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Jug r 6 but not Jug r 2 is the allergenic vicilin present in walnut kernels responsible for IgE cross-reactivities to other tree nuts and seeds

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Background

Walnuts like other tree nuts are ranked high in the list of the culprit foods inducing severe allergic reactions. Jug r 2 has been identified as a major allergen in common walnut by cDNA cloning from a somatic cell line

Aims

We aimed to purify and deeply characterize natural Jug r 2 and to assess IgE cross-reactivity among vicilins from different tree nuts and seeds

Methods

Vicilin was purified from walnut kernels and characterized by highly sensitive mass spectrometry based methods. In parallel, recombinant Jug r 2 was expressed in *Pichia pastoris*. Optimized multi-enzymatic digestion was applied for extensive protein characterization including post-translational modifications analysis and *de novo* sequencing. IgE binding activity of vicilin was tested in ELISA and Western Blot using sera from 77 walnut allergic patients. Level of cross-reactivity between detected allergen and selected homologues was assessed by inhibition ELISA.

Results

Extensive mass spectrometry analysis of the purified vicilin provided a protein mass of 47.1–48.8 kDa and allowed identification of the protein sequence that displayed only 44% identity to Jug r 2. The newly identified vicilin was designated by IUIS committee as Jug r 6. Sequence analysis revealed typical for vicilin two cupin domains and high sequence identity with homologues other tree nuts and seeds. Allergen was recognized by IgE of 26% in walnut allergic patients' sera tested. Jug r 6 displayed a remarkable level of cross-reactivity when tested with homologues from hazelnut, sesame and pistachio.

Conclusions

This is the first report on the purification of walnut vicilin from kernels, designated Jug r 6. Our data also provide evidence that Jug r 6 is involved in the cross-reactivities among tree nuts and seeds.

Conflict of interest: Paweł Dubiela received the salary from Medical University of Vienna, and currently is paid by GSK Poland. The other authors declare no conflict of interest.