

Profiles of birch sensitization (Bet v 1, Bet v 2, Bet v 4) and oral allergy syndrome across Italy.

Short title: Birch allergen molecules and OAS in Italy

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Abstract

Background Birch allergy (BA) is a common pollinosis. Bet v 1, Bet v 2, and Bet v 4 are birch allergen molecules. Oral allergic syndrome (OAS) is frequently associated with BA. Previously, it has been reported that there is a gradient of birch allergen sensitization profile across Europe. Thus, this study aimed to investigate the birch sensitization profile, including OAS, across Italy.

Methods This retrospective study considered a series of 854 patients (391 males, mean age 35.9 years, range 18-93): 196 patients were recruited in Genoa, 188 in Northern Italy, 359 in Central Italy, and 111 in Southern Italy. Serum IgE to Bet v 1, Bet v 2, and Bet v 4 were assessed. OAS was analysed.

Results. Considering the geographical path *Genoa-North-Center-South Italy*, Bet v 1 sensitization significantly ($p<0.0001$) decreased from Genoa (95.41%) to Southern Italy (58.56%). Bet v 2 sensitization significantly ($p<0.0001$) increased from Genoa (6.12%) to Southern Italy (52.25%). Also Bet v 4 significantly ($p=0.0002$) increased from Genoa (6.12%) to Southern Italy (14.41%). There was a significant ($p<0.0001$) different distribution of OAS positive patients across the geographical areas, the highest difference being between 33.5% in Genoa and 76.9% in Northern Italy. The frequencies of birch allergen molecules correlated with the OAS only in Central Italy.

Conclusions The present study demonstrated that there is a significant difference about birch associated molecules and the related clinical expression across Italy.

Key words : Bet v 1, Bet v 2, Bet v 4, oral allergic syndrome, birch allergy, serum IgE, Italy

Resumen

Introducción El síndrome de alergia oral (SAO) se encuentra frecuentemente asociado a la alergia al polen de abedul. Se han descrito diferentes gradientes de sensibilización a polen de abedul en Europa. Este estudio pretende determinar el perfil de sensibilización a polen de abedul, incluyendo la presencia de SAO, en Italia.

Métodos: Estudio retrospectivo con 854 pacientes (391 hombres, edad media 35.9 años, rango 18-93 años): 196 pacientes procedían de Génova, 188 del Norte de Italia, 359 de Italia Central y 111 del Sur de Italia. Se determinó la IgE específica a Bet v 1, Bet v 2 y Bet v 4, así como la presencia de SAO.

Resultados: De acuerdo a la procedencia geográfica Génova-Norte-Centro-Sur de Italia, la sensibilización a Bet v 1 disminuye significativamente ($p < 0,0001$) desde Génova (95,41%) hasta el Sur de Italia (58,56%). La sensibilización a Bet v 2 aumenta significativamente ($p < 0,0001$) desde Génova (6,12%) hasta el Sur de Italia (52,25%). También la sensibilización a Bet v 4 aumenta significativamente ($p < 0,0002$) desde Génova (6,12%) hasta el Sur de Italia (14,41%). Existe una distribución del SAO significativamente diferente entre las diferentes áreas geográficas consideradas, siendo la máxima diferencia la presentada entre Génova (33,5%) y el Norte de Italia (76,9%). Las frecuencias de sensibilización a las diferentes moléculas del polen de abedul se correlacionan con el SAO solo en la región Central de Italia.

Conclusiones El presente estudio demuestra la existencia de diferencias significativas entre la sensibilización a las diferentes moléculas del polen de abedul y su expresión clínica en diferentes regiones italianas.

Palabras Clave: Bet v 1, Bet v 2, Bet v 4, síndrome de alergia oral, alergia a polen de abedul, IgE sérica, Italia.

Introduction

IgE is the hallmark of allergy [1,2]. Birch allergy (BA) is one of the most frequent pollinosis in Europe [3-5]. Actually, BA is characterized by IgE response to *Betula verrucosa* pollen allergens. Birch tree belongs to the *Fagales* order which accounts for 8 families, including *Betulaceae*, that can be in turn divided in *Betuloideae* (mainly the genera birch and alder) and *Coryloideae* (mainly the genera hazel, hornbeam, hop-hornbeam, and *Ostryopsis*).

Recently, several allergen molecules from birch source have been identified, and the main molecules are at present Bet v 1, Bet v 2, and Bet v 4. The major birch pollen allergen is Bet v 1, which is a pathogenesis-related molecule (PR-10). Bet v 1 shares a molecular homology with many plant of *Betulaceae* family [6]. In addition, oral allergic syndrome (OAS) is a common allergic disorder sustained by a pollen-fruit cross-reaction and is very common in BA patients with an average prevalence of 70% [7-11]. The most frequent fruits causing OAS in BA patients belong to the *Rosaceae* family, including essentially stone fruits (mainly apple), and also hazelnut [7,8]. Interestingly, it has been proposed that the BA-associated OAS may depend on the molecular homology between the Bet v 1 of *Betulaceae* family pollens and the Bet v 1 homologs of hazelnut (Cor a 1) or of *Rosaceae* family fruits, including apple, peach, pear, apricot, and cherry [12,13]. Bet v 2 is a profilin that is able to bind to actin, so regulating cellular division, growth, and differentiation [14]. Profilins are widely cross-reactive, due to high sequence conservation, allergens also between pollen and food, as well as between pollen and latex [15]. The wide spread cross-reactivity of profilins has led to the designation of profilins as 'pan-allergens' [16]. In particular, it has been defined a so called Bet v 2 family [17]. Bet v 4 is a polcalcin, such as a calcium-binding protein. Bet v 4 has been also considered a pan-allergenic molecule [18].

An intriguing gradient of sensitization profile across Europe has been reported: Bet v 1 is predominant in Northern Europe, whereas Bet v 2 and Bet v 4 are more relevant in Southern Europe [19-22]. Therefore, this study aimed to evaluate the sensitization profile to birch allergen molecules in a large sample of Italian allergic patients, considering the living geographic area and also the presence of associated OAS.

Materials and Methods

This retrospective study considered a series of 854 patients (391 males, mean age 35.9 years, range 18-93) who referred to Allergy Departments of different Hospitals for suspected AR between 2011 and 2014. In particular, 196 patients were recruited in Genoa, 188 in Northern Italy, 359 in Central Italy, and 111 in Southern Italy. Inclusion criteria were sensitization to birch, documented by skin prick test positivity and/or serum allergen-specific IgE > 0.35 kUA/L, and reported nasal symptoms consistent with pollen exposure. The study was performed according to the Review Board rules and all patients signed an informed consent.

The presence of OAS was assessed by the patient's history positive for experiencing oropharyngeal itching immediately after eating any type of fresh fruits or vegetables related to Bet v 1 allergens, according to previous reports [13,14,16].

Serum IgE assay

Serum levels of specific IgE were detected by the IFMA procedure (ImmunoCAP Thermo Fisher Scientific, Uppsala, Sweden) in peripheral blood samples from patients. Serum was collected into gel-separator tubes, centrifuged and stored at -20°C until analysis for Bet v 1.

Measurement of circulating specific IgE antibodies was performed according to manufacturer's instructions [23]. Specific IgE concentrations were expressed in kUA/L according to the traceable

calibration to the 2nd IRP WHO for Human IgE and 0.35 kUA/L has been considered as a cut-off [24].

Statistical analysis

Statistical analysis was performed using the statistical software package Medcalc 9 (Frank Schoonjans, BE). All the variables were dichotomized (positive/negative, present/absent) and prevalence were reported as percentage. Variable association among different areas, as well as association between positivity combinations of allergens and OAS, were investigated using the Chi-Squared Test. A p-value <0.05 was considered statistically significant. The odds ratio has been used as an estimate for the risk of developing OAS in patients who were positive to allergens.

Results

Figure 1 shows the frequencies of sensitizations for Bet v 1, Bet v 2, and Bet v 4, and the percentage of patients with OAS, considering the 4 geographical areas evaluated, i.e. Genoa, Northern Italy, Central Italy, and Southern Italy.

About Bet v 1, there was a significant ($p < 0.0001$) decreasing gradient of percentage of sensitized patients from Genoa (95.41%) Southern Italy (58.56%). About Bet v 2, there was a significant ($p < 0.0001$) increasing gradient of percentage of sensitized patients from Genoa (6.12%) to Southern Italy (52.25%). About Bet v 4, there was a significant ($p = 0.0002$) increasing gradient of percentage of sensitized patients from Genoa (6.12%) to Southern Italy (14.41%).

About OAS, there was a significant ($p < 0.0001$) different distribution of OAS positive patients across the geographical areas, the highest difference being between 33.5% in Genoa and 76.9% in Northern Italy.

Furthermore, the different profiles of sensitization were analyzed. Figure 2 shows the possible combinations: there were significant differences both considering the different patterns ($p < 0.0001$) and the geographical areas (p values ranging from 0.2 to < 0.0001). In particular, the most frequent pattern was Bet v 1 positive, Bet v 2 and Bet v 4 negative, that was more evident in Genoa. On the contrary, the sensitization only to Bet v 2 and Bet v 4 was rare in Genoa, but relatively frequent in other areas ($p < 0.0001$ for both).

The association between positive combination of allergens and presence/absence of OAS showed a significant relationship only for two combinations of allergens: Bet v1 and Bet v2 positive, Bet v4 negative ($p < 0.0001$), and Bet v1 and Bet v4 positive, Bet v2 negative ($p = 0.0033$). Considering the global sample, and dividing patients according to the positivity or negativity to allergens, odds ratios showed an increased risk of OAS for patients who were positive to recombinants. When considering the different geographical areas, odds ratios did not show a significant risk, except for Bet v2 (OR= 1.5591, $p = 0.0390$) and Bet v4 (OR= 0.3455, $p = 0.0006$) for Central Italy.

The different profiles of sensitizing pollens are reported considering the different geographical areas as well as the most frequent fruits cause of OAS (Table 1).

Discussion

Allergy to *Betulaceae* pollen allergens is one of the most common pollinosis in our geographic area [20]. Bet v 1 is the major birch pollen allergen. The clinical relevance of Bet v 1 is supported by the evidence that more than 95% of patients living in Northern Europe and allergic to birch are sensitized to Bet v 1. Therefore, routine diagnosis and therapy of *Betulaceae* pollen-allergic patients are practically based on the assessment of Bet v 1 [25]. However, two other allergens related to birch, such as Bet v 2 and Bet v 4, are commonly evaluated in the clinical practice. Both define pan-

allergens that should be adequately considered when the diagnosis of true BA has to be made, mainly concerning the prescription of allergen immunotherapy.

It is also to note that Bet v 1 sensitization may occur also in absence of the Bet v 1 natural source, such as the presence of birch trees, as it has been reported the existence of Bet v 1 sensitization also in patients living in a birch-free area of central Italy [21]. This phenomenon may be explained why Bet v 1 is able to identify a patients population with co-reactivity to *Fagales* order (such as birch, hazel, hornbeam, hop-hornbeam, and oak) [26].

OAS is frequently associated with BA. However, the management of OAS is still debated, mainly concerning the role of molecular diagnosis [27]. In this regard, it has been reported that sensitization to Bet v 1 was frequently associated with OAS in patients with pollen allergy [28]. In addition, it was demonstrated that micro-array, measuring PR-10 proteins, was a reliable tool for diagnosis apple-mediated OAS in birch pollen allergy [29]. BA-related OAS may be believed as depending on cross-reactivity between *Betulaceae* pollen allergens and structurally homologous food proteins. In particular, Bet v 1 cross-reacts with several proteins contained in some *Rosaceae* fruits, such as apple (Mal d 1), hazelnut (Cor a 1), peach (Pyr c 1), apricot (Pru ar 1), and cherry (Pru av 1) [30]. So, molecular diagnostics approach based on birch associated molecules is fruitful for the allergy workup.

The first part of the current study showed that there is a significant sensitization gradient across Italy for all birch molecules confirming previous studies [19-22]. In particular, it is surprising the findings concerning Genoa both considering sensitization profile and OAS prevalence. Patients living in Genoa show the highest percentage of Bet v 1 positivity and the lowest percentage of both Bet v 2 and Bet v 4 sensitization as well as OAS. This result could be explained considering the

particular characteristic of the flora in the Genoa territory. Actually, Genoa may be considered a birch-free area, whereas there is a wide distribution of hop-hornbeam (*Ostrya carpinifolia*), which belongs to *Betulaceae* family. In this regard, pollen count for hop-hornbeam show relevant levels between March-April in Genoa. The allergen of hop-hornbeam has been identified and characterized: Ost c 1, that is a Bet v 1 homologous [31]. In particular, on the basis of IgE-binding experiments, it has been speculated that the allergy to *Fagales* allergens may be initiated by an independent sensitization against a specific member of the *Betuloideae* or *Coryloideae* family [32]. Further, a cross-reactivity could progress to Bet v 1-homologous proteins. Therefore, the allergenic characteristic of Ost c 1, such as very close to Bet v 1, could explain the low percentage of OAS positive patients in our area. This concept is consistent with a previous study that demonstrated a different pattern of sensitization comparing Italian patients, living in a birch-free area, with Austrian ones exposed to Bet v 1 [33]. These outcomes therefore could suggest that Bet v 1 could be one of the allergenic proteins present in the *Ostrya* pollen possibly being responsible for a later potential cross-reactivity with other members of taxonomically-related families, including pollens and fruits. However, Ost c 1 should be responsible for the primary sensitization accounting for respiratory symptoms. In addition, *Fagales* tree pollens show sequence identities with Bet v 1 above 70% among each other, but much lower similarities to homologues from typical birch pollinosis-related fruits and vegetables [34]. Cross-reactive Bet v 1 homologues share indeed only 37% to 67% of their sequences with Bet v 1 [35].

In addition, this study underlined the relevance of sensitization profile on OAS occurrence. There was a different OAS prevalence on the basis of sensitization profile. This fact is consistent with a different exposure to priming pollens that differ across Italy. In particular, sensitization to Bet v 2 is a significant risk factor for having OAS in the Central Italy.

In addition, we considered the different profiles of sensitization to other pollens as well as the frequencies of fruits cause of OAS considering the different geographical areas. Actually, there are differences that may depend on climate characteristics. However, this issue deserves an appropriate and adequate investigation in more in-depth studies.

It has also to consider that this study has some limitations: it is retrospective and cross-sectional, and did not consider the profile of sensitization to the most relevant food allergen molecules. Thus, further investigations should address these issues. Moreover, it has to consider that food causing OAS in the patients with sensitization to Bet v 1 could overlapping with profilin sensitization. This issue should be extensively investigated in an appropriate study.

In conclusion, the present study demonstrated that there is a significant difference about birch associated molecules and the related clinical expression across Italy.

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Table 1: Percentages of sensitized patients to other pollens and percentages of the most frequent fruits cause of OAS in the 4 geographical areas.

	Genoa	Northern Italy	Central Italy	Southern Italy
Par j 2	42.1	17.7	26.7	35.8
Ole e l	27.9	25.9	27	30.7
Phl p1+ Phl p 5	27.3	52.4	55.5	44.6
Cup a l	6.7	4.1	3.7	5.4
Amb a l	1.4	7.4	5.4	5.8
apple	83	68	55	53
peach	41.5	28	22	24
apricot	17	16	14	17
cherry	15	10	12	13

Figures Legend

Figure 1: Percentages of patients sensitized to Bet v 1, Bet v 2, and Bet v 4 in 4 Italian geographical areas, and percentages of patients with oral allergic syndrome (OAS) in these areas.

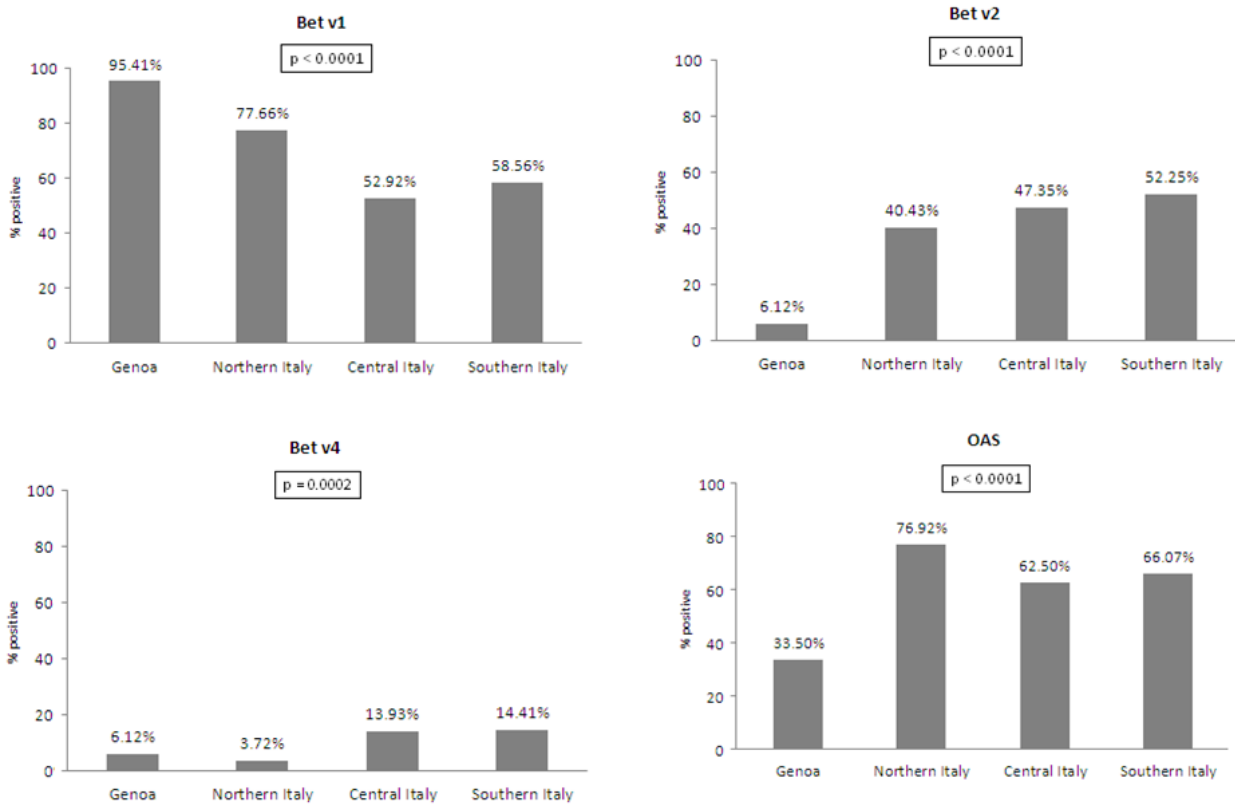
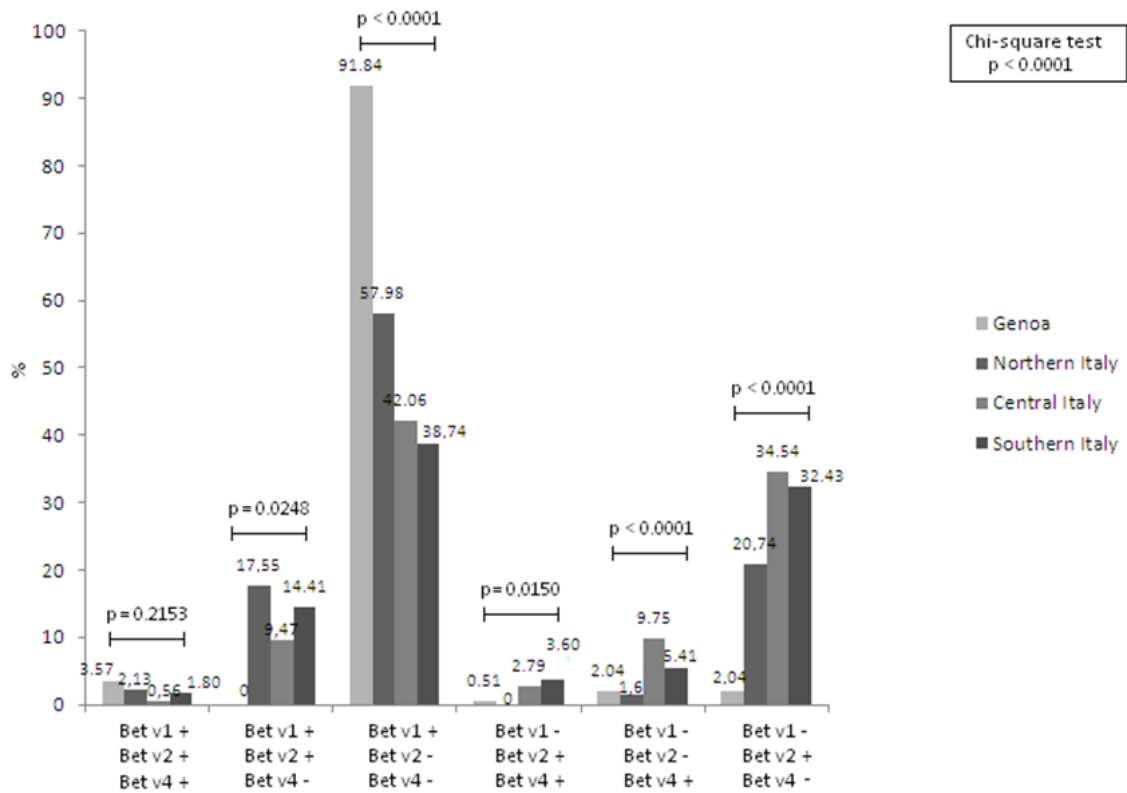


Figure 2: Percentages of patients with different patterns of Bet v1, Bet v 2, and Bet v 4 sensitization in 4 Italian geographical areas.



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