

Could Bet v 1 affect sensitization molecular pattern in children?

Maria Angela Tosca¹, Michela Silvestri², Angela Pistorio³, Roberta Olcese⁴, Paolo Del Barba⁴, Giorgio Ciprandi⁵

¹Pediatric Allergy, IRCCS Istituto Giannina Gaslini, Genoa, Italy; ²Pediatric Pneumology, IRCCS Istituto Giannina Gaslini, Genoa, Italy; ³Epidemiology and Biostatistics Service, IRCCS Istituto Giannina Gaslini, Genoa, Italy; ⁴Università Vita Salute San Raffaele, Milan, Italy; ⁵IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Summary. *Background:* Allergy is characterized by allergen-specific IgE production. Molecular-based allergy diagnostic allows to define the precise sensitization profile. Bet v 1 is the major allergen of the PR-10 family. It has been reported that pan-allergens could affect the sensitization panel in adults. *Objective:* This study aimed to evaluate the impact of Bet v 1 sensitization on clinical presentation in a sample of children with Bet v 1-sensitization; oral allergy syndrome (OAS) or anaphylaxis (ANA) were considered. *Methods:* Serum IgE molecular components were assessed by ISAC method. Sera and clinical data from 132 children, 91 males (68.94%) and 41 females (31.06%), mean age 9.08 years (3.45 years), were analyzed. *Results:* Bet v 1-sensitized children were frequently, but not exclusively, sensitized to other molecules belonging to PR-10 family. However, there was no significant difference concerning IgE levels between children with or without food allergy and between children with OAS and ANA, but hazelnut only for generic food allergy. *Conclusions:* The present study demonstrates that Bet v 1 sensitization may affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area, but it is unable to discriminate patients from a clinical point of view. So, ISAC test should be integrated with more precise IgE assay. (www.actabiomedica.it)

Key words: allergen-specific IgE, Bet v 1, molecular component, oral allergy syndrome, anaphylaxis

Introduction

Sensitization, such as the production of allergen-specific IgE, has to be considered the main biomarker of allergic disorders. Sensitized subjects usually produce IgE to more allergens over time (1,2). So, polysensitization is an important phenomenon which may occur in 90% of sensitized people (3,4).

The knowledge of the allergenic molecular profile has impressively changed the work-up in allergic patients. The molecular-based allergy diagnosis is built on the assessment of allergen molecules. This methodology allows precise definition and characterization of the sensitization profile by detecting the genuine major allergens and excluding false reactivity to pan-allergens

(5,6). Pan-allergen could be defined as allergen molecules shared by different allergen sources. The main pan-allergens involved in pollen allergy are: pathogenesis-related protein group 10 (PR-10), profilin, and lipid transfer protein (LTP); however, profilin is without clinical relevance in most patients (7-9). PR-10 was first identified in pollens of *Fagales* order, mainly birch, and further in cross-reacting fruits and vegetables (10). In the PR-10 family, the major allergen is Bet v 1, mainly contained in the pollens of the European white birch (*Betula verrucosa*) and cross-reacting with other tree pollens of the *Betulaceae* family, including alders, hazels, hornbeams, hazel-hornbeam, and hop-hornbeams (11).

In our geographic area, Genoa city overlooking the Mediterranean Sea, *Betulaceae* allergy (BA) is

very common (12). However, this area is paradoxically birch-free, but other PR-10-related pollen allergens are present, i.e. hazelnut and hornbeam, that may act as primary sensitizer.

Interestingly from a clinical point of view, the serum level of IgE to Bet v 1 may be able to discriminate mere sensitization from true allergy (13). In addition, it has been reported that patients with pollen allergy and oral allergy syndrome (OAS) have a peculiar molecular pattern depending on the geographical area they live (14). On the other hand, patients with pollen allergy and anaphylaxis are usually sensitized to LTP (15). So, we tested the hypothesis concerning the definition of different molecular patterns in children with BA and OAS or anaphylaxis (ANA). Therefore, the present study investigated the allergenic molecular profile in children living in Genoa and allergic to Bet v 1 with the aim of analyzing their molecular patterns also considering OAS or anaphylaxis to foods comorbidity.

Material and Methods

Patients

This retrospective study considered children suffering from respiratory allergy. They went to the Laboratory of the Istituto Giannina Gaslini of Genoa (Italy) for serologic assessment between July 2012 and April 2014. We analyzed the findings of serum allergen-specific IgE assessed by the ISAC method. We selected children with allergic rhinitis and/or asthma and Bet v 1 positivity.

OAS and ANA to foods were diagnosed as previously defined according to validated criteria (15).

The Review Board of the Istituto Giannina Gaslini approved the procedure. The patients' parents gave a written informed consent.

IgE Assay

Serum IgE were measured by ISAC test according to the manufacturer's recommendations (ThermoFisher Italy, Milan, Italy). Twenty μ L of the patient's serum were incubated on the microchip containing 112 allergen spots. After 1-hour incubation, slides

were washed and a monoclonal anti-IgE antiserum labeled with a fluorochrome was added and incubated for 1 hour. Then, slides were re-washed and the chips were analyzed by a Laser Scan Confocal microarray reader (LuxScan 10K/A, CapitalBio, Beijing, China). A microarray Image Analyser immediately analyzed the findings. All samples were identified using a single barcode. The results were calculated by the software. The ISAC score was expressed as ISAC Standardized Units (ISU), ranging from 0 to 100.

Data and Statistical analysis

The ISAC score was reported as ISAC Standardized Units (ISU-E), which ranges from 0 to 100 ISU. Positive finding, such as sensitization, was defined a value >0.3 ISU, according to the manufacturer's rules.

Within each group i.e. patients without OAS nor ANA (OAS/ANA- patients), patients with OAS only or ANA only (OAS/ANA + patients), patients with OAS only (OAS + patients) and patients with ANA only (ANA+ patients), the number of positive tests was evaluated. IgE levels were non-normally distributed (as evaluated by the Shapiro-Wilk test) and summarized as medians with lower and upper quartiles (LQ and UQ). IgE levels in sensitized patients (i.e. those with a positive test toward a specific allergenic molecule) were compared using the Mann U Whitney test. All the tests were two-sided and a p value <0.05 was considered as statistically significant. Statistica software 9.0 (StatSoft Corp., Tulsa, OK, USA) was used for all the analyses.

Results

Sera and clinical data from 132 patients, 91 males (68.94%) and 41 females (31.06%), mean age 9.08 years (3.45 years, range 0-17 years), were analyzed.

In the whole Bet v 1-positive population, rMal d 1, rCor a1.01, rPru p 1 represented the most commonly recognized PR-10, with over 80% of Bet v 1 positive patients sensitized to at least one of these allergenic molecules, with high or moderate median levels of IgE towards these molecules (Table 1). Sensitization to other PR-10 proteins were less frequent with 38% of

Table 1A. Frequency of positivity (sensitization) to different plant food allergenic molecules and IgE median serum levels (ISU-E) in allergenic-specific sensitized patients

Allergenic molecule	No.	%	Median (LQ-UQ) levels in positive pts
rMal d 1 - PR-10 protein	105	79.55	5.3 (1.6-12.85)
rCor a1.01 - PR-10 protein	87	65.91	5 (2.5-12.25)
rPru p 1 - PR-10 protein	87	65.91	4.6 (1.85-9.7)
rAra h 8 - PR-10 protein	50	37.88	1.85 (1.2-4.95)
rGly m 4 - PR-10 protein	50	37.88	2.75 (0.95-6.1)
rPru p 3 - Lipid transfer protein (LTP)	41	31.06	1.3 (0.65-3.2)
nJug r 3 - Lipid transfer protein (LTP)	37	28.03	1.6 (0.8-4.25)
rApi g 1 - PR-10 protein	32	24.24	1.8 (1-3.95)
nJug r 2 - Cupin	31	23.48	0.9 (0.5-2.1)
nAct d 1 - Cysteine protease	27	20.45	1.7 (1.25-3.25)
nJug r 1 - 2S albumin	27	20.45	2.3 (1.75-6.25)
rAra h 9 - Lipid transfer protein (LTP)	25	18.94	1.1 (0.6-4.85)
rCor a 8 - Lipid transfer protein (LTP)	23	17.42	1.1 (0.55-6.1)
nCor a 9 - Cupin	15	11.36	0.9 (0.65-1.5)
nAct d 8 - PR-10 protein	13	9.85	0.7 (0.6-1.1)
nAra h 6 - 2S albumin	12	9.09	2.6 (0.9-14.45)
rAra h 2 - 2S albumin	12	9.09	2.4 (1.15-13.55)
nSes i 1 - 2S albumin	10	7.58	3.65 (0.7-6.35)
nGly m 6 - Cupin	9	6.82	2.4 (0.65-4.35)
rAra h 1 - Cupin	8	6.06	4.3 (0.8-9.1)
nAct d 2 - Thaumatin-like protein	7	5.3	5.6 (3.65-8.55)
nGly m 5 - Cupin	7	5.3	1.4 (0.95-3.05)
rTri a14 - Lipid transfer protein (LTP)	7	5.3	0.8 (0.6-7.3)
rAna o 2 - Cupin	7	5.3	0.4 (0.4-2.25)
nAra h 3 - Cupin	4	3.03	6.95 (-)
nTri aaA - Alfa-amilase/trypsin inhibitor	4	3.03	1.2 (-)
rBer e 1 - 2S albumin	2	1.52	0.6 (-)

pts sensitized to rAra h 8 or to rGly m 4, 24% to rApi g 1, and less than 10% to nAct d 8 (Table 1A). No patient was sensitized to nAct d 5, rTri a19 nor to nFag e 2 (data not shown).

In addition, it was calculated how many patients were at least positive to one molecule belonging to the most important pan-allergen families, including PR-10, LTP, storage proteins, Cysteine protease, Thaumatin-like protein, and α -amilase/trypsin inhibitor. Table 1B shows the frequency of sensitizations: 89.39% of Bet v 1-positive children were sensitized to one PR-10 molecule; 39.39% to Storage proteins; 34.09% to LTP; 20.45% to Cysteine protease.

Comparison between OAS/ANA- and OAS/ANA+ subjects

Table 2A shows the median levels of plant food allergenic molecules in allergenic-specific sensitized

Table 1B. Frequency of positivity (sensitization) to different plant food allergenic molecule families in allergenic-specific sensitized patients

Allergenic molecule family	No.	%
PR-10 protein	118	89.39
Storage protein (Cupin and/or 2S albumin)	52	39.39
Lipid transfer protein (LTP)	45	34.09
Cysteine protease	27	20.45
Thaumatin-like protein	7	5.30
Alfa-amilase/trypsin inhibitor	4	3.03

patients. We found higher levels of IgE towards rCor a1.01 in OAS/ANA+ patients as compared to OAS/ANA- patients: 6.9 (3.25-14.15) and 3 (1.35-8.05), respectively (p=0.041). Similarly, all the other PR-10 proteins i.e. rMal d 1, rPru p 1, rAra h, 8 rGly m 4, rApi g 1, and two LTPs, i.e. rAra h 9, rCor a 8, were higher in OAS/ANA+ as compared to OAS/ANA-

Table 2A. IgE serum levels (ISU-E) to different plant food allergenic molecules among the different groups of allergenic-specific sensitized patients

	OAS/ANA-	OAS/ANA+	P value	OAS+	ANA+	P value
rMal d 1 - PR-10 protein [No. 105]	3.3 (1.7-11.75)	7.1 (1.55-14.95)	0.27	7.2 (1.65-16.3)	4.8 (0.8-16.55)	0.44
rCor a1.01 - PR-10 protein [No. 87]	3 (1.35-8.05)	6.9 (3.25-14.15)	0.041	6.5 (3.05-12.25)	8.6 (5.2-16.9)	0.46
rPru p 1 - PR-10 protein [No. 87]	3.15 (1.1-8.2)	5.2 (2-11.15)	0.10	4.9 (2.1-11.35)	6.9 (1.65-9.8)	0.89
rAra h 8 - PR-10 protein [No. 50]	1.7 (0.85-4.45)	2.1 (1.3-5.05)	0.24	2.95 (1.2-5.35)	1.7 (-)	0.41
rGly m 4 - PR-10 protein [No. 50]	2.5 (0.68-6.38)	3.1 (1.0-5.9)	0.64	3.5 (1.1-6.25)	1.3 (0.75-3.7)	0.19
rPru p 3 - LTP [No. 41]	1.2 (0.7-2.35)	1.3 (0.6-9.45)	0.51	1.1 (0.75-9.45)	2.2 (0.5-14.55)	0.53
nJug r 3 - LTP [No. 37]	1.6 (0.7-4.25)	1.6 (0.85-6.65)	0.70	1.35 (0.65-6.3)	3.55 (-)	0.08
rApi g 1 - PR-10 protein [No. 32]	1.0 (0.8-3.4)	2.4 (1.3-4.8)	0.10	2.5 (1.2-4.8)	18 (-)	0.55
nJug r 2 - Cupin [No. 31]	0.8 (0.4-2.85)	0.9 (0.55-1.25)	0.95	0.9 (0.55-1.25)	0.85 (0.45-2.9)	0.93
nAct d 1 - Cysteine protease [No. 27]	1.7 (1.35-3.25)	1.3 (0.9-3.25)	0.30	1.3 (0.9-2.15)	5.5 (-)	0.42
nJug r 1 - 2S albumin [No. 27]	2.85 (1.9-6.25)	2.3 (1.6-7.45)	0.61	2.05 (1.2-3.25)	11.1 (-)	0.16
rAra h 9 - LTP [No. 25]	0.8 (0.45-3.05)	1.45 (0.65-7.6)	0.17	0.7 (0.6-9.15)	4.1 (-)	0.20
rCor a 8 - LTP [No. 23]	0.85 (0.45-2.3)	1.2 (0.55-8.45)	0.35	0.7 (0.45-11.75)	3.65 (-)	0.33
nCor a 9 - Cupin [No. 15]	0.7 (-)	0.9 (0.65-2.75)	0.37	0.85 (-)	1 (0.55-4.85)	0.53

Table 2B. Frequency of positive test to different plant food allergenic molecule families among the different groups of patients.

	OAS/ANA-	OAS/ANA+	P value	OAS+	ANA+	P value
PR-10 proteins [No. 118]	47 (81.0%)	71 (96.0%)	0.0058	57 (98.3%)	14 (87.5%)	0.12#
Storage proteins [No. 52]	22 (37.9%)	30 (40.5%)	0.76	19 (32.8 %)	11 (14.9 %)	0.015
LTP [No. 45]	21 (36.2 %)	24 (32.4 %)	0.65	17 (29.3 %)	7 (43.8 %)	0.27
Cysteine protease [No. 27]	11 (19.0 %)	16 (21.6 %)	0.71	13 (22.4 %)	3 (25 %)	0.75
Thaumatococcus-like protein [No. 7]	2 (3.5 %)	5 (6.8 %)	0.46#	5 (8.6 %)	0	0.58#
Alfa-amylase/trypsin inhibitor [No. 4]	1 (1.7 %)	3 (4.1%)	0.63#	1 (5.2%)	2 (12.5 %)	0.12#

but without reaching the statistically significance. No other statistically significant difference was found between the two group of patients.

Comparison between OAS+ and ANA+ subjects

Table 2A also reported median levels of plant food allergenic molecules in sensitized patients who had OAS or ANA, analyzed separately. No statistically significant difference was observed between the two groups of patients however, OAS+ patients tended to have higher IgE levels towards some PR-10 proteins such as rMal d 1, rAra h 8, rGly m 4 whereas ANA+ patients tended to have higher IgE levels towards PR-10 proteins such as rCor a1.01, rPru p 1, rApi g 1, towards LTPs i.e. rPru p 3, nJug r 3, rAra h 9, rCor a 8 or towards other families of allergens (i.e. nAct d 1 and nJug r 1).

Considering the pan-allergen families, sensitization to PR-10 molecules was more frequent in children with OAS and/or anaphylaxis than in OAS/ANA- group ($p=0.0058$), as reported in Table 2B. In addition, there was a difference between OAS+ and ANA+ children about sensitization to Storage protein family ($p=0.015$).

Discussion

The assessment of IgE to pan-allergens may be useful in the allergy work-up. In this context, a clinical question is: can pan-allergens affect the sensitization pattern? A previous study, conducted in adults, showed that sensitization to a pan-allergen (i.e. Bet v 1, Pru p 3, and Bet v 2) entails higher odds to have other sensitizations (12). In addition, the co-sensitization pattern

depended on the basis of the sensitizing pan-allergen family primer. As *Betulaceae* allergy is very common in Genoa, curiously a birch-free geographical area (14), we focused our attention on Bet v 1 to test the hypothesis that sensitization to the major allergen of PR-10 family, such as Bet v 1, could affect the sensitization pattern in children and the clinical outcomes.

The current study shows that children with Bet v 1 sensitization very frequently present associated sensitization to other PR-10 plant food allergens. However, sensitization also to other allergenic molecular families was detectable in these Bet v 1-positive children, mainly concerning LTP. On the other hand, the serum levels measurement showed a single statistical difference between children with or without food allergy, concerning Cor a 1 (hazelnut): in fact, children with food allergy had higher level than children without food allergy. However, there was no difference between children with OAS and children with ANA.

So, the current pediatric study provided findings consistent with a previous one, conducted on adult patients living in central and southern Italy (birch-free area), demonstrating that there are specific relationships between sensitization patterns and clinical characteristics in subjects with Bet v 1 sensitization (15).

Anyway, the current study had some limitations: it was retrospectively conducted on a selected patient population sample, such as living in a particular geographic area, and there was no follow-up. In addition, this study did not consider possible confounding factors, such as passive smoking status, parasite infestation, environmental exposures, and seasonal variations. Finally, it has to be considered that ISAC is an immunoassay and that the result can be conditioned not only by the entity of the immune-response, but also by the homology of the sequence, by the amount of allergen in the assay, by the folding of the recombinant protein and the availability of epitopes, and the correlation between component homology and percentage of positive results is not very highly significant. Therefore, the most important message of this study is that ISAC method is not a precise diagnostic tool in clinical practice. In other words, ISAC test may be useful for a preliminary evaluation of molecular pattern in allergic subjects, but the work-up should be ever integrated by

more precise IgE assessment, for example by ImmunoCap assay.

In conclusion, the present study demonstrates that Bet v 1 sensitization may affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area, but it is unable to discriminate patients from a clinical point of view. So, ISAC test should be necessarily integrated with more precise IgE assay, e.g. ImmunoCap method.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

References

1. Fasce L, Tosca MA, Baroffio M, et al. Atopic infants with wheezing start always with monosensitization. *All Asthma Proc* 2007; 28: 449-53.
2. Melioli G, Marcomini L, Agazzi A, et al. The IgE repertoire in children and adolescents resolved at component level: A cross-sectional study. *Pediatr Allergy Immunol* 2012; 23: 433-40.
3. Arbes SJ, Jr., Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005; 116: 377-83.
4. Baatenburg de Jong A, Dikkeschel LD, Brand PL. Sensitization patterns to food and inhalant allergens in childhood: a comparison of non-sensitized, monosensitized, and polysensitized children. *Pediatr Allergy Immunol* 2011; 22: 166-71.
5. Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy* 2010; 40: 1442-60.
6. Sastre J, Landivar ME, Ruiz-Garcia M, et al. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. *Allergy* 2012; 67: 709-11.
7. McKenna OE, Asam C, Araujo GR, et al. How relevant is panallergen sensitization in the development of allergies? *Pediatr Allergy Immunol* 2016; 27: 560-8.
8. Asero R, Tripodi S, Dondi A, Di Rienzo Businco A, Sfika I, Bianchi A. et al. Prevalence and Clinical Relevance of IgE Sensitization to Profilin in Childhood: A Multicenter Study. *Int Arch Allergy Immunol* 2015; 168: 25-31.
9. Mastrorilli C, Tripodi S, Caffarelli C, et al. Endotypes of pollen-food syndrome in children with seasonal allergic rhinoconjunctivitis: a molecular classification. *Allergy* 2016; 71: 1181-91.
10. Jensen-Jarolim E. Happy 25th birthday, Bet v 1! *WAO Journal* 2014; 7: 14-7.

11. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen Bet v I, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989; 8: 1935-1938.
12. Ciprandi G, Comite P, Ferrero F, Bignardi D, Minale P, Voltolini S, Troise C, Mussap M. Birch allergy and oral allergy syndrome: the practical relevance of serum IgE to Bet v 1. *Allergy Asthma Proc* 2016; 37: 43-9.
13. Ciprandi G, Comite P, Ferrero F, et al. Can serum Bet v 1 IgE measurement distinguish between sensitization and allergy? *Int Forum Allergy Rhinol* 2015; 5: 1151-5.
14. Ciprandi G, Comite P, Mussap M, et al. Profiles of birch sensitization (Bet v 1, Bet v 2, Bet v 4) and oral allergy syndrome across Italy. *J Inv All Clin Immun* 2016; 26: 244-8.
15. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. *J Allergy Clin Immunol* 2018; 141: 11-9

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Correspondence:

Giorgio Ciprandi

Largo R. Benzi 10, 16132 Genoa

E-mail: gio.cip@libero.it