G. Ciprandi[1], M. Silvestri[2], A. Pistorio[3], R. Olcese[4], P. del Barba[5], M.A. Tosca[2]

Bet v 1 sensitization modulates allergenic molecular immune response

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

Key words
allergen-specific IgE; Bet v 1; molecular component; pan-allergen; ISAC; serum

Corresponding author
Giorgio Ciprandi
Largo R. Benzi 10
16132 Genoa, Italy
E-mail: gio.cip@libero.it

Doi
10.23822/EurAnnACI.1764-1489.73

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. This study aimed to evaluate the impact of Bet v 1 sensitization on sensitization pattern in a large sample of children.* ***Methods.*** *Serum IgE molecular components were assessed by ISAC method. Sera from 1,205 children, 708 males (58.76%) and 497 females (41.24%), median age 8.61 years (4.93 - 12.54 years) were analyzed.* ***Results.*** *A total of 354 PR-10-positive subjects were detected out of 1,205 subjects. Bet v 1 positive children were significantly more frequently sensitized to other molecules belonging to PR-10 family and noteworthy also to other allergenic families than Bet v 1 negative children.* ***Conclusions.*** *The present study demonstrates that Bet v 1 sensitization may significantly affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area.*

Introduction

Allergen-specific IgE production, such as sensitization, is the main biomarker of allergy. The natural history of allergy is usually characterized by a progressive increase of the number of sensitizations: the so-called poly-sensitization (1,2). From a clinical point of view, poly-sensitization is relevant, as its prevalence may be up to 90% of allergic subjects (3,4). The work-up in poly-sensitized patients, mainly concerning allergen immunotherapy prescription, has considerably advanced since the introduction of molecular-based allergy diagnostic, that is built on the assessment of allergen molecules (5,6). This methodology allows to precisely define and exactly characterize the sensitization profile by detecting the major allergens and excluding false reactivity to pan-allergens. Pan-allergen is an allergen molecule shared by different allergen sources. The main pan-allergens involved in pollen allergy are: PR-10, profilin, and LTP (7-9). PR-10 (pathogenesis-related protein group 10) molecules are appointed to defend plants against harmful microorganisms. PR-10 proteins were initially detected in pollens of *Fagales* order, mainly concerning birch, and further in cross-reacting fruits and vegetables (10). In the context of PR-10 family, the major allergen is Bet v 1, mainly contained in the pollens of the European white birch (*Betula verrucosa*) and in other trees of the *Betulaceae* family, including alders, hazels, hornbeams, hazel-hornbeam, and hop-hornbeams (11).

In our geographic area, *Betulaceae* allergy (BA) is very common: about 50% of patients with respiratory allergy are sensitized to *Betulaceae* pollens (12). However, this area is curiously birch-free: other pollen allergens related to PR-10 act as sensitizing primer, i.e. hazelnut and hornbeam. Previously, it has been demonstrated that serum IgE to Bet v 1 measurement may be useful to discriminate mere sensitization from true allergy in clinical practice (13). Recently, it has been reported that Bet v

1 sensitization is frequently associated with well-defined co-sensitizations and a peculiar sensitization pattern in adult subjects (14). Therefore, the present study aimed at testing the hypothesis that sensitization to Bet v 1 could affect the sensitization pattern also in children.

Material and methods

Patients

This retrospective study considered subjects suffering from respiratory complaints suggestive for allergy, as previously tested by skin prick test and/or serum IgE measurement, for allergen extracts, with positive findings. They went to the Laboratory of the Istituto Giannina Gaslini in Genoa (Italy) for serologic molecular assessment between July 2012 and April 2014. We analyzed the findings of serum allergen-specific IgEassessed by the ISAC method. The Review Board of the Istituto Giannina Gaslini approved the procedure. The patients' parents gave a written informed consent.

IgEAssay

Serum IgE were measured by ISAC test according to the manufacturer's recommendations (Thermo-Fisher Italy, Milan, Italy). Synthetically, 20 µL 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 considered as ISAC Standardized Units (ISU), ranging from 0 to 100. Positive finding, such as sensitization, was defined by a value > 0.3 ISU, according to the manufacturer's rules.

Data and statistical analysis

Within each group (i.e. Bet v 1 positive and Bet v 1 negative), the number of positive tests, expressed as percentage, was evaluated. IgE levels were non-normally distributed and were summarized as medians with lower and upper quartiles. The Shapiro-Wilk test was used to evaluate the normality of the distributions. Frequency of positivity towards each allergenic molecule in Bet v 1 positive and Bet v 1 negative groups was compared by the Chi-square or Fisher's Exact test (as appropriate). IgE levels 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.

To identify and graphically display patterns of positivity to allergenic molecules sensitization, multiple correspondence analysis (MCA) (15) was performed. This method is a multivariate descriptive technique used for modeling a set of categorical variables modalities (positivity or negativity to a specific allergenic molecule) as points on a plane. The entire data set comprised Bet v 1, Act d 8, Aln g 1, Api g 1, Ara h 8, Cor a 1.01, Cor a 1.04, Gly m 4, Mal d 1, and Pru p 1.
To perform this analysis, a disjunctive table (Burt table) has been constructed (table not shown); in this table absolute frequency of the subjects presenting each modality of the variables (positive vs negative) either for each singular allergenic molecule (diagonal elements) (ex: number of subjects Bet v 1 positive) or combination of pairs of different allergenic molecules (ex: number of subjects Bet v 1 positive and Mal d 1 positive or number of subjects Bet v 1 positive and Mal d 1 negative, etc.).
The MCA technique converts this data set into a particular type of a 2-dimensional graphical display known as "factor plane" in which the 2 generated axes are the ones that explain the majority of variability ("inertia"). Therefore, the two axis are generated on the basis of the relationships between the variables included in the specific data set, and the first one (F1) is the Factor that explains most of the variability and the second one (F2) is the second important Factor. In the "factor plane", points represent different modalities of the sensitization variables and they are closely displayed when they share similar profiles with a subset of other sensitization variables and are displayed as distant when they are mostly dissimilar. Only the most representative factor plane (F1 and F2) according to the total inertia explained is presented in the following analysis.
Statistica software 9.0 (StatSoft Corp., Tulsa, OK, USA) was used for all the analyses and XLStat 2.0.1 (Addinsoft Co., New-York, USA) for the MCA.

Results

Sera from 1,205 patients, 708 males (58.76%) and 497 females (41.24%), median age 8.61 years (4.93 - 12.54 years) and age range 0 - 17 years, were analyzed. A total of 354 PR-10-positive allergic individuals [221 males (62.5%) and 133 females (37.5%), median age 10.47 (7.59 - 13.97) years] were detected out of 1,205 subjects. The analysis included all subjects: they were subdivided in two sub-groups: Bet v 1 positive and Bet v 1 negative. Bet v 1 represented the most commonly recognized PR-10, being 340 out of 354 (96.04%) PR-10-positive participants, followed by Cor a 1.01 (292; 82.49%).
Concerning possible gender-related difference, no significant difference was found in molecule recognition profile (p = 0.27). In addition, the age did not significantly impact on molecular spreading, i.e. the number of sensitizations (p = 0.33): the median age in mono-sensitized subjects was 9.54 (5.53 - 13.8)

years, whereas in poly-sensitized ones was 10.54 (7.84 - 13.97). Mal d 1, Cor a 1.04, Aln g 1, and Pru p 1 were positive in more than half of the patients.

Comparison between Bet v 1 positive and Bet v 1 negative subjects

Table I shows the frequency of positive tests to PR-10s in the whole population, and in Bet v 1 positive and Bet v 1 negative. We found that the frequency of sensitization to the other 9 PR-10s was significantly higher in Bet v 1 positive patients than in Bet v 1 negative patients (**table I**). The vast majority of Bet v1 positive subjects was also sensitized to other allergenic molecules (i.e, other than PR-10s), whereas two-third of Bet v 1 negative subjects had positive tests to other allergenic molecules (99.4% and 66.5%, respectively, $p < 0.0001$) (**table I**). Of 865 patients negative for Bet v 1, only 14 (1.6%) subjects were positive to the other 9 PR-10s, and particularly to Cor a 1.01 and Cor a 1.04 (**table I**).

Analyzing only sensitization to PR-10s, 40 out of 354 (11.3%) reacted to only one of the 10 PR-10s studied, of which 27 (67.5%) reacted only to Bet v 1. The second most frequent mono-reactivity was to Cor a 1.01 (no. 4; 10%) and to Cor a 1.04 (no. 4; 10%). In Bet v 1 positive group, sensitization to different pollen allergenic molecules such as Cor a 1.01 Aln g 1 (PR-10 proteins), nOle e 1, rPar j 2 and rPhl p 1 (**table II**) or to few plant food allergenic molecules such as rMal d 1, rCor a 1.04, rPru p 1 (all PR-10 molecules) (**table III**) was found in at least 50% of the studied subjects. In Bet v 1 negative group, only a limited number of subjects was sensitized to pollen (**table II**) or to plant food (**table III**) allergenic molecules, being the frequency of positive tests always below 20%. The most common profilin in our sample was rMer a 1.

As compared to Bet v 1 negative group, Bet v 1 positive subjects showed significantly more frequently high (i.e. > 15 ISU) or moderate (i.e. between 1 and 15 ISU) levels of IgE towards rCor a1.01, rPar j 2, rPhl p 5b, nAmb a 1, nCup a 1, nOle e 1, rMal d 1, nAct d 1 and nJug r 3 ($p < 0.05$, each comparison). A high proportion of Bet v 1 positive subjects also showed high or moderate levels of IgE towards some pollen (nAmb a 1) or plant food (rGly m 4, rAra h 8, nAct d 8) allergenic molecules that was totally absent in Bet v 1 negative group.

Median levels of IgE towards rPar j 2, nAmb a 1, nOle e 1, rCor a 1.01, nCup a 1, nAra h 3 (**table IV**), rMal d 1, nAct d 2, nJug r 3 (**table V**) were significantly higher in Bet v 1 positive than in Bet v 1 negative subjects with the only exception of rMer a 1.

Multiple correspondence analysis was performed to evaluate the possible mutual interrelationships among different PR-10s on the basis of IgE reactivity. Since this technique places variables based on their levels of reciprocal relationship (i.e., highly related variables are close to each other, while unrelated variables are placed far away from each other), we found that Bet v 1 positivity is frequently associated to sensitization to all the other pollen-derived molecules (i.e., Cor a 1.01, Aln g 1) and to some plant-food allergens (i.e. Mal d 1, Pru p 1 and Cor a 1.04). A

Table I *- Demographic and allergic sensitization characteristics of Bet v1 1-positive and Bet v 1-negative children.*

	Bet v 1 pos (no. 340)	**Bet v 1 neg (no. 865)**	**p value**
age (yrs) [median (LQ-UQ)]	10.6 (7.7 - 14.0)	7.5 (4.0 - 11.9)	**< 0.001**
gender [no. (% male)]	210 (61.8%)	498 (57.6%)	0.18
sensitization to other PR-10 proteins [no. (%)]	313 (92.1%)	14 (1.6%)	**< 0.0001**
rCor a1.01, protein PR-10	287 (84.4%)	5 (0.6%)	**< 0.001**
rMal d 1, protein PR-10	276 (81.2%)	3 (0.3%)	**< 0.001**
rAln g 1, protein PR-10	248 (72.9%)	0	**< 0.001**
rCor a1.04, protein PR-10	246 (72.4%)	4 (0.5%)	**< 0.001**
rPru p 1, protein PR-10	237 (69.7%)	2 (0.2%)	**< 0.001**
rGly m 4, protein PR-10	127 (37.4%)	0	**< 0.001**
rAra h 8, protein PR-10	126 (37.1%)	0	**< 0.001**
rApi g 1, protein PR-10	87 (25.6%)	2 (0.2%)	**< 0.001**
nAct d 8, protein PR-10	42 (12.4%)	0.0	**< 0.001**
sensitization to other allergenic molecules (other than PR-10 proteins) [no. (%)]	338 (99.4%)	575 (66.5%)	< 0.0001

Table II *- Frequency of positive test to different pollen allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rCor a1.01, PR-10 protein	287 (84.4%)	5 (0.6%)	**< 0.001**
rAln g 1, PR-10 protein	248 (72.9%)	0 (0%)	**< 0.001**
nOle e 1, group 1 *Oleaceae*	215 (63.2%)	139 (16.1%)	**< 0.001**
rPar j 2, LTP	173 (50.9%)	98 (11.3%)	**< 0.001**
rPhl p 1, group 1 *Graminae*	170 (50%)	131 (15.1%)	**< 0.001**
nCup a 1, pectate lyase	157 (46.2%)	94 (10.9%)	**< 0.001**
nCyn d 1, group 1 *Graminae*	152 (44.7%)	100 (11.6%)	**< 0.001**
nCry j 1, pectate lyase	116 (34.1%)	49 (5.7%)	**< 0.001**
nPhl p 4, berberine bridge enzyme-like protein	86 (25.3%)	55 (6.4%)	**< 0.001**
rPhl p5b, group 5 *Graminae*	73 (21.5%)	45 (5.2%)	**< 0.001**
rPla a 3, LTP	63 (18.5%)	50 (5.8%)	**< 0.001**
nPla a 2, polygalacturonase	61 (17.9%)	28 (3.2%)	**< 0.001**
rMer a 1, profilin	59 (17.4%)	18 (2.1%)	**< 0.001**
nArt v 3, LTP	53 (15.6%)	48 (5.5%)	**< 0.001**
rPhl p 2, group 2 *Graminae*	48 (14.1%)	31 (3.6%)	**< 0.001**
rPhl p 6, group 6 *Graminae*	42 (12.4%)	23 (2.7%)	**< 0.001**
nHev b 8, profilin	38 (11.2%)	20 (2.3%)	**< 0.001**
nOle e 7, LTP	31 (9.1%)	24 (2.8%)	**< 0.001**
rBet v 2, profilin	30 (8.8%)	17 (2%)	**< 0.001**
rPhl p11, ole e 1-related protein	22 (6.5%)	19 (2.2%)	**< 0.001**
rPhl p12, profilin	22 (6.5%)	15 (1.7%)	**< 0.001**
nArt v 1, defensin-like protein	16 (4.7%)	14 (1.6%)	**0.002**
rOle e 9, 1,3-beta-glucanase	12 (3.5%)	8 (0.9%)	**0.001**
rPla l 1, ole e 1-related protein	12 (3.5%)	13 (1.5%)	**0.026**
rChe a 1, ole e 1-related protein	8 (2.4%)	8 (0.9%)	0.088[1]
rPhl p 7, pocalcin	8 (2.4%)	3 (0.3%)	**0.003**[1]
nAmb a 1, pectate lyase	7 (2.1%)	11 (1.3%)	0.31
rBet v 4, pocalcin	6 (1.8%)	4 (0.5%)	**0.035**[1]
nSal k 1, pectin methylesterase	2 (0.6%)	4 (0.5%)	0.677
rPla a 1, invertase inhibitor	0	1 (0.1%)	1.000[1]

LTP, lipid transfer protein; - [1]Fisher's Exact test

Table III - *Frequency of positive test to different plant food allergenic molecules among Bet v 1 positive and Bet v 1 negative children.*

	Frequency (%) of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rMal d 1, PR-10 protein	276 (81.2%)	3 (0.3%)	**< 0.001**
rCor a1.04, PR-10 protein	246 (72.4%)	4 (0.5%)	**< 0.001**
rPru p 1, PR-10 protein	237 (69.7%)	2 (0.2%)	**< 0.001**
rGly m 4, PR-10 protein	127 (37.4%)	0 (0%)	**< 0.001**
rAra h 8, PR-10 protein	126 (37.1%)	0 (0%)	**< 0.001**
rPru p 3, lipid transfer protein (LTP)	90 (26.5%)	84 (9.7%)	**< 0.001**
rApi g 1, PR-10 protein	87 (25.6%)	2 (0.2%)	**< 0.001**
nJug r 3, lipid transfer protein (LTP)	84 (24.7%)	64 (7.4%)	**< 0.001**
nJug r 2, cupin	66 (19.4%)	26 (3%)	**< 0.001**
rAra h 9, lipid transfer protein (LTP)	58 (17.1%)	8 (0.9%)	**< 0.001**
nJug r 1, 2S albumin	54 (15.9%)	47 (5.4%)	**< 0.001**
nAct d 1, cysteine protease	52 (15.3%)	24 (2.8%)	**< 0.001**
rCor a 8, lipid transfer protein (LTP)	49 (14.4%)	39 (4.5%)	**< 0.001**
nAct d 8, PR-10 protein	42 (12.4%)	0 (0%)	**< 0.001**
nAra h 6, 2S albumin	26 (7.6%)	20 (2.3%)	**< 0.001**
nSes i 1, 2S albumin	25 (7.4%)	22 (2.5%)	**< 0.001**
rAra h 2, 2S albumin	23 (6.8%)	13 (1.5%)	**< 0.001**
nAct d 2, thaumatin-like protein	20 (5.9%)	27 (3.1%)	**0.026**
rAra h 1, cupin	17 (5%)	12 (1.4%)	**< 0.001**
nGly m 6, cupin	16 (4.7%)	18 (2.1%)	**0.013**
rTri a14, lipid transfer protein (LTP)	16 (4.7%)	10 (1.2%)	**< 0.001**
nGly m 5, cupin	15 (4.4%)	7 (0.8%)	**< 0.001**
rAna o 2, cupin	14 (4.1%)	19 (2.2%)	0.066
nAra h 3, cupin	9 (2.6%)	6 (0.7%)	**0.016**[1]
nTri aaA, alfa-amilase/trypsin inhibitor	5 (1.5%)	5 (0.6%)	0.156[1]
rBer e 1, 2S albumin	3 (0.9%)	3 (0.3%)	0.359[1]
nAct d 5, kiwellin	2 (0.6%)	1 (0.1%)	0.194[1]
nFag e 2, 2S albumin	0 (0%)	0 (0%)	-
rTri a19, omega-5 gliadin	0 (0%)	3 (0.3%)	0.563[1]

LTP, lipid transfer protein; - [1]Fisher's Exact test.

Table IV *- IgE levels to different pollen allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
rBet v 4, pocalcin	16.5 (2.4 - 34.4)	5.8 (3.5 - 9.5)	0.92
rPar j 2, LTP	14.8 (6.1 - 31.9)	5.7 (1.8 - 11.8)	**< 0.001**
nAmb a 1, pectate lyase	14.1 (1.5 - 40.1)	0.9 (0.6 - 3.4)	**0.018**
rPhl p5b, group 5 *Graminae*	9.0 (3.0 - 22.4)	6.4 (1.2 - 23.8)	0.38
rPhl p 1, group 1 *Graminae*	6.9 (2.7 - 18.4)	4.3 (1.1 - 21.2)	0.084
nOle e 1, olive group 1	6 (2.6 - 17.8)	1.9 (0.8 - 6.5)	**< 0.001**
rCor a1.01, **PR-10 protein**	5.7 (2.1 - 13.2)	0.5 (0.4 - 0.6)	**< 0.001**
rPhl p 7, pocalcin	5.3 (2.2 - 23.9)	4.2 (1.4 - 22.9)	0.92
nCup a 1, pectate lyase	4.8 (1.3 - 15.2)	1.9 (0.6 - 8.0)	**< 0.001**
nSal k 1, pectin methylesterase	4.2 (0.3 - 8.0)	7.8 (3.7 - 12.8)	0.64
rAln g 1, **PR-10 protein**	3.4 (1.1 - 8.4)	(-)	-
rBet v 2, profilin	3.1 (0.9 - 10.0)	8.9 (3.0 - 13.7)	0.09
rPhl p 2, group 2 *Graminae*	2.9 (1.5 - 8.3)	2.7 (0.8 - 7.9)	0.45
rMer a 1, profilin	2.9 (0.7 - 6.3)	8.1 (3.1 - 14.4)	**0.016**
rPla l 1, ole e 1-related protein	2.8 (0.8 - 10.8)	1.2 (0.7 - 8.7)	0.72
rPhl p11, ole e 1-related protein	2.7 (2.0 - 8.2)	2.7 (1.0 - 19.6)	0.70
nCyn d 1, group 1 *Graminae*	2.7 (0.9 - 9.2)	3.3 (1.2 - 13.9)	0.19
rPhl p12, profilin	1.7 (0.8 - 2.5)	3.3 (0.7 - 5.4)	0.54
nArt v 1, defensin	1.6 (0.6 - 4.4)	3.0 (1.0 - 5.2)	0.35
rPhl p 6, group 6 *Graminae*	1.5 (0.8 - 7.1)	3.3 (0.7 - 14.8)	0.34
nOle e 7, LTP	1.2 (0.7 - 7)	1.0 (0.5 - 2.3)	0.28
nPhl p 4, berberine bridge enzyme-like protein	1 (0.5 - 2.6)	1.3 (0.5 - 4)	0.31
nPla a 2, polygalacturonase	1 (0.5 - 1.6)	1 (0.5 - 1.3)	0.68
nArt v 3, LTP	1 (0.5 - 2)	0.8 (0.5 - 1.5)	0.74
nCry j 1, pectate lyase	1 (0.6 - 2)	0.8 (0.5 - 2.6)	0.82
rOle e 9, 1,3-beta-glucanase	0.9 (0.5 - 2.4)	1.3 (0.6 - 1.5)	0.97
rPla a 3, LTP	0.9 (0.6 - 2.4)	0.8 (0.6 - 1.4)	0.26
rChe a 1, ole e 1-related protein	0.8 (0.5 - 2.2)	0.8 (0.6 - 0.9)	0.96
rPla a 1, invertase inhibitor	(-)	1.2 (1.2 - 1.2)	-

LTP, lipid transfer protein;

Table V *- IgE levels to different plant food allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
nAct d 5, kiwellin	11.4 (10.6 - 12.3)	14.1 (14.1 - 14.1)	--
nAra h 3, cupin	6.3 (2.8 - 11.2)	1.8 (1.1 - 2.1)	**0.008**
rAra h 2, 2S albumin	5.1 (1.8 - 20.3)	5.1 (0.9 - 9.7)	0.25
nAra h 6, 2S albumin	4.5 (1 - 11.6)	2.5 (1.1 - 5.7)	0.36
rMal d 1, **PR-10 protein**	4.5 (1.6 - 11.0)	0.4 (0.3 - 0.6)	**0.006**
rCor a1.04, **PR-10 protein**	4.3 (1.7 - 8.8)	1.5 (0.5 - 2.8)	0.055
nAct d 2, thaumatin-like protein	3.1 (1.3 - 5.9)	2.1 (0.7 - 2.9)	**0.021**
rAra h 1, cupin	3.1 (1.5 - 7.4)	2.3 (1.5 - 5.2)	0.55
rPru p 1, **PR-10 protein**	3.0 (1.1 - 8.0)	0.7 (0.4 - 1.0)	0.08
nGly m 6, cupin	2.6 (0.8 - 6.8)	0.8 (0.7 - 2.5)	0.17
nJug r 1, 2S albumin	2.5 (1.4 - 7.9)	1.6 (1.1 - 5.6)	0.14
rGly m 4, **PR-10 protein**	2.0 (0.8 - 5.5)	(-)	-
nSes i 1, 2S albumin	1.9 (0.7 - 3.9)	1.3 (0.7 - 6.8)	0.94
rAra h 8, **PR-10 protein**	1.7 (0.8 - 3.5)	(-)	-
nJug r 3, LTP	1.4 (0.8 - 3.5)	0.9 (0.5 - 1.9)	**0.032**
rApi g 1, **PR-10 protein**	1.4 (0.8 - 3.0)	1.4 (0.7 - 2.1)	0.74
nAct d 1, cysteine protease	1.3 (0.8 - 2.4)	1.0 (0.6 - 3.3)	0.46
nGly m 5, cupin	1.3 (0.8 - 4.1)	1.3 (0.4 - 3.7)	0.78
rAra h 9, LTP	1.15 (0.6 - 3.1)	0.8 (0.6 - 1.4)	0.07
nTri aaA, alfa-amilase/trypsin inhibitor	1.1 (0.4 - 1.3)	1.6 (0.7 - 2.2)	0.21
rPru p 3, LTP	1.1 (0.6 - 2.8)	1.0 (0.6 - 2.1)	0.56
rAna o 2, cupin	1.1 (0.4 - 2.8)	1 (0.4 - 2.1)	0.69
rCor a 8, LTP	1.0 (0.5 - 1.9)	0.7 (0.4 - 1.3)	0.10
nJug r 2, cupin	0.9 (0.5 - 1.4)	1.2 (0.7 - 2.4)	0.09
rBer e 1, 2S albumin	0.9 (0.3 - 3.5)	1.6 (0.6 - 2)	-
rTri a14, LTP	0.8 (0.5 - 1.8)	1.1 (0.9 - 1.7)	0.30
nAct d 8, **PR-10 protein**	0.7 (0.5 - 1.2)	(-)	-
nFag e 2, 2S albumin	11.4 (10.6 - 12.3)	(-)	-
rTri a19, omega-5 gliadin	6.3 (2.8 - 11.2)	1 (0.3 - 1.6)	-

LTP, lipid transfer protein;

Multiple correspondence analysis was performed to evaluate the possible mutual interrelationships among different PR-10s on the basis of IgE reactivity. Since this technique places variables based on their levels of reciprocal relationship (i.e., highly related variables are close to each other, while unrelated variables are placed far away from each other), we found that Bet v 1 positivity is frequently associated to sensitization to all the other pollen-derived molecules (i.e., Cor a 1.01, Aln g 1) and to some plant-food allergens (i.e. Mal d 1, Pru p 1 and Cor a 1.04). A different behavior was found for the remaining pollen-derived

molecules: Gly m 4 positivity was in fact associated to Ara h 8 and Api g 1positivity, whereas Act d 8 is less associated to other PR-10s (**figure 1**).

In addition, we analysed the frequency of sensitization and the IgE level to other molecules belonging the most common allergens in our region, i.e. house dust mites, cat, dog, caw milk, and egg, as reported in **table VI** and **VII**, respectively. Interestingly, Bet v 1 positive children showed higher frequency and serum IgE level than Bet v 1 negative children about most of the molecules.

Figure 1 *- Multiple correspondence analysis plot showing the mutual interrelationships between PR-10s (each molecule is represented by one dot) in terms of IgE reactivity (positivity/negativity). The horizontal axis (F1) explains 97.66% of total variability (inertia); the vertical axis (F2) explains 0.04% of total variability. See methods' section for details.*

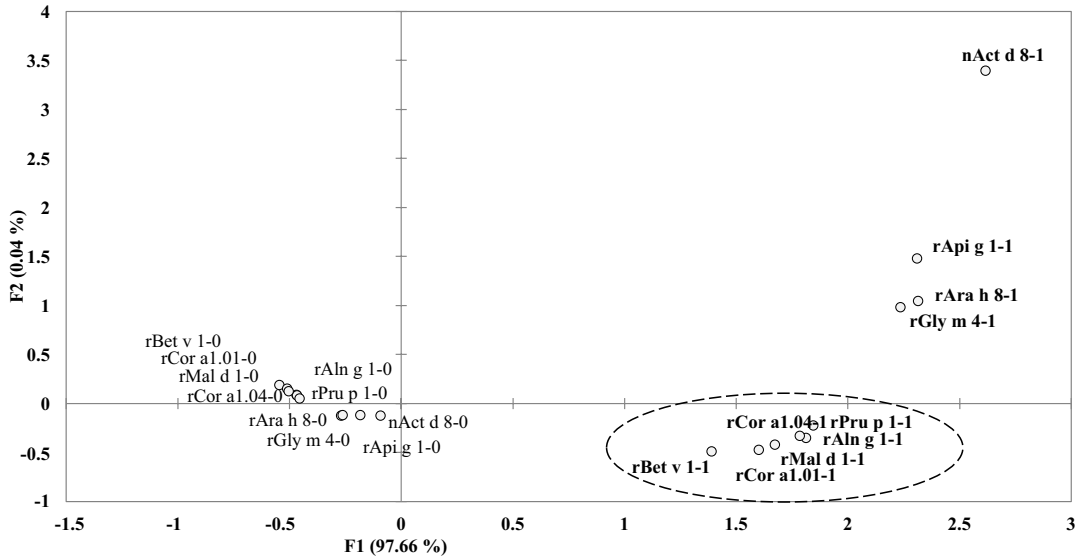

Table VI *- Frequency of positive test to different other allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rDer f 2, mite group 2 family	210 (61.8%)	291 (33.6%)	**< 0.001**
rFel d 1, uteroglobin	200 (58.8%)	158 (18.3%)	**< 0.001**
nDer p 2, mite group 2 family	197 (57.9%)	276 (31.9%)	**< 0.001**
nDer f 1, cystein protease	178 (52.4%)	255 (29.5%)	**< 0.001**
nDer p 1, cystein protease	178 (52.4%)	241 (27.9%)	**< 0.001**
rCan f 1, lipocalin	82 (24.1%)	70 (8.1%)	**< 0.001**
rCan f 5, arginin esterase	59 (17.4%)	45 (5.2%)	**< 0.001**
rLep d 2, mite group 2 family	51 (15.0%)	64 (7.4%)	**< 0.001**
nGal d 2, ovalbumin	34 (10.0%)	50 (5.8%)	**0.010**
rDer p 10, tropomyosin	27 (7.9%)	32 (3.7%)	**0.002**
nGal d 1, ovomucoid	25 (7.4%)	37 (4.3%)	**0.030**
nGal d 3, conalbumin	23 (6.8%)	30 (3.5%)	**0.012**

	Frequency [N (%)] of positive test among		
Allergenic molecules	**Bet v 1 pos children**	**Bet v 1 neg children**	**P**
rCan f 2, lipocalin	22 (6.5%)	18 (2.1%)	**< 0.001**
nBos d 4, alfa-lactalbumin	19 (5.6%)	47 (5.4%)	0.92
rFel d 4, lipocalin	16 (4.7%)	17 (2.0%)	**0.009**
nFel d 2, serum albumin	15 (4.4%)	16 (1.8%)	**0.011**
nBos d 8, casein	14 (4.1%)	40 (4.6%)	0.70
nCan f 3, serum albumin	14 (4.1%)	12 (1.4%)	**0.003**
nBos d 5, beta-lactoglobulin	13 (3.8%)	41 (4.7%)	0.49
nBos d 6, serum albumin	8 (2.4%)	24 (2.8%)	0.68
nBos d I, trasferrin	5 (1.5%)	9 (1.0%)	0.554[1]
nGal d 5, serum albumin	5 (1.5%)	10 (1.2%)	0.773[1]

[1]Fisher's Exact test

Table VII *- IgE levels to different other allergenic molecules among Bet v 1- positive and Bet v 1-negative children.*

	Bet v 1 pos children	**Bet v 1 neg children**	**p value**
rDer f 2, mite group 2 family	27.6 (11.2 - 55.4)	17.3 (5.5 - 43.1)	**< 0.001**
nDer f 1, cystein protease	12.5 (5.0 - 26.4)	7.3 (3.0 - 19.2)	**< 0.001**
nDer p 2, mite group 2 family	11.3 (4.4 - 24.2)	7.8 (2.9 - 17.7)	**0.002**
nDer p 1, cystein protease	7.8 (3.2 - 18.1)	5.5 (2.2 - 11.8)	**0.010**
rFel d 1, uteroglobin	7.1 (2.4 - 17.7)	4.45 (1.4 - 13.5)	**0.010**
rFel d 4, lipocalin	4.6 (2.4 - 9.6)	1.9 (0.6 - 8.5)	0.28
rCan f 2, lipocalin	3.1 (1.3 - 13.4)	4.6 (1.1 - 10.7)	0.90
rCan f 1, lipocalin	3.0 (1.1 - 12.5)	3.0 (1.1 - 10.6)	0.84
rDer p 10, tropomyosin	2.7 (0.8 - 18.9)	2.8 (0.6 - 14.2)	0.91
nBos d 4, alfa-lactalbumin	2.7 (0.95 - 7.45)	1.6 (0.7 - 4.05)	0.29
nBos d 5, beta-lactoglobulin	2.7 (0.75 - 15.1)	1.2 (0.5 - 3.3)	0.13
nBos d 8, casein	2.65 (0.5 - 12.25)	1.1 (0.65 - 3.9)	0.46
rCan f 5, arginin esterasi	2.2 (0.8 - 5.8)	1.8 (0.9 - 5.3)	0.97
nGal d 3, conalbumin	1.7 (0.8 - 7)	1.9 (0.7 - 6.6)	0.80
rLep d 2, mite Group 2 family	1.4 (0.8 - 4.5)	1.7 (0.8 - 7.2)	0.40
nBos d 6, serum albumin	1.2 (0.6 - 2.9)	1.1 (0.6 - 2.2)	0.78
nGal d 2, ovalbumin	1.0 (0.6 - 2.85)	0.8 (0.45 - 3.8)	0.60
nGal d 1, ovomucoid	1.0 (0.5 - 2.2)	1.8 (0.7 - 6.8)	**0.026**
nFel d 2, serum albumin	0.8 (0.6 - 5.2)	0.6 (0.4 - 1.5)	0.27
nCan f 3, serum albumin	0.8 (0.4 - 5.1)	1.25 (0.4 - 2.5)	0.76
nGal d 5, serum albumin	0.6 (-)	2.3 (0.8 - 22.6)	0.16
nBos d, lactoferrin-trasferrin	0.4 (-)	2.1 (1.5 - 6.3)	**0.007**

different behavior was found for the remaining pollen-derived molecules: Gly m 4 positivity was in fact associated to Ara h 8 and Api g 1positivity, whereas Act d 8 is less associated to other PR-10s (**figure 1**).
In addition, we analysed the frequency of sensitization and the IgE level to other molecules belonging the most common allergens in our region, i.e. house dust mites, cat, dog, caw milk, and egg, as reported in **table VI** and **VII**, respectively. Interestingly, Bet v 1 positive children showed higher frequency and serum IgE level than Bet v 1 negative children about most of the molecules.

Discussion

The assessment of IgE to pan-allergens is 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 (14). In addition, the co-sensitization pattern depended on the basis of the sensitizing pan-allergen family primer. As birch allergy is very common in Genoa, curiously a birch-free geographical area (16), 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.
The current study shows that Bet v 1 sensitization is significantly associated with sensitization to other PR-10 allergens, both pollens and fruits/vegetables. In contrast, children not sensitized to Bet v 1 very rarely are sensitized to other PR-10 allergens, including pollens and fruits/vegetables. Interestingly, both age and gender did not significantly impact on findings. The most common profilin was rMer a 1 in our sample. Curiously, Bet v 4 induced the highest serum level, but this findings could be due to the very low number of subjects, thus out-layers could interfere with results. This finding may be obviously explained by the homology shared by PR-10 molecules. More interestingly, Bet v 1 sensitization is also associated to sensitization to allergens belonging to other families, namely LTP-family, 2S albumin-family, cupin-family, polcalcin-family, and also to other pollens, mainly grasses and olive tree. This phenomenon could be considered as a "priming" effect where the primary Bet v 1 sensitization may promote the development of successive sensitizations to other allergens. Probably this effect could be initially limited to PR-10 family and then extended also to other allergen clusters. This finding is underlined by the multivariate analysis, that clearly highlights the close homologous behavior of PR-10 sensitization: the preserved molecular sequence of pollen and food molecules may explain the high frequency of co-sensitization patterns. Interestingly, the effect of Bet v 1 sensitization has an impact also on other molecules belonging to other allergens, including house dust mites, cat, dog, caw milk, and egg. This finding could mean that the pan-allergen Bet v 1 could be a sensitization primer, even though further longitudinal studies should confirm this cross-sectional outcome.
So, the current pediatric study provided findings that are consistent with a previous one conducted on adult patients living in the same geographic area. Moreover, a recent study conducted on allergic patients living in central and southern Italy (birch-free area), demonstrated that there are specific relationships between sensitization patterns and clinical characteristics in subjects with Bet v 1 sensitization (17). In this regard, previous studies investigated the relevance of *Betulaceae* pollens counts about sensitization pattern and clinical expression (18,19). In fact, allergy to *Betulaceae* represents a primary cause of sensitization and allergic symptom severity in our geographic area.
Anyway, the current study had some limitations: it was retrospectively conducted on a selected patient population sample, subjects referring for serologic assessment, there was no follow-up, and there are no clinical data. This issue is particularly relevant, as sensitization does not always correspond to allergy: this fact probably further reduces the percentages of subjects really "positive" to tests, such as truly allergic. 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 outcomes from ISAC may be not completely precise as many factors may interfere, such as the amount of allergen in the assay, the semi-quantitative analysis, and the chemical-physical characteristics of allergen molecules. Therefore, there is need to conduct cohort studies and long-term follow up trials to confirm these preliminary findings. However, the strength of the present study may be represented by the large size of the sample.
Another relevant issue is the possibility of investigating the potential role exerted by other pan-allergenic molecules, mainly concerning Pru p 3, the major allergen belonging to the lipid transfer protein family. In this regard, a study is ongoing in our geographic area, analyzing data deriving from the more precise ImmunoCap method.
Moreover, this study was cross-sectional, reflecting a single time point without analysing the evolution of sensitization over time. However, we very recently published two studies, concerning the present cohort and adults, analyzing the ISAC findings for pollen and food sensitizations (20,21). We demonstrated that Bet v 1 sensitization frequency progressively increased from 2% at < 2 years of age to a peak of 43.3% at 20 years of age. Equally, IgE serum levels progressively increased from 0.5 ISU at < 2 years of age to a peak of 8 ISU at 20 years. Furthermore, Cor a 1 sensitization frequency increased from < 1% at < 2 years of age to a peak of about 40% at 20 years. Likewise, IgE serum levels progressively increased from 4 ISU to a peak of 13 ISU at about 10 years.

It has also to be noted that our findings are consistent with previous studies that highlighted Bet v 1 as the strongest or most prevalent allergen in the PR-10 group (22,23). On the other hand, we focused our attention on Bet v1 as potential primer, but also Der f 2 and Fel d 1 could be protagonists in priming sensitization. In this regard, there is a longitudinal study that is ongoing. Another unmet need concerns the curious preeminence of Bet v 1 sensitization on other PR-10 molecules, including Cor a 1; molecular studies should be conducted to provide adequate explanation.

In conclusion, the present study demonstrates that Bet v 1 sensitization may significantly affect the sensitization pattern in children living in Genoa, a Mediterranean city located in a birch-free area.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Fasce L, Tosca MA, Baroffio M, et al. Atopic infants with wheezing start always with monosensitization. All Asthma Proc 2007; 28:449-453.
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; 23:433-440.
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; 116:377-383.
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; 22:166-171.
5. Sastre J. Molecular diagnosis in allergy. ClinExp Allergy 2010; 40:1442-1460.
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; 67:709-711.
7. McKenna OE, Asam C, Araujo GR, et al. How relevant is panallergen sensitization in the development of allergies? Pediatr Allergy Immunol. 2016; 27:560-568.
8. 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; 71:1181-1191.
10. Jensen-Jarolim E. Happy 25th birthday, Bet v 1! WAO Journal 2014; 7:14-17.
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 1IgE measurement distinguish between sensitization and allergy? Int Forum Allergy Rhinol 2015; 5:1151-1155.
14. Ciprandi G, Comite P, Bruzzone M, Fontana M. Can pan-allergens affect the sensitization pattern? Immunobiology 2017; 222:726-9.
15. Greenacre M, Blasius J. Multiple Correspondence analysis and related methods. Chapman and Hall/CRC London 2006: 1-581
16. 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.
17. Scala E, Abeni D, Cecchi L, et al. Molecular recognition profiles and clinical patterns of PR-10 sensitization in a birch-free Mediterranean area. Int Arch Allergy immunol 2017; 173:138-146.
18. Negrini AC, Negrini S, Giunta V, Quaglini S, Ciprandi G. Thirty year survey on airborne pollen concentrations in Genoa (Italy): relationship with sensitizations, meteorological data, and air pollution. Am J Rhinol Allergy 2011; 25:232-241.
19. Ruffoni S, Passalacqua G, Ricciardolo F, Furgani A, Negrini AC, DeAmici M, Ciprandi G. A 10-year survey on asthma exacerbations: relationships among emergency medicine calls, pollens, weather, and air pollution. Rev Franc Allergologie 2013; 44:111-116.
20. Tosca MA, Silvestri M, Olcese R, Pistorio A, Rossi GA, Ciprandi G. The impact of age on serum allergen-specific IgE to inhaled molecular components. Allergologia et Immunopathologia 2017; 45265-45271.
21. Tosca MA, Silvestri M, Olcese R, Sacco O, Pistorio A, Rossi GA, Ciprandi G. Allergen-specific IgE to food molecular components and age: from early childhood to adulthood. Allergol Immunopathol 2017; 45:87-89.
22. Tolkki L, Alanko K, Petman L, Skydtsgaard MB, Milvang PG, Seppälä U, Ranki A. Clinical characterization and IgE profiling of birch (Betula verrucosa)--allergic individuals suffering from allergic reactions to raw fruits and vegetables. J Allergy Clin Immunol Pract 2013; 1:623-631.
23. Siroux V, Lupinek C, Resch Y, Curin M, Just J, Keil T, Kiss R, et al. Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: The EGEA study. J Allergy Clin Immunol 2017; 139:643-654.