
Peanut Bioinformatics: Tools and Applications for Developing More Effective Immunotherapies for Peanut Allergy and Improving Food Safety

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Abstract

Advanced tools of bioinformatics have been employed to assess the features critically required for allergenicity and cross-reactivity. A tremendous accumulation of data on plant proteins in recent years has made it possible to classify allergens in different protein families, with most food allergens grouped into four protein families. These families can be grouped together into superfamilies by comparing sequences and related structures. This information makes it possible to identify a wide range of related proteins that may result in the development of multiple food allergies that initiate the development of cross-reactive antibodies in susceptible individuals. Since peanut allergies are responsible for most episodes of food-induced anaphylaxis, a detailed immunological and molecular characterization of these allergenic components is essential to develop suitable immunotherapies. This would also allow us to screen transgenic plants for the possible development of allergens similar to those allergenic components in peanuts. Homology modeling in combination with residue-wise solvent accessibility of monomers and biological assemblies of allergens certainly gives valuable information about antigenic determinants on protein allergens. Through this review, we discuss the applications of bioinformatics tools toward the mitigation of peanut allergy.

Keywords

Peanut • Allergens • Allergen database • Ara h and transgene allergenicity

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Abbreviations

BLAST	Basic Local Alignment Search Tool
DNA	Deoxyribonucleic acid
FARRP	Food Allergy Research and Resource Program
FASTA	Fast alignment
GM crops	Genetically modified crops
IgE	Immunoglobulin E
IUIS	International Union of Immunological Societies
kDa	Kilodalton
MW	Molecular weight
ORF	Open reading frames
PR	Pathogenesis-related genes
SDAP	Structural Database of Allergenic Proteins
URL	Uniform resource locator

1 Introduction

Food allergy can be described as adverse reactions to certain foods because of immunological mechanisms (Matsuda and Nakamura 1993), and it seems to be increasing when it comes to legumes. Peanut allergy affects approximately 1 % of the population (Burks 2003; Sampson 2004) and up to 8 % of children with IgE mediation (Ortolani et al. 2001; Woods et al. 2002; Mills et al. 2004).

The vast majority of anaphylactic reactions from adults and children can be traced back to the seed storage proteins, especially those from peanuts (Sicherer et al. 2003). There is no cure for peanut allergy, and management is therefore focused on allergen avoidance and prompt treatment (Simons 2008). Peanut allergy is one of the major prevalent food allergies triggered by several proteins known as allergens. About 10–47 % of food-induced anaphylactic reactions and more than 50 % of food allergy fatalities are accounted for peanut allergy (Bock et al. 2001).

The computational and bioinformatics tools in relevance to the study of allergy that helps to probe databases and data repositories belong to different categories: sequence analysis and comparison for classification, characterization of

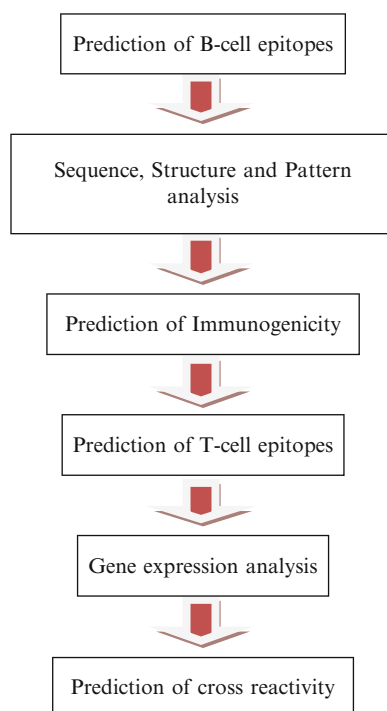


Fig. 1 Different steps of allergic cascade with the scope of bioinformatics tools applications (Brusic et al. 2003)

genes and proteins of confirmed and putative allergens, and prediction of function and structural analysis of genes and proteins (Fig. 1) (Midoro-Horiuti et al. 2001; Yang et al. 2000).

DNA sequence analysis focuses largely on finding conserved patterns of sequence, prediction of protein secondary structure, 3D structure, and function of whole molecules or their domains (Table 1).

The three major peanut allergenic proteins responsible for the allergenic response in susceptible individuals are Ara h 1 (vicilin), assembled in di- and trimeric complexes (Buschmann et al. 1996; Shin et al. 1998), two of its genes encoding 626 and 614 amino acids (Burks et al. 1995a, b; Wichers et al. 2004); Ara h 2 (2S albumin conglutin), two isoforms with masses of ca. 16 and 17 kDa (Chatel et al. 2003; Hales et al. 2004); and Ara h 3/Arah 4 (glycinin) – consisting of an acidic (40–45 kDa) and a basic (25 kDa) subunit (Piersma et al. 2005; Koppelman et al. 2003; Restani et al. 2005) linked by an intermolecular disulfide bridge (Table 2).

Table 1 Bioinformatics tools used in the identification of structural motifs and 3D structure analysis

Bioinformatics tools	Purpose	URL	References
Predict protein	Secondary structure prediction of a selection of pollen, fruit, and vegetable allergens. Identification of similar structural elements like P-loop region as a common domain of pollen and related food allergens	http://www.predictprotein.org/#	Cuff and Barton (2000) and Scheurer et al. (1999)
Jpred2	Predicts more than 75 % of amino acids that form α -helices, β -sheets, or coils in allergen groups	http://www.compbio.dundee.ac.uk/www-jpred/	Cuff and Barton (2000)
PAIRCOIL and COILS	Prediction of coil structure	http://groups.csail.mit.edu/cb/paircoil/cgi-bin/ http://embnet.vital-it.ch/software/COILS_form.html	Berger et al. (1995) and Lupas (1996)

Table 2 Peanut allergens identified until January 2013

Allergen	Protein family	MW (kDa)	Isoallergens	References
Ara h 1	Cupin: 7S vicilin-like globulins	64.0	Ara h 1.0101	Burks et al. (1995a)
Ara h 2	Prolamin: 2S albumin, conglutinin	17.0	Ara h 2.0101, Ara h 2.0201	Kleber-Janke et al. (1999)
Ara h 3	Cupin: legumin-like (11S globin, glycinin)	60.0	Ara h 3.0101, Ara h 3.0201	Rabjohn et al. (1999)
Ara h 4	Cupin: legumin-like (11S globin, glycinin)	37.0	Ara h 4.0101	Kleber-Janke et al. (1999)
Ara h 5	Profilin	15.0	Ara h 5.0101	Kleber-Janke et al. (1999)
Ara h 6	Prolamin: 2S albumin, conglutinin	15.0	Ara h 6.0101	Kleber-Janke et al. (1999)
Ara h 7	Prolamin: 2S albumin, conglutinin	15.0	Ara h 7.0101, Ara h 7.0201, Ara h 7.0202	Kleber-Janke et al. (1999)
Ara h 8	Pathogenesis-related protein, PR-10	17.0	Ara h 8.0101, Ara h 8.0101	Mittag et al. (2004)
Ara h 9	Prolamin: nonspecific lipid-transfer protein	9.8	Ara h 9.0101, Ara h 9.0201	Krause et al. (2009)
Ara h 10	Oleosin	16.0	Ara h 10.0101, Ara h 10.0102	Pons et al. (1998, 2002)
Ara h 11	Oleosin	14.0	Ara h 11.0101	IUIS/Allergome: UniProtKB/TrEMBL: Q45W87
Ara h 12	Defensin (PR-10)	8.0	Ara h 12.0101	Allergome: GenBank Acc: EY396089 EST name: 5J9
Ara h 13	Defensin (PR-10)	8.0	Ara h 13.0101	Allergome: GenBank Acc: EY396019 EST name: 1N15

Isoallergens are variants which may be recognized differently by patient IgE which either increases or decreases the severity of the allergenic response (Christensen, et al. 2010). Ara h 3 and Ara h 4 are 91 % homologous and are regarded as isoforms of each other and are considered to be the same allergen (Allergome, Boldt et al. 2005 and Koppelman et al. 2003). Ara h 8 is homologous to *Betula verrucosa* (birch) allergen Bet v 1 (Mittag et al. 2004). With regard to Ara h 11, 7 patients with peanut ingestion-related symptoms recognized the 14 kDa band (IUIS). Ara h 12 and Ara h 13 were isolated by analysis of expressed sequence tags (GenBank). Databases used were Allergome (<http://www.allergome.org>) and IUIS (<http://www.allergen.org/index.php>)

Abbreviations: MW molecular weight, kDa kilodalton, IUIS International Union of Immunological Societies

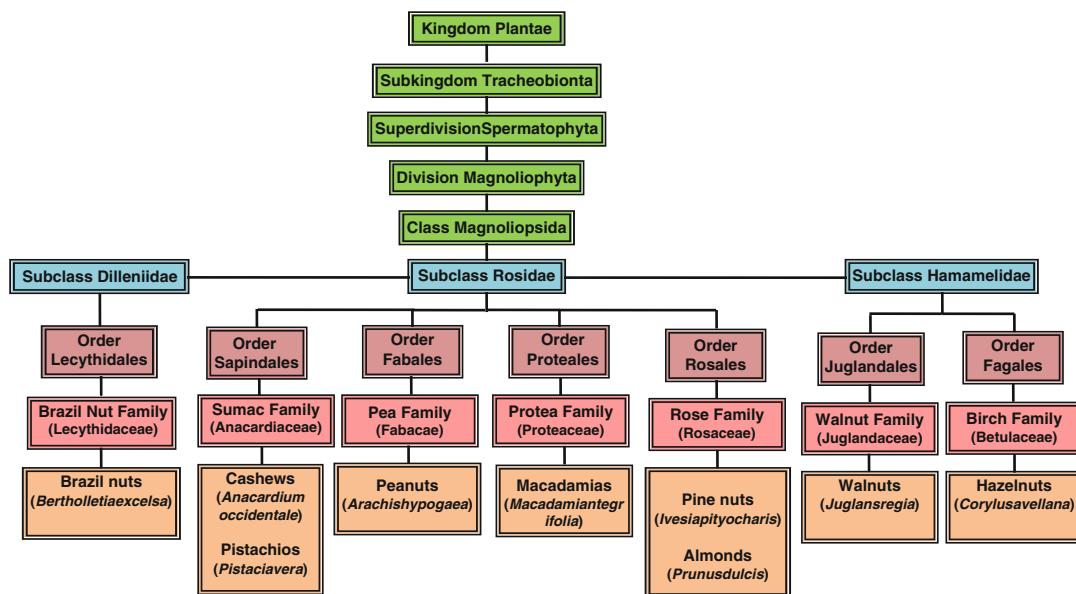


Fig. 2 Taxonomic classification of tree nuts and peanuts. Although they may belong to different families, peanuts and a number of tree nuts arise from the same class, Magnoliopsida, and for this reason may contain

homologous proteins responsible for allergenic cross-reactivity. Therefore, it is not surprising that many people suffering from peanut allergy are also allergic to a number of tree nuts as well

The allergen Ara h 1 is the most abundant (20 % of the total protein) followed by Ara h 2 (~10 %) and Ara h 3/4 (Burks et al. 1998; Koppelman et al. 2001).

To elucidate molecular features related to allergenicity and cross-reactivity and for designing hypoallergenic derivatives, allergen structures are necessary. Theoretical methods and computational techniques are used in molecular modeling to mimic the behavior of molecules (Sonika and Anil 2013).

Furthermore, many of the people with allergies to peanuts also have allergies to similar proteins in other tree nuts (Schein et al. 2007). This is not surprising considering the degree of relatedness between these plants as seen in Fig. 2 (Oezguen et al. 2008).

2 ALLERDB Database and Integrated Bioinformatics Tools

Bioinformatics has proven to play a key role in the management of allergen data, analysis of the properties of allergens, protein allergenicity, and

cross-reactivity (Aalberse 2000; Hileman et al. 2002). Sequences of allergenic proteins are spread out across a variety of databases, including general-purpose protein databases and specialist allergen databases. Each of these databases has a different focus, and they contain different subsets of allergen sequences (Table 3).

The most authoritative data source in relation to content is the IUIS Nomenclature Subcommittee Allergen Database (IUIS allergens), which defines allergens according to a set of well-defined criteria. Bioinformatics search and software tools have been developed to define the characteristics which distinguish allergens from nonallergens (Zhang et al. 2006; Power et al. 2013). This kind of categorization helps to organize allergenic proteins so that they can be better with further research in facilitating specific immunotherapies that could possibly be used to help allergic individuals to develop immunological tolerance to peanuts (Stadler and Stadler 2003; Hoffman et al. 2005).

Identification of allergic potential is a key issue whenever new proteins are brought in contact with humans, either through food or other modes of exposure. The entire ALLERDB

Table 3 List of different websites with information about allergen database and allergenicity prediction servers (Schein et al. 2007)

Website name	URL
<i>Nomenclature and general information database</i>	
AllFam	http://www.meduniwien.ac.at/allergens/allfam/
All allergy	http://allallergy.net/
IUIS (International Union of Immunological Societies)	http://www.allergen.org
Allergome	http://www.allergome.org
CSL (Central Science Laboratory, UK)	http://www.csl.gov.uk/allergen/index.htm
National Center for Food Safety and Technology	Information on Allergens: ftp://ftp.embl-heidelberg.de/ftp.ebi.ac.uk/pub/delete_databases/swissprot/release/allergen.txt
Protall	http://www.ifr.ac.uk/Protall/
Inform all	http://foodallergens.ifr.ac.uk/
<i>Cross-referenced databases with tools to compare sequences</i>	
FARRP	Allergen Online at Nebraska: http://www.allergenonline.org/
ADFS (Allergen Database for Food Safety)	http://allergen.nihs.go.jp/ADFS/
The Allergen Database	http://allergen.csl.gov.uk/index.htm
<i>Cross-referenced database with tools for prediction of allergenicity</i>	
SDAP (Structural Database of Allergenic Proteins) and SDAP-Food	http://fermi.utmb.edu/SDAP
ALLERDB	Zhang et al. (2007)
<i>Web servers for allergenicity prediction</i>	
WebAllergen	Riaz et al. (2005)
AllerTool	Zhang et al. (2007)
Allermatch	www.allermatch.org/
Algpred	http://www.imtech.res.in/raghava/algpred/

database has been created and maintained using Bioware data warehousing system (Koh et al. 2004). To assist allergen data analysis and retrieval, ALLERDB has five integrated tools, BLAST, Search, XRblast, AllerPredict, and XRgraph.

ALLERDB (Zhang et al. 2007) is a database of allergenic proteins with integrated bioinformatics tools for the assessment of allergenicity and allergic cross-reactivity (Schein et al. 2007). It facilitates the assessment of allergenic potential of anonymous proteins that might be introduced in food chain or potential close contact with allergic individuals. This database is, along with SDAP, a representative of a new brand of allergen databases which include a rich set of tools, including those for sequence comparison, pattern identification, and visualization of results (Ivanciuc et al. 2003, 2011). The progress in clinical allergology, genomics, and proteomics generates huge amounts of data for the study of allergens. The molecular basis for allergic cross-

reactivity is in structural similarity among proteins from diverse sources, and the clinical relevance appears to be influenced by several factors including the IgE response against the allergen, route of exposure, and the sequence and structure of the allergen (Ferreira et al. 2004). The main purpose of ALLERDB is the support of molecular analysis of allergenicity, assessment of allergic responses, and allergic cross-reactivity of clinically relevant protein allergens. This coded program proved to be accurate, but it still needs more programs to be created to help reduce the allergenicity of human foods.

3 Molecular Modeling of Peanut Allergen

Molecular modeling of Ara h 1, Len c 1, and Pis s 1 was carried out on a Silicon Graphics O2 R10000 workstation, using the programs Insight II, Homology, and Discover 3 (Accelrys, San

Diego CA, USA) (Viquez et al. 2003). It is an X-ray method used to look at the percentage of identity and homology of the vicilin monomers of peanut, lentil, and pea share with β -conglycinin allowed to build accurate 3D models for the vicilin monomers (Barre et al. 2008). We looked at the homology-based molecular modeling to characterize the structural features responsible for the epitopic community among the major vicilin allergens from peanuts (Ara h 1), lentils (Len c 1), and peas (Pis s 1). The B-cell epitopes share sequence similarity and conformation, suggesting they could be recognized by the same IgE antibodies. The B-cell epitopes most probably account for the IgE-binding cross-reactivity toward different seed storage vicilins and readily participate in the allergic response of sensitized individuals to multiple legume seeds (Mills et al. 2002). In addition, the resistance of the homotrimeric arrangement of the cupin monomers to both thermal denaturation and proteolysis preserved the most binding sites. This greatly increased the propensity of allergenicity.

The remaining B-cell epitopes characterized in the C-terminal region of Ara h 1 share a high degree of homology and most probably correspond to B-cell epitopes common to the seed storage protein family. In this respect, most of these homologous regions were predicted as being exposed B-cell epitopes along the amino acid sequences of Len c 1 and Pis s 1 using the classical hydrophilicity/ exposure/ flexibility-based predictive methods. Ara h 1 and other vicilin allergens were tentatively assigned as homotrimers displaying an arrangement of the cupin motifs similar to that found in β -conglycinin, phaseolin, or canavalin (Burks et al. 1995a, b). In vicilin allergens most of the amino acid residues are highly conserved at the interfaces of the cupin motifs of the β -conglycinin homotrimer (Singh and Raghava 2001). The cross-reactivity implication has proven to be a suppressant to legume seed allergy. Research today still resumes, trying to find better methods on shutting down allergenicity completely.

4 Food Allergenicity Associated with Hypothetical Open Reading Frames

Genetically modified crops have successfully been grown and consumed across the world from the past 16 years. Water scarcity, energy resources, and food have become a matter of concern with the growing population (Bruinsma 2009; Gregory and George 2011). To avoid confusion with expressed or predicted protein-coding sequence, open reading frames (ORFs) are referred to as novel polypeptides, even though at times the novel polypeptide sequence may overlap with an expressed or protein-coding sequence (Vrtala 2008; Silvanovich et al. 2009). Translation of stop-to-stop frames, alignments to the allergen online database using FASTA, and search for eight residue identical matches were used (Silvanovich et al. 2009). The novel polypeptide sequences were created by scanning all six DNA reading frames (starting at base 1, 2, or 3 of each DNA sequence and translating each non-overlapping nucleotide triplet into an amino acid using the standard genetic codon table and then reversing the sequence and repeating the process).

5 Assessment of the Potential Allergenicity of Transgenes

GM foods are developed using recombinant DNA technology by the transfer of foreign genes from one organism to another for human or animal consumption. The expression of proteins corresponding to foreign gene may cause allergenic reactions, and this potential has been seized upon by critics and regulatory bodies of GM crops. Gene suppression technology as an alternative GM approach can reduce or eliminate allergens. In silico approach to assess the allergenic potential of transgenes is gaining importance nowadays (Mishra et al. 2012). Most of the novel gene products showing cross-reactivity with allergens have demonstrable homology. To evaluate the

allergenicity of the transgene, there is a need to perform an initial homology search of the transgene against the database and final validation with relevant experiments.

6 Results

Gene	Length of the protein sequence (amino acids)	Full-length FASTA alignment in three databases (% sequence identity)	Sequence identity with
Manganese superoxide dismutase	228	85.09 86.207 86.20	<i>Hevea brasiliensis</i>
Rice chitinase	175	68.57 (Hev b 1.0102) 63.102 (Pers a 1) 63.40 (chitinase Ib)	Known allergens
Glycine betaine aldehyde dehydrogenase	490	40.00 41.02 43.70	Known allergens
Alfalfa beta-1, 3-glucanase	507	34.32 38.96 40.86 40.90	Known allergens
Wheat beta-1, 3-glucanase	399	33.83 36.33 41.20	Known allergens
Osmotin gene	246	91.87 (Lyc e NP24) 88.21 (Cap a 1w) 66.50 (Act d 2)	Known allergens

In many cases inhalation or ingestion of food allergens has resulted in severe systemic reactions (Helm and Burks 2000). In Asia there is a gradual increase in food allergy, particularly in North America and Europe; peanut allergy is exhibiting a rising trend (Sharma et al. 2011; Singh et al. 2006; Chandra 2003; Grundy et al. 2002; Sicherer et al. 2003).

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