Original Article

Walnut Allergy Across Europe: Distribution of Allergen Sensitization Patterns and Prediction of Severity

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What is already known about this topic? Although walnut is one of the tree nuts most often reported to elicit food-allergic reactions in Europe and worldwide, data on sensitization to individual walnut components and their geographical and clinical relevance are scarce.

What does this article add to our knowledge? Patterns of IgE sensitization to 7 walnut components in 12 European countries are presented, along with a highly discriminative model combining serological and clinical information for prediction of walnut allergy severity.

How does this study impact current management guidelines? Molecular diagnostics in walnut allergy reveal varied patterns of sensitization across Europe, and can help accurately distinguish mild to moderate from severe walnut allergy when considered in combination with extract-based testing and clinical background.

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Abbreviations used
AUC- Area under the curve
CRD- Component-resolved diagnostics
Lasso- Least Absolute Shrinkage and Selection Operator
LTP- Lipid transfer protein
PR-10- Pathogenesis-related protein family 10

BACKGROUND: Walnut allergy is common across the globe, but data on the involvement of individual walnut components are scarce.

OBJECTIVES: To identify geographical differences in walnut component sensitization across Europe, explore cosensitization and cross-reactivity, and assess associations of clinical and serological determinants with severity of walnut allergy.

METHODS: As part of the EuroPrevall outpatient surveys in 12 European cities, standardized clinical evaluation was conducted in 531 individuals reporting symptoms to walnut, with sensitization to all known walnut components assessed in 202 subjects. Multivariable Lasso regression was applied to investigate predictors for walnut allergy severity.

RESULTS: Birch-pollen–related walnut sensitization (Jug r 5) dominated in Northern and Central Europe and lipid transfer protein sensitization (Jug r 3) in Southern Europe. Profilin sensitization (Jug r 7) was prominent throughout Europe. Sensitization to storage proteins (Jug r 1, 2, 4, and 6) was detected in up to 10% of subjects. The walnut components that showed strong correlations with pollen and other foods differed between centers. The combination of determinants best predicting walnut allergy severity were symptoms upon skin contact with walnut, atopic dermatitis (ever), family history of atopic disease, mugwort pollen allergy, sensitization to cat or dog, positive skin prick test result to walnut, and IgE to Jug r 1, 5, 7, or carbohydrate determinants (area under the curve = 0.81; 95% CI, 0.73-0.89).

CONCLUSIONS: Walnut-allergic subjects across Europe show clear geographical differences in walnut component sensitization and cosensitization patterns. A predictive model combining results from component-based serology testing with results from extract-based testing and information on clinical background allows for good discrimination between mild to moderate and severe walnut allergy.

Key words: *Walnut allergy; IgE sensitization; Allergen components; Severity; Prediction; Europe; EuroPrevall; iFAAM*

INTRODUCTION

Walnut is one of the tree nuts most often reported to elicit food-allergic reactions in European countries and globally.[1-3] Ongoing developments in food allergy diagnostic testing make it possible to assess IgE sensitization to a broadening spectrum of specific food allergens, commonly referred to as component-resolved diagnostics (CRD). At the time of this study, 7 components of the "English" walnut, *Juglans regia*, had been characterized: Jug r 1 (2S albumin), Jug r 2 (vicilin-like 7S globulin), Jug r 3 (lipid transfer protein [LTP]), Jug r 4 (legumin-like 11S globulin), Jug r 5 (pathogenesis-related protein family 10 [PR-10] protein), Jug r 6 (vicilin-like 7S globulin), and Jug r 7 (profilin).

Studies on geographical differences in sensitization patterns to walnut components across Europe are scarce.[4] One study investigated sensitization to walnut components in 91 walnut-allergic patients from 3 European regions, and described a particularly high occurrence of Jug r 3 sensitization in Spain, and Jug r 5 sensitization in Germany and Switzerland.[5] However, geographical comparisons were limited by the fact that only children were included in Germany, and only adults in Switzerland. Larger studies, with standardized cross-border inclusion criteria and a broader geographical distribution including Northern and Eastern Europe, are needed to substantiate previous findings and expand data on international comparisons.

CRD can be of help not only in distinguishing primary from cross-reactive walnut sensitization[6,7] but also in predicting severity of food-allergic reactions.[8,9] For walnut, literature suggests that IgE to the seed storage proteins Jug r 1, Jug r 2, Jug r 4, and Jug r 6 is associated with more severe reactions,[5,10] but data are limited. A recent study evaluated CRD data in combination with other serological measurements and clinical factors for

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predicting severity of hazelnut allergy, and found that a model combining IgE to Cor a 14, IgE to walnut extract, atopic dermatitis, and pollen allergy performed well.[9] Such a predictive model has not yet been elaborated for walnut allergy.

In this study, we explored walnut allergy through data collected during the standardized EuroPrevall outpatient project, from 12 geographically, culturally, and socioeconomically diverse regions across Europe. Our aim was 3-fold: (1) to identify differences in sensitization patterns to walnut components across Europe; (2) to assess relationships between IgE to walnut components and IgE to pollen and foods other than walnut, providing insight into possible primary sensitizers; and (3) to optimally predict severity of walnut allergy using data from clinical history and IgE responses to walnut and walnut components.

METHODS

Study design, setting, and subjects

Participants of the EuroPrevall outpatient clinic study reporting adverse reactions within 2 hours of ingestion of walnut were evaluated in this study. A detailed methodology of the standardized EuroPrevall outpatient food allergy workup was published previously.[11]

Data were collected between 2006 and 2009 in 12 European allergy clinics, in Athens (Greece), Łódź (Poland), Madrid (Spain), Manchester (United Kingdom), Milan (Italy), Prague (Czech Republic), Reykjavik (Iceland), Sofia, (Bulgaria), Strasbourg (France), Utrecht (The Netherlands), Vilnius (Lithuania), and Zürich (Switzerland).

Ethical approval and written informed consent were obtained in each center and from each participating subject.

Data collection

A detailed questionnaire was completed for each subject by a trial physician, and focused on demographic data, reaction characteristics, and personal and family history of atopy.

IgE sensitization was assessed through skin prick test (SPT) and serum analyses, according to the same standardized approach in all centers (see details in the Supplementary Methods section in this article's Online Repository at www.jaci-inpractice.org), using extracts from food (including walnut) and inhalant allergens that are commonly implicated in food allergy across Europe. Additional prick-to-prick testing with fresh walnut was performed in case of negative SPT result with walnut extract, as indicated by local practice. Additional testing of sera for IgE to walnut components Jug r 1, Jug r 2, a low-molecular-weight fragment of Jug r 2, Jug r 3, Jug r 4, Jug r 5, Jug r 6, and Jug r 7 was performed in January 2008 with all sera collected at that time. The low-molecular-weight fragment of Jug r 2 is described in the Supplementary Methods section in this article's Online Repository at www.jaci-inpractice.org. SPT results were expressed as allergen/histamine wheal ratios, and a ratio greater than or equal to 0.5 was considered positive. IgE levels greater than or equal to 0.35 kU_A/L were considered positive.

Definitions

Probable walnut allergy was defined as a combination of reported symptoms to walnut and matching IgE sensitization, as demonstrated by a positive walnut SPT result, prick-to-prick testing, and/or presence of serum IgE against walnut extract and/or 1 or more individual walnut components as tested by ImmunoCAP.

Reactions to walnut were classified as *severe* if subjects reported dysphagia, dysphonia, lower airway, cardiovascular, or neurological symptoms, or anaphylaxis (specifically severe laryngeal edema, severe bronchospasm, or hypotensive shock). All other symptoms were considered *mild to moderate*: isolated oral allergy symptoms, symptoms of the skin, eyes, upper airway, or gastrointestinal system (see details in this article's Supplementary Methods section in this article's Online Repository at www.jaci-inpractice.org).[12,13]

Allergy to inhalant allergen sources and to latex was defined as symptoms and matching IgE sensitization in SPT and/or ImmunoCAP to the respective allergen source.

Statistical analyses

Walnut sensitization patterns across Europe. Demographic characteristics, reaction severity, and proportions of positive test results were explored for each participating center. Medians and interquartile ranges were calculated to evaluate IgE levels for walnut extract and walnut components. Differences between centers in levels of IgE to walnut extract were tested using the Kruskal-Wallis test with Bonferroni correction.

Relationship between IgE to walnut components and other allergens. Spearman rho coefficients were calculated to evaluate relationships between levels of IgE to walnut components and levels of IgE to food, latex, and pollen extracts. Bonferroni correction was used to correct for multiple comparisons.

Predictors for severity of walnut allergy. Only subjects conforming to the definition of "probable walnut allergy" were included for prediction of severity of walnut allergy. Univariable logistic regression was performed to explore crude associations between demographic characteristics, clinical history variables, walnut sensitization patterns, and severity of walnut allergy.

To identify the most discriminative combination of predictors for severity of walnut allergy, Least Absolute Shrinkage and Selection Operator (Lasso) regression was applied. Lasso regression is a form of penalized regression, which selects only the most contributive predictors and applies shrinkage of regression coefficients through cross-validation to limit overfitting.[14] To enable the use of all data and increase power for this predictive analysis, multiple imputation of sporadically missing data on predictor variables was performed (10 imputations by chained equations using the R package *mice*).[15] Missing data are described in Table E1 in this article's Online Repository at www.jaci-inpractice.org.

A 3-step approach to model building was taken. In model 1, all demographic and clinical variables were entered, and Lasso regression selected the most discriminative combination of predictors. In model 2, variables on IgE sensitization to walnut extract as assessed by SPT and ImmunoCAP were entered, along with the variables selected in model 1. In model 3, ImmunoCAP results for walnut components, and IgE to Ana c 2 (bromelain) as a measure for cross-reactive carbohydrate determinants, were added to the variables remaining after selection in model 2. Predictor variables selected in at least 7 of the 10 imputed data sets were included in each model, and their coefficients and 95% CIs were pooled, using Rubin's rules.

To assess how well each model could discriminate between mild to moderate and severe walnut allergy, the area under the curves (AUCs) of the receiving-operating characteristics and corresponding 95% CIs were calculated and pooled over the 10 imputed data sets. DeLong's test was used to compare AUC values.[16]

TABLE I. Characteristics of subjects with self-reported walnut allergy across Europe

Characteristic	Total	Athens	Madrid	Manchester	Milan	Łódź	Prague
Self-reported walnut allergy, n	531	44	25	30	39	74	19
Age (y), mean ± SD	30.4 ±13.9	27.8 ± 10.3	23.8 ± 12.9	30.7 ± 13.3	34.7 ± 10.9	29.5 ± 18.4	15.9 ± 11.7
Age <18 y	82 (15.4)	4 (9.1)	7 (28.0)	5 (16.7)	0 (0.0)	22 (29.7)	11 (57.9)
Female sex	344 (64.8)	17 (38.6)	18 (72.0)	23 (76.7)	29 (74.4)	59 (79.7)	10 (52.6)
Symptom severity*							
Mild	214 (40.3)	14 (31.8)	9 (36.0)	3 (10.0)	27 (69.2)	14 (18.9)	5 (26.3)
Moderate	184 (34.7)	18 (40.9)	9 (36.0)	15 (50.0)	6 (15.4)	41 (55.4)	6 (31.6)
Severe	133 (25.0)	12 (27.3)	7 (28.0)	12 (40.0)	6 (15.4)	19 (25.7)	8 (42.1)
Sensitization to walnut†							
SPT walnut positive	211 (40.8)	36 (81.8)	13 (54.2)	9 (30.0)	21 (53.8)	12 (16.9)	7 (38.9)
ImmunoCAP walnut positive	182 (35.5)	35 (81.4)	20 (87.0)	11 (39.3)	19 (48.7)	10 (13.9)	7 (43.8)
CRD walnut performed	202	19	13	5	18	15	8
CRD walnut positive‡	158 (79.4)	13 (68.4)	10 (76.9)	4 (80.0)	15 (83.3)	9 (64.3)	8 (100.0)
Jug r 1	21 (10.4)	0 (0.0)	3 (23.1)	1 (20.0)	0 (0.0)	1 (6.7)	3 (37.5)
Jug r 2	19 (9.6)	0 (0.0)	3 (23.1)	1 (20.0)	1 (5.6)	1 (7.1)	2 (25.0)
Jug r 2 LMW	43 (22.1)	5 (26.3)	4 (30.8)	1 (20.0)	3 (16.7)	3 (23.1)	3 (37.5)
Jug r 3	28 (13.9)	9 (47.4)	3 (23.1)	0 (0.0)	4 (22.2)	0 (0.0)	2 (25.0)
Jug r 4	18 (9.2)	0 (0.0)	3 (23.1)	2 (40.0)	1 (5.6)	0 (0.0)	2 (25.0)
Jug r 5	115 (58.1)	1 (5.3)	1 (7.7)	1 (20.0)	12 (66.7)	7 (50.0)	7 (87.5)
Jug r 6	12 (6.2)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (25.0)
Jug r 7	47 (23.3)	4 (21.1)	4 (30.8)	0 (0.0)	7 (38.9)	1 (6.7)	1 (12.5)
Probable walnut allergy	336 (65.8)	43 (97.7)	23 (95.8)	15 (53.6)	32 (82.1)	22 (31.4)	13 (81.3)

Characteristic	Reykjavik	Sofia	Strasbourg	Utrecht	Vilnius	Zürich	*P*
Self-reported walnut allergy, n	9	10	50	74	50	107	
Age (y), mean ± SD	36.4 ± 17.6	23.2 ± 14.3	33.8 ± 12.8	31.2 ± 11.3	27.9 ± 14.0	33.8 ± 12.8	<.001
Age <18y	1 (11.1)	4 (40.0)	5 (10.0)	3 (4.1)	14 (28.0)	6 (5.6)	<.001
Female sex	6 (66.7)	7 (70.0)	34 (68.0)	54 (73.0)	22 (44.0)	65 (60.7)	<.001
Symptom severity*							
Mild	2 (22.2)	0 (0.0)	33 (66.0)	33 (44.6)	18 (36.0)	56 (52.3)	<.001
Moderate	2 (22.2)	7 (70.0)	9 (18.0)	20 (27.0)	22 (44.0)	29 (27.1)	
Severe	5 (55.6)	3 (30.0)	8 (16.0)	21 (28.4)	10 (20.0)	22 (20.6)	
Walnut sensitization†							
SPT walnut positive	4 (44.4)	2 (20.0)	13 (26.5)	25 (37.3)	38 (77.6)	31 (29.0)	<.001
ImmunoCAP walnut positive	3 (33.3)	3 (30.0)	13 (26.5)	19 (25.7)	15 (34.1)	27 (25.5)	<.001
CRD walnut performed	3	4	16	20	14	67	
CRD walnut positive‡	1 (33.3)	1 (33.3)	15 (93.8)	19 (95.0)	11 (84.6)	52 (77.6)	.065
Jug r 1	1 (33.3)	0 (0.0)	1 (6.3)	7 (35.0)	0 (0.0)	4 (6.0)	.001
Jug r 2	1 (33.3)	1 (25.0)	1 (6.7)	6 (30.0)	1 (7.7)	1 (1.5)	.007
Jug r 2 LMW	1 (33.3)	1 (33.3)	3 (20.0)	8 (40.0)	3 (23.1)	8 (12.3)	.527
Jug r 3	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.0)	1 (7.1)	6 (9.0)	.002
Jug r 4	1 (33.3)	0 (0.0)	1 (6.7)	6 (30.0)	0 (0.0)	2 (3.1)	.001
Jug r 5	0 (0.0)	0 (0.0)	14 (93.3)	18 (90.0)	11 (84.6)	43 (62.5)	<.001
Jug r 6	1 (33.3)	0 (0.0)	0 (0.0)	5 (25.0)	0 (0.0)	3 (4.6)	.005
Jug r 7	0 (0.0)	0 (0.0)	1 (6.3)	9 (45.0)	1 (7.1)	19 (28.4)	.041
Probable walnut allergy	5 (55.6)	5 (50.0)	29 (60.4)	37 (54.4)	42 (85.7)	70 (66.0)	<.001

LMW, Low molecular weight.

All measurements are in n (%) unless otherwise specified.

*Symptom severity: mild = isolated oral allergy symptoms; moderate = symptoms of the skin, eyes, upper airway, or gastrointestinal system; severe = dysphagia, dysphonia, lower respiratory, cardiovascular or neurological symptoms, or anaphylaxis.

†The results show the number and percentage of subjects with positive sensitization according to each test. SPT with walnut extract was performed in 517 subjects; ImmunoCAP with walnut extract in 513 subjects.

‡For some centers (Łódź, Sofia, Strasbourg, Vilnius, and Zürich), the results of 1 or 2 of the individual CRD tests were missing. The percentage given in parentheses is the percentage of the total number of available CRD results. *P* values were determined for exploratory purposes (no correction for multiple testing) using the Pearson χ^2 test for categorical variables and the ANOVA or Kruskall-Wallis test for continuous variables.

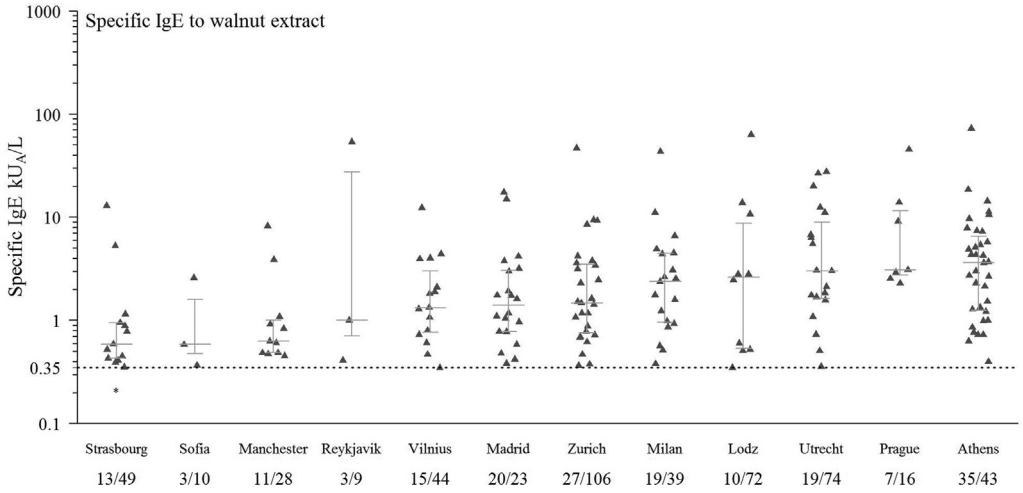

FIGURE 1. IgE to walnut extract across Europe. Walnut specific IgE levels in subjects with positive serology to walnut extract in ImmunoCAP ($\geq$0.35 kU_A/L). The triangles represent individual subjects, and the lines indicate medians and interquartile ranges. n/N = number of subjects with positive serology/number of subjects in whom ImmunoCAP with walnut extract was performed. *Significantly different from Prague, Athens, and Utrecht.

Analyses were conducted with SPSS version 25 and R version 3.4.1.

RESULTS

Population characteristics

As the fourth most commonly reported causative food in the EuroPrevall outpatient clinic study, walnut was reported to elicit symptoms in 531 (23.4%) subjects, most often in Utrecht (37.0%) and least often in Reykjavik (6.3%). Most were female (64.8%) and older than 18 years (84.6%) (Table I).

The most commonly reported symptoms were oral allergy symptoms in 426 of 531 (80.2%) subjects, of which 214 had no other symptoms. Symptoms of the upper airway, skin, and digestive system were reported by respectively 33.3%, 32.0%, and 23.2% of subjects. Fewer subjects reported lower airway (15.1%), cardiovascular (2.4%), or neurological (3.2%) symptoms. Anaphylaxis was reported by 15 subjects (2.8%).

Walnut sensitization patterns across Europe

SPT and ImmunoCAP with walnut extract were positive in 40.8% and 35.5% of subjects (Table I). Positive serology to walnut extract was found in less than 30% of subjects reporting symptoms to walnut from Łódź, Strasbourg, Utrecht, and Zürich, but in more than 80% of subjects from Athens and Madrid. In subjects with positive serology to walnut extract, median IgE levels were lowest in Strasbourg, Sofia, and Manchester, and highest in Milan, Łódź, Utrecht, Prague, and Athens (Figure 1).

Sensitization by CRD was assessed in 202 subjects, and 79.4% of the 199 subjects with complete CRD results were found to be sensitized to at least 1 individual walnut component by ImmunoCAP. The distribution of IgE levels in subjects sensitized to a specific walnut component is shown in Figure 2. Median IgE levels for PR-10 protein Jug r 5 were highest.

Of the subjects with *negative* SPT result and ImmunoCAP to walnut extract (N = 237), in whom CRD with all walnut components was completed (N = 79), 70.9% were sensitized to at least 1 component (N = 56 of 79), most frequently to Jug r 5 (N = 50 of 79 [63.3%]) (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

For international comparison of walnut component sensitization patterns, only those centers where CRD results were available for at least 10 subjects were taken into account (Table I; Figure 3). Sensitization to PR-10 protein Jug r 5 was most prevalent everywhere except in Athens and Madrid. In Athens, sensitization to LTP Jug r 3 dominated. Besides Athens, LTP sensitization occurred most frequently in other Southern centers, Madrid and Milan. Sensitization to profilin Jug r 7 was most common after sensitization to Jug r 5, and was particularly recognized in Utrecht, Milan, Madrid, Zürich, and Athens. Storage proteins Jug r 1, 2, 4, and 6 were recognized in up to 10% of subjects overall, all most frequently in Utrecht, followed by Madrid.

Relationship between IgE to walnut components and other allergens

Figure 4 and Figure E1 in this article's Online Repository at www.jaci-inpractice.org reveal how IgE levels to walnut components correlated with IgE levels to pollen and other foods.

Regarding pollen, the strongest correlation overall was between IgE to Jug r 5 and birch (see Table E3 in this article's Online Repository at www.jaci-inpractice.org; ρ = 0.92). This positive correlation was prominent in all evaluated centers (ρ = 0.75-0.97), except Madrid and Athens. In Madrid, the strongest correlation between a walnut component and pollen was between Jug r 7 and grass pollen (ρ = 0.70). In Athens, the correlations between Jug r 3 and mugwort, *Chenopodium*, and plane tree pollen (ρ = 0.76-0.86) were most remarkable.

Regarding IgE levels to food extracts other than walnut, the overall strongest correlations were found between Jug r 5 and hazelnut (ρ = 0.88), and between Jug r 3 and lentil (ρ = 0.80). However, the walnut components most likely to show strong correlations with the various foods differed per center (see Table E4 in this article's Online Repository at www.jaci-inpractice.org). For example, IgE levels to hazelnut correlated strongly with Jug r 5 IgE levels in most centers, but with Jug r 3

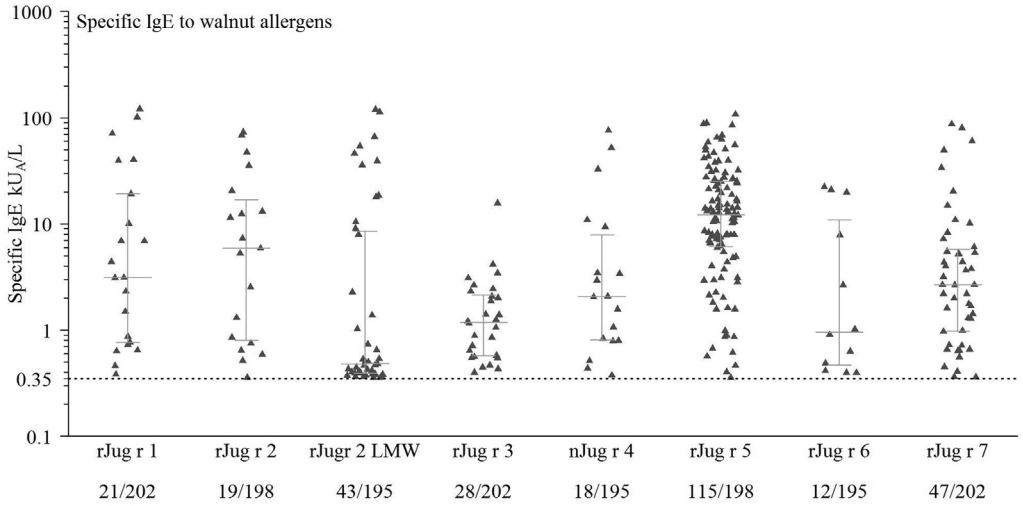

FIGURE 2. IgE to walnut allergens. Walnut allergen specific IgE levels in subjects with positive serology to the respective walnut allergens in ImmunoCAP (≥0.35 kU$_A$/L). The triangles represent individual subjects, and the lines indicate medians and interquartile ranges. n/N = number of subjects with positive serology/number of subjects in whom ImmunoCAP with walnut allergen was performed.

IgE levels in Athens. Lentil IgE levels were found to correlate strongly with different walnut components in each center, but never with Jug r 5 or Jug r 7.

Predictors for severity of walnut allergy

Probable walnut allergy, where reported symptoms were supported by IgE sensitization, was identified in 336 subjects (Table I). Of these 336 subjects, 246 (73.2%) had mild to moderate symptoms and 90 (26.8%) had severe symptoms.

The results from univariable analyses are listed in Table II. Regarding clinical history, subjects with severe walnut allergy were significantly more likely to have mugwort allergy, and significantly less likely to have birch pollen allergy or IgE sensitization to cat or dog, than subjects with mild to moderate walnut allergy. Although not statistically significant, severely allergic subjects were more often sensitized to walnut in SPT, and had higher median IgE levels to walnut extract in ImmunoCAP. No significant differences between severity groups were found regarding the percentage of subjects sensitized to specific walnut allergens, or median IgE levels, although trends among sensitized subjects suggested higher IgE levels to storage proteins and LTP in severely allergic and to PR-10 and profilin in mild to moderately allergic subjects (see Table E5 in this article's Online Repository at www.jaci-inpractice.org).

CRD was performed in 177 of 336 subjects with probable walnut allergy. These 177 subjects were included in the multivariable analyses for prediction of severity of walnut allergy. Table III presents the results of the Lasso regression analysis. Of all the demographic and clinical history variables included in model 1, Lasso regression selected "symptoms upon skin contact with walnut," "family history of atopic disease," "atopic dermatitis," and "mugwort pollen allergy," which were positively associated with severe walnut allergy, and "IgE sensitization to cat or dog," which was inversely associated with severe walnut allergy. In model 2, all the variables selected in model 1 remained. In addition, SPT positivity to walnut was selected as an extra predictor (positive association). Finally, in model 3, IgE levels to Jug r 1, Jug r 5, Jug r 7, and Ana c 2 were found to further contribute to prediction of severity of walnut allergy.

Although walnut SPT positivity was selected as an additional predictor in model 2, model accuracy remained similar to model 1 (AUC = 0.74 in both models). Addition of CRD in model 3 significantly increased the AUC to 0.81 (P_{DeLong}= .002).

Additional analyses of the performance of individual tests revealed that combinations of tests as defined in the Lasso regression models better predicted severity than SPT to walnut, ImmunoCAP to walnut extract, or ImmunoCAP to individual walnut allergens (evaluated separately or combined), for which AUCs ranged from 0.48 to 0.66 (see Table E6 in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

The current study is the largest European multicenter study on walnut allergy to date. Clear geographical differences were observed in walnut component sensitization and cosensitization patterns, and our predictive model combining demographic, clinical, and serological variables attained good accuracy with an AUC of 0.81 for distinguishing mild to moderate from severe walnut allergy.

Walnut allergy across Europe: Distribution of allergen (co)sensitization patterns

The distribution of sensitization to walnut components across Europe was found to follow the same pattern as many other plant-source foods, including other tree nuts[17]: sensitization to PR-10 proteins (Jug r 5) in Northern and Central Europe,[18] sensitization to profilin (Jug r 7) throughout Europe,[19] and sensitization to LTPs (Jug r 3) in the Mediterranean.[20]

The highest overall sensitization rates were found for Jug r 5 and Jug r 7. Pollen exposure helps explain their geographical distribution, because sensitization to plant food PR-10 proteins and profilins is induced by similar proteins in pollen.[6,21] Jug r 5 is homologous with Bet v 1, the major allergen of birch pollen, the dominating pollen in Northern and Central Europe.[18] Jug r

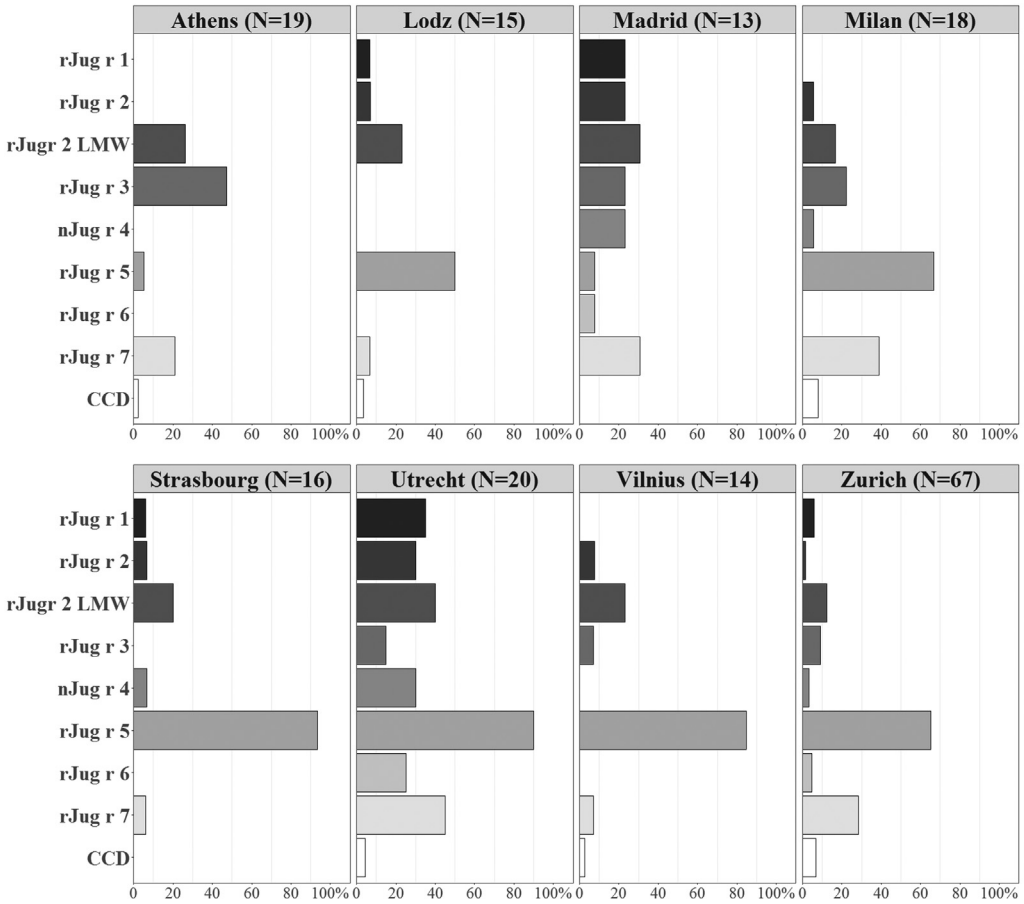

FIGURE 3. IgE sensitization to walnut components across Europe. N = the total number of subjects in whom CRD was performed. The number of subjects in whom CRD was positive is visible for each center in Table I. Only those centers where CRD was completed in at least 10 subjects are shown. The length of the bars corresponds with the percentage of subjects with positive serology to each specific walnut allergen.

7 sensitization, on the other hand, could be secondary to sensitization to almost any type of pollen, because all pollen contains profilin. Our findings were consistent with these patterns of cross-reactivity (Figure 4; Table E3): IgE to Jug r 5 showed strong correlations with IgE to birch pollen ($\rho = 0.92$), and IgE to Jug r 7 moderate to strong correlations ($\rho > 0.60$) with IgE to almost all pollen.

Sensitization to Jug r 3 is generally thought to occur through peach as primary sensitizer,[20,22-24] although plane tree and mugwort pollen have also been suggested as primary sources of sensitization to LTP.[25-27] Indeed, IgE to Jug r 3 correlated with IgE to peach, plane tree, and mugwort in our data ($\rho > 0.60$), but also to other LTP-containing pollen (eg, *Chenopodium, Parietaria*, and cypress), fruits (tomato, apple, kiwi), and legumes (lentil, soybean, peanut).[20] Future studies with IgE inhibition assays could help further differentiate between independent cosensitization and cross-reactivity, and identify primary sources of sensitization to Jug r 3 and other walnut components.

Similar distributions of Jug r 3 and Jug r 5 sensitization were observed by Ballmer-Weber et al,[5] in Germany, Switzerland, and Spain.[5] However, occurrence of sensitization to walnut storage proteins was more frequent in their data (48%-57%) than in ours (7%-10%). This is likely due to the diverse study populations, which in the study of Ballmer-Weber et al included more severely allergic subjects, more pediatric subjects, and more subjects with onset of symptoms before the age of 14 years, all of which make primary sensitization more likely.

Notably, a high proportion of subjects sensitized to Jug r 5 tested negative to walnut extract (Tables I and E2), as has also been observed previously.[28] This finding substantiates that the concentration of Jug r 5 is low in walnut extract, causing a low sensitivity of extract-based tests for subjects with birch-pollen–related walnut allergy.

Walnut allergy across Europe: Prediction of severity

A model combining symptoms upon skin contact with walnut, history of atopic dermatitis, family history of atopic disease, mugwort pollen allergy, sensitization to cat or dog, positive SPT result for walnut, and IgE to Jug r 1, Jug r 5, Jug r 7, and cross-reactive carbohydrate determinant was found to have the highest accuracy for predicting severity of walnut allergy (AUC, 0.81; 95% CI, 0.73-0.89).

Our findings suggest that sensitization via the cutaneous route may be associated with severity of walnut allergy. Several studies have established that atopic dermatitis predisposes to food sensitization and allergy, presumably as a result of skin

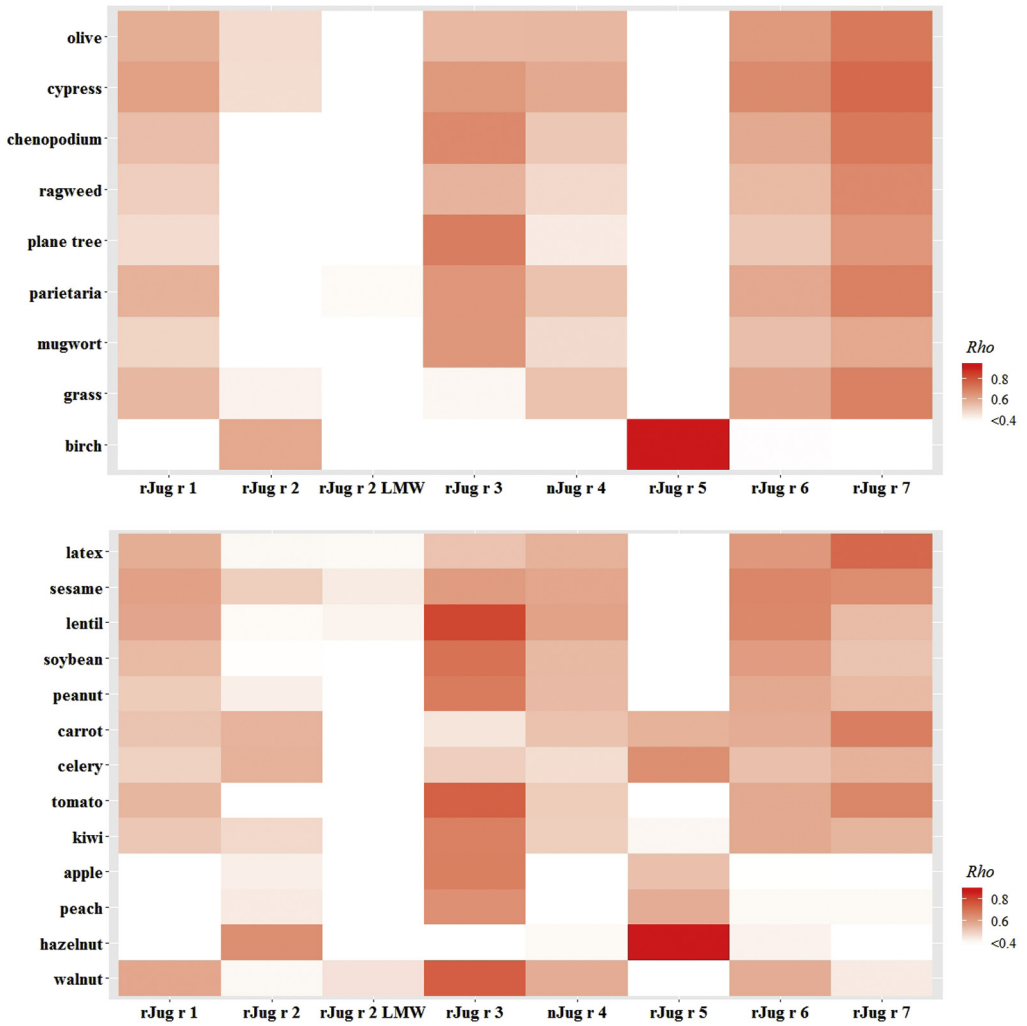

FIGURE 4. Correlation between IgE levels to walnut components and pollen and other foods. The numeric values of the Spearman rho correlation coefficients are available from Table E3.

barrier impairement.[29] In line with our findings, having atopic dermatitis was previously found to be associated with severe hazelnut allergy.[9] One could speculate that sensitization via the skin leads to primary (non—cross-reactive) food sensitization, which is thought to be associated with more severe reactions.[30]

In cross-reactive food allergy, pollen is generally the primary sensitizer, with sensitization most probably occurring through the respiratory tract. Symptomatic subjects generally present with mild symptoms.[18,21] As remarked previously, subjects with a birch-pollen—related walnut allergy are poorly detected by diagnostic tests with walnut extract, explaining the positive association between SPT and severe walnut allergy.

Remarkably, mugwort pollen allergy almost quadrupled the odds of severe walnut allergy. LTP sensitization, which is associated with severe allergic reactions to plant-source foods,[31] could be the link. It has been suggested that sensitization to mugwort LTP (Art v 3) can facilitate subsequent sensitization to LTP in plant-source foods, and the other way around.[26,32] However, the observation that Jug r 3 IgE levels were not predictive of walnut allergy severity makes this explanation less likely. Another plausible explanation is that other still uncharacterized mugwort allergens are associated with severe walnut allergy.

Addition of walnut component testing was found to considerably improve prediction of walnut allergy severity. Our expectations were that sensitization to PR-10 proteins and profilins would be associated with mild to moderate walnut allergy, and that sensitization to seed storage proteins and LTPs would predict severe walnut allergy.[5,6,9] The former associations were indeed confirmed in our data; IgE levels to Jug r 5 and 7 were predictive of mild to moderate walnut allergy. IgE to walnut storage proteins appears to be of lesser importance in prediction of walnut allergy severity in subjects from the general population, in whom such sensitization occurs infrequently. We have no clear explanation for why IgE to Jug r 1 was inversely associated with severity in our data.

Overall, the prediction models in this study provide insight into the clinical profile of subjects more likely to have mild to moderate or severe reactions to walnut, and suggest some particular focus areas during diagnostic workup of walnut allergy. Besides obtaining information on allergic comorbidities and family atopy, as is standard in clinical history for food allergy,

TABLE II. Characteristics of subjects with probable walnut allergy related to severity

Characteristic	Mild to moderate probable walnut allergy (N = 246)	Severe probable walnut allergy (N = 90)	*P*	Univariable OR (95% CI)
Demographic				
Age (y), mean ± SD	29.9 ± 13.0	28.4 ± 12.5	.972	0.99 (0.97-1.01)
Female sex	147 (59.8)	47 (52.2)	.216	0.74 (0.45-1.98)
Clinical history				
Age onset of symptoms <14 y	97 (39.8)	38 (42.2)	.683	1.11 (0.67-1.81)
Symptoms upon skin contact with walnut	9 (4.1)	7 (8.8)	.117	2.23 (0.77-6.19)
Family history of atopic disease	152 (67.6)	60 (71.4)	.514	1.20 (0.70-2.11)
Atopic dermatitis (ever)	68 (28.2)	32 (36.4)	.155	1.45 (0.86-2.43)
Asthma (ever)	229 (97.0)	86 (96.6)	.851	0.88 (0.24-4.14)
Birch pollen allergy	153 (64.6)	44 (51.8)	.038	0.59 (0.36-0.97)
Grass pollen allergy	138 (58.5)	53 (62.4)	.532	1.18 (0.71-1.97)
Mugwort pollen allergy	31 (13.3)	20 (23.0)	.035	1.95 (1.03-3.62)
Planetree pollen allergy	17 (7.4)	8 (9.2)	.595	1.27 (0.50-2.97)
House-dust mite allergy	66 (28.1)	23 (26.7)	.812	0.94 (0.53-1.61)
Latex allergy	12 (5.1)	5 (5.7)	.813	1.14 (0.35-3.17)
Cat/dog sensitization	173 (73.6)	53 (60.9)	.027	0.56 (0.33-0.94)
Sensitization to walnut*				
SPT walnut positive	150 (61.5)	61 (68.5)	.236	1.37 (0.82-2.31)
IgE level walnut extract	0.39 (0.05-1.70)	0.73 (0.15-3.63)	.018	1.02 (0.99-1.05)
IgE level Jug r 1	0.01 (0.00-0.06)	0.01 (0.00-0.05)	.719	1.00 (0.95-1.02)
IgE level Jug r 2	0.05 (0.02-0.13)	0.04 (0.01-0.08)	.516	1.02 (0.98-1.06)
IgE level Jug r 2 LMW	0.24 (0.17-0.36)	0.23 (0.15-0.32)	.571	1.01 (0.99-1.04)
IgE level Jug r 3	0.04 (0.01-0.17)	0.05 (0.01-0.12)	.739	0.93 (0.54-1.21)
IgE level Jug r 4	0.03 (0.01-0.09)	0.02 (0.01-0.06)	.215	1.00 (0.93-1.05)
IgE level Jug r 5	6.69 (0.03-16.83)	1.60 (0.02-9.11)	.118	0.97 (0.94-1.00)
IgE level Jug r 6	0.03 (0.01-0.07)	0.02 (0.01-0.07)	.399	1.04 (0.91-1.16)
IgE level Jug r 7	0.02 (0.00-0.65)	0.02 (0.00-0.18)	.503	0.92 (0.75-1.00)

LMW, Low molecular weight; *OR*, odds ratio.
All measurements are in n (%) or median (Q1-Q3) unless otherwise specified. All IgE levels were measured in kU_A/L on ImmunoCAP.
*For subjects with mild to moderate and severe probable walnut allergy, SPT was performed in respectively 244 and 89 subjects; ImmunoCAP with walnut extract in 240 and 89 subjects; and CRD in 136 and 41 subjects.

physicians assessing walnut allergy should find out whether presenting patients are allergic to mugwort or have symptoms elicited by skin contact with walnut. Information on cross-reactive sensitization (Jug r 5, Jug r 7, cross-reactive carbohydrate determinant) contributes to prediction of a more mild phenotype. Because Jug r 5 is underrepresented in walnut extract, diagnostic workup in birch-endemic areas would benefit from additional testing of Jug r 5. After validation, the prediction of a mild to moderate phenotype using our final model could potentially translate into performance of fewer challenge tests in clinical practice (Table E6).

Strengths and limitations

All in all, this is the largest study to map walnut sensitization across Europe. The consistent and standardized approach to data collection makes our results particularly valuable. We did not include subjects with walnut allergy determined by food challenge, but all subjects presenting to an allergy clinic with symptoms to walnut within 2 hours of ingestion, and corresponding IgE sensitization. Through this approach, we likely captured more subjects with pollen-related walnut allergy, who form a significant proportion of walnut-allergic subjects in Europe. We have also, for the first time, suggested a prediction model for assessing severity of walnut allergy, taking both clinical evaluation and serology testing into account. The main limitation of our study was that complete CRD data were available for only 177 of 336 walnut-allergic subjects. Multiple imputation and penalized regression were applied to appropriately deal with sparse data, and models 1 and 2 were also developed in the total population of 336 walnut-allergic subjects, revealing no relevant differences. However, it is important to realize that we could not adjust the multivariable analyses for center due to sparsity of data. Although we do not expect the effect of predictors on severity to depend on center, we do observe geographically varying baseline prevalence of severe walnut allergy (Table I).

CONCLUSIONS

We confirm that cross-reactivity with pollen is a major cause of walnut sensitization and allergy across Europe, leading to molecular recognition patterns similar to those of other plant-source foods. PR-10 protein and profilin sensitization occur frequently, and predict a mild to moderate walnut allergy phenotype. Sensitization to walnut storage proteins is less common. The information obtained from walnut CRD, in combination with results from extract-based testing and clinical background evaluation, allows for good discrimination between mild to moderate and severe walnut allergy. A prediction model

TABLE III. Prediction models for walnut allergy severity

	Model 1: Demographics and clinical history		Model 2: Model 1 selection + sensitization to walnut		Model 3: Model 2 selection + sensitization to walnut components	
	OR	95% CI	OR	95% CI	OR	95% CI
Symptoms upon skin contact with walnut	1.95	1.51-2.53	2.32	1.48-3.63	2.43	1.58-3.75
Family history atopic disease	1.65	1.49-1.82	1.97	1.74-2.23	2.69	2.35-3.07
Atopic dermatitis	1.89	1.64-2.19	2.12	1.82-2.48	2.68	2.26-3.18
Mugwort pollen allergy	1.96	1.66-2.32	2.28	1.93-2.69	3.75	3.18-4.42
Cat/dog sensitization	0.41	0.36-0.48	0.34	0.30-0.40	0.40	0.35-0.46
SPT walnut positive			1.06	0.94-1.18	1.07	0.96-1.20
IgE level Jug r 1					0.99	0.98-1.00
IgE level Jug r 5					0.97	0.97-0.97
IgE level Jug r 7					0.98	0.97-0.98
IgE level Ana c 2					0.63	0.55-0.73
Intercept	−1.32		−1.45		−1.52	
AUC (95% CI)	0.74 (0.65-0.83)		0.74 (0.65-0.83)		0.81 (0.73-0.89)	

OR, Odds ratio.
All IgE levels were measured in kU_A/L on ImmunoCAP. The 95% CIs for each coefficient were calculated from SEs obtained for each imputed data set through bootstrapping, and pooled over the 10 imputed data sets using Rubin's rules.
Unselected variables model 1: age, sex, age at onset of symptoms to walnut (<14 vs ≥14 y), asthma, birch/grass/plane tree pollen allergy, house-dust mite allergy, latex allergy. Unselected variables model 2: IgE level walnut extract. Unselected variables model 3: IgE level Jug r 2, Jug r 3, Jugr4, and Jug r 6.

combining this information performs significantly better than CRD, extract-based testing, or clinical background alone.

Acknowledgments

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ONLINE REPOSITORY

SUPPLEMENTARY METHODS

Skin prick testing

SPT was performed with commercially available extracts (ALK-Abelló, Madrid, Spain) following guidelines of the European Academy of Allergology and Clinical Immunology.[E1]

IgE testing

IgE levels in serum were measured by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP analyses with extracts were performed at the Paul-Ehrlich-Institut (Langen, Germany). ImmunoCAP analyses with walnut components were carried out at the Amsterdam University Medical Centers (Location AMC, Amsterdam, The Netherlands).

Low-molecular-weight fraction of Jug r 2

The low-molecular-weight fraction of Jug r 2 consists of the N-terminal region of Jug r 2, which is removed during maturation. It does not contain any of the mature Jug r 2 cupin domains. In the nut, the N-terminal region is found as 6 individual peptides. Here, they are expressed as 1 polypeptide chain. IgE to low-molecular-weight fraction of Jug r 2 was not included as a candidate predictor for prediction of severity of walnut allergy, because a considerable number of walnut-allergic subjects without sensitization to Jug r 2 were sensitized to low-molecular-weight fraction of Jug r 2 at an IgE level below 1.0 kU_A/L, which in part may be due to an elevated background of this experimental assay.

Symptom severity classification

For classification of severe symptoms, *lower airway symptoms* included dyspnea, wheezing, cough, or chest tightness; *cardiovascular symptoms* consisted of cardiac arrhythmia, myocardial ischemia, or hypotension; *neurological symptoms* comprised disorientation/confusion, dizziness, seizures, incontinence, or loss of consciousness; and *anaphylaxis* included reactions with severe laryngeal edema, severe bronchospasm, or hypotensive shock. For classification of mild to moderate symptoms, *skin symptoms* included urticaria, angioedema, erythema/flushing, or itching; *eye symptoms* comprised conjunctivitis; *upper airway symptoms* consisted of rhinitis, conjunctivitis, or tightness of throat; and *gastrointestinal symptoms* comprised stomach pain, cramps, nausea, vomiting, and diarrhea.[E2,E3]

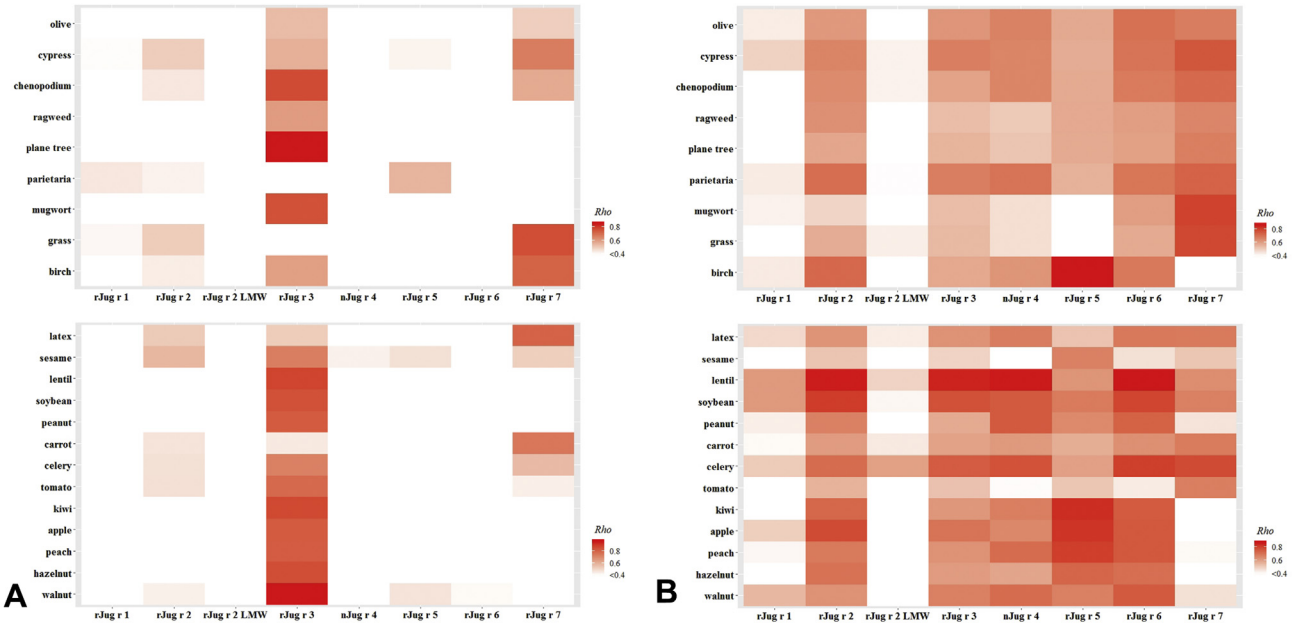

FIGURE E1. Correlation between IgE levels to walnut components and pollen and other foods per center with at least 10 subjects completing CRD testing. (**A**) Athens (N = 19). (**B**) Łódź (N = 15). (**C**) Madrid (N = 13).(**D**) Milan (N = 18). (**E**) Strasbourg (N = 16). (**F**) Utrecht (N = 20). (**G**) Vilnius (N = 14). (**H**) Zürich (N = 67). Only those centers with at least 10 subjects completing CRD were evaluated separately. Too few subjects completed CRD in Prague (N = 8), Manchester (N = 5), Reykjavik (N = 3), and Sofia (N = 4) to determine valid correlations.

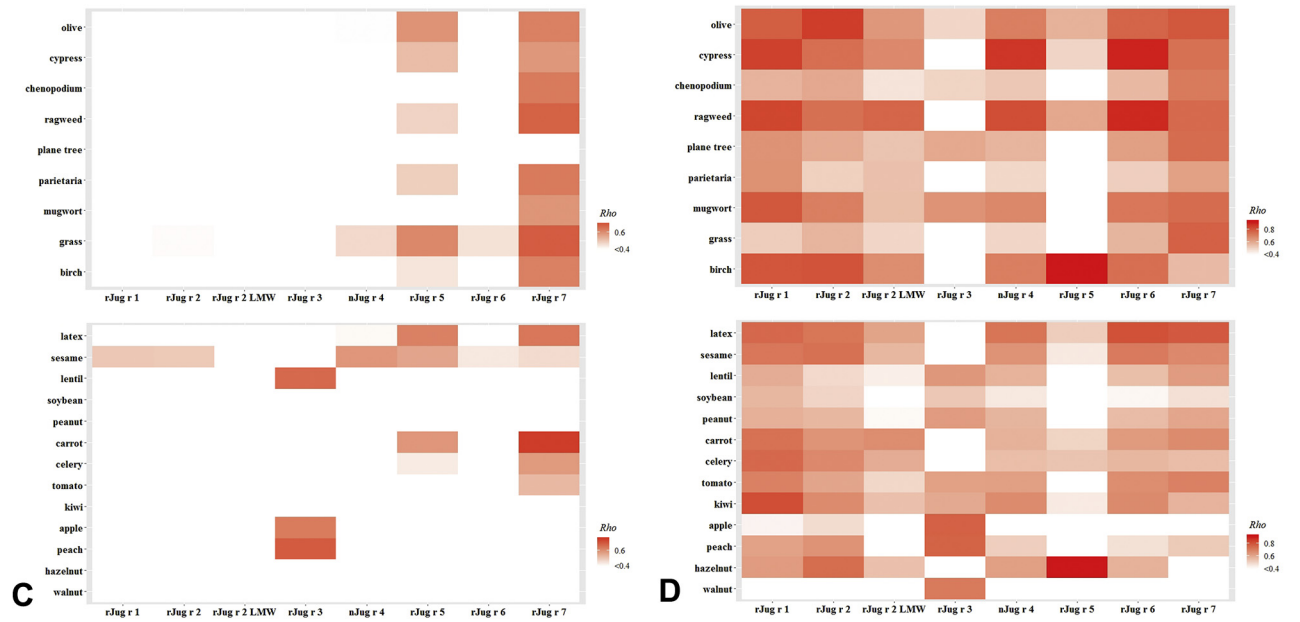

FIGURE E1. (*CONTINUED*).

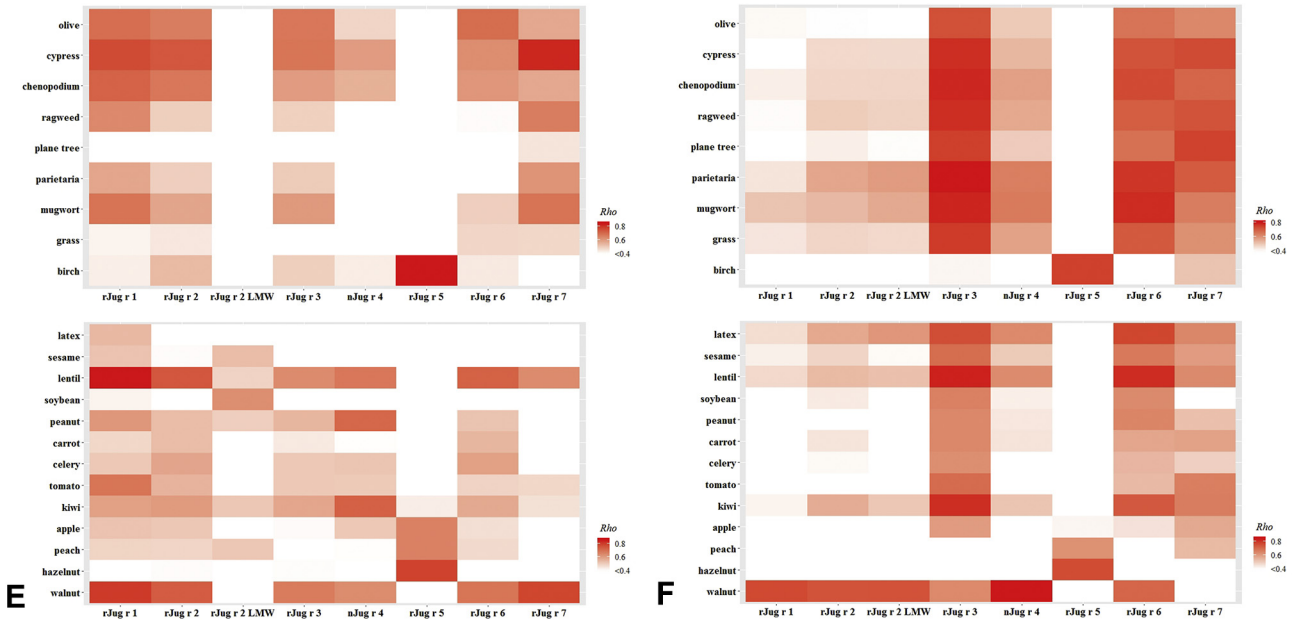

FIGURE E1. (*CONTINUED*).

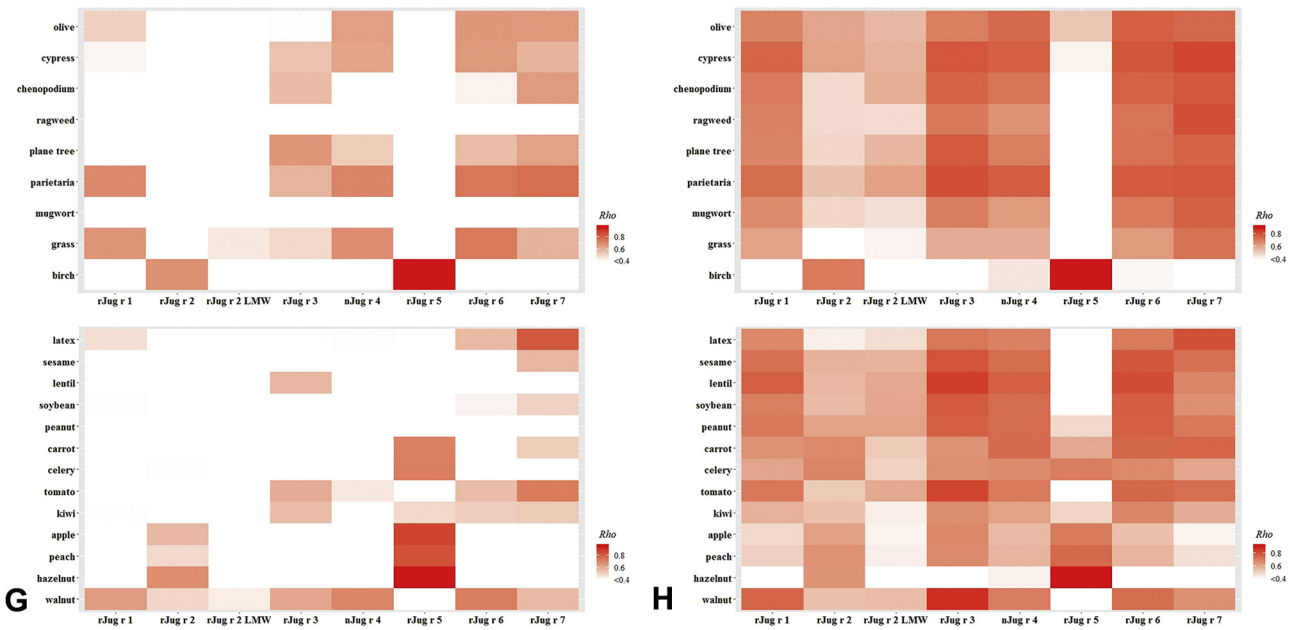

FIGURE E1. (*CONTINUED*).

TABLE E1. Missing data in variables included for Lasso regression

Characteristic	Missing (total N = 177)
Age	0
Female sex	0
Clinical history	0
Age at onset of symptoms	21
Symptoms upon skin contact with walnut	14
Family history of atopic disease	6
Atopic dermatitis	3
Asthma	2
Birch pollen allergy	5
Grass pollen allergy	7
Mugwort pollen allergy	4
Planetree pollen allergy	7
House-dust mite allergy	6
Latex allergy	0
Cat/dog sensitization	0
SPT walnut positive	0
IgE level walnut extract	0
IgE level Jug r 1	0
IgE level Jug r 2	2
IgE level Jug r 2 LMW	4
IgE level Jug r 3	0
IgE level Jug r 4	4
IgE level Jug r 5	2
IgE level Jug r 6	4
IgE level Jug r 7	0

LMW, Low molecular weight.
Values for these missing data were estimated using multiple imputation procedures, for which all the above determinants were included as covariates, along with severity of walnut allergy, IgE levels to other foods (hazelnut, peach, apple, kiwi, tomato, carrot, celery, peanut, soybean, lentils, sesame seed), and center.

TABLE E2. IgE to walnut components in subjects with negative walnut SPT and ImmunoCAP result

	Negative ImmunoCAP result			Negative SPT result			Negative ImmunoCap and SPT result		
		IgE level (kU_A/L)			IgE level (kU_A/L)			IgE level (kU_A/L)	
Variable	N	Median	IQR	N	Median	IQR	N	Median	IQR
Total	331			306			237		
CRD performed	120			115			82		
CRD positive	88 of 117			85 of 112			56 of 79		
Jug r 1	3 of 120	0.73	0.69-0.80	5 of 115	1.50	0.63-3.14	0 of 82	—	—
Jug r 2	3 of 117	0.75	0.70-0.80	5 of 112	0.59	0.52-0.65	1 of 79	0.65	—
Jug r 2 LMW	15 of 115	0.40	0.37-0.50	15 of 110	0.42	0.38-0.50	7 of 77	0.38	0.36-0.41
Jug r 3	1 of 120	0.89	—	9 of 115	0.89	0.55-1.41	1 of 82	0.89	—
Jug r 4	1 of 115	0.79	—	6 of 110	0.66	0.46-0.83	1 of 77	0.84	—
Jug r 5	79 of 117	11.46	5.20-23.46	67 of 112	10.75	5.76-20.57	50 of 79	9.44	4.90-19.13
Jug r 6	1 of 115	0.91	—	4 of 110	0.52	0.41-0.73	0 of 77	—	—
Jug r 7	21 of 120	1.62	0.72-4.02	29 of 115	3.91	1.31-6.39	16 of 82	1.91	0.72-5.01

LMW, Low molecular weight.

TABLE E3. Correlations between IgE levels to walnut components and pollen and other foods

	Walnut allergen							
IgE	**Jug r 1**	**Jug r 2**	**Jugr 2 LMW**	**Jug r 3**	**Jug r 4**	**Jug r 5**	**Jug r 6**	**Jug r 7**
Birch	0.33	0.60	*0.18*	*0.22*	0.35	0.92	0.40	0.39
Grass	0.57	0.43	0.32	0.42	0.54	0.27	0.61	0.70
Mugwort	0.50	0.38	0.33	0.64	0.48	*0.21*	0.55	0.61
Parietaria	0.58	0.37	0.41	0.65	0.54	*0.19*	0.60	0.70
Plane tree	0.48	0.32	0.34	0.71	0.45	*0.18*	0.53	0.65
Ragweed	0.51	0.36	0.31	0.58	0.49	*0.24*	0.56	0.68
Chenopodium	0.55	0.36	0.38	0.68	0.53	*0.18*	0.60	0.72
Cypress	0.62	0.48	0.37	0.64	0.60	0.33	0.67	0.75
Olive	0.59	0.48	0.37	0.56	0.57	0.37	0.64	0.72
Latex	0.57	0.42	0.41	0.53	0.57	*0.20*	0.62	0.73
Sesame seed	0.61	0.50	0.44	0.61	0.59	0.27	0.67	0.65
Lentil	0.60	0.41	0.43	0.80	0.60	*0.14*	0.66	0.54
Soybean	0.55	0.40	0.40	0.71	0.55	*0.20*	0.61	0.53
Peanut	0.51	0.44	0.38	0.69	0.55	0.31	0.58	0.55
Carrot	0.53	0.56	0.33	0.45	0.53	0.57	0.58	0.68
Celery	0.50	0.57	0.30	0.51	0.47	0.65	0.53	0.57
Tomato	0.56	0.38	0.37	0.75	0.51	*0.20*	0.58	0.66
Kiwi	0.52	0.48	0.32	0.68	0.50	0.42	0.58	0.56
Apple	0.36	0.44	*0.21*	0.68	0.33	0.54	0.40	0.38
Peach	0.36	0.44	*0.23*	0.64	0.32	0.58	0.42	0.41
Hazelnut	0.37	0.64	*0.23*	0.28	0.41	0.88	0.43	0.29
Walnut	0.59	0.42	0.46	0.75	0.58	*0.01*	0.58	0.44

LMW, Low molecular weight.

All correlations are Spearman rho correlations. Italics: NOT statistically significant after Bonferroni correction ($P < .007$ for pollen and $P < .00025$ for food/latex). For all other correlations, the P values were smaller than the Bonferroni-corrected P values.

TABLE E4. Food extract IgE levels correlating strongly with walnut components

Center	Jug r 1	Jug r 2	Jug r 2 LMW	Jug r 3	Jug r 4	Jug r 5	Jug r 6	Jug r 7
Zürich	Tomato Peanut Lentil Sesame	—	—	**Tomato** Peanut **Lentil** Soy **Sesame**	Carrot Tomato Peanut Lentil Soy Sesame	HN Peach Apple Celery	Carrot Tomato Peanut **Lentil** Soy **Sesame**	Carrot Tomato Peanut Sesame
Madrid	—	—	—	Peach	—	—	—	Carrot
Athens	—	—	—	**HN** **Peach** **Apple** **Kiwi** Tomato Celery **Peanut** **Soy** **Lentil** Sesame	—	—	—	Carrot
Utrecht				**Kiwi** Tomato **Lentil** Sesame		HN	Kiwi **Lentil**	—
Łódź	—	HN Apple Kiwi Celery **Soy** **Lentil**	—	Celery **Lentil** Soy	Peach Celery Peanut Soy **Lentil**	HN **Peach** **Apple** **Kiwi**	HN Peach Apple Kiwi **Celery** Peanut **Soy** **Lentil**	Celery
Vilnius	—	—	—	—	—	**HN** **Peach** **Apple** Celery Carrot	—	Tomato
Milan	**Kiwi** Celery Carrot Sesame	HN Sesame	—	Peach Apple	—	**HN**	Sesame	—
Strasbourg	**Lentil**	Lentil	—	—	Kiwi Peanut	HN	Lentil	

LMW, Low molecular weight.
This table shows the food extracts, other than walnut, of which the IgE levels correlated strongly with IgE levels to walnut components in each center. Only those foods with $\rho \geq 0.7$ and $\rho \geq 0.8$ (bold) are shown. Only those centers with at least 10 subjects completing CRD were evaluated.

TABLE E5. IgE levels related to severity of walnut allergy in subjects with positive serology

	Mild to moderate probable walnut allergy (N = 246)			Severe probable walnut allergy (N = 90)			
ImmunoCAP	Total tested (N)	Total positive,* N (%)	Median IgE level (kU_A/L) (IQR)	Total tested (N)	Total positive,* N (%)	Median IgE level (kU_A/L) (IQR)	*P*
Walnut extract	240	127 (52.9)	1.34 (0.73-3.84)	89	55 (61.8)	2.31 (1.02-7.77)	.049
Jug r 1	136	14 (10.3)	3.13 (0.68-32.30)	41	7 (17.1)	4.40 (1.59-13.12)	.765
Jug r 2	135	13 (9.6)	5.31 (0.75-13.15)	40	6 (15.0)	9.44 (2.48-29.62)	.726
Jug r 2 LMW	134	35 (26.1)	0.46 (0.39-1.66)	39	8 (20.5)	5.97 (0.47-46.21)	.126
Jug r 3	136	23 (16.9)	1.17 (0.56-2.05)	41	5 (12.2)	1.89 (1.06-2.65)	.529
Jug r 4	134	14 (10.4)	1.57 (0.79-3.29)	39	4 (10.3)	6.42 (2.99-15.25)	.167
Jug r 5	135	91 (67.4)	12.99 (6.63-27.59)	40	24 (60.0)	7.92 (2.63-27.59)	.101
Jug r 6	134	9 (6.7)	0.91 (0.41-2.67)	39	3 (7.7)	7.88 (4.18-13.92)	.518
Jug r 7	136	38 (27.9)	3.42 (1.07-6.97)	41	9 (22.0)	2.00 (0.55-2.68)	.176

LMW, Low molecular weight.
*IgE $\geq$ 0.35 kU_A/L.

TABLE E6. Accuracy of individual diagnostic tests and models for severity of walnut allergy

Individual test	AUC	Positivity threshold		Mild to moderate	Severe	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Walnut SPT	0.54 (0.44-0.65)	0.50	<	77	20	51.2	35.1-67.1	56.6	47.9-65.1	26.3	17.0-37.3	79.4	70.0-86.9
			≥	59	21								
Walnut ImmunoCAP	0.54 (0.43-0.64)	0.35	<	75	20	51.2	35.1-67.1	55.2	46.4-63.7	25.6	16.6-36.4	79.0	69.4-86.6
			≥	61	21								
		0.002	<	8	5	**87.8**	**73.8-95.9**	5.9	2.3-11.3	22.0	15.9-29.1	61.5	31.6-86.1
			≥	128	36								
		12.46	<	130	39	4.9	0.6-16.5	**95.6**	**90.6-98.4**	25.0	3.2-65.1	76.9	69.8-83.1
			≥	6	2								
Jug r 1	0.52 (0.41-0.62)	0.35	<	122	34	17.1	7.2-32.1	89.7	83.3-94.3	33.3	14.6-57.0	78.2	70.9-84.4
			≥	14	7								
		0.002	<	38	12	**70.7**	**54.5-83.9**	27.9	20.6-36.3	22.8	15.9-31.1	76.0	61.8-86.9
			≥	98	29								
		3.14	<	130	37	9.8	2.7-23.1	**95.6**	**90.6-98.4**	40.0	12.2-73.8	77.8	70.8-83.9
			≥	6	4								
r Jug r 2	0.53 (0.43-0.64)	0.35	<	122	34	15.0	5.7-29.8	90.4	84.1-94.8	31.6	12.6-56.6	78.2	70.9-84.4
			≥	13	6								
		0.005	<	7	3	**92.5**	**79.6-98.4**	5.2	2.1-10.4	22.4	16.3-29.6	70.0	34.8-93.3
			≥	128	37								
		5.31	<	129	36	10.0	2.8-23.7	**95.6**	**90.6-98.4**	40.0	12.2-73.8	78.2	71.1-84.2
			≥	6	4								
r Jug r 2 LMW	0.53 (0.42-0.63)	0.35	<	99	31	20.5	9.3-36.5	73.9	65.6-81.1	18.6	8.4-33.4	76.2	67.2-83.2
			≥	35	8								
		0.11	<	13	3	**92.3**	**79.1-98.3**	9.7	5.3-16.0	22.9	16.6-30.3	81.3	54.4-96.0
			≥	121	36								
		9.12	<	128	35	10.3	2.9-24.2	**95.5**	**90.5-98.3**	40.0	12.2-73.8	78.5	71.4-84.6
			≥	6	4								
r Jug r 3	0.48 (0.38-0.58)	0.35	<	113	36	12.2	4.1-26.2	83.1	75.7-89.0	17.9	6.1-36.9	75.8	68.2-82.5
			≥	23	5								
		0.006	<	17	5	**87.8**	**73.8-95.9**	12.5	7.5-19.3	23.2	16.8-20.7	77.3	54.6-92.2
			≥	119	36								
		2.01	<	130	39	4.9	0.6-16.5	**95.6**	**90.6-98.4**	25.0	3.2-65.1	76.9	69.8-83.1
			≥	6	2								
n Jug r 4	0.57 (0.46-0.68)	0.35	<	120	35	10.3	2.9-24.2	89.6	83.1-94.2	22.2	6.4-47.6	77.4	70.0-83.7
			≥	14	4								
		0.003	<	4	3	**92.3**	**79.1-98.4**	3.0	0.8-7.5	21.7	15.7-28.7	57.1	18.4-90.1
			≥	130	36								
		2.07	<	128	36	7.7	1.6-20.9	**95.5**	**90.5-98.3**	33.3	7.5-70.2	78.0	70.9-84.1

(continued)

TABLE E6. *(Continued)*

Individual test	AUC	Positivity threshold		Mild to moderate	Severe	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
			≥	6	3								
Jug r 5	0.58 (0.49-0.68)	0.35	<	44	16	60.0	43.3-75.1	32.6	24.8-41.2	20.9	13.9-29.4	73.3	60.3-83.9
			≥	91	24								
		0.003	<	6	3	**92.5**	**79.6-98.4**	4.4	1.7-9.4	22.3	16.2-29.4	66.7	29.9-92.5
			≥	129	37								
		62.73	<	129	40	0.0	0.0-8.8	**95.6**	**90.6-98.4**	0.0	0.0-45.9	76.3	69.2-82.5
			≥	6	0								
Jug r 6	0.54 (0.44-0.65)	0.35	<	125	36	7.7	1.6-20.9	93.3	87.6-96.9	25.0	5.5-57.2	77.6	70.4-83.8
			≥	9	3								
		0.005	<	8	3	**92.3**	**79.1-98.4**	6.0	2.6-11.4	22.2	16.1-29.4	72.7	39.0-94.0
			≥	126	36								
		0.41	<	128	36	7.7	1.6-20.7	**95.5**	**90.5-98.3**	33.3	7.5-70.1	78.0	70.9-84.1
			≥	6	3								
Jug r 7	0.53 (0.44-0.63)	0.35	<	98	32	22.0	10.6-37.6	72.1	63.7-79.4	19.1	9.2-33.3	75.4	67.1-82.5
			≥	38	9								
		0.004	<	42	12	**70.7**	**54.5-83.9**	30.9	23.2-39.4	23.6	16.4-32.1	77.8	64.4-88.0
			≥	94	29								
		15.00	<	130	41	0.0	0.0-8.6	**95.6**	**90.6-98.4**	0.0	0.00-45.9	76.0	68.9-82.2
			≥	6	0								

Models	AUC	Positivity threshold		Mild to moderate	Severe	Sensitivity	95% CI	Specificity	95% CI	PPV	95% CI	NPV	95% CI
Model CRD only*	0.66 (0.57-0.75)	0.50	<	128	35	5.4	0.7-18.2	100.0	97.2-100.0	100.0	15.8-100.0	78.5	71.4-84.6
			≥	0	2								
		0.16	<	33	3	**91.9**	**78.1-98.3**	25.8	18.5-34.3	26.4	19.0-34.8	91.7	77.5-98.3
			≥	95	34								
		0.30	<	123	33	10.8	3.0-25.4	**96.1**	**91.1-98.7**	44.4	13.7-78.8	78.9	71.6-85.0
			≥	5	4								
Model 1	0.74 (0.65-0.83)	0.50	<	103	29	9.4	2.0-25.0	100.0	96.5-100.0	100.0	29.2-100.0	78.0	70.0-84.8
			≥	0	3								
		0.17	<	56	8	**75.0**	**56.6-88.5**	54.4	44.3-64.2	33.8	23.0-46.1	87.5	76.85-94.45
			≥	47	24								
		0.34	<	99	23	28.1	13.8-46.8	**96.1**	**90.4-98.9**	69.2	38.6-90.9	81.2	73.1-87.7
			≥	4	9								
Model 2	0.74 (0.65-0.83)	0.50	<	102	25	21.9	9.3-40.0	99.0	94.7-100.0	87.5	47.4-99.7	79.5	71.5-96.8
			≥	1	7								
		0.14	<	56	7	**78.1**	**60.0-90.7**	54.4	44.3-64.2	34.7	23.9-46.9	88.9	78.4-95.4
			≥	47	25								
		0.36	<	99	23	28.1	13.8-46.8	**96.1**	**90.4-98.9**	69.2	38.6-90.9	81.2	73.1-87.7
			≥	4	9								

Model 3	0.81 (0.73-0.89)	0.50	<	97	23	23.3	9.9-42.3	98.0	92.9-99.8	77.8	40.0-97.2	80.8	72.6-87.4
			≥	2	7								
		0.14	<	39	3	**90.0**	**73.5-97.9**	39.4	29.7-49.7	31.0	21.6-41.9	92.9	80.5-98.5
			≥	60	27								
		0.39	<	95	14	53.3	34.3-71.7	**96.0**	**90.0-99.9**	80.0	56.3-94.8	87.2	79.4-92.8
			≥	4	16								

LMW, Low molecular weight; *NA*, not applicable.
Measures of accuracy were calculated for each of the individual diagnostic tests, and for the models on clinical background variables (model 1), clinical background variables + sensitization to walnut extract in SPT or ImmunoCAP (model 2), and clinical background variables + sensitization to walnut extract + sensitization to walnut components (model 3). The 3 rows of threshold values given for each diagnostic test respectively indicate the cutoff points generally used in clinical practice, corresponding with a high sensitivity (closest to 95%), and corresponding with a high specificity (closest to 95%).
Bold indicates the sensivity and specificity estimates closest to 95%.
*Model including Jug r 1, 2, 3, 4, 5, 6, 7 and Ana c 2 (not Jug r 2 LMW).

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