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## *Phleum pratense* molecular pattern across Italy

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### KEY WORDS

*Phleum pratense*; grass pollen;  
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### Summary

**Introduction.** *Phleum pratense* (Timothy grass) is the most frequent cause of grass allergy in Europe. Molecular-based allergy diagnostics have been recently introduced in the clinical practice, allowing to define and characterize exactly the sensitization profile. **Aim of the study.** The present study was aimed to investigate the possible relationships between Gramineae pollen data and the pattern of IgE reactivity to different allergen components across Italy. **Methods.** Pollen data, including duration and quantity, were recorded over a 10-year period in 24 Italian centres located along the Italian peninsula. Phl p molecular patterns (Phl p 1, 5, 7, 12) were evaluated in 4 different Italian geographical areas. **Results.** There were significant differences about pollen count and sensitization prevalence across Italy. Different molecular patterns were defined considering the different Italian locations. **Conclusion.** This study demonstrates that *Phleum pratense* sensitization is relevant in Italy, but there are significant geographical variations as far as pollen exposure and pattern of IgE reactivity to the considered allergen components are concerned. This information may have clinical relevance in managing patients allergic to grass pollen.

### Introduction

Sensitization, such as the on-going production of allergen-specific IgE, is the peculiarity of allergy. Sensitization can be demonstrated *in vitro* by measuring specific IgE levels. Traditionally, natural allergen extracts from allergenic sources are used, but they are usually heterogeneous, because they may include not only the major allergens, but also cross-re-

active allergens, non-allergenic antigens, and interfering substances (1). Thus, molecular-based allergy diagnostics, which allows the evaluation of IgE binding to single allergen components, have been recently introduced in the clinical practice, allowing to define and characterize exactly the sensitization profile (2). Thus, the allergy workup is changing, inasmuch it is highly useful in allergen-specific immunother-

apy prescription: positivity to major allergens excludes false reactivity to pan-allergens (3).

Pollens are the most common allergenic sources causing respiratory allergy, and grass pollen is the most relevant in Europe (4,5). Grass pollens belong to *Poaceae* family and are classified in many genera. *Phleum pratense* (Timothy grass) is the most frequent cause of grass allergy in Europe (6), so *Phleum pratense* (Phl p) is widely employed in diagnostics and immunotherapy (7). There are several molecules belonging to Phl p species. Phl p 1 and Phl p 5 are genuine marker of grass pollen sensitization, i.e. they are species-specific molecules (8). Instead, Phl p 7 and Phl p 12 are the main cross-reactive components: Phl p 7 is a calcium-binding protein and Phl p 12 is a profilin (9). There are variants of Phl p reactivity to different allergen molecules, depending on geographic location (10). In this regard, three studies evaluated Phl p molecular profile in Italy. The first study was conducted in Northern Italy and allowed to define a diagnostic prediction model (11). The second study, performed in Rome, enrolling children candidate to allergen immunotherapy (AIT), demonstrated that IgE sensitization profiles to Phl p are very heterogeneous, but molecular profile characterization allowed to tailor a personalized AIT (12). The last study, done in Central Italy, analysed the sensitization pattern to Phl p 1, 5, 7, and 12, in a group of allergic children (13). The author concluded that assessment of molecular profile is useful for giving a more specific and effective AIT. However, these studies were conducted in very restricted geographic areas, and did not consider the impact of pollen exposure both concerning duration and quantity. Therefore, the present study aimed to investigate the possible relationship between pollen data, including duration and quantity recorded over a 10-year period in 24 Italian centres located along the Italian peninsula, and Phl p molecular pattern, such as Phl p 1, 5, 7, and 12, in 4 different Italian geographical areas.

## Materials and Methods

### Pollen data

We retrospectively analysed the data, concerning 24 Italian centres (Acqui Terme, Alassio, Bologna, Bordighera, Busto Arsizio, Cagliari, Caltanissetta, Casate Novo, Città di Castello, Cosenza, Crotone, Cuneo, Desenzano, Faenza, Foggia, Gallarate, Genova, Milano, Novi Ligure, Perugia, Pesaro, Pescara, Rimini, Verona), as shown in **figure 1**, recorded during a 10-year period (2004-2013). The grass pollens were collected, assessed, and analysed according to validated methods (14).

Duration of pollen season (days) and annual pollen count (number/mc) were evaluated. The period from which the sum of daily mean pollen concentrations reaches 1% of the total sum corresponds to start of pollen season, the time when the sum reaches 99% of the whole pollen amount corresponds to the end of pollen season. The data were expressed as the mean of 10 consecutive years: from 2004

to 2013, considering 4 different geographical areas: Northern, Central, Southern Italy, and Liguria as previously defined (15).

### Patients data

Sensitization prevalence, assessed by SPT (using the allergen extract manufactured by Stallergenes Italia, Milan, Italy), to *Phleum pratense* were considered in the 4 areas. Data were the mean of the different centers, concerning the global outpatient records in the last 10 years.

### Serum IgE assay

This retrospective study considered a series of 11,235 patients who referred to Allergy Departments of different Hospitals for suspected AR between 2011 and 2014. In particular, 3567 patients resided in Liguria, 2284 in Northern Italy, 3804 in Central Italy, and 1580 in Southern Italy. Inclusion criteria were allergen-specific IgE to *Phleum pratense* > 0.35 kUA/L. The study was performed according to the Review Board rules and all patients signed an informed consent.

Serum levels of specific IgE were detected by the FEIA (fluorescence enzyme immune assay) 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. Measurement of circulating specific IgE antibodies was performed according to manufacturer's instructions (16). Specific Ig E concentrations were expressed in kUA/L according to the traceable calibration to the 2<sup>nd</sup> IRP WHO for Human IgE and 0.35 kUA/L was considered as a cut-off (17).

### Statistical analysis

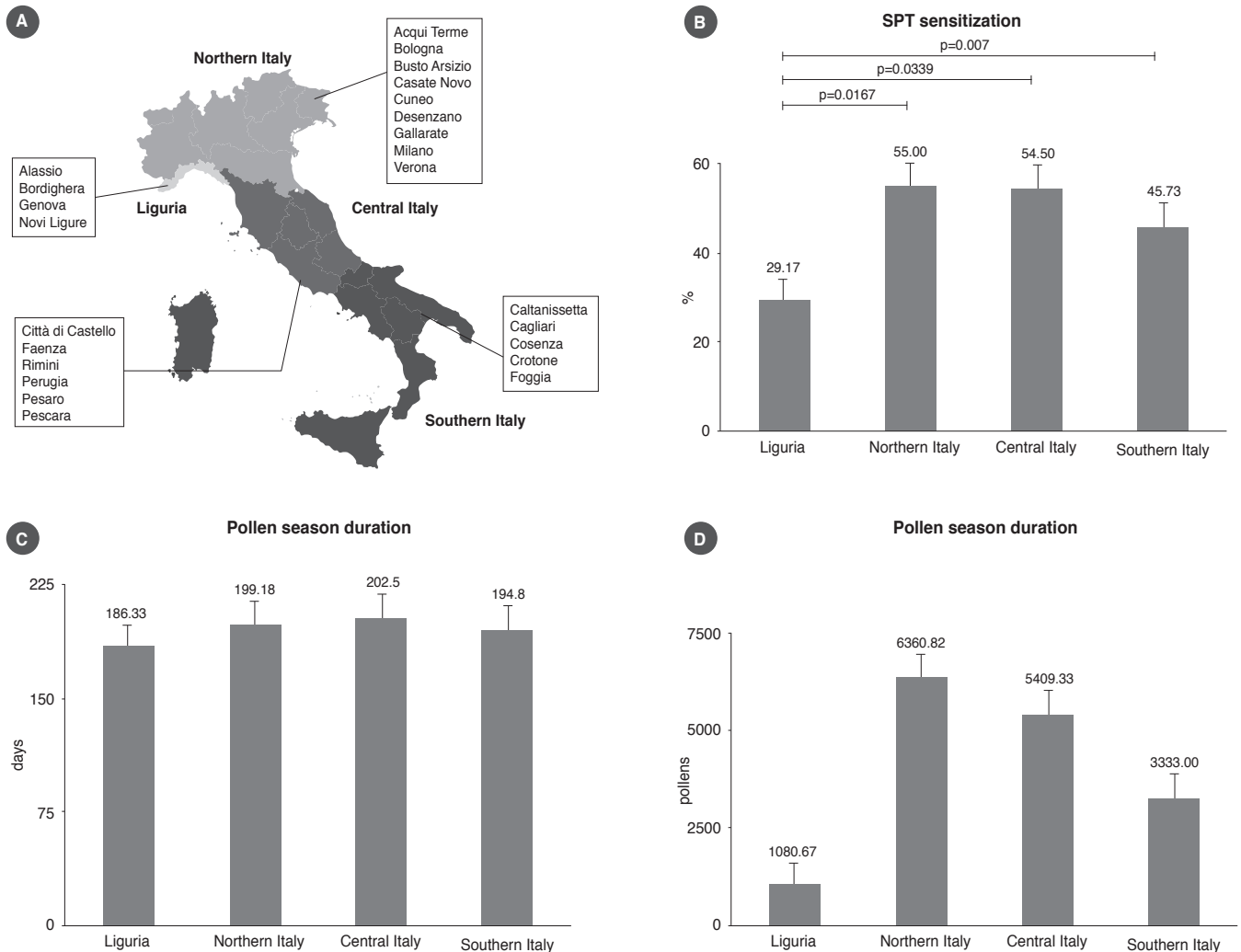
Statistical analysis was performed using the statistical software package Medcalc 9 (Frank Schoonjans, Belgium). All the variables regarding patients' sensitization were dichotomized (positive/negative, present/absent) and prevalence was reported as percentage. Variable association of pollen count, pollen season duration and sensitization, as assessed by SPT, among different areas, were investigated using the one-way analysis of variance (ANOVA test), for IgE a  $\chi^2$  Test was used. The correlation between pollen count and pollen season duration with sensitization at SPT or IgE for all the different areas was evaluated through Spearman correlation analysis. A p-value  $\leq 0.05$  was considered statistically significant.

## Results

### Pollen data

The pollen season duration was no significantly different among geographical areas as well as the pollen count (**figure 1**). How-

**Figure 1** - A: Involved centers in the 4 Italian geographical areas; B: SPT sensitization in Liguria, Northern, Central, and Southern Italy; C: Pollen season duration; D: pollen count. Data are expressed as mean + S.D.



ever, Liguria showed the lowest annual pollen count in comparison with the other areas.

#### Patients data

Among study subjects, grass sensitization was significantly different among 4 areas: the highest percentage (55%) was in the Northern Italy, the lowest in Liguria (29.2%), as shown in **figure 1**.

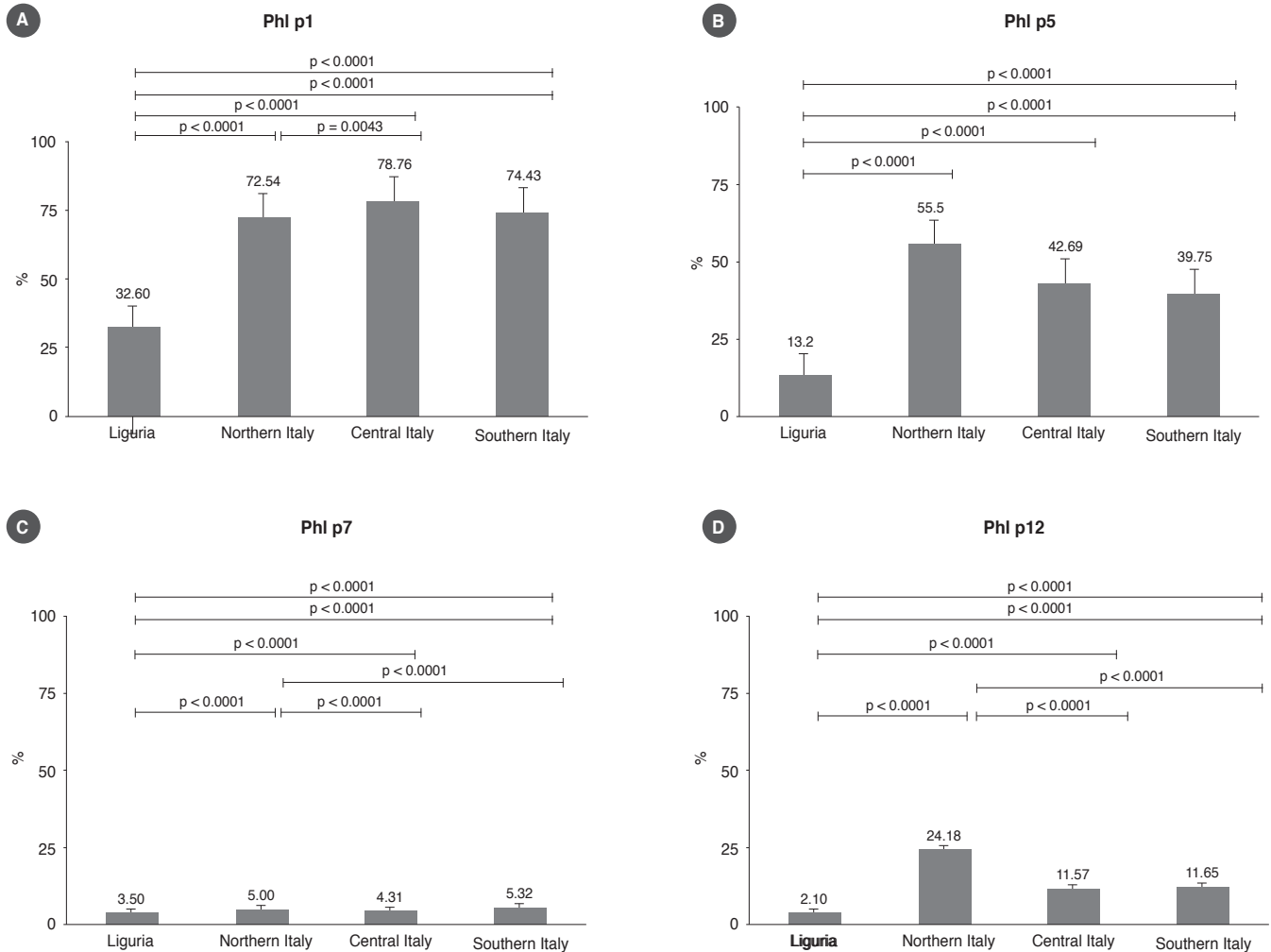
#### Molecular pattern

There were significant differences for Phl p 1, 5, 7 and 12 in the 4 areas with the lowest frequencies in Liguria (**figure 2**). Phl p

1 sensitization was the most relevant in all areas, even though the prevalence was lower in Liguria. Phl p 5 sensitization was also common with significant differences. Phl p 1 sensitization was consistent with Phl p 5. Phl p 7 sensitization was low everywhere, but with significant differences. Phl p 12 sensitization was 24% in Northern Italy, but only 2.1% in Liguria, with significant differences among areas.

There was moderate inverse correlation between pollen season duration and prevalence of *Phleum pratense* sensitization, assessed by SPT, in Liguria and Central Italy ( $r = -0.43$  and  $-0.48$ , respectively) and between pollen count and sensitization in Central and Southern Italy ( $r = -0.32$  and  $-0.37$ , re-

**Figure 2** - A: *Phl p 1* sensitization distribution in Liguria, Northern, Central, and Southern Italy; B: *Phl p 5*; C: *Phl p 7*; *Phl p 12*. Data are expressed as mean + S.D.



spectively). There was very strong relationship between pollen count and serum IgE positivity in Liguria and Central Italy ( $r = -0.81$  and  $-0.94$ , respectively) and moderate in Northern Italy ( $r = -0.51$ ).

**Discussion**

Pollen allergy, such as hay fever, is the most common allergic disorder, and grass allergy is the most frequent in Europe (5). Previously, a geographic-dependent variability of assessment of pattern of IgE reactivity to different allergen components of *Phleum pratense* has been reported. Therefore, it is clinically relevant to correctly define the sensitization profile, mainly in view of AIT prescription.

The present study addressed the assessment of *Phl p* molecular pattern in Italy, also considering grass pollen season duration and count, recorded during a 10-year observation period.

The findings show that grass pollination lasts 6-7 months without remarkable differences among geographic areas. On the contrary, the pollen quantity significantly varied with a gradient from North to South with the exception of Liguria, where very low values were observed.

The present molecular outcomes are substantially consistent with previous surveys conducted in Italy, that showed the pre-eminence of IgE reactivity to *Phl p 1* and *Phl p 5* (11-13). In particular, *Phl p 1* appears as the most important species-specific *Phl p* allergen. *Phl p 5* is also species-specific, but IgE to

this allergen are relatively less frequent and display a North-South gradient consistent with pollen count. Phl p 7 reactivity is around 5% across the whole Country. In contrast, Phl p 12 sensitization reaches up to 25% in Northern Italy, but it is only 2% in Liguria.

These results highlight the peculiarity of Liguria region, as the lowest sensitization prevalence both for SPT and IgE pattern were demonstrated, and this outcome complies with pollen data. Possible explanations may consist in a particular botanic milieu where Betulaceae predominates. This is in agreement with the high prevalence of birch allergy with a Bet v 1 polarized pattern (18). This Bet v 1 preponderance influences the low prevalence of oral allergy syndrome in this region (19). Bet v 1 predominance might affect grass pattern. We speculate that there may be a competition between Bet v 1 and Phl p sensitization, depending on botanical differences, but further studies should investigate these issues.

The main limitation of this study is that it was conducted without considering clinical outcomes, such as allergic symptoms. On the other hand, the strength of this study is the number of contributing centers, the long period of pollen recording, and the evaluation across Italy.

In conclusion, this study demonstrates that *Phleum pratense* sensitization is relevant in Italy, with significant geographical variations in the pattern of reactivity to the considered allergen components. This information may have clinical relevance in managing patients allergic to grass pollen.

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