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An atlas of IgE sensitization patterns in different Italian areas. A multicenter, cross-sectional study

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KEYWORDS

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Abbreviations

NW, North-West; NE, North-East;
C, Centre; S, South; Is, Islands subset.

Summary

Background. The development of recombinant technology supported the allergy diagnostic work-up in the daily clinical practice, representing a useful tool for epidemiological studies. **Methods.** An atlas of the IgE sensitization profiles found throughout Italy was prepared from a nationwide, multicenter, cross-sectional study. **Results.** 6052 unselected consecutive individuals, belonging to North-West [NW], North-East [NE], Centre [C], South [S], and Islands subset [Is] were evaluated by means of the ImmunoCAP ISAC test. The top-ranked sensitizations found were Cup a 1 in [C] (58.1%) and [S] (53.6%), Phl p 1 in the North (from 46.1% to 49%), and Cyn d 1 in [Is] (44.2%). High frequency of house dust mite group 2 molecules sensitization was found in [C] (36.9%) and [S] Italy (40.8%), whilst low level of reactivity was recorded in [NW] (20%). Pellitory hypersensitivity was mainly found in [C], [S], and [Is], whilst ragweed Amb a 1 sensitivity was particularly found in [NW] Italy. IgE recognition of PR-10, profilin, and nsLTP was mutually exclusive in 69.1% of cases, PR-10 reactivity mostly occurring in [NE], profilin in [NW], and nsLTP molecules recognition mainly recorded in [C] and [S]. **Conclusions.** Divergent IgE sensitization patterns were found along Italy, possibly linked to the distinct geographical locations, indicating multiplex system IgE analysis as a reliable approach for epidemiological evaluation even in small geographical areas.

Introduction

Allergic diseases are rising worldwide, affecting about one quarter of the total population in industrialized countries mainly as a consequence of genetic, epigenetic, and environmental factors affecting predisposed individuals (1). In recent years, the development of recombinant technology has led to important achievements in allergen characterization, purification and production, thus supporting the allergy diagnostic work-up in the daily clinical practice (2,3). The microarray technology, which

allows the simultaneous detection of IgE reactivity towards multiple allergens while using a minimum amount of serum (4), is considered a useful tool for epidemiological studies (5), and in particular for IgE profiling of patients affected by respiratory symptoms (6), atopic dermatitis (7,8), and food allergy (9,10), leading to a more accurate diagnosis of sensitization (11).

It is widely known that allergic sensitization profiles strongly reflect the lifestyle and the environment of the studied population (12). In this regard, several studies have shown that different IgE sensitiza-

tion patterns can be demonstrated, by means of molecular diagnostic approaches, for grasses or tree pollen allergy in different regions of Spain (13,14) or Italy (15,16,17), thus representing possible loco-regional signatures of sensitization. This has also been demonstrated in other countries, such as Africa and Asia (18,19,20).

Italy, located in South-Central Europe, includes a boot-shaped peninsula and two large islands, i.e. Sardinia and Sicily. The Alps form the northern boundary, and the Apennine mountain range runs along the entire peninsula, with the Po river valley, the largest plain found in Italy, placed between them. Due to such geographical characteristics, Italy has a multiplicity of climate systems. Following the Köppen-Geiger classification system, the most widely used climate categorization scheme, the North-East of Italy, the Po and the Adige valleys show a “Humid subtropical climate [Cfa]”, a “Mediterranean climate [Csa]” involves all the coastal areas, whilst a “Mediterranean mild climate [Csb]” is found in-land and in Southern Italy. A number of other microclimates can be found in Italy, such as an “Oceanic climate [Cfb]” in the Apennines, a “Humid continental climate [Dfb]” in the Alps (but above the tree line a “Tundra climate” is registered), and a “Cold continental climate [Dfc]” in the alpine valleys. Some regions in Italy have a type of weather presenting elements belonging to both Cfa and Csa, and therefore cannot be uniquely classified. Such climatic variety largely affects the vegetation, thus allowing distinct types of cultivation, e.g. olive trees, in some regions and not in other ones. Such characteristics represent a wonderful model to study the impact of climate and geographical location on IgE sensitization.

In this light, we present a multicenter study designed to comprehensively analyze the IgE sensitization profile evaluated by means of multiplex testing performed during routine clinical care, in samples from different regions throughout Italy, to verify the impact of geographical and climatic characteristics on the distribution of allergic sensitization.

Methods

Patients

Six allergy units (Florence, Milan, Palermo, Pavia, Pordenone, and Rome) distributed in different Italian areas participated to this multicenter study. Between September 2015 and August 2016, doctors enrolled unselected patients presenting with a history of at least one of the following: adverse reactions to foods, allergic rhinitis, bronchial asthma, and/or atopic eczema. Blood samples were taken from all patients; after centrifugation, properly coded sera were kept at -20 °C until their use for in-vitro analyses.

All ISAC tests were performed during routine care, and the samples were anonymized, so that no personal data, with the exception of age and sex, was available. The Institutional Review Board of IDI-IRCCS confirmed that an ethical approval was not required (n. 493.1).

IgE antibody measurements

Serum IgE reactivity was analyzed using the ISAC platform (Thermo Fisher Scientific, Uppsala, Sweden) by means of the latest commercially available ImmunoCAP-ISAC as per manufacturer's instructions. The ISAC microarray in the current versions is a fully commercial product. Allergens spotted on the chip are determined by the manufacturer, and presently this mainly depends on molecule availability from a number of different providers, i.e. either companies or research labs.

In brief, ImmunoCAP-ISAC 112 slides were washed, rinsed and dried at room temperature (RT). Undiluted serum (30 µl) from each patient was pipetted on to the slide, and after 120 min incubation at RT in a humid chamber, slides were washed, rinsed and dried. IgE binding was detected by addition of an anti-human secondary antibody (Thermo Fisher Scientific). Slides were then washed, rinsed, dried, and stored in the dark until scanning. Images were acquired by scanning allergen biochips with a CapitalBio LuxScan® 10K microarray scanner. IgE values were expressed as ISU arbitrary units (ISAC Standardized Units), corresponding to IgE antibody levels in the ng/ml range (detection limit: 0.01 ISU-E) (21-23).

Statistics

All data were analyzed using the IBM SPSS Statistics, version 21 statistical package (Armonk, NY, USA) for statistical evaluation. The TD-Synergy Laboratory Information System was used to search and collect demographic information, i.e. age and gender, and clinical and laboratory data for Allergy Clinic patients who attended the outpatient Allergy clinic and underwent specific IgE testing.

Categorical variables were analyzed using the Pearson's χ^2 or Fisher's exact test. Differences between prevalence were evaluated using the nonparametric Mann-Whitney U-test. The degree of relationship between the quantitative variables studied was analyzed using the Pearson Correlation (r) coefficient. Statistical significance cut-off level has been set for $p < 0.05$. The degree of relationship between quantitative variables was analyzed using the Spearman's correlation (r) coefficient, given the non-parametric distribution of the observed values.

Separate modelling was performed for each condition including all molecules, in addition to sex and age. P values < 0.05 were considered significant.

Result

Study cohort

A total of 6052 ISAC-positive adult individuals (53.3% female; mean age 33 ± 19 years) were recruited by the participating centers. Patients were subdivided according to the classification of terri-

torial units for statistics, (NUTS, for the French *Nomenclature d'Unités Territoriales Statistiques*), i.e. a geocode standard developed by the European Union for referencing the administrative divisions of Italy and other countries for statistical purposes. Briefly, patients were assigned to one of the following macro-regions: (i) 1217 participants from the Aosta Valley, Liguria, Lombardy, and Piedmont were grouped in the North-West [NW], (ii) 1450 participants from Emilia-Romagna, Friuli-Venezia Giulia, Trentino-South Tyrol, and Veneto in the North-East [NE], (iii) 2332 participants from Lazio, Marche, Tuscany, and Umbria in the Centre [C], (iv) 802 participants from Abruzzo, Apulia, Basilicata, Calabria, Campania, and Molise in the South [S], and (v) 251 participants from Sardinia and Sicily in the Islands subset [Is].

Prevalence of allergen IgE recognition

Table I shows the proportion of the first twenty positive allergens found in the five macro-regions. Cypress pollen aller-

gen Cup a 1 was the most common sensitization in C and S Italy, whilst the timothy grass major-pollen allergen Phl p 1, and its homologous bermuda grass allergen, Cyn d 1, were the most prevalent in the other macro-regions. In Centre and South Italy, cypress pollen and grass pollen sensitizations were followed by the house dust mite allergen Der f 2, in Is by pellitory Par j 2, by birch pollen Bet v 1 in NE, and by cat uteroglobin (Fel d 1) in NW. Interestingly, the peach lipid transfer protein (LTP) Pru p 3 was the most common food allergen in C, S and Is, whilst PR10 molecules were the most frequent food allergens found in NE and NW. Profilin allergens and the major ragweed pollen allergen, Amb a 1, were included among the first twenty positive allergens only in NW. No difference in cross-reactive carbohydrate determinants (CCD) IgE recognition was observed throughout Italy, with a prevalence ranging from 9.8% to 12.7% of the IgE sensitized participants.

Table I. Frequency of IgE sensitization to the top ranked 20 allergenic molecules tested on ISAC-112 microarray in Italy macro-regions. Prevalence have been calculated on the respective tested subjects positive to at least one of the 112 allergens on the microarray.

	NE		NW		C		S		Is	
	n= 1450		n= 1217		n= 2332		n= 802		n= 251	
1	Phl p 1	46,1%	Phl p 1	49,0%	Cup a 1	58,1%	Cup a 1	53,6%	Cyn d 1	44,2%
2	Cyn d 1	41,9%	Cyn d 1	44,9%	Phl p 1	54,6%	Phl p 1	47,1%	Cup a 1	41,0%
3	Cup a 1	35,4%	Phl p 5	33,9%	Cyn d 1	48,6%	Cyn d 1	45,4%	Phl p 1	38,2%
4	Bet v 1	32,8%	Phl p 4	30,9%	Cry j 1	41,4%	Der f 2	40,8%	Par j 2	28,7%
5	Phl p 5	31,6%	Fel d 1	26,8%	Der f 2	35,9%	Der p 2	39,0%	Fel d 1	28,3%
6	Fel d 1	31,5%	Amb a 1	26,7%	Der p 2	35,8%	Cry j 1	38,2%	Cry j 1	27,9%
7	Der f 2	30,9%	Phl p 2	25,7%	Phlp 4	34,8%	Par j 2	35,7%	Der f 2	27,9%
8	Der p 2	29,9%	Phl p 6	25,1%	Fel d 1	32,4%	Der f 1	31,2%	Der p 2	25,5%
9	Cor a 1.01	28,3%	Bet v 1	23,9%	Phl p 5	31,9%	Der p 1	30,3%	Ole e 1	25,5%
10	Mal d 1	28,1%	Cor a 1.01	22,1%	Der f 1	31,6%	Phl p 4	29,9%	Der f 1	24,7%
11	Aln g 1	27,7%	Ole e 1	21,5%	Der p 1	30,9%	Ole e 1	28,1%	Der p 1	23,9%
12	Phl p 4	27,6%	Cor a 1.04	20,8%	Par j 2	27,9%	Pru p 3	27,2%	Phl p 4	22,7%
13	Cor a 1.04	26,6%	Mal d 1	20,7%	Phl p 2	27,5%	Fel d 1	26,6%	Pru p 3	22,3%
14	Der p 1	25,7%	Der p 2	20,0%	Ole e 1	27,5%	Phl p 5	26,1%	Jug r 3	18,3%
15	Der f 1	25,6%	Aln g 1	19,9%	Phl p 6	22,7%	Jug r 3	24,3%	Pla a 2	17,9%
16	Pru p 1	24,1%	Der f 2	19,7%	Pru p 3	21,3%	Pla a 3	22,4%	Phl p 5	15,5%
17	Cry j 1	23,7%	Pru p 1	19,2%	Pla a 2	18,5%	Ara h 9	20,1%	Pla a 3	15,5%
18	Ole e 1	23,4%	Cup a 1	18,7%	Jug r 3	18,3%	Phl p 2	19,6%	Art v 3	13,5%
19	Phl p 2	22,2%	Hev b 8	18,2%	Pla a 3	16,5%	Art v 3	19,5%	Phl p 2	13,1%
20	Phl p 6	16,4%	Mer a 1	18,0%	Phl p 11	15,5%	Phl p 6	19,1%	Alt a 1	15,5%

A. Species specific molecules

1. Grass pollen allergy

Table II reports proportion for each of the grass pollen allergens in the different areas. Overall, patients from Central Italy showed a higher reactivity compared to the other Italian regions, whereas a higher occurrence of profilin, Phl p 12, reactivity was recorded in NW Italy. Interestingly, Cyn d 1 IgE recognition overcame Phl p 1 recognition in Is, whilst in the other regions Phl p 1 reactivity was always highly prevalent. No difference in the occurrence of polcalcin, Phl p 7, sensitization was recorded throughout Italy.

2. House dust mite allergy

House dust mite sensitization was higher in C and S Italy, particularly the group 2 allergens (**table II**). Interestingly, the

highest level of Lep d 2 was recorded in the South. Der p 10 reactivity was rarely observed in the NW part of the country. No difference in Blo t 5 prevalence sensitization was recorded throughout Italy.

3. Tree pollen allergy

A higher reactivity to *Fagales* was detected in the North (particularly in NE), similar to the sensitization profile of central Europe (**table II**). On the other hand, cypress pollen sensitization was most prevalent in C and S Italy. Olive and plane tree reactivity was highest in C.

4. Weed pollen allergy

Pellitory was one of the most important causes of IgE sensitization in S, C, and I, whereas it was rarely observed in the

Table II - Prevalence of IgE sensitization to Inhalant allergens. Results are based on two-sided tests assuming equal variances with significance level 0.01. For each significant pair, the key of the smaller category appears under the category with larger mean.

	A Centre	B Isles	C Nord-East	D Nord-West	E South
Grasses					
Cyn d 1	1133 48,6% C	111 44,2%	607 41,9%	546 44,9%	364 45,4%
Phl p 1	1274 54,6% BCDE	96 38,2%	669 46,1%	596 49,0%	378 47,1%
Phl p 2	642 27,5% BCE	33 13,1%	322 22,2%	313 25,7%	157 19,6%
Phl p 4	812 34,8% BC	57 22,7%	400 27,6%	376 30,9%	240 29,9%
Ulp 5.0204	743 31,9% BE	39 15,5%	458 31,6%	412 33,9%	209 26,1%
Phl p 6	529 22,7% BC	22 8,8%	238 16,4%	306 25,1%	153 19,1%
Phl p 7	82 3,5%	5 2,0%	48 3,3%	34 2,8%	25 3,1%
Phl p 11	362 15,5% B	16 6,4%	196 13,5%	186 15,3%	97 12,1%
Phl p 12	243 10,4%	14 5,6%	119 8,2%	172 14,1%	77 9,6%
House Dust Mite					
Der f 1	738 31,6% CD	62 24,7% D	371 25,6% D	184 15,1%	250 31,2% CD
Der p 1	721 30,9% CD	60 23,9% D	373 25,7% D	182 15,0%	243 30,3% D
Der f 2	838 35,9% CD	70 27,9% D	448 30,9% D	240 19,7% BCD	327 40,8% BCD
Der p 2	836 35,8% BCD	64 25,5% D	434 29,9% D	243 20,0%	313 39,0% BCD
Lep d 2	252 10,8% D	24 9,6% D	131 9,0% D	60 4,9% ACD	122 15,2% ACD
Der p 10	133 5,7% D	11 4,4% D	93 6,4% D	21 1,7% D	62 7,7% D
Blo t 5	138 5,9%	10 4,0%	73 5,0%	60 4,9%	59 7,4%
	A Centre	B Isles	C Nord-East	D Nord-West	E South
Tree pollen allergens					
Aln g 1	181 7,8% ABDE	8 3,2%	401 27,7% ABE	242 19,9% B	79 9,9% B
Bet v 1	268 11,5% ABDE	15 6,0%	475 32,8% ABE	291 23,9% B	104 13,0% B
Cor a 1.0101	261 11,2% B	12 4,8%	410 28,3% ABDE	269 22,1% ABE	101 12,6% B
Cry j 1	965 41,4% BCD	70 27,9% D	343 23,7% D	192 15,8%	306 38,2% BCD
Cup a 1	1355 58,1% BCD	103 41,0% D	513 35,4% D	227 18,7% BCD	430 53,6% BCD
Ole e 1	641 27,5% D	64 25,5%	340 23,4%	262 21,5%	225 28,1% D
Ole e 7	168 7,2% CD	17 6,8%	60 4,1%	44 3,6%	92 11,5% ACD
Ole e 9	91 3,9% CD	6 2,4%	50 3,4%	27 2,2%	41 5,1% D
Pla a 1	74 3,2% CD	3 1,2%	9 0,6%	12 1,0%	11 1,4% D
Pla a 2	431 18,5% CD	45 17,9% D	182 12,6% D	133 10,9% D	133 16,6% D
Pla a 3	384 16,5% CD	39 15,5% CD	121 8,3% D	100 8,2% D	180 22,4% ACD
Weed pollen allergens					
Amb a 1	28 1,2% AE	6 2,4%	79 5,4% ABCE	325 26,7% ABCE	11 1,4% A
Art v 1	131 5,6% AB	10 4,0%	169 11,7% A	112 9,2% A	69 8,6% A
Art v 3	284 12,2% CD	34 13,5% D	128 8,8% C	79 6,5% C	156 19,5% ACD
Par j 2	650 27,9% CD	72 28,7% CD	77 5,3% C	140 11,5% C	286 35,7% ACD
Pla l 1	167 7,2% B	4 1,6%	198 13,7% ABDE	61 5,0%	39 4,9% B
	A Centre	B Isles	C Nord-East	D Nord-West	E South
Animal allergens					
Can f 1	234 10,0% BD	9 3,6%	155 10,7% BD	78 6,4%	73 9,1% B
Can f 2	61 2,6% D	2 0,8%	58 4,0% D	20 1,6%	22 2,7% D
Can f 3	55 2,4% D	2 0,8%	48 3,3% D	19 1,6%	21 2,6% D
Can f 5	291 12,5% D	20 8,0%	198 13,7% D	123 10,1%	94 11,7% D
Equ c 1	112 4,8% D	5 2,0%	64 4,4% D	32 2,6%	36 4,5% D
Equ c 3	37 1,6% D	2 0,8%	33 2,3% D	10 0,8%	12 1,5% D
Fel d 1	756 32,4% DE	71 28,3%	457 31,5% D	326 26,8%	213 26,6% ACD
Fel d 2	69 3,0% D	3 1,2%	53 3,7% D	21 1,7%	23 2,9% D
Fel d 4	100 4,3% D	4 1,6%	71 4,9% D	37 3,0%	34 4,2% D
Mus m 1	54 2,3% AD	3 1,2%	66 4,6% AD	19 1,6%	22 2,7% D
Latex allergens					
Hev b 1	15 0,6% D	1 0,4%	9 0,6% D	12 1,0%	6 0,7% D
Hev b 3	19 0,8% D	1 0,4%	12 0,8% D	8 0,7%	6 0,7% D
Hev b 5	15 0,6% D	0 0,0%	19 1,3% D	12 1,0%	6 0,7% D
Hev b 6.01	55 2,4% D	3 1,2%	42 2,9% D	16 1,3%	16 2,0% D
Hev b 8.0204	291 12,5% D	22 8,8%	182 12,6% D	221 18,2% ABCE	92 11,5% D

Table III - Prevalence of IgE sensitization to Pan-Allergens. Results are based on two-sided tests assuming equal variances with significance level 0.01. For each significant pair, the key of the smaller category appears under the category with larger mean.

	A Centre	B Isles	C North-East	D North-West	E South
PR-10 allergens					
Act d 8	52 2,2%	4 1,6%	70 4,8%	62 5,1%	22 2,7%
Aln g 1	181 7,8%	8 3,2%	401 27,7%	242 19,9%	79 9,9%
Api g 1	59 2,5%	2 0,8%	129 8,9%	82 6,7%	25 3,1%
Ara h 8	105 4,5%	6 2,4%	197 13,6%	146 12,0%	48 6,0%
Bet v 1	268 11,5%	15 6,0%	475 32,8%	291 23,9%	104 13,0%
Cor a 1.0101	261 11,2%	12 4,8%	410 28,3%	269 22,1%	101 12,6%
Cor a 1.0401	207 8,9%	10 4,0%	385 26,6%	253 20,8%	77 9,6%
Gly m 4	85 3,6%	3 1,2%	172 11,9%	116 9,5%	36 4,5%
Mal d 1	222 9,5%	12 4,8%	407 28,1%	252 20,7%	87 10,8%
Pru p 1	173 7,4%	9 3,6%	350 24,1%	234 19,2%	78 9,7%
Lipocalin					
Can f 1	234 10,0%	9 3,6%	155 10,7%	78 6,4%	73 9,1%
Can f 2	61 2,6%	2 0,8%	58 4,0%	20 1,6%	22 2,7%
Equ c 1	112 4,8%	5 2,0%	64 4,4%	32 2,6%	36 4,5%
Fel d 4	100 4,3%	4 1,6%	71 4,9%	37 3,0%	34 4,2%
Mus m 1	54 2,3%	3 1,2%	66 4,6%	19 1,6%	22 2,7%
Polcalcin					
Bet v 4	70 3,0%	5 2,0%	41 2,8%	28 2,3%	18 2,2%
Phl p 7	82 3,5%	5 2,0%	48 3,3%	34 2,8%	25 3,1%
Lipid Transfer Proteins (nsLTPs)					
Ara h 9	333 14,3%	31 12,4%	103 7,1%	100 8,2%	161 20,1%
Art v 3	284 12,2%	34 13,5%	128 8,8%	79 6,5%	156 19,5%
Cor a 8	265 11,4%	28 11,2%	77 5,3%	87 7,1%	133 16,6%
Jug r 3	426 18,3%	46 18,3%	124 8,6%	118 9,7%	195 24,3%
Ole e 7	168 7,2%	17 6,8%	60 4,1%	44 3,6%	92 11,5%
Pla a 3	384 16,5%	39 15,5%	121 8,3%	100 8,2%	180 22,4%
Pru p 3	497 21,3%	56 22,3%	157 10,8%	140 11,5%	218 27,2%
Tri a 14	121 5,2%	8 3,2%	50 3,4%	35 2,9%	60 7,5%
Profilin allergens					
Bet v 2	255 10,9%	16 6,4%	168 11,6%	210 17,3%	87 10,8%
Hev b 8.0204	291 12,5%	22 8,8%	182 12,6%	221 18,2%	92 11,5%
Mer a 1	287 12,3%	13 5,2%	175 12,1%	219 18,0%	95 11,8%
Phl p 12	243 10,4%	14 5,6%	119 8,2%	172 14,1%	77 9,6%
Tropomyosin allergens					
Ani s 3	88 3,8%	9 3,6%	79 5,4%	16 1,3%	42 5,2%
Bla g 7	89 3,8%	9 3,6%	111 7,7%	17 1,4%	41 5,1%
Der p 10	133 5,7%	11 4,4%	93 6,4%	21 1,7%	62 7,7%
Pen m 1	124 5,3%	14 5,6%	77 5,3%	19 1,6%	62 7,7%

North, particularly in NE (**table II**). Hypersensitivity to Amb a 1, the major ragweed pollen allergen, was highest in NW Italy, whilst it was rarely observed in Central and Southern Italy. The major mugwort pollen allergen, Art v 1 was frequent in

NE, whilst the nsLTP Art v 3 showed a different behavior (see LTP section) with a higher incidence in C and S. Pla l 1 reactivity, from plantain (*Plantago lanceolata*) was largely found in North-East Italy.

5. Cat, dog and horse

No differences in the prevalence of sensitization to animal dander allergens were observed, with the exception of a significant higher occurrence of Can f 1 reactivity in C, S and NE (**table II**).

6. Latex

No differences in latex molecules reactivity were observed throughout Italy, whilst the reactivity to the profilin, Hev b 8, was mainly found in NW (**table II**) according to the other members of that superfamily (see above).

B. Cross reactive molecules

The overall distribution of profilin, PR-10, nsLTP and tropomyosin prevalence throughout Italy is shown in **figure 1**.

1. Plant food panallergens

Overall, plant food panallergens reactivity was found in 2935 (48.5%) participants. The IgE recognition of PR-10, profilin, and nsLTP was mutually exclusive in 69.1% of cases, ranging from 65% in NE to 83% in the Islands.

Figure 1 - Pollen food panallergens distribution in Italy macro-regions. Prevalence have been calculated on the respective tested subjects (2332 in C, 251 in I, 1450 in NE, 1217 in NW and 802 in S)

Profilin reactivity affected around 10% of the entire allergic population throughout Italy (**table III**), but in NW the frequency was double than in all other regions (**figure 1**). Interestingly, profilin from latex, Hev b 8, was the top ranked molecule in all macro-regions but the S, where the annual mercury profilin, Mer a 1, was the most prevalent.

In Northern Italy, particularly in the NE, PR-10 reactivity was frequent, with Bet v 1 recognition levels from two to three times higher than in the rest of Italy (**table III**). Accordingly, all the molecules belonging to the PR-10 family showed a comparable consistent pattern.

nsLTP molecules recognition showed an opposite trend if compared to PR-10 (**table III**), prevailing in Center, Islands and, particularly, in the South of Italy where Prt p 3 recognition affected around 1/3 of the entire allergic population (**figure 1**).

Figure 2 recapitulates the results of the principal correspondence analysis, showing three-dimensionally the mutual relationship of Plant food panallergens in terms of IgE co-recognition. Since this statistical approach places variables in a tridimensional space based on their levels of reciprocal relationship (i.e., highly correlated variables are close to each other, while uncorrelated variables are placed far away from each other), we observe here the significantly divergent behavior between PR-10, profilin and LTP molecules in 69% of participants, suggesting the presence of a mutual exclusive IgE recognition in the majority of cases.

2. Tropomyosins, polcalcin, and lipocalins

Similar levels of tropomyosin recognition were recorded throughout Italy, with the exception of NW where such reactivity was rarely observed (**table III**). Der p 10 was the tropomyosin most frequent in C and S, whilst Bla g 7 was the top ranked in NE and Pen m 1 was most common in Is and S. No, or scant differences in the recognition proportion of polcalcin and lipocalins were recorded (**table III**), thus representing sensitization affecting a restricted subset of patients.

Discussion

We studied, by means of a microarray system, the relative frequency of IgE sensitization in several macro-regions in Italy. Extreme heterogeneity in the IgE sensitization profile was found across Italy, largely depending on the geo-climatic features and the distribution of the allergenic pollen. On the bases of these two criteria, a map of exposure to pollen derived allergens was recently published (24). An increasing body of evidences shows that other factors might play a role in increasing the risk of sensitization and allergy to pollen allergens, including air pollution and climate change, all of them comprised in the new concept of "exposome" (25).

Figure 2 - Multiple correspondence analysis plot, showing the mutual inter-relationships among plant-food panallergens (each molecule is represented by one dot) in term of IgE reactivity. The plot illustrates the divergent behavior of IgE recognition between PR-10, Profilin and nsLTP.

Overall, the most frequent cause of sensitization found in Italy was the cypress pollen in C and S (26), and the grass pollen in NE and NW (15). Only in the main Islands, the Bermuda grass (*Cynodon dactylon*) sensitization surpassed timothy grass pollen IgE recognition, thus suggesting differences in the plant distribution or of other local genetic and/or environmental-related factors affecting the IgE mediated response to the allergen. No difference in CCD reactivity was registered throughout Italy.

The group 1 grass allergens always overcame the other grass pollen molecules sensitization across the entire country, thus confirming the possible role of Phl p 1 as the initiator of grass

pollen reactivity (27) and its role as genuine marker of grass sensitization in Europe (28).

Par j 2 reactivity was found more often in C, S, and I, but it was virtually absent in the North (29), where birch pollen Bet v 1 and, consequently, the PR-10 related molecules, were among the most common elicitors of sensitization, also as food allergen. On the other hand, the peach nsLTP, Pru p 3, was the most important food allergen found in C, I, and, particularly, in the S, where about 1/3 of the entire population studied was affected, therefore confirming previous observation regarding the relevance of nsLTP sensitization in the Mediterranean area (17).

A surprising low level of house dust mite reactivity was recorded in NW, if compared to the rest of Italy, probably because of the climatic differences, the type of buildings, or a higher prevalence of other HDM allergens like Der p 23.

The high occurrence of ragweed Amb a 1 IgE recognition represented a main feature of the NW sensitization profile, consistently with the area of ragweed distribution in Italy (30).

Cypress and *Fagales* pollen reactivity showed a divergent behavior, the first being mainly distributed in C and S (31) and the former in NE and NW, accordingly with the areas of distribution of these plants and of their pollen (32).

Reactivity for the major allergen of olive tree, Ole e 1, was evenly distributed across Italy, but the putative nsLTP, Ole e 7, was mainly found in the S, similarly to what observed in Spain (33), but probably also related to the higher frequency of nsLTP sensitization observed in the Southern areas of Italy (34,17).

No geographical variability influence was registered for latex sensitization, confirming that such reactivity is apparently not related to environmental exposure.

A particular observation in this study was the radically diverse profile of panallergen sensitization found throughout Italy (**figure 1**): nsLTP reactivity was prevalently found in C and S where the source of sensitization is food, as also suggested by the higher prevalence of IgEs to nsLTP Art v 3 than Art v 1 (the major mugwort allergen) in those regions, compared to the opposite results found in the NE and NW (**table II**). Profilin sensitization was higher in NW and PR10 IgE recognition in the North, particularly in NE, possibly because of the high exposure to the grass profilin and birch PR-10 (Bet v 1), respectively. A mutual exclusive IgE recognition of the three plant food panallergens studied was observed in large part of our Italian cohort. More than 69% percent of patients, in fact, produced specific IgE to a group of plant food panallergens but not to the others. Polcalcin reactivity was equally distributed along the entire Peninsula. Tropomyosin was rarely found among the most common causes of food allergy in NW, where sensitization to the major HDM allergens was low as well. This finding suggests that in that area the major source of sensitization to tropomyosin is HDM, and not crustaceans.

In summary, the present study provides a clear picture of how geographical location could influence sensitization profiles and their clinical expression, even in comparatively small geographical areas.

Conflict of Interest

The authors declare that they have no conflict of interest.

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