Chitinases as a new group of pan allergens: an *in silico* approach to their structural and immunological basis

Quitinasas como un nuevo grupo de panalérgenos: un enfoque in silico desde sus bases estructurales e inmunológicas

Marlon Múnera[1], Neyder Contreras[1], Andrés Sanchez[1,2], Jorge Sanchez[2], Yuliana Emiliani[1]

[1] Health Faculty, Medical Research group (GINUMED) Universitary Corporation Rafael Nuñez.
[2] Group of Clinical and Experimental Allergy (GACE), IPS Universitaria, University of Antioquia, Medellin, Colombia.

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Autor de correspondencia:
Marlon Múnera
marmunera@gmail.com

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ABSTRACT

Introduction: chitinases are chitin-modifying enzymes that have been reported as allergens in plants and, to a lesser extent, in animals, though they possess cross-reactivity due to their high conservation.

Objectives: to explore the allergenic potential and molecular mimicry among chitinases from common allergenic sources using bioinformatics.

Methods: ElliPro and BepiPred were used to predict B and T cell epitopes. Phylogenetic, identity, and structural conservation studies were conducted using MEGA 5, PRALINE, and Consurf. 3D models of chitinases not reported in the Protein Data Bank were obtained using Swiss model. Ligand binding capacity was explored with AutoDock Vina, using Bisdionin C, Bisdionin F, and Montelukast as ligands.

Results: the chitinase from *P. americana* (Per a 12) shares 44% identity with homologs in *P. vannamei*, mites, and insects, and moderate identity with human chitinase. High structural homology was revealed. A linear epitope between residues 127 and 144 is highly conserved in all chitinases. Three conserved T cell epitopes were predicted. Molecular docking simulations revealed the active site and ligand-binding potential, identifying critical residues.

Conclusions: we propose chitinases as a potential new group of panallergens, explaining sensitization cases to various allergenic sources. Given their homology to human proteins, they deserve immunological exploration to support their implication in autoimmune responses.

Keywords: allergen; chitinase; cross reactivity; bioinformatics; epitope; docking.

RESUMEN

Introducción: las quitinasas son enzimas modificadoras de quitina y se han reportado como alérgenos en plantas y poco en animales, aunque poseen reactividad cruzada debido a su alta conservación.

Objetivo: explorar el potencial alergénico y el mimetismo molecular entre quitinasas de fuentes alergénicas comunes mediante bioinformática.

Métodos: se utilizaron ElliPro y BepiPred para predecir epítopos de células B y T. Se realizaron estudios filogenéticos, de identidad y de conservación estructural con MEGA 5, PRALINE y Consurf. Se obtuvieron modelos 3D de quitinasas no reportadas en el Protein Data Bank mediante Swiss model. La capacidad de unión de ligandos se exploró con AutoDock Vina, utilizando Bisdionina C, Bisdionina F y Montelukast como ligandos.

Resultados: la quitinasa de *P. americana* (Per a 12) comparte un 44% de identidad con homólogos en *P. vannamei*, ácaros e insectos, y una identidad moderada con la quitinasa humana. Se reveló una alta homología estructural. Un epítopo lineal entre los residuos 127 y 144 está altamente conservado en todas las quitinasas. Se predijeron tres epítopos de células T conservados. Las simulaciones de acoplamiento molecular revelaron el sitio activo y el potencial de unión de varios ligandos, identificando residuos críticos.

Conclusión: proponemos a las quitinasas como un nuevo grupo potencial de panalérgenos, explicando casos de sensibilización a varias fuentes alérgenas. Dado su homología con proteínas humanas, merece una exploración inmunológica para apoyar su implicación en la respuesta autoinmune.

Palabras Clave: alérgeno; quitinasas; reactividad cruzada; bioinformática; epitope; acoplamiento molecular.

INTRODUCTION

Chitinases are enzymes that assist in catalyzing the degradation of chitin, a long-chain polymer of N-acetylglucosamine located as a primary component of cell walls in several organisms (1). Chitinases consist of a few conserved repeats of amino acids and enzyme core, which has 8 strands of parallel β sheets, creating a barrel positioned down α helices, in turn forming a ring towards the outside, with a molecular weight range between 40 to 85 KDa (2). Chitinases are well known as allergen in plants (3). They are considered panallergens from fruits (4). Frequency of IgE reactivity in populations sensitized to these allergenic sources range between 67 to 72%(4, 5). These enzymes have been identified in mammals, which belong to the family 18 of glycosyl hydrolases (GH18), which can be divided into chitinase-like proteins with no enzymatic activity, and enzymatically active true chitinases (6). Also, chitinases are reported in some allergenic sources, such as: cockroaches and mites (7, 8). For the species *Periplaneta americana*, a chitinase with a molecular weight of 45 KDa and frequency of reactivity of 63% in a population of allergic subjects sensitized to cockroach was registered in Allergome database under name Per a 12 (7). The purified allergen inhibited IgE reactivity to extract cockroaches by 40% and shared a 33% amino acid sequence identity to group 15 mite allergens. Allergens from *P. americana* have been classified into nine groups with different physical and biochemical properties (9). However, there remains characterization about their antigenic potential and presence of homologous in other allergenic sources.

In 2016, a new allergen belonging to the chitinase family was reported. Der p 18, from the mite species *Dermatophagoides pteronyssinuss* had a frequency of reactivity of 10% (8). According to characterization experiments, Der p 18 showed a molecular weight of 51 kDa and a topology according to chitinase class (8). Cockroaches and

mites are considered important allergenic sources to develop allergic responses in atopic subjects (10, 11).

Due to their relative abundance and homology in nature, chitinases could represent an emergent group of allergens with significant role in allergy. Exploration of new sources and possible cross reactivity is important to determine new cases of sensitization. Before, it has been demonstrated homology among environmental allergens with human proteins and its relevance for autoreactive response in allergy (12). Here, we explored chitinases from several allergenic sources in advance to understand this new class of allergens and possible implications in autoreactive response in humans.

METHODS

Searching homologous with BLAST (Basic Local Alignment Search Tool)

The amino acid sequence from allergen Per a 12 (Uniprot accession: A0A059WIM0), an allergenic chitinase from *Periplaneta americana* was used as query to performance a search with PSI-BLAST tool to search for homologous. Parameters were set up as default.

Sequence Retrieval and Phylogenetic Analysis

The amino acid sequences of lipocalins from six allergenic sources and human: *P. vannamei, Dermatophagoides pteronyssinus* (Q86R84), *Drosophila melanogaster, Blomia tropicalis* (A1KXI8), *Aspergillus fumigatus* (2XVP), *Hevea brasiliensis (CAC42881.1) and Homo sapiens* (Q13231) were selected based on the reported allergenic capacity, with exception that was used to comparison purpose (Table 1). The sequences were obtained from the UniProt database. Sequences that were reported by the World Health Organization (WHO)/International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee and had complete sequences were used. Identity grades among chitinases used in this study were determined by using the tool Jalview 2.1. Parameters to perform alignment were set up to use BLOSUM62 as an exchange matrix. Three iterations were used, with an E-value of 0.01. Structural homology and Root Median Square Deviation values were determined using UCSF Chimera (V. 1.13.1) and PDB Viewer software (https://spdbv.vital-it.ch/)(13).

Construction of 3D model

The 3D models of chitinases from *P. americana, P. vannamei, Drosophila melanogaster and D. pteronyssinus* were generated by homology using the SWISS-MODEL server. The quality of the model was analyzed by ProSA-web. The model was refined in DeepView (energy minimization and rotamer replacements). Its quality was evaluated by several tools, including Ramachandran graphs, WHATIF, QMEAN4 index, and energy values (GROMOS96 force field). Three-dimensional structures (PDB: 3FXY) of the human chitinase were retrieved from the Protein Data Bank.

Search of epitopes in database

Immune Epitope Database and Analysis Resource was used to search epitopes from chitinases reported previously with experimental data. Parameters were set up as default. Chitinase word was used as input for searching.

B and T cell epitope prediction

ElliPro and BepiPred tools were used to predict B and T cell epitopes on Per a 12[18]. With ElliPro, the 3D structure of Per a 12 was used to predict epitopes. Minimum score and maximum distance (Angstrom) were set to 0.5 and 6.

Generation of motifs

Motifs were generated in an iterative way starting with allergen sequences of two Allergome databases, namely the original one from February 2003 (779 sequences) and the follow up one from April 2005 (1102 sequences). In the iterative

procedure, the algorithm MEME (Multiple EM for Motif Elicitation) was used for identifying motifs in the allergen sequences. Briefly, in the first step, the allergen database was analyzed with MEME to determine a motif representing a set of related sequences. These sequences were removed from the allergen database. The remaining sequences were subjected to MEME analysis for the generation of a motif representing a new set of related sequences. This procedure was repeated until no more statistically relevant motifs (E-value less than 0.01) were detected. The motif size was defined as 50 amino acids in order to represent the mean length of a protein domain and to prevent the generation of multi-domain motifs.

Identification of domains

Identification of domains in proteins was performed using the CATH system. Amino acid sequence of Per a 12 was used as a query to analyze.

Conservation analysis

The 3D structure of Per a 12 was submitted to the ConSurf server in order to generate evolutionarily related conservation scores to help to identify functional regions in the proteins.

Selection of ligand

From the studies on the experimental inhibitory activity against AMcase, a database was realized with the main molecules reported (14), whose structures were search through the obtained from Protein Data Bank (PDB) (15) reported as ligands associated to crystallographic structure, and PUBCHEM database (16). The structures were identified with common name and accession number, respectively. 7a (95N), 7g (95K), 7i (95Q), Argifin (449124), Bisdionin C (5025063), Bisdionin F (15602759) and Montelukast (5281040).

Preparation of ligand and receptor

BIOVIA Discovery Studio software version 4.5 (17) was used to correct the structures through the conversion of the format of the structure, addition of hydrogen atoms, neutralization of charges, and clean geometry. The representative crystallographic structure of Acidic mammalian chitinase (AMCase) (Accession number 2YBT, resolution of 2.22 Å) was obtained from Protein Data Bank (PDB) (15), and homologous structures as Per a 12 and Der p 18 was selected. For the preparation of the receptors, the proteins in PDB format obtained were prepared through the addition of hydrogen atoms, the elimination of solvent molecules (water), extraction of ligands and the allocation of partial charges using the software packages UCSF Chimera version 1.13 (13).

Molecular docking

The molecular docking was performed with AutoDock Vina 4.2.1 (18), based at PyRx 0.8 as a graphic interface (19). Prior to molecular docking, a virtual screening of inhibitors and drug as references in treatment of asthma was carried out to determine the molecules with the best structural affinity against AMcase studied; Thereby, were minimized by a universal force field (uff) and conjugate gradients including 200 steps using an algorithm supported for Open Babel tools (20). AMcase and inhibitors were developed in a grid space of x = 39.2 Å, y = 37.1 Å, z = 36.5 Å. Subsequently, each docking simulation was executed for a single molecule and obtained nine conformations predetermined by software based on effectiveness value and free energy of binding, which are classified according to the energy level of each conformation. The best conformation structures according to affinity energy were visualized using PyMOL software version 2.3.2 (21), and finally, saved in PDB format. BIOVIA Discovery Studio visualizer version 4.5 (22) was used for interaction forces and amino acids.

RESULTS

Per a 12 and chitinases exhibited identity

BLAST analysis performed with amino acid sequence from Per a 12 found chitinases

homologous from several allergenic sources such as: *D. pteronyssinus*, *P. vannamei* and *B. tropicalis* (Table 1). A human chitinase was reported in the search and added to analysis. Molecular weights from chitinases ranged from 31 to 73 KDa (Table 1). Phylogenetic analysis using Maximum Likelihood method suggests that Per a 12 is closest to *D. pteronyssinus*, *P. vannamei* and human (Figure 1). Algorithm MEME identified three motifs in all chitinases, except *A. fumigatus* and *H. brasiliensis* (Figure 2). Supporting lack of identity. Multiple alignment among amino acid sequences from all chitinases showed a moderated level of identity among amino acid sequences of 44% (Figure 3). Identity analysis revealed that *Per a 12* and chitinases from human and other allergenic sources share an identity level ranging between 30 and 43% (Table 2) in amino acid sequences. A low identity of only 16% was found for chitinases from *A. fumigatus* and *H. brasiliensis*, two important allergenic sources.

Table 1. Chitinases used in study, proteins show differences in amino acid sequence length and other properties

Chitinase name	length	pI	Mw (Daltons)
P. americana	407	5.94	45669.58
H. sapiens	387	5.11	42594.90
A. fumigatu	310	4.32	34067.12
D. melanogaster	469	5.36	52572.30
D. pteronyssinus	751	8.42	85094.59
P. vannamei	659	6.10	73005.25
B. tropicalis	461	5.62	51647.71
H. brasilensis	295	5.21	31647.24

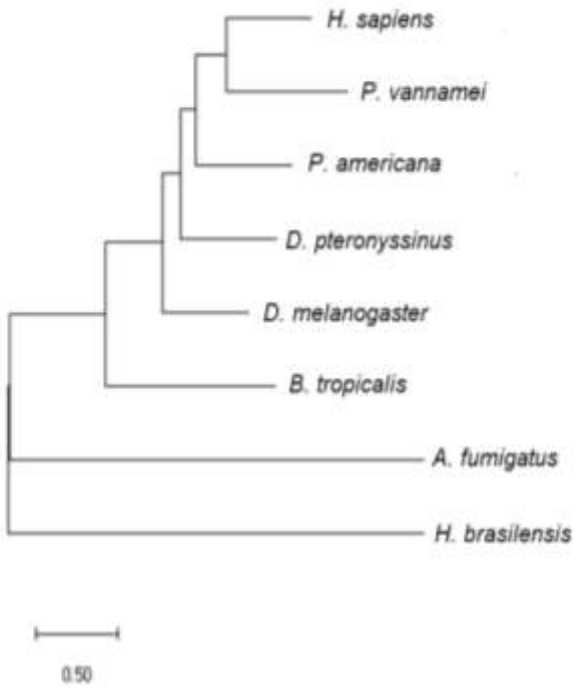

Figure 1. Evolutionary history of chitinase used in study inferred by using the Maximum Likelihood method. According to results, chitinase from P. americana exhibited a major relation with homologous *P. vannamei* and humans.

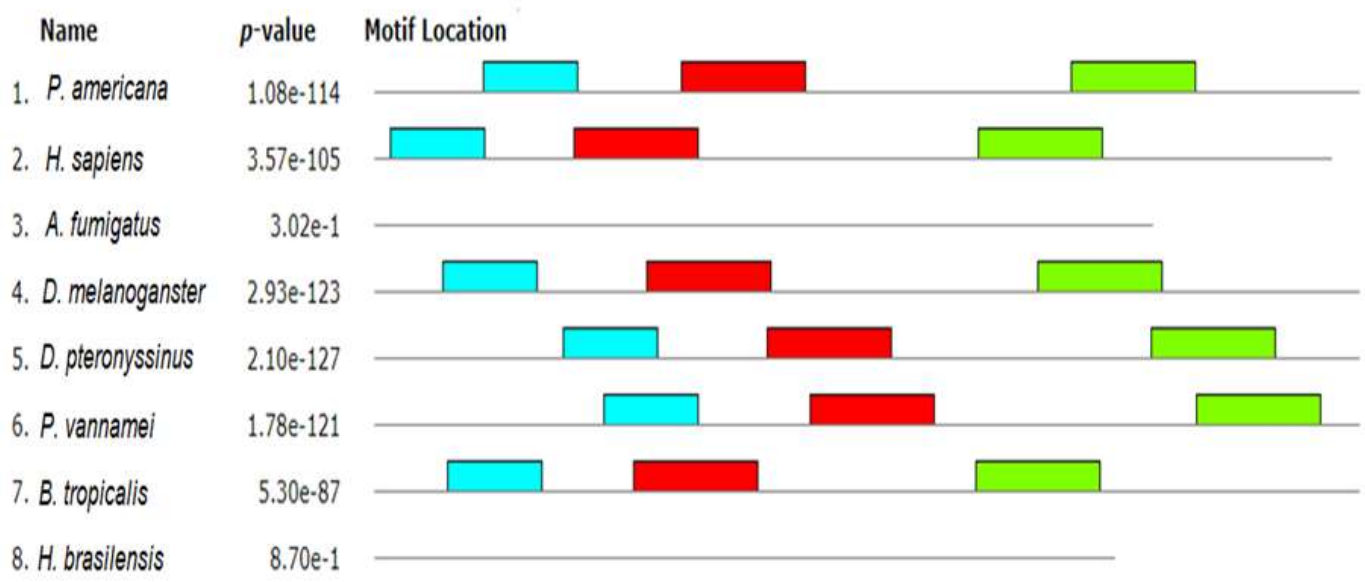

Figure 2. Analysis for motif location. It shows numbers of motifs found on chitinases.

Table 2. Identity matrix among amino acid sequences of chitinases. *H. brasilensis* and *A. fumigatus* share the lowest degree identity among chitinases tested

	P. americana	*H. sapiens*	*A. fumigatus*	*D. melanogaster*	*D. pteronyssinus*	*P. vannamei*	*B. tropicalis*	*H. brasiliensis*
P. americana	100	41	16	43	43	43	30	13
H. sapiens	41	100	17	41	44	43	25	12
A. fumigatus	16	17	100	16	16	15	13	15
D. melanogaster	43	41	16	100	42	39	29	17
D. pteronyssinus	43	44	16	42	100	35	29	12
P. vannamei	43	43	15	39	35	100	27	11
B. tropicalis	30	25	13	29	29	27	100	12
Hevea brasiliensis	13	12	15	17	12	11	12	100

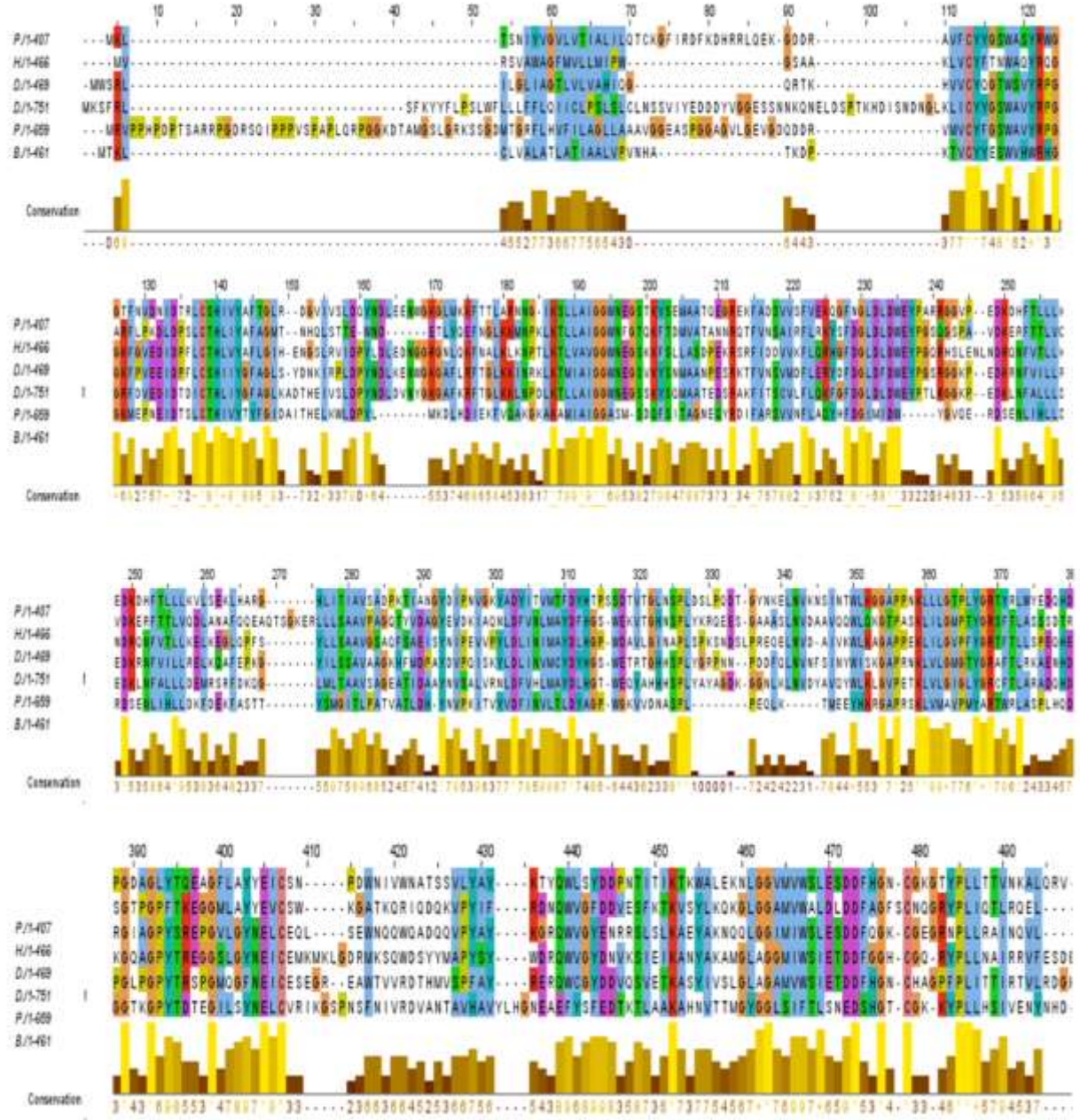

Figure 3. Multiple alignment among chitinases from allergenic sources. Chitinases share a 47% of identity in their amino acid sequences.

Structural homology

In this study, 3D models of chitinase from *P. Americana, D. melanogaster, P. vannamei* and *D. pteronyssinus* were obtained by homology modeling. According to CATH results, chitinases from allergenic sources tested exhibited a typical fold of TIM barrel and have a three chitinase domain well conserved in its family (Figure 4A-E). All chitinases were conformed by eleven alpha helix and ten beta strands forming a cavity for accommodation of ligands. When Per a 12 and human chitinase structures are compared, a RMSD of 0.5 Å (Figure 4F-I). Indicating structural homology. Finally, Consurf results revealed that chitinases are highly conserved across nature (Figure 5).

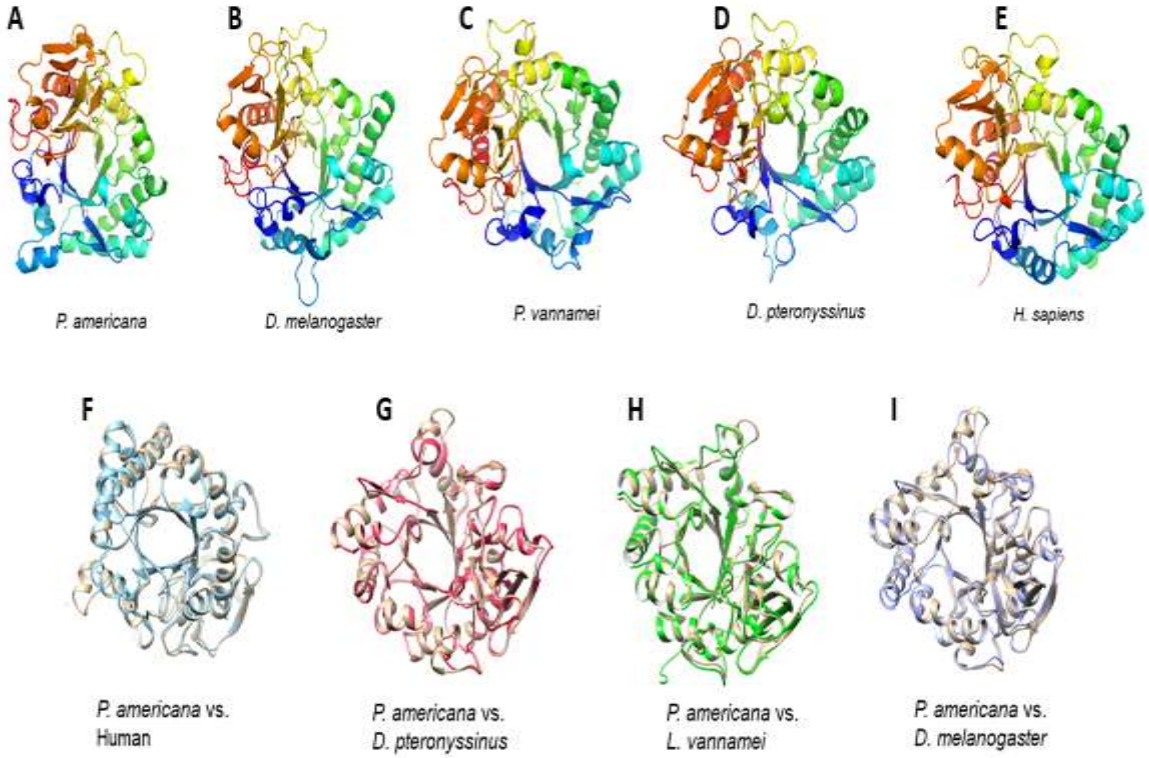

Figure. 4. Structural representation of chitinases used in the study. A-E: 3D cartoon models of chitinases from several allergenic sources. Showing a typical α/β fold of chitinase family. F-I: superposition for comparisons of 3D structures of chitinase from *P. americana* (Brown) and human (blue), *D. pteronyssinus* (red), *L. vannamei* (green) and *D. melanogaster* (violet). Analysis revealed structural homology with RMSDvalues below to 0.8 Å.

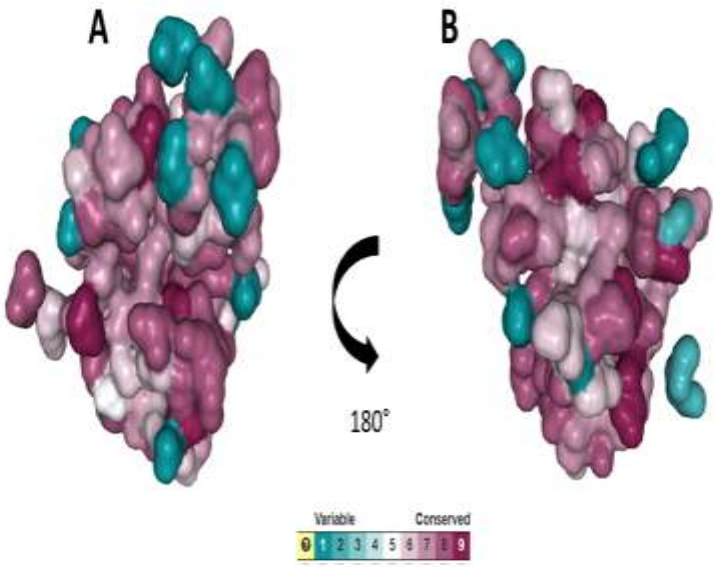

Figure 5. Phylogenetic analysis of lipocalins by using Consurf. (A–B) Surface models showing conserved regions among lipocalins.

B and T epitopes were predicted on chitinases

Using ElliPro and BepiPred servers, three B and T epitopes on Per a 12 were predicted. First B epitope was located between residues 52 to 66 in a loop with a surface area of 230 Å. This epitope is conserved among all chitinases tested. Meanwhile, for second and third epitopes positions 86 to 106 and 127 to 144, correspondingly, were located on alpha helix with surface area of 250 Å (Table 3), (Figure 6A-B).

Table 3. Linear epitopes predicted by Ellipro and Bepipred servers. LE (Lineal epitope).

Epitope	Sequence	Position
LE1	SYRWGVGTFNVDNI	52-66
LE2	IVSLDQYNDLEENWGKGLMKK	86-106
LE3	EGSTKYSEMAATQEGREK	127-144

For T cell epitope, first and second were located on beta strands spanning residues 69 to 83 and 111 to 125. Third T cell epitope located in an alpha helix cover residue 149 to 163 (Figure 6C-D), (Table 4).

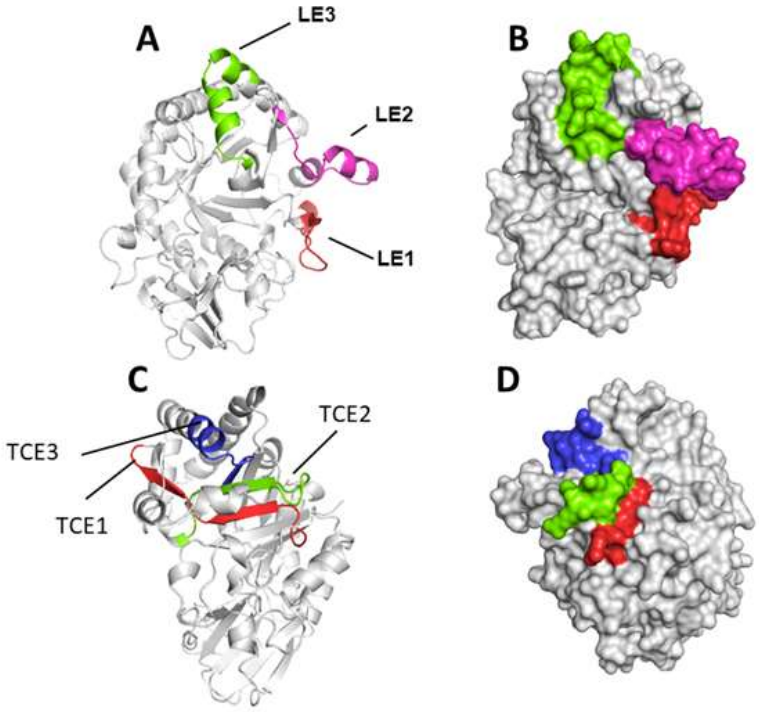

Figure 6. Cartoon and Surface models showing epitopes predicted. A-B: location of linear epitopes predicted, and area covered by them. C-D: location of T cell epitopes and area covered by them.

Table 4. T cell epitope predicted on Per a 12

Number epitope	Sequence	Allele	Start	End
TCE1	RLCSHIVYAFTGLRD	HLA-DRB1*01:01	69	83
TCE2	ARNNGIKSLLAIGGW	HLA-DRB1*01:02	111	125
TCE3	VVSFVEKQGFNGLDL	HLA-DRB1*01:01	149	163

Docking

Molecular Docking interactions between the inhibitors with binding affinity to *H. sapiens*, Der p 18, Per a 12 are shown in Figure 7A-I, which two inhibitors and drug reference showed the interaction and affinity for each protein. In the Table 5, is evidenced binding energies ≥8.9 Kcal/mol, affinity values that are minor comparatively to the reference molecules. Different types of interactions were observed between molecules and the residues of the pocket formed in the proteins, such as van der Waals, H-bond (conventional and carbon), π-sigma, alkyl and π-alkyl, π-π-Stacked, π-π-T-shaped, Attractive charge, π-sulfur, π-anion. Likewise, for 2YBT are shown common amino acids involved in binding such as Glu123, Met193, Tyr195 and Asp196; for Der p 18 were identified Trp83, Glu200, Tyr201, Tyr265 and Asp266; in Per a 12 were identified interaction with Asp232, Tyr287, Phe320 and Trp378. The binding affinity of selected inhibitors where it can be seen that the 7i compound and Argifin demonstrated affinity values of -9.8 Kcal/mol for 2YBT; -9.6 and -8.9 Kcal/mol for Argifin and Bisdionin F; and for Per a 12 were obtained high interaction with 7i compound and Argifin, which were register values of -10.2 and -9.8 Kcal/mol, respectively. On the other hand, drugs reference (Montelukast) showed energies of binding of 10.4, -10.2 and -11.1 Kcal/mol, for 2YBT, Der p 18 and Per a 12, respectively. The residues of interaction between inhibitors, drug and AMCases demonstrate that alkyl, π-alkyl, π-π-Stacked, H-bond (conventional and C-bond) were found to strengthen. However, interaction types π-sulfur, π-anion and π-σ were observed in the three proteins that bind to Montelukast and only determine halogen interaction with Glu127 in Per a 12. Equally, it was found that aromatic interactions between the active site of proteins and molecules involved in the binding as described in Figure 8.

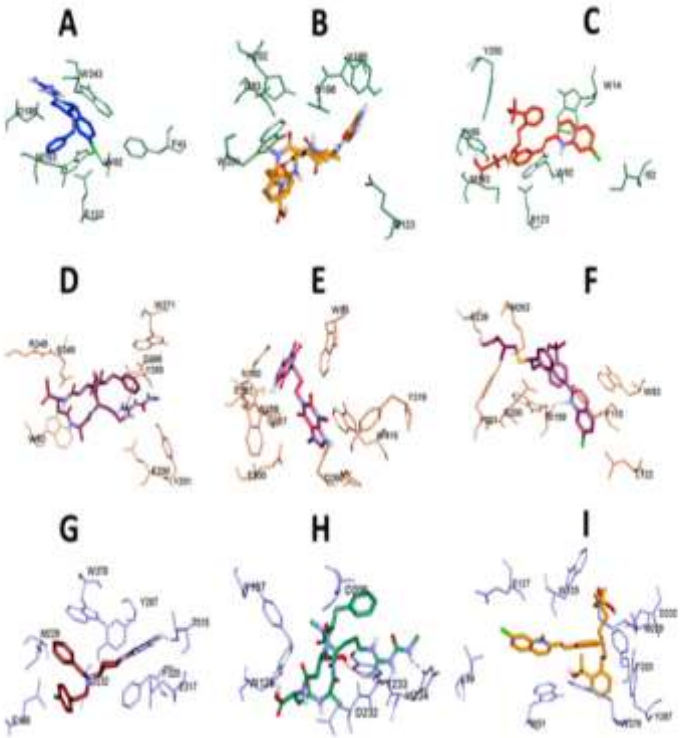

Figure 7. Residues of interaction in AMCases with inhibitors and Montelukast (Drug). A. 2YBT and 7i compound. B. 2YBT and Argifin. C. 2YBT and Montelukast (Drug). D. Der p 18 and Argifin. E. Der p 18 and Bisdionin F. F. Der p 18 and Montelukast (Drug). G. Per a 12 and 7i compound. H. Per a 12 and Argifin. I. Per a 12 and Montelukast (Drug).

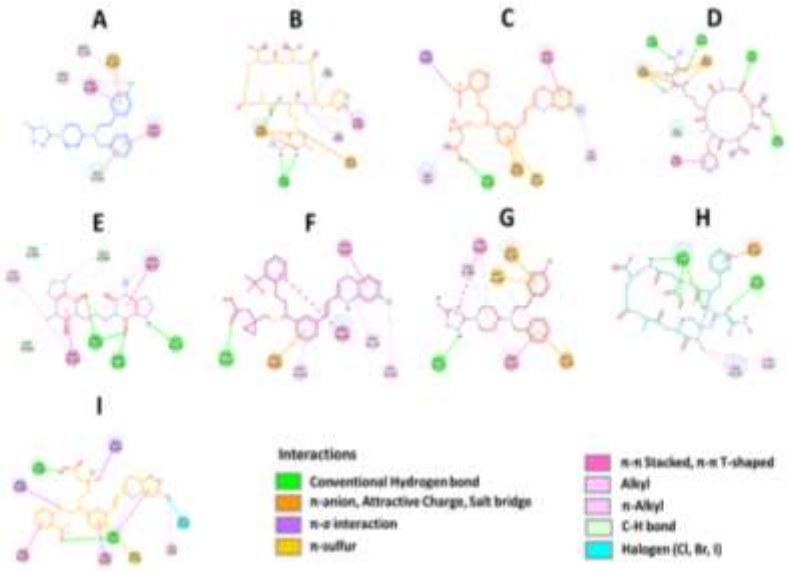

Figure 8. Interactions between active site of AMCases with inhibitors and Montelukast (Drug). A. 2YBT and 7i compound. B. 2YBT and Argifin. C. 2YBT and Montelukast (Drug). D. Der p 18 and Argifin. E. Der p 18 and Bisdionin F. F. Der p 18 and Montelukast (Drug). G. Per a 12 and 7i compound. H. Per a 12 and Argifin. I. Per a 12 and Montelukast (Drug).

Table 5. Molecular interactions of AMcases active site with selective inhibitors highest binding energy and Montelukast (drug for asthma treatment).

Protein	Ligands	Binding energy (Kcal/mol)	Residues	Type interacting
2YBT	7i compound	-9.8	Phe41, Trp82, Glu123, Met193, Asp196, Trp343	Alkyl, π-alkyl, π-anion, H-bond (C-bond), π-π-Stacked
	Argifin	-9.8	Glu123, Tyr195, Asp196, Trp201, His252, Ile283	Alkyl, π-alkyl, π-π-Stacked, H-bond (Conventional and C-bond), Attractive charge
	Montelukast	-10.4	Trp14, Ile52, Trp82, Glu123, Met193, Tyr195, Tyr250	Alkyl, π-alkyl, π-π-Stacked, H-bond (Conventional), π-anion, π-sulfur, π-σ
Der p 18	Argifin	-9.6	Trp83, Gly157, Trp159, Asn160, Glu200, Tyr265, Asp266, Tyr319, Trp415	π-alkyl, π-π-Stacked, H-bond (Conventional and C-bond)
	Bisdionin F	-8.9	Trp83, Glu200, Tyr201, Tyr265, Asp266, Trp271, Arg348, Glu349	H-bond (Conventional and C-bond), Salt bridge, π-π-T-shaped, Attractive charge
	Montelukast	-10.2	Trp93, Phe110, Leu122, Trp159, Glu200, Tyr201, Met263	Alkyl, π-alkyl, π-π-Stacked, π-π-T-shaped, H-bond (Conventional), π-anion
Per a 12	7i compound	-10.2	Glu166, Met229, Asp232, Tyr287, Glu317, Phe320, Trp378	π-alkyl, π-π-T-shaped, Attractive charge, π-sulfur, H-bond (Conventional)
	Argifin	-9.8	Trp125, Tyr167, Asp206, Asp232, Tyr233	π-alkyl, π-anion, H-bond (Conventional)
	Montelukast	-11.1	Trp51, Leu89, Trp125, Glu127, Met229, Asp232, Tyr287, Phe320, Trp378	Alkyl, π-alkyl, π-π-T-shaped, π-π-Stacked, H-bond (Conventional), π-sulfur, π-σ, Halogen

DISCUSSION

Identification and characterization of new allergenic sources is important to improve diagnosis and potential therapeutic treatment of allergic diseases. Here, we explored using a bioinformatic approach the cross reactivity between chitinases from allergenic sources with clinical impact in developing allergic symptoms, such as: shellfish, mites and insects (23-25). In this study, a group of chitinases was analyzed and we found moderate identity among their amino acid sequences and structural homology according to RMSD analysis. A lineal epitope spanning residues 52 to 66 was predicted by Ellipro and Bepipred tools.

According to multi alignment analysis, epitope sequence is highly conserved among chitinases analyzed and could be involved in cross reactivity. This fact is supported by analysis performed with the Consurf tool. It revealed that the position occupied by cross reactive epitopes is conserved in the chitinase family. Our results provided first evidence that shows chitinase as an important pan allergen family. Also, we provided evidence that exhibit chitinase from humans as a homologous to environmental chitinases. This is important because many allergens homologous to human proteins have been implicated in autoallergy response (12, 26, 27). And the fact that three conserved T epitopes were predicted, could be expected to participate in cell-mediated autoimmunity.

According to our results, chitinases from *A. fumigatus* and *H. brasilensis* don't share identity or structural homology with other chitinases tested here. Considering that *H. brasilensis* is an important allergenic source containing chitinases as allergens (28, 29), they don't have capacity to cross react with chitinases in animals. For *A. fumigatus*, allergenic capacity of chitinase had not been elucidated, however, similar results with chitinases from *H. brasilensis* were found.

Shrimp is a high demand food, a high frequency of sensitization is reported among allergic patients to shellfish, and sometimes with fatal results (30). Several allergens have been characterized, with tropomyosin as major allergen (24). However, full allergome spectra remain to be characterized. Here, we showed chitinases as potential allergens from *P. vannamei.* It's important to elucidate its role as an allergenic component to improve diagnosis and treatment of allergic diseases. Also, sensitization to shrimp is correlated with cockroaches and mites (30).

According to frequency of IgE reactivity reported for Der p 18 (63%) (8), this new class of allergens from animals could be involved in new cases of cross sensitization. For this, epitope mapping is crucial to understand antigenic properties of allergens. For chitinases, just are mapped epitopes for just a few organisms, such as: *Onchocerca volvulus, Leishmania mexicana, Plasmodium gallinaceum* and *Sarcoptes scabiei* (31-34). However, none of epitopes mapped share identity with chitinases reported in this study (Data not shown). So, this is the first approach in reporting potential cross reactive antigenic regions in chitinase with clinical relevance for allergic disease.

The role of chitinases in mammals is still unknown, however, different experimental approaches have established that their expression could regulate innate and adaptive immune responses, and it has even been indicated that CHIT1, expressed in phagocytes, contributes to the innate immune response. of mammals against fungi, bacteria and other pathogens (35, 36). Likewise, AMCase has been linked to lung production during allergic inflammation and asthma, where it has been demonstrated in models of antigen-induced pulmonary allergic inflammation in mice and also in humans AMCase increases in the lungs of patients

with asthma exposed to allergens and in alveolar macrophages in cases of fatal asthma (37, 38). Therefore, due to low certainty of the mechanisms associated with the induction of allergy, inflammation and responses such as asthma, molecular docking studies have been implemented as an alternative to establish the possible interaction of inhibitory agents at the decrease in clinical conditions related to chitinase mediating mechanisms.

Three AMCases were evaluated against reported inhibitors, in which a considerable affinity was established against this type of chitinase enzymes, an energy binding > 8.9 Kcal/mol was indicated, demonstrating a high interaction with compounds 7i, Argifin and Bisdionin F. From this Thus, it has been described that molecules such as Bisdionin demonstrate a selective strategy with considerable response *in vitro* and *in vivo* in the function of AMCases related to inflammatory responses directed by Th2 (38). In addition, experimental inhibition studies have stated that Bisdionin F and C in hAMCase in vitro recombinant models appear to reduce lung chitinase production and eosinophilic cell levels in allergic mice (39).

Thus, Bisdionin analogues interact with the active site of Der p 18 by means of methylxanthine groups with aromatic residues Trp83 and Trp415 establishing connections type π-π-Stacked, as well as conventional H-bond with residues of Trp159 and Asn160 with Oxygens of the carbonyl group of methylxanthines present in Bisdionin F (14). On the other hand, Argifin has a selective inhibitory activity against AMcases given a complex structure of natural origin characterized by a cyclic peptide in which it includes an N-methylcarbamoyl-L-arginine chain, which demonstrates ionic interactions, attractive charges and dependent on the protonation states of the guanidine group and the glycine group that maintain a union with the residues such as Glu123 and Asp126 in 2YBT; Glu200 and Asp266 in Der p18 and Asp206 in Per a 12 (40-42). Likewise, H-bond interactions are observed by Tyr195 in 2YBT, Tyr265 and Tyr201 in Der p 18; Tyr233 in Per a 12.

On the other hand, other types of inhibitors resulting from synthetic development such as those proposed in the studies of Mazur et al., Indicate that compounds 7i (1- (3-Amino-1H-1,2,4 -triazol-5-yl) -N-benzyl-N- (4-chlorophenethyl) piperidin-4-amine), have a high selectivity in the experimental and theoretical bases against AMcase, in which a remarkable anti-inflammatory effect has been demonstrated in based on chitin-containing house dust (HDM) allergens models. The *in silico* studies obtained indicate that hydrophobic interactions occur at the binding site, specifically with Phe41, Trp82, Met193, Trp343 residues that are related to the phenethyl and chlorobenzyl substituents in the enzyme 2YBT (43) and additionally, the residues Glu166 and Asp292 establish attractive interactions, as well as hydrophobic bonds with Met220 and Trp378 are established with these substituents in the enzyme Per a 12.

Currently the use of Montelukast has been widely described as a therapeutic agent in clinical conditions related to asthma and as a major alternative in the treatment of these patients. Although this drug indicates other mechanisms of action such as the antagonistic or blocking effect of leukotriene receptors and their selective secretion blockade, with a decrease in the infiltration effect of inflammatory cells and therefore a decrease in bronchoconstriction mechanisms (44).

In our study, it was selected as a drug reference at the molecular docking and as an element of comparison with AMcase inhibitors. However, a high affinity for the active site was obtained, demonstrating a higher energy of 10.2 Kcal/mol. Independent of not being associated with its physiological mechanism. In a study proposed by Dymek et al., In which the therapeutic efficiency of

a dual agent (AMcase/CHIT1 inhibitor) was compared, Montelukast was used in inhibition assays in chronic rodent models HDM-induced airway inflammation, in the which indicated that it had chitinolytic inhibition activity at values of 30 mg/Kg, which could partly relate the interaction obtained with the enzymes studied (45).

CONCLUSION

We provided a bioinformatic basis to propose to the chitinases as a new potential group of pan allergens that could explain new cases of sensitization to several allergenic sources. Given the fact that they are homologous to humans, they deserve an immunological exploration to support its implication in autoreactive response.

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REFERENCES

1. Oyeleye A, Normi Yahaya M. Chitinase: diversity, limitations, and trends in engineering for suitable applications. Bioscience Reports. 2018;38(4): BSR2018032300.

2. Rathore AS, Gupta RD. Chitinases from Bacteria to Human: Properties, Applications, and Future Perspectives. Enzyme Research. 2015; 2015:8.

3. O'Riordain G, Radauer C, Hoffmann-Sommergruber K, Adhami F, Peterbauer CK, Blanco C, et al. Cloning and molecular characterization of the Hevea brasiliensis allergen Hev b 11, a class I chitinase. Clin Exp Allergy. 2002;32(3):455-62.

4. Blanco C, Diaz-Perales A, Collada C, Sanchez-Monge R, Aragoncillo C, Castillo R, et al. Class I chitinases as potential panallergens involved in the latex-fruit syndrome. J Allergy Clin Immunol. 1999;103(3 Pt 1):507-13.

5. Volpicella M, Leoni C, Fanizza I, Placido A, Pastorello EA, Ceci LR. Overview of plant chitinases identified as food allergens. J Agric Food Chem. 2014;62(25):5734-42.

6. Hamid R, Khan MA, Ahmad M, Ahmad MM, Abdin MZ, Musarrat J, et al. Chitinases: An update. J Pharm Bioallied Sci. 2013;5(1):21-9.

7. Fang Y, Long C, Bai X, Liu W, Rong M, Lai R, et al. Two new types of allergens from the cockroach, Periplaneta americana. Allergy. 2015;70(12):1674-8.

8. Resch Y, Blatt K, Malkus U, Fercher C, Swoboda I, Focke-Tejkl M, et al. Molecular, Structural and Immunological Characterization of Der p 18, a Chitinase-Like House Dust Mite Allergen. PLoS One. 2016;11(8): e0160641.

9. Pomés A, Mueller GA, Randall TA, Chapman MD, Arruda LK. New Insights into Cockroach Allergens. Current allergy and asthma reports. 2017;17(4):25-.

10. Pomes A, Mueller GA, Randall TA, Chapman MD, Arruda LK. New Insights into Cockroach Allergens. Curr Allergy Asthma Rep. 2017;17(4):25.

11. Huss K, Adkinson NF, Jr., Eggleston PA, Dawson C, Van Natta ML, Hamilton RG. House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program. J Allergy Clin Immunol. 2001;107(1):48-54.

12. Hradetzky S, Werfel T, Rösner LM. Autoallergy in atopic dermatitis. Allergo J Int. 2015;24(1):16-22.

13. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13): 1605-12.

14. Liu T, Chen L, Ma Q, Shen X, Yang Q. Structural insights into chitinolytic enzymes and inhibition

mechanisms of selective inhibitors. Curr Pharm Des. 2014;20(5): 754-70.

15. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. Nucleic Acids Res. 2000;28(1): 235-42.

16. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem Substance and Compound databases. Nucleic Acids Res. 2016;44(D1): D1202-13.

17. BIOVIA DS. Discovery Studio Modeling Environment, Release 2017: San Diego: Dassault Systèmes; 2016.

18. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2): 455-61.

19. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2015;1263: 243-50.

20. Contreras-Puentes N, Mercado-Camargo J, Alvíz-Amador A. In silico study of ginsenoside analogues as possible BACE1 inhibitors involved in Alzheimer's disease [version 1; peer review: 1 approved]. F1000Research. 2019;8(1169).

21. DeLano WL. Pymol: An open-source molecular graphics tool. CCP4 Newsletter On Protein Crystallography. 2002;40.

22. BIOVIA DS. Discovery Studio Visualizaer, 4.5: San Diego: Dassault Systèmes; 2016

23. Yang Z, Zhao J, Wei N, Feng M, Xian M, Shi X, et al. Cockroach is a major cross-reactive allergen source in shrimp-sensitized rural children in southern China. Allergy. 2018;73(3): 585-92.

24. Múnera M, Gómez L, Puerta L. El camarón como una fuente de alérgenos. Biomédica. 2013;33: 161-78.

25. Fernandez-Caldas E, Puerta L, Caraballo L. Mites and allergy. Chem Immunol Allergy. 2014;100: 234-42.

26. Roesner LM, Ernst M, Chen W, Begemann G, Kienlin P, Raulf MK, et al. Human thioredoxin, a damage-associated molecular pattern and Malassezia-crossreactive autoallergen, modulates immune responses via the C-type lectin receptors Dectin-1 and Dectin-2. Scientific Reports. 2019;9(1): 11210.

27. Fluckiger S, Mittl PR, Scapozza L, Fijten H, Folkers G, Grutter MG, et al. Comparison of the crystal structures of the human manganese superoxide dismutase and the homologous Aspergillus fumigatus allergen at 2-A resolution. J Immunol. 2002;168(3): 1267-72.

28. Radauer C, Adhami F, Fürtler I, Wagner S, Allwardt D, Scala E, et al. Latex-allergic patients sensitized to the major allergen hevein and hevein-like domains of class I chitinases show no increased frequency of latex-associated plant food allergy. Mol Immunol. 2011;48(4): 600-9.

29. O'Riordain G, Radauer C, Hoffmann-Sommergruber K, Adhami F, Peterbauer CK, Blanco C, et al. Cloning and molecular characterization of the Hevea brasiliensis allergen Hev b 11, a class I chitinase. Clinical & Experimental Allergy. 2002;32(3): 455-62.

30. McGowan EC, Peng R, Salo PM, Zeldin DC, Keet CA. Cockroach, dust mite, and shrimp sensitization correlations in the National Health and Nutrition Examination Survey. Ann Allergy Asthma Immunol. 2019;122(5): 536-8.e1.

31. Drabner B, Reineke U, Schneider-Mergener J, Humphreys RE, Hartmann S, Lucius R. Identification of T helper cell-recognized epitopes in the chitinase of the filarial nematode Onchocerca volvulus. Vaccine. 2002;20(31-32): 3685-94.

32. Joshi MB, Rogers ME, Shakarian AM, Yamage M, Al-Harthi SA, Bates PA, et al. Molecular characterization, expression, and in vivo analysis of LmexCht1: the chitinase of the human pathogen, Leishmania mexicana. J Biol Chem. 2005;280(5): 3847-61.

33. Langer RC, Li F, Popov V, Kurosky A, Vinetz JM. Monoclonal antibody against the Plasmodium falciparum chitinase, PfCHT1, recognizes a malaria transmission-blocking epitope in Plasmodium gallinaceum ookinetes unrelated to the chitinase PgCHT1. Infect Immun. 2002;70(3): 1581-90.

34. Shen N, Zhang H, Ren Y, He R, Xu J, Li C, et al. A chitinase-like protein from Sarcoptes scabiei as a candidate anti-mite vaccine that contributes to immune protection in rabbits. Parasit Vectors. 2018;11(1): 599.

35. Alrifai M, Marsh LM, Dicke T, Kılıç A, Conrad ML, Renz H, et al. Compartmental and temporal dynamics of chronic inflammation and airway remodelling in a chronic asthma mouse model. PLoS One. 2014;9(1): e85839.

36. Boot RG, Blommaart EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, et al. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. J Biol Chem. 2001;276(9): 6770-8.

37. Okawa K, Ohno M, Kashimura A, Kimura M, Kobayashi Y, Sakaguchi M, et al. Loss and Gain of Human Acidic Mammalian Chitinase Activity by Nonsynonymous SNPs. Mol Biol Evol. 2016;33(12): 3183-93.

38. Kim LK, Morita R, Kobayashi Y, Eisenbarth SC, Lee CG, Elias J, et al. AMCase is a crucial regulator of type 2 immune responses to inhaled house dust mites. Proc Natl Acad Sci U S A. 2015;112(22): E2891-9.

39. Sutherland TE, Andersen OA, Betou M, Eggleston IM, Maizels RM, van Aalten D, et al. Analyzing airway inflammation with chemical biology: dissection of acidic mammalian chitinase function with a selective drug-like inhibitor. Chem Biol. 2011;18(5): 569-79.

40. Andersen OA, Nathubhai A, Dixon MJ, Eggleston IM, van Aalten DM. Structure-based dissection of the natural product cyclopentapeptide chitinase inhibitor argifin. Chem Biol. 2008;15(3): 295-301.

41. Hirose T, Sunazuka T, Sugawara A, Endo A, Iguchi K, Yamamoto T, et al. Chitinase inhibitors: extraction of the active framework from natural argifin and use of in situ click chemistry. J Antibiot (Tokyo). 2009;62(5):277-82.

42. Hirose T, Sunazuka T, Omura S. Recent development of two chitinase inhibitors, Argifin and Argadin, produced by soil microorganisms. Proc Jpn Acad Ser B Phys Biol Sci. 2010;86(2): 85-102.

43. Mazur M, Olczak J, Olejniczak S, Koralewski R, Czestkowski W, Jedrzejczak A, et al. Targeting Acidic Mammalian chitinase Is Effective in Animal Model of Asthma. J Med Chem. 2018;61(3): 695-710.

44. Langlois A, Ferland C, Tremblay GM, Laviolette M. Montelukast regulates eosinophil protease activity through a leukotriene-independent mechanism. J Allergy Clin Immunol. 2006;118(1):113-9.

45. Mazur M, Dymek B, Koralewski R, Sklepkiewicz P, Olejniczak S, Mazurkiewicz M, et al. Development of Dual Chitinase Inhibitors as Potential New Treatment for Respiratory System Diseases. J Med Chem. 2019;62(15): 7126-45.