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Towards a global vision of molecular allergology: a map of exposure to airborne molecular allergens

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Summary

Allergy diagnostics have changed in the last 10–15 y, moving from the use of extracts for in vivo *and* in vitro *diagnosis to the Component Resolved Diagnosis, based on purified or recombinant allergens. As expected, aerobiology developed similarly, and measurement of allergens in both outdoor and indoor air is now feasible. With the aim of promoting a global view of molecular allergy, we have drawn a map of exposure to molecular aeroallergens in Italy on the bases of geo-climatic regions, maps of pollen distribution, and published data on the molecular profile of sensitization in Italian patients. Given the latitudinal extension of Italy, the profile of exposure to some allergens, such as those of the "Birch Group" and weeds, varies greatly from North to South, while the distribution of exposure to grass allergens is more homogeneous. This map can contribute to a global molecular vision of allergy, helping clinicians to view exposure to pollen in a new way. The exposure profile of the area where patients live can also indicate the correct choice of molecular diagnostics and, therefore, of the appropriate allergen immunotherapy.*

Introduction

In the last 20 years, allergology has changed markedly, moving from the use of extracts to a molecular-based diagnosis. Thanks to the identification and purification of allergens and their production as recombinant molecules, molecular diagnostics are now available in daily clinical practice. This change prompted a new way of looking at the sources of allergens and at the mechanisms linking exposure, sensitization, and symptoms of respiratory allergic diseases. This new approach also involved aerobiology, inducing researchers to apply molecular methods aimed at improving the assessment of airborne allergens. Although pollen count has been used for over 50 years for the assessment of allergen exposure both in clinical practice and in experimental studies, proof that pollen count represents allergen exposure is lacking. In this context, measurement of the allergen content of pollen, the so called "pollen potency", may better contribute improvement of our knowledge of the relationship between exposure to airborne allergens and allergic sensitization and symptoms.

After the early studies at the end of the 1990s (1), a number of papers in this field have been published in the last few years, from Germany and Spain (2-7), thanks to a substantial improvement in both devices and methods used for analysis. More recently, the EU-funded HIALINE (Health Impacts of airborne ALlergen Information Network) project (8) set the standard for the assessment of pollen potency in ten countries over three pollen seasons. Measurement of Phl p 5, Ole e 1 (9), and Bet v 1 (10) in the pollen of grass, olive tree, and birch, respectively, confirmed that allergen count deviates from pollen count.

These findings implied that pollen potency varies, even on a daily basis, and that the pollen quantity may not represent the amount of allergen to which a patient is exposed.
To the best of our knowledge, we here present the first map of the distribution of aeroallergens (Figure 1), with the aim of disseminating the concepts of molecular allergology and aerobiology within the community of allergy specialists.

Materials and methods

Pollen and aeroallergen data

The geo-climatic classification proposed by the Italian Association of Aerobiology (AIA) (11), specifically aimed at describing the complexity of pollen distribution in Italy, was selected as a background map. Data on the distribution of aeroallergens have not been published in Italy to date; we assumed that the exposure to the source of allergen (i.e. pollen) could be used as a proxy for exposure to the allergens, which are actually contained in the source. Therefore, data on pollen distribution were gathered from the European Aeroallergen Network (12) and from the regional networks' websites (13,14). Data were organized according to the classification of allergens based on sequence homology, as proposed by Lorenz in 2009 (15): grass, trees ("Birch group", *Oleaceae* and *Cupressaceae*), and weeds. Although this classification largely overlaps with the classical botanical classification, the molecular approach is particularly useful in the case of the "Birch group", in which homology and cross-reactivity are both high among the major allergens, Bet v 1 (birch), Cor a 1 (hazelnut), Car b 1 (hornbeam), Aln g 1 (alder), and Que a 1 (oak). As the distribution of allergens from the "Birch group" varies over the Italian peninsula, a symbol (">") was introduced to visualize a gradient of exposure to these specific allergens. According to the aerobiological characteristics of the Italian peninsula, Par j 1 and 2 (wall pellitory), Art v 1 (mugwort), and Amb a 1 (ragweed) were selected in the weeds group, Ole e 1 (olive tree) and Fra a 1 (ash) in the *Oleaceae* group, and Cup a 1 (cypress) in the *Cupressaceae* group. Phl p 1 and 5 (*Phleum pratense*) were chosen as the major allergens representing allergen groups 1 and 5, respectively, of grass pollen; due to its low cross-reactivity with the other grass pollen allergens, Cyn d 1 (*Cynodon dactylon*) was also included. "Minor allergens" like Pla a 1 from the plane tree and Sal k 1 from *Salsola kali* were also considered.

Although factors affecting the production and dispersion of indoor allergens and moulds differ from those of pollen, house dust mite and *Alternaria* allergens have also been included in the map. Specifically, the major allergens of *Dermatophagoides pteronyssinus* (Der p 1 and Der p 2) and *Dermatophagoides farinae* (Der f 1 and Der f 2) and the major allergen of *Alternaria alternata* (Alt a 1) were also taken into consideration.
Given the educational and practical purposes of this project, the allergens were chosen on the basis of the availability of *in vitro* diagnosis for respiratory allergic diseases, other than of their epidemiological and clinical importance, as seen on the Allergome (16) and AllFam (17) web-based databases.

Data on sensitization

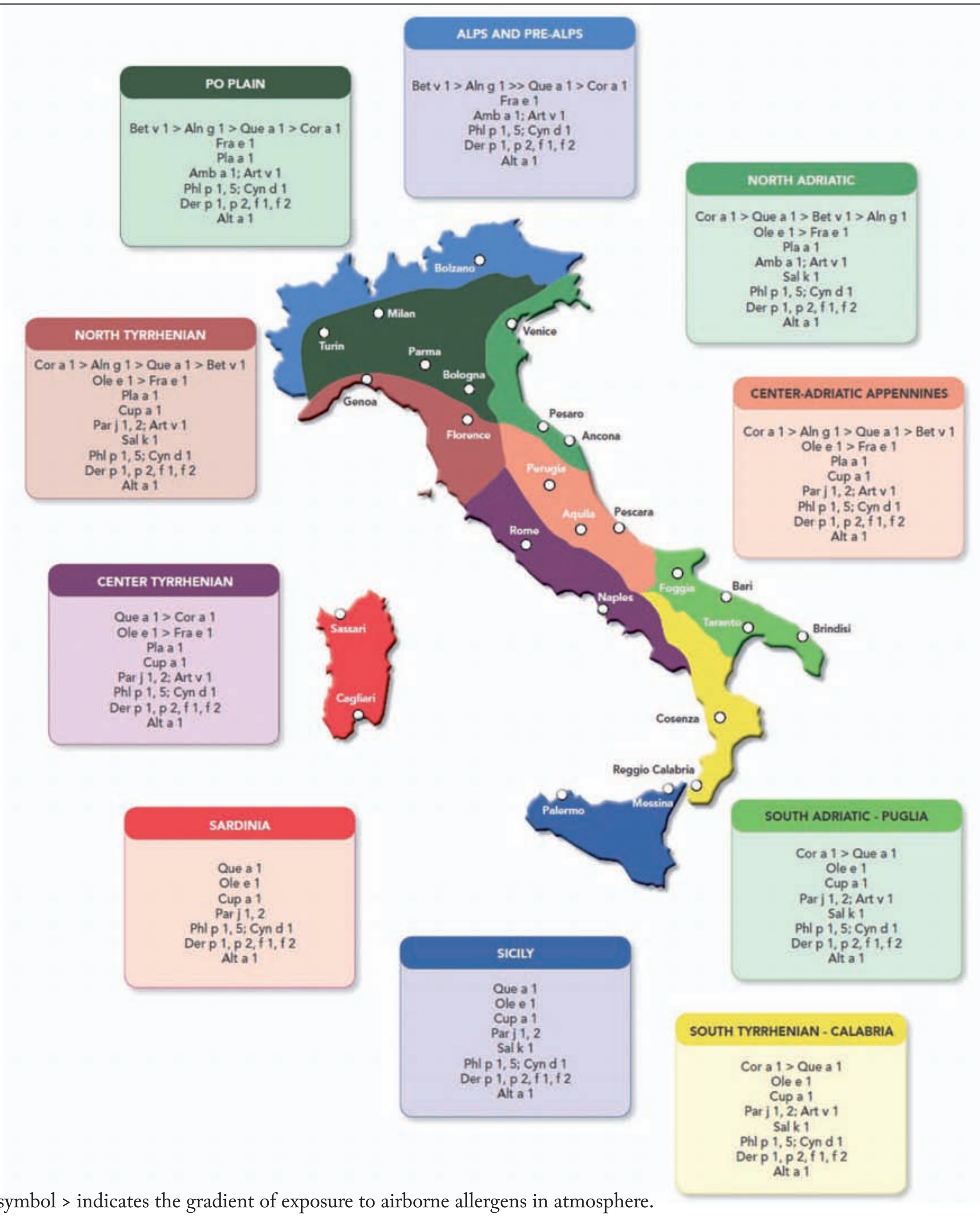
An extensive study of the molecular profile of sensitisation of the Italian population remains lacking. Therefore, data obtained from different studies were gathered from the scientific literature for the purposes of this project. Only papers published in peer-reviewed journals were considered. The underlying assumption of this method is the direct relationship between exposure to the source of allergen and development of sensitization towards the particular allergen. In other words, we assumed that the molecular profile of resident patients is related to the type of allergens to which they are exposed.

Results

Grass pollen allergens

Among the 11 groups of allergens of the approximately 20 species of grasses that are implicated in pollinosis, Phl p 1, Phl p 5, and Cyn d 1 were selected. Due to the high cross-reactivity among the grass allergen groups 1 and 5, the two major allergens of *Phleum pratense*, Phl p 1 and Phl p 5, were used as representative of the exposure to grass pollen allergens in Italy. This assumption was supported by the data on cross-reactivity between Phl p 1, Lol p 1 (*Lolium perenne*), Poa p 1 (*Poa pratensis*), Dac g 1 (*Dactylis glomerata*), and Hol l 1 (*Holcus lanatus*) (18). An earlier epidemiological study confirmed the higher prevalence of sensitization to Phl p 1 and 5 than to Phl p 2, 3, 4, 6, 7, and 11, which can be considered as minor allergens in Italy (19). Later studies showed similar findings both in the northern (20) and in the central (21) Italian regions.

Figure 1 - The Italian molecular map of exposure to airborne allergens



The symbol > indicates the gradient of exposure to airborne allergens in atmosphere.

Sources:
• Geo-fitoclimatic map proposed by The Italian Association of Aerobiology (AIA) and available at the following site:
- http://www.ilpolline.it/bollettino-pollinico/.Accessed 29 November 2012.

• Data on aeroallergen distribution gathered from the following sites:
- www.POLLnet.it (Italian network of aerobiological monitoring);
- www.pollinieallergia.net (AAITO aerobiological network, AAITO: Italian Association of Hospital Allergists and Immunologists);
- www.polleninfo.org (European Aeroallergen Network).

Data on the distribution of sensitization rates to the major allergens of grass pollen are still lacking, and previous findings of high regional variability of the prevalence of Phl p 1- and Phl p 5-specific IgEs in Spain (22) cannot be confirmed. However, findings from a small number of paediatric patients showed limited variation in terms of the sensitization rate to grass pollen allergens; in particular, the rate of sensitization to Phl p 5 is similar to that for Phl p 2 and Phl p 6 (23). Although some minor variation is expected, exposure to the single allergens from grasses seems to be similar throughout the country, in general.

Weed allergens

Weed allergens can be grouped together, as a number of them belongs to the LTP family, i.e. Amb a 6, Art v 3, and Par j 1 and 2 (24). Par j 1 and Par j 2 (25) are the major allergens of *Parietaria judaica* (wall pellitory), an important cause of respiratory allergy in the Mediterranean area (26). Two panallergens, the profilin Par j 3 (27) and the calcium binding protein (CBP) Par j 4 (28), were identified subsequently. Exposure to *P. judaica* allergens shows a clear north–south gradient, with the central–southern areas most affected. A study performed in north-western Italy, where wall pellitory is rare, supports this observation. Only 33% of patients with skin prick tests positive for *P. judaica* were actually sensitized to Par j 2 (the genuine marker of sensitization), while the remaining group was sensitized to the panallergens CBP and/or profilins (29). Given the high homology and crossreactivity between Par j 1 and Par j 2 (24), both allergens were shown in the map.
Exposure to ragweed (*Ambrosia artemisiifolia*) shows a completely opposite gradient, as northern Italy lies in the area of highest impact of this allergenic pollen (Central and Eastern Europe) (26). Ragweed pollen is virtually absent south of the Po River, except for instances of long-distance transport (30). Amb a 1 is the major allergen, as IgEs specific to this allergen are detected in 90% of patients allergic to ragweed (31). Amb a 5, Amb a 6 (LTP), and panallergens Amb a 8 (profilin), Amb a 9, and Amb a 10 (CBPs) are identified as minor allergens (32). Unlike ragweed, mugwort (*Artemisia vulgaris*), which belongs to the same botanical family *Asteraceae/Compositae*, grows throughout the peninsula, as shown by the distribution of the major allergen Art v 1 in the map. In the northern areas, where the pollen season overlaps, sensitization to both ragweed and mugwort pollen is common, as shown by both in *in vitro* and *in vivo* diagnoses (33), possibly because of the extensive homology between Amb a 1 and Art v 6, a minor mugwort allergen (34,35). However, recent data suggested a more complex mechanism (36). Despite its wide distribution throughout the country, mugwort allergy seems to be of little clinical importance.

Birch group allergens

The diversity of exposure is particularly evident for "Birch Group" (Bet v 1-like family) allergens, being supposedly different in the north (prevalence of exposure to Bet v 1 and Aln g 1), the centre (Cor a 1 and Aln g 1), and the south of the peninsula, and the islands (Cor a 1 and Que a 1). The high crossreactivity between the *in vivo* and *in vitro* extract-based diagnostics prevented greater clarity regarding the heterogeneity of exposure to "Birch group" allergens; major allergens of birch (Bet v 1), alder (Aln g 1), hornbeam (Car b 1), and hazel (Cor a 1) are indeed highly crossreactive (37,38). As observed in Northern Europe (39), patients allergic to birch were prevalently sensitized to Bet v 1 in the northern Italian regions, although at a lower rate (56% in the study by Rossi et al. (40)).
As shown in the map, the distribution of "Birch group" aerollergens in Italy is complex, because of the diverse phytoclimatic conditions. The molecular approach to the issue of exposure represents the only way to disentangle the specific pattern of allergens to which the resident patients are exposed. In this regard, the crossreactivity between Que a 1, the major allergen of oak (*Quercus*), and other members of the "Birch group" (37) further complicates the picture. In fact, it has less homology to the other "Birch group" members, but extract-based diagnostics and pollen count are not able to evaluate the clinical relevance of allergy to oak, as shown in a recent paper (41).

Oleaceae group

Olive tree pollen is the major source of allergens of the *Oleaceae* group in Italy, particularly in central–southern regions and in the islands (42). Ole e 1 is the major allergen in this group and represents the genuine marker of sensitization to olive pollen in the Mediterranean (43). Two panallergens, Ole e 2 (profilin) and Ole e 3 (CBP), have been identified, together with the minor allergens Ole e 7 and Ole e 9 (44), which seemed to play a relevant clinical role in a Spanish study (45). The homology (89%) between Ole e 1 and Fra e 1 (the major allergen of ash [*Fraxinus*]) (46) explains the reports of sensitization to olive tree pollen extracts in areas where the plant is virtually absent (47).

Therefore, in the "*Oleaceae* group", exposure to Fra a 1 is prevalent in the North, exposure to Ole e 1 and Fra a 1 in the Centre, and that to Ole e 1 only in the South.

Other pollen allergens

Cypress pollen is an important cause of respiratory allergy in large areas of central Italy, i.e. Tuscany, Lazio, and Umbria. Major allergens are Cup a 1 (*Cupressus arizonica*) and Cup s 1 (*Cupressus sempervirens*), which are highly crossreactive and homologous. Cup a 1 ranked first among the 103 allergens tested in a large study in Rome (48).
Pla a 1 (49) and Pla a 2 (50) are the major allergens of plane tree (*Platanus acerifolia*), which seemed to play a relevant clinical role in Spain (51), while its importance in Italy is still debated. Although the plants are widely distributed in Italian cities, a high rate of sensitization may be partly ascribed to Pla a 3 (LTP), which cross reacts with other food-associated LTPs (52).
Salsola kali, belonging to the *Chenopodiaceae* family, has been reported as a cause of allergy in Spain (53) and in southern Italy (54). Sal k 1 is the marker of sensitization and is available for *in vitro* diagnosis (55).

House dust mite and moulds

House dust mite group 1 (Der p 1 and Der f 1) and group 2 (Der p 2 and Der f 2) allergens are the most prevalent allergens worldwide (56), while a recent study showed that *D. farinae* allergens are more important in the Mediterranean countries (57). Although Der p 1, 2 and Der f 1, 2 were included among the first ten most prevalent allergens in an ISAC-based study (21), data on possible differences in terms of sensitization profile as well as of exposure in Italy are still lacking; we assumed, however, that distribution is presumably homogeneous throughout the peninsula.
Among fungi, *Alternaria alternata* is one of the principal species associated with respiratory allergic diseases. Its major allergen is Alt a 1 (58), which was identified and purified more than 20 years ago (59). Like all fungal spores, *A. alternata* is widely distributed in the outdoor and indoor environment and its environmental levels depend on several factors, such as geographical areas, weather, and climate conditions (60). Use of Alt a 1 as marker of exposure and sensitization to *A. alternata* in the map is an obvious oversimplification, as is the homogenous distribution of this allergen throughout the country. However, we felt the need to cover the types of allergen sources comprehensively.

Discussion

Both allergy diagnosis and aerobiology are moving toward a "molecular era", which allows allergy specialists to look at the issues of diagnosis and exposure to the allergens in a completely new way. This change prompted a new type of reasoning, which is based on the knowledge of the physicochemical properties of the allergens and their geographical distribution. The latter issue is one of the main achievements of the molecular allergology, because it contributes to a better definition of the sensitization profile in the different regions, leading to an improvement in both prevention and treatment of food and respiratory allergies. In this regard, the effects of exposure to aeroallergens are relevant, for it is also involved in the sensitization to key food allergens, as in the case of LTPs (61).
The main result of this study is a map of the distribution of aeroallergens in Italy, from a molecular perspective. To our knowledge, this is the first time that the concepts of molecular aerobiology and allergology have been applied to constructing a map of exposure to airborne allergens. Clearly, the map is an oversimplification and offers a partial view of a very complex mixture of aeroallergens that are distributed over a diverse and heterogeneous geographical area, both from a climatic and vegetation point of view. However, the aim of this study was to provide allergy specialists with a new view of airborne allergens and to disseminate a "molecular reasoning" approach. In this regard, the map shows the allergens for which molecular diagnoses are actually available, thus practically contributing to daily clinical practice, particular with regard to the choice of the appropriate allergen immunotherapy.

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Conflicts of interest
Franco Frati and Ilaria Dell'Albani are employees of Stallergenes Italy. The other authors don't have any conflicts of interest that are directly relevant to the content of the study.

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