Identification and implication of allergenic PR10 protein from walnut in birch pollen associated walnut allergy

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Background: Beside hazelnut, the English walnut (Juglans regia) belongs to the most important allergenic tree nuts across Europe. So far, four walnut allergens (2S albumin, vicilin, nsLTP, 11S globulin) are listed in the official IUIS allergen database. Although an association of allergic reactions to walnut with birch pollen sensitization has been reported, no cross-reactive culprit walnut allergen has been described. The aim of the present study was to identify a Betv1-like protein in walnut and to investigate its allergenic properties.

Methods: Using a Betv1-homologous cDNA sequence from iron walnut leaves (KJ598787) as template, a cDNA encoding a corresponding protein from Juglans regia kernels (Jug r PR10) was cloned by RT-PCR and 5'RACE. Recombinant® Jug r PR10 protein was expressed in E. coli and purified by a two-step chromatographic procedure. Purity and secondary structure were analyzed by SDS-PAGE and CD spectroscopy. Specific IgE levels to walnut extract, rBetv1 and rJug r PR10 were measured by ImmunoCAP™ in birch pollen allergic with concomitant allergy to walnut (n=15), confirmed by a positive open or double-blind placebo-controlled food challenge test. The presence of natural Jug r PR10 in walnut extract was analyzed by IgE immunoblot competition experiments using rJug r PR10 as inhibitor.

Results: Jug r PR10 (KX034087) was 100% identical in amino acid sequence to KJ598787, 67% to Bet v 1.01 and up to 74% to PR10 proteins from fruits, e.g. Mal d 1 (apple), Pru av 1 (cherry) and Pru p 1 (peach). Recombinant Jug r PR10 displayed secondary structures similar to those of Bet v 1. Walnut sensitization was detected in 40% (6/15) and 47% (7/15) of the patients studied, by ImmunoCAP and skin testing, respectively. In contrast, 93% (14/15) were reactive to rJug r PR10 and 100% to Bet v 1. The Bet v 1 and Jug r PR10 specific IgE values correlated strongly (r²=0.93), even though lower IgE levels were observed for Jug r PR10 (median 12.9 kU/L) than for Bet v 1 (median 21.5 kU/L). The presence of an IgE-reactive PR10 protein in walnut kernels was confirmed by immunoblot inhibition.

Conclusion: According to the established criteria, the PR10 protein from English walnut qualifies as major allergen. Low diagnostic sensitivity of walnut extract for patients with birch pollen associated walnut allergy might be due to small amounts of Jug r PR10 in walnuts. Recombinant Jug r PR10 may therefore become a useful tool for component-resolved diagnosis.