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Ara h 6 sensitization in peanut allergy: friend, foe or innocent bystander?

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KEY WORDS

Ara h 6; Ara h 2; Ara h 9; component resolved diagnostic; peanut allergy

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Summary

The clinical role of Ara h 6 sensitization in peanut allergy is a current matter of debate. We investigated the role of Ara h 6 sensitization patterns in a sample of young adults from different Italian cities. Sera of 33 patients with specific IgE against Ara h 6 were selected. According to clinical symptoms upon peanut ingestion, patients were divided into severe reaction (SR) and mild-tolerant (MT) subgroups. While the SR group mainly showed sensitization patterns involving Ara h 2 and other major allergenic components, a previously undescribed association between Ara h 6 and Ara h 9 was found in the MT group. This pattern seems to be clustered in Mediterranean Italy and associated with Pru p 3 sensitization. This finding might shed a new light on the role of Ara h 6 sensitization in peanut allergy.

Introduction

Food allergy is a growing epidemic in Western countries, affecting mostly children and young adults, with a severe impairment of the quality of life and a potentially fatal outcome.

In clinical practice there is a strong need for prognostic markers to better identify subsets of patients at high risk of anaphylaxis, allowing earlier recognition and proper treatment. Current research is trying to unveil the association between the severity of food allergy and the detection of IgE antibodies against specific allergenic components. Hence component-resolved diagnostic (CRD) is playing a greater role in the diagnostic workup of food allergy.

Concerning peanut allergy (PA), several studies showed how the sensitization to various seed protein families has a diverse impact on the clinical outcome upon allergen exposure. The sensitization to 2S albumins, like Ara h 2, is predominant in peanut allergic children from USA and continental Europe, and

is highly linked to severe allergic reactions compared to PR-10 (Ara h 8) and Lipid Transfer Protein (LTP) (Ara h 9) sensitizations (1). In the Mediterranean area the sensitization to Ara h 9, a non-specific LTP allergenic molecule, is the most frequently observed cause of PA (1-4). Ara h 9 sensitization was seen to occur mostly in areas where the sensitization to other LTP molecules, in particular Pru p 3, were also observed (1). Furthermore, a cross-inhibition study performed on sera of patients with peanut and peach allergy suggested that Pru p 3 sensitization acts as a primary sensitizer for Ara h 9, confirming the strong correlation between these two molecules (3).

Ara h 6 is a seed storage protein belonging to the 2S albumin family that shares structural homology with Ara h 2. Ara h 2 and Ara h 6 sensitizations occur often simultaneously and both share the same clinical features and prognostic value in peanut allergic subjects (5-7). The detection of both allergenic molecules is considered by some to be redundant, especially in adults

(1,5). However, the combined Ara h 6 - Ara h 2 determination yielded a better diagnostic performance than single sensitizations in high-risk children (7,8) and there is small evidence that anaphylaxis can occur even in Ara h 6 mono-sensitization (9). Although there are still some gray areas in the use of microarray technology in CRD (10,11), multiplex assay offers exciting opportunities for broad range IgE testing and identification of sensitization patterns in PA. Moreover, a commercial microarray panel including the Ara h 6 molecule is currently available for diagnostic purposes in clinical practice. The aim of this study was to assess the sensitization patterns involving Ara h 6 and their clinical role in a sample of children and young adults from different Italian cities.

Materials and Methods

We retrospectively analyzed microarray test results of sera collected from three Italian allergy centers of different climatic regions: northern/continental area (Pordenone) and central/southern Mediterranean areas (Ancona and Naples).

Specific IgE against Ara h 6 were assessed using ImmunoCAP® Immuno Solid-phase Allergen Chip 112 (Thermo Fisher, Uppsala, Sweden).

We selected patients with specific IgE against Ara h 6 and absence of sensitization to cross-reactive carbohydrate determinants (MUXF3) and we collected data on clinical history, type and severity of allergic reaction upon peanut exposure and skin prick test (SPT) results for peanut extract.

Patients were then divided according to clinical history into two groups: the severe reaction group (SR) included any patient who reported at least two of the following symptoms upon peanut exposure: hypotension, syncope, urticaria, dyspnea, vomiting.

In the mild reaction or tolerant group (MT) were included all subjects with mild local symptoms (i.e. oral allergy syndrome) or no symptoms at all after peanut ingestion.

A commercial peanut extract (ALK-Abelló, Madrid, Spain) was used for SPT, along with positive (histamine 10 mg/mL) and negative (saline solution) controls. A positive SPT was defined as ≥ 3 mm wheal diameter compared to negative control.

Patients whose SPT results or clinical history were unavailable, or tested negative for peanut extract were excluded from the study. Positivity threshold were set to ≥ 0.30 ISU for Ara h 6 and ≥ 0.00 ISU for MUXF3.

Statistical analysis was performed using Microsoft® Excel 2007 (Microsoft, Redmond, USA).

Results

Sera of 74 patients were analyzed, though only 33 subjects fulfilled the enrollment criteria (mean age 16.5 ± 9.4 years; 11

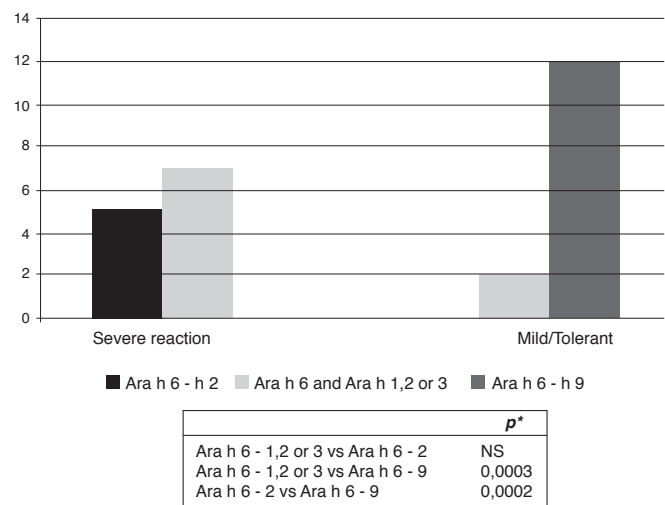
females). Among these, 16 subjects experienced severe reaction to peanut, 3 mild local symptoms and 14 tolerated peanut consumption. Five of 16 SR patients showed sensitization to Ara h 6 and Ara h 2, while in the MT group this association was not seen. By contrast, Ara h 6 and Ara h 9 co-sensitization was present in 12 out of 17 MT patients and none of the SR group (P value = 0.002, Fisher exact test).

The statistical difference between SR and MT groups was significant even when Ara h 6 and the co-sensitization to other major peanut allergens associated with severe PA (Ara h 1, Ara h 2, Ara h 3) was considered (**figure 1**).

Mean Ara h 6 IgE levels in the SR group were significantly higher compared to the MT group (8.3 ± 9.1 vs 2.8 ± 3.3 ISU, P value < 0.005) (**table 1**). Conversely, the Ara h 9/Ara h 6 IgE ratio was considerably higher in MT patients compared to SR (1.8 vs 0.1). Each patient co-sensitized to Ara h 6 and Ara h 9 showed specific IgE against Pru p 3 and the Ara h 6 - h 9 pattern was present in the Ancona and Naples centers only. On the contrary, the Ara h 6 - Ara h 2 co-sensitization pattern was scattered all over Italy (**figure 2**) and only three patients of the SR group from Naples showing the Ara h 6 - h 2 pattern were also sensitized to Ara h 9/Pru p 3.

No difference in age (median age SR vs. MT: 12 vs 15 years; $p > 0.05$, Student T-test) or sex was seen between Ara h 6 - h 9 and Ara h 6 - h 2 co-sensitized groups.

Figure 1 - Distribution of patients according to the sensitization pattern and clinical severity of peanut allergy.



* Fisher exact test, SR vs MT

Abbreviations: MT, mild/tolerant group; NS, not statistically significant; SR, severe reaction group.

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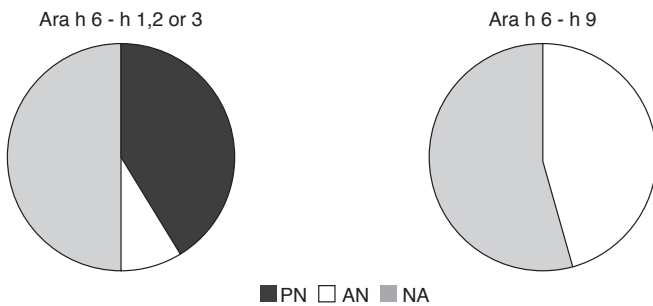
Table 1 - Specific IgE levels for Ara h 2, Ara h 6, Ara h 9 and Pru p 3 seen in the severe reaction and mild / tolerant groups.

		SR	MT	P value
Ara h 2	mean	11.1	0.9	< 0.05
	(ISU) SD	15.3	3.1	
Ara h 6	mean	8.3	2.8	< 0.05
	(ISU) SD	9.1	3.3	
Ara h 9	mean	1.1	5.2	< 0.05
	(ISU) SD	3.7	5.2	
Pru p 3	mean	1.1	9.4	< 0.005
	(ISU) SD	2.6	11.0	

¹Student T test, SR vs MT.

Abbreviations: ISU, ISAC Standardized units; MT, mild/tolerant group; SR, severe reaction group.

Figure 2 - Distribution of main Ara h 6 co-sensitization patterns observed according to center.



Abbreviations: PN, Pordenone; AN, Ancona; NA, Naples.

Discussion

Our study confirms the known association between severe allergic reaction to peanut and the Ara h 6 - h 2 sensitization pattern. Furthermore, it suggests the presence of a new pattern, namely Ara h 6 - h 9, associated with mild or none clinical symptoms upon peanut exposure, clustered in Mediterranean Italy.

These results therefore question the close relationship between Ara h 6 sensitization and severe reaction to peanut. Two aspects of this finding are noteworthy: the geographical clustering of the sensitization patterns and the impact of the co-sensitization profile on the prognostic outcome of Ara h 6. Italy is largely considered a Mediterranean country, however there are marked differences between northern and southern regions in terms of climate. These regional differences are mirrored by the diverse

clinical manifestations and sensitization patterns seen in both respiratory and food allergy (12,13).

Peanut allergy apparently makes no exception; we observed a strong resemblance between the sensitization patterns seen in Pordenone and continental Europe in peanut allergic subjects (1). While the Ara h 6 - h 2 pattern was evenly distributed throughout the country, we speculate that the observed predominance of this pattern in continental areas is due to the lack of sensitization to Pru p 3, as seen in Northern Italy. On the other hand, the distribution of Pru p 3 sensitization clearly overlapped Ara h 6 - h 9 sensitization in Southern Italy, but surprisingly the Pru p 3/Ara h 9 co-sensitization was seen to seldom occur in Ara h 6 - h 2 positive patients from southern regions. Therefore, our data support that sensitization patterns, rather than single sensitizations, are better means to assess the prognostic value of IgE positivity in food allergy, a concept that was already described in peach allergy (14,15). Several hypothesis can be drawn to better explain the prognostic shift of Ara h 6 according to the concomitant sensitization pattern. We observed an inversion of the Ara h 9/Ara h 6 ratio in the SR vs MT group, and this might suggest that the presence of a high level of specific IgEs against LTP molecules might have a role on the sensitization to Ara h 6. Although it has never been demonstrated to date, some degree of cross-reactivity between Ara h 6 and Ara h 9 could ensue, being both proteins belonging to the prolamine superfamily (16). Furthermore, we noted a marked difference in Ara h 6-specific IgE levels between the Ara h 6 - h 2 and Ara h 6 - h 9 patterns, and this may be an additional reason for the different clinical outcomes observed. We applied a positive cut-off value for Ara h 6 of ≥ 0.30 ISU as suggested by the manufacturer, although other authors showed better sensitivity and specificity of microarray Ara h 6 IgE assay when applying a higher threshold (≥ 2.00 ISU) (8). Eventually, we cannot exclude the possibility of a false positive result linked to the detection system in the presence of high level LTP sensitization.

Since the Ara h 6 - Ara h 9 pattern was not reported in recent studies on Ara h 6 sensitization in both pediatric and adult patients from Mediterranean areas (1,9), the reasons for this discrepancy are worthy of discussion. The different age groups considered might explain the inconsistency between our results and those shown by Pedrosa et al (9), where Ara h 9 sensitization was present only in a small percentage of children with no correlation with Ara h 6. Our sample belongs to an older age group compared to Pedrosa's pediatric population (mean age 16.5 ± 9.4 years vs 7.62 ± 3.23 years) (9). Although conflicting results on Pru p 3 allergy in children were documented (17,18), the onset of Pru p 3 sensitization seems to occur predominantly after early childhood (4). A second discrepancy was found between our study and the results of the EuroPrevall cohort, designed to describe the sensitization patterns in PA across Eu-

rope (1). In this survey 18 patients (12% of the overall sample) were enrolled in Mediterranean areas (Spain, Italy and Greece) (1). Though our cohort shares with this selected subgroup the same age and geography, the Ara h 6 - Ara h 9 sensitization pattern was not reported. We speculate two possible reasons for divergence, namely a selection bias or a difference in Ara h 6 IgE detection techniques. Concerning the first, in the EuroPrevall survey patients were selected by oral peanut challenge and the number of Mediterranean patients, both tolerant and non-tolerant, was small and this could have led to an underestimation of the Ara h 6 - h 9 pattern. On the contrary, in our study the selection was driven by the detection of specific IgE against Ara h 6, which could have magnified the Ara h 6 - h 9 subset.

As for IgE detection techniques, for the present study we performed a multiplex assay available for diagnostic purpose in clinical setting, while the EuroPrevall group developed an experimental singleplex assay for Ara h 6 IgE testing and concerns on the reliability of EuroPrevall's experimental assay have already been expressed (9).

Conclusion

Multiplex CRD performed in Italian Ara h 6-positive patients showed two main sensitization patterns, namely Ara h 6 - h 2 and Ara h 6 - h 9, with different clinical outcomes. Our study offered new insights on PA and Ara h 6 sensitization in Mediterranean areas, emphasizing the role of sensitization patterns, rather than single IgE positivity, for prognostic purposes. Limitations of this study were the retrospective design, the small number of observations and the absence of an oral peanut challenge in self-reported tolerant subjects. Further studies are needed to properly elucidate the impact of LTP allergy on Ara h 6 sensitization.

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