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Research Article

Analysis of the Interaction between Household Animal Lipocalin in Simulator

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Abstract

One class of allergens originating from domestic animals that is clinically significant for the emergence of allergic reactions is lipocalins. They have been described in various animal species. Little is known about the epitopes involved in allergy responses despite the fact that allergenic capability has been characterised. Here, bioinformatics technologies were used to investigate putative antigenic areas involved in lipocalin cross-reactivity. Using phylogenetic analyses, the amino acid sequences of many lipocalins from various domestic animals (mouse, dog, cat, bull, hamster, horse, and pig) were utilised to ascertain the level of kinship. Using MEGA programme, the groups with the highest phylogenetic relations were found. To find putative antigenic areas weakened in cross-reactivity, homology was used to create 3D lipocalins not listed in the protein data bank. The maximum likelihood tree and the alignment of the whole database of allergenic lipocalins separate lipocalins into five monophyletic clades (referred to as A, B, C, D, and E in this context). The numerous pairing analyses revealed that their amino acid sequences had the highest level of identity (58%). Three antigenic sites that group C shares, which may help explain some of its cross-reactivity, were discovered through the examination of conserved and exposed residues. For the purpose of generating cross-reactivity between the various lipocalins examined in this investigation, potential antigenic sites were found. These findings bolster the need for directed mutagenesis experiments to verify their applicability in determining lipocalins' allergenic capacity.

Keywords : *Bioinformatics, Immunology, Prediction, Epitope, Allergen, Antigen*

Introduction

"A hypersensitivity reaction initiated by immunological mechanisms" is how the WAO defines an allergy [1]. Cells or antibodies can mediate this; immunoglobulin E (IgE) is typically the causative antibody. Allergens are described as structurally variable compounds that have the ability to bind IgE and are typically associated with a carbohydrate [2] [3]. Patients may be more sensitive to certain allergens than others, and certain genetic factors predispose them to certain diseases. Environmental allergens can cause allergy illnesses such as rhinitis, conjunctivitis, asthma, and atopic dermatitis.

Lipocalins are one type of these allergens; they are the most significant group of allergens derived from hairy mammals. Moreover, the variety and quantity of pets that are kept in homes has increased lipocalin exposure, which has increased sensitivity [4] [5]. The lipocalins are a diverse family of proteins found in bacteria, plants, and invertebrate and vertebrate animals. Their structures and roles vary greatly throughout species. These proteins are comparatively small, ranging in size from 150 to 250 amino acid residues in their basic structure. They can perform a variety of tasks, including attaching to surface receptors and acting as a transporter of small hydrophobic compounds like retinol. Numerous proteins, as well as other members of this family, have been described based on their structure or sequence. The main sequences within the lipocalins have a poor degree of conservation, with some comparisons showing values as low as 20% identity.

Over the past ten years, there has been an increase in the identification of allergenic lipocalins from domestic animals, and co-sensitization to various species is common among pet allergy sufferers. While lipocalins, as panallergens, may account for the co-sensitization to many pets, the various epitopes responsible for cross-reactivity resulting from these proteins have not received much attention. In this work, we discover many antigenic sites that might be implicated in lipocalin cross-reactivity utilising bio-computational methods.

Materials and Methods

Lipocalin Selection and Alignment

Based on the reported allergenic capability, the amino acid sequences of lipocalins from seventeen domestic animals were chosen. All lipocalins meeting this requirement were selected, regardless of the animal source from which they originated. Using the Praline Web Server, the identity grade between the lipocalins employed in this investigation was ascertained. Alignment parameters were configured to use BLOSUM62 as the exchange matrix. Three iterations were employed, and the E-value was 0.01.

Analysis of Phylogeny

The trees were constructed with the aid of the software Molecular Evolutionary Genetic Analysis (MEGA) version 7, utilising the Neighbour-This model employs a comparative matrix to determine the similarity between amino acids of seventeen sequences in order to establish the evolutionary proximity between the species. It joins with support by Bootstrap with 500 replications as a measure of reliability and robustness under the assumption of minimal evolution in the topology. All of the published lipocalin amino acid sequences that were obtained from the Uniprot database were used to generate the matrix.
As a result, sequences will be positioned closer together in the tree and have a stronger association if more positive identity values are detected between them. Every vacant space was removed (complete removals). The sum of the length of branches (SBL), which determines the number of nodes and their position, including the "clus-ters" of the evolutionarily nearest sequences, will be reported based on the global comparison and homologies. None of the phylogenetic sub-analyses were carried out because of the large number of sequences involved. The CLUSTAL W. programme, which performs alignments, was used to carry out the alignment for the phylogenetic study.

Building Three-D Models

The lipocalin models that were not listed in the protein data bank were created using homology. Using ProSA-web, the models' quality was evaluated. Deep-View was used to enhance the models (rotamer replacements and energy minimization). A number of methods were used to assess its quality, including energy values (GROMOS96 force field), Ramachandran graphs, WHATIF, and the QMEAN4 index. ASA-view was used to calculate the relative values of the area of accessible solvent (r-ASA). To find conserved residues, the lipocalin sequences were aligned. In order to detect pooled areas (>4 residues) and potential cross-reactivity, those that were conserved and the residues accessible to the solvent (rASA > 0.25) were located in the 3D model.

Results

Analysis of Phylogenetics

With 152 locations in the final dataset, a total of 17 sequences were analysed. An ideal tree would have a total branch length of 16.5. The lipocalin sequences formed five nodes with the strongest evolutionary link among them, according to analysis. The analyses show that group A has the greatest concentration of lipocalins that are phylogenetically related, including Cav p 6, Can f 6, Bos d 5, Mus m 1, and Can f 2. Group D, on the other hand, only had two members: Can f 4 and Bos d 2. Among the groups, group A exhibits the strongest association.

Building Three-D Models

With the exception of Bos d 2, Can f 2, and Equ c 1, which were retrieved from the SDAP database, the 3D models of those lipocalins not reported in the protein data bank were constructed by modelling homology. These models show the classic folding of lipocalins, following the pattern of eight antiparallel β strands and an α helix, which help to form a cavity for the union of lipid ligands.

Finding Possible Sites for Cross-Reactive Antigenics

The lipocalins from the various groupings that were discovered through phylogenetic analyses were aligned more than once. The amino acid sequences of lipocalins from Group A lipocalins are 30% similar. Twenty residues in all were found to be identified and conserved in the lipocalins under analysis.

An analysis of the amino acid sequences of the lipocalins examined for group B revealed a 28% identity. 26 residues in all were found to be conserved among the several lipocalins from group B. Upon examination of the antigenic patches, it is noted that they are distributed throughout the structure. This implies that not every residue found would contribute to these antigens' cross-reactivity. In the meantime, group C's amino acid sequences showed 60% identity. Moreover, it displayed the greatest quantity of conserved and exposed residues—33 in total—concentrated in three antigenic patches identified by

the lipocalin structure. Analogous outcomes were attained. The groups' amino acid sequences were 22% and 25% similar, respectively.

Discussion

We describe putative antigenic areas shared by several domestic animal lipocalins in the current investigation. Determining the function of cross-reactivity in sensitization to various allergenic sources requires epitope identification. Cross reactivity in this set of allergens was examined in a number of research [8] [11] [12]. The allergens Can f 1 and Fel d 7 have been shown to exhibit cross reactivity in experiments, and their amino acid sequences share 60% of identity [13]. Despite the fact that both allergens belonged to group B, we only detected a 28% identity between both lipocalins—a low enough identity to predict cross-reactivity.

Because Ory c 4 and Phod s 1 have an identity grade with Can f 1 and Fel d 7, our data indicate that cross reactivity between these allergens is improbable. Even with extracts from allergenic sources like to the Siberian hamster (Phodopus sungorus), experimental evidence suggests that the Phod s 1 allergen is not cross-reactive [14]. Mus m 1 is the allergen that is most closely linked to Phod s 1. An examination of their amino acid sequences showed a considerable degree of homology, with 56% identity. When inhibition experiments were conducted, Nilsson et al. discovered cross reactivity between Can f 6, Fel d 4, and Equ c 1 [12]. Equ c 1 and Fel d 4 are grouped together in group C according to our data, and they have a phylogenetic relationship with Rat n 1, a lipocalin from Rattus novergicus [15]. The amino acid sequences of these lipocalins are 42% identical. 33 residues that were surface exposed and conserved formed three antigenic patches on the 3D model, according to our research. Can f 6 has most of the antigenic residues found in the C group preserved, and it has 54% identity with Rat n 1. Rat n 1 may be a lipocalin with cross reactivity to Can f 6, Fel d 4, and Equ c 1, according to this finding. Additionally, we discovered putative antigen-containing sites implicated in cross-reactivity that were discovered experimentally [15].

It has been determined that bos d 2 is a weak immunogen. has a T cell epitope in the C-terminal region, and mouse experiments have shown that its amino acid sequence is homologous to the allergens Can f 1 and Rat n 1 [11].

Bos d 2 binds to IgG and IgE antibodies in allergy subjects' serum and causes Th2 proliferative responses in mouse cell lines. The allergen's C-terminal region triggered an IgG reaction. This implies that mice and humans recognised Bos d 2 in a comparable manner. In our investigation, we discovered thirty residues that were surface exposed and conserved, however we only discovered two antigenic areas that were shared by Boss 2 and Can f 1. Perhaps as a result of the poor identity. Cav p 2, Cav p 3, and Ory c 1 allergen are not well characterised for the E group. Further research on these allergens is warranted because Cav p 2 and Cav p 3 were reacted to by IgE in 65 and 44 percent of guinea pig allergic patients, respectively [6]. Here, despite a low degree of identification, we suggest that Ory c 1 may play a part in the cross reactivity with Cav p 2 and Cav p 3.

In summary, we have detected a few putative antigenic sites in a few lipocalins; on the other hand, the low identity among these proteins from various species indicates that, while cross-reactivity between them is conceivable, it occurs seldom. These findings corroborate the necessity of doing mutagenicity tests to verify their applicability in determining lipocalins' allergenic potential.

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