Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults

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Background: Component-resolved diagnosis has been shown to improve the diagnosis of food allergy.
Objective: We sought to evaluate whether component-resolved diagnosis might help to identify patients at risk of objective allergic reactions to hazelnut.
Method: A total of 161 hazelnut-sensitized patients were included: 40 children and 15 adults with objective symptoms on double-blind, placebo-controlled food challenges (DBPCFCs) and 24 adults with a convincing objective history were compared with 41 children and 41 adults with no or subjective symptoms on DBPCFCs (grouped together). IgE levels to hazelnut extract and single components were analyzed with ImmunoCAP.
Results: IgE levels to hazelnut extract were significantly higher in children with objective than with no or subjective symptoms. In 13% of children and 49% of adults with hazelnut allergy with objective symptoms, only sensitization to rCor a 1.04 was observed and not to other water-soluble allergens. Sensitization to rCor a 8 was rare, which is in contrast to rCor a 1. Sensitization to nCor a 9, rCor a 14, or both was strongly associated with hazelnut allergy with objective symptoms. By using adapted cutoff levels, a diagnostic discrimination between severity groups was obtained. IgE levels to either nCor a 9 of 1 kU_A/L or greater or rCor a 14 of 5 kU_A/L or greater (children) and IgE levels to either nCor a 9 of 1 kU_A/L or greater or rCor a 14 of 1 kU_A/L or greater (adults) had a specificity of greater than 90% and accounted for 83% of children and 44% of adults with hazelnut allergy with objective symptoms.
Conclusion: Sensitization to Cor a 9 and Cor a 14 is highly specific for patients with objective symptoms in DBPCFCs as a marker for a more severe hazelnut allergic phenotype.
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Key words: *Component-resolved diagnosis, diagnostics, double-blind, placebo-controlled food challenge, hazelnut allergy, severity, food allergy*

The clinical presentation of hazelnut allergy is highly variable and ranges from local and mild to systemic and severe allergic reactions.[1] The prevalence and severity of hazelnut allergy are different between children and adults. The reported prevalence of hazelnut allergy is approximately 0.2% among children[2] and up to 4.5% among adults from birch-endemic areas.[3,4] In a birch-endemic area children with hazelnut allergy have often objective symptoms (67%)[5-7] in contrast to mainly subjective oral symptoms in adults (93%).[8,9] A differential sensitization profile might be responsible for this difference. Sensitization to the cross-reactive lipid transfer protein Cor a 8 has mainly been described in patients from the Mediterranean area,[10,11] with a strong association with severe allergic symptoms. Evidence that sensitization to Cor a 9, an 11S globulin, might be associated with severe hazelnut allergy in children has been reported both in the United States and Europe.[5,12] In birch pollen–related hazelnut allergy,[10,13] sensitization to Cor a 1, the Bet v 1 homolog belonging to the pathogenesis-related protein 10 family, has been implicated as a cause of typically mild and local symptoms, mainly in adults.[5,9,10] The clinical relevance of sensitization to profilin in hazelnut (Cor a 2, another pollen-related allergen) seems limited,[14] and sensitization to cross-reactive carbohydrate determinants (CCDs) is now well established as being clinically inert.[15,16] More recently identified allergens in hazelnut are the oleosins Cor a 12 and Cor a 13[17] and Cor a 14, belonging to the 2S albumins.[18] The clinical relevance for sensitization to the oleosins and Cor a 14 from hazelnut still needs to be evaluated.

Sensitization to hazelnut extract can occur regardless of whether patients react to hazelnut with severe or mild symptoms or even at all and is therefore not discriminative.[5,7] Previous studies have suggested that component-resolved diagnosis might increase the diagnostic specificity and might predict the severity of an allergic reaction to hazelnut.[5,10-12] The number of patients with hazelnut allergy with objective symptoms was limited in

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Abbreviations used
AUC: Area under the curve
CCD: Cross-reactive carbohydrate determinant
DBPCFC: Double-blind, placebo-controlled food challenge

previous studies, especially in adults from birch-endemic areas. The aim of this study was to analyze the sensitization to the currently available components rCor a 1, rCor a 8, nCor a 9, and rCor a 14 in a population of hazelnut-sensitized children and adults from a birch-endemic area. A large group of children, as well as adults, responding with objective symptoms on a double-blind, placebo-controlled food challenge (DBPCFC) or having a convincing history of hazelnut allergy with objective symptoms was enrolled to evaluate whether component-resolved diagnosis can help identify patients with objective allergic reactions to hazelnut. This group was compared with a group of hazelnut-sensitized children and adults with a negative DBPCFC result or with only subjective symptoms.

METHODS

Patient selection

Patients sensitized to hazelnut (≥0.35 kU_A/L) were retrospectively recruited. The aim was to enroll equally powered groups of approximately 40 children and adults with a more severe phenotype, as judged based on objective symptoms in DBPCFCs (or, if the DBPCFC was declined, based on a convincing history of hazelnut allergy with objective symptoms) and similar numbers of sensitized children and adults without allergy or a mild phenotype as judged based on no or subjective symptoms in DBPCFCs, respectively. Forty patients per group were necessary to build a prediction model with IgE to 4 hazelnut components as variables. A total of 164 hazelnut-sensitized patients were enrolled. Three patients were excluded from further analyses because they lost their previously determined hazelnut sensitization. The final study population therefore consisted of 161 sensitized patients: 81 children and 80 adults. On the basis of the challenge outcome (performed in all 81 children and 56 adults) or a convincing objective history (24 adults), the population was divided into 79 patients (40 children and 39 adults) with an objective phenotype and 82 patients (41 children and 41 adults) without an objective phenotype (negative DBPCFC results or subjective symptoms on DBPCFCs, Fig 1). For those reporting adverse reactions to hazelnut in daily life, a detailed clinical history was recorded. In addition, data were collected on asthma, birch pollinosis, and atopic dermatitis.

The study was performed at the University Medical Center Utrecht between 2010 and 2012. The study was approved by the local ethics committee, and all patients provided written informed consent before entering the study.

DBPCFCs

DBPCFCs with hazelnut were performed during diagnostic workup, as previously described,[6] and discontinued if objective symptoms occurred. Suggestive oral symptoms appearing at the last open dose resulted in a positive assessment of the challenge in 5 patients. In 8 patients the DBPCFC was prematurely discontinued after subjective symptoms on 3 consecutive dosages. In patients with birch pollinosis, the DBPCFC was performed outside the pollen season. A total of 24 adults declined to undergo a DBPCFC. All had a convincing history of an objective phenotype, with generalized urticaria, dyspnea, or wheezing.

Classification of symptoms during DBPCFCs

Symptoms recorded during DBPCFCs were used to stratify patients into severe and nonsevere phenotypes. The approach chosen for this stratification was to grade subjective symptoms (oral allergy, nausea, abdominal discomfort, and throat tightness) as nonsevere and objective symptoms (generalized urticaria or angioedema, emesis, diarrhea, rhinoconjunctivitis, hoarseness, stridor, and wheezing) as severe. Although it could be argued that, for example, throat tightness might be seen as severe and rhinoconjunctivitis as mild, we have chosen this approach because it is least influenced by patient and observer bias, and overall, the division of subjective versus objective has acceptable overlap with the division of mild versus severe. Sensitized patients with negative DBPCFC results were stratified together with the mild subjective group. This clinical stratification was finished before serology data were generated or any further analysis was done to ensure blinding.

IgE measurements

IgE to hazelnut extract, rCor a 1, rCor a 8, nCor a 9, rCor a 14, birch pollen extract, rBet v 1, rBet v 2, and CCDs was determined by using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Experimental tests were used for nCor a 9 and rCor a 14. nCor a 9 was purified from defatted hazelnut extract by means of ion exchange chromatography and reversed-phase chromatography. rCor a 14[18] was expressed as a hexahistidine-tagged protein and purified by means of immobilized metal ion affinity chromatography and ion exchange chromatography. A hazelnut extract depleted of Cor a 1 was prepared by means of immunoadsorption with an affinity column carrying an mAb directed against Cor a 1. IgE test results of 0.35 kU_A/L or greater were considered positive. Values of less than 0.35 kU_A/L were analyzed statistically as 0.34 kU_A/L, and values of greater than 100 kU_A/L were analyzed statistically as 101 kU_A/L.

Data analysis

The prevalence of asthma, atopic dermatitis, and birch pollinosis was compared between children and adults and between patients with a hazelnut allergy with objective and no or subjective symptoms by using the χ^2 test. The Mann-Whitney *U* test (continuous IgE values) and χ^2 test (binary, ≥0.35 kU_A/L) were used to compare IgE levels between patients with hazelnut allergy with objective and no or subjective symptoms. The diagnostic value of IgE levels (continuous values) for discrimination between hazelnut allergy with objective and no or subjective symptoms was determined by calculating the area under the curve (AUC) of the receiver operating characteristic. Sensitivity and specificity in the discrimination between hazelnut allergy with objective and no or subjective symptoms were calculated for different cutoff values of IgE and different combinations of IgE. A *P* value of less than .05 was considered significant. All analyses were performed with SPSS software (version 20.0; SPSS, Chicago, Ill).

RESULTS

Patients' characteristics

Clinical characteristics of children and adults are shown in Table I. There was a male predominance in children and a female predominance in adults. Asthma was more common among adults with hazelnut allergy with objective than those with no or subjective symptoms ($P = .03$) and more common among children with no or subjective symptoms than adults with no or subjective symptoms ($P = .04$). Atopic dermatitis was more common in children than in adults ($P < .001$), whereas birch pollinosis was less common in children than in adults ($P = .01$) and less common in children with objective than those with no or subjective symptoms ($P = .01$). Clinical characteristics were comparable within the group of children and adults with hazelnut allergy with no or subjective symptoms, whereas age was significantly higher in children and adults with subjective symptoms compared with those with no hazelnut allergy (data not shown). Eliciting doses during DBPCFCs were lower in children and adults with hazelnut allergy with objective symptoms than those with no or subjective symptoms ($P = .02$).