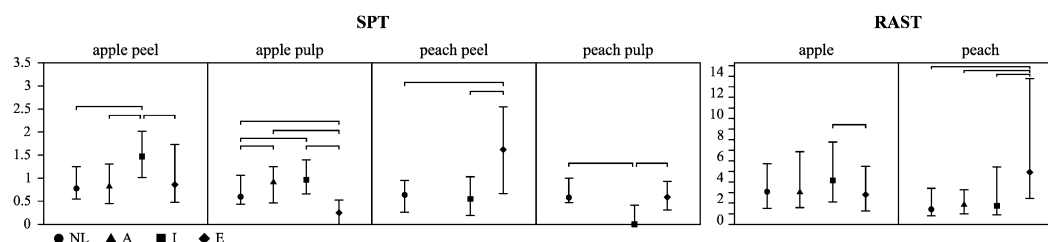


FIG 2. SPTs and RASTs to apple and peach. The variable used in SPTs was the ratio to the histamine response (allergen wheal area/histamine wheal area). Median value and 25th and 75th percentiles are given. Differences between countries were analyzed by using a median test. In the RAST panels the y axis is in international units per milliliter. Only the significant ($P < .05$) paired comparisons are shown. NL, The Netherlands; A, Austria; I, Italy; E, Spain.

TABLE I. SPT results: pollens and plant foods

	Median (P25-P75)				Median test
	NL	A	I	E	Paired comparisons (with $P < .05$)
<i>Betula verrucosa</i>	1.0 (0.61-1.33)	1.12 (0.81-1.51)	1.54 (0.97-2.24)	0 (0-0.33)	I > NL; I > A; I > E; NL > E; A > E
<i>Phleum pratense</i>	0.56 (0.06-1.04)	0.87 (0-1.52)	0.66 (0-1.63)	1.20 (0.51-2.16)	E > NL; E > A; E > I; A > NL
<i>Olea europea</i>	0.26 (0-0.55)	0.37 (0-0.93)	0.46 (0-1.12)	0.94 (0.31-1.50)	E > NL; E > A; E > I; I > NL
<i>Artemisia vulgaris</i>	0 (0-0)	0 (0-0.72)	0.18 (0-0.78)	0 (0-0.33)	I > NL; I > E; E > NL; A > NL
<i>Ambrosia elatior</i>	0 (0-0)	0 (0-0.40)	0.49 (0-1.20)	0 (0-0.23)	I > NL; I > A; I > E; A > NL; E > NL
<i>Parietaria judaica</i>	0 (0-0)	0 (0-0)	0 (0-0.33)	0 (0-0)	I > NL; I > A; I > E; E > NL; E > A
Hazelnut	0.50 (0.31-0.72)	0.70 (0.38-0.91)	0.71 (0.50-1.0)	0.20 (0-0.47)	E < NL; E < A; E < I; I > NL
Peanut	0.20 (0-0.55)	0.31 (0-0.88)	0.67 (0.24-1.11)	0.38 (0-0.66)	I > NL; I > A; I > E; E > NL
Walnut	0 (0-0)	0 (0-0)	0 (0-0.18)	0 (0-0.54)	I > NL; I > A; E > NL; E > A
Celery	0.37 (0-0.74)	0.55 (0-1.0)	0.68 (0.34-1.13)	0 (0-0.36)	E < NL; E < A; E < I; I > NL

The variable used in SPTs was the ratio to the histamine response (allergen wheal area/histamine wheal area).

P25-P75, 25th percentile-75th percentile; NL, The Netherlands; A, Austria; I, Italy; E, Spain.

reached significance compared with that seen in Spain. The higher SPT reactivity to peach observed in Spain was supported by the RAST results (Fig 2). This was also the case for the differences observed for SPTs to BP, grass pollen, and hazelnut. RAST results to *Artemisia*, *Parietaria*, *Chenopodium*, and *Platanus* species were higher in Spain (Table II).

In the Netherlands, Austria, and Italy SPT responses and RAST results to BP were higher ($P < .001$) than those to apple, which in turn were higher ($P < .001$) than those to peach (Table II and Fig 2). In contrast, in Spain SPT responses and RAST results to peach were significantly higher than those to apple ($P < .001$, (Fig 2).

IgE responses against individual apple allergens

The apple allergen profile (component-resolved diagnosis) is presented in Fig 3. In the Netherlands, Austria, and Italy RAST results to nMal d 1 and nBet v 1 were higher than in Spain. RAST results to rMal d 2 were higher in Spain and Italy compared with those in the Netherlands and Austria, but titers were generally low. RAST results to nMal d 3 were higher in Spain than in the other 3 countries.

In turn, Italian patients demonstrated higher IgE reactivity to this allergen than those in the Netherlands and Austria. For rMal d 4, RAST results were higher in Spain.

In the Netherlands, Austria, and Italy RAST results to apple were correlated ($r = 0.6-0.9$, $P < .001$) to RAST results to birch, nBet v 1, and nMal d 1. Only in Spain were significant ($P < .0001$) correlations found between SPT responses to apple peel and peach peel ($r = 0.77$), RAST results to apple and peach ($r = 0.85$), and RAST results to nMal d 3 and peach ($r = 0.60$).

RASTs to rPru p 3 were performed in 91 of 99 Spanish patients, with a median value of 0.80 (25th percentile, 0.20; 75th percentile, 2.60). Titers of 1.0 IU/mL or greater were found in 45%. RAST results to rPru p 3 were correlated with those to apple ($r = 0.66$, $P < .0001$) and nMal d 3 ($r = 0.68$, $P < .0001$).

Individual apple allergens and the clinical presentation of apple allergy

Only the IgE responses to nMal d 1 and nMal d 3 were associated with the clinical presentation of apple allergy. Sensitization to nMal d 3 was a risk factor for having systemic reactions (adjusted OR, 7.76; 95% CI, 3.87-15.56), whereas sensitization to nMal d 1 was a protective factor

TABLE II. RAST results: pollens and plant foods

	Median (P25-P75)				Median test
	NL	A	I	E	Paired comparisons (with $P < .05$)
<i>Betula verrucosa</i>	10.19 (5.07-18.21)	15.36 (8.34-29.69)	11.07 (5.29-18.51)	1.08 (0.34-3.23)	A > NL; A > I; A > E; NL > E; I > E
<i>Phleum pratense</i>	4.96 (0.73-21.47)	3.65 (0.56-40.21)	3.70 (0.36-19.79)	41.23 (8.79-100.48)	E > NL; E > A; E > I
<i>Olea europea</i>	1.0 (0.43-2.43)	2.92 (0.70-7.15)	1.15 (0.30-3.29)	4.62 (1.85-11.41)	E > NL; E > A; E > I; A > NL; A > I
<i>Artemisia vulgaris</i>	0.48 (0.26-1.53)	1.09 (0.34-3.10)	1.05 (0.32-3.34)	1.67 (0.79-3.81)	E > NL; E > A; A > NL; I > NL
<i>Ambrosia elatior</i>	0.29 (0.22-0.45)	0.41 (0.21-1.17)	2.37 (0.29-9.64)	0.56 (0.29-1.29)	NL < A; NL < I; NL < E; I > A; I > E
<i>Parietaria judaica</i>	0.42 (0.33-1.06)	0.57 (0.24-1.13)	0.76 (0.39-3.89)	1.61 (0.66-3.91)	E > NL; E > A; E > I; I > NL
<i>Platanus acerifolia</i>	0.32 (0.22-0.75)	0.64 (0.25-1.04)	0.37 (0.19-1.71)	1.68 (0.54-4.18)	E > NL; E > A; E > I
<i>Chenopodium album</i>	0.64 (0.37-1.87)	1.18 (0.69-3.72)	0.99 (0.50-2.09)	2.72 (1.04-4.77)	E > NL; E > A; E > I
Hazelnut	2.50 (1.13-4.99)	3.93 (2.18-6.97)	2.95 (1.72-6.13)	1.98 (0.67-5.33)	A > NL; A > E; I > E; I > NL
Peanut	1.58 (0.66-4.19)	2.80 (1.18-5.01)	1.94 (1.08-4.81)	2.73 (0.84-5.59)	A > NL
Walnut	0.50 (0.31-1.49)	1.17 (0.62-1.90)	0.72 (0.43-2.41)	2.16 (0.61-5.05)	E > NL; E > A; E > I; A > I; A > NL
Carrot	0.52 (0.24-1.54)	1.31 (0.52-2.25)	0.92 (0.42-3.84)	1.64 (0.50-5.60)	E > NL; E > NL; A > NL
Celery	0.50 (0.29-1.38)	1.09 (0.60-1.94)	0.96 (0.51-2.82)	2.03 (0.66-5.45)	E > NL; E > A; E > I; A > NL; I > NL

P25-P75, 25th percentile-75th percentile; NL, The Netherlands; A, Austria; I, Italy; E, Spain.

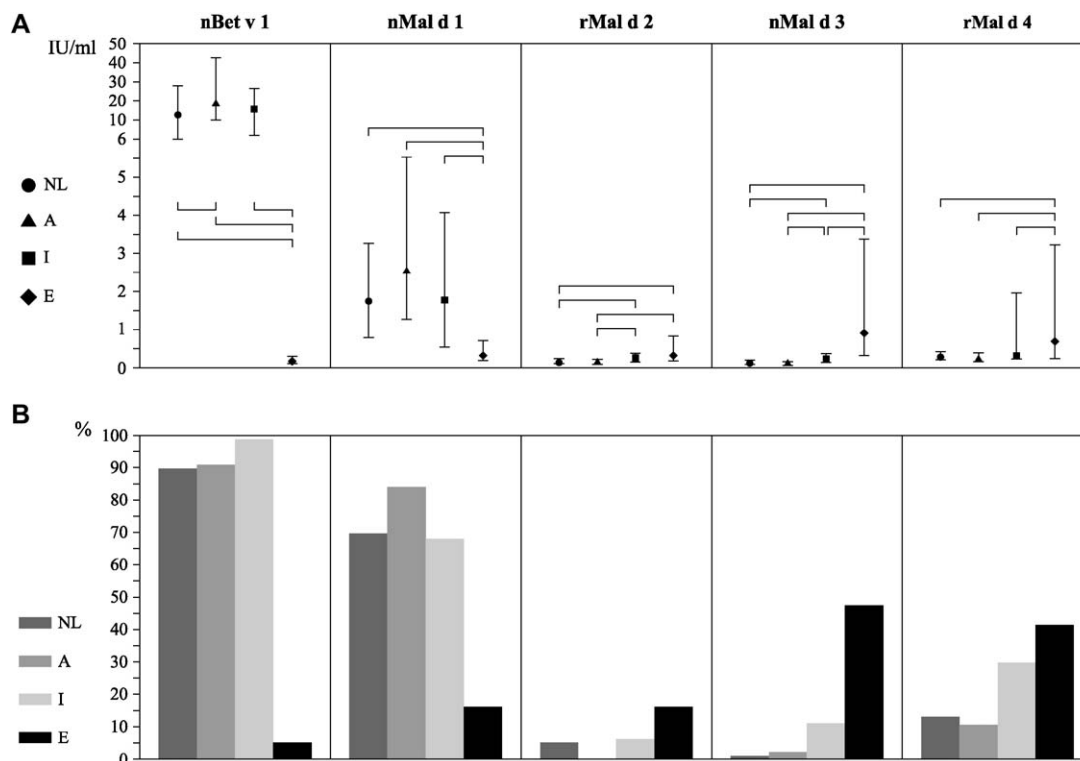


FIG 3. RASTs to apple allergens and nBet v 1. **A**, Median value and 25th and 75th percentiles are given. Differences between countries were analyzed by using a median test. Only the significant ($P < .05$) paired comparisons are shown. **B**, Frequency (percentage) of patients with RAST results of 1.0 IU/mL or greater. NL, The Netherlands; A, Austria; I, Italy; E, Spain.

(adjusted OR of systemic reactions, 0.35; 95% CI, 0.18-0.68; if estimated OR of local reactions, 2.85, 95% CI, 1.47-5.55; Fig 4). When this analysis was performed

in the 103 Dutch and Spanish patients with challenged-confirmed apple allergy, a similar effect was obtained: the adjusted OR of systemic reactions was 0.57 (95% CI,

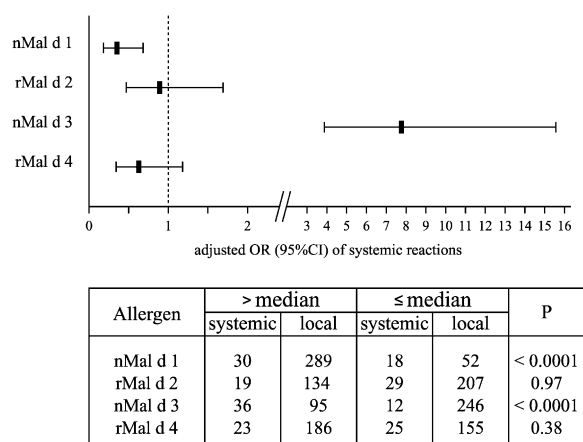


FIG 4. Sensitization to individual apple allergens and clinical expression of apple allergy.

0.24-1.35) for nMal d 1 and 7.89 (95% CI, 2.20-28.34) for nMal d 3.

Factors associated with the IgE response to nMal d 3

Only the IgE responses to nBet v 1 and to the pollens of mugwort and plane tree were significantly associated with the sensitization to nMal d 3 in the logistic regression analysis (Table III). Sensitization to nBet v 1 had a protective effect (decreased OR of 0.29), whereas sensitization to mugwort and plane tree pollens had the opposite effect, with an increased OR of 2.38 and 2.81, respectively.

DISCUSSION

This study has analyzed the relation between sensitization to individual apple allergens and the resulting clinical presentation of apple allergy in a comprehensive multi-center approach across Europe. In 4 clinical centers in the Netherlands, Austria, Italy, and Spain, 389 patients with a history of apple allergy and specific IgE to this fruit were included. With the exception of Italy, female subjects dominated. This has been observed in other studies on allergies to plant foods, but an explanation has thus far not been proposed. The age distribution showed striking differences as well, with the Spanish patients being the youngest. This might reflect that food allergies linked to pollens, as is the case in the Netherlands, Austria, and Italy, start later than those not related to pollen, such as in Spain. The older age of Italian patients might be related to the relatively recent introduction of birch trees in this area, which can favor the late appearance of birch pollinosis in adult patients.²⁹

In this study we have shown the existence of 2 patterns of apple allergy across Europe: one in the Netherlands, in Austria, and in 90% of the (northern) Italian patients and another one in Spain and in the remaining Italian subjects. Whether the pattern in southern Italy would be closer to the Spanish one needs to be investigated.

TABLE III. Adjusted OR of Mal d 3 sensitization in relation to RAST to pollens

RAST	OR	95% CI	P value
nBet v 1			
<8.79 IU/mL	1.00		
≥8.79 IU/mL	0.29	0.17-0.50	<.0001
<i>Artemisia vulgaris</i> (mugwort)			
<1.04 IU/mL	1.00		
≥1.04 IU/mL	2.38	1.26-4.47	.007
<i>Platanus acerifolia</i> (plane tree)			
<0.56 IU/mL	1.00		
≥0.56 IU/mL	2.81	1.50-5.24	.001

ORs are adjusted for age, sex, and RAST results to nBet v 1 and the pollens of birch and *Phleum*, *Olea*, *Artemisia*, *Ambrosia*, *Parietaria*, *Platanus*, and *Chenopodium* species. Age and RAST values were categorized by their median, with the values less than the median as the reference category.

In the Netherlands, Austria, and Italy apple allergy is mild (>90% present exclusively oral symptoms) and is preceded by (birch) pollen allergy, and the main apple allergen involved is Mal d 1, the homologue of Bet v 1. The IgE responses to apple, BP, Mal d 1, and Bet v 1 are strongly correlated, with those to BP and Bet v 1 being higher. Altogether, these results support the theory that patients from areas rich in birch trees become allergic to BP and its major allergen, Bet v 1, through the inhalant route.^{6,8} The apple allergy arises later as a result of the cross-reactivity between Bet v 1 and Mal d 1.^{15,30} The lability of Mal d 1 makes sensitization through the oral route highly unlikely and explains the restriction of symptoms to the mouth.^{7,8} The latter is further supported by the finding that sensitization to Mal d 1 favors local reactions (adjusted OR, 2.85; 95% CI, 1.47-5.55). This model of BP and apple can be extended to the remaining allergies to plant foods linked to birch pollinosis because of sensitization to Bet v 1 homologues.^{6,15,16}

The scenario in Spain is strikingly different. Apple allergy is severe (>35% of systemic reactions), and the main apple allergen is Mal d 3. In contrast to the other 3 countries, peach allergy in Spain starts earlier than apple allergy, and the onsets of peach and pollen allergies are not different. SPT responses to peach and apple peels (where the nsLTPs are located²⁰) and RAST results to peach, apple, and the nsLTPs nMal d 3 and rPru p 3 were strongly correlated. Altogether, these results suggest that apple allergy in Spain is a result of a primary sensitization to peach and its major allergen, Pru p 3. Apple allergy starts later, as a result of the cross-reactivity between Pru p 3 and Mal d 3.^{28,31} In contrast to Bet v 1 homologues, nsLTPs resist the proteolytic attack in the digestive tract and can thus induce sensitization and symptoms on oral exposure, behaving as true food allergens.^{9,11-13,25} This is further supported by our finding that sensitization to Mal d 3 is a risk factor for systemic reactions (OR, 7.76; 95% CI, 3.87-15.56).

Although exposure to BP is virtually absent in Madrid, some patients demonstrated IgE reactivity to BP, Bet v 1, and Mal d 1. It is highly unlikely that these antibodies were

induced by exposure to Bet v 1 because RAST results to Mal d 1 were higher than those to Bet v 1. In areas in which BP exposure is high (ie, Vienna, Utrecht, or Milan) the IgE response to Bet v 1 is always higher than that to Mal d 1. Possibly another source of Fagales pollen causing exposure to Bet v 1–like allergens³² is at the basis of Bet v 1–Mal d 1 sensitization. A candidate might be holm oak pollen (*Quercus ilex*), which is a Fagales tree common in Central Spain.

One question remains open. Why is sensitization to nsLTPs (Mal d 3) not observed in the Netherlands or Austria? Geographic differences in the sensitization to nsLTPs might be related to genetic factors (not reported thus far), differences in dietary habits and pollen exposure, or both. Apple consumption is not less in the Netherlands and Austria than in Spain, but perhaps the difference relies on the consumption rate of peaches. Because comparable consumption data for peach were not available, a pilot study was carried out by our group in the Netherlands, Austria, Italy, Spain, and the United Kingdom, looking for the presence of peach in ready-made meals and drinks for babies and toddlers. A significantly higher chance of finding peach in these products was observed for Spain (data not shown). Additionally, fresh peach is probably given to Spanish children more frequently than to northern and central European children. Regarding pollen exposure, we have found that sensitization to the major allergen of BP, Bet v 1, reduces the risk of an IgE response to nMal d 3 by a factor of 3.5, whereas sensitization to the pollens of mugwort and plane tree increases the risk by a factor of 2.3 and 2.8, respectively. These results support the epidemiologic finding that sensitization to nsLTP appears (predominantly) in areas free of birch trees and are in agreement with previous studies that have found an association between IgE responses to mugwort and plane tree pollens and nsLTP sensitization.^{31,33,34} In the light of these results, we might hypothesize that exposure at a young age to peach and certain pollens, such as those of mugwort and plane tree, together with a lack of exposure to BP, might contribute altogether to the appearance of sensitization to nsLTP in Spain. Further studies in animal models of allergy to nsLTPs (peach and apple) are needed to fully elucidate the sensitization pathway or pathways in these puzzling food allergies.

In conclusion, the analysis of individual apple allergens in a clinical context has provided valuable information about the sensitization pathway and about the intrinsic risk an allergen bears to induce severe or mild food allergy. Information on the sensitization pathway is essential to develop preventive strategies in food allergy. The application of individual food allergens with a known intrinsic risk will improve the prognostic value of the diagnostic tests in food allergy compared with the current generation of tests based on whole food extracts.

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