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REVIEW

Non-specific lipid-transfer proteins: Allergen structure and function, cross-reactivity, sensitization, and epidemiology

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Abstract

Background: Discovered and described 40 years ago, non-specific lipid transfer proteins (nsLTP) are present in many plant species and play an important role protecting plants from stressors such as heat or drought. In the last 20 years, sensitization to nsLTP and consequent reactions to plant foods has become an increasing concern.

Aim: The aim of this paper is to review the evidence for the structure and function of nsLTP allergens, and cross-reactivity, sensitization, and epidemiology of nsLTP allergy.

Materials and Methods: A Task Force, supported by the European Academy of Allergy & Clinical Immunology (EAACI), reviewed current evidence and provide a signpost for future research. The search terms for this paper were "Non-specific Lipid Transfer Proteins", "LTP syndrome", "Pru p 3", "plant food allergy", "pollen-food syndrome".

Results: Most nsLTP allergens have a highly conserved structure stabilised by 4-disulphide bridges. Studies on the peach nsLTP, Pru p 3, demonstrate that nsLTPs are very cross-reactive, with the four major IgE epitopes of Pru p 3 being shared by nsLTP from other botanically related fruits. These nsLTP allergens are to varying degrees resistant to heat and digestion, and sensitization may occur through the oral, inhaled or cutaneous routes. In some populations, Pru p 3 is the primary and sole sensitizing allergen, but many are poly-sensitised both to botanically un-related nsLTP in foods, and non-food sources of nsLTP such as Cannabis sativa, Platanus acerifolia, (plane tree), Ambrosia artemisiifolia (ragweed) and Artemisia vulgaris (mugwort). Initially, nsLTP sensitization appeared to be limited to Mediterranean countries, however more recent studies suggest clinically relevant sensitization occurs in North Atlantic regions and also countries in Northern Europe, with nsLTP sensitisation profiles being broadly similar.

Discussion: These robust allergens have the potential to sensitize and provoke symptoms to a large number of plant foods, including those which are raw, cooked or processed. It is unknown why some sensitized individuals develop clinical symptoms to foods whereas others do not, or indeed what other allergens besides Pru p 3 may be primary sensitising allergens. It is clear that these allergens are also relevant in non-Mediterranean populations and there needs to be more recognition of this.

Conclusion: Non-specific LTP allergens, present in a wide variety of plant foods and pollens, are structurally robust and so may be present in both raw and cooked foods. More studies are needed to understand routes of sensitization and the world-wide prevalence of clinical symptoms associated with sensitization to these complex allergens.

KEYWORDS
allergy, epidemiology, food, lipid transfer protein, LTP, sensitization

1 | BACKGROUND

Non-specific lipid-transfer proteins (nsLTPs) are a ubiquitous multigene family of molecules expressed in the Plant kingdom, starting life 470 million years ago in Marchantiophyta, the first plants grown on land after red and green algae.[1] Despite their longevity, nsLTPs were first discovered in plants only about 40 years ago, by Jean-Claude Kader, whose numerous and fundamental studies advanced the botanical knowledge regarding this family of proteins.[2] The biological longevity of nsLTPs throughout time is probably also due to their important role in defence of plants against biotic and abiotic stress and their expression in different plant tissues under different environmental conditions.[3] Their immunoglobulin E (IgE)-binding capacity was first demonstrated in peach fruits and Parietaria pollen, and then

was detected by IgE immunoblotting in peach, cherry, apricot and plum. It was found not to be birch (*Betula verrucosa*) cross-reactive, unlike the Bet v 1 homologues, which at the time were considered to be the main trigger of allergic symptoms to fruits. The novel nsLTP allergens seemed initially to be an important cause of symptoms only in southern Europe.[4] The peach nsLTP, Pru p 3, was first fully sequenced and characterized simultaneously in Italy and Spain, identifying a 9 kDa protein, of 91–93 amino acids long, with a high p (isoelectric point) and a conserved 8 cysteine motif, now considered as the major structural characteristic shared by all members of the nsLTP protein family.[5,6]

Many further studies have demonstrated that nsLTPs are relevant allergens of several pollens and plant foods belonging to both the widespread Rosaceae family and many other distantly related species and can elicit systemic reactions in nsLTP sensitized subjects.[7] Unlike Bet v 1-related or profilin-related food allergy, reactions involving LTP allergens have also been demonstrated to occur with cereals.[8] It has also been observed that nsLTP sensitization could induce other food-related allergic conditions, including 'Baker's' asthma and food-dependent, exercise-induced anaphylaxis (FDEIA).[9,10] Sensitization to nsLTP and the elicited clinical reactions were initially mostly reported to affect subjects living in the Mediterranean area, with those who were not pollen-sensitized typically experiencing more severe reactions.[11,12]

In some countries (notably Italy and Spain), y Pru p 3, the nsLTP from peach (*Prunus persica*), is the first sensitizing allergen in children who may subsequently develop sensitization to a larger number of nsLTP-containing foods.[13] According to the data published from a number of different countries, Pru p 3 is also a marker allergen in adult-onset symptoms attributed to nsLTP allergens. However, nsLTPs present in different sources also seem to be able to initiate sensitization via different routes of exposure and contact and different climate zones.[14,15] The mechanism underlying the induction of sensitization to Pru p 3 has recently been demonstrated, identifying the role of the ligand in the allergic immune response which may lead to further insights into how nsLTP sensitization may develop.[16]

Over the last 30 years, many researchers have contributed to our understanding of the structure, function and IgE-binding properties of nsLTPs, providing evidence of the clinical entity of nsLTP syndrome(s), now well recognized by the entire International Scientific Community. However, the challenging nature of this condition, the characterization of an increasing number of nsLTP proteins in different foods and the complexities surrounding sensitization mechanisms led to the formation of a Task Force, supported by the European Academy of Allergy & Clinical Immunology (EAACI). The Task Force's aim was to produce a summary of the current evidence and provide a signpost for future research needs to ensure that this fascinating condition can be more effectively managed in the future. This paper is the first of the two papers, with the second one reviewing the evidence for the diagnosis and management of symptoms linked to nsLTP sensitization. The search terms for this paper were 'Non-specific Lipid Transfer Proteins', 'LTP syndrome', 'Pru p 3', 'plant food allergy', 'pollen-food syndrome'.

2 | MOLECULAR STRUCTURE AND BIOLOGICAL FUNCTION OF nsLTPs

nsLTPs form a large protein family, known to occur in land plants, where they are abundantly expressed in most tissues.[1,17] These nsLTPs are small, non-glycosylated proteins of 6–9 kDa and basic isoelectric points of 9.[2] They are encoded by multigene families and occur in many flowering plants.[18] Currently, 46 nsLTPs have been listed by the WHO/IUIS Allergen Nomenclature Sub-Committee (Table 1). Most nsLTPs are synthesized with an N-terminal signal peptide, which directs the proteins to the extracellular, apoplastic space. Several nsLTPs also contain a C-terminal sequence motif for post-translational addition of a glycosylphosphatidylinositol (GPI)-anchor, such GPI-anchors that target the nsLTPs to the plasma membrane surfaces.[19,20] Based on their molecular mass, nsLTPs are grouped into two types, LTP1 (9–10 kDa; around 90 amino acids) and LTP2 (6-7 kDa; around 70 amino acids).[21] However, the majority of allergenic nsLTPs belong to the nsLTP1 type.

Structural studies on nsLTPs from wheat, maize, barley and rice were performed by x-ray and / or nuclear magnetic resonance analyses[22–26] and the crystal structure of Pru p 3 was described in 2005 by Pasquato and colleagues[27] (see Figure 1). These studies confirmed the overall highly conserved structure of nsLTPs, consisting of four alpha-helices, which are stabilized by four highly conserved disulphide bridges and connected by flexible loops. The characteristic of the nsLTP structure is an internal, tunnel-like, hydrophobic cavity, which allows binding and transportation of various lipids[2,17] (see Figure 2). The major biological role of nsLTPs seems to be the transfer and deposition of lipids for the assembly of complex barrier polymers on the surface of plant tissues and organs, such as waxes on leaves[28], suberin in seeds and roots, and sporopollenin in pollen grains.[29] Such barriers are required to control the fluxes of gases and liquids, but are also important for the protection of the plants from biotic and abiotic stress. Indeed, the involvement of nsLTPs in signalling during pathogen attack[30,31] and in the defence against temperature extremes or drought has been shown.[32]

Structural studies of liganded nsLTPs provided evidence that the hydrophobic internal cavity can bind to a range of different ligands including fatty acids, acyl-coenzyme A and phospholipids, thus showing great flexibility of the cavity. Furthermore, ligand binding affects the orientation of certain conserved amino acid residues at the C-terminal region and induces a conformational change as shown for Pru p 3, Jug r 3 and Mal d 3.[33–35] These structural modifications can also modify the IgE-binding capacity of nsLTPs.[33–35] Moreover, evidence has accumulated that the lipid–ligand bound to Pru p 3 can act as an adjuvant to promote sensitization to Pru p 3 via CD1d-mediated activation of invariant natural killer T-cells (iNKTs).[16] These findings show that nsLTP–lipid interaction can increase the allergenic properties of nsLTPs and might promote sensitization to these proteins.[36]

TABLE 1 Allergen nomenclature as agreed by the WHO/IUIS Allergen Nomenclature Sub Committee

	Species	Allergen	Biochemical name	MW	UniProt
1	*Ambrosia artemisiifolia* (Short ragweed)	Amb a 6	nsLTP 1	10 kDa	O04004
2	*Apium graveolens* (Celery)	Api g 2	nsLTP 1	9 kDa	E6Y8S8
3	*Apium graveolens* (Celery)	Api g 6	nsLTP 2	7 kDa	P86809
4	*Arachis hypogaea* (Peanut, groundnut)	Ara h 9	nsLTP 1	9.8 kDa	B6CEX8
5	*Arachis hypogaea* (Peanut, groundnut)	Ara h 16	nsLTP 2	8.5 kDa	-
6	*Arachis hypogaea* (Peanut, groundnut)	Ara h 17	nsLTP 1	11 kDa	-
7	*Artemisia vulgaris* (Mugwort, wormwood)	Art v 3	nsLTP 1	12 kDa	P0C088
8	*Asparagus officinalis* (Asparagus)	Aspa o 1	nsLTP 1	9 kDa	-
9	*Brassica oleracea* (Cabbage and others)	Bra o 3	nsLTP 1	9 kDa	-
10	*Cannabis sativa* (Indian hemp)	Can s 3	nsLTP 1	9 kDa	W0U0V5
11	*Castanea sativa* (Chestnut)	Cas s 8	nsLTP 1	9 kDa (red)	-
12	*Citrus limon* (Lemon)	Cit l 3	nsLTP 1	9.6 kDa	P84160
13	*Citrus reticulata* (Tangerine)	Cit r 3	nsLTP 1	9 kDa	P84161
14	*Citrus sinensis* (Sweet orange)	Cit s 3	nsLTP 1	9.46 kDa	P84161
15	*Corylus avellana* (Hazelnut)	Cor a 8	nsLTP 1	9 kDa	Q9ATH2
16	*Fragaria ananassa* (Strawberry)	Fra a 3	nsLTP 1	9 kDa	Q8VX12
17	*Helianthus annuus* (Sunflower)	Hel a 3	nsLTP 1	9 kDa	Q7X9Q5
18	*Hevea brasiliensis* (Para rubber tree (latex))	Hev b 12	nsLTP 1	9 kDa	Q8RYA8
19	*Juglans regia* (English walnut)	Jug r 3	nsLTP 1	9 kDa	C5H617
20	*Lactuca sativa* (Cultivated lettuce)	Lac s 1	nsLTP 1	9 kDa	-
21	*Lens culinaris* (Lentil)	Len c 3	nsLTP 1	9 kDa	A0AT29
22	*Lupinus angustifolius* (Narrow-leaved blue lupin)	Lup an 3	nsLTP 1	11 kDa	-
23	*Malus domestica* (Apple)	Mal d 3	nsLTP 1	9 kDa	Q5J026
24	*Morus nigra* (Mulberry)	Mor n 3	nsLTP 1	10 kDa	P85894
25	*Musa acuminata* (Banana)	Mus a 3	nsLTP 1	9 kDa	P86333
26	*Olea europaea* (Olive)	Ole e 7	nsLTP 1	9.5 kDa	P81430
27	*Parietaria judaica* (Wall Pellitory)	Par j 1	PhosphoLTP	15 kDa	P43217
28	*Parietaria judaica* (Wall Pellitory)	Par j 2	PhosphoLTP	10-14 kDa	P55958
29	*Parietaria officinalis* (Pellitory)	Par o 1	PhosphoLTP	15 kDa	-
30	*Phaseolus vulgaris* (Green bean, French bean)	Pha v 3	nsLTP 1	8.8-9.0 kDa	D3W146
31	*Platanus acerifolia* (London plane tree)	Pla a 3	nsLTP 1	10 kDa	-
32	*Prunus armeniaca* (Apricot)	Pru ar 3	nsLTP 1	9 kDa	P81651
33	*Prunus avium* (Sweet cherry)	Pru av 3	nsLTP 1	10 kDa	Q9M5X8
34	*Prunus domestica* (European plum)	Pru d 3	nsLTP 1	9 kDa	P82534
35	*Prunus dulcis* (Almond)	Pru du 3	nsLTP 1	9 kDa	C0L0I5
36	*Prunus persica* (Peach)	Pru p 3	nsLTP 1	10 kDa	P81402
37	*Punica granatum* (Pomegranate)	Pun g 1	nsLTP 1	9 kDa	A0A059STC4
38	*Pyrus communis* (Pear)	Pyr c 3	nsLTP 1	9 kDa	Q9M5X6
39	*Rubus idaeus* (Red raspberry)	Rub i 3	nsLTP 1	11 kDa	Q0Z8V0
40	*Sinapis alba* (Yellow mustard)	Sin a 3	nsLTP 1	12.3 kDa	E6Y2L9

TABLE 1 (Continued)

	Species	Allergen	Biochemical name	MW	UniProt
41	*Solanum lycopersicum (Lycopersicon sculentum)* (Tomato)	Sola l 3	nsLTP 1	9 kDa	P93224
42	*Solanum lycopersicum (Lycopersicon sculentum)* (Tomato)	Sola l 6	nsLTP 2	7 kDa	K4BBD9
43	*Triticum aestivum* (Wheat)	Tri a 14	nsLTP 1	9 kDa	D2T2K2
44	*Triticum turgidum ssp durum* (Durum wheat)	Tri tu 14	nsLTP 1	9.2 kDa	-
45	*Vitis vinifera* (Grape)	Vit v 1	nsLTP 1	9 kDa	Q850K5
46	*Zea mays* (Maize)	Zea m 14	nsLTP 1	9 kDa	P19656-1

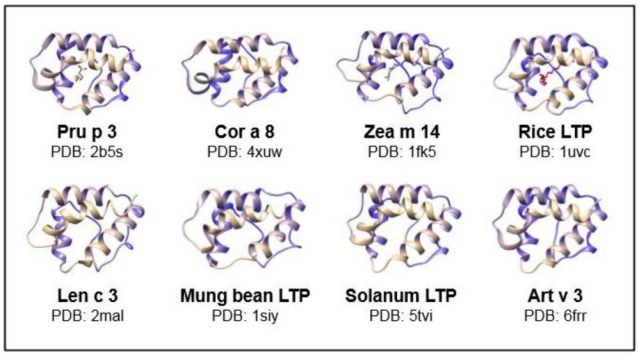

FIGURE 1 Structure of lipid-transfer protein allergens

BOX 1. Definitions

nsLTP sensitization—sensitization to one or more nsLTPs

nsLTP allergy—clinically relevant sensitization to one or more nsLTPs

nsLTP syndrome—multiple plant food allergies attributed to nsLTP sensitization

So far, it has been shown that four major (immunodominant) IgE epitopes of Pru p 3 are shared by LTPs of fruits from the *Rosaceae* family.[37,38] In a cohort of Spanish and Italian peach allergic patients, a Th2-dominated response was identified, and among several T-cell epitopes, immunodominant T-cell-activating regions of Pru p 3 were detected.[39] In contrast, other nsLTPs from more distantly related species, such as Cor a 8 in hazelnut (*Corylus avellane*) and Hel a 3s in sunflower seed (*Helianthus annuus*), do not contain these conserved epitope sequences. In a study on patients allergic to hazelnut and peach, the immunodominant role of Pru p 3 was confirmed, but no Cor a 8-specific T-cell epitopes were detected suggesting cross-reactivity rather than genuine primary sensitization to Cor a 8.[40] These molecular data suggest that Pru p 3 could be the primary sensitizer behind nsLTP-driven food allergy in some populations.

The disulphide bridges contribute to the folding of nsLTPs into a compact structure with high thermal and proteolytic stability, which might explain the high allergenicity of nsLTPs.[41,42] *In vitro* experiments confirmed the stability of Pru p 3 and its resistance to digestion and heat treatment in an acidic environment by circular dichroism (CD) spectroscopy . However, when performing these experiments under neutral pH conditions, the stability of these proteins is reduced as shown for Pru p 3 and Cor a 8 which might be due to the cleavage of disulphide bonds at neutral pH and high temperature.[43–45] Interestingly, stability to endolysosomal degradation also seems to differ within the nsLTP protein family, since Pru p 3 showed higher resistance as compared to Cor a 8.[40] These data point towards a selective resistance of individual nsLTPs. It remains to be shown, whether the binding of lipids or the structural differences among individual members of the nsLTP family might cause this selective resistance [44]

The abundancy of allergenic nsLTPs in the culprit food can also contribute to the allergenic risk. While the concentration of Pru p 3 in peach peel is comparatively high (approx. 6 mg/g peach peel; it is less in apple peel (approx. 66 µg/g tissue).[45,46] Regarding tree nuts, nsLTPs from walnut and hazelnut are accumulated in the brown skin of the embryo and the concentration per nut is comparatively low compared to peach.[47]

3 | HIERARCHY OF CROSS-REACTIVITY

Patients suffering from an 'nsLTP syndrome' frequently display symptoms with multiple plant-derived foods due to the ubiquitous distribution of these pan-allergens.[48,49] The clinical relevance of these allergens, as well as the primary sensitizer, varies greatly depending on the patient's age and geographic area.[50] Initial epidemiological studies on nsLTP sensitization have mainly been conducted in southern Europe on adult or adolescent populations.[49] In this region, most allergists agree that peach is the primary driving source behind nsLTP sensitization, although nsLTPs of some pollen, particularly plane tree and mugwort, have been proposed as contributing to sensitization.[51] According to Palacin's study, where the IgE-binding capacity of a number of purified LTPs was analysed using sera from nsLTP syndrome patients; Pru p 3 (peach) was the most frequently recognized, followed by Mal d 3 (apple), Cit r 3 (orange), Bra o 3 (cabbage) Sin a 3 (mustard) and the tree nut LTPs, Jug r 3 (walnut) and Cas s 8 (chestnut). In contrast, Tri a 14, the wheat nsLTP, was seldom recognized in the population studied.[52]

Furthermore, IgE binding frequently does not predict clinical cross-reactivity; most patients did not report any clinical reaction to lentil, soybean, maize and sesame, despite specific IgE levels to the nsLTPs in these foods. This might suggest either that in these foods, nsLTPs are less abundant or thermal processing reduces allergenicity.[53,54] So, patients with an 'nsLTP syndrome' are usually polysensitized to multiple foods, to some extent, influenced by the amount of allergen present in the sources, the route of sensitization, geographical patterns and the sensitizing agent. Primary sensitization through Can s 3 exposure might, for example, result in a different array of hierarchical order of food nsLTP reactivity.[55]

4 | ROUTES OF SENSITIZATION TO nsLTP

Sensitization to nsLTP can occur in several different ways. The characterization of lipid ligands bound to nsLTPs has shed light on the potential innate stimulatory properties of nsLTPs, which might account for their high sensitization potential.[16,56] In early life, sensitization to foods such as milk, egg and peanut may occur through cutaneous exposure due to skin barrier disruption and exposure via the epi-cutaneous route, with IgG1+ B-cell immunity preceding the initiation of IgE responses.[57,58] Sensitization to nsLTP may also occur through the cutaneous route; is reported that peach-induced contact urticaria has been linked to nsLTP sensitization.[59] Sensitization to foods via the gastrointestinal tract is a common cause of food-allergic reactions, and this is also true of nsLTP allergens. Tordesillas et al. demonstrated that Pru p 3 has a high capacity to cross the gastric barrier, a factor that could be relevant to the capacity for sensitization by the gastric route.[60]

Just as reactions to plant foods also frequently occur through sensitization to inhaled pollen aeroallergens provoking cross-reactivity to plant foods, sensitization to nsLTP in foods via inhalation may also occur.[61] Case reports describe a patient who developed respiratory symptoms when exposed to peach tree crops, and a second patient who developed rhinitis after working in a wholesale fruit warehouse, both of whom subsequently developed severe reactions to eating peaches and other foods.[62,63] Garcia and colleagues subsequently showed that peach leaves contain Pru p 3, which might explain up to 10% of residents in an area with extensive orchard-tree crops were mono-sensitized to Pru p 3, and the finding that Pru p 3 is an occupational respiratory allergen in peach crop workers.[19,64] nsLTP in a variety of foods has also been shown to be relevant in occupational allergy in those involved in processing other plant food crops including asparagus, wheat and maize.[9,65–69]

Rodriguez and colleagues generated animal models of anaphylaxis to Pru p 3 following sensitization via the nasal route in combination with lipopolysaccharide (LPS).[70] Recently, Rosace et al. have described a progressive remodelling process of the oral mucosa, along with respiratory allergy progression, using profilin as an allergen.[71] Co-localization of CD11+ and CD4+ cells demonstrates that the oral mucosa is an immunocompetent organ and can contribute to the progression from respiratory to food allergy in Bet v 1 homologues, profilin and nsLTP allergic syndromes.

Respiratory exposure and subsequent clinical reactivity to nsLTP can also occur due to inhalation of *Cannabis sativa*. The first description of a case of a patient sensitized to Pru p 3, with nsLTP sensitization mediated by smoking marijuana, was published by Gamboa in 2007 and has been followed by other publications supporting the relevance of *C. sativa* as a potential source of nsLTP sensitization and cannabis-related allergy.[14,72–74] Research from northern/central Europe suggests marijuana inhalation is an effective entry route to nsLTP sensitization in the absence of Pru p 3-mediated allergy.[75–77] Decuyper and colleagues reported that 45% of 120 patients allergic to *C. sativa* and sensitized to Can s 3, and the nsLTP in *C. sativa* had severe and generalized allergy to plant foods.[78] Passive inhalation of smoke from *C. sativa* can also lead to sensitization to Can s 3, and other nsLTP allergens.[79] However, sensitization to Can s 3 may not be universal; a publication from the United States, reporting on the sequencing of allergens from 23 patients with a positive skin prick test to a crude extract of *C. sativa*, did not find anything sensitized to Can s 3.[80]

5 | SENSITIZATION TO POLLEN nsLTP ALLERGENS

Sensitization to nsLTP in plant foods via the inhaled route may also involve nsLTP in pollens.

The existence of pollen food syndrome mediated by nsLTPs was first described by García-Sellés in 2002.[81] Pollen nsLTPs such as Art v 3 from *Artemisia vulgaris* (mugwort) and Pla a 3 from *Platanus acerifolia* (plane tree) share partial cross-reactivity with *Rosaceae* nsLTPs, and could thus initiate the sensitization process and/or contribute to the progressive recognition of multiple nsLTPs.[82] In some areas, high exposure to nsLTP-containing pollens could result in the development of allergic respiratory symptoms, with a proportion of individuals also affected by nsLTP-driven food allergy.[15,83–85] For example, the pollen allergen Pla a 3 was first noted to play a clinically relevant role by clinicians in Barcelona, an area with high *Platanus* pollen exposure where a significant percentage of pollen-allergic patients (12%) are mono-sensitized to *Platanus*.[86,87] Thus, Pla a 3 sensitization is associated with nsLTP food sensitization, and in inhibition assays plane pollen extracts inhibited IgE binding to food nsLTPs by between 50% and 100%.[88]

Likewise, some with nsLTP sensitization to foods are also affected by respiratory symptoms due to nsLTP allergens in the pollen, with cross-sectional studies suggesting that in some cases the nsLTP within the pollen is the primary sensitizer.[84–86] Studies suggest that Art v 3 could also act as a primary sensitizer, demonstrating the clinical relevance of pollen nsLTPs in areas of high exposure to Artemisia pollen.[85,89] For example, *Artemisia* is the dominant allergenic pollen in China, and studies have demonstrated that this might be a mechanism of sensitization to Pru p 3, and the cause of peach allergy in the Chinese population.[83] In areas with high exposure to either Artemisia pollen as Gran Canaria Island[4] or to Platanus pollen as Barcelona,

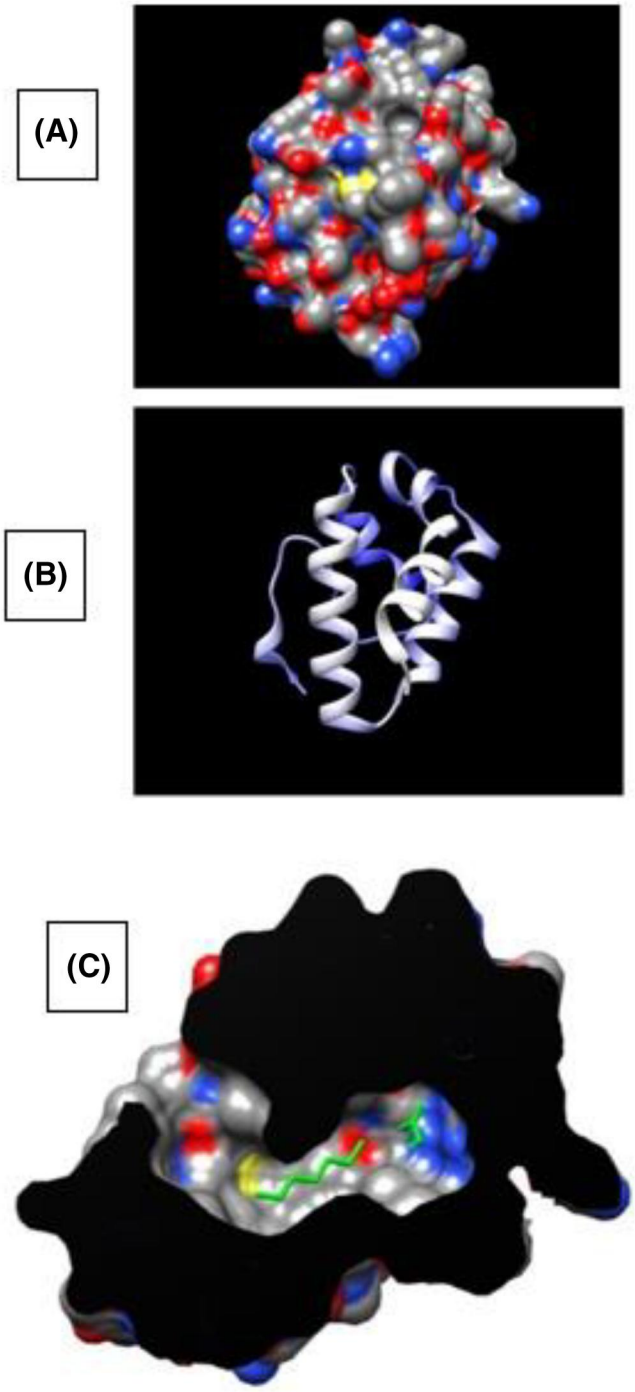

FIGURE 2 Structure of Pru p 3: (A) surface model, (B) ribbon model, (C) internal cavity with ligand binding

epidemiological studies have also demonstrated a broader recognition pattern to nsLTPs in sensitized and symptomatic patients.[52]

It has also been demonstrated that patients sensitized to Pru p 3 can show sensitization to Art v 3 and/or Pla a 3 even in geographical areas lacking Artemisia/plane tree pollen, presumably due to IgE cross-reactivity.[84,85] Despite a lack of Artemisia pollen, a recent UK study reported a strong correlation between Pru p 3 and both Pla a 3 and Art v 3, with the *r*-value being greater than that for a matched group of Italian subjects.[90]. A study performed in patients with a clinical history of peach anaphylaxis demonstrated dual sensitization to both Pru p 3 and Art v 3, and therefore, even in the absence of atmospheric mugwort pollen, there is a possibility that these patients could still develop a respiratory allergy if exposed to mugwort.[88,91] The presence of nsLTP sensitization in the United Kingdom, and other parts of more northerly European latitudes suggests the primary sensitizing nsLTP might be from a pollen rather than from a food.[90,92]

Allergenic pollen nsLTPs have also been described from other sources including *Parietaria judaica* (Par j 2) and olive tree (*Oleum Europea*) (Ole e 7). Sensitization to *Parietaria* has been reported in subjects from northern European countries free from this weed, suggesting this is another expression of cross-reactivity and probably to relevant.[90,92] A study by Tordesillas and colleagues in 2011 reported that there was no cross-reactivity between nsLTP allergens from olive and parietaria, or between the pollen nsLTP and nsLTP allergens from foods.[93] However, a more recent study from Ciprandi and colleagues reported that in a group of children with allergic rhinitis due to Parietaria pollen allergy and sensitization to Pru p 3, 50% reported a food allergy or oral allergy symptoms, and 25% reported anaphylaxis after ingesting nsLTP-containing foods.[94] It is also unclear how pertinent Ole e 7 is in terms of diagnostic relevance. Although it has less than 20% amino acid sequence identity with Pru p 3, recognition of Ole e 7 is associated with reactions to plant-derived food (mainly peach, walnut and peanut), and 80% of Ole e 7 reactors are sensitized to food nsLTPs versus only 30% of Ole e 1 reactors.[95] A recent study reported that common IgG and IgE epitopes were identified in Ole e 7 and Pru p 3, with inhibition studies suggesting that primary sensitization to Ole e 7 could lead to sensitization to Pru p 3.[96]

There has been some suggestion that the greater the atmospheric burden of birch pollen, the lower the incidence of nsLTP sensitization, leading to a hypothesis that high levels of birch pollen protect against nsLTP allergy.[97] It is suggested one potential explanation could be lower reactivity of effector cells exposed to the nsLTPs, as described by Christensen et al., on the basis of interference by other IgE molecules bound to the high-affinity IgE receptors on effector cells.[98] However, a recent study from Belgium demonstrate that many subjects are sensitized to both nsLTP allergens in foods and pollens.[92] A study on nsLTP sensitized adults in the UK study reported that co-sensitization to birch pollen allergens did not seem to be protective against severe reactions, with the use of adrenaline and subsequent hospital attendance being significantly reported.[90]

6 | EPIDEMIOLOGY

Epidemiological data have most often involved the reporting of the rates of sensitization to the peach nsLTP Pru p 3. Using ISAC microarray, Scala et al. reported that 9% of 23,000 patients living in central and southern Italy were sensitized to Pru p 3.[99] In Spain, Barber et al. detected Pru p 3 sensitization in 12% of 2000 Spanish

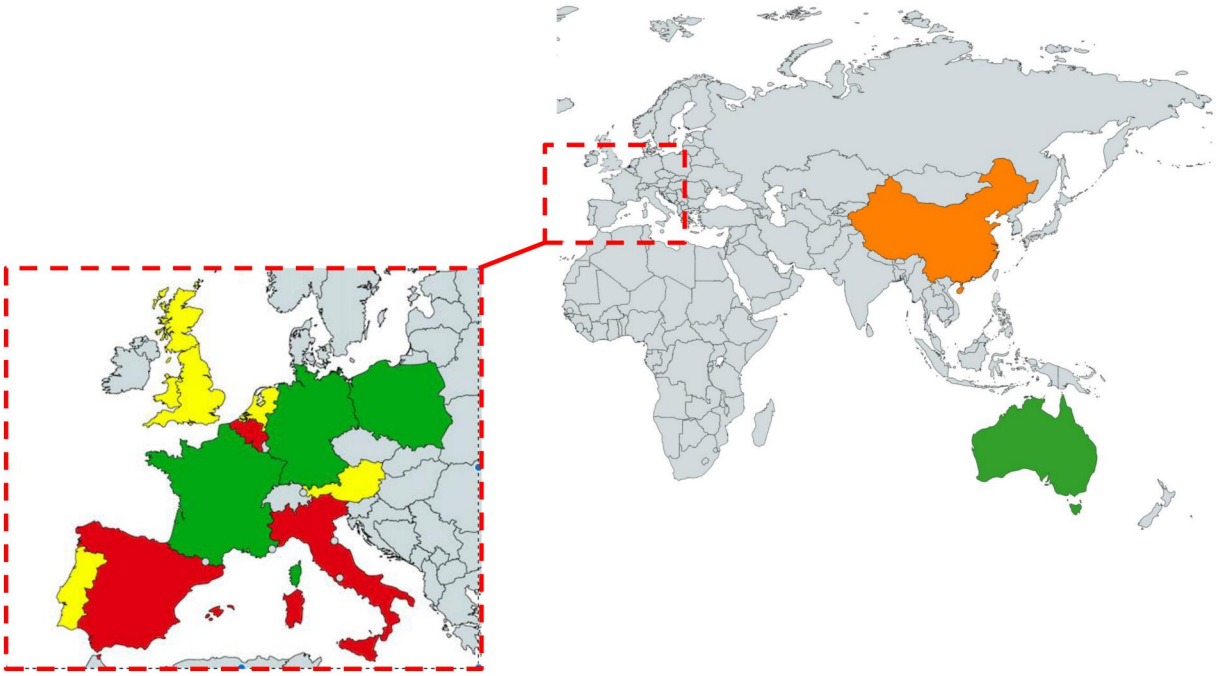

FIGURE 3 World-wide reported lipid-transfer protein sensitization

patients with pollen allergy.[100,101] This proportion was confirmed by Gonzalez-Mancebo et al. who reported that 53/430 (12.3%) of outpatients seen in their allergy unit for any reason were sensitized to Pru p 3.[102] Those sensitized to Pru p 3 may not always be symptomatic and the symptom severity may vary. A study on nsLTP syndrome reported that in a group of 87 subjects sensitized to Pru p 3, 44% experienced anaphylaxis, 43% skin or oropharyngeal reactions, and 13% were asymptomatic.[103] It is generally accepted that nsLTP sensitization often occurs early in life.[104,105] Pru p 3 sensitization among children in a Spanish cohort was twice as high as that in adults.[100] Another study reported that the level of Pru p 3 sensitization in a large population of Italian subjects peaks around the start of the third decade of life.[106] Further, a study in LTP-allergic adults suggested that the younger the age of onset, the higher the titres of specific IgE to Pru p 3 that are detected in patients.[13]

In addition to those who are mono-sensitized to Pru p 3, studies have also investigated subjects with sensitization to multiple nsLTP and usually have clinically relevant allergy to different plant foods. These individuals have the so-called LTP syndrome.[107] Rosaceae fruits and nuts are the main plant food allergens involved in this syndrome.[108] In a nationwide review of 17 Italian allergy clinics, 210 subjects sensitized to nsLTP reported reactions to multiple food triggers including peach (85%), apple (35%) and tree nuts (20%). Offending foods included also other Rosaceae (apricot, plum, pear, almond, strawberry), cereals (maize, beer, rice), kiwifruit, grapes, lettuce, cauliflower and broccoli.[12]

Following initial identification around 20 years ago, nsLTP sensitization appears to be most prevalent in the 'Mediterranean basin', a climate area which is defined by a southern European latitude. Although precise epidemiologic data from the general population are lacking, there is little doubt that sensitization to nsLTPs is a significant cause of primary food allergy in adults living in the Mediterranean basin[12,109] where it accounts for a large—if not the largest—proportion of food-induced anaphylaxis.[8] Interestingly, there are marked geographic variations in the prevalence of LTP hypersensitivity within single countries. This has been best shown for Italy due to its very different climate zones, that is, northern (continental climate) and central southern (Mediterranean climate), with nsLTP sensitization dominating in the latter.[12,110] Similar observations have been seen in two Spanish studies, where nsLTP-sensitization prevalence among patients with seasonal allergy in different areas was 2% and 40%, respectively.[52,101] However, a Mediterranean climate on its own is not the only factor in the development of sensitization to nsLTP. A high prevalence of nsLTP sensitization is also found in Portugal, which, despite being part of the Iberian Peninsula, is an Atlantic country.[111] In addition, those living in areas of Spain and Italy characterized either by a continental or Atlantic climate may also be sensitized to nsLTP.[12,112]

The geographic variability of nsLTP sensitization can be best appreciated in multinational studies, where the differences between northern and southern European countries are evident.[113] However, the presence of nsLTP sensitization in European countries other than the traditional 'endemic' Mediterranean areas is also emerging—see Figure 3. A study from Belgium reported nsLTP sensitization rates in selected pollen and/or plant food allergic children and adults to exceed 20% (albeit clinically irrelevant in most cases).[92] Other reports have been published from Austria,[114,115] France,[75] Belgium,[116] Germany,[117,118] Poland[119] and the United Kingdom.[90,120]. Outside Europe, nsLTP hypersensitivity and allergy seem relevant in certain areas of China.[83,121] A single report has been published from

Australia.[122] Remarkably, nsLTP sensitization has not yet been reported in the Americas or Africa.

7 | FUTURE RESEARCH NEEDS

Although experimental and clinical studies of nsLTP sensitization have increased significantly in the last decade, there are still several issues not fully understood. Although some definitions have been agreed by the Task Force, it is important to develop internationally agreed criteria and definitions for nsLTP sensitization and nsLTP syndromes, especially when determining the prevalence of nsLTP sensitization. In paediatric populations, nsLTP sensitization has some peculiarities: the lower risk of severe reactions compared to adults and the common occurrence of subclinical sensitization. Continent-wide epidemiological prevalence studies in both adults and children are urgently needed to evaluate the prevalence of nsLTP sensitization, its geographical distribution, risk/protective factors, genetic predisposition, clinical features and natural history.

The variation in the severity of symptoms also warrants investigation. It has already been demonstrated in one study that there is a link between multiple nsLTP sensitizations and systemic reactions.[92] It is possible that patients sensitized to multiple nsLTPs, with strong concomitant exposure to pollen-derived nsLTP allergens, could display a broad T-cell stimulatory repertoire, similar to that reported for profilin allergy.[123,124] The reports of more severe reactions in those with nsLTP sensitization but without pollinosis confirms the complex nature of food-allergic mechanisms and identifies a need for studies into such mechanisms that may ameliorate severe nsLTP reactions. Evaluation of sensitization profiles to pollen and food in different regions of Europe might contribute to the identification of primary sensitizers, whether they be from plant foods, pollens or other inhalants such as cannabis, thus paving the way for prevention and control measures.

Key Messages

1. nsLTPs are a large group of allergens found in land plants; the majority of allergenic nsLTPs belong to LTP (type)1 (9–10 kDa; around 90 amino acids).
2. nsLTPs are highly cross-reactive with particular hierarchies of sensitization depending on the primary sensitizer.
3. In Italy and Spain, sensitization frequently occurs in childhood, with peach nsLTP (Pru p 3) often being the sole sensitizing nsLTP.
4. Sensitization may be both via the oral, inhaled or cutaneous route, and may be initiated by a pollen nsLTP.
5. Although first described in Italy and Spain, nsLTP sensitization occurs in many European countries and has also been reported in China.
6. When an allergy to a plant food is suspected, nsLTP allergens should not be ruled out as a trigger, wherever the geographical location

8 | CONCLUSION

Investigating the structure and function of nsLTP has led to an understanding of their allergenic potential, which is exacerbated due to the high degree of cross-reactivity between nsLTP from different plant species. These allergens are present in fruits, vegetables, nuts, seeds, legumes and cereals, and food nsLTPs have been shown to be able to sensitize through the oral, respiratory and cutaneous routes. Exposure to peaches has been linked to the development of primary sensitization to the peach nsLTP, Pru p 3, which dominates in the Mediterranean areas of Europe. However, the sensitization process might depend on both individual (dietary/occupational) and/or geographical factors. Although data suggest it is an important cause of primary food allergy in the southern European countries, the extent of nsLTP sensitization could also be significant in the rest of Europe. In this context, the role of exposure to pollen needs further study, with some pollens, such as those of mugwort and plane tree, potentially playing a major role in sensitization to food nsLTPs. Despite high levels of sensitization to Pru p 3, many children remain asymptomatic or experience only mild skin symptoms. Moderate or severe clinical symptoms are much more likely to present in teenage years or in adults, despite levels of Pru p 3 falling. Sensitization to nsLTP is common and likely to be much more widespread than has been previously thought. It is therefore likely to be an important cause of plant food allergy in many adults in Europe and beyond.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Isabel Skypala established the Task Force, set up and chaired the meetings, determined the sections of the manuscript, merged all of the sections from individual contributors, added key points, wrote the abstract, made amendments to the manuscript following both internal and external reviews and submitted the manuscript in its final format. Ricardo Asero, Domingo Barber, Lorenzo Cecchi, Arazeli Diaz Perales, Karin Hoffmann-Sommergruber, Elide A. Pastorello, and Ines Swoboda attended and contributed to the meetings, wrote individual sections of the manuscript, reviewed it once all the sections had been put together and following internal and external review. Joan Bartra, Didier G. Ebo, Margaretha A. Faber, Montserrat Fernández-Rivas, Francesca Gomez, A. P. Konstantinopoulos, Olga Luengo, Ronald van Ree, Enrico Scala and Stephen J. Till attended and contributed to the meetings and reviewed the manuscript following review.

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