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Exploring the repertoire of cross-reactive allergens among *Aspergillus fumigatus* and *D. pteronyssinus* mites: an in-silico approach

Exploración de los alérgenos de reactividad cruzada entre los ácaros *Aspergillus fumigatus* y *D. pteronyssinus*: un enfoque *in silico*

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Abstract

Objective: To examinate the potential cross reactivity among A. fumigatus and mites, both important allergenic source in tropical regions, using mainly a bioinformatic approach.

Methods: Amino acid sequences from allergens from *Aspergillus fumigatus* reported in the allergome database were retrieved and used as input to perform PSI-BLAST against *Dermatophagoides pteronyssinus´*s proteome. Results with similitudes and query cover values above 25% and 80%, respectively, were selected to further analysis. B cell epitope prediction was done by using the Ellipro tool. Just epitopes conserved between both allergenic sources were informed and displayed on 3D model surfaces obtained by modeling based on homology.

Results: Twelve allergens from *Aspergillus fumigatus* shared homology with proteins reported in *D. pteronyssinus*. All 3D models obtained showed typical folding to the protein family they belonged. Ribosomal protein L3, Molecular chaperone Mod-E/Hsp90, Acidic ribosomal protein P2, Enolase, and peptidyl-propyl cis-trans isomerase were allergens with the highest identity score (>60%). At least four B linear epitopes were predicted to be shared between allergens and homologous in *D. pteronyssinus*.

Conclusion: Results indicated that cross-reactivity between Aspergillus fumigatus and *D. pteronyssinus* is feasible. At least twelve allergens could be involved, and this could explain how molds increase sensitization to mites. In vitro analyses are needed to confirm these results.

Keywords: Allergy; Cross-reactivity; IgE antibodies; Mites; Molds; Bioinformatic.

Resumen

Objetivo: Examinar la posible reactividad cruzada entre *A. fumigatus* y los ácaros, ambas importantes fuentes de alérgenos en regiones tropicales, mediante un enfoque bioinformático.

Métodos: Se recuperaron las secuencias de aminoácidos de alérgenos de *Aspergillus fumigatus* registradas en la base de datos del alergoma y se utilizaron como entrada para realizar un análisis PSI-BLAST contra el proteoma de *Dermatophagoides pteronyssinus*. Se seleccionaron para su posterior análisis los resultados con similitudes y valores de cobertura de consulta superiores al 25 y al 80%, respectivamente. La predicción de epítopos de células B se realizó mediante la herramienta Ellipro. Solo se identificaron los epítopos conservados entre ambas fuentes alergénicas y se visualizaron en superficies de modelos 3D obtenidas mediante modelado basado en homología.

Resultados: Doce alérgenos de *Aspergillus fumigatus* compartieron homología con proteínas descritas en *D. pteronyssinus*. Todos los modelos 3D obtenidos mostraron el plegamiento típico de la familia proteica a la que pertenecen. La proteína ribosomal L3, la chaperona molecular Mod-E/Hsp90, la proteína ribosomal ácida P2, la enolasa y la peptidil-propil cis-trans-isomerasa fueron los alérgenos con mayor puntuación de identidad (>60%). Se predijo que al menos cuatro epítopos lineales B eran compartidos entre los alérgenos y homólogos en *D. pteronyssinus*.

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Conclusión: Los resultados indicaron que la reactividad cruzada entre *Aspergillus fumigatus* y *D. pteronyssinus* es factible. Al menos doce alérgenos pueden estar implicados, lo que explica cómo los mohos aumentan la sensibilización a los ácaros. Se requieren análisis in vitro para confirmar estos resultados.

Palabras clave: Alergia; Reactividad cruzada; Anticuerpos IgE; Ácaros; Mohos; Bioinformática.

INTRODUCTION

Allergic diseases are a broad spectrum of inflammatory hypersensitive reactions, and their prevalence has significantly risen, representing one of the most important challenges worldwide today. Aspergillus fumigatus is a ubiquitous fungus, can cause from invasive disease to allergic disease allergic bronchopulmonary aspergillosis (ABPA),[1] its allergens are important sensitizers and trigger symptoms in allergic individuals with other allergic conditions.[2-4] For example, this fungi have been frequently found in nasal and skin microbiota of allergic patients[5] and is an important inducer of allergic and auto reactive responses in patients suffering atopic dermatitis.[6,7] Fungal sensitization in allergic subjects with respiratory diseases range from 2.3% to even 80%, according to Mirabi et al,[8] in particular for *A. fumigatus* various allergens have been characterized, such as manganese superoxide dismutase (MnSOD),[1] thioredoxin[9] and Cyclophilin B,[10] making the study of its allergens of pivotal importance to understand fungal allergy and the designing of specific allergen recombinants for immunotherapy and component resolved diagnosis. In the tropics mite's sensitization is the main risk factor for asthma and rhinitis and co-exposure with Aspergillus is common, both are colonizers of human skin and abundant in the tropical environment, thus potential of cross sensitization and cross reactivity for these two allergenic sources needs to be explored.

Cross-reactivity among allergenic sources is an important immunological mechanism involved in cross sensitization and exacerbation of allergic responses.[11] This is a phenomenon described for allergy to foods and mites.[11,12] For *A. fumigatus*, cross-reactivity with other fungus such as: *Malassezia simpodialis, Penicillium notatum, Malassezia furfur, Alternaria alternata and Cladosporium herbarum*, have been studied.[13] For these fungus species, allergens related to MnSOD, cyclophilins, thioredoxins, serin proteases, enolases, P1 and P2 ribosomal proteins, heat shock protein and peroxisomal proteins are involved in cross reactivity,[13] being these allergens highly conserved, which is of relevance when studying cross-reactivity in the related *A. fumigatus* specie. Nowadays, eighty fungus genomes are available,[14] constituting a powerful tool to study cross reactivity using in silico approaches. However, cross-reactivity among fungus and other allergenic sources has been poorly explored. Here, we examined potential cross reactivity among *A. fumigatus* and mites, both important allergenic sources in tropical regions, using mainly a bioinformatic approach.[15]

METHODS

Selection of allergens and homologous search

Aminoacid sequence from allergens derived of *Aspergillus fumigatus* and *Dermatophagoides pteronyssinus* were retrieved from database Allergome.[16] Allergens used for this study are listed in **Tables 1 and 2**. Aminoacid sequences from *A. fumigatus* were used as input in BLASP (https://blast.ncbi.nlm.nih.gov/) to search similar sequences in mite's proteome using the term and taxid "house-dust mites (taxid:6952)". Aminoacid sequences with similarity upper 25% were selected for furthers analysis. A similar approach to extend analysis of cross reactivity, but in this situation, aminoacid sequences from allergen characterized in allergenic source *D. pteronyssinus* was used as input to perform BLASTP against *A. fumigatus*, using term and taxid "Aspergillus fumigatus (taxid:746128)". Upon sequences were selected, binary alignment was performed and identity level was determined with IBIVU PRALINE tool.[17]

Modeling 3D models of allergens from *A. fumigatus*

All allergens without experimental structures resolved and reported in protein data bank were modeled using Swiss-model server. For this, aminoacid sequences for each allergen without PDB reported were used as input in server for modeling. Templates were selected based on highest identity level share with aminoacid sequence used for it, and Angstrom value. Quality model was assessed by Prosa web.[18] Visualization of models was performed with Pymol software.[19]

Epitope prediction

B cell epitope prediction was done using Ellipro v 3.0 server. 3D allergen models were used as input. Minimum score and maximum distance (Angstrom) were set to 0.5 and 6, respectively. Epitopes with high conserved rates were visualized on 3D model.

Evolutionary analysis of Aspergillus allergen

Evolutionary or conservation analysis of each aminoacid for each allergen from *A. fumigatus* among invertebrates was estimated by using Consurf server.[20]

RESULTS

Allergens selected

In total, twenty aminoacid sequences reported in allergome database were retrieved for analysis (**Table 1**). Thirteen proteins shared conservation and identities with some sequence in proteome from *D. pteronyssinus*. In this, we found that Ribosomal protein L3, from *A. fumigatus* exhibited the highest identity level (69%) with a homologous from *D. pteronyssinus* (60S ribosomal protein L3-like).

Modelling allergen

Allergens from *A. fumigatus* were modeled to predict B cell epitopes. All models showed typical folding related to the family protein they belonged. Seven allergens didn't exhibit any identity with proteome from *D. pteronyssinus* but were modeled to further comparative analysis (**Table 1** and **Figure 1**).

Conservation of Epitopes among *A. fumigatus* and *D. pteronyssinus*

To determine cross-reactivity, B cell epitope prediction was performed. We informed only epitopes conserved between allergens from *A. fumigatus* and *D. pteronyssinus*. Of thirteen allergens modeled from Aspergillus, seven were used for cross-reactivity exploration, these were selected based on identity level shared with homologous in mite's proteome (> 40%). Allergens used were: Ribosomal protein L3, Molecular chaperone Mod-E/Hsp90, Enolase, Acidic ribosomal protein P2, peptidyl-propyl cis-trans isomerase, Superoxide dismutase Mn and Thioredoxin (**Table 1** and **Figure 2**).

Ribosomal protein L3 showed 69% in identity level with a homologous in *D. pteronyssinus* identified as 60S ribosomal protein L3-like. According to epitope prediction, these proteins shared four conserved epitopes. This was the highest number of predicted epitopes shared for allergens

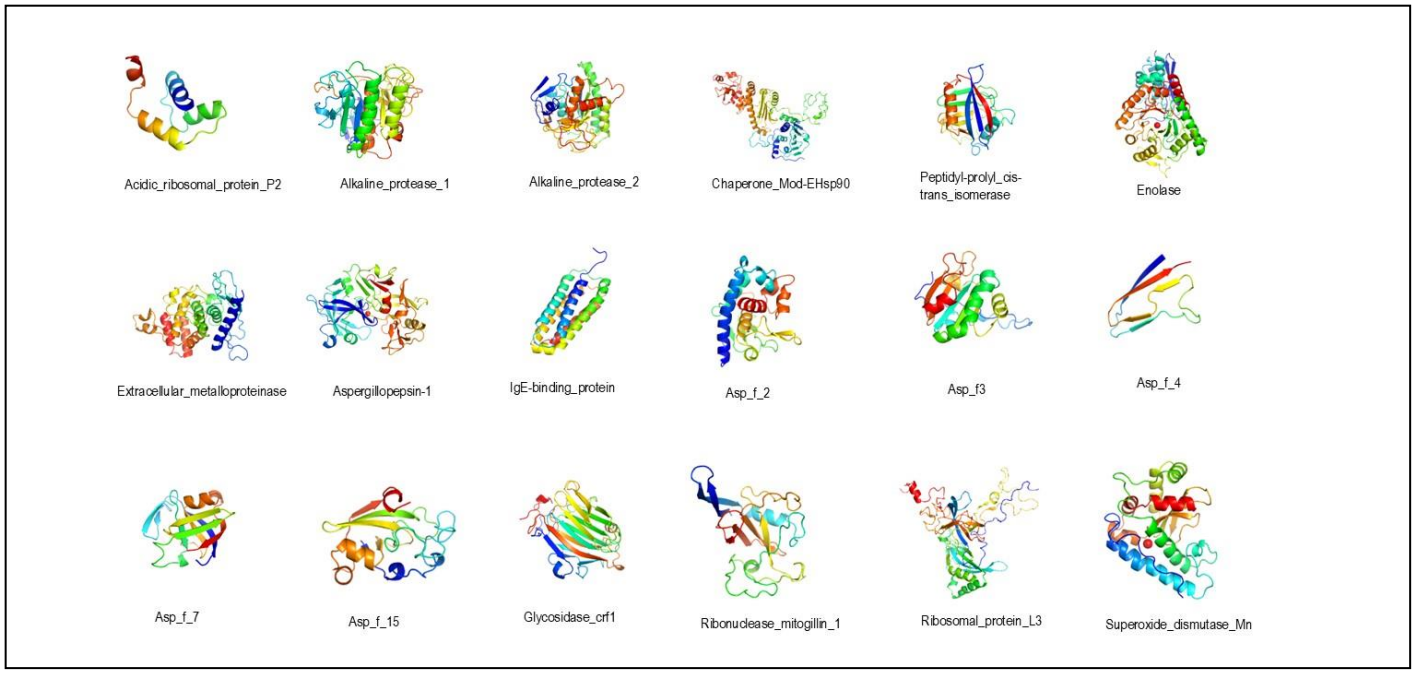

Figure 1. It shows all allergens modeled and characterized from A. fumigatus used for analysis in this study. All models had typical folding about the family they belonged to.

Table 1. Allergens characterized from *A. fumigatus* with homology and identity shared with *D. pteronyssinus*.

Allergen N°	Alergenos de Aspergillus Fumigatus	Uniprot acceso	PDB template	Homologous	Access code	Identity with *D. pteronyssunis*
1	Ribosomal protein L3	Q8NKF4	6y2l.2.A	60S ribosomal protein L3-like	XP_027199289.1	69
2	Molecular chaperone Mod-E/Hsp90	B0Y324	4z1f.1.A	Heat shock protein 83-like	XP_027197940.1	66
3	Enolase	Q96X30	4g7f.1.A	Enolase-like	XP_027197319.1	66
4	Acidic ribosomal protein P2	Q9UUZ6	4v6i.72.A	Ribosomal protein P2-like protein	AUX14772.1	65
5	peptidyl-propyl cis-trans isomerase	Q9Y7F6	2cfe.1.A	Cis-trans isomerase F (Der f 29)	XP_027204080.1	64

...continuation table 1.

Allergen N°	Alergenos de Aspergillus Fumigatus	Uniprot acceso	PDB template	Homologous	Access code	Identity with *D. pteronyssunis*
6	Superoxide dismutase Mn	Q92450	2cdy.1.A	Superoxide dismutase [Mn]	XP_027200320.1	52
7	Thioredoxin Asp	Q1RQJ1	2ypm.1.A	Thioredoxin-like protein 1	XP_027200765.1	43
8	Aspergillopepsin-1	P41748	1ibq.1.A	Lysosomal aspartic protease-like	XP_027204642.1	33
9	Alkaline protease 1	P28296	3f7m.1.A	Membrane-bound transcription factor site-1 protease-like	XP_027194529.1	33
10	Alkaline protease 2	P87184	3f7o.1.A	Membrane-bound transcription factor site-1 protease-like	XP_027194529.1	29
11	Major allergen Asp f 2	P79017	1eb6.1.A	Uncharacterized protein LOC113788378	XP_027193641.1	28
12	Peroxiredoxin Asp f3	O43099	1h4o.8.A	Peroxiredoxin 1-like	XP_027200335.1	28
13	Allergen Asp f 7	O42799	5ntb.1.A	Sister chromatid cohesion protein PDS5 homolog B-like	XP_027205714.1	24
14	Ribonuclease mitogillin	P67875	1jbr.1.C	N/A		
15	Allergen Asp f 15	O60022	3m3g.1.A	N/A		
16	Probable glycosidase crf1	Q8J0P4	6ibu.1.A	N/A		
17	Cell wall protein phiA	A4FSH5		N/A		
18	Allergen Asp f 4	O60024	3qis.1.A	N/A		

...continuation table 1.

Allergen N°	Alergenos de Aspergillus Fumigatus	Uniprot acceso	PDB template	Homologous	Access code	Identity with *D. pteronyssunis*
19	Extracellular metallopro-teinase mep	P46075	4m65.1.A	N/A		
20	IgE-binding protein	O60025	5csd.3.A	N/A		

Table 2. Allergens characterized *D. pteronyssinus* with homology and identity shared with *A. fumigatus*.

Mites	Allergen	Uniprot code	Identidad	*A. fumigatus*	Homologous
Der p 28	Heat Shock Proteins	A0A291KZD8	77.89%	XP_750490.1	molecular chaperone Hsp70
Der f 26	Myosins	A0A291KYZ8	33.54%	XP_751821.2	Calmodulina
Der p 25	Triosephosphate isomerase	A0A291KYZ7	54.81%	XP_753309.1	triosephosphate isomerase
Der p 24	Ubiquinol-Cytochrome C Reductase Binding Protein	A0A0K2GUJ4	29.89%	XP_752147.2	ubiquinol-cytochrome c reductase complex 14 kDa
Der p 18	Chitinases	Q4JK71	27.03%	EDP48562.1	class V chitinase

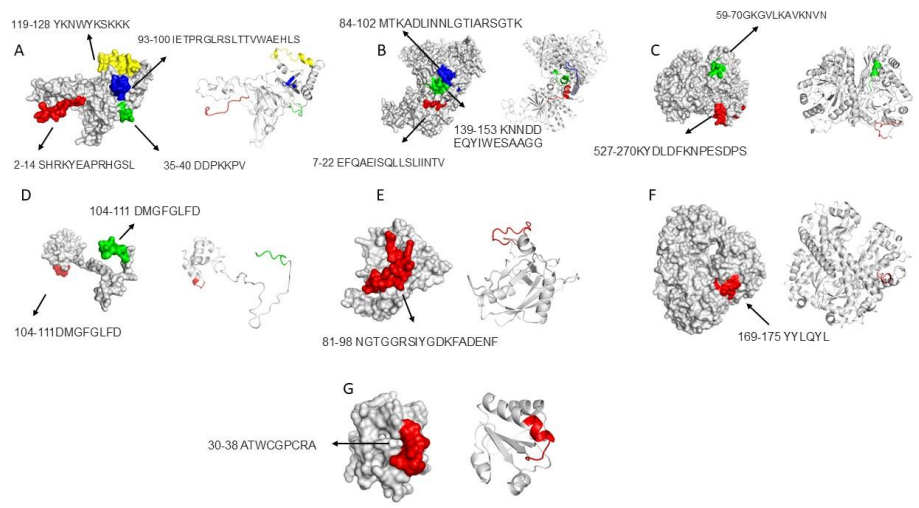

Figure 2. Surface and cartoons models showing predicted epitopes between *A. fumigatus* and *D. pteronyssinus*. A: Ribosomal protein L3, B: HSP90, C: Enolase, D: Acidic ribosomal protein P2, E: peptidyl-propyl cis-trans isomerase, F: MnSOD and G: Thioredoxins.

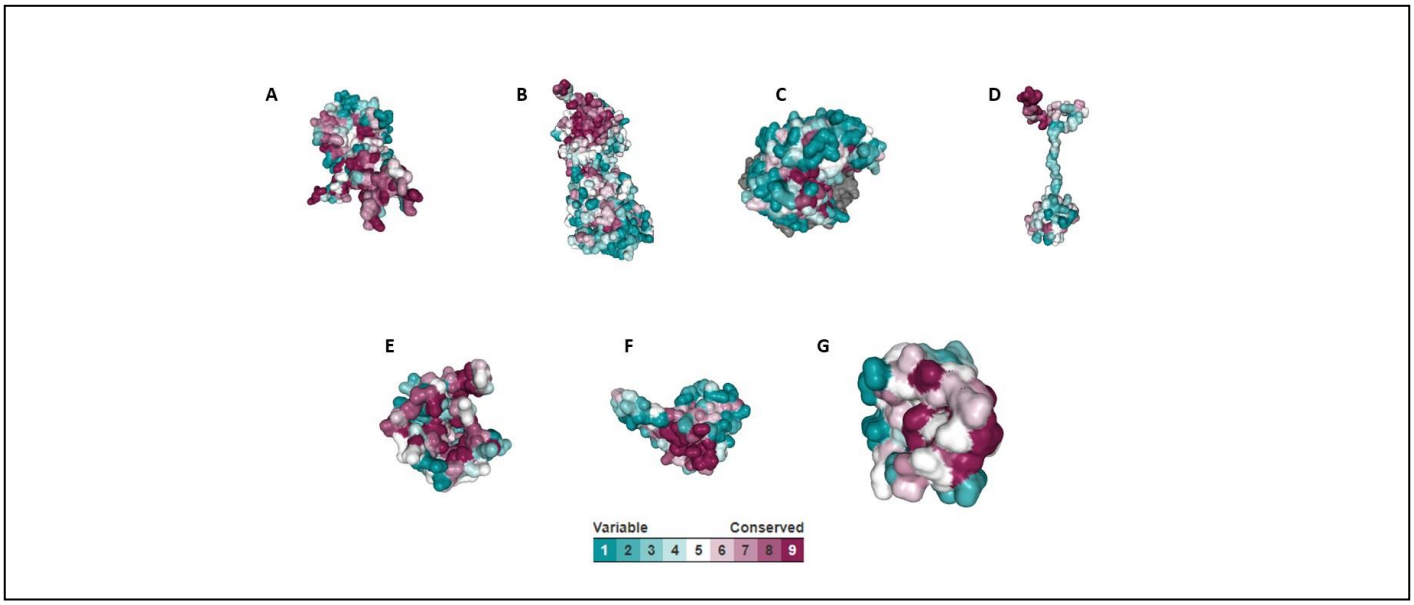

Figure 3. Consurf results represented on surface models for allergens conserved between A. fumigatus and D. *pteronyssinus*. A: Ribosomal protein L3, B: HSP90, C: Enolase, D: Acidic ribosomal protein P2, E: peptidyl-propyl cis-trans isomerase, F: MnSOD and G: Thioredoxins.

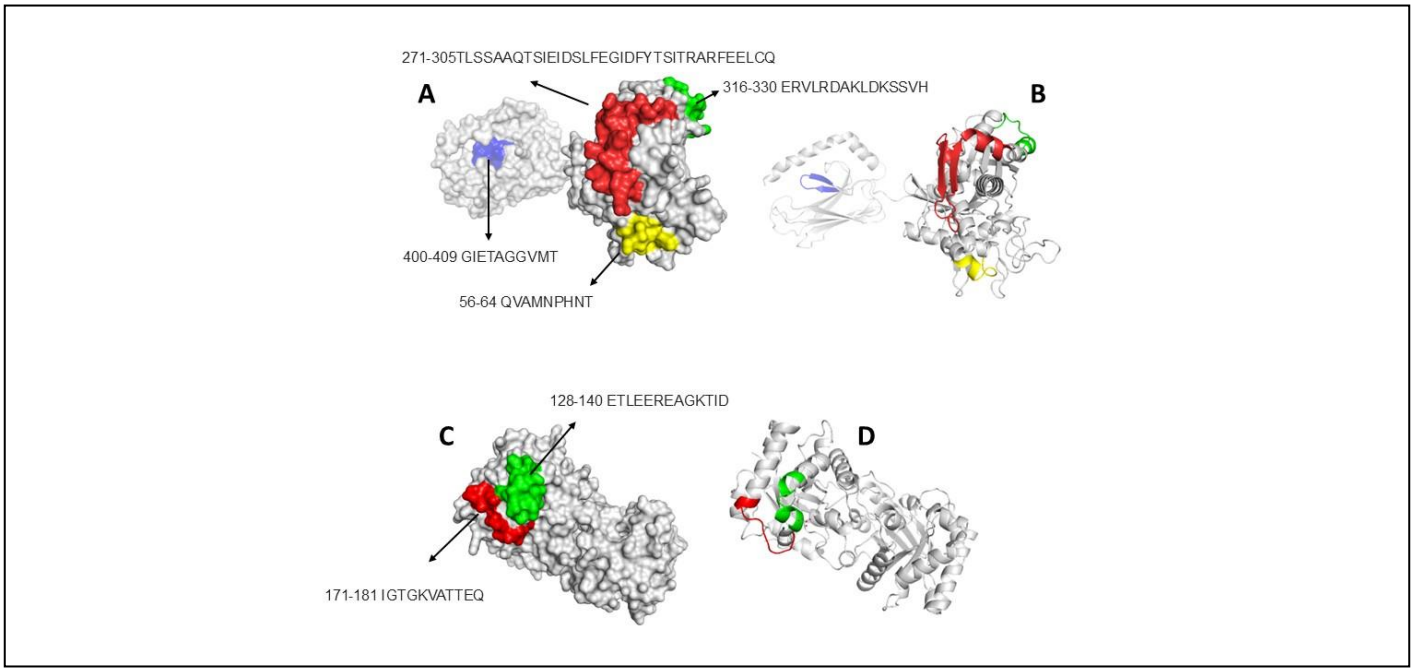

Figure 4. Surface and cartoon models showing conserved epitopes between allergen characterized in D. *pteronyssinus* and Homologous of *A. fumigatus*. A: Surface model showing epitopes on Der p 28, B: Cartoon model to show epitopes on model of Der p 28, C and D: Surface and cartoon models of allergen Der p 25.

used. Next, Hsp90 was the second allergen with an identity level above 60%, and bioinformatic approaches predicted three epitopes conserved with homologous in *D. pteronyssinus*. For enolase and Acidic ribosomal protein P2, two epitopes were predicted. Both allergens exhibited 66 and 65%, correspondly, with their Homologous in mites. Finally, for allergens peptidyl-propyl cis-trans isomerase, MnSOD and, thioredoxins, just one epitope was conserved. These allergens shared different identity levels, for peptidyl propyl was 64% with the allergen Der f 29 from *D. pteronyssinus*, MnSOD is 52% and 43% for the thioredoxin allergen in *A. fumigatus*.

Conservation of Epitopes among allergens of *D. pteronyssinus* and homologous in *A. fumigatus*

We found five allergens of *D. pteronyssinus* with identity in their aminoacid sequences with homologous in *A. fumigatus* (**Table 2**). In **Figure 4**, two representative allergens (Der f 28 and Der f 25) that shared 77.8 and 54.8% of identity are shown. For first allergen, Der p 28, three conserved epitopes with molecular chaperone Hsp70 of *A. fumigatus* were predicted. For Der p 25, two epitopes are informed (**Figure 4 C-D**).

DISCUSSION

Aspergillus fumigatus has wide distribution worldwide. Due to this, it is difficult to approximate a real prevalence. There is estimated prevalence data depending on the context (asthma, cystic fibrosis, immunocompromised) or some regions of the world. In low- and middle-income countries such as Colombia, there is not enough data to calculate its prevalence. It is highlighted that it is not a fungus that is only in the environment, but also on surfaces and is dependent on changes in climate and environmental regulation mechanisms. In humans, cause significant allergic manifestations in sensitized subjects. Fungi and dust mite species cohabit in household dust, yet the precise dynamics of their interaction remain poorly understood. Existing literature offers limited evidence, indicating a potential interplay between fungal presence and *Dermatophagoides pteronyssinus*, with suggestions of both synergistic and antagonistic relationships. In this study, twelve allergens from *A. fumigatus* were found sharing homology with proteins reported in *D. pteronyssinus*. The 3D models obtained showed typical folding to the protein family they belonged. Ribosomal protein L3, Molecular chaperone Mod-E/Hsp90, Acidic ribosomal protein P2, Enolase, and peptidyl-propyl cis-trans isomerase were the allergens with the highest score identity (>60%). At least four B linear epitopes were predicted to be shared between allergens and homologous in *D. pteronyssinus*.The immune response to the different allergens currently described is variable which compromises the immune response in sensitized individuals.

Research on fungal allergies faces significant challenges due to the complexity of the elicited immune response, which produces a variety of intracellular metabolites, cell wall components and extracellular secretomes, inducing humoral and cellular immune responses. This diversity makes the clinical interpretation of sensitization test results difficult, since sensitization to total fungi extracts does not always reflect precise sensitization to a specific species, due to the variability in the composition and allergenicity of the final products, resulting of different cultivation and extraction processes. Standardization in the production of reliable fungal extracts is presented as a key challenge to advance in the diagnosis and treatment of fungal allergies. This would involve establishing uniform protocols for the cultivation and extraction of fungi, with the aim of minimizing variability between batches of products.

Respiratory diseases caused by *Aspergillus* are classified according to the immune mechanism: Allergic aspergillosis (IgE-mediated): allergic bronchopulmonary aspergillosis (ABPA), severe asthma with fungal sensitization (SAFS) and severe allergic bronchopulmonary aspergillosis. Aspergillus allergic sinusitis, and hypersensitivity pneumonitis. Diseases due to saprophytic colonization: they include simple or complex aspergilloma (chronic cavitary pulmonary aspergillosis), chronic pulmonary aspergillosis (CPA), fungal sinus aspergillomas. Invasive disease: invasive pulmonary aspergillosis, which can be acute subacute, called chronic necrotizing pulmonary aspergillosis (CNPA), acute fulminant invasive sinusitis, chronic invasive sinusitis, invasive granulomatous sinusitis.[21,22] From the perspective of clinical diagnosis traditionally, in the evaluation of ABPA and Aspergillus sensitive asthma (ASA), derivatives of crude extracts of *A. fumigatus* are used, presenting limitations in their purity and consistency. The differentiation between ASA and ABPA can be complicated and requires multiple approaches, since both can share symptoms between them: difficult-to-manage asthma, high IgE levels and bronchiectasis, the use of antigens could improve the current diagnosis for ABPA.

A. fumigatus is a well allergenic source characterized, several allergens with molecular and immunological different are reported in IUIS. For example, cyclophilins, L3 ribosomal proteins, thioredoxin and MnSOD are important allergens related to trigger allergic reactions.[1,23,24] Cross-reactivity of this allergenic source with other fungi or yeast is well reported in the literature,[25] *Penicillium sp*., *Alternaria alteria*, and *Malassezia simpodialis* are referred to be cross reactivity with *A. fumigatus*.[26] In this study we demonstrate that another important challenge in the diagnosis of fungal hypersensitivity is distinguishing between genuine sensitization and cross-reactivity. Evaluation of the sensitization profile to specific allergens and panallergens is crucial, since crude allergenic extracts from different fungal sources can have significant cross-reactivity by structural homology, which can complicate the interpretation of sensitization test results.

This is important to understand the cross sensitization that can suffer the patients, and this can lead to exacerbated symptoms. However, cross reactivity of *A. fumigatus* with other allergenic source different to fungi or yeast is not reported. This is the first study to determine the potential of *A. fumigatus* in cross reactivity with other allergenic source, for example, mites. Here, using a bioinformatic approach we have identified thirteen allergens with identity (above 20%) in their amino acid sequences between *A. fumigatus* and *D. pteronyssinus*. In this study, we focused on bioinformatic characterization of seven allergens (Ribosomal protein L3, HSP90, Enolase, Acidic ribosomal protein P2, peptidyl-propyl cis-trans isomerase, MnSOD and Thioredoxins). Epitope prediction suggests that several antigenic regions would be involved in cross reactivity between allergens from *A. fumigatus* and their homologous in *D. pteronyssinus*.

Thioredoxin [Trx] has antioxidant and protein regulation functions of the photosynthetic mechanism of the chloroplast, such as NADP-malate dehydrogenase [NADP-MDH] are identified in humans and in allergenic sources such as Aspergillus spp where the allergens known as ASPID F28, ASPID F29 characterized as thioredoxins, reported in other allergenic sources such as *Malassezia sympodialis* and *Coprinus comatus*, also observing cross-reactivity between the human Trx enzyme and those present in *Aspergillus spp, Malassezia sympodialis* and *Coprinus comatus*, due to molecular mimicry between the thioredoxin of the allergenic sources and human, presenting cross-reactivity mediated by IgE, thus generating autoreactive responses that exacerbate the symptoms of allergic diseases. Therefore, it is necessary to evaluate this CR in the clinic and if it presents with *D. pteronyssinus*.

Manganese super oxide dismutase [MnSOD], another cross-reactive allergen, are important enzymes for the physiological response to oxygen toxicity, dismutating

toxic superoxide free radicals into oxygen and hydrogen peroxide, oxidative stress and lung parenchymal damage. MnSOD is involved in the IgE-mediated autoreactive immune response. Structural homology has been found with human MnSOD, of Af especially the allergen ASPID F 6, *Drosophila melanogaster* and *Saccharomyces cerevisae*, which in previously sensitized individuals favors the crossed and autoreactive immune response due to structural homology.

Is important to define that homologous identified in mites, just Cis-trans isomerase is reported as allergen in *D. farinae* but not in *D. pteronyssinus*, registered as Der f 29.[27] However, homology and epitopes predicted indicated that homologous in *D. pteronyssinus* need to be explored in future to determine allergenic potential.

Mites are an important allergenic source in the tropics, and species such as *Blomia tropicalis*, *D. farinae* and *D. pteronyssinus* have been implicated in allergic sensitization.[15,28] Cockroach, *Anisakis simplex* and shrimp are the sources implicated in cross reactivity with mites,[29,30] and tropomyosin is considered the major allergen in cross reactivity among invertebrates.[31] In **Table 2**, we reported identity between five allergens from *D. pteronyssinus* and homologous in *A. fumigatus*. This increases the repertoire of allergens involved in cross reactivity between these sources.

Methodology used here was based on bioinformatic, we considered this was adequate for initial explorations to determine cross reactivity between the allergenic sources studied. We know that results obtained in our study need to be validated by experimentation. However, bioinformatic tools used in were robust and previously validated for allergen characterization and study.[32,33]

CONCLUSION

At least 12 allergens could be implicated in cross reactivity between *A. fumigatus* and *D. pteronyssinus*, two allergenic sources with clinical relevance. Proteomic and serological studies are needed to confirm these results.

DECLARATIONS

Author contribution´s

JV conceived the study, performed data curation, conducted the bioinformatic analyses, and drafted the initial manuscript. AS contributed to methodology development, structural comparison, validation of results, and critical manuscript revision. JS provided scientific supervision, supported evolutionary and structural analyses, contributed to clinical interpretation, and revised the manuscript for intellectual content. EG assisted with sequence analyses, 3D structural visualization, epitope evaluation, and technical editing. MM led the overall study design and project administration, supervised all stages of the investigation, contributed to immunological interpretation and methodological decisions, critically reviewed the manuscript, approved the final version, and assumes full responsibility for the integrity of the work.

Conflict of interest

The authors declare not to have any conflict of interest.

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Ethics responsibilities

The study was carried out in adherence to ethical standards, the Regulation of the General Health Law on Health Research, and the Declaration of Helsinki.

Human and animal rights and informed

Consent This article does not contain any studies with human, or animal subjects performed by any of the authors.

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