



Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Tree nut allergens

Sabine Geiselhart, Karin Hoffmann-Sommergruber*, Merima Bublin

Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Keywords:

Allergy
Component resolved diagnosis
Prevalence
Recombinant allergens
Tree nuts

ABSTRACT

Tree nuts are considered as part of a healthy diet due to their high nutritional quality. However, they are also a potent source of allergenic proteins inducing IgE mediated hypersensitivity often causing serious, life-threatening reactions. The reported prevalence of tree nut allergy is up to 4.9% worldwide. The general term “tree nuts” comprises a number of nuts, seeds, and drupes, derived from trees from different botanical families. For hazelnut and walnut several allergens have been identified which are already partly applied in component resolved diagnosis, while for other tree nuts such as macadamia, coconut, and Brazil nut only individual allergens were identified and data on additional allergenic proteins are missing. This review summarizes the current knowledge on tree nut allergens and describes their physicochemical and immunological characterization and clinical relevance.

1. Introduction

Tree nuts are a potent and frequent allergen source inducing IgE mediated food allergic reactions which can cause serious and sometimes even fatal reactions. Tree nut allergy has been reported from all over the world with a prevalence of up to 4.9% of the general population (McWilliam et al., 2015).

Botanically, a tree nut is composed of a dry hard shell protecting the seed or dormant embryo such as the nuts from trees from the botanical order Fagales. The strict botanical definition is not always in line with what is generally accepted as a tree nut – an edible seed originating from a tree. Tree nuts are found in both, dicots and monocots, some of them are phylogenetically closely related, while others are not. This relationship is also reflected by sequence similarity which in turn provides the molecular background for potential IgE cross reactivity.

Usually, these seeds contain a high number of essential nutrients to provide the germinating seeds with energy for survival and proliferation. For example, seed storage proteins are highly abundant in tree nuts packed in protein bodies. Due to their high nutritional quality tree nuts are also regarded as part of a healthy diet and their consumption is recommended. This is also reflected by the annual worldwide production of tree nuts, which ranges from 4.9 M tons of cashew nuts to 1.1 M tons of pistachios (FAOSTAT, 2018).

Tree nuts can be consumed as a snack alone, as a nut mix, or as an ingredient in a range of dishes. Furthermore, they are also present in different food products such as chocolate, nougat, marzipan, nut milk and oil, pesto, cereals, baked goods, flavored coffee, and salads.

Exposure to tree nuts can also occur via using cosmetics, handling pet food, and bean bags. Food processing such as roasting, heating or enzymatic treatment may affect the allergenicity of food allergens. It has been shown that heating and dry roasting of hazelnuts and walnut can destroy some allergenic fractions, while other proteins remain stable and maintain their IgE binding activity, thus still posing a risk for the tree nut allergic patient (Downs et al., 2016; Hansen et al., 2003; Muller et al., 2000).

2. Protein families involved in tree nut allergy

The majority of proteins involved in tree nut allergy belong to protein families of 2S albumins, vicilins, legumins, and nsLTPs. Bet v 1-homologues and profilins are involved in pollen associated tree nut allergy (Fig. 1). Also, oleosins and thaumatin-like proteins were shown to be important allergens. An overview of the already identified allergens is given in Tables 1–3.

2.1. 2S albumins

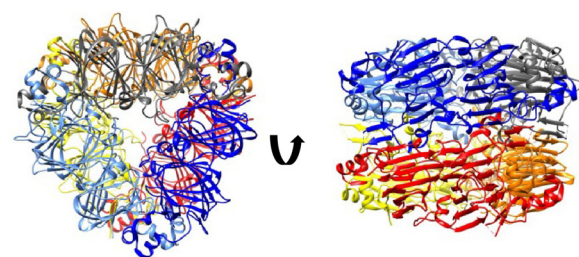
2S albumins together with nsLTPs belong to the prolamin superfamily. These proteins provide an overall alpha helical dominating structure and share 6–8 conserved cysteine residues forming disulfide bridges facilitating a compact structure. Besides these conserved structural determinants sequence similarities are rather low among members of these protein families. 2S albumins are encoded by a multigene family and contain a high number of sulfur rich amino acids.

* Corresponding author at: Department of Pathophysiology and Allergy Research, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.
E-mail address: karin.hoffmann@mv.ac.at (K. Hoffmann-Sommergruber).

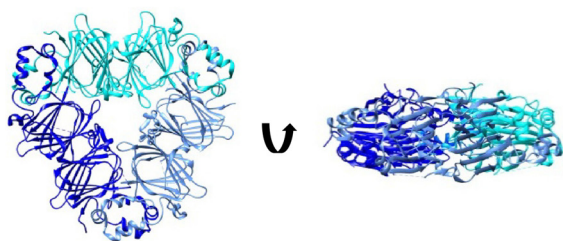
<https://doi.org/10.1016/j.molimm.2018.03.011>

Received 15 March 2018; Accepted 19 March 2018

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Legumin: Coconut (PDB: 1XGF)



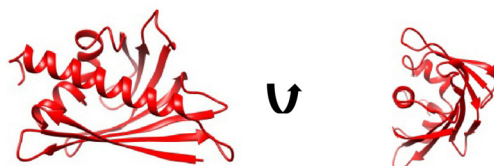
Vicilin: Pecan (PDB: 5E1R)



2S albumin: Brazil nut (PDB: 2LVF)



nsLTP: Hazelnut (PDB: 4XUW)



Bet v 1: Birch pollen (PDB: 1BV1)

Fig. 1. Representative 3D structures of tree nut allergens as well as Bet v 1 from birch pollen as a representative for Bet v 1-like proteins identified in hazelnut and walnut.

Most of them are cleaved into a large subunit of approx. 9 kDa and a small subunit of approx. 4 kDa (Moreno and Clemente, 2008). Post-translational modifications such as C-terminal truncations of the small subunit contribute to the heterogeneity of 2S albumin fractions. Their compact structure accounts for a high resistance when subjected to thermal and enzymatic treatment.

2.2. Non-specific lipid transfer proteins (nsLTPs)

NsLTPs are thought to transport phospholipids across membranes and are upregulated during plant defence upon fungal and bacterial

infections (De Lucca et al., 2005). NsLTPs are characterized by a shared structure based on 4 disulfide bridges forming an internal cavity that allows carrying ligands. It has been shown that this cavity can host different types of ligands such as fatty acids, fatty acyl CoA, phospholipids, glycolipids, hydroxylated fatty acids, and prostaglandin B2 (Carvalho and Gomes, 2007; Zachowski et al., 1998). 3D-structures of several nsLTPs have been resolved, e.g. from hazelnut, peach, and barley by either X-ray crystallography or NMR (Lerche and Poulsen, 1998; Offermann et al., 2015; Pasquato et al., 2006). Recent studies provided evidence for nsLTPs from apple, peach, and walnut that they preferentially bind unsaturated fatty acids and via this binding the C-terminal part of the tunnel undergoes a conformational change thus exposing amino acid residues to the surface (Dubiele et al., 2017). NsLTPs have been identified as allergens from a vast range of dicot plants, present in pollen, fruits, tree nuts, and seeds. Primarily, these allergens are regarded as true food allergens, indicating that sensitization occurs via the gastrointestinal tract. These allergens are accumulated in the outer layers of fruits and seeds. They display a remarkable stability against heat treatment and enzymatic degradation, however, their stability is pH dependent.

2.3. Vicilins

Vicilins (7S globulins), together with legumins (11S globulins), belong to the cupin superfamily characterized by a β -barrel structure. Typically, these glycoproteins are proteolytically processed thus providing a range of isoforms. In their native form vicilins appear as trimers with an overall molecular mass of approx. 150–190 kDa. They display an intermediate stability when subjected to heat and enzymatic treatment.

2.4. Legumins

Legumins are high molecular weight proteins (approx. 350 kDa) consisting of six subunits. The precursor protein is processed to form trimers in the endoplasmic reticulum and then transferred to the protein storage vacuoles. There each subunit is cleaved into an acidic (40 kDa) and basic polypeptide (20 kDa). These subunits remain linked by a disulfide bridge and finally form a hexameric structure (Fig. 1) (Shewry et al., 1995).

2.5. Oleosins

Oleosins, abundantly expressed in seeds and nuts, are structural proteins of oil bodies. These bodies consist of neutral lipids (92–98%), predominantly triacylglycerols, an outer monolayer of phospholipids (1–4%) and proteins (1–4%), mostly oleosins (Alexander et al., 2002). The proteins (~15–26 kDa) share similar structures, with a hydrophobic central well conserved domain of about 68–74 amino acid residues which is inserted into the triacylglycerol matrix, flanked by hydrophilic N- and C-terminal domains of variable length (50–70 and 55–98 residues). In addition to their role as structural proteins in the oil bodies there is growing evidence that they may also assist in the biosynthesis and mobilization of oils (Parthibane et al., 2012). Although oleosins have been identified as allergens from hazelnut, peanut, and seeds, further information is missing due to their low solubility in aqueous solutions (Akkerdaas et al., 2006; Leduc et al., 2006; Schwager et al., 2017).

2.6. Bet v 1-like proteins

Bet v 1-related proteins have been identified from species all over the plant kingdom. Proteins from the Bet v 1 family (154–160 amino acid residues) are non-glycosylated, share high sequence similarity and are usually encoded by a multigene family. The overall structure is also highly conserved, formed by seven stranded antiparallel β -sheets

Table 1
Hazelnut allergens.

Allergen	Isoallergens	Protein family	Accession No. (Uniprot)	Molecular mass (SDS-PAGE)	Resolved structure (PDB)	Native purified (Ref)	Recombinant: expression system / vector	IgE reactivity (% of tested patients)
Cor a 1	Cor a 1.0401	Bet v 1-like (PR-10)	Q9SWR4	17	no	Pastorello et al. (2002)	<i>E. coli</i> / pTYB11 (Lüttkopf et al., 2002)	65 of 65 (100%) – CAP-RAST (Pastorello et al., 2002)
	Cor a 1.0402		Q9FPK4					310 of 423 (74.3%) – ImmunoCAP (Datema et al., 2015)
	Cor a 1.0403		Q9FPK3					
	Cor a 1.0404		Q9FPK2					
Cor a 2	Cor a 2.0101	Profilin	Q9AXH5	14	no	no	<i>E. coli</i> / pET30a (Lauer et al., 2008)	83 of 423 (19.6%) – ImmunoCAP (Datema et al., 2015)
	Cor a 2.0102		Q9AXH4					
Cor a 8	Cor a 8.0101	nsLTP	Q9ATH2	9	4XUW	Rigby et al., (2008)	<i>E. coli</i> / pET-16b (Schocker et al., 2004) <i>P. pastoris</i> / pPICZαA (Lauer et al., 2008)	48 of 423 (11.3%) – ImmunoCAP (Datema et al., 2015)
							no	12 of 14 ^a (86%) Immunoblot (Beyer et al., 2002)
Cor a 9	Cor a 9.0101	Legumin	Q8W1C2	40	no	Rigby et al. (2008)		42 of 423 (9.9%) – ImmunoCAP (Datema et al., 2015)
Cor a 11	Cor a 11.0101	Vidilin	Q8S4P9	48	no	Rigby et al. (2008)	<i>E. coli</i> / pET100D (Lauer et al., 2004)	15 of 423 (3.5%) – ImmunoCAP (Datema et al., 2015)
								60 of 423 (14.2%) – ImmunoCAP (Datema et al., 2015)
Cor a 12	Cor a 12.0101	Oleosin	Q84T21	17	no	Zuidmeer-Jongejan et al. (2014)	<i>E. coli</i> / pET-19b (Akkerdaas et al., 2006)	
Cor a 13	Cor a 13.0101	Oleosin	Q84T91	14–16	no	Zuidmeer-Jongejan et al. (2014)	<i>E. coli</i> / pET-19b (Akkerdaas et al., 2006)	
Cor a 14	Cor a 14.0101	2S albumin	D0PWG2	12	no	Garino et al. (2010), Pfeifer et al. (2015)	<i>E. coli</i> / pPAL7 (Garino et al., 2010)	25 of 423 (5.9%) – ImmunoCAP (Datema et al., 2015)

^aHazelnut allergic patients with systemic reactions.

Table 2
Walnut allergens.

Allergen	Isoallergens	Protein family	Accession No. (Uniprot/ GeneBank Nucleotide)	Molecular mass (SDS-PAGE)	Resolved structure (PDB)	Native purified	Recombinant/ Expression system / vector	IgE reactivity (% of tested patients)
Jug r 1	Jug r 1.0101	2S albumin	P93198	15–16	no		<i>E. coli</i> / pGEX (Teuber et al., 1998)	12 of 16 (75%) – immunoblot (Teuber et al., 1998)
Jug r 2	Jug r 2.0101	Vicilin	Q9SEW4	44	no		<i>E. coli</i> / pTH19 (Sordet et al., 2009)	25 of 31 (80.6%) – ImmunoCAP (Pedrosa et al., 2015)
Jug r 3	Jug r 3.0101	nsLTP	C5H617	9	no	Pastorello et al. (2004)	<i>E. coli</i> / pGEX (Teuber et al., 1999)	9 of 15 (60%) – ImmunoCAP (Teuber et al., 1999)
Jug r 4	Jug r 4.0101	Legumin	Q2TPW5		no		<i>E. coli</i> / pMAL-c2X (Wallowitz et al., 2006)	37 of 46 (80%) – immunoblot (Pastorello et al., 2004)
Jug r 5	Jug r 5.0101	Bet v 1-like	APD76154.1	20	no		<i>E. coli</i> / pMAL-c2X (Wallowitz et al., 2006)	15 of 23 (65%) – immunoblot (Teuber et al., 2003)
Jug r 6	Jug r 6.0101	Vicilin	XM_018959147.1	47	no	Dubiela 2017 (www.allergen.org)		21 of 37 (57%) – immunoblot (Wallowitz et al., 2006)
Jug r 7	Jug r 7.0101	Profilin	MG366484	13	no			5 of 9 (55%) – ImmunoCAP (Wangorsch 2016, www.allergen.org)
								19 of 76 (25%) – ELISA (Dubiela 2017, www.allergen.org)
								48 of 217 (22%) – ImmunoCAP (Versteeg 2017, www.allergen.org)

curving around a long C-terminal helix and two additional helices, thus providing a large hydrophobic pocket which is able to bind ligands such as plant steroids. Several structures of these proteins have been resolved either by crystallography or NMR approaches. Bet v 1-related proteins are expressed in a range of plant organs, predominantly in mature pollen, fruits, and seeds. Together with homologues from closely related trees these allergens are causing inhalant allergies. After this primary sensitization to the pollen allergen most of the patients develop allergic symptoms upon ingestion of Bet v 1-related proteins from food. Usually, the symptoms of this pollen related food allergy tend to be restricted to the oral cavity and rarely induce severe generalized symptoms. Bet v 1-related proteins get rapidly degraded when subjected to heating or enzymatic treatment.

2.7. Profilins

These small non-glycosylated cytosolic proteins (12–15 kDa) are expressed in all eukaryotic cells and fulfill a number of key functions such as regulating intracellular transport, cellular movement, and signaling (Witke, 2004). Sequences from profilins from higher plants are highly homologous. These proteins have a globular structure with a central seven stranded antiparallel β -sheet enclosed by N- and C-terminal α -helices. These proteins have intermediate stability when subjected to heating, ultrahigh pressure, and enzymatic treatment. Profilins have been identified as allergens from pollen and plant food. Due to their high sequence similarity they account for frequent IgE cross-reactivity, however, not always linked with clinical relevance.

3. Allergenic tree nuts and their allergens

3.1. Hazelnut

Hazelnuts (*Corylus avellana*) are classified as tree nuts belonging to the family of Betulaceae. They are consumed worldwide due to their nutritional value and beneficial health effect. Although there are significant geographic and age-related variations regarding the severity of symptoms, hazelnut allergy has been reported to be the most common tree nut allergy in Europe (Burney et al., 2014; McWilliam et al., 2015).

Currently, eight allergens from hazelnut are registered in the database of the International Union of Immunological Societies (IUIS; www.allergen.org). In birch endemic areas, allergy to hazelnut mainly correlates with cross-reactive IgE to Bet v 1 and Bet v 2, usually causing mild symptoms (Datema et al., 2015). The cross-reactive components in hazelnut, Cor a 1 (17 kDa, Bet v 1-homologue) and Cor a 2 (14 kDa, profilin), are described to be abundant in both, pollen and seeds. So far, four different variants of Cor a 1 from the kernels, Cor a 1.0401–Cor a 1.0404, have been cloned and expressed in *E. coli* showing an IgE reactivity of 95%–74% in a hazelnut patients, cohort (Lüttkopf et al., 2002). In a larger set of hazelnut allergic patients across Europe, 74% (310 of 423) were sensitized (Datema et al., 2015). Two variants of hazelnut profilin have been described, Cor a 2.0101 and Cor a 2.0102 (www.allergen.org). Among patients with hazelnut allergy 42% had specific IgE to rCor a 2 (Hansen et al., 2009).

In Mediterranean areas, hazelnut allergy is mainly linked to the nsLTP, Cor a 8 (9 kDa), and associated with more severe reactions (Schocker et al., 2004). To date, this is the first resolved structure of a hazelnut allergen (Offermann et al., 2015). Although structurally similar to Pru p 3, the nsLTP from peach, there are differences in epitope binding areas between the two molecules, which may contribute to limited cross-reactivity between Pru p 3 and Cor a 8.

In 2002, Beyer et al. identified a 40 kDa IgE binding protein belonging to the legumin family. The protein was recognized by serum IgE from 12 of 14 (86%) patients with severe reactions (Beyer et al., 2002). Recently, another hazelnut 55 kDa legumin was identified that turned out to be an isoform of Cor a 9. However, only the basic subunit exhibited IgE-affinity (Nitride et al., 2013).

Table 3
Other officially recognized tree nut allergens.

Allergen	Isoallergens	Protein family or function	Acc. No. (UniProt)	kDa - SDS-PAGE/ theoret.	Native/recombinant	Expression system/ vector	Resolved structure (PDB)	IgE reactivity % of tested patients	References
Pecan (<i>Carya illinoensis</i>)									
Car i 1	Car i 1.0101	2S albumin	Q84XA9	16/16.5	Recombinant	<i>E. coli</i> /pMAL-c4x	no	79% of 25	Sharma et al. (2011a)
Car i 2	Car i 2.0101	Vicilin	B3STU4	55/55	Recombinant	<i>E. coli</i> /pMAL-c4x	5E1R	24% of 25	Lee et al. (2014) and Zhang et al. (2016)
Car i 4	Car i 4.0101	Legumin	B5KVH4	55.4	Recombinant	<i>E. coli</i> /pMAL-c4x	no	59% of 27	Sharma et al. (2011b)
Almond (<i>Prunus dulcis</i>)									
Pru du 3	Pru du 3.0101	nsLTP	COL015	9/10.4	Recombinant	<i>E. coli</i> /pMAL-c4x	no	16% of 31	www.allergen.org
Pru du 4	Pru du 4.0101	Profilin	Q8GSL5	14/13.9	Both	<i>E. coli</i> /pMAL-c2	no	Rec. 33% of 18 Nat. 44% of 18	Tawde et al. (2006)
Pru du 5	Pru du 5.0101	60 s acidic ribosomal prot. P2	Q8GSL5 Q8H2B9	10/11.4	Recombinant	<i>E. coli</i> /pMAL-c2	no	6 pooled sera	Abolhassani and Roux (2009)
Pru du 6	Pru du 6.0101	Legumin	E3SH28	65/61	Both	<i>E. coli</i> /pMAL-c4x	3FZ3	46% of 13	Albillos et al. (2008) and Jin et al. (2009)
	Pru du 6.0102		E3SH29	65/55.9				30% of 13	www.allergen.org
Cashew (<i>Anacardium occidentale</i>)									
Ana o 1	Ana o 1.0101	Vicilin	Q8L5L5	50/61.8	Both	<i>E. coli</i> /pMAL-c2	no	50% of 20	Wang et al. (2002)
	Ana o 1.0102		Q8L5L6	50/59					
Ana o 2	Ana o 2.0101	Legumin	Q8GZP6	55/52	Both	<i>E. coli</i> /pMAL-c2	no	62% of 21	Robotham et al. (2005)
Ana o 3	Ana o 3.0101	2S albumin	Q8H2B8	14/16.3	Both	<i>E. coli</i> /pMAL-c2	no	81% of 28	Reisma et al. (2016) and Robotham et al. (2005)
Pistachio (<i>Pistacia vera</i>)									
Pis v 1	Pis v 1.0101	2S albumin	B7P072	7/17.3	Recombinant	<i>E. coli</i> /pET24b	no	68% of 28	Ahn et al. (2009)
Pis v 2	Pis v 2.0101	Legumin	B7P073	32/56.5	Recombinant	<i>E. coli</i> /pET24b	no	50% of 28	Ahn et al. (2009)
	Pis v 2.0201		B7P074	32/53.3					
Pis v 3	Pis v 3.0101	Vicilin	B4X640	55/59.8	Recombinant	<i>E. coli</i> /pMAL-c2	no	36% of 14	Willison et al. (2008)
Pis v 4	Pis v 4.0101	Manganese superoxide dismutase	B2BDZ8	25.7/25.8	Recombinant	<i>E. coli</i> /pET-32b	no	59% of 27	Noorbakhsh et al. (2010)
Pis v 5	Pis v 5.0101	Legumin	B7SLJ1	36/51.1	Native	-	no	36% of 28	www.allergen.org
Brazil nut (<i>Bertholletia excelsa</i>)									
Ber e 1	Ber e 1.0101	2S albumin	P04403	9/16.9	Both	<i>P. pastoris</i> /pPIC9	2LVF	89% of 9	Alcocer et al. (2002) and Pastorello et al. (1998)
Ber e 2	Ber e 2.0101	Legumin	Q84ND2	29/52.3	Both	<i>E. coli</i>	no	44% of 27	Beyer et al. (2008) and Guo et al. (2007)

The first evidence of a 48 kDa glycosylated allergenic seed storage protein came from Muller et al. in 2000. The protein was recognized by IgE from 41% of adults and 61% of children allergic to hazelnut (Muller et al., 2000). The heat-resistant protein did not show cross-reactivity to birch pollen allergens. The characterization of the hazelnut vicilin Cor a 11 revealed two potential glycosylation sites and a prevalence of IgE reactivity below 50%. Recombinant and natural Cor a 11, sharing similar secondary structures induced a similar mediator release suggesting that the glycans do not contribute to the allergenic activity. In a birch-endemic region, sensitization to Cor a 11 correlated with severe hazelnut allergy (Verweij et al., 2012).

The hazelnut 2S albumin Cor a 14 together with Cor a 9 has been described as a high risk marker for severe reactions. Masthoff et al. showed that IgE to Cor a 14 had a diagnostic specificity of greater than 90% and accounted for 83% of children and 44% of adults with hazelnut allergy (Masthoff et al., 2013). Also, a Belgian study demonstrated that IgE to Cor a 14 and Cor a 9 enables to identify almost 90% of preschool and 80% of school-aged children, but only 29% of adults with generalized reactions to hazelnut (Faber et al., 2014). Natural Cor a 14 shares the typical features of 2S albumins with high thermostability and resistance to gastrointestinal digestion (Pfeifer et al., 2015). Further, the existence of two different isoforms and both, linear and conformational epitopes were shown.

A group of hazelnut allergens that have been isolated from oil bodies are the so called oleosins. Insoluble in water, these proteins have long been overseen in diagnostic tests. The first identification of hazelnut oleosins (Cor a 12 and Cor a 13, 14, and 17 kDa, respectively) came from Akkerdaas et al., who cloned Cor a 12 from a cDNA library and expressed it in *E. coli* (Akkerdaas et al., 2006). They showed that sensitization to hazelnut oleosins is associated with severe systemic reactions and a negative skin-prick test (Zuidmeer-Jongejan et al., 2014). Sensitization to Cor a 12 ranged between 10 and 25% across Europe. Children (34%) were more often sensitized to oleosins than adults (11.4%). Overall, 14.2% of hazelnut allergic patients had specific IgE to Cor a 12 (Datema et al., 2015). Sensitization to oleosins correlated with sensitization to other oil-rich foods such as soybean, sesame, peanut, sunflower, but also to latex and diverse pollen. However, it has to be considered that in most studies, the experiments were carried out with oil body fractions which were virtually contaminated with other allergens (Zuidmeer-Jongejan et al., 2014).

Hazelnut thaumatin-like protein (TLP), not listed in the IUIS database, has also been linked to severe allergic reactions. First data about the existence of a hazelnut TLP came from a study evaluating the allergen composition of commercial extracts for diagnosis (Akkerdaas et al., 2003). To analyze their Cor a TLP content, extracts were analyzed by immunoblotting using a polyclonal serum against recombinant apple TLP, resulting in 2 bands of 25 and 50 kDa, representing the mono- and dimeric forms, respectively. A Spanish study revealed that Cor a TLP was recognized by less than 10% of hazelnut allergic patients (Palacín et al., 2012).

3.2. Walnut

The common walnut (*Juglans regia*) belongs to the family of Juglandaceae and is cultivated around the world, predominantly in temperate climates. The edible kernels are part of healthy, balanced food diets because of their high content of polyunsaturated fatty acids, phytochemicals, and antioxidants. Generally, there is little information concerning the prevalence of walnut allergy. In contrast to Europe, where the prevalence of walnut allergy is rather low with an overall incidence of 2.2% (Burney et al., 2014), it is the most frequently reported tree nut allergy in the US (Sicherer et al., 2010).

Currently, seven allergens from walnut (Jug r 1–7) are officially recognized (www.allergen.org). Jug r 1 was cloned from a cDNA expression library prepared from walnut somatic embryos. It belongs to the 2S albumin protein family and shows 46.1% sequence identity with

Ber e 1, the 2S albumin from Brazil nut. Seventy five percent of walnut allergic patients had specific IgE against rJug r 1 expressed in *E. coli* (Teuber et al., 1998). Robotham et al. found one linear immunodominant IgE epitope of Jug r 1 which was recognized by 75% of walnut allergic patients (Robotham et al., 2002). In a subsequent study, four additional IgE epitopes were identified (Sordet et al., 2009). Further, they reported cross-reactivity between Jug r 1 and sesame seed extract but not for other tree nuts and peanut. Tested by ImmunoCAP ISAC, Jug r 1 showed a better diagnostic performance than Jug r 2 and Jug r 3 (Pedrosa et al., 2015).

Teuber et al. identified and cloned a second walnut allergen, Jug r 2 a vicilin with a molecular mass of 47 kDa, by using the same methodology as for Jug r 1 (Teuber et al., 1999). Nine of 15 patients (60%) had specific IgE, thus identifying Jug r 2 as a major allergen. Inhibition experiments showed minimal or no cross-reactivity between Jug r 2 and pea, peanut, and cacao. Jug r 2 proved to be responsible for T cell responses in patients with walnut allergy (Archila et al., 2015). Just recently, another member of the vicilin family has been deposited in the IUIS database, designated Jug r 6. The natural protein was purified from walnuts and showed a molecular mass of 47 kDa. Nineteen of 76 (25%) sera from walnut sensitized subjects demonstrated IgE binding to the natural purified protein (www.allergen.org).

In a study analyzing low molecular weight allergens from walnut, Downs et al. identified allergenic peptides from the N-terminal region of the vicilin proprotein (Downs et al., 2014). IgE immunoblotting revealed that 3 of 11 (27%) sera from walnut-allergic subjects showed reactivity to these fragments.

Another allergenic walnut protein belonging to the legumin family was identified by Teuber et al. and designated Jug r 4. Of the 23 patients tested, 15 (65%) patients had specific IgE against *E. coli* derived rJug r 4 (Teuber et al., 2003). Later on, they showed that serum IgE from 21 of 37 (57%) patients recognized the rJug r 4-pMAL fusion protein. In vitro cross reactivity was shown for hazelnut, cashew, and peanut (Wallowitz et al., 2006).

In 2004, a walnut nsLTP was identified and designated Jug r 3 (Pastorello et al., 2004). In an Italian cohort, sera from 37 of 46 (80%) walnut allergic patients had Jug r 3-specific IgE. This specific binding could be inhibited by Pru p 3, the nsLTP from peach.

Also in walnut a PR-10-related protein was recently identified and designated Jug r 5. Wangorsch et al. used a cDNA clone encoding the Bet v 1 homologue from walnut kernels by RT-PCR and produced recombinant Jug r 5 in *E. coli* (Wangorsch et al., 2017). Screening of 16 sera from birch pollen allergic subjects with concomitant walnut allergy revealed that although only 44% were tested positive to walnut, 94% were reactive to rJug r 5. Recombinant Jug r 5 will be a useful diagnostic tool, compensating the low sensitivity of walnut extracts.

Recombinant walnut profilin Jug r 7 was recognized by 48 of 217 (22%) sera from patients sensitized to walnut (www.allergen.org).

3.3. Pecan

Pecan tree (*Carya illinoensis*) belongs to the family of Juglandaceae along with walnut. The high sequence identity among three identified pecan allergens Car i 1 (2S albumin), Car i 2 (vicilin), and Car i 3 (legumin) with their counterparts from walnut explain the frequently observed association between walnut and pecan allergies (Andorf et al., 2017; Elizur et al., 2018; Maloney et al., 2008).

Sequences coding for the three pecan allergens were obtained by PCR from the maturing pecan nuts cDNA library using specific primers based on the available mRNA sequences in GenBank and the recombinant proteins were produced in *E. coli*.

Amino acid sequence comparison of Car 1.0101 with other 2S albumins showed the highest identity to Jug r 1 (88%) and lowest for Pis v 1 (35%). Recombinant Car i 1 was produced as a protein of 17 kDa including a 20 amino acid N-terminal signal peptide which is usually not present in the mature protein (Sharma et al., 2011a). The presence

of Car i 1.0101 in pecan extract was confirmed by purifying natural pecan 2S albumin and subsequent identification of matching peptides by MALDI-TOF-MS/MS sequencing. The recombinant protein was recognized by 79% of 28 sera from pecan allergic subjects. However, rCar i 1 showed very low ability to inhibit IgE binding to natural purified pecan 2S albumin (Sharma et al., 2011a).

Two recombinant variants of Car i 2.0101 were produced: one form containing both, the C-terminal domain comprising the structural core of the vicilin as well as the N-terminal low complexity region, while the second form represented only the structural core (Zhang et al., 2016). Both proteins displayed similar IgE binding indicating the localization of IgE-epitopes in the protein core. The crystal structure revealed a typical cupin fold with three vicilin molecules forming a doughnut-shaped trimer through head-to-tail association. Similarly to Korean pine nut vicilin, Car i 2, in its native trimeric state, has three copper ligands (Zhang et al., 2016). The IgE binding was observed in 24% (6 of 25) of tested sera from pecan allergic subjects (Lee et al., 2014; Zhang et al., 2016).

Car i 4.0101 has high sequence identity to other tree nut legumins ranging from 95% with Jug r 4 from walnut to 54% with Pru du 6 from almond. The mapping of Car i 4 identified sequential IgE epitopes indicating cross-reactivity with legumins from walnut and hazelnut. The recombinant protein was recognized by 57% of 28 tested sera from pecan allergic patients (Sharma et al., 2011b).

3.4. Almond

Almond is a drupe of *Prunus dulcis*, a tree from the Rosaceae family. It is one of the most commonly produced and consumed nuts all over the world (FAOSTAT, 2018). The nuts can be consumed either raw or processed in a wide variety of forms, which are more or less related to dietary habits specific for populations from different geographic regions. According to studies on the prevalence of tree nut allergies, almond allergy usually ranks fourth (Elizur et al., 2018; McWilliam et al., 2015).

Out of six identified almond allergens, Pru du 3 (nsLTP), Pru du 4 (profilin), Pru du 5 (60S ribosomal protein), and Pru du 6 (legumin), are included in the WHO-IUIS allergen list (www.allergen.org). Pru du 3 (Costa et al., 2012), Pru du 4 (Tawde et al., 2006), and Pru du 5 (Abolhassani and Roux, 2009) were identified by screening an almond cDNA library with sera from almond allergic patients. These allergens were produced in *E. coli* using the pMAL expression vector and IgE reactivity was evaluated by dot blot analysis. Pru du 3.0101 displays 99% amino acid sequence identity to a nsLTP from peach (UniProtKB-M5W0S9, not listed in the IUIS) and 57% to two other peach nsLTP isoforms, Pru p 3.0101 and Pru p 3.0102. Association of almond and peach allergy due to nsLTP-sensitization has been confirmed in a case study, since a 9 kDa IgE-binding protein present in almond fruit mesocarp/epicarp was recognized by a monoclonal anti-Pru p 3 antibody (Garrido-Fernandez et al., 2009). Two clones of almond profilin (Pru du 4) differing in only two silent mutations were identified. In parallel, 2D immunoblotting revealed the presence of at least two native isoforms in almond extract (Tawde et al., 2006). Recombinant Pru du 4 was recognized by 33% (6 of 18) of patients with pollinosis and self-reported almond allergy suggesting its relevance in pollen-associated almond allergy. Pru du 5 has been identified as an 11.4 kDa 60S ribosomal protein involved in protein biosynthesis (Abolhassani and Roux, 2009). IgE reactivity of the recombinant protein was confirmed using pooled or individual sera of almond allergic patients. Nevertheless, the clinical relevance of this allergen is unknown and requires further research.

Pru du 6, a legumin also known as almond major protein or amandin (Osborne and Campbell, 1896) is the first and best biophysically characterized allergen in almond to date. The crystal structure of natural Pru du 6 (prunin-1) was determined, showing a conserved typical legumin fold (Albillos et al., 2008; Jin et al., 2009). Two cDNA clones encoding almond legumins named prunin-1 (Pru du 6.0101) and

prunin-2 (Pru du 6.0201) have been isolated. Pru du 6.0101 (61.0 kDa) and Pru du 6.0201 (55.9 kDa) share 57% amino acid sequence identity. *E. coli* derived rPru du 6.0101 was recognized by 6 and rPru du 6.0201 by 4 sera from 13 almond allergic subjects as evaluated by dot blot analysis (www.allergen.org). In addition to the allergens officially deposited in the WHO-IUIS database, two IgE binding proteins were identified in almond protein extract by immunoblotting using 5 sera of almond allergic patients (Poltronieri et al., 2002). The 45 kDa IgE binding protein was purified and identified by N-terminal sequencing as vicilin (UniProtKB-P82952). Another 12 kDa IgE binding protein was identified as 2S albumin (UniProtKB-P82944).

3.5. Cashew

Cashew is the seed of the cashew plant (*Anacardium occidentale*), a member of the Anacardiaceae family. Cashew allergens can induce severe reactions at minute doses (Blom et al., 2013; van der Valk et al., 2016a), and the prevalence of cashew nut allergy appears to be increasing paralleled by the increasing consumption of this nut (Johnson et al., 2014).

To date, three cashew allergens have been recognized officially: Ana o 1, a vicilin-like protein, Ana o 2, a legumin-like protein, and Ana o 3, a 2S albumin. These cashew allergens are well characterized regarding their sequence data and recombinant proteins are available (Robotham et al., 2005; Wang et al., 2002, 2003).

Two Ana o 1 encoding clones and one single Ana o 2 clone were isolated by screening cashew cDNA library with sera from cashew allergic patients and rabbit antisera raised against cashew proteins (Robotham et al., 2005; Wang et al., 2002). Ana o 1.0102 represents the truncated 5' end of Ana o 1.0101 differing in only a single nucleotide substitution in the overlapping region. Both Ana o 1 clones were expressed in *E. coli* as recombinant proteins of 62 and 59 kDa, respectively (Wang et al., 2002). Recombinant Ana o 1 was recognized by 50% of 20 sera from cashew allergic subjects. The amino acid sequence of Ana o 1.0101 exhibits 81% identity to the vicilin, Pis v 3.0101, from pistachio and 53% to Cor a 11 from hazelnut. Natural purified Ana o 1 was characterized as a glycoprotein appearing as a monomer of 50 kDa or as a dimer of approx. 100 kDa (Reitsma et al., 2016). One single isoform of Ana o 2 was expressed as a 52 kDa protein in *E. coli* recognized by 13 of 21 sera from cashew allergic patients (Robotham et al., 2005). Ana o 2 shares 79% sequence identity with legumin from pistachio Pis v 5, 59% with legumins from pecan, Car i 2 and walnut, Jug r 4. The monomer of natural purified Ana o 2 appeared as a protein of 53 kDa consisting of a 30 kDa large subunit and a 21 kDa small subunit (Reitsma et al., 2016). One isoform of cashew 2S albumin, Ana o 3.0101, was amplified from a cDNA library and the recombinant protein of approx. 16 kDa was produced in *E. coli* (Robotham et al., 2005). Cashew 2S albumin shows 64% amino acid sequence identity to Pis v 1 from pistachio and 43% to Cor a 14 from hazelnut. Natural purified Ana o 3 was shown to be proteolytically processed into two subunits of approx. 8 and 4 kDa held by 4 disulfide bridges with N- and C-terminal micro-heterogeneity characteristic for 2S albumins (Reitsma et al., 2016).

Recombinant Ana o 3 (available for routine serological diagnostic) has been reported as a highly accurate diagnostic marker for cashew nut allergy, showing higher diagnostic specificity (94.4%) as compared to total cashew extract (58.3%). Additionally, rAna o 3 was also considered as a diagnostic marker for pistachio allergy (Savvatanos et al., 2015). In a study from van der Valk et al., sIgE levels to natural purified Ana o 1, 2 and 3 were individually predictive for the positive outcome of food challenge tests in cashew-allergic children because of the high correlation of IgE levels between these components (van der Valk et al., 2016b). Therefore, application of one of the components was suggested to be probably sufficient.

3.6. Pistachio

Belonging to the same Anacardiaceae family the seeds of pistachio (*Pistacia vera*) and cashew plant, share a similar protein expression profile. Expected cross-reactivity of IgE binding proteins was confirmed by different studies (Andorf et al., 2017; Elizur et al., 2018; Maloney et al., 2008; Noorbakhsh et al., 2011). Pistachio allergens identified so far belong to the protein families of 2S albumins (Pis v 1), legumins (Pis v 2 and Pis v 5), vicilins (Pis v 3), and iron/manganese superoxide dismutase (Pis v 4).

Pis v 1 and Pis v 2 have been identified as 7 kDa and 32 kDa IgE binding proteins in 68% and 50% of 28 pistachio allergic sera, respectively (Ahn et al., 2009). In parallel, these purified allergens were characterized by N-terminal sequencing. Subsequently, one single full length Pis v 1 cDNA clone and two Pis v 2 clones were isolated from a cDNA library generated from immature pistachios by PCR screening with primers designed on the basis of the internal amino acid sequences (Ahn et al., 2009). Pis v 1.0101 shares 64% sequence identity with Ana o 3, a cashew 2S albumin. Pis v 2.0101 and Pis v 2.0201 show 84% amino acid sequence identity. Both Pis v 2 isoforms display 48% identity to cashew legumin Ana o 2. However, another pistachio legumin designated Pis v 5 (www.allergen.org), more closely related to Ana o 2 (79% amino sequence identity), shares only 51% amino acid sequence identity to Pis v 2. Both recombinant *E. coli*-derived proteins, rPis v 1 and rPis v 2, were recognized by 6 selected sera from pistachio allergic patients (Ahn et al., 2009).

Full length clones of Pis v 3 and Pis v 4 were isolated from a cDNA library generated from mature pistachios by screening with degenerated primers specific for conserved sequences from vicilins or plant manganese superoxide dismutases (MnSOD) (Noorbakhsh et al., 2010; Willison et al., 2008). Recombinant Pis v 3 was expressed in *E. coli* as a maltose-binding fusion protein using the pMAL vector and Pis v 4 as a thioredoxin fusion protein using pET-32b(+) (Willison et al., 2008). Pis v 3 shares 80% amino acid sequence identity to cashew vicilin, Ana o 1, as well as a common sequential IgE epitope (Rouge et al., 2011), explaining high IgE cross-reactivity of these two proteins (Willison et al., 2008). The recombinant protein was recognized by 36% of 14 sera from pistachio allergic subjects. The amino acid sequence of Pis v 4 exhibits 88% identity to MnSOD from latex suggesting potential IgE cross-reactivity but the clinical relevance is yet to be confirmed. IgE binding activity of natural Pis v 4 (23 kDa) protein was confirmed in 59% (16 out of 27) of tested sera by IgE immunoblotting with pistachio extract (Ayuso et al., 2007). Recombinant Pis v 4 fusion protein was recognized by 40% (10 of 25) of allergic patients' sera tested by IgE ELISA (Noorbakhsh et al., 2010).

3.7. Brazil nut

Brazil nut is the seed of a giant tree (*Bertholletia excelsa*) native to South America. Allergy to Brazil nut is common in the US and UK (Roberts et al., 2005; Sicherer et al., 2010) and may be associated with severe reactions (Borja et al., 1999; Ewan, 1996; Pastorello et al., 1998). To date, Ber e 1 (2S albumin) and Ber e 2 (legumin) are characterized allergens from this source.

Ber e 1 aroused interest as a prime example of the unintended consequences of cloning an allergen encoding gene to create a genetically modified crop. Initially, this 2S albumin has attracted scientific attention due to its unusual large content of sulfur rich amino acids, methionine (18%) and cysteine (8%) (Altenbach et al., 1992; Sun et al., 1987). To improve the nutritional quality with methionine, Brazil nut 2S albumin has been inserted into transgenic soybeans (Townsend and Thomas, 1994). However, the newly expressed protein in this transgenic soybean retained its allergenic activity as has been shown by IgE immunoblotting and skin-prick testing with Brazil nut allergic patients (Nordlee et al., 1996). That study underlined the importance of assessing the allergenic risk of foods derived from genetically engineered

plants.

Purified native Ber e 1 is composed of two polypeptides with molecular masses of about 9 and 3 kDa which are joined by disulfide bonds, a structure specific for 2S albumins. Encoded by a multigene family, several isoforms of the protein were determined (Altenbach et al., 1992; Ampe et al., 1986). Ber e 1 shows low amino acid sequence identity to other tree nut 2S albumins such as 47% to Cor a 14 and 41% to Jug r 1. For determination of the 3D structure, recombinant properly folded and processed Ber e 1 was produced in *Pichia pastoris* (Alcocer et al., 2002). The best expressing clone yielded around 200 mg of the protein per liter (Rundqvist et al., 2012). The overall fold of rBer e 1 was similar to other 2S albumins. The NMR spectra demonstrated the protein interaction with lipids and Cu²⁺ ions (Rundqvist et al., 2012). The interaction of Ber e 1 with lipids has been shown to be relevant to induce an allergic response in mice (Dearman et al., 2007).

It has been shown that *in vitro* determination of rBer e 1-specific IgE has the potential to diagnose Brazil nut allergy, with a higher sensitivity and no loss of specificity as compared to measuring IgE specific to Brazil nut extract (Pastorello et al., 1998; Rayes et al., 2016). However, the significance of these findings remains to be determined in more cohort studies in different areas.

Ber e 2, also known as excelsin, is not yet fully characterized. Purification of the natural protein and initial crystallographic characterization showed that pre-Ber e 2 (52 kDa) is post-translationally cleaved into multiple polypeptides held by disulfide bonds (Guo et al., 2007). The cloning and positive IgE-reactivity of the recombinant protein in 44% of sera from Brazil nut sensitized patients has been reported by Beyer et al. (Beyer et al., 2008).

3.8. Coconut

Coconut is the drupe of the monocotyledonous palm tree (*Cocos nucifera*) from the Arecaceae family. So far, coconut allergy was only described in a small number of case reports (Benito et al., 2007; Manso et al., 2010; Nguyen et al., 2004; Tella et al., 2003). IgE cross-reactivity among nuts, coconut and sesame was observed *in vitro* (Nguyen et al., 2004; Teuber and Peterson, 1999). However, a US study provided evidence of no significant risk of allergy to coconut in peanut and tree nut allergic children (Stutius et al., 2010). In a recent study, IgE sensitization to coconut was highly associated with sensitization to almond and macadamia (Polk et al., 2016). Currently, only one allergen, Coc n 1, belonging to the vicilin protein family is accepted. It was identified as an IgE binding protein in coconut palm pollen by using 10 sera from patients sensitized to coconut palm pollen (Saha et al., 2015). In a previous study, a 29 kDa IgE binding protein was detected in coconut extract using 2 sera from coconut allergic individuals. This protein was further identified by MALDI-TOF-MS as a vicilin (Benito et al., 2007).

3.9. Macadamia

Macadamia nuts are seeds of the evergreen tree *Macadamia integrifolia* of the Proteaceae family. Several case reports have described anaphylaxis in macadamia allergic patients but there was no information regarding the identity and characterization of the relevant allergens. IgE reactivity to a 17 kDa protein in raw and roasted macadamia extract not specified in any further detail has been described. A low IgE cross-reactivity of this protein with proteins from hazelnut extract was demonstrated (Sutherland et al., 1999). In a case report of a patient with severe symptoms after ingestion of macadamia a 12 kDa IgE binding protein was identified from macadamia extract in immunoblots under non-reducing conditions (Herbst et al., 2010). Interestingly, specific IgE assays frequently provided false negative IgE antibody results for macadamia nut (De Knop et al., 2010; Ekbote et al., 2010; Knott et al., 2008; Lerch et al., 2005).

4. Conclusions

Although tree nut allergy is regarded as a relevant food allergy globally, reliable prevalence data are scarce and no immunotherapy is available. Therefore, large clinical studies on tree nut allergies in different geographic regions are needed. So far, a number of food allergens have been identified from tree nuts, some of which are available as purified natural proteins, while others are recombinant products derived from prokaryotic and eukaryotic expression systems. Their physicochemical characterization ranges from primary sequence analyses up to detailed structural analyses, determination of IgE epitopes and stability assessment of food allergens upon food processing. To date, some allergen panels such as from hazelnut and walnut include both, seed storage proteins and pollen related allergens, while other allergen panels are incomplete and lack major components. Application of refined analytical methods will help to decide which molecules to include for component resolved diagnosis as well as to design hypoallergenic variants for immunotherapeutic options.

Funding

This work was supported by the Austrian Science Fund (FWF): [SFB F4603] and by the Jubiläumsfonds der Österreichischen Nationalbank [No. 17560].

Conflict of interest

The authors declare no commercial or financial conflict of interest.

References

- Abolhassani, M., Roux, K.H., 2009. cDNA cloning, expression and characterization of an allergenic 60s ribosomal protein of almond (*Prunus dulcis*). *Iran J. Allergy Asthma Immunol.* 8, 77–84.
- Ahn, K., Bardina, L., Grishina, G., Beyer, K., Sampson, H.A., 2009. Identification of two pistachio allergens, Pis v 1 and Pis v 2, belonging to the 2S albumin and 11S globulin family. *Clin. Exp. Allergy* 39, 926–934.
- Akkerdaas, J.H., Schocker, F., Vieths, S., Versteeg, S., Zuidmeer, L., Hefle, S.L., Aalberse, R.C., Richter, K., Ferreira, F., Van Ree, R., 2006. Cloning of oleosin, a putative new hazelnut allergen, using a hazelnut cDNA library. *Mol. Nutr. Food Res.* 50, 18–23.
- Akkerdaas, J.H., Wensing, M., Knulst, A.C., Krebitz, M., Breiteneder, H., De Vries, S., Penninks, A.H., Aalberse, R.C., Hefle, S.L., Van Ree, R., 2003. How accurate and safe is the diagnosis of hazelnut allergy by means of commercial skin prick test reagents? *Int. Arch. Allergy Immunol.* 132, 132–140.
- Albillos, S.M., Jin, T., Howard, A., Zhang, Y., Kothary, M.H., Fu, T.J., 2008. Purification, crystallization and preliminary x-ray characterization of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. *J. Agric. Food Chem.* 56, 5352–5358.
- Alcocer, M.J.C., Murtagh, G.J., Bailey, K., Dumoulin, M., Meseguer, A.S., Parker, M.J., Archer, D.B., 2002. The disulphide mapping, folding and characterisation of recombinant Ber e 1, an allergenic protein, and SFA8, two sulphur-rich 2S plant albumins. *J. Mol. Biol.* 324, 165–175.
- Alexander, L.G., Sessions, R.B., Clarke, A.R., Tatham, A.S., Shewry, P.R., Napier, J.A., 2002. Characterization and modelling of the hydrophobic domain of a sunflower oleosin. *Planta* 214, 546–551.
- Altenbach, S.B., Pearson, K.W., Sun, S.S.M., 1992. Nucleotide-sequences of cDNAs encoding 2 members of the Brazil nut methionine-rich 2S albumin gene family. *Plant Phys.* 98, 1520–1522.
- Ampe, C., Vandamme, J., Decastro, L.A.B., Sampaio, M.J.A.M., Vanmontagu, M., Vandekerckhove, J., 1986. The amino-acid-sequence of the 2S sulfur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H.B.K.). *Eur. J. Biochem.* 159, 597–604.
- Andorf, S., Borres, M.P., Block, W., Tupa, D., Bollyky, J.B., Sampath, V., Elizur, A., Lidholm, J., Jones, J.E., Galli, S.J., Chinthrajah, R.S., Nadeau, K.C., 2017. Association of clinical reactivity with sensitization to allergen components in multifood-allergic children. *J. Allergy Clin. Immunol. Pract.* 5, 1325–1334.
- Archila, L.D., Jeong, D., Pascal, M., Bartra, J., Juan, M., Robinson, D., Farrington, M.L., William, W., 2015. Jug r 2-reactive CD4+ T-cells have a dominant immune role in walnut allergy. *J. Allergy Clin. Immunol.* 136, 983–992.
- Ayuso, R., Grishina, G., Ahn, K., Bardina, L., Beyer, K., Sampson, H., 2007. Identification of a MnSOD-like protein as a new major pistachio allergen. *J. Allergy Clin. Immunol.* 119, 115.
- Benito, C., Gonzalez-Mancebo, E., de Durana, M.D., Tolon, R.M., Fernandez-Rivas, M., 2007. Identification of a 7S globulin as a novel coconut allergen. *Ann. Allergy Asthma Immunol.* 98, 580–584.
- Beyer, K., Bardina, L., Grishina, G., Ashraf, A., Teuber, S., Niggemann, B., Sampson, H.A., 2008. Identification of a new Brazil nut allergen - Ber e 2. *J. Allergy Clin. Immunol.* 121, 247.
- Beyer, K., Grishina, G., Bardina, L., Grishina, A., Sampson, H.A., 2002. Identification of an 11S globulin as a major hazelnut food allergen in hazelnut-induced systemic reactions. *J. Allergy Clin. Immunol.* 110, 517–523.
- Blom, W.M., Vlieg-Boerstra, B.J., Kruizinga, A.G., van der Heide, S., Houben, G.F., Dubois, A.E., 2013. Threshold dose distributions for 5 major allergenic foods in children. *J. Allergy Clin. Immunol.* 131, 172–179.
- Borja, J.M., Bartolome, B., Gomez, E., Galindo, P.A., Feo, F., 1999. Anaphylaxis from Brazil nut. *Allergy* 54, 1007–1008.
- Burney, P.G.J., Potts, J., Kummeling, I., Mills, E.N.C., Clausen, M., Dubakiene, R., Barreales, L., Fernandez-Perez, C., Fernandez-Rivas, M., Le, T.M., Knulst, A.C., Kowalski, M.L., Lidholm, J., Ballmer-Weber, B.K., Braun-Fahlander, C., Mustakov, T., Kralimarkova, T., Popov, T., Sakellariou, A., Papadopoulos, N.G., Versteeg, S.A., Zuidmeer, L., Akkerdaas, J.H., Hoffmann-Sommergruber, K., van Ree, R., 2014. The prevalence and distribution of food sensitization in European adults. *Allergy* 69, 365–371.
- Carvalho, A.D., Gomes, V.M., 2007. Role of plant lipid transfer proteins in plant cell physiology—a concise review. *Peptides* 28, 1144–1153.
- Costa, J., Mafra, I., Carrapatoso, I., Oliveira, M.B., 2012. Almond allergens: molecular characterization, detection, and clinical relevance. *J. Agric. Food Chem.* 60, 1337–1349.
- Datema, M.R., Zuidmeer-Jongeban, L., Asero, R., Barreales, L., Belohlavkova, S., De Blay, F., Bures, P., Clausen, M., Dubakiene, R., Gislason, D., Jedrzejczak-Czechowicz, M., Kowalski, M.L., Knulst, A.C., Kralimarkova, T., Le, T.M., Lovegrove, A., Marsh, J., Papadopoulos, N.G., Popov, T., Del Prado, N., Purohit, A., Reese, G., Reig, I., Seneviratne, S.L., Sinaniotis, A., Versteeg, S.A., Vieths, S., Zwinderman, A.H., Mills, C., Lidholm, J., Hoffmann-Sommergruber, K., Fernández-Rivas, M., Ballmer-Weber, B., Van Ree, R., 2015. Hazelnut allergy across Europe dissected molecularly: a EuroPrevall outpatient clinic survey. *J. Allergy Clin. Immunol.* 136, 382–391.
- De Knop, K.J., Hagendorens, M.M., Bridts, C.H., Stevens, W.J., Ebo, D.G., 2010. Macadamia nut allergy: 2 case reports and a review of the literature. *Acta Clin. Belg.* 65, 129–132.
- De Lucca, A.J., Cleveland, T.E., Wedge, D.E., 2005. Plant-derived antifungal proteins and peptides. *Canad. J. Microbiol.* 51, 1001–1014.
- Dearman, R.J., Alcocer, M.J.C., Kimber, I., 2007. Influence of plant lipids on immune responses in mice to the major Brazil nut allergen Ber e 1. *Clin. Exp. Allergy* 37, 582–591.
- Downs, M.L., Semic-Jusufagic, A., Simpson, A., Bartra, J., Fernandez-Rivas, M., Rigby, N.M., Taylor, S.L., Baumert, J.L., Mills, E.N.C., 2014. Characterization of low molecular weight allergens from English walnut (*Juglans regia*). *J. Agric. Food Chem.* 62, 11767–11775.
- Downs, M.L., Simpson, A., Custovic, A., Semic-Jusufagic, A., Bartra, J., Fernandez-Rivas, M., Taylor, S.L., Baumert, J.L., Mills, E.N.C., 2016. Insoluble and soluble roasted walnut proteins retain antibody reactivity. *Food Chem.* 194, 1013–1021.
- Dubiela, P., Aina, R., Polak, D., Geiselhart, S., Humeniuk, P., Bohle, B., Alessandri, S., Del Conte, R., Cantini, F., Borowski, T., Bublin, M., Hoffmann-Sommergruber, K., 2017. Enhanced Pru p 3 IgE-binding activity by selective free fatty acid-interaction. *J. Allergy Clin. Immunol.* 140, 1728–1731.
- Ekbote, A., Hayman, G., Bansal, A., 2010. Macadamia nut allergy: potentially misleading specific IgE results. *Allergy* 65, 1345.
- Elizur, A., Appel, M.Y., Nachshon, L., Levy, M.B., Epstein-Rigbi, N., Golobov, K., Goldberg, M.R., 2018. NUT Co Reactivity – ACquiring Knowledge for Elimination Recommendations (NUT CRACKER) study. *Allergy* 73, 593–601.
- Ewan, P.W., 1996. Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *Brit. Med. J.* 312, 1074–1078.
- Faber, M.A., De Graag, M., Van Der Heijden, C., Sabato, V., Hagendorens, M.M., Bridts, C.H., De Clerck, L.S., Ebo, D.G., 2014. Cor a 14: missing link in the molecular diagnosis of hazelnut allergy? *Int. Arch. Allergy Immunol.* 164, 200–206.
- FAOSTAT, 2018. Food and Agriculture Organization of the United Nations – Statistics Division, Rome, Italy.
- Garino, C., Zuidmeer, L., Marsh, J., Lovegrove, A., Morati, M., Versteeg, S., Schilte, P., Shewry, P., Arlorio, M., van Ree, R., 2010. Isolation, cloning, and characterization of the 2S albumin: a new allergen from hazelnut. *Mol. Nutr. Food Res.* 54, 1257–1265.
- Garrido-Fernandez, S., Garcia, B.E., Sanz, M.L., Ariz, S., Tabar, A.L., 2009. The role of lipid transfer proteins in the almond tree and almond fruit as contact and airborne allergens. *J. Invest. Allergol. Clin. Immunol.* 19, 61–63.
- Guo, F., Jin, T.C., Howard, A., Zhang, Y.Z., 2007. Purification, crystallization and initial crystallographic characterization of Brazil-nut allergen Ber e 2. *Acta Crystallogr. F: Struct. Biol. Commun.* 63, 976–979.
- Hansen, K.S., Ballmer-Weber, B.K., Lüttkopf, D., Skov, P.S., Wüthrich, B., Bindslev-Jensen, C., Vieths, S., Poulsen, L.K., 2003. Roasted hazelnut-allergenic activity evaluated by double-blind, placebo-controlled food challenge. *Allergy* 58, 132–138.
- Hansen, K.S., Ballmer-Weber, B.K., Sastre, J., Lidholm, J., Andersson, K., Oberhofer, H., Luch-Bernal, M., Östling, J., Mattsson, L., Schocker, F., Vieths, S., Poulsen, L.K., 2009. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J. Allergy Clin. Immunol.* 123, 1134–1141.
- Herbst, R.A., Wahl, R., Frosch, P.J., 2010. Specific IgE reactivity and identification of potential allergens in macadamia allergy. *J. Eur. Acad. Dermat. Venereol.* 24, 1361–1363.
- Jin, T.C., Albillos, S.M., Guo, F., Howard, A., Fu, T.J., Kothary, M.H., Zhang, Y.Z., 2009. Crystal structure of prunin-1, a major component of the almond (*Prunus dulcis*) allergen amandin. *J. Agric. Food Chem.* 57, 8643–8651.
- Johnson, J., Malinovsky, A., Alving, K., Lidholm, J., Borres, M.P., Nordvall, L., 2014. Ten-year review reveals changing trends and severity of allergic reactions to nuts and other foods. *Acta Paediatr.* 103, 862–867.
- Knott, E., Gurer, C.K., Ellwanger, J., Ring, J., Darsow, U., 2008. Macadamia nut allergy. *J.*

- Eur. Acad. Dermatol. Venereol. 22, 1394–1395.
- Lauer, I., Foetisch, K., Kolarich, D., Ballmer-Weber, B.K., Conti, A., Altmann, F., Vieths, S., Scheurer, S., 2004. Hazelnut (*Corylus avellana*) vicilin Cor a 11: molecular characterization of a glycoprotein and its allergenic activity. *Biochem. J.* 383, 327–334.
- Lauer, I., Alessandri, S., Pokoj, S., Reuter, A., Conti, A., Vieths, S., Scheurer, S., 2008. Expression and characterization of three important panallergens from hazelnut. *Mol. Nutr. Food Res.* 52, 262–271.
- Leduc, V., Moneret-Vautrin, D.A., Tzen, J.T.C., Morisset, M., Guerin, L., Kanny, G., 2006. Identification of oleosins as major allergens in sesame seed allergic patients. *Allergy* 61, 349–356.
- Lee, B., Zhang, R.H., Du, W.X., Grauke, L.J., McHugh, T.H., Zhang, Y.Z., 2014. Expression, purification and crystallization of pecan (*Carya illinoensis*) vicilin. *Acta Crystallogr. Sec. F: Struct. Biol. Commun.* 70, 1049–1052.
- Lerch, M., Egger, C., Bircher, A.J., 2005. Allergic reactions to macadamia nut. *Allergy* 60, 130–131.
- Lerche, M.H., Poulsen, F.H., 1998. Solution structure of barley lipid transfer protein complexed with palmitate. Two different binding modes of palmitate in the homologous maize and barley nonspecific lipid transfer proteins. *Protein Sci.* 7, 2490–2498.
- Lüttkopf, D., Müller, U., Skov, P.S., Ballmer-Weber, B.K., Wüthrich, B., Skamstrup Hansen, K., Poulsen, L.K., Kästner, M., Haustein, D., Vieths, S., 2002. Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol. Immunol.* 38, 515–525.
- Maloney, J.M., Rudengren, M., Ahlstedt, S., Bock, S.A., Sampson, H.A., 2008. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. *J. Allergy Clin. Immunol.* 122, 145–151.
- Manso, L., Pastor, C., Perez-Gordo, M., Cases, B., Sastre, J., Cuesta-Herranz, J., 2010. Cross-reactivity between coconut and lentil related to a 7S globulin and an 11S globulin. *Allergy* 65, 1487–1488.
- Masthoff, L.J.N., Mattsson, L., Zuidmeer-Jongejan, L., Lidholm, J., Andersson, K., Akkerdaas, J.H., Versteeg, S.A., Garino, C., Meijer, Y., Kentie, P., Versluis, A., Jager, C.F.D., Bruijnzeel-Koomen, C.A.F.M., Knulst, A.C., van Ree, R., van Hoffen, E., Pasmans, S.G.M.A., 2013. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. *J. Allergy Clin. Immunol.* 132, 393–399.
- McWilliam, V., Koplin, J., Lodge, C., Tang, M., Dharmage, S., Allen, K., 2015. The prevalence of tree nut allergy: a systematic review. *Curr. Allergy Asthma Rep.* 15, 54.
- Moreno, F.J., Clemente, A., 2008. 2S albumin storage proteins: what makes them food allergens? *Open Biochem. J.* 2, 16–28.
- Muller, U., Lüttkopf, D., Hoffmann, A., Petersen, A., Becker, W.M., Schocker, F., Niggemann, B., Altmann, F., Kolarich, D., Haustein, D., Vieths, S., 2000. Allergens in raw and roasted hazelnuts (*Corylus avellana*) and their cross-reactivity to pollen. *Eur. Food Res. Technol.* 212, 2–12.
- Nguyen, S.A., More, D.R., Whisman, B.A., Hagan, L.L., 2004. Cross-reactivity between coconut and hazelnut proteins in a patient with coconut anaphylaxis. *Ann. Allergy Asthma Immunol.* 92, 281–284.
- Nitride, C., Mamone, G., Picariello, G., Mills, C., Nocerino, R., Berni Canani, R., Ferranti, P., 2013. Proteomic and immunological characterization of a new food allergen from hazelnut (*Corylus avellana*). *J. Proteom.* 86, 16–26.
- Noorbakhsh, R., Mortazavi, S.A., Sankian, M., Shahidi, F., Assarehzadegan, M.A., Varasteh, A., 2010. Cloning, expression, characterization, and computational approach for cross-reactivity prediction of manganese superoxide dismutase allergen from pistachio nut. *Allergol. Int.* 59, 295–304.
- Noorbakhsh, R., Mortazavi, S.A., Sankian, M., Shahidi, F., Tehrani, M., Azad, F.J., Behmanesh, F., Varasteh, A., 2011. Pistachio allergy-prevalence and in vitro cross-reactivity with other nuts. *Allergol. Int.* 60, 425–432.
- Nordlee, J.A., Taylor, S.L., Townsend, J.A., Thomas, L.A., Bush, R.K., 1996. Identification of a Brazil-nut allergen in transgenic soybeans. *New Engl. J. Med.* 334, 688–692.
- Offermann, L.R., Bublin, M., Perdue, M.L., Pfeifer, S., Dubiel, P., Borowski, T., Chruszcz, M., Hoffmann-Sommergruber, K., 2015. Structural and functional characterization of the hazelnut allergen Cor a 8. *J. Agric. Food Chem.* 63, 9150–9158.
- Osborne, T.B., Campbell, G.F., 1986. Conglutin and vitellin. *J. Am. Chem. Soc.* 108, 609–623.
- Palacin, A., Rivas, L.A., Gómez-Casado, C., Aguirre, J., Tordesillas, L., Bartra, J., Blanco, C., Carrillo, T., Cuesta-Herranz, J., Bonny, J.A.C., Flores, E., García-Alvarez-Eire, M.G., García-Núñez, I., Fernández, F.J., Gamboa, P., Muñoz, R., Sánchez-Monge, R., Torres, M., Losada, S.V., Villalba, M., Vega, F., Parro, V., Blanca, M., Salcedo, G., Díaz-Perales, A., 2012. The involvement of thaumatin-like proteins in plant food cross-reactivity: a multicenter study using a specific protein microarray. *PLoS One* 7, e44088.
- Parthibane, V., Rajakumari, S., Venkateshwari, V., Iyappan, R., Rajasekharan, R., 2012. Oleosin is bifunctional enzyme that has both monoacylglycerol acyltransferase and phospholipase activities. *J. Biol. Chem.* 287, 1946–1954.
- Pastorello, E.A., Vieths, S., Pravettoni, V., Farioli, L., Trambaioli, C., Fortunato, D., Lüttkopf, D., Calamari, M., Ansaloni, R., Scibilia, J., Ballmer-Weber, B.K., Poulsen, L.K., Wüthrich, B., Hansen, K.S., Robino, A.M., Ortolani, C., Conti, A., 2002. Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J. Allergy Clin. Immunol.* 109, 563–570.
- Pasquato, N., Berni, R., Folli, C., Folloni, S., Cianci, M., Pantano, S., Helliwell, J.R., Zanotti, G., 2006. Crystal structure of peach Pru p 3, the prototypic member of the family of plant non-specific lipid transfer protein pan-allergens. *J. Mol. Biol.* 356, 684–694.
- Pastorello, E.A., Farioli, L., Pravettoni, V., Ispano, M., Conti, A., Ansaloni, R., Rotondo, F., Incorvaia, C., Bengtsson, A., Rivolta, F., Trambaioli, C., Previti, M., Ortolani, C., 1998. Sensitization to the major allergen of Brazil nut is correlated with the clinical expression of allergy. *J. Allergy Clin. Immunol.* 102, 1021–1027.
- Pastorello, E.A., Farioli, L., Pravettoni, V., Robino, A.M., Scibilia, J., Fortunato, D., Conti, A., Borgonovo, L., Bengtsson, A., Ortolani, C., 2004. Lipid transfer protein and vicilin are important walnut allergens in patients not allergic to pollen. *J. Allergy Clin. Immunol.* 114, 908–914.
- Pedrosa, M., Boyano-Martínez, T., García-Ara, C., Caballero, T., Quirce, S., 2015. Microarrayed Jug r 1 shows the best diagnostic performance in walnut allergy. *Clin. Trans. Allergy* 5, P20.
- Pfeifer, S., Bublin, M., Dubiel, P., Hummel, K., Wortmann, J., Hofer, G., Keller, W., Radauer, C., Hoffmann-Sommergruber, K., 2015. Cor a 14, the allergenic 2S albumin from hazelnut, is highly thermostable and resistant to gastrointestinal digestion. *Mol. Nutr. Food Res.* 59, 2077–2086.
- Polk, B.I., Dinakarandian, D., Nanda, M., Barnes, C., Dinakar, C., 2016. Association of tree nut and coconut sensitizations. *Ann. Allergy Asthma Immunol.* 117, 412–416.
- Poltronieri, P., Cappello, M.S., Dohmae, N., Conti, A., Fortunato, D., Pastorello, E.A., Ortolani, C., Zacheo, G., 2002. Identification and characterisation of the IgE-binding proteins 2S albumin and conglutinin gamma in almond (*Prunus dulcis*) seeds. *Int. Arch. Allergy Immunol.* 128, 97–104.
- Rayes, H., Raza, A.A., Williams, A., Matthews, S., Arshad, S.H., 2016. Specific IgE to recombinant protein (Ber e 1) for the diagnosis of Brazil nut allergy. *Clin. Exp. Allergy* 46, 654–656.
- Reitsma, M., Bastiaan-Net, S., Sforza, S., van der Valk, J.P.M., van Wijk, R.V., Savelkoul, H.F.J., de Jong, N.W., Wichers, H.J., 2016. Purification and characterization of *Anacardium occidentale* (cashew) allergens Ana o 1, Ana o 2, and Ana o 3. *J. Agric. Food Chem.* 64, 1191–1201.
- Rigby, N.M., Marsh, J., Sancho, A.I., Wellner, K., Akkerdaas, J., van Ree, R., Knulst, A., Fernández-Rivas, M., Brettlova, V., Schilte, P.P., Summer, C., Pumphrey, R., Shewry, P.R., Mills, E.N.C., 2008. The purification and characterisation of allergenic hazelnut seed proteins. *Mol. Nutr. Food Res.* 52, S251–S261.
- Roberts, G., Peckitt, C., Northstone, K., Strachan, D., Lack, G., Henderson, J., Golding, J., Team, A.S., 2005. Relationship between aeroallergen and food allergen sensitization in childhood. *Clin. Exp. Allergy* 35, 933–940.
- Robotham, J.M., Teuber, S.S., Sathe, S.K., Roux, K.H., 2002. Linear IgE epitope mapping of the English walnut (*Juglans regia*) major food allergen, Jug r 1. *J. Allergy Clin. Immunol.* 109, 143–149.
- Robotham, J.M., Wang, F., Seamon, V., Teuber, S.S., Sathe, S.K., Sampson, H.A., Beyer, K., Seavy, M., Roux, K.H., 2005. Ana o 3, an important cashew nut (*Anacardium occidentale* L.) allergen of the 2S albumin family. *J. Allergy Clin. Immunol.* 115, 1284–1290.
- Rouge, P., Thibaut, F., Bourrier, T., Saggio, B., Culerrier, R., Rance, F., Barre, A., 2011. Clinical relevance of IgE-binding cross-reactivity of allergens from cashew nut (*Anacardium occidentale*). *Revue Française D Allergologie* 51, 31–35.
- Rundqvist, L., Tengel, T., Zdunek, J., Björn, E., Schleucher, J., Alcocer, M.J.C., Larsson, G., 2012. Solution structure, copper binding and backbone dynamics of recombinant Ber e 1—the major allergen from Brazil nut. *PLoS One* 7, e46435.
- Saha, B., Sircar, G., Pandey, N., Bhattacharya, S.G., 2015. Mining novel allergens from coconut pollen employing manual *de novo* sequencing and homology-driven proteomics. *J. Proteome Res.* 14, 4823–4833.
- Savvatanos, S., Konstantinopoulos, A.P., Borge, A., Stavroulakis, G., Lidholm, J., Borres, M.P., Manousakis, E., Papadopoulos, N.G., 2015. Sensitization to cashew nut 2S albumin, Ana o 3, is highly predictive of cashew and pistachio allergy in Greek children. *J. Allergy Clin. Immunol.* 136, 192–194.
- Schocker, F., Lüttkopf, D., Scheurer, S., Petersen, A., Cisteró-Bahima, A., Enrique, E., San Miguel-Moncin, M., Akkerdaas, J., Van Ree, R., Vieths, S., Becker, W.M., 2004. Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for *in vitro* diagnosis of potentially severe hazelnut allergy. *J. Allergy Clin. Immunol.* 113, 141–147.
- Schwager, C., Kull, S., Behrends, J., Rockendorf, N., Schocker, F., Frey, A., Homann, A., Becker, W.M., Jappe, U., 2017. Peanut oleosins associated with severe peanut allergy—importance of lipophilic allergens for comprehensive allergy diagnostics. *J. Allergy Clin. Immunol.* 140, 1331–1338.
- Sharma, G.M., Irsigler, A., Dhanarajan, P., Ayuso, R., Bardina, L., Sampson, H.A., Roux, K.H., Sathe, S.K., 2011a. Cloning and characterization of 2S albumin, Car i 1, a major allergen in pecan. *J. Agric. Food Chem.* 59, 4130–4139.
- Sharma, G.M., Irsigler, A., Dhanarajan, P., Ayuso, R., Bardina, L., Sampson, H.A., Roux, K.H., Sathe, S.K., 2011b. Cloning and characterization of an 11S legumin, Car i 4, a major allergen in pecan. *J. Agric. Food Chem.* 59, 9542–9552.
- Shewry, P.R., Napier, J.A., Tatham, A.S., 1995. Seed storage proteins - structures and biosynthesis. *Plant Cell* 7, 945–956.
- Sicherer, S.H., Munoz-Furlong, A., Godbold, J.H., Sampson, H.A., 2010. US prevalence of self-reported peanut, tree nut, and sesame allergy: 11-year follow-up. *J. Allergy Clin. Immunol.* 125, 1322–1326.
- Sordet, C., Culerrier, R., Granier, C., Rancé, F., Didier, A., Barre, A., Rougé, P., 2009. Expression of Jug r 1, the 2S albumin allergen from walnut (*Juglans regia*), as a correctly folded and functional recombinant protein. *Peptides* 30, 1213–1221.
- Stutus, L.M., Sheehan, W.J., Rangsitienchai, P., Bharamane, A., Scott, J.E., Young, M.C., Dioun, A.F., Schneider, L.C., Phipatanakul, W., 2010. Characterizing the relationship between sesame, coconut, and nut allergy in children. *Pediatr. Allergy Immunol.* 21, 1114–1118.
- Sun, S.S., Altenbach, S.B., Leung, F.W., 1987. Properties, biosynthesis and processing of a sulfur-rich protein in Brazil nut (*Bertholletia excelsa* H.B.K.). *Eur. J. Biochem.* 162, 477–483.
- Sutherland, M.F., O’Hehir, R.E., Czarny, D., Suphioglu, C., 1999. Macadamia nut anaphylaxis: demonstration of specific IgE reactivity and partial cross-reactivity with hazelnut. *J. Allergy Clin. Immunol.* 104, 889–890.
- Tawde, P., Venkatesh, Y.P., Wang, F., Teuber, S.S., Sathe, S.K., Roux, K.H., 2006. Cloning

- and characterization of profilin (Pru du 4), a cross-reactive almond (*Prunus dulcis*) allergen. *J. Allergy Clin. Immunol.* 118, 915–922.
- Tella, R., Gaig, P., Lombardero, M., Paniagua, M.J., Garcia-Ortega, P., Richart, C., 2003. A case of coconut allergy. *Allergy* 58, 825–826.
- Teuber, S., Peterson, W., Uratsu, S., Dandekar, A., Roux, K., Sathe, S., 2003. Identification and cloning of Jug r 4, a major food allergen English walnut belonging to the legumin group. *J. Allergy Clin. Immunol.* 720.
- Teuber, S.S., Dandekar, A.M., Peterson, W.R., Sellers, C.L., 1998. Cloning and sequencing of a gene encoding a 2S albumin seed storage protein precursor from english walnut (*Juglans regia*), a major food allergen. *J. Allergy Clin. Immunol.* 101, 807–814.
- Teuber, S.S., Jarvis, K.C., Dandekar, a.M., Peterson, W.R., Ansari, A.A., 1999. Identification and cloning of a complementary DNA encoding a vicilin-like proprotein, Jug r 2, from english walnut kernel (*Juglans regia*), a major food allergen. *J. Allergy Clin. Immunol.* 104, 1311–1320.
- Teuber, S.S., Peterson, W.R., 1999. Systemic allergic reaction to coconut (*Cocos nucifera*) in 2 subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: new coconut and walnut food allergens. *J. Allergy Clin. Immunol.* 103, 1180–1185.
- Townsend, J.A., Thomas, L.A., 1994. Factors which influence the agrobacterium-mediated transformation of soybean. *J. Cell. Biochem.* 78.
- van der Valk, J.P.M., van Wijk, R.G., Baumert, J.L., Nordlee, J.A., Vlieg-Boerstra, B.J., de Groot, H., Dubois, A.E.J., de Jong, N.W., 2016a. Threshold dose distribution and eliciting dose of cashew nut allergy. *Ann. Allergy Asthma Immunol.* 117, 712–714.
- van der Valk, J.P.M., van Wijk, R.G., Dubois, A.E.J., de Groot, H., Reitsma, M., Vlieg-Boerstra, B., Savelkoul, H.F.J., Wichers, H.J., de Jong, N.W., 2016b. Multicentre double-blind placebo-controlled food challenge study in children sensitised to cashew nut. *PLoS One* 11.
- Verweij, M.M., Hagendorens, M.M., Trashin, S., Cucu, T., de Meulenaer, B., Devreese, B., Bridts, C., de Clerck, L.S., Ebo, D.G., 2012. Age-dependent sensitization to the 7S-vicilin-like protein cor a 11 from hazelnut (*Corylus avellana*) in a birch-endemic region. *J. Invest. Allergol. Clin. Immunol.* 22, 245–251.
- Wallowitz, M., Peterson, W.R., Uratsu, S., Comstock, S.S., Dandekar, A.M., Teuber, S.S., 2006. Jug r 4, a legumin group food allergen from walnut (*Juglans regia* Cv. Chandler). *J. Agric. Food Chem.* 54, 8369–8375.
- Wang, F., Robotham, J.M., Teuber, S.S., Sathe, S.K., Roux, K.H., 2003. Ana o 2, a major cashew (*Anacardium occidentale* L.) nut allergen of the legumin family. *Int. Arch. Allergy Immunol.* 132, 27–39.
- Wang, F., Robotham, J.M., Teuber, S.S., Tawde, P., Sathe, S.K., Roux, K.H., 2002. Ana o 1, a cashew (*Anacardium occidentale*) allergen of the vicilin seed storage protein family. *J. Allergy Clin. Immunol.* 110, 160–166.
- Wangorsch, A., Jamin, A., Lidholm, J., Gräni, N., Lang, C., Ballmer-Weber, B., Vieths, S., Scheurer, S., 2017. Identification and implication of an allergenic PR-10 protein from walnut in birch pollen associated walnut allergy. *Mol. Nutr. Food Res.* 61, 1–9.
- Willison, L.N., Tawde, P., Robotham, J.M., Penney, R.M., Teuber, S.S., Sathe, S.K., Roux, K.H., 2008. Pistachio vicilin, Pis v 3, is immunoglobulin E-reactive and cross-reacts with the homologous cashew allergen, Ana o 1. *Clin. Exp. Allergy* 38, 1229–1238.
- Witte, W., 2004. The role of profilin complexes in cell motility and other cellular processes. *Trends Cell Biol.* 14, 461–469.
- Zachowski, A., Guerbette, F., Grosbois, M., Jolliot-Croquin, A., Kader, J.C., 1998. Characterisation of acyl binding by a plant lipid-transfer protein. *Eur. J. Biochem.* 257, 443–448.
- Zhang, Y., Lee, B., Du, W.X., Lyu, S.C., Nadeau, K.C., Grauke, L.J., Wang, S., Fan, Y., Yi, J., McHugh, T.H., 2016. Identification and characterization of a new pecan [*Carya illinoensis*(Wangenh.) K. Koch] allergen, Car i 2. *J. Agric. Food Chem.* 64, 4146–4151.
- Zuidmeer-Jongejan, L., Fernández-Rivas, M., Winter, M.G.T., Akkerdaas, J.H., Summers, C., Lebens, A., Knulst, A.C., Schilte, P., Briza, P., Gadermaier, G., Van Ree, R., 2014. Oil body-associated hazelnut allergens including oleosins are underrepresented in diagnostic extracts but associated with severe symptoms. *Clin. Trans. Allergy* 4, 1–10.