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REVIEW

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Tree nut allergies: Allergen homology, cross-reactivity, and implications for therapy

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Summary

Tree nut allergy is a potentially life-threatening disease that is increasing in prevalence, now affecting 1% of the general population in the United States. While other food allergies often resolve spontaneously, tree nut allergies are outgrown in less than 10% of cases. Due to the likelihood of cross-sensitization to multiple tree nut allergens, the current treatment guideline is strict avoidance of all nuts once one tree nut allergy has been diagnosed. For example, walnut and pecan are highly cross-reactive, along with cashew and pistachio, but the extent of clinical, IgE-mediated cross-reactivity among other tree nuts remains unclear, therefore making avoidance of all tree nuts a safe approach. There have been recent advances in immunotherapy for food allergies. For instance, there are investigational immunotherapies for milk, egg and peanut allergies, specifically oral immunotherapy, sublingual immunotherapy and epicutaneous immunotherapy. However, there are no large randomized controlled clinical trials for tree nut allergies. Even though there has been less research into tree nut allergy immunotherapies, the evidence of T-cell cross-reactivity among tree nuts exists in animal models and in T cells from allergic patients indicates that immunotherapeutic interventions may be possible. Here, we review the literature regarding epidemiology, allergen homology and cross-reactivity among tree nuts, and explore how current findings can be employed for effective therapy.

1 | INTRODUCTION

Food allergies are potentially fatal and affect 8% of children and 5% of adults in the United States.[1] Tree nut allergy affects an estimated 1% of children in the United States, and prevalence has increased in the past decade.[2,3] Tree nuts are defined as any nut grown on trees, including cashew, walnut, pistachio, almond, pecan, Brazil nut, pine nut, hazelnut and macadamia nut.[4] The tree nuts that most commonly cause allergic reactions are walnut, hazelnut, cashew and almond.[5,6] Although peanuts are often grouped with tree nuts, they grow underground and are considered legumes. Tree nut allergies are rarely outgrown; one study found that only 9% of 101 children outgrew their tree nut allergy. Similarly, another found that only 14.3% of individuals with self-reported tree nut allergy outgrew their allergy.[2] The same study found that a slightly higher proportion of peanut allergy sufferers outgrow their allergies (~20%).[2] According to telephone surveys in 1997, 2002 and 2008, the number of children with self-reported tree nut or peanut allergies increased over an 11-year span from 0.6% to 2.1%, while the prevalence among adults remained constant over the same time frame.[7] The majority of those allergic to tree nuts are allergic to more than one tree nut; after the diagnosis of one tree nut allergy, most individuals (86%) develop an allergy to another tree nut by age 14.[8]

Tree nut allergy accounts for 18%-40% of fatalities from food-induced anaphylaxis, and in some cases, allergic reactions to tree nuts have been reported to be more severe than reactions to peanut.[4,9] Due to the anxieties associated with the potentially fatal consequences of inadvertently consuming allergens, allergic individuals

and their families have reduced qualities of life.[10] Despite labelling requirements for prepackaged food, ambiguity exists when phrases that state potential cross-contamination are included. To help with this concern, recent studies have determined eliciting dose (ED) amounts for allergic reactions for several major allergens, including cashew, hazelnut, peanut, milk and egg. The ED predicted to cause allergic reactions in 50% of the allergic population (ED_{50}) is the lowest for peanut at 67.3 mg, followed by hazelnut (80.6 mg), cashew (120 mg), milk (156 mg) and egg (199 mg).[11] A recent study showed that the ED_{50} for walnut was 625 mg, much higher than the allergens previously mentioned.[12] Although the ED_{50} for walnut was higher, peanut, hazelnut and cashew elicit reactions after consumption of a very small amount of allergen, contributing to the severity of reactions to peanuts and tree nuts.

Several promising clinical trials have been initiated to treat food allergies, but the current recommendation is strict avoidance of the offending allergens.[13] Of the therapies under investigation, most are desensitization therapies that include oral immunotherapy (OIT), sublingual immunotherapy (SLIT) and epicutaneous immunotherapy (EPIT).[14-16] These desensitization therapies have shown promise in animal models and in Phase I and II human trials; Phase III trial data for OIT and EPIT is forthcoming, which may lead to the first FDA-approved therapy. OIT has been tested for egg, milk, and peanut, whereas SLIT and EPIT have been primarily evaluated for peanut allergy. SLIT has been investigated for a small group of 23 hazelnut allergic individuals, with promising results indicating that SLIT can increase tolerance of hazelnut.[17] Despite these results, there have been no large randomized clinical trials for tree nut allergies.

2 | ALLERGEN BIOCHEMISTRY

During an allergic reaction, tree nut allergens bind to IgE on the surface of mast cells and basophils. The cross-linking of the IgE causes these cells to degranulate and release allergic mediators including histamine. The majority of allergens in tree nuts are seed storage proteins, including vicilins (7S globulins), 2S albumins and legumins (11S globulins). Additional tree nut allergens include profilins, heveins and lipid transfer proteins, which are considered pan-allergens and have high IgE-mediated cross-reactivity with pollen and food homologues.[18,19] Seed storage proteins are also the major allergens in peanuts and other legumes, including the peanut allergens Ara h 1 (vicilin), Ara h 2 and Ara h 6 (2S albumins) and Ara h 3 (legumin).[20,21]

These seed storage allergen proteins are more stable and resistant to proteolytic digestion compared to other proteins within these foods, which is thought to contribute to their allergenicity. These allergens must remain at least partially intact in the gastrointestinal (GI) tract in order for them to be taken up by the gut and subsequently sensitize an individual. Several studies have shown that these seed storage proteins, especially 2S albumins, are resistant to digestion with pepsin and trypsin, which mimics digestion in the GI tract. Disulphide bonds between cysteine residues in the 2S albumin allergens of Brazil nut (Ber e 1), cashew (Ana o 3) and peanut (Ara h 2 and Ara h 6) have been shown to protect these allergens against digestion.[22-24] Thermal processing also influences tree nut allergen immunogenicity and has been summarized elsewhere.[25] Briefly, the allergenicity of PR-10 proteins in hazelnut and almond may be reduced by thermal processing, while non-specific lipid transfer proteins and seed storage proteins are shown to be resistant to heat. In a more recent study, heat and pressure treatments were seen to decrease IgE-binding properties of cashew and pistachio protein extracts.[26]

Component-resolved diagnostic tests have become a valuable tool to determine which allergens a patient is sensitized and will likely react to. Contradictory findings regarding the effectiveness of component-specific IgE at distinguishing cross-sensitization vs allergy have been reported for tree nuts, and efficacy seems to vary between tree nuts. For example, Ana o 1-, 2- and 3-specific IgE have been used to distinguish allergic and tolerant children that were sensitized to cashew.[27] Contrarily, Jug r 1-specific IgE was not shown to be any more effective than extract-specific IgE in diagnosing walnut-allergic adults.[28] Recently, allergen components have been investigated to determine correlations with clinical reactivity during double-blind, placebo-controlled food challenges in children with multiple food allergies. Increased levels of IgE against 2S albumins in cashew (Ana o 3), walnut (Jug r 1) and hazelnut (Cor a 14) were correlated with patients experiencing GI reactions, which correspond well with the digestion-resistant characteristics of 2S albumins.[29] Additionally, component-resolved diagnostics for peanut-allergic subjects recently showed that sensitization to seed storage proteins in tree nuts was uncommon, suggesting that many peanut-allergic patients could tolerate tree nuts without reactions.[30]

3 | CROSS-REACTIVITY

3.1 | Cross-reactivity vs cross-sensitivity

Due to the high cross-reactivity, patients are typically instructed to avoid all tree nuts once one nut allergy has been diagnosed.[13] However, there is an important distinction between cross-reactivity and cross-sensitization. Cross-reactivity occurs when a patient has clinical reactivity (ie allergic symptoms) to a closely related food. By contrast, cross-sensitization occurs when a patient has a positive IgE or skin test to a closely related food, but does not necessarily exhibit allergic symptoms upon ingestion of the food.[31] Therefore, it is important to distinguish if a patient is cross-reactive or cross-sensitized, to minimize unnecessary food avoidance. For example, a recent study found that 49 of 83 individuals (59%) with suspected tree nut allergy were sensitized to almond (reactive via skin prick test), but only one individual was allergic to almond.[32]

However, true cross-reactivity between tree nut allergens is high between cashew and pistachio, which are both members of the Anacardiacea family, and walnut and pecan, which are both members of the Juglandaceae family.[29,33] A clinical trial in Europe (ProNuts) has begun to address the question of whether the avoidance of all nuts is necessary (Clinicaltrials.gov ID: NCT01744990). The study is

employing allergy testing, including skin prick tests, specific IgE and basophil activation tests (BATs), to predict cross-reactivity vs tolerance in 150 individuals. The results of this trial could lead to individualized recommendations for nut avoidance in tree nut-allergic patients. Furthermore, the results from allergy testing could lead to the implementation of BATs to differentiate tree nut cross-sensitization vs cross-reactivity while avoiding cumbersome double-blind, placebo-controlled food challenges. Previously, BATs have been shown to be more effective at distinguishing peanut allergic from tolerant individuals, compared to skin prick tests and peanut-specific IgE.[34] Another study showed the usefulness of basophil CD203c expression as an ex-vivo outcome measure for nut-allergic subjects.[35] Finally, basophil reactivity in peanut-allergic subjects was found to be associated with reaction severity, and basophil sensitivity to be associated with peanut threshold doses that elicit allergic reactions during oral food challenge.[36] These results in peanut-allergic individuals show promise for the application of BATs in tree nut-allergic patients.

3.2 | Cross-reactivity with pollen and lipid transfer proteins

Oral allergy syndrome (OAS) occurs when an individual is initially sensitized to pollen or another plant inhalant allergen, and has a cross-reaction to raw fruit, vegetables or nuts.[37] These reactions are caused by pan-allergens, are often localized to the oropharynx and mouth, and usually do not lead to anaphylaxis.[38] In rare cases, however, Ara h 8 and other pan-allergens cause systemic reactions and anaphylaxis.[39,40] Some families of pathogenesis-related (PR) proteins in plants are homologous to proteins in fruit and vegetables, and are often responsible for the IgE cross-reactivity leading to OAS. Ribosome-inactivating proteins (PR-10), lipid transfer proteins (PR-14) and thaumatin-like proteins (PR-5) are most commonly involved. The birch pollen Bet v 1, a PR-10 protein, is one of the major pan-allergens in OAS. Homologous proteins have been identified in walnut (Jug r 5), hazelnut (Cor a 1) and peanut (Ara h 8), and shown to be cross-reactive to Bet v 1.[41-43] The peach lipid transfer protein, Pru p 3, is allergenic and has been shown to be the primary sensitizing allergen for cross-reactivity with other lipid transfer proteins, including peanut (Ara h 9), hazelnut (Cor a 8), walnut (Jug r 3) and almond (Pru du 3).[44] Lipid transfer proteins are more heat stable and resistant to proteolytic digestion compared to other pan-allergens, and therefore can cause severe, systemic reactions.[45]

3.3 | Homology

Bioinformatics approaches have been implemented to compare seed storage allergen protein sequences and predict cross-reactivity based on homology.[46] For example, modelling studies have been performed to map the linear IgE-binding epitopes for walnut and hazelnut 11S globulins and compare them to other allergens, including cashew, peanut and soybean. Several allergenic "hot spots" were identified, including some structural motifs that could be involved in IgE elicitation and binding.[47] Other studies have also modelled linear IgE-binding epitopes on legumins and vicilins from peanut and tree nuts. Some structural homology was observed between surface-exposed epitopes, which could contribute to the cross-reactivity seen between peanut and tree nut allergens, despite not belonging to the same botanical family.[48,49] These findings have been supported by inhibition assays in which Brazil nut and almond extract were used to inhibit binding of IgE in peanut-allergic serum to Ara h 2, further demonstrating that Ara h 2 shares common IgE-binding epitopes with these tree nuts.[50]

Per cent sequence identities between common tree nut, legume and other food allergens are shown for vicilins in Table 1, 2S albumins in Table 2 and legumins in Table 3. Briefly, allergen amino acid sequences were obtained from PubMed protein searches using codes from the Allergen Nomenclature website (allergen.org), and per cent sequence identities between allergens was determined using the BLAST protein comparison tool. There is consistently high identity between allergens from walnut and pecan, and pistachio and cashew. Legumin allergens in walnut (Jug r 4) and pecan (Car i 4) have 95% identity, and vicilin allergens in cashew (Ana o 1) and pistachio (Pis v 3) have 79% identity. Hazelnut allergens have high sequence identity with walnut vicilin (Cor a 11 and Jug r 6, 72%) and legumin (Cor a 9 and Jug r 4, 73%), and pecan legumin (Cor a 9 and Car i 4, 71%), respectively. Interestingly, none of the peanut allergens have sequence identity greater than 70% with any tree nut or seed. Phylogenetic trees have been generated to visually represent the homology between vicilin, 2S albumin and legumin allergens in nuts and related seeds/legumes (Figure 1). The high similarity between walnut and pecan as well as cashew and pistachio can be seen for all three types of seed storage protein allergens. The other food proteins that are not tree nuts or allergens shown in red in Tables 1 and 3 are included to demonstrate how well-conserved some allergenic proteins can be across many plant species. Interestingly, although these proteins are relatively well-conserved, they are known allergens in some species but not in others. It is possible that tree nuts, peanuts and other seeds that are sensitizers have sensitizing linear and/or conformational epitopes that are missing or altered in other plant species that are not sensitizers.

Protein sequences and sequence identity alone cannot accurately predict cross-reactivity between allergens, as structural epitopes may also play a role when the allergen is properly folded. For example, previous work has shown that cross-reactivity is unlikely between vicilin seed storage allergens in walnut (Jug r 2) and peanut (Ara h 1) despite nearly a 40% sequence identity.[51] Similarly, pea vicilin (Pis s 1), which has greater than 50% sequence identity with peanut vicilin (Ara h 1), is rarely allergenic.[52] After a thorough comparison of structural properties of allergens, Aalberse suggests that at least 70% overlap is required for cross-reactivity to take place.[53] To more accurately assess cross-reactivity among allergens, IgE-binding epitope regions need to be taken into account when interpreting sequence identities. The location within the protein sequence that accounts for high percentages of sequence identities is important for

TABLE 1 Per cent sequence identity of the vicilin allergens for tree nuts and other foods

	Walnut Jug r 2	Walnut Jug r 6	Pecan Car i 2	Cashew Ana o 1	Pistachio Pis v 3	Hazelnut Cor a 11	Coconut Coc n 1	Peanut Ara h 1	Pea Pis s 1	Sesame Ses i 3	Lentil Len c 1	Soybean Gly m 5	Mung bean Vig r 2	Buckwheat Fag e 3	Castor bean	Peach
Walnut Jug r 2	100	44	92[a]	36	38	47	23	37	40	43	39	37	36	36	40	41
Walnut Jug r 6	44	100	44	49	54	72[a]	22	36	36	60	36	34	33	31	55	59
Pecan Car i 2	92[a]	44	100	36	38	47	24	34	40	43	40	36	35	33	38	40
Cashew Ana o 1	36	49	36	100	79[a]	53	23	30	33	44	49	31	30	32	51	46
Pistachio Pis v 3	38	54	38	79[a]	100	56	24	32	35	48	34	33	32	35	53	49
Hazelnut Cor a 11	47	72[a]	47	53	56	100	24	35	37	58	36	32	35	43	59	57
Coconut Coc n 1	23	22	24	23	24	24	100	24	24	23	23	23	24	33	22	24
Peanut Ara h 1	37	36	34	30	32	35	24	100	53	35	54	49	49	46	33	32
Pea Pis s 1	40	36	40	33	35	37	24	53	100	34	90[a]	55	54	28	36	35
Sesame Ses i 3	43	60	43	44	48	58	23	35	34	100	33	33	33	42	42	49
Lentil Len c 1	39	36	40	49	34	36	23	54	90[a]	33	100	55	55	28	36	35
Soybean Gly m 5	37	34	36	31	33	32	23	49	55	33	55	100	66	31	31	30

Foods marked in RED are not known to be allergens but have relatively high sequence homology to some tree nut allergens in the same family.
[a]Greater than 70%. Duplicate values are shaded for easier viewing.

TABLE 2 Per cent sequence identity of the 2S albumin allergens for tree nuts and other foods

	Walnut Jug r 1	Pecan Car i 1	Cashew Ana o 3	Pistachio Pis v 1	Brazil nut Ber e 1	Hazelnut Cor a 14	Peanut Ara h 2	Peanut Ara h 6	Peanut Ara h 7	Soybean Gly m 8	Sesame Ses i 1	Sesame Ses i 2	Castor bean Ric c 1	Mustard Sin a 1
Walnut Jug r 1	100	88[a]	39	38	43	60	33	33	29	31	41	34	41	26
Pecan Car i 1	88[a]	100	41	36	40	57	31	29	26	31	43	33	46	27
Cashew Ana o 3	39	41	100	66	29	43	33	28	28	20	43	30	42	26
Pistachio Pis v 1	38	36	66	100	33	36	25	26	<20	25	37	28	37	27
Brazil nut Ber e 1	43	40	29	33	100	45	31	33	26	24	38	41	36	23
Hazelnut Cor a 14	60	57	43	36	45	100	33	32	30	30	47	36	36	29
Peanut Ara h 2	33	31	33	25	31	33	100	55	49	36	29	26	27	<20
Peanut Ara h 6	33	29	28	26	33	32	55	100	46	33	26	26	32	<20
Peanut Ara h 7	29	26	28	<20	26	30	49	46	100	29	<20	25	28	<20
Soybean Gly m 8	31	46	20	25	24	30	36	33	29	100	24	28	23	<20
Sesame Ses i 1	41	33	43	37	38	47	29	26	< 20	24	100	37	37	<20
Sesame Ses i 2	34	31	30	28	41	36	26	26	25	28	37	100	25	<20

[a]Greater than 70%. Duplicate values are shaded for easier viewing.

TABLE 3 Percent sequence identity of the legumin allergens for tree nuts and other foods

	Walnut Jug r 4	Pecan Car i 4	Cashew Ana o 2	Pistachio Pis v 2	Pistachio Pis v 5	Almond Pru du 6	Brazil nut Ber e 2	Hazelnut Cor a 9	Peanut Ara h 3	Soybean Gly m 6	Sesame Ses i 6	Mustard Sin a 2	Castor bean	Buckwheat	Peach
Walnut Jug r 4	100	95[a]	57	51	58	53	51	73[a]	45	46	51	40	59	41	54
Pecan Car i 4	95[a]	100	57	51	58	54	51	71[a]	45	45	51	40	60	42	54
Cashew Ana o 2	57	57	100	50	79[a]	43	48	54	43	46	42	44	51	44	48
Pistachio Pis v 2	51	51	51	100	51	46	48	47	38	40	41	39	56	42	45
Pistachio Pis v 5	58	58	79[a]	51	100	45	48	55	43	47	42	44	51	42	48
Almond Pru du 6	53	54	43	46	45	100	42	51	41	40	37	42	49	38	57
Brazil nut Ber e 2	51	51	48	48	48	42	100	48	37	39	41	38	50	46	42
Hazelnut Cor a 9	73[a]	71[a]	54	47	55	51	48	100	45	46	42	42	51	41	51
Peanut Ara h 3	45	45	43	38	43	41	37	45	100	56	35	30	38	34	39
Soybean Gly m 6	46	45	46	40	47	40	39	56	56	100	32	36	40	34	41
Sesame Ses i 6	51	51	42	41	42	37	41	42	35	32	100	34	39	37	39
Sesame Sin a 2	40	40	44	39	44	42	38	42	30	36	34	100	40	36	44

Foods marked in RED are not known to be allergens but have relatively high sequence homology to some tree nut allergens in the same family. Duplicate values are shaded for easier viewing.
[a]Greater than 70%.

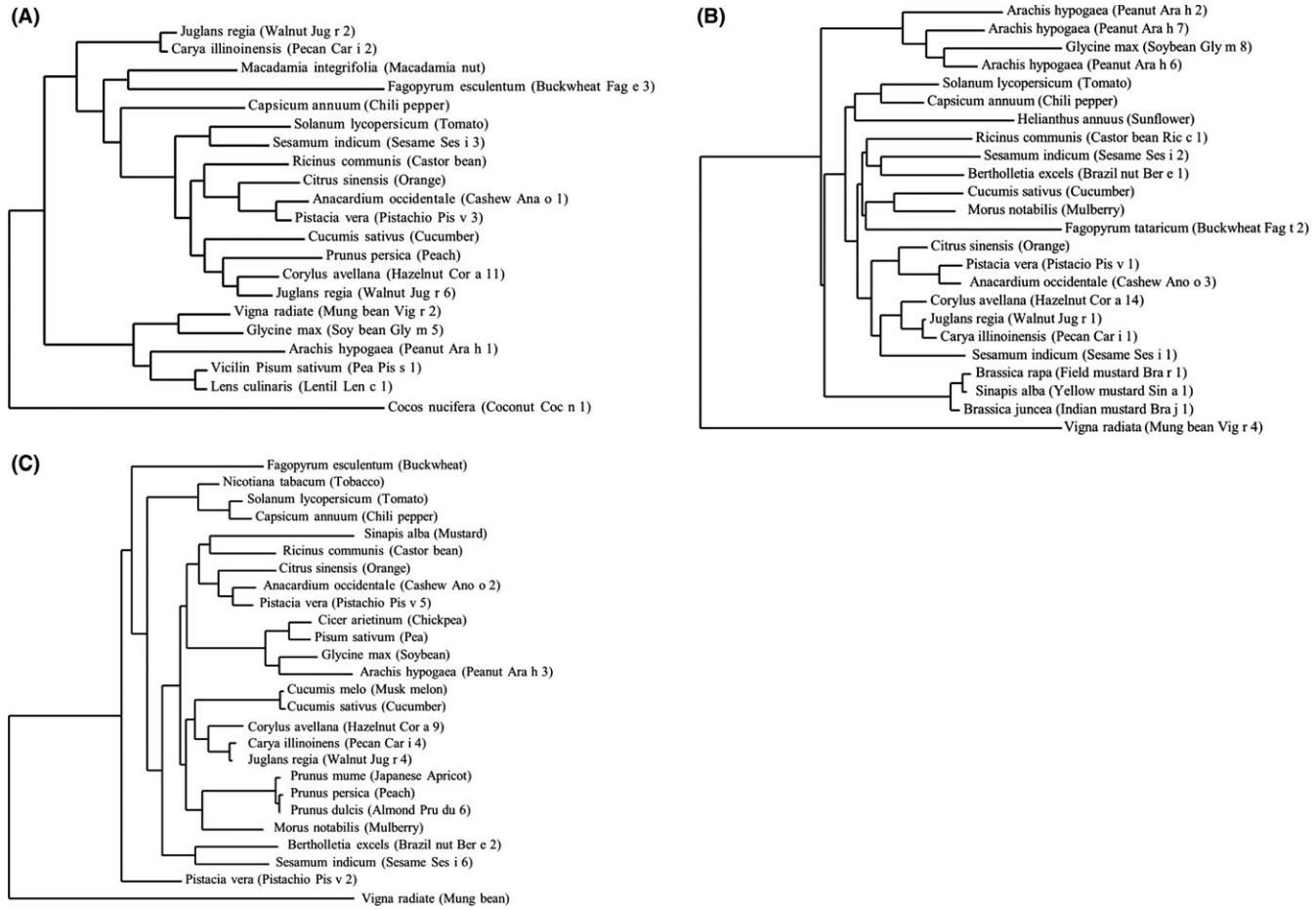

FIGURE 1 Phylogenetic trees for (A) vicilin, (B) 2S albumin, and (C) legumin allergens in tree nuts, legumes and other foods. Allergen amino acid sequences were obtained from PubMed protein searches using codes obtained from the Allergen Nomenclature web site (http://www.allergen.org) or from homology searches performed using BLAST. The phylogenic trees were generated by inputting the amino acid sequences in FASTA format into the online tool on the Phylogeny.fr web site (http://www.phylogeny.fr/simple_phylogeny.cgi)[72-76]

determining whether these similarities will lead to cross-reactive IgE binding. Future work incorporating this type of analysis is needed.

3.4 | IgE cross-reactivity

A previous study measured the correlation between peanut-, tree nut- and seed-specific IgE in patients with Spearman rank order correlation coefficients. The highest correlations were between walnut and pecan (0.96), cashew and pistachio (0.95) and almond and hazelnut (0.84). Interestingly, peanut was not highly correlated with any tree nut or seed; the highest correlation was with almond (0.53).[33] These findings highlight the high cross-reactivity that exists in individuals with certain tree nut allergies. This study also investigated the use of food-specific IgE to diagnose symptomatic nut and seed allergy. They found that the majority of peanut-allergic patients (86%) were also sensitized to tree nuts, but only 34% were clinically reactive to tree nuts.[33] However, inhibition ELISAs have shown that peanut-specific IgE can cross-react with pecan, almond, Brazil nut and hazelnut allergens, indicating that cross-reactivity is patient-specific.[54,55] Similarly, peanut-specific IgE has been shown to sensitize basophils to almond and Brazil nut allergens.[56] There are still disparities regarding the properties of these allergens that allow this cross-reactivity in vitro, but not in vivo.

One possible contributor to the disparity between IgE cross-reactivity and clinical reactivity is that not all IgE can trigger allergic reactions. For instance, peanut allergens have been categorized as major allergens or minor allergens.[57] Previously, the major allergens were defined as allergens that bind IgE from >90% of subjects and cause allergic symptoms as purified proteins. Based on this designation, Ara h 1, 2 and 3 were considered the major allergens. However, mounting evidence questioned the validity of this definition of a major allergen. More recently, the ability of peanut allergens to cross-link IgE and its high-affinity receptor FcεRI (termed allergic effector activity) has been used as a new measure.[57] Using this measure, it was found that Ara h 2 and the related protein Ara h 6 together account for the majority of the effector activity in crude peanut extracts.[57,58] Murine studies also demonstrated that Ara h 2 and Ara h 6 are the major elicitors of anaphylaxis and can desensitize peanut-allergic mice.[57,58] Therefore, although some IgE may cross-react between allergens of different tree nuts, if the cross-

binding IgEs do not cross-link IgE and FcεRI, they likely will not cause allergic symptoms.

Other potential contributors to the false positives seen in IgE tests are cross-reactive carbohydrate determinants (CCDs).[59] These carbohydrates are present on glycoproteins, mainly in plants, and bind to IgE, which are independent from peptide epitopes in allergens that contain binding sites specific to IgE.[60] Studies have shown that CCDs contribute to the false-positive IgE reactivity to peanut.[61] However, there are currently no studies that investigate the prevalence of CCDs contributing to tree nut allergies.

IgE cross-reactivity has also been investigated in mouse models of tree nut allergy. A previous study showed that mice sensitized only to cashew react upon food challenge with not only with cashew, but to walnut and to a lesser extent, peanut. Cashew-specific IgE was higher in these mice compared to cross-reactive walnut-specific and peanut-specific IgE. In a parallel experiment, mice sensitized to cashew and walnut reacted upon challenge with cashew, and more strongly when challenged with walnut. Walnut-specific IgE was significantly higher in multisensitized mice compared to monosensitized mice; peanut-specific IgE levels were low in both.[62] These findings from a mouse model demonstrate potential cross-reactivity between cashew and walnut, which further emphasize that sequence identity is not the only requirement for cross-reactivity.

3.5 | T-cell cross-reactivity

T cells play a major role in allergic responses, including during the initial sensitization of an individual. T-cell epitopes have been identified for the peanut allergen Ara h 1, walnut allergen Jug r 2 and cashew allergens Ana o 1 and 2.[63-65] Little is known about cross-reactivity between T-cell epitopes in humans, but one study has shown that T cells that reacted to cashew allergens were cross-reactive with hazelnut and pistachio, which elicited Th2 responses.[65]

In mouse models, T-cell cross-reactivity has been investigated in mono- and multisensitized mice (IgE results discussed above). Mice sensitized to cashew reacted upon challenge to cashew, walnut and less severely to peanut. Their T cells also released the Th2 cytokines IL-4 and IL-5 in response to cashew, walnut and peanut, showing clear cross-reactivity at the T-cell level.[62] Further experiments in mice and humans are needed to better understand T-cell cross-reactivity of tree nut allergens.

4 | IMPLICATIONS FOR THERAPY

Although the routes of administration and doses used vary between therapies in clinical trials, that is sublingual, OIT and EPIT, their mechanisms are broadly the same. Generally, the allergen is administered every day with increasing doses over several months to years to desensitize an allergic individual. As a result, allergen-specific Treg cells are generated, and allergen-specific Th1 and Th2 cells are suppressed. Allergen-specific IgE levels initially increase but then decrease over time, whereas IgG4 increases throughout therapy. Mast cell and basophil degranulation often decrease in response to antigen within the first few months of therapy, indicating desensitization has occurred.[66]

Based on the tree nut cross-reactivity described above, there is great potential for single allergen therapy to have therapeutic effects for multiple cross-reactive allergens. Kulis et al showed that immunotherapy with a single tree nut can prevent allergic reactions in mice with multiple tree nut allergies. They tested a cross-reactivity model where mice were sensitized to cashew, underwent immunotherapy for cashew and were able to tolerate cashew and pistachio upon challenge. In a multisensitization model, mice were sensitized to walnut and cashew and underwent immunotherapy with cashew-alone or walnut-alone; both groups were significantly protected from reactions to cashew.[67] These exciting results demonstrate the possibility to simultaneously target similar allergens during immunotherapy, and should be investigated in clinical trials.

A small preliminary study with subjects allergic to walnut and another tree nut investigated the effects of long-term walnut OIT on desensitization to walnut and the other tree nut. Oral food challenges after 142 weeks on therapy showed that the majority (7 of 8, 88%) of subjects were desensitized to walnut and the other tree nut. After 4 weeks of therapy, subjects were challenged again to determine sustained unresponsiveness; 4 of 7 (57%) were unresponsive to walnut and the other tree nut. Decreased skin prick tests to walnut and the other tree nut, along with increased walnut-specific IgG4 levels, were also observed.[68] These promising results reaffirm what animal models demonstrate and indicate that these methods can be further employed to develop OIT for patients with multiple tree nut allergies. Another recent study has shown that anti-IgE treatment paired with OIT in multifood-allergic individuals has potential for desensitizing individuals to cross-reactive allergens, including walnut and pecan, and cashew and pistachio. All subjects who underwent OIT with walnut passed an oral food challenge to pecan after 36 weeks, and the majority (83%) of subjects who underwent OIT with cashew passed an oral food challenge to pistachio at the same time point, highlighting the potential to desensitize individuals to multiple cross-reactive allergens.[69]

Another potential type of immunotherapy aims at developing hypoallergenic tree nut allergens that avoid IgE reactivity and maintain T-cell reactivity, thereby improving the safety and maintaining efficacy of immunotherapy. This approach was employed in a Phase 1 clinical trial for peanut allergens Ara h 1, 2 and 3, where recombinant Ara h 1, 2 and 3 were modified by amino acid substitution within IgE-binding epitopes and administered rectally over 13 weeks. However, many subjects experienced adverse reactions and 20% had severe allergic reactions, so the trial was discontinued.[70] Further development of this approach is required to effectively develop hypoallergenic tree nut allergens.

DNA vaccines are also being investigated for the treatment of allergies; human trials are underway for Japanese red cedar allergy (JRC-LAMP-Vax) and peanut allergy (ARA-LAMP-Vax). ARA-LAMP-Vax targets Ara h 1, 2 and 3 and has shown promise in treating peanut-allergic mice. After vaccination over 4 weeks, peanut-allergic

mice had higher peanut-specific IgG2a levels, and lower peanut-specific IgE levels compared to placebo-treated mice. Mice also had lower symptom scores and plasma histamine levels during peanut challenges compared to the placebo group.[71] These results demonstrate promise for DNA-based vaccines targeting well-defined allergens including those in tree nuts. As there is high cross-reactivity between walnut and pecan, as well as cashew and pistachio, single vaccines may be feasible for the treatment of two or more tree nut allergies.

5 | CONCLUSIONS AND OUTLOOK

Tree nut allergies have become increasingly prevalent in children, and are rarely outgrown in adulthood. Tree nut allergies are responsible for up to 40% of documented anaphylactic reactions, and in some cases have been shown to be more severe than allergic reactions to peanut. Seed storage proteins are the major allergens identified in tree nuts and other legumes/seeds, including vicilins, 2S albumins and legumins. There is high sequence identity between tree nuts, and even between tree nuts and some legumes and seeds, resulting in potential cross-reactivity between these allergens. It is currently unclear the extent of similarities required to warrant true cross-reactivity at the IgE or T-cell level. Several studies have shown how these properties can be exploited for immunotherapy against multiple tree nuts. Future work should explore the extent and efficacy of cross-reactive immunotherapy in animal models and tree nut-allergic individuals.

AUTHOR CONTRIBUTIONS

J.S., K.B. and M.K. all contributed to reviewing the published literature on tree nut allergies and drafting the manuscript. All authors have read and approved the final version.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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