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TITLE: A retrospect study into the utility of allergen components in walnut allergy

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To the Editor

Walnut (*Juglans regia*) and Pecan (*Carya illinoensis*) are members of the Juglandaceae family. Walnut and pecan appear similar and share high protein homology with the result that most walnut allergic individuals are also allergic to pecan nut. Walnut is a common allergy in tree nut allergic individuals in the United States and the United Kingdom (1) and has been associated with severe and fatal reactions. The diagnosis of a nut allergy has been shown to negatively influence quality of life (2). However, walnut and pecan nut are a healthy source of protein and found in a variety of foods. Unlike many other nuts, walnut and pecan are easily shelled and therefore free from contamination with other nuts and safe for consumption if walnut and pecan tolerant. It is important therefore to accurately diagnose the presence of allergy or tolerance to walnut and/or pecan in individuals where food allergy is suspected.

A diagnosis of walnut allergy is made on the basis of one or more of the following diagnostic modalities; clinical history, specific Immunoglobulin E (IgE) testing and skin prick testing to walnut. However, it may not be possible to unequivocally confirm or refute a diagnosis of walnut allergy based on the above modalities, in which case, food challenges are required. Food challenges are costly, logistically demanding and not without risk to the patient. The use of IgE molecular allergen components has been shown to increase the specificity of the diagnosis of peanut (3), hazelnut (4), cashew and pistachio allergy (5). In a Mediterranean population, walnut components were shown to be a useful marker of walnut allergy (6), however, the utility and clinical significance of walnut component testing has not been extensively explored in a northern European population. The molecular components identified and recognized as allergens in English walnut, *Juglans regia*, are Jug r 1, Jug r 2, and Jug r 3, Jug r 4 (7). Jug r 1 is a 2S albumin seed storage protein and is inherently allergenic (8). Jug r 2 is a vicilin storage protein (7). Jug r 3, a commonly recognised lipid transfer protein (LTP) in Italian populations

(9), is associated with local symptoms and systemic reactions. Jug r 4 is a 11s globulin seed storage protein (7). Jug r 1, Jug r 2 and Jug r 3 are commercially available walnut components.

We performed a retrospective observational study to investigate the utility of walnut 2S albumin Jug r 1 and walnut LTP Jug r 3 in the diagnosis of walnut allergy with a secondary aim to explore the relevance of closely related pecan specific IgE sensitisation in walnut allergy. For this study, walnut allergic and tolerant individuals were identified from patients referred to two Paediatric Allergy Specialists in a single London centre with suspected food allergy over an 18month period (14th September 2014- 20th March 2015). The age distribution was 6 months – 19 years at the time of consultation. All individuals were investigated with a standardised panel of specific IgE nut components [2S Albumin (Jug r 1, Cor a 14, Ara h 2); 7S Vicilin-like globulin (Ara h 1); 11S Globulin (Cor a 9, Ara h 3); PR-10 (Cor a 1, Ara h 8); LTP (Jug r 3, Cor a 8, Ara h 9)] and specific IgE to complete nuts (walnut, pecan, hazelnut and peanut) as routine investigation of potential nut allergy. The hospital's Research and Development group approved this retrospective audit. Serum IgE reactivity was measured using commercially available ImmunoCAP components (Thermo Fisher) and was analysed as per manufacturers instructions. Corresponding clinic letters, with a full history from one of two Specialists of an allergic reaction to walnut or tolerance of walnut, were identified.

A priori study definitions, principally 'walnut allergic' and 'walnut tolerant' groups, were defined. Walnut allergy was defined as a documented Specialist determined history, in a clinic letter, of an immediate reaction to walnut plus evidence of IgE sensitization measured with walnut specific IgE greater than or equal to 0.10kU/L. Walnut tolerance was defined as a documented Specialist determined history, of tolerance after ingesting an age-appropriate portion of walnut. IgE evidence of walnut sensitization was not included in the definition of tolerance. Statistical analysis was performed on 45 patients who were characterised as either tolerant (29 individuals) or allergic (16 individuals). One patient had a reaction after a kiss

contact when the mother ate a walnut and was included in the allergic group. No cases of anaphylaxis were reported and all reactions to walnut occurred in the community.

Comparisons between the walnut allergic and tolerant data set were made to address the aims of this study. Mann-Whitney's U-test was used to perform a comparison between allergic and tolerant individuals for walnut specific IgE, Jug r 1, Jug r 3 and pecan specific IgE. A statistically significant difference (p-value <0.001) was shown for walnut specific IgE, Jug r 1 and pecan specific IgE between allergic and tolerant groups (Table 1). There was no significant difference in Jug r 3 values between the allergic and tolerant groups. The profile of a walnut allergic individual showed IgE reactivity to walnut, Jug r 1 and pecan. The profile of a walnut tolerant individual did not show IgE reactivity to walnut, Jug r 1 or pecan. Jug r 3 was not significant in the profile of either walnut tolerant or allergic individuals. The overall ability of the components (Jug r 1 and Jug r 3) and complete nuts (walnut and pecan) to discriminate between allergic and tolerant individual was judged using Receiver Operator Curve (ROC) analysis. For the data set, area under the curve (AUC) demonstrated that Jug r 1, walnut, and pecan all performed similarly well (Table 1). Calculations regarding combined diagnostic performance did not show an improvement compared to the individual performance of the tests. Jug r 3 performed poorly and did not appear relevant to walnut allergy in this study population from a northern European country. Jug r 3 may be of greater significance in different populations, such as those from Mediterranean countries, as demonstrated in other studies (6, 9).

The diagnostic performance of walnut, pecan, and walnut components at specific IgE assay cut-off 0.35kU/L is shown in Table 2. At this low assay cut-off, walnut was found to be a more sensitive test than components however notably Jug r 1 showed higher specificity and a higher positive predictive value. This data suggests that Jug r 1 is the dominant component in the diagnosis of walnut allergy, with good diagnostic accuracy and indicates that Jug r 1 has potential as a useful diagnostic test in walnut allergy because of its higher specificity above walnut specific IgE. Clinically a test needs to both identify those with true allergy but also

identify those without allergy to prevent over diagnosis and the burden to an individual of an incorrect walnut allergy diagnosis. In this data set, at an assay cut-off of 0.35kU/L walnut specific IgE only has a specificity of 48.3%. 15 out of 29 tolerant individuals would also have been identified incorrectly as allergic. In comparison at the same assay cut-off of 0.35kU/L, Jug r 1 has a sensitivity of 87.5% and a specificity of 75.9%. As a test it would have correctly identified 14 out of 16 patients in the allergic group and incorrectly identified 7 individuals as allergic out of the 29 in the tolerant group.

High correlations between walnut and pecan IgE have previously been observed in individuals with suspected nut allergy (10). In our data, pecan specific IgE reactivity was also shown to be predictive of walnut allergy however pecan specific IgE reactivity does not necessarily represent clinical pecan allergy.

The findings of our study should ideally be validated in a larger set of patients undergoing oral food challenge. However, this data demonstrates that Jug r 1 is a significant walnut allergen in northern countries and may potentially assist in effectively discriminating walnut allergic from tolerant patients.

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FIGURES/TABLES:

Table 1: Comparison between walnut allergic and tolerant groups

	Allergic n=16	Tolerant n=29	p-value*	AUC**
Median IgE kU/l (range)				
Walnut	7.7 (0.6-73)	0.4 (0.1-30)	< 0.001	0.89
Jug r 1	4.9 (0.1-60)	0.1 (0.1-20)	< 0.001	0.86
Jug r 3	0.1 (0.1-0.1)	0.1 (0.1-6.2)	ns	0.38
Pecan	6.5 (0.1-24)	0.1 (0.1-6.4)	< 0.001	0.86

* Comparison between groups was performed using Mann-Whitney's U-test. ns = not significant

**Area under the curve (AUC) from Receiver Operator Curve (ROC) analysis.

Table 2: Diagnostic performance of walnut, pecan and walnut components at specific IgE assay cut-off 0.35kU/L.

Diagnostic performance	Components and complete IgE			
	Walnut	Jug r 1	Jug r 3	Pecan
Sensitivity, Specificity (%)	100, 48.3	87.5, 75.9	0, 86.2	87.5, 69.0
PPV, NPV (%)	51.6, 100	66.7, 91.7	0, 61.0	60.9, 90.9