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Research Article

The severity and frequency of systemic reactions to hazelnut are significantly higher in hazelnut allergic patients monosensitized to *Cor a 8* than in patients polysensitized to *Cor a 1*, *Cor a 8* and *Cor a 9.*

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Short Title: Systemic reactions to hazelnut in mono- and plurisensitized patients

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Abstract

Introduction: Hazelnuts are a leading trigger of food allergy. To date, several molecular components of hazelnut are available for component-resolved diagnosis. However, little is known about how simultaneous sensitization to multiple allergens affects the severity of the hazelnut-induced reaction. In a previous study, our group demonstrated a lower risk of systemic reactions to peach in patients sensitized to both *Pru p 3* and *Pru p 1* than in the patients monosensitized to peach LTP. We aimed to assess whether this was also true in hazelnut allergy in a cohort of adult patients.

Methods: Patients were selected based on a history of symptoms such as urticaria, vomiting, diarrhea, asthma, and anaphylaxis indicative of hazelnut IgE-mediated food allergy and graded according to a clinical severity scale. For all patients, specific IgE was determined for *Cor a 1* and *Cor a 8* and, for most patients, also *Cor a 9*. Patients were offered an oral food challenge in open format (OFC) with a cocoa-based roasted hazelnut spread on a voluntary basis in order to prescribe an appropriate diet.

Results: A total of two hundred and fourteen patients were recruited. Among these, 43 patients were monosensitized to *Cor a 8*. One hundred and seventy-one patients were sensitized to *Cor a 1* (79.9%) and, among them, 48/171 (28.1%) were also *Cor a 8* positive. *Cor a 9* was evaluated in 124/214 patients, testing positive in 21/124 (16.9%). Patients monosensitized to *Cor a 8* experienced systemic reactions more frequently than those sensitized to *Cor a 1* ± *Cor a 8* ($p < 0.00001$), with significantly more severe reactions ($p < 0.0005$), and testing more frequently positive at OFC ($p < 0.0001$).

Regarding *Cor a* 9, the sensitized patients were significantly younger (p= 0.0013) and showed reactions of similar severity to patients who tested *Cor a 9* negative, and these reactions were milder than in patients monosensitized only to *Cor a* 8 .

Discussion/Conclusion: Sensitization to *Cor a 1* seems to protect from the development of the severe systemic reactions induced by *Cor a* 8 sensitization, *Cor a 9* does not influence the severity of symptoms in adult patients. The OFC with roasted hazelnut may help in dietary guidance.

Introduction

Food allergy represents a relevant health problem and its prevalence, after a steep increase in the last decades, appears to have now stabilized in many areas worldwide [1], [2], [3].

“Nuts” as a group are one of the most prevalent allergenic foods. Among them, hazelnut (*Corylus avellana*) is the most frequent trigger of allergic reactions in Europe [4], [5]. More precisely, it is one of the five major triggers of food allergy overall, and the second most common in school-age [6], [7].Comparing the 31 most important hazelnut-producing countries in the world, in 2021 Italy ranks second after Turkey, with a hazelnut production which reached 84,670 tonnes, according to FAOSTAT [8]. Moreover, in a study conducted by Uzundumlu *et al*, the world hazelnut production is expected to increase constantly until 2025 and Turkey and Italy will keep their positions as the leading countries in the export and production of hazelnuts [9].

Italy is also the largest consumer country of hazelnuts with respect to the world population [10]. For all these reasons it is not surprising that hazelnut is a relevant allergenic food in Italy and Turkey.

In a recent Turkish study hazelnut was the most common tree nut causing food allergy in a population of 227 children aged 6-10 years with allergy to tree nuts and/or peanuts [11]. Similarly in Italy a recent epidemiological study conducted by the Italian Society of Pediatric Allergology and Immunology, has shown that hazelnut was the second cause of anaphylaxis, after milk, in a population of 191 food allergic children [12]. Thus, the diagnosis of hazelnut allergy, when suspected, calls for promptness and has to consider the relevant role of all the single allergens in relationship to the severity of symptoms.

The clinical manifestations of hazelnut allergy are of variable severity, ranging from mild forms limited to the oral mucosa, to potentially fatal forms such as anaphylactic shock [13], [14] . Furthermore, this food allergy is generally persistent [15] and the presence of a high degree of cross-reactivity of hazelnut allergens with those of other nuts further complicates its management [16].

Promising new management strategies currently under study in children with hazelnut allergy are the selection of hypoallergenic cultivars and the development of oral desensitization protocols (OIT)

[16], [17].

At present 12 hazelnut allergens belonging to different allergenic families have been identified, of which 9 are food allergens and 3 respiratory allergens (WHO/IUIS) [18]. Their molecular characteristics are associated with variable resistance to physical-chemical agents and with different clinical severity, according to the

sensitization profile with the limit being the availability of allergens for *"in vitro"* testing [19].

Thus the sensitization to *Cor a 1*, the cross-reactive homolog of *Bet v 1*, is associated mostly with mild forms of food allergy, being limited to the oral cavity [20], while sensitization to *Cor a 8*, a lipid transfer protein (LTP), is associated with more severe clinical manifestations [21]. This molecule displays features of marked chemical-physical stability, as opposed to *Cor a 1* [22], [23]. Sensitization to *Cor a 8* is usually secondary to sensitization to the peach LTP, *Pru p 3* [24]. The sensitization to seed storage proteins, *Cor a 9*, *Cor a 11* and *Cor a 14*, has a relevant role in determining severe systemic reactions in the pediatric population [25] but its role in the adult population has not been fully elucidated at present. Indeed, in adults only a limited number of cases of allergy to these allergens have been described and these are geographically restricted to Northern and Central European countries [26], [27],[28].

Finally, the value of sensitization to oleosins, *Cor a 12*, *Cor a 13* and *Cor a 15*, [18] for which reliable *in vitro* or *in vivo* tests are currently lacking, and to profilin, *Cor a 2* [20], still remains elusive.

Additionally, a still unknown aspect of food allergy needs to be elucidated, *i.e.,* the possible clinical role of simultaneous sensitization to multiple allergens. In a study published by our group [29] , confirmed by other studies conducted in the Mediterranean area [30], [31], the simultaneous sensitization to *Pru p 1 and Pru p 3* was associated with less severe symptoms than sensitization to *Pru p 3* alone.

We therefore, sought to evaluate whether it was possible to confirm this phenomenon, *i.e.,* the modulation of the severity of the symptoms triggered by the sensitization to LTP by an allergen belonging to the PR-10, also in the model of patients allergic to hazelnuts. For this purpose, we selected individuals with a clinical history of allergy to hazelnut and sensitized to *Cor a 8* or *Cor a 1* or both and we characterized the severity of clinical reactions in relation to the different sensitizations. Moreover, we looked for the role of *Cor a 9* in modulating the clinical reactivity in a limited group of patients.

Materials and Methods

Patients

During the period from February 2016 to February 2019, all consecutive patients referred to the Department of Allergology and Clinical Immunology of the ASST Grande Ospedale Metropolitano Niguarda (Milan, Italy) for suspected allergy to hazelnut were selected for further evaluation as regards the presence of a convincing and documented history of clinical reactivity to hazelnut. The clinical history was considered convincing only when the patient reported symptoms that were in keeping with IgE-mediated food allergy after the ingestion of hazelnut, i.e. urticaria, angioedema, gastrointestinal symptoms (OAS, nausea, vomiting, abdominal pain), respiratory symptoms, anaphylaxis [13], [14]. Patients with a convincing clinical history and positive specific IgE for hazelnut were considered true food allergic and were investigated for the presence of specific IgE to the recombinant allergens *Cor a 8* and *Cor a 1*.

Symptoms were classified as oral allergy syndrome (OAS) when they were localized to the oral mucosal and as severe symptoms when systemic reactions (SR) were documented. These, in turn, were classified in four grades of severity as follows: grade I (urticaria), grade II (urticaria + gastrointestinal reactions (*i.e.*, vomiting, diarrhea, and severe abdominal pain), grade III (urticaria + angioedema and/or rhino-conjunctivitis and/or asthma), grade IV (life-threatening symptoms such as hypotension with syncope and anaphylactic shock). Patients were then subdivided into one group of *Cor a 8* positive *Cor a 1* negative patients and another group of *Cor a 1* positive patients which was further subdivided into two groups, one of *Cor a 1* + *Cor a 8* + patients and one of *Cor a 1* + *Cor a 8* - ones.

In 35/48 (72.9%) *Cor a 8* + *Cor a 1* + patients, we measured also specific IgE to *Cor a 9*, as well as in 64/123 (52.0%) *Cor a 1* only positive patients and in 23/43 (53.5%) *Cor a 8* only positive patients. All sampling was random.

Study Design

The major objective of the study was to compare the frequency and severity of SR induced by hazelnut among the group of adult patients sensitized to *Cor a 8* but not to *Cor a 1,* and the group of patients sensitized to *Cor a 1* with or without simultaneous sensitization to *Cor a 8.*

A second objective was to compare the frequency and severity of SR among the patients *Cor a 8* + Cor a 1 - and the two subgroups of *Cor a 1* + patients formed by patients *Cor a 1* + *Cor a 8* + and patients *Cor a 1* + *Cor a 8* -. An additional task was to evaluate a possible role of *Cor a 9* in modulating the severity of the reactions in the same populations of patients.

For the study, we selected patients with a documented history of allergic reactions to hazelnut, and specific IgE to hazelnut and to at least one among *Cor a 1* and *Cor a 8*.

***In vivo* tests**

Open food challenge

In order to plan a diet with or without roasted hazelnut-enriched products we suggested that patients undergo an OFC with a commercial roasted hazelnut-based chocolate spread on voluntary adherence.

It is known that food-allergic patients often develop unwarranted fear toward the ingestion of harmless food items and often eliminate most vegetable foods, negatively affecting their diet. The OFC was performed in consideration of the fact that *Cor a 1* is a labile allergen; the aim of the OFC was to convince *Cor a 1*-positive patients that they did not need to eliminate toasted hazelnut from the diet, hence avoiding the useless elimination of an important food item. Moreover, OFC with roasted hazelnut was performed on *Cor a 8* patients to ascertain whether or not they reacted to roasted hazelnut.

The challenge was accepted by 13 *Cor a 8 + Cor a 1* - patients, by 31 patients to *Cor a 1 + Cor a 8 +* and 69 *Cor a 1 + Cor a 8 -*.

The challenge product recipe contained 13 grams of roasted hazelnut per 100 grams of cocoa-based cream.

Patients were challenged with a total of 100 grams of spread using an incremental protocol. The challenge began with the ingestion of 1/3 teaspoon of the product after 10 minutes 2/3 teaspoon was ingested; the dose was then doubled every 10 minutes until the entire dose of spread was consumed.

The challenge was stopped when objective symptoms developed or the maximum dose was reached.

***In vitro* tests**

Serum levels of anti-hazelnut, anti-*Cor a 1*, anti-*Cor a 8*, and anti-*Cor a 9* specific IgE were determined using the Immuno-CAP System (Thermo Scientific, Uppsala, Sweden) according to the manufacturer's instructions. IgE values were considered positive for values greater than 0.10 kUA/L.

Analysis of subpopulations

Based on positive (+) or negative (-) specific IgE levels against one or both the two major allergens of hazelnut, the studied populations were the following;

(1) *Cor a 8 + Cor a 1* - (N° 43)

(2) *Cor a 1* + (N° 171) subdivided in:

(2a) *Cor a 1* + *Cor a 8* - (N° 123)

(2b) *Cor a 1* + *Cor a 8* + (N°48)

Group-2b was further subdivided into two subgroups: *Cor a 1* + *Cor a 8 + Cor a 9* -

and *Cor a 1* + *Cor a 8* + *Cor a 9* +

Statistical analysis

A comparison of categorical variables between groups was carried out with Fisher's exact test. Continuous variables were compared with Student's t-test in case of normal distribution and Mann-Whitney's U test with exact algorithm (two groups) or Kruskal-Wallis test (more than two groups) in case of non-normal distribution. Statistical significance was assumed at $p < 0.05$. All statistical analyses were performed with Stata/SE 17.0 (Stata Corp, College Station, USA).

Results

Patients

A total of two hundred and fourteen patients with a mean age of 41.4 years (95% CI, 26 - 56.9 years) were selected for a convincing and documented history of clinical reactions to hazelnut. Women were 153 with a mean age of 43.8 years (95% CI, 28.3-59.3 years) and men were 61 with a mean age of 35.3 years (95% CI, 21.8 - 48.8 years). The age difference was statistically significant, as women were significantly older than men (p= 0.0001, Student's t-test).

A total of 43/214 (20.1%) patients were *Cor a 8 + Cor a 1* - while 171/214 (79.9%) were *Cor a 1* positive. We did not find any statistically significant difference between the two groups with regard to sex and age (p= 0.708, Fisher's exact test, and p= 0.1758 Student's t-test, respectively). Out of the 171 *Cor a 1* + patients, 48 (28.1%) were also *Cor a 8 +* while 123 (71.9%) were *Cor a 8* -, as shown in Table 1 .

Among the 214 studied patients, *Cor a 9* specific IgE were evaluated in 124 randomly selected patients. A total of 103/124 were *Cor a 9* negative with a mean age of 41.8 ± 16 years while 21/124 were *Cor a 9* positive with a mean age of 29.7 ± 9.4 years; this difference was highly statistically significant (Mann-Whitney U test: p= 0.0013).

As regards the 21 *Cor a 9* + patients, 17/21 (81%) were in the subgroup of patients *Cor a 1 + Cora a 8 +,* 3/21 (14%) were in the group of 43 *Cor a 8 + Cor a 1* - and 1/21 (5%) in the subgroup of *Cor a 1* single positive. Considering the different subgroups, the positivity was as follows: of the 123 *Cor a 1 + Cor a 8* - patients, *Cor a 9* was evaluated in 64 patients and was positive in 1 case only (2%). In the group of 43 *Cor a 8* + *Cor a 1* - patients, 25 were evaluated for *Cor a 9* and only 3/25 (12%) had positive IgE. Thus, we compared the reactions of the 17 *Cor a 9* + patients out of the 48 *Cor a 1 + Cor a 8* + group with those of the 18 *Cor a 9* - patients in the same group of *Cor a 1 + Cor a 8 +* patients.

Distribution of the severity of reactions

Regarding symptom severity, in the overall study population 139/214 (65.0%) patients had only OAS (considered in the figures as SR 0) and 75/214 (35.0%) had SR. The distribution of the reactions is reported in Table 1.

Comparison of the frequency of systemic reactions

As shown in Figure 1 and 2, the prevalence of SR was significantly higher in the 43 *Cor a 8 + Cor a 1* – group than in the 171 patients *Cor a 1 +* even if considering the subpopulations (2a) of 123 *Cor a 1 + Cor a 8* – patients and (2b) of 48 *Cor a 1 + Cor a 8 +* ones.

In particular, a patient taken at random in the group of the *Cor a 8 + Cor a 1- patients* had a 74.4 % higher probability of developing more frequent systemic symptoms than a patient taken at random among *Cor a 8* + *Cor a 1* + sensitized patients ($p < 0.00001$, Mann-Whitney U test).

We then compared the prevalence of systemic reactions between the 43 *Cor a 8* only positive patients, the 17 *Cor a 1* + *Cor a 8* + *Cor a 9* + patients and the 18 *Cor a 1* + *Cor a 8* + *Cor a 9* - ones and found that the prevalence of SR among *Cor a 8* + *Cor a 1* - patients was significantly higher than that of the *Cor a 1* + *Cor a 8* + ones even if positive to *Cor a 9* ($p= 0.0001$, Fisher's exact test) (Figure 3). Finally, we did not find any difference between the 17 *Cor a 9 +* and the 18 *Cor a 9* - subgroups as regards the prevalence of SR ($p > 0.99$, Fisher's exact test).

Results regarding the severity of SR

The development of grade 3 + 4 SR was significantly more frequent in *Cor a 8 + Cor a 1* - patients than among *Cor a 1* + patients (26/43, 60.5% vs 15/171, 8.8%; $p <0.0005$, Fisher's exact test). The same result was found considering separately the subpopulations of *Cor a 8* + *Cor a 1* + patients (26/43, 60.5% vs 11/48, 22.9%; $p= 0.001$, Fisher's exact test) and the *Cor a 8* + *Cor a 1* - ones (26/43, 60.5% vs 4/12, 33.3%; $p <0.0005$, Fisher's exact test).

IgE values

Table 1 shows that the value of specific IgE was significantly higher in the group of patients only positive to *Cor a 8*, as compared to the group of *Cor a 1* + *Cor a 8* + ($p < 0.00001$, Mann-Whitney U test). On this basis, we can say that the severity of symptomatology was probably correlated with the levels of specific IgE to *Cor a 8*.

Results of OFC with roasted hazelnut

OFC with roasted hazelnut spread gave the following results: in the group of 43 *Cor a 8 + Cor a 1* - patients, 13 OFCs were performed, of which 3 were negative (23.1%) and 10 were positive (76.9%). In the group of 171 *Cor a 1* positive patients, 100 OFCs were performed of which 84 were negative (84%) and 16 were positive (16%) with a statistically significant difference ($p < 0.0005$, Fisher's exact test) indicating that only the *Cor a 8* positive group had significantly more frequent positive OFC. In particular, 31 OFCs were performed in *Cor a 1 + Cor a 8* + patients, of which 10 OFC were positive (32.3%) and 21 were negative (67.7%). Nearly all of the patients with positive challenge developed symptoms either during the challenge or immediately after test completion. The number was too low to find statistical differences.

Discussion/Conclusion

In this work, we explored the role of allergenic multi-positivity in the hazelnut food allergy model. With this aim, we selected a group of patients based on a well-documented clinical history of hazelnut allergy and IgE positivity for hazelnut and for at least one of the two major allergenic proteins *Cor a 1* and *Cor a 8*. We collected data from each patient regarding past clinical manifestations and compared patients with only OAS with those reporting also severe SR. We then compared the frequency of these two types of reactions, between patients sensitized to *Cor a 8* but not to *Cor a 1* (n=43) and patients sensitized to *Cor a 1* with or without *Cor a 8 +* (n=171).

As we have already reported for hazelnut [14] and was subsequently confirmed by many other studies [15], [28], in the present study, we found that patients sensitized to hazelnut LTP, *Cor a 8,* had not only a higher prevalence of SR (p <0.0001) but also showed higher degrees of severity, as compared to patients sensitized to the PR-10 *Cor a 1* with or without sensitization to *Cor a 8* (p <0.0001). We then compared the prevalence of SR among the 43 *Cor a 8 + Cor a 1* - patients and *Cor a 1* + patients, subdivided into the two population of *Cor a 1* + *Cor a 8* - and *Cor a 1 + Cor a 8* +. The comparison showed that *Cor a 8 + Cor a 1* - patients had a clinical history of more frequent systemic reactions than patients who were simultaneously *Cor a 1* + *Cor a 8*+. Therefore, it is confirmed that sensitization to the major birch allergen that invariably conditions sensitization to *Cor a 1* is also protective against LTP-driven symptoms in the case of hazelnut as well as that of peach [29]. Interestingly, the results of this study thus fully confirm the apparent protective role of sensitization to *Cor a 1* over that to *Cor a 8* and allow us to extend the protective role of PR-10 sensitization to other plant-foods containing both the allergens that is to all the foods involved in LTP-syndrome if the patients are also sensitized to birch pollen. It should be emphasized here that double positivity to PR-10 and LTP allergens in Italy is particularly frequent also because of the coexisting exposure to birch and peach, which represent the allergenic sources that condition the main types of hazelnut sensitization. This means that if sensitization and thus allergy to hazelnut in Central Europe depends essentially on sensitization to the main birch allergen, in our country and especially in Northern Italy, allergy to hazelnut is a consequence of not only *Bet v 1* sensitization but also of *Pru p 3*, which is extremely common in our area.

Interestingly this apparent protection due to pollen allergen concerns not only PR-10 allergens but also profilin. This has been demonstrated by Bogas *et al* who found that in a population of LTP allergic patients the simultaneous sensitization to a pollen allergen such as profilin could identify patients with a significantly lower frequency of systemic anaphylactic reactions [31].

It should be emphasized that *Cor a 1* and *Cor a 8* sensitizations individually have their own precise clinical role, as sensitization to *Cor a 1* results in a mild clinical picture, characterized by OAS, whereas allergy to *Cor a 8* can determine more important and severe symptoms although it can rarely cause only OAS. Interestingly, *Cor a 8* allergy was not considered in the recently published papers regarding hazelnut allergy in central and Northern Europe, in which the discussion and comparison was limited to *Cor a 1*, *Cor a 9*, and *Cor a 14* [27], [28]. It should be stressed that the two latter allergens have no clear role in the adult population and are only rarely positive. Without wishing to create further separation between Southern and Northern Europe, it is nevertheless clear how a single allergy to LTP may hardly exist in a population as large as Northern Europe where birch allergy certainly represents the most prevalent respiratory allergy. In fact, it was not found in any of the patients selected [7], [20]. An observation that is however difficult to explain given the Dutch work published a few years ago [23].

In the present work, we also sought to understand the reason for this discrepancy in SR in subjects sensitized to *Cor a 8* alone or to *Cor a 8* and *Cor a 1*. To this aim, we compared the level of specific IgE against *Cor a 8* between the two groups. We thus found a significant difference *i.e*. as *Cor a 8* specific IgE titer was significantly higher in the pure *Cor a 8* group than in the *Cor a 8* + *Cor a 1* + group. This significant difference seems to show that the difference in symptom severity between the two groups is linked precisely to the protective effect of *Cor a 1* sensitization on the intensity of *Cor a 8* sensitization.

The study of the correlation between sensitization to various allergenic proteins and symptomatology has been the focus of many studies published in recent years in Europe. These works, carried out mainly on children have shown that the most important allergens for the diagnosis of hazelnut allergy would be *Cor a 9* and *Cor a 14* [20], [23]. Indeed, sensitization to *Cor a 9* has been shown to have a strong impact on the severity of symptoms in children as shown in an Italian paper cited in Nilsson's recent review demonstrating that severe hazelnut allergy in children is determined by sensitization to *Cor a 9* and *Cor a 14* [32], [33], [34].

However, subsequent work has shown that sensitization to these allergens can be asymptomatic, thus diminishing their assumed diagnostic value. Surprisingly, in Uotila's work, about half of the children sensitized to *Cor a 9* had no symptoms, and only simultaneous sensitization *to Cor a 14* resulted in a definite clinical manifestation [35]. However, the enrollment criteria were not specified, i.e., whether only on the basis of SPT and RAST positivity or previous symptoms. Moreover, the role of these allergens in adults has never been thoroughly investigated, especially with regard to the frequency of sensitization.

The role of *Cor a 9* sensitization was only marginally evaluated as the primary objective of the study was the comparison of the two *Cor a 8* monosensitized groups with the *Cor a 8 + Cor a 1 +* group. We randomly sampled 124 patients for *Cor a 9* sensitization and found it positive in only 21 of the 124 tested. Its role was best studied in the *Cor a 1 + Cor a 8 +* group: we found that *Cor a 9* positivity does not affect the severity of symptoms in this group.

The distribution showed that most of the *Cor a 9* positive subjects were simultaneously positive for *Cor a 1* and *Cor a 8*. Thus, we were able to distinguish subjects positive for *Cor a 1*, *Cor a 8* and *Cor a 9* from subjects positive only for *Cor a 1* and *Cor a 8* and negative for *Cor a 9* and then compared them to those positive only for *Cor a 8*. Comparing the frequency of systemic symptoms and their severity with respect to the group of patients purely sensitized to *Cor a 8*, we found that the symptoms reported by the latter were always significantly more severe even when compared to the subgroup of subjects with triple positivity for *Cor a 1, 8* and *9*. Moreover, the symptoms reported by this subgroup were superimposable to those of the *Cor a 1* + *Cor a 8* + but *Cor a 9* - subjects. Thus, it seems that in adults, sensitization to *Cor a 9* does not contribute to modulating symptomatology and is not particularly useful at the diagnostic level. Another aspect worthy of mention was that in the patients sensitized only to *Cor a 1* nearly none were sensitized to *Cor a 9*. In fact, about 64 patients had been evaluated and all but one were negative. This figure will need to be better investigated, as we believe that the low frequency of *Cor a 9* sensitization is characteristic of the adult population. In fact, an interesting finding was that the *Cor a 9* negative group was significantly older than the *Cor a 9* positive group. We might therefore conclude that *Cor a 9* sensitization may be a childhood expression perhaps destined to wane with age. If *Cor a 9* is a significant allergen only in children, it will be important to monitor allergic children and assess clinical changes in reactivity. Thus, it does not appear from our data that

sensitization to *Cor a 9* is of extreme relevance in adults.

With the aim of improving the quality of life of these patients, we also assessed for allergy to hazelnut found in a well-known roasted hazelnut chocolate-based spread.

We challenged subjects with 10g of this spread (corresponding to 13 g of roasted hazelnut by weight) and found that it was perfectly tolerated by the vast majority of *Cor a 1* + subjects. Whereas the group of *Cor a 8* only positive patients, in which only 13 agreed to take the challenge, only 3 were negative. This implies that most *Cor a 1* sensitized individuals do not need to avoid roasted hazelnut, hence maintaining this important food item in their diet. OFC with roasted hazelnut also was performed on *Cor a 8* patients to ascertain whether or not they reacted to roasted hazelnut. We found that in most cases of *Cor a 8* positive subjects roasted hazelnut can cause symptoms. However, this is not always the case because 3/13 *Cor a 8* patients had a negative challenge to 13 g of roasted hazelnut, corresponding to about 25% of those that underwent challenge. Thus, if we keep in mind that these 3 subjects had a clinical history of only local reactions to hazelnut despite being *Cor a 8* positive, it is possible that some *Cor a 8* patients may tolerate roasted hazelnut on its own or in shelf products. Therefore, an oral food challenge with roasted hazelnut may improve dietary quality in some patients exclusively *Cor a 8* +. The majority of the cases however reacted even to the roasted hazelnut. The thermal resistance of LTPs is the main reason of the difficult dietary management of these patients. The ultimate solution should be to try specific immunotherapy (SIT) for *Pru p 3* as it has been recently reported that in a group of patients sensitized to both *Pru p 3* and *Ara h 9* of peanut, the SIT with *Pru p 3* protected significantly not only to peach reactivity but also to peanut *Ara h 9* reactivity [36]. As a matter of fact we know that the first sensitizing allergen is peach LTP and this sensitization should be considered a pre-requisite before directing patients to SIT. Unfortunately, the extracts for *Pru p 3* immunotherapy are not available in Italy as well as in many European countries [36].

The data of the present study lead us to conclude that the severity and frequency of the reactions to hazelnut are significantly higher in hazelnut allergic patients monosensitized to *Cor a 8* than in patients sensitized to *Cor a 1* even when simultaneously sensitized to *Cor a 8* and *Cor a 9.*

Statement of Ethics

In this cross-sectional observational study, patients' written informed consent was obtained for participation and for the processing of personal data; the study was approved on 31 January 2015 by the Niguarda Hospital Ethics Committee under protocol number 31-012015.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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Author Contributions Statement

EAP designed the study, wrote and revised the draft paper. JS and CMR analyzed the data and wrote the draft paper. AT collected the data and helped writing the paper. LMS and MGA helped writing the draft paper. MN performed the statistical analysis. LF analyzed the data and revised the draft paper. All authors approved the final version.

Data Availability Statement

The data that support the findings of this study are not publicly available due to the content of information that could compromise the privacy of research participants but are available from the corresponding author EAP upon reasonable request.

References

1 Clarke AE, Elliott SJ, St. Pierre Y, Soller L, La Vieille S, Ben-Shoshan M. Temporal trends in prevalence of food allergy in Canada. J Allergy Clin Immunol Pract. 2020;8:1428-1430.e5.

2 Rangkakulnuwat P, Lao-Araya M. The prevalence and temporal trends of food allergy among preschool children in Northern Thailand between 2010 and 2019. World Allergy Organ J. 2021;14:100593.

3 Ma Z, Chen L, Xian R, Fang H, Wang J, Hu Y. Time trends of childhood food allergy in China: Three cross-sectional surveys in 1999, 2009, and 2019. Pediatr Allergy Immunol 2021;32:1073–9.

4 McWilliam V, Koplin J, Lodge C, Tang M, Dharmage S, Allen K. The Prevalence of Tree Nut Allergy: A Systematic Review. Curr Allergy Asthma Rep 2015;15:54.

5 Lyons SA, Clausen M, Knulst AC, Ballmer-Weber BK, Fernandez-Rivas M, Barreales L, et al. Prevalence of Food Sensitization and Food Allergy in Children Across Europe. J Allergy Clin Immunol Pract; 2020;8:2736-2746.e9.

6 Valcour A, Lidholm J, Borres MP, Hamilton RG. Sensitization profiles to hazelnut allergens across the United States. Ann Allergy Asthma Immunol; 2019;122:111-116.e1.

7 Grabenhenrich L, Trendelenburg V, Bellach J, Yürek S, Reich A, Fiandor A, et al. Frequency of food allergy in school-aged children in eight European countries—The EuroPrevall-iFAAM birth cohort. Allergy Eur J Allergy Clin Immunol. 2020;75:2294–308.

8 http://www.fao.org/faostat

9 Ahmet Semih Uzundumlu, Seval Kurtoglu, Coskun Serefoglu, Zafer Algur The role of Turkey in the world hazelnut production and exporting Emirates Journal of Food and Agicultural 2022; 34(2): 117-127).

10 Worldwide Hazelnut Market Analysis & Projections (2019-2024): In Terms of Consumption, Europe is Expected to Register the Highest CAGR, at 7.6% (prnewswire.com)

11 Pinar Gur Cetinkaya, Betul Buyuktiryaki, Ozge Soyer, Umit Murat Sahiner, Cansin Sackesen, Bulent Enis Sekerel. Phenotypical characterization of tree nuts and peanut allergies in east Mediterranean children. Allergol Immunophatol 2020 Jul-Aug;48(4):316-322.)

12 Elisabetta Calamelli, Alessia Trozzo, Elisabetta Di Blasi, Laura Serra and Paolo Bottau. Hazelnut allergy., Medicina 2021 January 14, 57 (1) 67.

13 Sarah A Lyons, Peter G J Burney, Barbara K Ballmer-Weber, Montserrat Fernandez-Rivas, Laura Barreales, Michael Clausen, Ruta Dubakiene, Cristina Fernandez-Perez, Philipp Fritsche, Monika Jedrzejczak-Czechowicz, Marek L Kowalski, Tanya Kralimarkova, Ischa Kummeling, Tihomir B Mustakov, Ans F M Lebens, Harmieke van Os-Medendorp, Nikolaos G Papadopoulos, Todor A Popov, Alexandros Sakellariou, Paco M J Welsing, James Potts, E N Clare Mills, Ronald van Ree, André C Knulst, Thuy-My Le. Food Allergy in Adults: Substantial Variation in Prevalence and Causative Foods Across Europe. J Allergy Clin Immunol Pract 2019 Jul-Aug;7(6):1920-1928.e11.

14 Ortolani C, Ballmer-Weber BK, Hansen KS, Ispano M, Wüthrich B, Bindslev-Jensen C, Ansaloni R, Vannucci L, Pravettoni V, Scibilia J, Poulsen LK, Pastorello EA. Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. J Allergy Clin Immunol. 2000 Mar;105(3):577-81

15 McWilliam VL, Perrett KP, Dang T, Peters RL. Prevalence and natural history of tree nut allergy. Ann Allergy, Asthma Immunol. 2020;124:466–72.

16 Moraly T, Pelletier de Chambure D, Verdun S, Preda C, Seynave M, Vilain AC, et al. Oral Immunotherapy for Hazelnut Allergy: A Single-Center Retrospective Study on 100 Patients. J Allergy Clin Immunol Pract; 2020;8:704-709.e4.

17 Sabouraud M, Biermé P, André-Gomez SA, Villard-Truc F, Payot F, Corréard AK, et al. Real-life experience with hazelnut oral immunotherapy. Ann Allergy Asthma Immunol [Internet]. Ann Allergy Asthma Immunol; 2022;128:432–8.

18 Richard E Goodman, Heimo Breiteneder. The WHO/IUIS Allergen Nomenclature. Allergy. 2019 Mar;74(3):429-431

19 Dramburg, S, Hilger, C, Santos, A, et al. EAACI Molecular Allergology User's Guide 2.0. Pediatr Allergy Immunol. 2023; 34(Suppl. 28):e13854.

20 Datema MR, van Ree R, Asero R, Barreales L, Belohlavkova S, de Blay F, et al. Component-resolved diagnosis and beyond: Multivariable regression models to predict severity of hazelnut allergy. Allergy [Internet]. Allergy; 2018;73:549–59.

21 Pastorello EA, Vieths S, Pravettoni V, Farioli L, Trambaioli C, Fortunato D, et al. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. J

Allergy Clin Immunol; 2002;109:563–70.

22 Lamberti C, Nebbia S, Antoniazzi S, Cirrincione S, Marengo E, Manfredi M, et al. Effect of hot air and infrared roasting on hazelnut allergenicity. Food Chem; 2020;342:128174–128174.

23 Masthoff LJ, Hoff R, Verhoeckx KCM, Van Os-Medendorp H, Michelsen-Huisman A, Baumert JL, et al. A systematic review of the effect of thermal processing on the allergenicity of tree nuts. Allergy; 2013;68:983–93.

24 Skypala IJ, Asero R, Barber D, Cecchi L, Diaz Perales A, Hoffmann-Sommergruber K, et al. Non-specific lipid-transfer proteins: Allergen structure and function, cross-reactivity, sensitization, and epidemiology. Clin Transl Allergy 2021;11: e12010.

25 Valbuena T, Reche M, Marco G, Toboso I, Ringauf A, Thuissard-Vasallo IJ, et al. Storage proteins are driving pediatric hazelnut allergy in a lipid transfer protein-rich area. Foods. 2021;10: 2463.

26 Masthoff LJN, Mattsson L, Zuidmeer-Jongejan L, Lidholm J, Andersson K, Akkerdaas JH, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol 2013;132:393–9.

27 Masthoff LJN, Blom WM, Rubingh CM, Klemans RJB, Remington BC, Bruijnzeel-Koomen CAFM, et al. Sensitization to Cor a 9 or Cor a 14 has a strong impact on the distribution of thresholds to hazelnut. J allergy Clin Immunol Pract 2018;6:2112-2114.e1.

28 Andrews T, Banks JR. Sensitization to cor a 9 and cor a 14 is highly specific for a hazelnut allergy with objective symptoms in dutch children and adults. Pediatrics 2014; 134:S152.

29 Pastorello EA, Farioli L, Pravettoni V, Scibilia J, Mascheri A, Borgonovo L, et al. Pru p 3-Sensitised Italian Peach-Allergic Patients Are Less Likely to Develop Severe Symptoms When Also Presenting IgE Antibodies to Pru p 1 and Pru p 4. Int Arch Allergy Immunol 2011;156:362–72.

30 Scala E, Till SJ, Asero R, Abeni D, Guerra EC, Pirrotta L, et al. Lipid transfer protein sensitization: reactivity profiles and clinical risk assessment in an Italian cohort. Allergy. 2015;70:933-43.

31 Bogas G, Muñoz-Cano R, Mayorga C, et al. Phenotyping peach-allergic patients sensitised to lipid transfer proteinand analysing severity biomarkers. Allergy. 2020;75(12):3228-3236.

32 Flinterman AE, et al. Lipid transfer protein-linked hazelnut allergy in children from a non-Mediterranean

birch-endemic area. J.Allergy Clin Immunol 2008.

33 Carraro S, Berardi M, Bozzetto S, Baraldi E, Zanconato S. Cor a 14-specific IgE predicts symptomatic hazelnut allergy in children. Pediatr Allergy Immunol. 2016;27:322–4.

34 Nilsson C, Berthold M, Mascialino B, Orme M, Sjölander S, Hamilton R. Allergen components in diagnosing childhood hazelnut allergy: Systematic literature review and meta-analysis. Pediatr Allergy Immunol.; 2020;31:186–96.

35 Uotila R, Röntynen P, Pelkonen AS, Voutilainen H, Kaarina Kukkonen A, Mäkelä MJ. For hazelnut allergy, component testing of Cor a 9 and Cor a 14 is relevant also in birch-endemic areas. Allergy. 2020;75:2977–80.

36 Gomez F, Bogas G, Gonzales M et al. The clinical and immunological effects of Pru p 3 immunotherapy on peach and peanut allergy in patients with systemic reactions. Clin Exp Allergy. 2017;47(3):339-350.

Figure Legends

Figure 1. Frequency of systemic reactions among the population of patients only *Cor a 8 +* compared to that of *Cor a 1* + patients. SR=systemic reactions. SR0 denotes reactions limited to the oral cavity.

Figure 2. Frequency of systemic reactions among the population of patients only *Cor a 8* + compared to that of *Cor a 1 + Cora a 8* - patients (a) and compared to that of *Cor a 1* + *Cor a 8* + patients (b). SR=systemic reactions. SR0 denotes reactions limited to the oral cavity.

Figure 3. Frequency of systemic reactions of *Cor a 8* + patients compared to that of the *Cor a 1*+ *Cor a 8* + *Cor a 9* + subgroup and of *Cor a 1* +, *Cor a 8* +, *Cor a 9* - group. SR= systemic reactions. SR0 denotes reactions limited to the oral cavity.