

Title: IgE-response to peanut allergens is related with age and onset of disease in paediatric population

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Short title: Response to peanut allergens in children and adolescents.

Abbreviations: SSP, seed storage proteins; LTP, lipid transfer proteins; SPT, skin prick test; PR-10, pathogenesis related-10; double-blind-placebo-controlled food challenge (DBPCFC); OAS, oral allergy syndrome.

Key Words: Food allergy, peanut, peach, allergens, sensitization, LTP, SSP

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What's Known on This Subject: The molecular response to vegetal food allergens varies with age, with allergens from food and inhalants playing an important role. This is important when evaluating allergic reactions to peanut.

What This Study Adds: Peanut allergy patients show different recognition patterns to peanut allergens according to age. Ara h 2 sensitization is more frequent at an early age, changing to a predominance of Ara h 9 in older patients.

Contributor's Statement:

Garcia-Blanca A: MD Garcia-Blanca collected patient's sera and analyzed the data

Aranda A: Dr. Aranda performed the experiments, wrote the manuscript, coordinated data collection approved the final manuscript.

Blanca-Lopez N and Perez D: Dr. Blanca-Lopez and Perez collected patient's sera.

Gomez F: Dr. Gomez analyzed the data

Mayorga C and Torres MJ: Dr. Mayorga and Dr. Torres conceived and designed the experiments and contributed reagents/materials/analysis tools

Diaz-Perales A and Villalba M: Dr. Diaz-Perales and Dr. Villalba revised the manuscript

Perkins JR: Dr. Perkins wrote the manuscript

Blanca M and Canto G: Dr. Blanca and Dr. Canto coordinated and supervised data collection approved the final manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ABSTRACT

Background: Peanut allergens are common responsible of food allergy. Analyses of sensitization patterns, relationships with other allergens, clinical symptoms and variation with age are needed. We studied sensitization to Ara h 2, Ara h 9 and Pru p 3 in a population of children/adolescents allergic to peanut, and the relation with peach and pollen.

Methods: Patients allergic to peanut were classified into two groups: A) allergic to peanut, and B) allergic to peach and peanut. The IgE response was measured to Ara h 2, Ara h 9 and Pru p 3.

Results: Of 456 subjects evaluated, 28% were allergic to peanut. From this group 69% were sensitized to pollen. Urticaria was the most frequent entity followed by anaphylaxis and OAS. Fifty-nine percent had Ara h 2 and/or Ara h 9 specific IgE. Over half reported symptoms with peanut alone (Group A); 46% to peanut and peach (Group B). We observed significant differences in sex, age, onset of symptoms and sensitization to *Artemisia*. IgE response to Ara h 2 was more frequent in Group A; Ara h 9 and Pru p 3 in Group B. We observed a decrease in sensitization to Ara h 2 and an increase to Ara h 9 and Pru p 3 with increasing age.

Conclusion: Peanut allergy is frequent in subjects with allergy to plant-foods, with Ara h 2 and Ara h 9 being two important allergens. In younger patients Ara h 2 predominates over Ara h 9. The reverse was observed in older patients.

INTRODUCTION

The prevalence of food allergy varies between 1% and 3% in the general population and is on the rise (1-4). Immediate reactions to food of plant origin are one of the most frequent causes of food allergy, with many cases starting in infancy (5,6). Peanut allergens are frequently implicated, affecting 1% of the population (7). Several studies have reported an allergy prevalence rate of 0.50%-1.85% in children (1,8). However, more studies need to be undertaken in other populations and with larger age ranges.

The major peanut allergens Ara h 1, Ara h 2 and Ara h 3 are vicilin, conglutin (2S Albumin) and legumin seed storage proteins (SSP), respectively (9-14). Ara h 8 is a pathogenesis-related protein, PR-10 (15,16); Ara h 9 belongs to the lipid transfer protein (LTP) family, many of which are important allergens in the Mediterranean area (17-19).

Many plant allergens are panallergens, present in many different sources. They are responsible for the cross-reactivity between homologous proteins from plant-derived foods and pollens (20-22). In central European countries a strong association exists between pollen sensitisation and food allergy. For example, apple and peach sensitization has been associated with primary sensitization to Bet v 1, the major birch pollen allergen, as well as Mal d 1 or Pru p 1, all belonging to the PR-10 family (23). Other associations between pollen and plant-derived foods have been described, along with details of the responsible allergens (24,25). This situation varies in the Mediterranean area where sensitization to Pru p 3 (LTP from peach) has been shown to be unrelated to pollen allergies (26,27).

Values of specific IgE to single molecular allergens may vary according to age. For example, peanut allergic children and adults have shown differences in allergen molecular recognition, with Ara h 2 the most prevalent in children (28) and Ara h 9 in adults (18). It is known that Ara h 9 sensitization is due to cross reactivity with Pru p 3,

both LTPs (19,29). However, the reasons for the age-related differences in allergen recognition are complex and cannot be explained in term of allergen exposure and the cross-reactivity phenomenon only. Detailed studies of how these patterns of sensitization occur at different ages in the same population and the relationship with related foods/inhalants are needed. This is particularly relevant in populations where primary sensitization occurs through the oral route.

In order to verify these variations in the molecular response to plant food allergens we undertook a cross-sectional study in a population ranging from 1 to 20 years of age. This provides a clearer view of sensitization patterns in different age groups. Those confirmed as allergic to peanut were further studied in order to verify the role of major peanut and peach molecular allergens in inducing sensitization and symptoms. Results indicate that the predominate allergen differs according to age: Ara h 2 for younger children and Ara h 9 for older children and adolescents. These findings have important clinical consequences.

MATERIAL AND METHODS

Subject selection

All patients were aged between 1 and 20 years old and referred to our service with suspicion of food allergy of plant origin were included in our study. The area of study was Madrid, Central Spain. All participants completed a written informed consent and the ethical committee of our institution approved the study.

The allergological evaluation included an examination of the patient's clinical history with implementation of an exhaustive questionnaire and skin prick test (SPT) as detailed below. In subjects with unclear history, double-blind-placebo-controlled food challenge (DBPCFC) was carried out.

The SPT was performed according to European guidelines (30) using a standardized panel of relevant inhalant allergenic extracts that included *Phleum*, *Olea*, *Platanus*, and *Artemisia* pollens plus peach and peanut from ALK-Abelló (Madrid, Spain). The SPT response was considered positive if the diameter of the wheal area was 3 mm greater than that induced by the negative control.

Patients were considered allergic if their clinical history showed at least two convincing reactions in their history within the first hour after exposure. In those with clear clinical history but only one episode to peanut, DBPCFC was performed to confirm the diagnosis. DBPCFC was performed as described previously (31). Briefly, meals containing 5g of amount of flour peanut with a mixture of yogurt, orange juice, dried coffee and oatmeal flakes were prepared. Placebo meals consisted of the same ingredients, without peanut. If cutaneous and/or respiratory symptoms or alterations in vital signs appeared, the procedure was stopped and the symptoms were evaluated and treated (32). We excluded subjects with pathologies such as genetic, autoimmune and metabolic diseases.

Quantification of IgE antibodies to whole peanut and peach extract and allergens

Specific IgE antibodies to whole peanut and peach extract and allergens Ara h 2, Ara h 9 and Pru p 3 were measured by ImmunoCAP following the manufacturer's recommendations (Phadia, Uppsala, Sweden). A positive result was defined as a value > 0.35 kUA/l.

Statistical analysis

Quantitative variables are shown as median and interquartile ranges (IR), while qualitative variables are shown as frequencies. Medians between groups were compared using Mann-Whitney or Kruskal-Wallis tests, while χ^2 was used to compare proportions. Differences with a $p < 0.05$ were considered significant.

RESULTS

Characteristics of peanut allergic patients

We evaluated a population of children and adolescents ranging from 2 to 20 years old who reported symptoms after peanut intake and/or contact. After proving hypersensitivity we studied peanut allergy in the context of sensitization to other relevant allergens including food and inhalants. We established the relationship between them by focusing on three relevant allergens in our population: Ara h 2, Ara h 9 and Pru p 3.

From the total number of 456 cases allergic to plant foods initially evaluated, 28% (128 cases) of the patients reported symptoms with peanut, 260 showed tolerance to peanut and the remaining 68 subjects had not had previous contact with peanut. Of the 128 patients with reported symptoms, 120 were confirmed as hypersensitive to peanut. Of these, 91 were diagnosed by clinical history and positive skin prick test and 29 by DBPCFC. The remaining 8 cases had good tolerance in spite of reported symptoms after peanut ingestion in their clinical history and were not used for further study. The median age of peanut hypersensitive patients was 9.86 (IR:2-20) years and 55% were male. Skin test response to inhalant allergens was positive for 96% of the cases, with 69% sensitive to pollens. The majority of patients (88%) had other allergic diseases in addition to food allergy. These were atopic dermatitis (63%), rhinoconjunctivitis (68%) and asthma (51%). The clinical symptoms most frequently reported by the patients was urticaria/angioedema in 58 patients (48%), followed by anaphylaxis (dyspnea, wheeze and hypotension) in 33 (28%) and OAS in 29 (24%).

Detailed analysis of these patients showed that 42 were also allergic to peach. The clinical symptoms reported by these patients were: OAS in 20 patients (48%), urticaria/angioedema in 18 (42%) and anaphylaxis in 4 (9%).

Skin prick test results

Most patients (91%) showed positive skin prick test results to peanut. More detailed analysis of these results showed that 90% of patients with anaphylaxis were skin test positive to peanut, as were 89% of patients with urticaria/angioedema. For patients with OAS, 79% of patients were skin test positive to peanut. Out of the 11 patients with negative SPT, 4 had urticaria/angioedema, 4 had OAS and 3 cases had anaphylaxis. With respect to pollen allergy, 87% of peanut allergic subjects were positive for *Phleum*, 76% for *Olea*, 73% for *Platanus* and 45% for *Artemisia*.

Specific IgE results

From 120 patients studied, 83% had specific IgE to whole peanut extract. When we measured specific IgE to whole peach extract to patients allergic to peanut and peach we observed that 90% of these had specific IgE to peach extract.

Measuring IgE response to peanut allergens showed that Ara h 2 antibodies were detected in 33% of patients and Ara h 9 in 42% (Figure 1A). Considering cases with specific IgE to only one allergen, 18% were positive to Ara h 2 and 27% to Ara h 9 only. Out of all peanut allergic patients tested, 59% were positive to at least one allergen (Figure 1B).

Of the patients with positive SPT to peanut, 68% showed IgE antibodies and of the 11 cases with negative SPT, 7% had IgE antibodies.

The proportion of patients with specific IgE to Ara h 2 and Ara h 9 was similar for patients with anaphylaxis and urticaria. For OAS, a higher proportion of patients showed IgE to Ara h 9 (48%) compared to Ara h 2 (24%) (Figure 2).

Comparison between peanut allergic patients with tolerance to peach and patients allergic to both peanut and peach.

In order to establish the relationship between peanut and peach allergy we compared the results in those patients with allergy to peanut but tolerant to peach (Group A, N=64) and those with symptoms induced by both peanut and peach (Group B, N=42) (Table 1). Those subjects who never took peach (N=14) were removed from this analysis. The patients of Group A were younger and the symptoms appeared earlier than in Group B. The overall pollen sensitization in Group A was lower than in Group B, but if we analyzed this response considering the pollens individually we detected that *Phleum*, *Olea* and *Platanus* were similar between both groups, however the percentage of *Artemisia* was significant higher ($p=0.033$) in Group B. Significant differences were observed between groups for patient age, age at symptom onset, sex, IgE specific to peach and sensitization to pollens.

When we compared the clinical symptoms induced, anaphylaxis appeared in 31% of the cases in Group A and in 19% of Group B ($p=0.014$), urticaria/angioedema was similar for both (52% in Group A and 44% in Group B). OAS was more common in Group B with 37% compared to 15% of Group A ($p=0.022$) (Figure 3).

The IgE response to individual peanut and peach allergens was analysed. When we compared Group A and Group B, we observed the number of individuals with IgE to Ara h 9 and Pru p 3 was significantly higher ($p=0.001$ and $p=0.008$, respectively) in Group B. Within Group A, Ara h 2 was the most frequent allergen, although this difference did not reach statistical significance. Within Group B Ara h 9 and Pru p 3 were significantly more prevalent than Ara h 2 ($p=0.003$ and $p=0.012$, respectively) (Figure 4).

When taking into account combinations of allergens we found that for Group A the cases positive to Ara h 2 were the most frequent ($p=0.050$) followed by the combination of Ara h 2, Ara h 9 and Pru p 3. Very few cases showed IgE to the combination of Ara h

9 and Pru p 3 only. On the other hand, for cases with allergy to both peanut and peach, the highest number of positive results corresponded to the combination of Ara h 9 and Pru p 3 ($p=0.018$) only; few cases were found that were positive to Ara h 2 only (Table 2).

Differences in the recognition of peanut and peach allergens according to age

We grouped patients by age intervals of 5 years from youngest to oldest in order to evaluate differences in the IgE response to Ara h 2, Ara h 9 and Pru p 3 (Figure 5). We observed that values of specific antibodies showed a significant decrease of Ara h 2 allergen prevalence with increasing age (Figure 5A). In contrast, the number of patients with antibodies to Ara h 9 and Pru p 3 increased with age (Figure 5B, 5C). We conclude that Ara h 2 positivity was much more prevalent in young children than Ara h 9 and Pru p 3. However the opposite was observed for older age groups.

DISCUSSION

In this work we have performed a cross-sectional study in a large group of children and adolescents with allergy to plant foods in a centre located in central Spain (Madrid). More than a quarter of patients reported symptoms with peanut, in agreement with previous studies in a similar population (3,34,35). We also observed association with other allergic diseases such as atopic dermatitis and asthma, as reported previously (36). Reactions to plant allergens are highly associated with pollen allergy (20,21,37). In our population we observed a variable percentage of sensitization to the pollens tested with *Phleum*, *Olea* and *Platanus* higher than 50%. Whether this association is related to cross-reactivity or represents a clinical property of atopic patients is a matter of speculation, however all these pollens contain panallergens with similar structures to peanut and peach proteins, such as profilins, PR-10 and LTPs. Focusing on the pollens with LTP allergens, we observed a strong association between patients allergic to both peanut and peach as food sources, and *Artemisia* pollen, suggesting cross-reactivity across LTP proteins (38-39).

Considering the clinical symptoms, over three quarters of peanut allergic patients observed in the study developed systemic symptoms, and more than a quarter developed anaphylaxis. These findings are similar to those reported by Pedrosa (28), where urticaria/angioedema was the most frequent clinical symptom followed by OAS and anaphylaxis.

The diagnosis of peanut allergy in our study was largely based on an unequivocal history of several episodes after ingestion of peanut. We detected that SPT was positive to peanut in 91% of these patients and 83% of them had specific IgE antibodies to peanut. Similar results have been described in other studies (40). For patients with anaphylaxis, 95% were SPT positive, and 75% had detectable IgE antibodies to

allergens peanut. For urticaria/angioedema 89% were SPT positive and 66% had detectable IgE antibodies. For those patients with OAS, 88% had a positive SPT and 76% had detectable IgE antibodies. Interestingly, we detected IgE antibodies to purified allergens for 7% of the cases that produced a negative SPT. This result may be due to a low concentration of the relevant allergen in the whole extract used for SPT, as suggested by others (41).

Regarding the sensitization profile to peanut allergens, we found that 42% of patients had IgE to Ara h 9, and 27% of the patients were monosensitized to this allergen.

A third of all patients recognized Ara h 2 and 18% were monosensitized. Our results are in line with a recent study from Calamelli (42) but are lower than those of Vereda et al (19). More than half (59%) of all patients had IgE antibodies to at least one allergen. Ara h 9 is more prevalent in the population studied here and appears to play an important role in peanut allergy among patients from the Mediterranean area (17,18). Ara h 2 has been implicated in other populations (28,43-45). For example, the work of Vereda et al. (19) showed that Ara h 2 was recognised by 42% of patients with peanut allergy.

Our study revealed an age dependent sensitization pattern in patients allergic to peanut extract. Interestingly, we found that the youngest group of children were more frequently monosensitized to Ara h 2: 32% at 1-5 years and 20% at 6-10 years. However the opposite pattern was found for Ara h 9: no patients in the 1-5 year group were monosensitized, however for 6-10 year old subject, 36% were monosensitised. Similar results have been described in a recent study where levels of IgE to Ara h 2, Ara h 8, Ara h 9 were measured in a pediatric population (42). It seems that natural loss of sensitisation occurs to Ara h 2 with increasing age, while awareness of LTP is gradually beginning. The presence of members of the LTP family in many species could be like a

maintenance dose of the allergen for these individuals. However, the absence of cross reactivity between 2S albumins due to the high difference in the primary structure of these proteins may lead a reduction in the allergic response to the allergen (46).

Regarding the relationship between clinical symptoms and the pattern of allergen recognition, fewer patients with OAS had IgE against Ara h 2 than Ara h 9. However, we did not find any differences for anaphylaxis or urticaria/angioedema. Our data agree with previous studies (28,47), although one study has shown anaphylaxis to be more highly associated with Ara h 2 (48).

The association of peach and peanut allergy is high in the Mediterranean region, due to cross-reactivity between both peanut and peach LTP (49). However this relation is less marked in other countries as described previously (19). So, our population could be divided into two major groups: patients with allergic reactions to peanut and not peach (Group A), and patients with allergic reactions to both peanut and peach (Group B) as described previously (29,49,50). Almost one third of the peanut allergic patients belonged to Group B. Symptoms with peach often appeared before peanut allergy. This may be due a primary sensitization to peach LTP, Pru p 3, as previously reported by our group in an adult population (29). Sensitization to pollen was higher in patients allergic to peanut and peach than in those only allergic to peanut, especially for *Artemisia*. This could be due to cross-reactivity between pollen and food as described previously (39,51).

Analysis of the sensitization pattern confirmed that allergen recognition differed between groups. In Group A, Ara h 2 was higher than Ara h 9 or Pru p 3 (peanut and peach LTPs). In Group B, Ara h 9 and Pru p 3 were significantly higher. These findings agree with other studies (17,18,45,47,50). However, only 22% of cases from Group A had systemic reactions (anaphylaxis and urticaria/angioedema) and only 12% of Ara h 2

positive cases Ara h 2 from Group B had systemic reactions. In 10% of cases in Group A, the subject had IgE antibodies to Ara h 2 and Ara h 9. Because these subjects developed symptoms to peanut only and tolerated peach, we conclude that Ara h 2 was responsible for the symptoms.

For some cases in Group B, specific IgE was detected for Ara h 9 but not Pru p 3, indicating that Ara h 9 can be the primary sensitizing agent. Ara h 9 has been described as the primary sensitizer in adults (29).

Summarizing, our study presents the largest series of child and adolescent peanut allergic patients and suggests that the sensitization profile is related to sensitivity to other foods and pollen. Moreover, the proportion of patients with specific IgE antibodies varies according to age: for older children and adolescents, fewer patients show IgE to the Ara h 2, whilst more patients show IgE to the LTP proteins Ara h 9 and Pru p 3.

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Table 1. Comparison of demographic and clinical characteristics of patients with symptoms to peanut and tolerance to peach (Group A) and patients with symptoms to peanut and peach (Group B). Significant differences ($P < 0.05$) are indicated in bold.

	SYMPTOMS TO PEANUT AND TOLERANCE TO PEACH (N=64)	SYMPTOMS TO PEANUT AND PEACH (N=42)	P
Age, median (IR)	8.77 (2-20)	11.87 (2-20)	0.010
Age at onset of symptoms, median (IR)	5.71 (1-17)	Peanut: 8.45 (1-19) Peach: 6.68 (1-19)	0.016
Sex, % Male	63	38	0.020
Caucasian, %	86	91	0.559
Specific IgE, % Peanut whole extract Peach whole extract	80 61	93 93	0.145 0.003
Atopy, %	95	84	0.324
Sensitization to pollen, % <i>Phleum</i> <i>Olea</i> <i>Platanus</i> <i>Artemisia</i>	61 93 70 73 33	84 82 82 79 57	0.031 0.156 0.301 0.660 0.033
Associated Allergic Diseases, %	86	91	0.559

Table 2. Comparison of percentages of positive cases to single or to combinations of allergens Ara h 2, Ara h 9 and Pru p 3 for patients with symptoms to peanut and tolerance to peach (Group A) and patients with symptoms to peanut and peach (Group B). Significant differences ($P < 0.05$) are indicated in bold.

	SYMPTOMS TO PEANUT AND TOLERANCE TO PEACH (N=64)	SYMPTOMS TO PEANUT AND PEACH (N=42)	p
Ara h 2	20%	9%	0.050
Ara h 9	2%	6%	0.564
Pru p 3	0%	3%	1
Ara h 9 + Pru p 3	6%	44%	0.018
Ara h 2 + Ara h 9	2%	3%	1
Ara h 2 + Pru p 3	2%	0%	1
Ara h 2 + Ara h 9 + Pru p 3	16%	9%	0.132

FIGURE LEGENDS

Figure 1.- Percentage of positive cases to peanut allergens (Ara h 2 and Ara h 9) in the population reporting symptoms to peanut (measured by ImmunoCAP). A) IgE response to peanut allergens Ara h 2 and Ara h 9. B) IgE response to single peanut allergens (Ara h 2 + Ara h 9 -, Ara h 2 - Ara h 9 +) and to any or both allergens (Ara h 2 + and/or Ara h 9 +).

Figure 2.- Percentage of positive cases to peanut allergens (Ara h 2 and Ara h 9) for patients presenting different clinical symptoms in the population reporting symptoms to peanut (measured by ImmunoCAP).

Figure 3.- Differences between clinical symptoms developed by ingestion and/or contact to peanut in patients with symptoms to peanut and tolerance to peach (Group A : N=64) and patients with symptoms to peanut and peach (Group B : N=42). $P < 0.05$ represents a significant difference.

Figure 4.- Percentage of positive cases to peanut and peach allergens (Ara h 2, Ara h 9 and Pru p 3) in patients with symptoms to peanut and tolerance to peach (Group A : N=64) and patients with symptoms to peanut and peach (Group B : N=42). $P < 0.05$ represents a significant difference.

Figure 5.- Percentage of positive cases showing specific IgE (A,B,C) to peanut and peach allergens (Ara h 2, Ara h 9 and Pru p 3) in patients, grouped by age intervals of 5 years from youngest to oldest. $P < 0.05$ represents a significant difference.

FIGURE 1

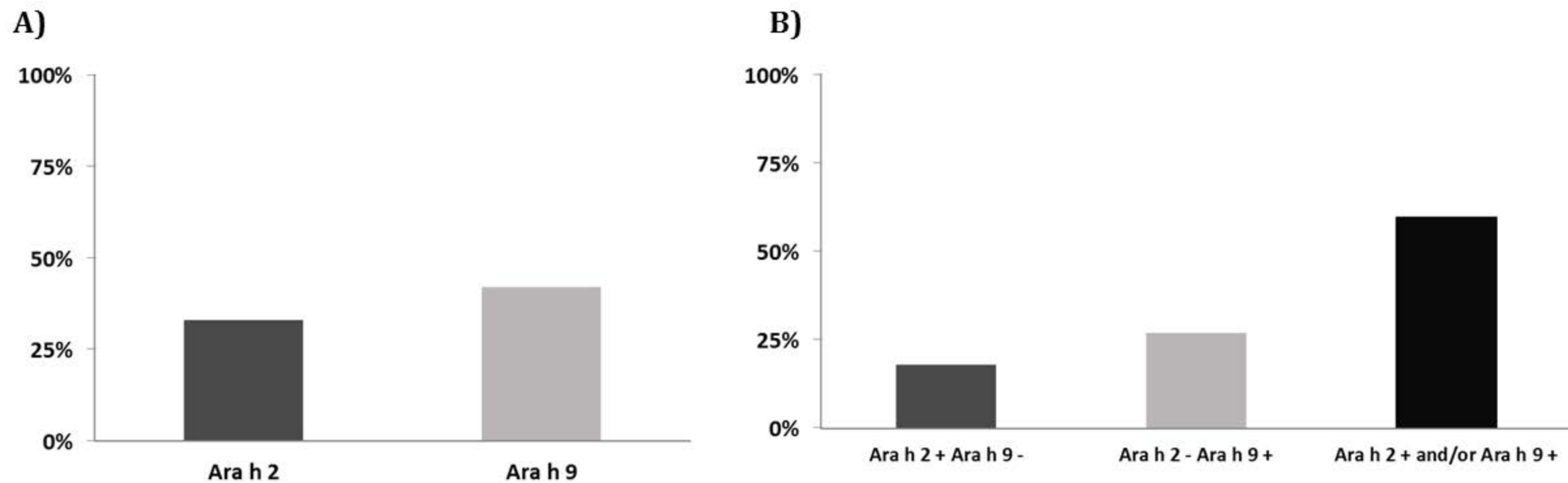


FIGURE 2

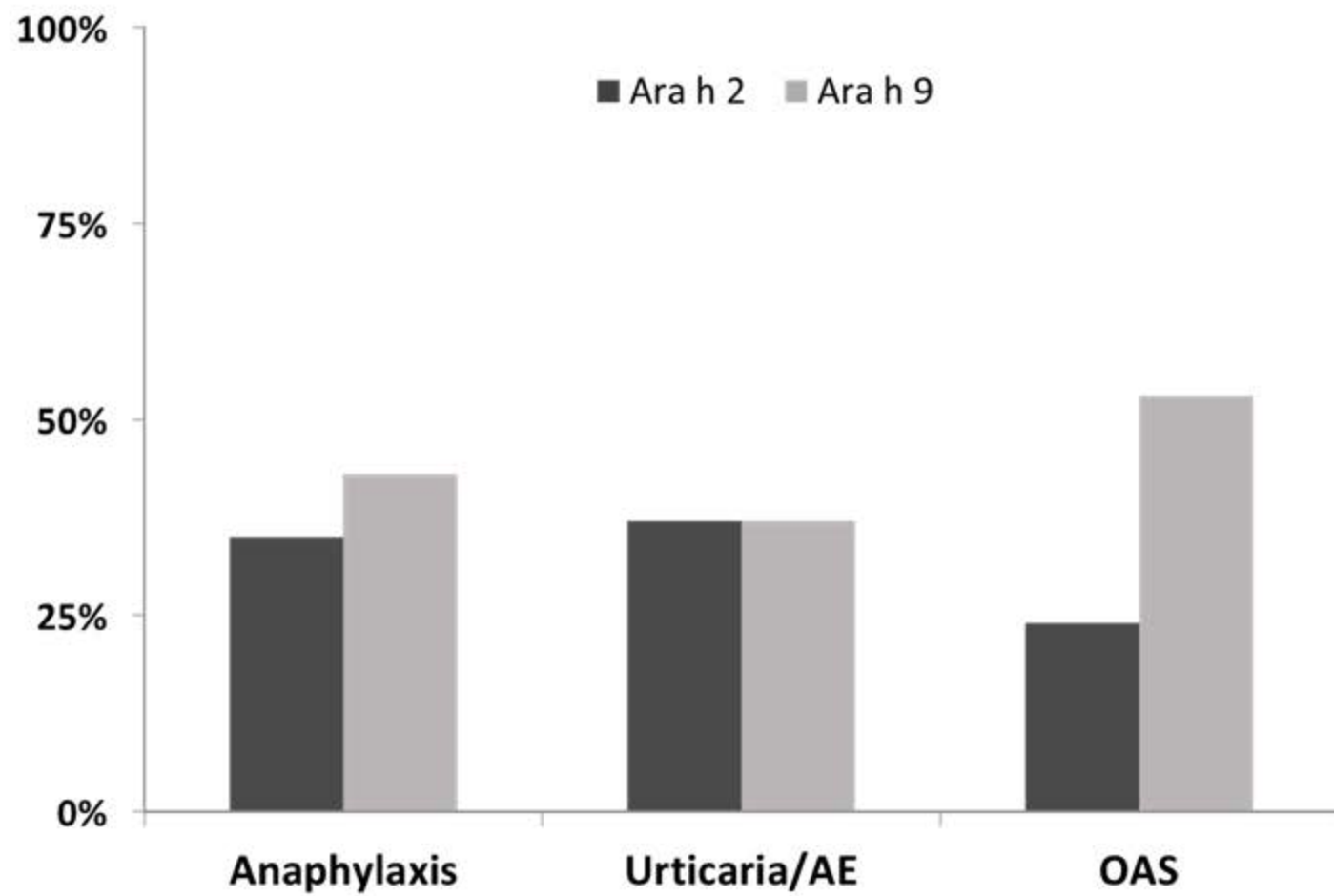


FIGURE 3

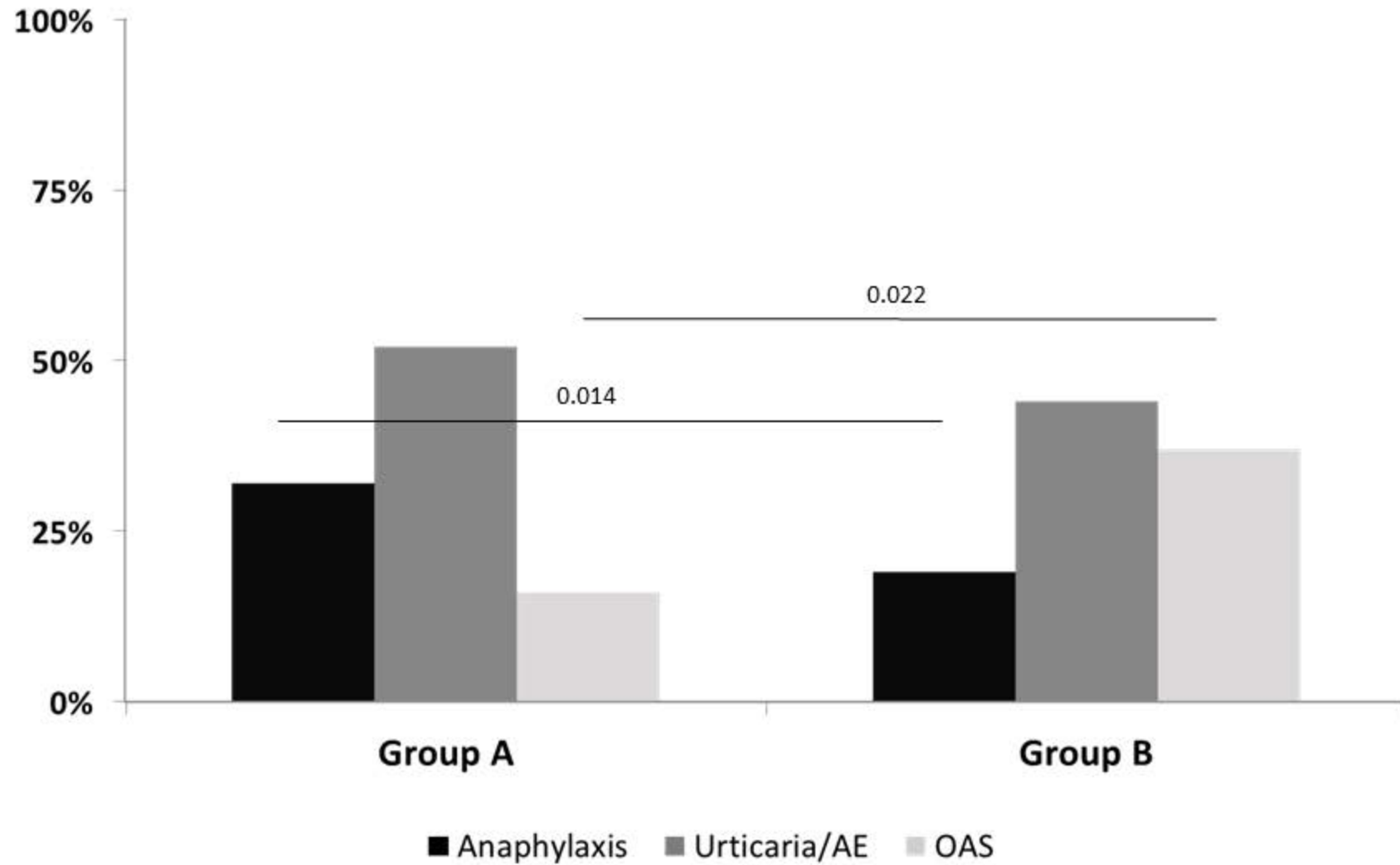


FIGURE 4

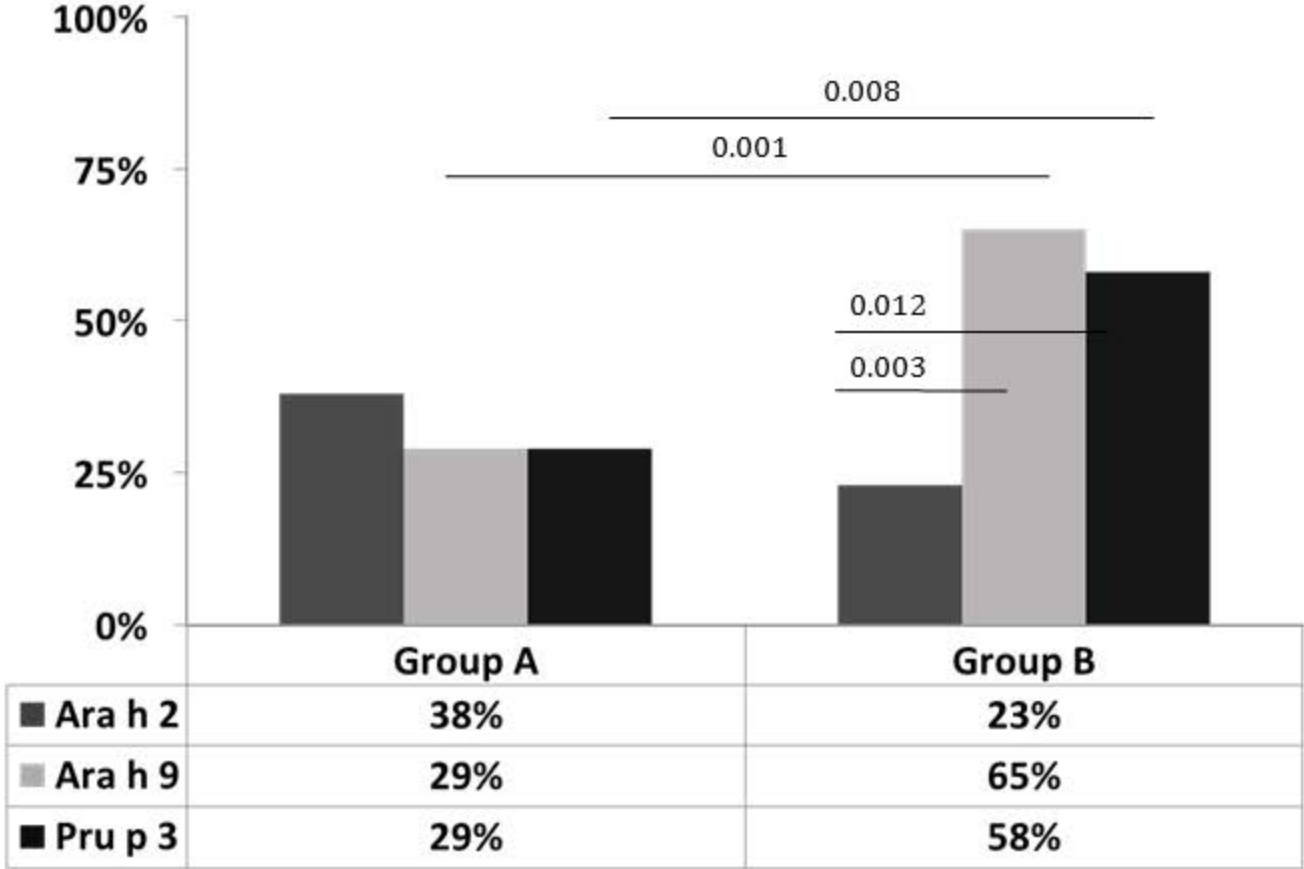
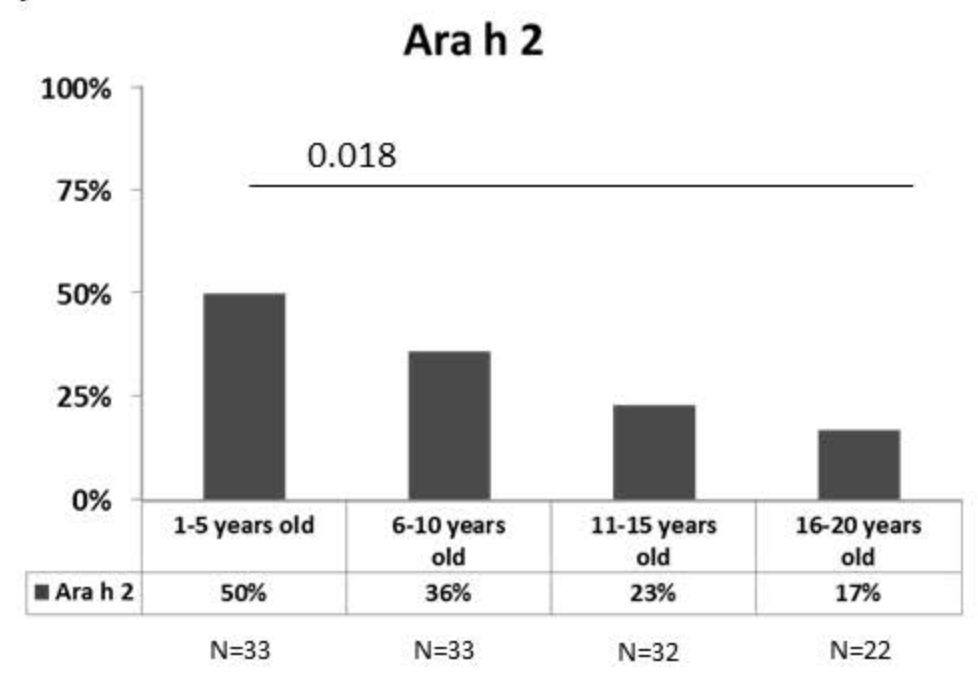
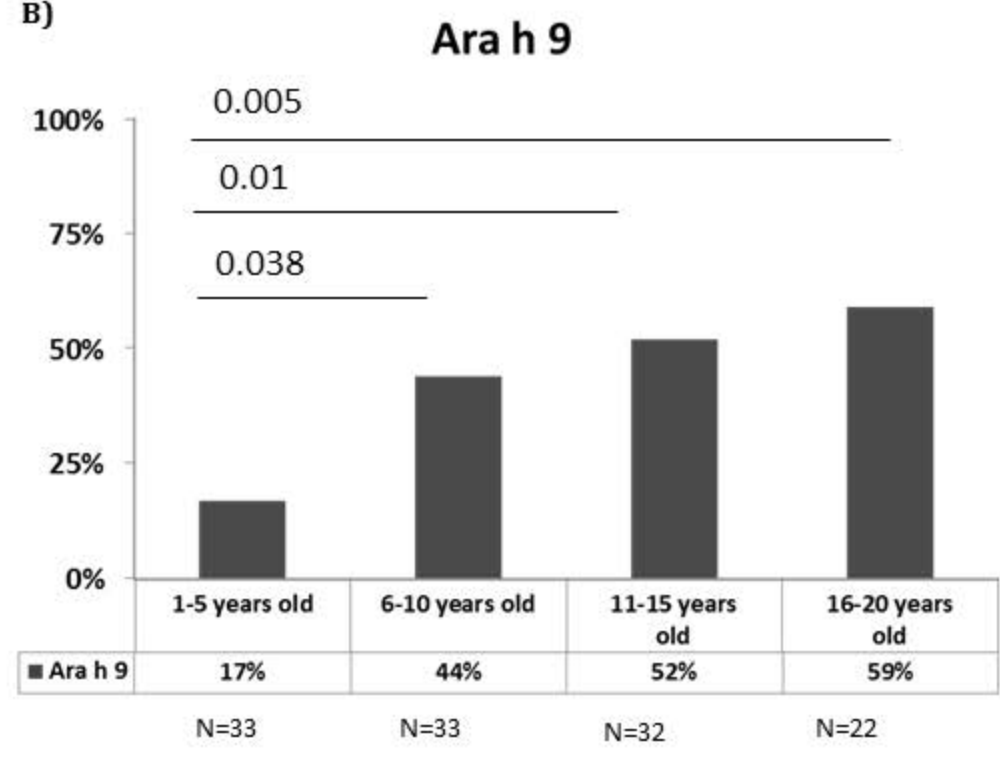


FIGURE 5

A)



B)



C)

