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Regional patterns of molecular sensitization to dust mites and pollens in a Portuguese pediatric population: a multicentric study

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Abstract

Molecular tools enable detailed analysis of molecular allergens. Understanding their regional variability, considering environmental factors, improves diagnostic accuracy and allows individualized approaches in pediatric allergy management. This multicenter retrospective study

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analyzed the results of ImmunoCAP ISAC® test (n = 1010) performed in pediatric patients from 14 Portuguese hospitals. Sensitization to dust mite (DM) molecular allergens was found in 64% of patients, mainly to major allergens Der p 1, Der p 2, and Der p 23. There were 96 patients (10%) monosensitized to one DM molecular allergen with almost half of them being sensitized to Der p 23 (48%), followed by Der p 1 (26%). Urbanization and coastal residence were associated with a higher risk of DM sensitization, particularly to *Dermatophagoides* species. Pollen sensitization affected 57% of patients, with grass pollens—mainly Phl p 1 and Cyn d 1—being the most prevalent pollens. Interior regions, characterized by greater vegetation, showed a significantly higher prevalence of pollen sensitization. Regional trends that reflected the native flora were noted, such as elevated sensitivity to olive pollen allergen Ole e 1 in Trás-os-Montes region and Arizona cypress pollen allergen Cup a 1 in Coimbra region. Sensitization to birch pollen allergen Bet v 1 and proteins of the pathogenesis-related class 10 plant protein family (PR-10) was associated with food allergy. The findings of this research emphasized the need for incorporating these regional variations into clinical practice and draw attention to notable regional variations in allergy sensitivity patterns among Portuguese children.

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molecular
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Introduction

Understanding which allergens are most common in a specific geographic location is critical for accurate diagnosis, treatment, and prevention of allergic disorders. Local environmental factors, such as plant species, urbanization level, and climate, influence the type and intensity of allergen exposure, resulting in regional variance in allergic responses.

Unlike traditional methods that test for whole allergen extracts, ImmunoCAP ISAC⁰ utilizes purified, recombinant, and native allergen molecules, allowing for a more detailed analysis of immunoglobulin E (IgE) sensitization patterns at molecular level. This approach enhances the ability to differentiate between primary sensitization and sensitization to cross-reactive allergens. Therefore, the understanding of molecular allergen sensitization is fundamental in the diagnosis and treatment of allergic diseases.

In Portugal, some studies were conducted that evaluated the molecular profile of patients, but most of these studies focused on adult patients with very few evaluating children.^{1,2}

The goal of this study was to identify the prevalence of sensitization to each molecular allergen in the Portuguese pediatric population and evaluate its association with geographic location, thereby offering a comprehensive picture of our country's allergen sensitization map.

Material and Methods

The retrospective study was conducted at 14 hospitals in Portugal. All pediatric patients (n = 1010) that had performed a ImmunoCAP ISAC®112 between January 2020 and January 2025 were included in the study. The collected variables included age, gender, coexisting allergic diseases (asthma, allergic rhinoconjunctivitis, atopic dermatitis, and food allergies) and their residency (defined by their reference hospital center). Missing data were present only in the variables regarding the coexisting allergic diseases,

ranging from 0.3% to 0.6%. As the proportion was low and assumed to be missing completely at random (MCAR), a complete-case analysis was conducted.

We defined ≥ 0.3 ISU-E (a positive test result of allergy diagnostics) as a cut-off for positivity as defined by the manufacturer and recommended in European Academy of Allergy & Clinical Immunology (EAACI) Molecular Allergology User's Guide 2.0.³

The hospitals were then grouped according to the Portuguese Nomenclature of Territorial Units for Statistics (NUTS) III divisions. All NUTS were classified as being either coastal or interior and as having either high urban density or low urban density according to their demographic characteristics. Pollinic maps were used to compare the results with the natural distribution of allergens in each region.

A descriptive analysis was conducted, and statistical analysis was carried out using SPSS version (25.0) (IBM Corp., Armonk, NY, USA) to estimate associations between molecular allergens and specific regions of Portugal as well as the risk of coexisting diseases.

Figure 1 shows a map of Portugal with the location of each NUT region.

Results

A total of 1010 patients were enrolled, with slightly male preponderance (63%) and a median age of 9 years (Interquartile range [IQR]: 5-13).

Most of the patients came from high-density regions (n = 665; 65%) and coastal locations (n = 609; 60%). The included NUTS III were: Porto Metropolitan Area (n = 401; coastal and high urban density), Trás-os-Montes e Alto Douro (n = 161; Interior and low urban density), Minho e Alto Minho (n = 126; coastal and high urban density), Guarda (n = 102; interior and low urban density), Santarém (n = 82; coastal and low urban density), Coimbra (n = 80; interior and high urban density), and Tâmega e Sousa (n = 58; interior and high urban density).

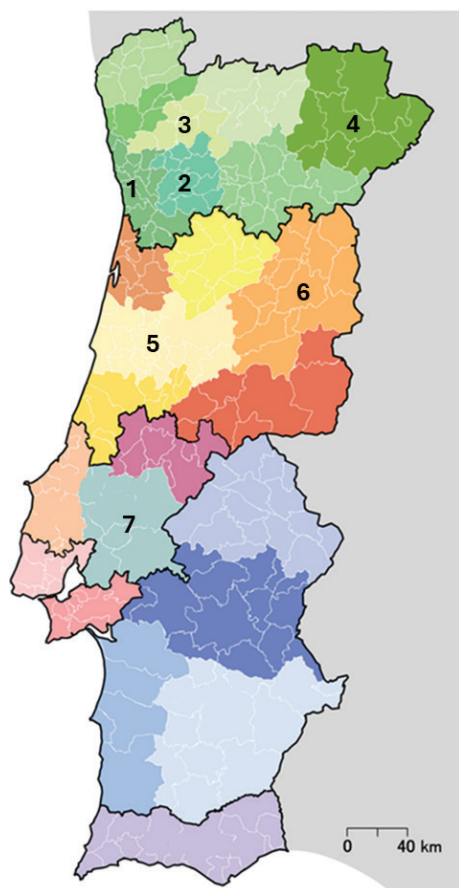


Figure 1 Map of Portugal with the different NUTS III included in our study. 1 - Porto Metropolitan Area (n = 401; coastal and high urban density), 2 - Tâmega e Sousa (n = 58; interior and high urban density), 3 - Minho e Alto Minho (n = 126; coastal and high urban density), 4 - Trás-os-Montes e Alto Douro (n = 161; interior and low urban density), 5 - Coimbra (n = 80; interior and high urban density), 6 - Guarda (n = 102; interior and low urban density), and 7 - Santarém (n = 82; coastal and low urban density).

The groups examined in this study exhibit similar demographic characteristics, including gender distribution, age range, and other relevant factors (e.g., coexisting allergic diseases).

The examination was primarily conducted as an adjunct to immunotherapy selection in patients with multiple sensitizations (54%), followed by those with multiple food allergies (35%).

The most common disease was allergic rhinoconjunctivitis, present in 62% of the patients, followed by food allergy and asthma (51% and 45%, respectively), and lastly atopic dermatitis present in 32% of the patients.

Sensitization to at least one dust mite (DM) molecular allergen was identified in 64% (n = 645) of the patients.

Among these patients, sensitization to *Dermatophagoides pteronyssinus* was the most prevalent one with the major allergen Der p 1 being the most common molecular allergen (74%), followed by allergens Der p 2 and Der p 23 (73% and 72% of the patients, respectively). *Dermatophagoides farinae* molecular allergens,

Der f 1 and Der f 2, were present in 67% and 71% of the patients, respectively. Sensitization to both *Lepidoglyphus destructor* and *Blomia tropicalis* was lower, each being present in 36% and 15% of the population. Regional differences of sensitization to DM are demonstrated in Figure 2.

Sensitization to allergen Der p 10, a minor allergen of DM but responsible for cross-reactivity with shrimps and other crustaceans, was found in 9% of the patients. Of these patients, 94% were also sensitized to Pen m 1, a troponomyosin responsible for the cross-reactivity between DM and shrimp.

There were 96 patients (10%) monosensitized to only one DM molecular allergen, with almost half of them being sensitized to Der p 23 (48%), followed by Der p 1 (26%).

Sensitization to at least one DM molecular allergen was associated with an increased risk of presenting allergic diseases, with patients having 2.8 (95% Confidence Interval [CI]: 2.2, 3.7) and 2.4 (95% CI: 1.8, 3.0) times the risk of having asthma or rhinoconjunctivitis, respectively. Patients sensitized to Der p 10 had 2.7 (CI 95%: 1.9, 3.9) times the risk of having food allergies.

The degree of urbanization was associated with an increased risk of sensitization to all DM molecular allergens as shown in Figure 3 and Table 1, with patients from these areas having 4.3 (95% CI: 3.3, 5.7) times the risk of being sensitized to at least one DM molecular allergen.

There was also an increased risk of sensitization to DM in patients living in coastal areas, but this increase was only significant to *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, as shown in Figure 3 and Table 1.

We found sensitization to at least one pollen molecular allergen in 57% of our patients. Grass pollens were the most prevalent group, especially Phl p 1 and Cyn d 1, being positive in 47% and 36% of the patients, respectively. As represented in Figure 4, we demonstrated that sensitization to grass pollens increased with age across the population. Phl p 1 was the major allergen in patients aged >3 years, but in younger patients, Cyn d 1 and Phl p 2 were the first allergens to appear, in 5% and 4% of the patients, respectively.

Most of the allergens were associated with an increased risk of allergic rhinoconjunctivitis, especially grass pollens, which increased the risk by at least four times (95% CI: 3.0, 5.7). Olive pollen allergen Ole e 1 was associated to asthma, increasing its risk by 1.8 times (95% CI: 1.2, 2.7).

The interior of the country was associated with a significantly increased risk of sensitization to pollens as demonstrated in Table 2.

There was significant variation in sensitization patterns to tree pollens among the regions shown in Figure 5. Ole e 1 was significantly more prevalent in the region of Trás-os-Montes e Alto Douro, with patients from this region having 6.9 (95% CI: 4.5, 10.5) times the risk of being sensitized. Patients from Coimbra also had 3.2 (95% CI: 1.9, 5.6) times the risk of being sensitized to allergen Cup a 1 from Arizona cypress pollen.

There were 61 patients (6%) sensitized to birch pollen allergen Bet v 1, which is a protein of the pathogenesis-related class 10 (PR-10) plant protein family that is sometimes associated with cross-reactivity with Bet v 1-related food proteins. In these patients, sensitization to Aln g 1

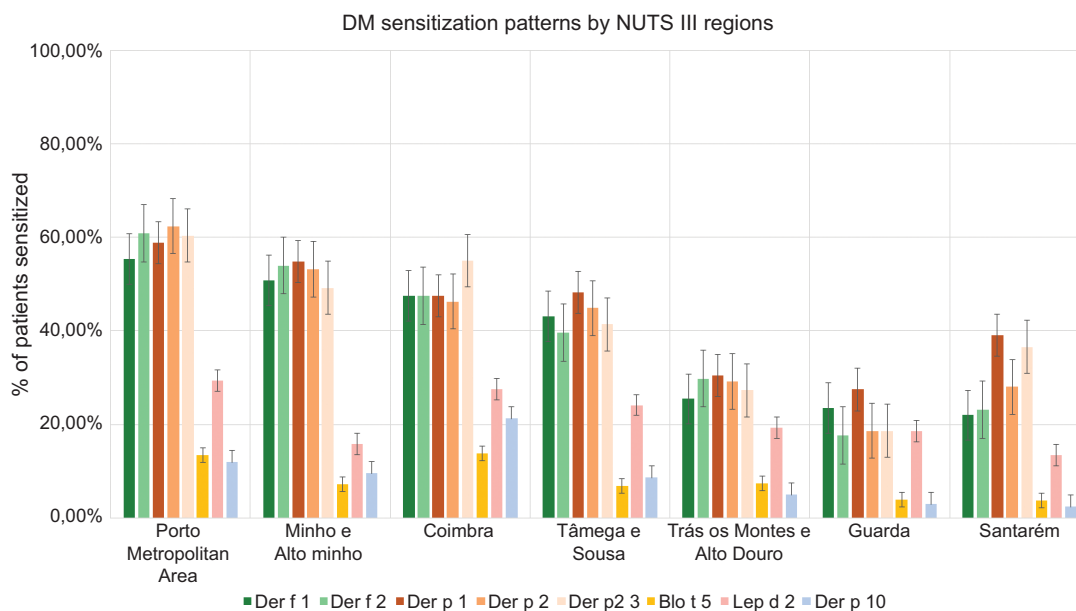


Figure 2 Sensitization pattern to each HDM molecular allergen in each NUT region. Each molecular allergen is represented with a different colour, and the graph represents the % of patients sensitized to that specific molecular allergen in that specific region.

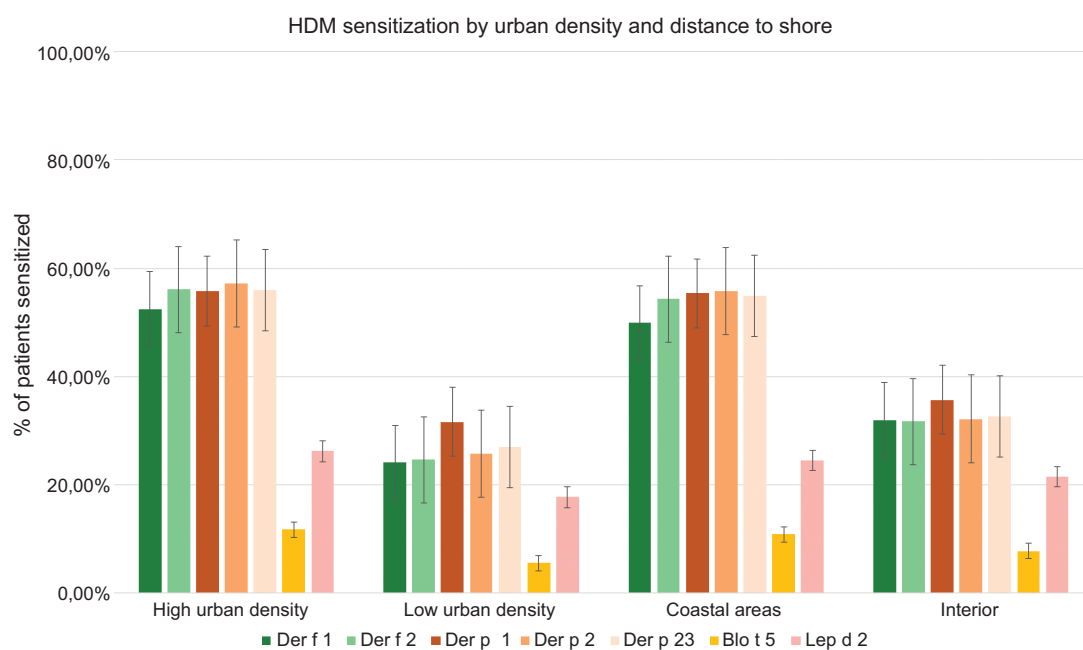


Figure 3 Difference in sensitization rate by urban density and distance to shore. Each molecular allergen is represented with a different color. There are significant differences with all dust mites (DM) when comparing the urban density, but when comparing distance to shore, both blo t 5 and lep d 2 show no significant differences, as summarized in Table 1.

and Cor a 1.0101 was found in 67% and 59% patients, respectively. When analysing sensitization values to evaluate possible primary sensitization, we identified that patients living in the interior regions had 4.1 (95% CI: 1.4, 12.1) the risk of being primarily sensitized to Bet v 1. Sensitization to Bet v 1 also increased the risk of developing food allergy (OR 2.1; 95% CI: 1.2, 3.6) in those living in the coastal areas, but there was no increased risk in those living in the interior regions (OR 1.2; 95% CI: 0.8, 1.3)

Discussion

As described in the literature, our results also demonstrate that DM allergens are more the most prevalent, being found in more than half of our patients.⁴ Persistent inflammation brought on by prolonged exposure to DM allergens increases the risk of developing respiratory diseases,⁴ as demonstrated in our population with patients sensitized to DM allergens having an increased

Table 1 Odds ratio of sensitization to house dust mites in relationship to geographic location (95% CI).

	Der f 1	Der f 2	Der p 1	Der p 2	Der p 23	Blo t 5	Lep d 2
High urbanization (n = 665, 66%)	3.4 (2.6-4.6)	3.8 (2.8-5.0)	2.7 (2.1-3.6)	5.0 (3.8-6.7)	4.3 (3.2-5.7)	4.9 (3.2-8.1)	2.5 (1.8-3.4)
Coastal areas (n = 609, 60%)	2.1 (1.6-2.8)	2.6 (1.9-3.3)	2.2 (1.7-2.9)	2.7 (2.1-3.5)	2.5 (1.9-3.3)	-	-

Note: Odds ratio (OR) of being sensitized to each molecular allergen; comparison between coastal vs. interior (interior as a reference) and high vs. low urban density (low density as a reference). High urbanization is associated with an increased risk of sensitization to all allergens, while coastal areas are associated with an increased risk of sensitization to all but Blo t 5 and Lep d 2.

risk of developing asthma, allergic rhinitis, and atopic dermatitis.

Molecular allergology is fundamental to choose the most adequate allergen-specific immunotherapy (AIT). Der p 23 is one of the clinically significant DM allergens.³ In our study, Der p 23 was the third most frequently discovered DM molecular allergen and was also the most frequently detected allergen in our monosensitized patients, making essential its inclusion in immunotherapy.

The presence of cross-reactive allergens, especially Der p 10, is another important factor in DM allergen sensitivity.³ Owing to its structural similarity to tropomyosin from other invertebrates, such as shrimp, Der p 10 is known to exhibit cross-reactivity that is clinically significant. Nearly 10% of our patients had sensitization to Der p 10, and almost all of them had cross-reactivity with Pen m 1, highlighting the importance of studying these molecular allergens, because identifying Der p 10 sensitization allows for better risk assessment, dietary recommendations, and potential avoidance strategies in affected individuals.

Dust mite sensitization patterns have been linked to environmental conditions, such as the level of urbanization and the distance from sea level.^{6,7} Furthermore, DM proliferation and allergen load are influenced by temperature and humidity fluctuations.⁷ DM populations are typically higher in coastal places with higher humidity levels, while concentrations of these allergens may be lower in drier inland areas. Understanding these geographical influences on DM exposure can help tailor preventive measures and guide public health strategies aimed at reducing prevalence of allergic diseases.

With this work we contributed to a better understanding of the distribution of sensitization to DM in our country as well as the influence of both urbanization level and distance to seashore. As described in the literature, we discovered a significant increase in sensitization to DM allergens in the patients that live in more urban regions.⁶ In our study, this translated an increased risk of sensitization that ranged from 2.7 to 7 times the risk.

Interestingly, when comparing patients living in the coastal areas to patients located more inland,

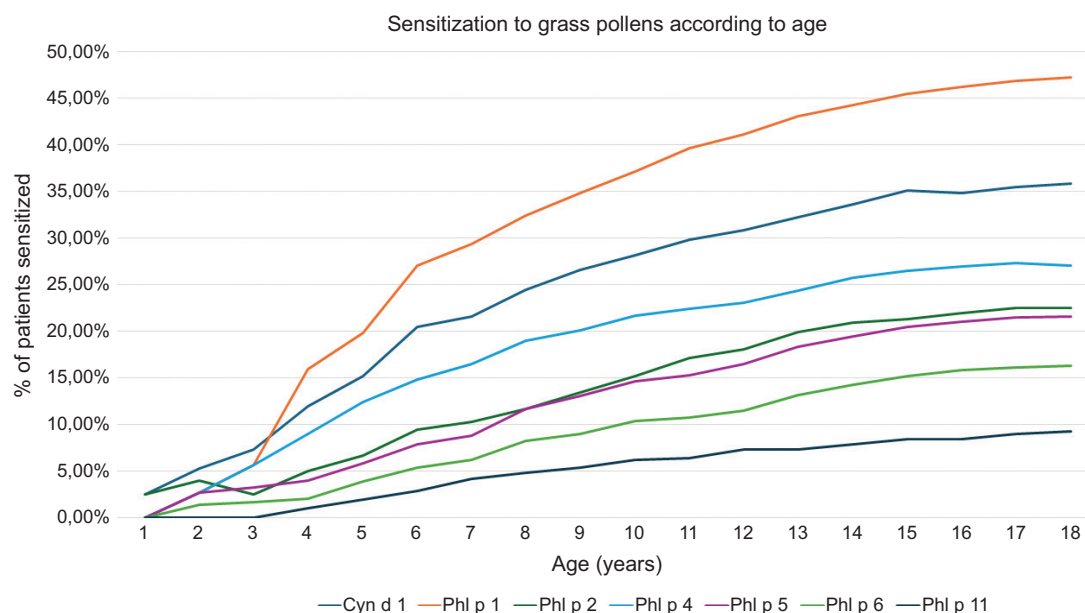


Figure 4 Percentage of patients sensitized to each grass pollen molecular allergen by age. There is a steady increase in sensitization to grass pollen molecular allergens as patients grow older. Even though Phl p 1 becomes the most common allergen in children older than 3 years old, in the first three years of life the most common allergens are both cyn d 1 and phl p 2.

Table 2 Odds ratio of sensitization to pollens in the interior of the country versus coastal areas (95% CI).

Cyn d 1	1.7 (1.3-2.3)	Cup a 1	2.3 (1.6-3.5)	No statistically significant difference for Phl p 1, Ole e 9, Par j 2, and Pla l 1
Phl p 2	1.7 (1.3-2.3)	Ole e 1	4.7 (3.1-7.3)	
Phl p 4	2.1 (1.6-2.9)	Cry j 1	2.2 (1.4-3.6)	
Phl p 5	2.6 (1.9-3.5)	Bet v 1	1.9 (1.1-3.1)	
Phl p 6	2.6 (1.9-3.8)	Art v 1	4.7 (1.7-13.1)	
Phl p 11	2.3 (1.5-3.5)	Che a 1	1.9 (1.1-3.8)	

Note: Odds ratio of being sensitized to each pollen molecular allergen in children living in the interior regions versus the coastal areas. There is an increased risk of sensitization to most of them in children living in the interior of the country, where these pollens are most prevalent.

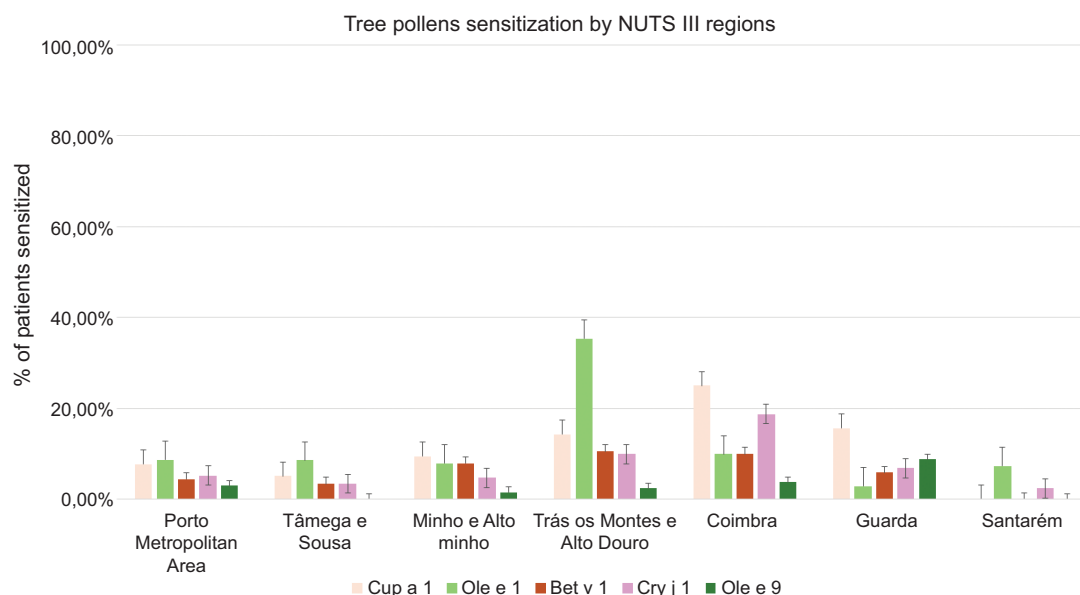


Figure 5 Sensitization to tree pollen molecular allergens by each region. Significant differences in sensitization are shown and match the distribution of each tree in our country.

the increased risk of sensitization was limited to both *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. *Lepidoglyphus destructor* and *Blomia tropicalis* did not show a statistically significant difference.

The absence of difference between inland and coastal areas to *Lepidoglyphus destructor* might be justified by the characteristics of each DM. *Lepidoglyphus destructor* was initially associated with occupational disease in workers of food storage in farms and agricultural lands; therefore, it was classified as storage DM, but it has since been demonstrated to be an important allergen in people living in both urban and rural areas.⁷ The inland of Portugal is usually more rural and associated with older structures, which create a perfect environment for the growth of storage DMs. These findings highlight the importance of considering these allergens when approaching patients sensitized to DMs living in these regions.

The sensitization pattern to *Blomia tropicalis* found in our population is also interesting. In tropical and subtropical areas, *Blomia tropicalis* is a common house DM that is known to be a major cause of indoor allergies.⁸ This mite grows best in warm, humid conditions with a relative

humidity (RH) of 70% and temperature ranging from 25°C to 30°C. The possible effects of global warming on the distribution of *Blomia tropicalis* were investigated in recent studies.^{5,8} Rising global temperatures and changed patterns of RH brought about by climate changes expand the habitats suitable for this DM species. In our patients, unlike what was expected, there was no significant difference between the coastal areas (which are typically considered as a suitable environment) and the more inland regions. Even though we have a small sample of patients sensitized to *Blomia tropicalis* and therefore may lack statistical power to infer any conclusion, we suggest that this could, in part, be explained by the climate changes that are observed globally. Our country has seen significant changes in the climate with the inland areas suffering from higher temperatures, increased heat wave frequency, and altered precipitation patterns.⁹ Another possible explanation is the increased migratory flow of patients from areas where *Blomia tropicalis* is common, such as South America.^{10,11} These changes might justify the higher-than-expected sensitization to *Blomia tropicalis* in our inland population and demonstrate the importance to further study the impact

of climate changes and migratory flows in our clinical practice.

In our study, sensitization to pollens was significant, with more than half the population being sensitized to at least one allergen. Grass pollens, in specific, were associated with a significant risk of developing rhinoconjunctivitis, reinforcing the role that these allergens have in the inflammation of nasal mucosa. As described in the literature, we demonstrated in our population that the proportion of grass pollens the patients are sensitized to increases with age.¹² This demonstrates that while in early childhood we might find patients monosensitized to Phl p 1, most patients are sensitized to all grass molecular allergens by the age of 18. However, interestingly, we demonstrated that during the first few years of life, patients are sensitized first to cyn d 1 and phl p 2 prior to phl p 1, raising the possibility of these two allergens having a role in the sensitization to grass pollens in the early childhood.

As expected, significant differences were discovered between the different regions of our country regarding sensitization. First, when comparing the interior to the coastal areas, we observed that there was a significant increase in sensitization to pollens in the interior, which was expected, given the increase in vegetation in these areas.¹³ Interestingly we discovered a direct correlation between the number of green spaces and the degree of sensitization, with Santarém being the region with least sensitization.

When considering specific regions, we discovered patterns that were direct translation of the pollinic maps of our country.¹⁴ The patients from Trásos Montes e Alto Douro (corresponding to the Douro region) had a significantly increased sensitization to Ole e 1, compared to other regions. This is explained when we look at the distribution of olive trees in our country, which are predominantly found in Alentejo and Douro Region. Similarly, there was a significant sensitization to Arizona cypress pollen Cup a 1 in Coimbra, which also matches the region of distribution of Cypress trees. These differences in sensitization pattern are important as they allow clinicians of each region to better understand the most important allergens prevalent in their area.

Sensitization to Bet v 1, often related to cross-reactivity, was associated with an increased risk of food allergy in our population. Interestingly, when evaluating primary sensitizer, we discovered that patients from the interior region had a significantly higher chance of being primarily sensitized by Bet v 1 and, unlike the remaining patients, demonstrated no increased risk of food allergy. This aligned with the distribution of *Betula tropicalis* in our country, found predominantly in the interior regions.¹⁴ These results are important as different sensitization pathways translate distinct clinical behaviours as postulated in some studies.¹⁵

Conclusions

The findings of this research emphasize the need of incorporating these regional variations into clinical practice and draw attention to notable regional variations in allergy sensitivity patterns among Portuguese children. The need for

a more individualized approach to the diagnosis and treatment of allergy illnesses is highlighted by the difference in sensitization profiles that are impacted by climatic, urbanization, and geographic factors.

The sensitization pattern to *Blomia tropicalis* presents a compelling case for considering the impact of climate change and migratory flows in clinical practice. Despite expectations of higher prevalence in coastal regions because of warmer and more humid conditions, our findings revealed no significant differences between coastal and inland areas. This unexpected pattern could be explained by rising global temperatures, altered precipitation patterns, and increased migratory flows from regions with a higher prevalence of *Blomia tropicalis*. These factors highlight the importance of ongoing surveillance and adaptability in clinical guidelines to address the evolving landscape of allergen exposure.

Additionally, the high prevalence of sensitization to Der p 23 among patients, particularly those who are monosensitized, underscores its clinical significance and the need for its inclusion in AIT formulations. The underrepresentation of Der p 23 in AIT could result in suboptimal treatment outcomes for a significant subset of patients, emphasizing the importance of incorporating region-specific allergen profiles into treatment protocols to enhance efficacy and achieve better clinical management of allergic diseases.

Another notable result is the sensitization to Bet v 1, primarily associated with birch pollen, which exhibited distinct patterns between coastal and interior regions. The higher prevalence of primary sensitization to Bet v 1 in the interior regions aligns with a high distribution of *Betula tropicalis* in this region and suggests that sensitization pathways differ significantly based on local vegetation. This differentiation is clinically significant, as patients from the interior regions showed no increased risk of food allergies, compared to those in birch-free areas, indicating the necessity for region-specific diagnostic and management strategies.

In summary, incorporating regional allergen profiles into clinical guidelines could lead to more effective prevention strategies and personalized treatment plans, ultimately reducing the burden of allergic diseases. Future research should continue to explore the impact of environmental changes, migratory patterns, and regional allergens, thus extending the study to the remaining country.

Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that no AI-assisted tools were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

Author Contributions

All authors contributed equally to this article.

Conflicts of Interest

Authors declared no conflict of interest.

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