

Vicilins: A case study in allergen pedigrees

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The seed storage proteins classified as 7S globulins occur in a wide range of plants and are often called *vicilins* because of their presence in the *Viciae* group of legumes. They typically exist as homo-trimers exhibiting molecular weights ranging from 150 to 190 kDa, each subunit of the trimer ranging from approximately 40 to 80 kDa. The vicilins are primarily produced in seed cotyledons, which are modified embryonic leaves that apparently evolved to provide nutrients during seed germination. Vicilins are synthesized within the rough endoplasmic reticulum before being transported to a vacuole, where they are deposited and form protein bodies of the seed.¹ The proportion of vicilin proteins is second only to that of the 11S proteins (legumins) in representing the majority of total protein in many seeds, many of which are consumed as foods. Vicilins from some food sources are also resistant to processing and digestion, making them prime candidates as potential allergenic proteins.^{2,3} Not surprisingly, the vicilins are major allergens found in both peanuts⁴ and walnuts⁵; as now reported by Wang et al⁶ in this issue of the Journal, vicilins are also a major allergen in cashews. However, as pointed out by the authors, identification of all vicilins as potential cross-reactive allergens might be premature. Not all vicilins are allergens—a fact that brings into sharp focus the debate about what makes a protein an allergen.

These new data shine new light on an old subject long discussed by allergists: Can clinical cross-reactivity between protein homologs from different, and often taxonomically distinct, food sources be predictable? The implications are clear for allergic patients who need to make informed choices about foods that they must avoid. The new data also extend the list of allergens that are potentially useful in immunotherapy, clinical screens, and immunoassays. Moreover, the data have relevance to the development of safe biotech crops, with respect to which it is essential to prevent the transfer of known or cross-reactive allergens between commodity crops. The allergy assessment testing strategy, as originally framed by the US Food and Drug Administration⁷ and further modified by Food and Agriculture Organization of the United

Nations/World Health Organization scientific panels,^{8,9} proposes that all proteins introduced into crops be assessed for their structural and biochemical similarity to known food allergens. Bioinformatic screens, which compare the primary amino acid sequence of such transferred proteins to those of known allergens and gliadins, are conducted as part of the safety assessment process.^{10,11} The logic is that homologous proteins are more likely to share allergenic cross-reactive conformational and linear epitopes than unrelated proteins. However, we know that the degree of similarity between allergen and nonallergen homologs varies widely in nature. Thus there is no consensus as to what degree of sequence identity/similarity is required to or predicted to elicit clinical cross-reactivity.

Before the report by Wang et al,⁶ data linking sequence homology and the potential for clinical cross-reactivity were largely limited to allergens involved in oral allergy syndrome.^{12,13} In addition, there have been very few reports comparing clearly determined IgE binding epitopes of protein homologs isolated from taxonomically distinct food sources.

As reported in the article by Wang et al, the amino acid sequence of the cashew vicilin exhibits a variable amount of sequence identity to other vicilins and depends on the source of the vicilin. For example, there is 27% identity between the amino acid sequences of the cashew vicilin and the peanut vicilin. Other similarities between these major allergens include the presence of multiple linear IgE binding epitopes, a lack of any common primary structural characteristics of the linear IgE binding epitopes, positional overlap of some of the IgE binding epitopes, and the presence of immunodominant IgE binding epitopes. However, the similarities end there. Even though cashew and peanut vicilins share significant amino acid sequence identity, the investigators found very little sequence conservation between epitopes of these 2 allergens. Four epitopes that showed positional overlap between the 2 proteins were examined more closely for any amino acid sequence similarity. Epitope 10 on the cashew allergen and epitope 17 of the peanut allergen shared positional overlap, and 5/9 amino acids were either similar or identical. However, at least 1 of the amino acids demonstrated to be critical for IgE binding in epitope 17 of Ara h 1 of peanut¹⁴ was not similar or identical to epitope 10 of Ana o 1 of cashew.⁶ As pointed out by the authors, these results might explain the lack of clinical cross-reactivity that is observed between tree nut-sensitive and peanut-sensitive patients' sera.^{6,15}

The authors do caution, however, that their work does not address cross-reactivity due to conformational epitopes. This could be significant, particularly for the vicilins, because these proteins have a characteristic quaternary structure that allows formation of large trimeric

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oligomers that might contain significant conformational epitopes.^{3,14} Once the tertiary structure of the cashew vicilin has been determined, it will be interesting to revisit the question of whether there is positional overlap of the cashew IgE binding epitopes with the peanut epitopes. If the IgE binding epitopes turn out to be similarly placed on the overall structure of these proteins, this will imply that specific protein structures play a larger role in defining those epitopes than previously believed.

The new data also highlight the difficulty in using a single criterion, amino acid sequence identity, as an indicator of potential allergenicity. The cashew vicilin shares only 27% identity with the highly allergenic peanut vicilin, indicating a low likelihood of allergenicity due to cross-reactivity.¹⁶ Furthermore, the IgE directed against the peanut vicilin epitopes would not cross-react with the cashew vicilin—a fact that suggests negligible risk of clinical reactions. All of this makes sense, given that trees (cashews) and legumes (peanuts) are distant relatives in the plant kingdom pedigree. However, in this situation, other tests in the safety assessment process would identify the cashew vicilin as an allergen on the basis of the knowledge that cashew allergy exists (ie, cashews are an allergenic source). Required testing of the cashew vicilin for IgE binding from cashew-sensitive patients' sera⁹ would lead to the correct conclusion: that cashew vicilin is indeed an important allergen. The work by Wang et al⁶ leaves open the question of the origin of cashew vicilin allergenicity, given the paradox that the highly similar homolog in pea (43% identity) is considered to be rarely allergenic. However, the characterization of Ana o 1 represents a significant step forward in defining the relationship between clinical cross-reactivity and sequence pedigree. For developers of agricultural biotechnology products, these data reinforce the need to design case-by-case safety assessment strategies that are informed by all available experimental data; results from sequence similarity analyses must be placed in the context of gene source and appropriate clinical data.

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