

## Original article

GA<sup>2</sup>LEN skin test study I: GA<sup>2</sup>LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe

**Background:** Skin prick testing is the standard for diagnosing IgE-mediated allergies. However, different allergen extracts and different testing procedures have been applied by European allergy centres. Thus, it has been difficult to compare results from different centres or studies across Europe. It was, therefore, crucial to standardize and harmonize procedures in allergy diagnosis and treatment within Europe.

**Aims:** The Global Asthma and Allergy European Network (GA<sup>2</sup>LEN), with partners and collaborating centres across Europe, was in a unique position to take on this task. The current study is the first approach to implement a standardized procedure for skin prick testing in allergies against inhalant allergens with a standardized pan-European allergen panel.

**Methods:** The study population consisted of patients who were referred to one of the 17 participating centres in 14 European countries ( $n = 3034$ , median age = 33 years). Skin prick testing and evaluation was performed with the same 18 allergens in a standardized procedure across all centres.

**Results:** The study clearly shows that many allergens previously regarded as untypical for some regions in Europe have been underestimated. This could partly be related to changes in mobility of patients, vegetation or climate in Europe.

**Conclusion:** The results of this large pan-European study demonstrate for the first time sensitization patterns for different inhalant allergens in patients across Europe. The standardized skin prick test with the standardized allergen battery should be recommended for clinical use and research. Further EU-wide monitoring of sensitization patterns is urgently needed.

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Skin prick testing is the standard method to assess immunoglobulin (Ig)E-mediated sensitization to inhalant allergens. It is a cheap, rapid, reproducible and accurate way of identifying the causative allergen of an IgE-mediated allergy. However, no standard consensus protocol exists. A previous study showed that procedures vary across Europe (1). Furthermore, in most European allergy centres, tests are limited to typical local allergens that have been tested in the past.

Mobility in the private and business sector is increasing within Europe and inter-European and inter-continental migration has become commonplace. Thus, allergologists are increasingly consulted by patients from other regions or by local individuals who have travelled abroad. The distribution of inhalant allergens is changing as a result of climate change. A short list of local allergens is probably insufficient. A pan-European prick testing core panel, similar to those which exist on a national level, e.g. for contact allergens (2), had until now not been developed.

The current study, therefore, aimed to define a pan-European standard prick test for inhalant allergens with harmonized operating procedures. The choice of allergens was based on a previous study reporting on the current use of allergens and sensitization rates in Europe (1). The Global Allergy and Asthma European Network (GA<sup>2</sup>LEN) is a consortium of 24 leading allergy centres and more than 50 collaborating centres with high clinical and experimental research competence across Europe providing an ideal platform for multidisciplinary research efforts. In the current study, a standard prick testing core panel with the 18 most frequent inhalant allergens within Europe was agreed upon, taking into consideration sensitization rates, European ecology, climate and cross-reactivities. Allergen extracts were chosen and supplied to

17 GA<sup>2</sup>LEN centres in 14 European countries. Standard operating procedures for performance, evaluation and data collection were defined and data collected over an 18-month period.

This strategy allowed the first true comparison and determination of sensitization rates for the most common inhalant allergens within Europe.

### Methods

#### Study design and patients

The pan-European prick test study was designed as a multicentre open label study covering a study period of 18 months. Initially, 25 centres in 16 European countries were asked to participate. Seventeen allergy centres in the following 14 countries participated: Austria (Medical University of Vienna, Vienna), Belgium (University Hospital, Ghent), Denmark (University Hospital, Odense), Finland (Helsinki University Central Hospital, Helsinki), France (University Hospital, Montpellier), Germany (Charité University Medicine, Berlin; Ludwig Maximilians University, Munich; Technical University, Munich), Greece (National and Kapodistrian University, Athens), Hungary (Semmelweis Medical University, Budapest), Italy (Consiglio Nazionale delle Ricerche, Palermo; University of Genoa, Genoa), Netherlands (Academic Medical Centre, Amsterdam), Poland (Medical University of Lodz, Lodz), Portugal (Coimbra University, Coimbra), Switzerland (Children's University Hospital, Zurich), United Kingdom (Royal Brompton Hospital, London).

The study was conducted in full accordance with the then-current revision of the Declaration of Helsinki. The multicentre study was approved beforehand by the institutional review board of the coordinating centre (Ethikkommission, Charité Universitätsmedizin, Berlin, Germany) as well as by the institutional review boards of each participating allergy centre.

Outpatients of participating allergy centres were included in the study following informed consent if they had a suspected present or former allergic reaction to inhalant allergens and if they were eligible for routine allergy testing. Patients with a suspected inhalant allergy and, in addition, a high likelihood of clinically relevant symptoms, were selected randomly to participate in the pan-European prick test study over a period of at least 1 year. Volunteers

*Abbreviations:* ECRHS, European Community Respiratory Health Survey; EU, European Union; GA<sup>2</sup>LEN, Global allergy and asthma European Network; IgE, immunoglobulin E; ISAAC, International Study of Asthma and Allergies in Childhood.

were identified only by study numbers on the data reported to the co-ordinating centre to guarantee confidentiality of patient data. Documents with patient information were kept strictly confidential.

#### Extracts and reagents

The following inhalant allergens were selected as standard prick testing core panel: alder, *Alternaria*, *Ambrosia*, *Artemisia*, *Aspergillus fumigatus*, birch, *Blatella*, cat, *Cladosporium herbarum*, cypress, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, dog, grass mix (smooth meadow grass/*Poa pratensis*, cock's foot grass/*Dactylis glomerata*, perennial rye grass/*Lolium perenne*, timothy grass/*Phleum pratense*, meadow fescue/*Festuca pratensis*, meadow oat grass/*Helictotrichon pratense*), hazel, olive, *Parietaria* and plane. Histamine dihydrochloride was used as a positive control, diluent as a negative control. As only very few direct comparisons exist for the different allergen extracts available, companies were chosen based on experience as well as to ensure a minimum of conflict-of-interest (see Table 1). Standardized extracts from ALK-Abelló (Hamburg, Germany), Allergopharma (Reinbek, Germany), Leti Pharma (Witten, Germany) and Stallergènes (Antony, France) were obtained and distributed by the co-ordinating centre.

#### Skin tests

Testing solutions were stored at +2 to +8°C when not in use. A testing grid was fixed on the volar forearm surface of the patient and the orientation of the grid marked on the patient's arm. Tests were placed 2 cm apart. Then, a small drop of each testing solution was placed in the centre of each grid square and the allergens applied in the same order for each test (single testing). For each allergen, a new lancet (ALK-Abelló; 3) was used which was then pressed against the skin in the centre of the allergen drop for at least 1 s without causing bleeding. Then, the testing grid was removed and any excess solution blotted with a tissue to

avoid cross-contamination. Results were recorded after 15 min. The largest and perpendicular diameter of the wheal for each of the allergens was measured and the following value calculated: largest + perpendicular diameter/2. A test was regarded positive if the value calculated was  $\geq 3$  mm and controls showed adequate reactions. Values calculated for each of the 18 allergens tested were recorded in standardized data collection forms.

#### Statistical analysis

Statistical analysis was performed using STATA 10.0 software (StataCorp LP, College Station, TX, USA). Direct standardization with regard to gender and age was used to calculate sensitization rates. The reference population for standardization which comprised all patients of the study population is depicted in Table 2. This population is an excellent representation of patients with allergic diseases. Differences in the study population in comparison with the population of the European Union in general (EU27 population, 01.01.2007, Eurostat, Luxembourg, <http://epp.eurostat.ec.europa.eu>) might be due to gender- and age-specific prevalence rates for allergic diseases (4). Compared with the EU27 population, older women and men are slightly underrepresented in the reference population used in this study, younger women are more frequent.

## Results

#### Demographic characteristics of study population

A total of 3034 valid data sets were analysed. The participating allergy centres are represented on the map in Fig. 1 which also includes climatic conditions (Köppen-Geiger climate map). The demographic characteristics of the study population with regard to patient frequency, gender and age are demonstrated in Table 3.

#### Standardized sensitization rates

The geographical distribution of sensitization rates for the different allergens tested is demonstrated in Table 4. To increase comparability, standardized sensitization rates are given. For Greece, Switzerland and the UK, crude sensitization rates are given to avoid age distribution biases.

#### Indoor allergens

Among the European countries investigated, sensitization to cat allergen was particularly high in Nordic countries

Table 1. Extracts for pan-European standard prick test panel

Allergen	Company	Standard
Negative control (diluent)	ALK-Abelló	–
Positive control (histamin dihydrochloride)	ALK-Abelló	10 mg/ml
Cat	ALK-Abelló	10 HEP
Dog	ALK-Abelló	10 HEP
Grass mix	ALK-Abelló	10 HEP
<i>Ambrosia</i>	ALK-Abelló	1 : 100 G/V
<i>Alternaria</i>	ALK-Abelló	1 : 20 G/V
<i>Parietaria</i>	ALK-Abelló	10 HEP
<i>Cladosporium herbarum</i>	Allergopharma	10 000 BE/ml
<i>Aspergillus fumigatus</i>	Allergopharma	10 000 BE/ml
Birch	Allergopharma	50 000 SBE/ml
Hazel	Allergopharma	50 000 BE/ml
Alder	Allergopharma	50 000 BE/ml
<i>Blatella</i>	Leti	1 mg/ml
<i>Dermatophagoides pteronyssinus</i>	Stallergenes	100 IR/ml
<i>Dermatophagoides farinae</i>	Stallergenes	100 IR/ml
Olive	Stallergenes	100 IR/ml
Cypress	Stallergenes	100 IC/ml
Plane	Stallergenes	100 IC/ml
<i>Artemisia</i>	Stallergenes	100 IR/ml

HEP, histamine equivalent prick; G/V, Gewicht/Volumen = weight/volume; BE, biologische Einheiten = biological units; SBE, standardisierte biologische Einheiten = standardized biological units; IR, index of reactivity; IC, index of concentration.

Table 2. Reference population

Gender	Age (years)	Weight
Male	$\leq 29$	0.20531
Male	30–59	0.18523
Male	$\geq 60$	0.04113
Female	$\leq 29$	0.21600
Female	30–59	0.27396
Female	$\geq 60$	0.07837
	Total	1.00000

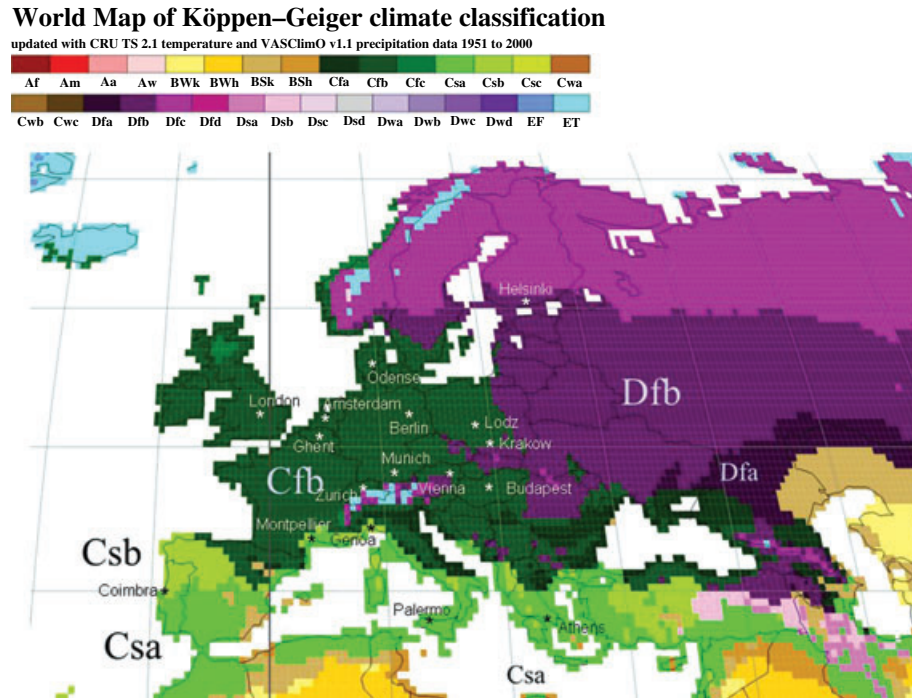


Figure 1. Participating study centres with characterization of the climatic conditions [as defined by the Köppen-Geiger climate map; <http://www.borntraeger-cramer.de>, (35)]. Finland is defined as Dfb (snow, fully humid, warm summer), Denmark, Austria, Belgium, Germany, Netherlands, Switzerland, UK, Poland and Hungary are characterized as Cfb (warm temperature, fully humid, warm summer) and the Mediterranean countries France, Greece, Italy and Portugal characterized as Csa (warm temperature, fully humid, hot summer).

Table 3. Demographic characteristics of study population

	Nordic countries	Central/Western Europe	Mediterranean	Poland	Hungary	Total
<b>Patients</b>						
Frequency (n)	377	1394	808	198	257	3034
Percentage (%)	12.4	45.9	26.6	6.5	8.5	100.0
<b>Gender (%)</b>						
Male	36.3	42.7	47.8	37.4	43.6	43.0
Female	63.7	57.3	52.2	62.6	56.4	57.0
<b>Age (years)</b>						
Median	39	35	25	26	39	33
Percentile 25	29	23	12	18	28	21
Percentile 75	52	49	41	38	55	47
<b>Age groups (%)</b>						
≤ 14	0.0	12.1	32.3	14.1	0.0	15.1
15–29	26.3	26.5	24.9	42.9	31.1	27.5
30–44	36.9	30.3	25.2	23.7	28.4	29.2
45–64	29.4	23.1	13.5	17.7	29.6	21.5
≥65	7.4	8.0	4.1	1.5	10.9	6.7

[Denmark (49.3%), Finland (30.4%)] and Hungary (32.5%) compared to the other European countries. The rate of sensitization to dog allergen was even higher in the Nordic countries with the highest sensitization rate in Denmark (56.0%). Low sensitization rates all over Europe were demonstrated for the indoor fungus

*A. fumigatus* ranging from 0.4% (Italy) to 6.9% (Portugal). Sensitization rates to house dust mite were the highest in Nordic and Mediterranean countries; specifically, sensitization to both *D. pteronyssinus* (Der p) and *D. farinae* (Der f) was most prominent in Portugal (Der p: 68.8%; Der f: 68.0%) and Denmark (Der p: 51.5%; Der f: 51.8%). The highest rates for *Blatella* sensitization were found in the Mediterranean countries with Portugal standing out with 33.4%. The other countries investigated showed sensitization rates ranging from 2.1% (Belgium) to 12.0% (Germany) (Table 4).

#### Outdoor allergens

Sensitization rates to typical trees of the northern hemisphere like hazel, alder and birch were, as expected, the highest in the Nordic countries. Sensitization to all three trees was most prominent in Denmark (hazel: 49.4%; alder: 47.0%; birch: 57.4%). High rates of sensitization to northern trees were also seen in Central/Western Europe with Germany showing the highest sensitization rates for hazel, alder and birch (hazel: 35.9%; alder: 34.8%; birch: 37.6%). Poland also shows high sensitization rates for northern trees which are most prominent for birch (27.7%). Sensitization to northern trees was also prevalent in the Mediterranean countries with hazel sensitization ranging from 7.4%

Table 4. Country-specific sensitization rates to inhalant indoor allergens

Allergen: Country	Sensitization rates [%]					
	Cat SR (95% CI)	Dog SR (95% CI)	<i>Aspergillus</i> SR (95% CI)	<i>Dermatophagoides pteronyssinus</i> SR (95% CI)	<i>Dermatophagoides farinae</i> SR (95% CI)	<i>Blatella</i> SR (95% CI)
Europe	26.3 (24.8–27.9)	27.2 (25.6–28.8)	4.4 (3.7–5.2)	31.3 (29.7–33.0)	28.9 (27.3–30.5)	8.9 (7.9–9.9)
Austria (Vienna)	16.8 (11.1–22.5)	16.1 (10.6–21.6)	0.5 (0.0–1.3)	24.6 (18.2–31.0)	20.5 (14.5–26.4)	4.0 (1.1–6.9)
Belgium (Ghent)	18.4 (13.0–23.8)	17.8 (12.5–23.2)	2.4 (0.3–4.5)	29.9 (23.6–36.1)	26.4 (20.4–32.4)	2.1 (0.3–4.0)
Denmark (Odense)	49.3 (41.3–57.2)	56.0 (48.3–63.7)	4.8 (1.2–8.4)	51.5 (44.1–58.8)	51.8 (44.3–59.4)	11.1 (5.7–16.5)
Finland (Helsinki)	30.4 (23.2–37.5)	36.5 (29.2–43.7)	2.5 (0.1–4.9)	16.8 (10.9–22.8)	15.5 (9.9–21.1)	10.0 (5.6–14.4)
France (Montpellier)	23.0 (17.2–28.8)	24.6 (18.6–30.6)	4.3 (1.6–7.1)	38.1 (31.5–44.8)	31.8 (25.4–38.2)	11.4 (7.0–15.9)
Germany (Berlin– Munich)	28.1 (23.0–33.1)	27.4 (22.4–32.3)	6.2 (3.5–8.8)	23.5 (18.8–28.2)	21.1 (16.5–25.6)	12.0 (8.3–15.8)
Greece* (Athens)	29.4 (23.4–36.0)	29.1 (23.1–35.7)	10.3 (6.6–15.2)	32.7 (26.5–39.4)	26.6 (20.8–33.1)	9.8 (6.2–14.6)
Hungary (Budapest)	32.5 (26.5–38.4)	32.8 (26.9–38.7)	2.5 (0.7–4.3)	31.3 (25.6–36.9)	26.3 (20.8–31.8)	4.6 (1.9–7.3)
Italy (Genoa, Palermo)	21.3 (16.1–26.6)	17.4 (12.8–22.0)	0.4 (0.0–1.1)	38.9 (32.6–45.1)	35.1 (28.9–41.2)	3.4 (1.0–5.7)
The Netherlands (Amsterdam)	18.5 (13.7–23.3)	29.9 (24.4–35.4)	4.6 (2.0–7.2)	29.0 (23.5–34.6)	30.9 (25.3–36.6)	8.8 (5.3–12.2)
Poland (Lodz)	23.8 (17.7–29.9)	34.7 (27.8–41.6)	4.8 (1.6–7.9)	22.2 (16.6–27.8)	19.1 (14.0–24.3)	8.2 (4.3–12.2)
Portugal (Coimbra)	25.4 (19.4–31.5)	20.7 (13.8–27.6)	6.9 (3.2–10.5)	68.8 (61.8–75.2)	68.0 (60.9–75.2)	33.4 (26.1–40.7)
Switzerland* (Zurich)	42.1 (33.9–50.5)	23.4 (16.8–31.2)	2.1 (0.4–5.9)	24.8 (18.0–32.7)	26.9 (19.9–34.9)	1.8 (0.2–6.2)
United Kingdom* (London)	31.7 (23.7–40.6)	21.4 (14.6–29.6)	7.9 (3.9–14.1)	39.7 (31.1–48.8)	34.9 (26.6–43.9)	0.8 (0.0–4.3)

All sensitization rates are standardized with regard to gender and age except where indicated.

SR, standardized sensitization rate/crude sensitization rate.

\*Crude sensitization rate (95% CI).

(Portugal) to 11.9% (France), alder sensitization ranging from 3.1% (Italy) to 10.4% (France) and birch sensitization ranging from 6.8% (Portugal) to 9.4% (Italy) (Table 5).

Sensitization to the typical southern tree plane was low all over Europe ranging from 0.9% (Finland) to 8.9% (Denmark). Furthermore, the overall European sensitization rate against the second southern tree investigated i.e. cypress was low ranging from 0.0% (Finland) to 8.7% (France). As expected, the highest cypress sensitizations were found in the Mediterranean ranging from 5.1% (Portugal) to 8.7% (France). Denmark as a Nordic country also showed comparable sensitization rates (5.8%). Olive sensitization was expectedly high in the Mediterranean ranging from 18.2% (France) to 23.3% (Italy). High sensitization rates were also found in the Central/Western European countries with Austria (13.3%) and Germany (9.7%) showing the highest rates. Olive sensitizations were also found in the Nordic countries Denmark (9.1%) and Finland (2.0%).

The rate of sensitization to grasses was high all over Europe ranging from 19.5% (Italy) to 69.9% (Denmark). *Ambrosia* sensitization was predominant in Hungary (53.8%). However, our data demonstrate that *Ambrosia* sensitization in the other European countries ranges from 2.3% (Finland) to 18.6% (Netherlands). *Artemisia* sensitization, one of the other weeds investigated, was also the highest in Hungary (44.3%). In Poland, the second eastern European country in this study, *Artemisia* sensitization was high as well (26.2%). High *Artemisia* sensitizations were also found in Denmark (28.3%) and Finland (17.6%). Lower but also significant sensitization rates were seen in Central/Western Europe and the

Mediterranean ranging from 6.2% (Netherlands) to 22.5% (Germany). Sensitization rates for *Parietaria* were low ranging from 0.9% (Finland) to 8.7% (Netherlands) except for the Mediterranean countries. Here, the countries with high rates were Italy (33.2%) and Portugal (17.5%).

Sensitization rates to both outdoor fungi investigated, i.e. *Alternaria* and *Cladosporium* were most prominent in Hungary (*Alternaria*: 18.6%; *Cladosporium*: 12.8%). In all other European countries, sensitization was lower ranging for *Alternaria* from 2.0% (Finland) to 11.0% (Germany) and ranging for *Cladosporium* from 0.5% (Finland) to 10.3% (Portugal).

Since sensitization rates from Greece, Switzerland and the UK could not be standardized with regard to age and gender because of age distribution biases (Greece: maximum age 30 years; Switzerland: maximum age 30 years; UK: maximum age 50 years), crude sensitization rates are given (Table 4).

## Discussion

Pan-European harmonization of prick testing was initiated to remedy the lack of standardization in prick test methodology and allergen selection for inhalant allergens in European allergy centres and the resulting insufficient comparability of sensitization data (1). The present study presents comparable data from across Europe obtained upon the harmonization of prick testing for inhalant allergens. Novel trends were identified regarding both sensitization rates and geographical distribution for inhalant allergens within Europe.

## Novel inhalant allergen sensitization trends in Europe

Table 5. Country-specific sensitization rates to inhalant outdoor allergens

Allergen: Country	Sensitization rates [%]					
	Hazel SR (95% CI)	Alder SR (95% CI)	Birch SR (95% CI)	Plane SR (95% CI)	Cypress SR (95% CI)	Olive SR (95% CI)
Europe	22.8 (21.3–24.3)	21.2 (19.8–22.7)	24.2 (22.7–25.7)	5.6 (4.7–6.4)	3.9 (3.2–4.6)	15.0 (13.7–16.3)
Austria (Vienna)	22.7 (16.4–29.0)	21.8 (15.6–28.0)	19.4 (13.4–25.4)	4.1 (1.5–6.7)	1.5 (0.0–2.9)	13.3 (8.4–18.2)
Belgium (Ghent)	16.6 (11.3–21.9)	16.1 (10.9–21.4)	17.6 (12.2–23.0)	1.2 (0.0–2.8)	2.0 (0.0–3.9)	4.0 (1.2–6.9)
Denmark (Odense)	49.4 (41.7–57.0)	47.0 (39.4–54.6)	57.4 (49.7–65.1)	8.9 (4.1–13.8)	5.8 (1.8–9.7)	9.1 (4.7–13.5)
Finland (Helsinki)	24.7 (18.0–31.4)	26.3 (19.5–33.2)	34.0 (26.8–41.2)	0.9 (0.0–2.5)	0.0 (0.0–1.5)	2.0 (0.0–4.5)
France (Montpellier)	11.9 (7.5–16.4)	10.4 (6.3–14.6)	8.4 (4.6–12.2)	7.6 (3.9–11.3)	8.7 (4.8–12.6)	18.2 (12.3–23.5)
Germany (Berlin, Munich)	35.9 (30.6–41.2)	34.8 (29.5–40.1)	37.6 (32.3–43.0)	5.3 (3.1–7.5)	2.8 (1.3–4.2)	9.7 (6.6–12.8)
Greece* (Athens)	10.3 (6.6–15.2)	8.4 (5.1–13.0)	9.8 (6.2–14.6)	6.5 (3.6–10.7)	5.6 (2.9–9.6)	35.0 (28.7–41.8)
Hungary (Budapest)	20.2 (15.2–25.2)	16.0 (11.5–20.5)	20.1 (15.1–25.0)	7.0 (4.0–10.0)	2.9 (0.9–4.9)	14.4 (9.9–18.9)
Italy (Genoa, Palermo)	9.3 (5.5–13.1)	3.1 (0.8–5.4)	9.4 (5.5–13.2)	3.2 (0.8–5.6)	8.1 (4.6–11.6)	23.3 (17.7–29.0)
The Netherlands (Amsterdam)	24.8 (19.6–30.0)	24.5 (19.3–29.7)	26.9 (21.5–32.2)	4.7 (2.1–7.2)	1.5 (0.0–2.9)	12.3 (8.2–16.4)
Poland (Lodz)	22.3 (16.4–28.2)	22.8 (16.9–28.7)	27.7 (21.4–34.0)	4.0 (1.6–6.5)	1.2 (0.0–2.6)	2.7 (0.7–4.7)
Portugal (Coimbra)	7.4 (3.6–11.2)	6.8 (3.2–10.5)	6.8 (3.2–10.4)	7.0 (2.4–11.7)	5.1 (1.9–8.3)	21.3 (14.7–27.8)
Switzerland* (Zurich)	51.7 (43.3–60.1)	44.1 (35.9–52.6)	50.3 (41.9–58.7)	3.4 (1.1–7.9)	1.4 (0.2–4.9)	45.5 (37.2–54.0)
United Kingdom* (London)	15.9 (10.0–23.4)	16.7 (10.6–24.3)	19.0 (12.6–27.0)	15.9 (10.0–23.4)	11.1 (6.2–17.9)	15.1 (9.3–22.5)

	Sensitization rates (%)					
	Grasses SR (95% CI)	Ambrosia SR (95% CI)	Artemisia SR (95% CI)	Parietaria SR (95% CI)	Alternaria SR (95% CI)	Cladosporium SR (95% CI)
Europe	37.8 (36.1–39.5)	14.1 (12.9–15.4)	16.8 (15.4–18.1)	9.2 (8.2–10.2)	8.9 (7.9–9.9)	4.9 (4.1–5.6)
Austria (Vienna)	30.8 (24.0–37.5)	8.5 (4.2–12.7)	10.6 (5.8–15.4)	2.0 (0.1–3.8)	6.5 (2.7–10.2)	1.1 (0.0–2.4)
Belgium (Ghent)	25.5 (19.5–31.4)	3.0 (0.05–5.6)	4.7 (1.6–7.9)	1.4 (0.0–3.0)	5.3 (2.2–8.3)	0.8 (0.0–1.8)
Denmark (Odense)	69.9 (62.8–77.0)	17.1 (11.1–23.2)	28.3 (21.0–35.7)	5.6 (1.8–9.3)	8.2 (4.0–12.3)	7.9 (3.7–12.2)
Finland (Helsinki)	23.8 (16.6–30.9)	2.3 (0.3–4.3)	17.6 (11.7–23.4)	0.9 (0.0–2.1)	2.0 (0.0–4.1)	0.5 (0.0–1.3)
France (Montpellier)	26.4 (20.3–32.6)	9.0 (5.0–12.9)	10.7 (6.4–15.1)	6.5 (3.1–9.9)	10.3 (6.1–14.6)	3.4 (0.9–5.9)
Germany (Berlin, Munich)	37.9 (32.6–43.3)	14.4 (10.3–18.5)	22.5 (17.7–27.3)	6.9 (4.0–9.9)	11.0 (7.4–14.6)	8.0 (4.9–11.2)
Greece* (Athens)	49.5 (42.6–56.4)	11.7 (7.7–16.8)	16.9 (12.1–22.6)	24.8 (19.1–31.1)	23.8 (18.3–30.1)	7.0 (4.0–11.3)
Hungary (Budapest)	40.6 (34.5–46.7)	53.8 (47.8–59.8)	44.3 (38.1–50.5)	3.1 (0.8–5.4)	18.6 (13.7–23.5)	12.8 (8.5–17.1)
Italy (Genoa, Palermo)	19.5 (14.3–24.7)	3.5 (1.1–5.9)	6.7 (3.4–10.0)	33.2 (27.0–39.5)	3.5 (1.1–6.0)	0.0 (0.0–1.3)
The Netherlands (Amsterdam)	35.5 (29.8–41.1)	18.6 (13.9–23.4)	6.2 (3.2–9.1)	8.7 (5.2–12.1)	5.5 (2.7–8.3)	3.9 (1.6–6.2)
Poland (Lodz)	38.0 (31.1–44.9)	10.8 (6.1–15.5)	26.2 (19.8–32.7)	3.0 (0.4–5.7)	6.2 (3.3–9.0)	1.8 (0.3–3.4)
Portugal (Coimbra)	34.4 (27.3–41.5)	12.4 (7.6–17.1)	16.3 (10.3–22.4)	17.5 (12.1–22.9)	8.5 (4.5–12.5)	10.3 (5.4–15.2)
Switzerland* (Zurich)	78.6 (71.0–85.0)	18.6 (12.6–25.9)	17.2 (11.5–24.4)	2.1 (0.4–5.9)	5.5 (2.4–10.6)	0.7 (0.0–3.8)
United Kingdom* (London)	54.0 (44.9–62.9)	7.9 (3.9–14.1)	5.6 (2.3–11.1)	17.5 (11.3–25.2)	0.8 (0.0–4.3)	7.1 (3.3–13.1)

All sensitization rates are standardized with regard to gender and age except where indicated.

SR, standardized sensitization rate/crude sensitization rate.

\*Crude sensitization rates (95% CI) are presented.

### Pan-European harmonization of prick testing – methodology and allergen selection

The only guideline for skin prick testing was established within the European Academy of Allergology and Clinical Immunology in 1989 (5). Prick testing methodologies in the European allergy centres investigated (1) were all consistent with published practice guidelines (5–10). However, as these recommendations allow for flexibility, some methodological details varied, which resulted in only limited comparability of prick testing data collected. While a number of common standards like the use of positive and negative controls could be documented, certain differences like unequal timing and rating of assessment as well as variable allergen selection were apparent (1). Furthermore, allergen extracts from various local manufacturers were

used for prick testing, resulting in a significantly decreased comparability of sensitization data obtained by prick testing (11).

Although EU regulatory boards have already recognized the pressing need to implement standardized allergen extracts for prick testing within Europe by launching the Certified Reference Materials for Allergic Products and Validation of Methods for their Quantification programme (12), manufacturers still only use in-house standardization of extract batches. There are clearly large differences between extracts and this study was not aimed at comparing extracts from various laboratories. The selection of allergens included in the prick testing core panel for the current study was a result of a previous GA<sup>2</sup>LEN survey, which investigated common and regionally relevant inhalant aller-

gens used by the different partners (1) followed by discussions and consensus between participating centres. All centres used the selected allergen extracts from specified manufacturers. For some allergens, only low potency extracts were available, e.g. the cypress extract from *Cupressus sempervirens* which has a low rate of reactivity.

A consensus procedure was initiated regarding the exact step-by-step prick testing and evaluation methodology to be implemented in all European allergy centres participating in the current study. For instance, the timing of assessment of the wheal reaction was set to 15 min and a positive test defined as a wheal reaction of more than 3 mm of the calculated value. Other factors which had been previously agreed on were reconfirmed, including the use of positive and negative controls or exclusion criteria for prick testing. Seasonal biases due to increased patient recruitment in the respective pollen seasons were omitted from the study reported here, as each centre recruited patients for at least a full year. However, a potential selection bias has to be taken into account when interpreting the present data: allergy centres may have recruited slightly different patient populations due to referral by general practitioners or specialists in departments of ear, nose and throat-diseases, respiratory diseases or dermatology. Furthermore, patients who were determined by the investigating allergologist to have an increased likelihood of clinically relevant symptoms were preferentially included into the study and may therefore thus, not constitute a random population of allergy patients.

#### Sensitization rates

Regional variations of sensitization rates to inhalant allergens in Europe have been investigated in several large European studies such as the European Community Respiratory Health Survey (ECRHS) (13, 14), the International Study of Asthma and Allergies in Childhood (ISAAC) (15) and other multi-country studies (16–18) or general population surveys in selected European countries (4, 19–28). As a landmark study of allergic diseases, the ECRHS was the first population-based study to make broad comparisons across Europe for nine common allergens (*Alternaria*, *Cladosporium*, grass, birch, olive, *Parietaria*, *Ambrosia*, *D. pteronyssinus* and cat) and some regional ones (29, 30). With respect to the skin prick test, the ECRHS referred to the respective guideline and provided training for the centres. Information regarding country-specific and region-specific sensitization rates was gathered demonstrating the need for further Europe-wide studies. Naturally, in the population-based ECRHS sample, the sensitization rates were lower compared with the ones detected in the current patient-based study with 7.9% positive sensitization for cat, 17.8% for *D. pteronyssinus*, 4.4% for moulds and 19.3% for pollen (31). Interestingly, the sensitization rate difference

between the population-based ECRHS sample and the patient-based current study was the highest for *D. pteronyssinus* and the lowest for moulds (31).

Patterns of sensitization also differed. In the current study, positive sensitizations against Der p and Der f were the lowest in Central/Western Europe and significantly higher in Nordic countries and the Mediterranean. This is in contrast to an ECRHS analysis in a subsample of asthmatic patients where the highest sensitization rates for *D. pteronyssinus* were seen in Central Europe compared with lower rates in northern and southern Europe (13). As the study reported here is not a population-based study, comparisons are possibly biased. Furthermore, there is a probable under-representation of cypress pollen allergy due to the low reactivity of the extract used.

All Oleaceae pollens are cross-reactive and olive extract tests positive for both olive (Mediterranean area) and ash (most areas of Europe): sensitizations to olive as a typical southern tree were high in the Mediterranean. They were also high in Central/Western Europe with highest sensitization rates in Austria (13.3. %) where ash is part of the vegetation. The major olive allergen Ole e 1 and the ash-counterpart Fra e 1 are cross-reacting (32, 33). Thus, olive sensitizations in Austria and other Central/Western European countries are most likely due to sensitizations to cross-reactive ash pollen (34). Interestingly, olive sensitizations were also found in Nordic countries (Denmark: 9.1%, Finland: 2%). Furthermore, increasing mobility within Europe in matters of work, leisure and migration might also have led to these unexpected sensitizations if allergen exposure occurred in the country of origin or travel.

Using a standardized skin test panel, large differences were seen between allergy centres. Many of the results were expected due to the geographical distribution of the centres. However, in many centres, sensitizations to allergens which were not thought to be present in the atmosphere were found in some patients. Taking into consideration cross-reactivities, this suggests that the European population is changing and it appears that more allergens need to be tested than common and regional ones.

In summary, our data demonstrate that the introduction of harmonized prick testing procedures in Europe is, indeed, necessary to identify novel sensitization trends for inhalant allergens. It is proposed that this standardized skin prick test that includes 18 inhalant allergens should be implemented at all centres in Europe to harmonize skin test procedures and for a better management of allergic patients.

#### Conclusion

Introducing a standardized prick testing core panel for inhalant allergens in European allergy centres as well as

the harmonization of standard operating and data collection procedures assured a significant increase in the comparability of data collected. This will, indeed, allow a better surveillance of inhalant allergen sensitization rates over time. By implementing this strategy, novel sensitization trends were revealed in selected GA<sup>2</sup>LEN-affiliated allergy centres identifying both unexpected sensitizations as well as so far unforeseen geographical distributions of inhalant allergens within Europe. Thus, optimal diagnosis and treatment for allergy patients whose sensitizations were previously not recognized will be assured and a reduction in costs attained.

As the current study, for the first time, provides truly comparable sensitization rates for the most frequent

inhalant allergens within Europe, we strongly recommend the timely implementation of standardized procedures for prick testing proposed here in allergy centres throughout Europe.

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