Marker Allergens and Panallergens in Tree and Grass Pollen Allergy

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Abbreviations

CCD	Cross-reactive carbohydrate determinants
cDNA	Complementary deoxyribonucleic acid
CRD	Component resolved diagnostics
Fab	Antigen-binding fragment
IgE	Immunoglobulin E
LTP	Lipid transfer protein
nsLTP	Nonspecific lipid transfer protein
OAS	Oral allergy syndrome
PR-10	Pathogenesis-related proteins

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10.1 Introduction

Many allergens from botanically related sources share structural similarities resulting in IgE cross-reactivity. As a consequence, allergens sharing similar structures are often also related on an immunological level, and patients sensitized to one specific allergen may show clinical or *in vitro* reactivity to structurally similar allergenic proteins of other allergen sources. Different IgE sensitization profiles can be identified in allergic patients according to reactivity to certain allergens. These allergens are defined as marker allergens (Kazemi-Shirazi et al. 2002; Suphioglu 2000; Valenta et al. 2007).

Today, genuine allergic sensitization can be differentiated from cross-reactivity using modern allergen component resolved diagnostics (CRD) (Valenta et al. 1999; Hiller et al. 2002; Lupinek et al. 2014). In grass and tree pollen allergy, CRD can identify the appropriate immunotherapy in poly-sensitized patients. Since specific immunotherapy is time-consuming (taking up to several years) and burdensome, early identification of patients suffering from genuine sensitization to grass or tree pollen who should benefit from a specific immunotherapy is important for clinical management of patients.

Allergens that pinpoint genuine sensitization and may be defined as marker allergens for specific tree and grass pollen allergies, will be described in this article.

10.2 Allergen Sources in Trees and Grasses

Tree and grass pollen from wind-pollinated plants are a frequent source of allergens. Between 12 and 17 % of the general population in Europe suffer from pollen allergy with almost 10 % suffering from tree pollen allergy (Blomme et al. 2013; Wüthrich et al. 1995). After hydration, tree and grass pollen rapidly release large amounts of allergens, i.e., defined IgE-binding proteins and glycoproteins. Upon contact with the mucosal surfaces of the respiratory tract, these allergens trigger allergic symptoms in susceptible patients (Grote et al. 2001; Vrtala et al. 1993).

10.2.1 Grasses

Most allergenic grasses belong to a botanical family of grasses called Poaceae, which is mainly found in temperate climate zones. As examples, timothy grass (*Phleum pratense*), perennial ryegrass (*Lolium perenne*), orchard grass (*Dactylis glomerata*), and Kentucky bluegrass (*Poa pratensis*) belong to the Pooideae subfamily and are closely related. Other grasses, such as Bermuda grass (*Cynodon dactylon*), Rhodes grass (*Chloris gayana*), love grass (*Eragrostis tenella*), rice (*Oryza sativa*), common reed (*Phragmites communis*), Bahia grass (*Paspalum notatum*), Johnson grass (*Sorghum halepense*), corn (*Zea mays*), and buffel grass

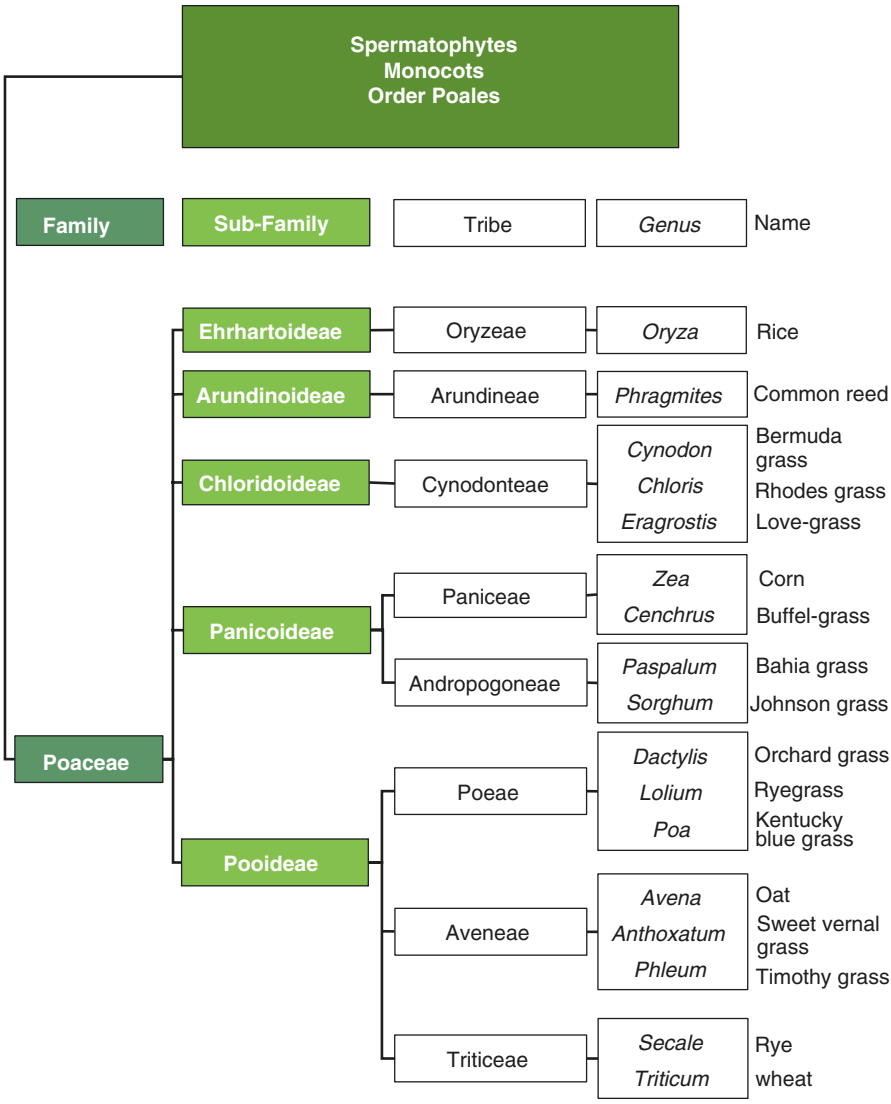

Fig. 10.1 Phylogenetic botanical relationship between important allergenic grasses (Adapted from Simon et al. (2011))

(*Cenchrus ciliaris*), belong to the Chloridoideae, Ehrhartoideae, Arundinoideae, and Panicoideae subfamilies, respectively, found in subtropical and tropical climate zones (Andersson and Lidholm 2003; Hejl et al. 2009; Johansen et al. 2009; Simon et al. 2011; Davies 2014). An overview of the botanical relationship between grasses is shown in ◉ Fig. 10.1.

10.2.2 Trees

Unlike grass pollen, allergenic tree pollen originates from different botanical groups of spermatophytes occurring in different geographical regions (Mothes et al. 2004; Swoboda et al. 2008; Marth et al. 2014). The following overview and ◉ Fig. 10.2 have been compiled according to the principles of phylogenetic classification (The Angiosperm Phylogeny Group 2009; Christenhusz et al. 2011).

The majority of trees are flowering plants (angiosperms). An important group of cross-reactive allergenic tree pollen originates from two families of the order Fagales:

- The Betulaceae family (birch, *Betula verrucosa*; alder, *Alnus glutinosa*; hazelnut, *Corylus avellana*; and hornbeam, *Carpinus betulus*)
- The Fagaceae family (oak, *Quercus alba*; common beech, *Fagus sylvatica*; and chestnut, *Castanea sativa*)

These trees are mainly found in temperate climate zones of Northern Europe, North America, and other continents (Wuthrich et al. 1995; Mothes et al. 2004; Asam et al. 2015).

Trees of the family Oleaceae (order Lamiales) are the source of a second important group of cross-reactive allergenic pollen. They are endemic all over Europe, but

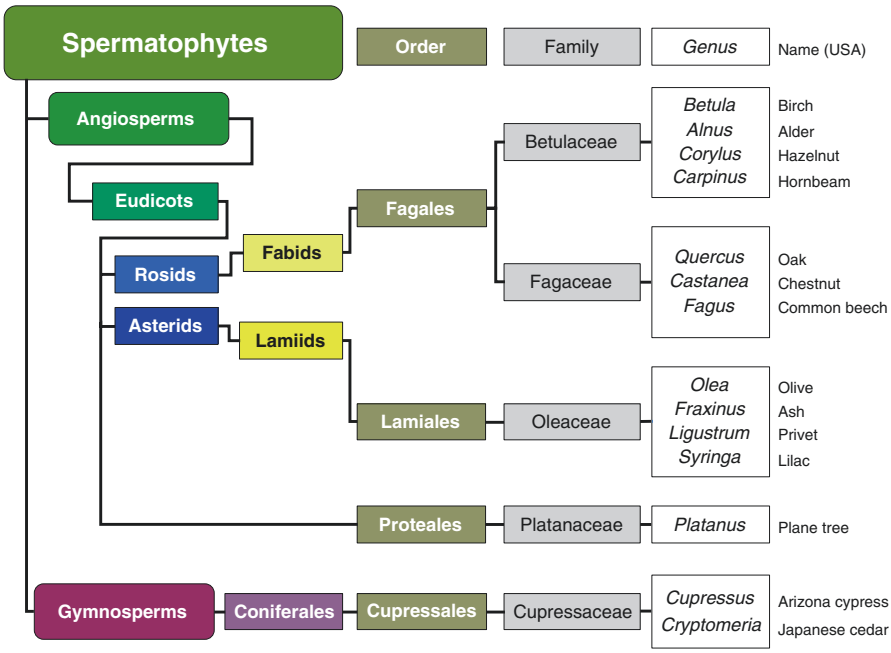

Fig. 10.2 Phylogenetic botanical relationship between important allergenic trees (Adapted from The Angiosperm Phylogeny Group (2009) and Christenhusz et al. (2011))

are also found in North America and other continents (Marth et al. 2014; Asam et al. 2015). The olive tree (*Olea europaea*) is the most widely spread species, especially in the Mediterranean region (Bousquet et al. 1984), but *Olea* ssp. is also found in other continents in areas with a Mediterranean climate, e.g., the Southwestern USA. Other allergenic members of the Oleaceae family are privet (*Ligustrum vulgare*), lilac (*Syringa vulgaris*), and ash trees (*Fraxinus excelsior*).

In some areas of the Mediterranean, different species of plane tree (order Proteales, family Platanaceae) represent a locally important source of allergens originating from angiosperms. Plane trees are also increasingly planted for ornamental purposes in many regions of Europe and North America and may cause allergic symptoms there (Asam et al. 2015).

Another important source of cross-reactive allergenic pollen originates from the botanical group of gymnosperms. The most important trees belong to the order of Cupressales (family Cupressaceae), such as the Arizona cypress (*Cupressus arizonica*), mountain cedar (*Juniperus ashei*), and the Japanese cedar (*Cryptomeria japonica*) (Swoboda et al. 2008; Di Felice et al. 2001; Marth et al. 2014). While the Arizona cypress has been widely exported from its native southwest of North America to Europe, and is frequently found in regions around the Mediterranean, the mountain cedar is of particular importance in southwestern North America, especially in Texas.

10.3 Important Grass Pollen Allergens

◉ Table 10.1 gives an overview of the most important grass pollen allergens.

10.3.1 Allergens Found in all Poaceae Grasses

10.3.1.1 Marker Allergen for all Poaceae Grasses: Group 1 (Phl p 1)

Group-1 allergens have been isolated and/or cloned from more than 20 Poaceae species (Andersson and Lidholm 2003; Griffith et al. 1991; Johnson and Marsh 1965; Laffer et al. 1994a; Perez et al. 1990). Phl p 1 is the group-1 allergen of timothy grass. It has a sequence identity of between 85 and 95 % with other members of the Pooideae subfamily. Most amino acid substitutions found in isoforms and in group-1 allergens of other Pooideae species (e.g., Hol l 1, Poa p 1, und Lol p 1) do not significantly alter allergenicity of the molecule (Andersson and Lidholm 2003; Johansen et al. 2009; Laffer et al. 1994a, b). Most IgE epitopes of Phl p 1 cluster at the c-terminus (Flicker et al. 2006), and grass pollen-specific IgE antibodies have been shown to bind with high density to the Phl p 1 molecule (Madritsch et al. 2015).

Up to 90 % of all grass pollen allergic patients show IgE reactivity to group-1 allergens of other grass species (Andersson and Lidholm 2003; Johansen et al. 2009; Laffer et al. 1994a, 1996; Van Ree et al. 1992). Phl p 1 is the most important group-1 allergen and represents an important cross-reactive major allergen.

Table 10.1 Important grass pollen allergens (Example: Timothy grass)

Protein	Significance	Example	Molecular weight (kDa)
Marker allergens for grass pollen			
Group-1 grass pollen allergen Glycosylated, β-expansin	Major allergen, all grasses	Phl p 1	31–35
Group-5 grass pollen allergen Unknown function	Major allergen, subfamiliy of Pooideae	Phl p 5	27–33
Other important allergens			
Allergens in all grasses			
Group-13 grass pollen allergen Glycosylated, polygalacturonase	Grass pollen specific	Phl p 13	~55
Allergens in some grasses			
Group-2 grass pollen allergen Expansin-related protein	Grass pollen specific	Phl p 2	10–12
Group-6 grass pollen allergen P-particle-associated-protein	Grass pollen specific	Phl p 6	~13
Group-11 grass pollen allergen Glycosylated, Ole-e-1-like protein	Little cross-reactivity	Phl p 11	~20
Allergens, not specific for grasses			
Polcalcin 2 EF-hand	Panallergen, cross-reactivity between different plant pollen	Phl p 7	~9
Profilin	Panallergen, cross-reactivity between many plant pollen, plant-derived food, and latex	Phl p 12	~14
Berberine bridge enzyme Glycosylated	Panallergen, clinically reduced relevance	Phl p 4	50–67

Cross-reactivity of group-1 allergens has been demonstrated in many studies with natural extracts of different Pooideae species and other Poaceae subfamilies (Johansen et al. 2009; Laffer et al. 1994b; Van Ree et al. 1992). Purified recombinant Phl p 1 inhibited binding of patient sera to natural extracts of eight different grasses (timothy grass, *Phleum pratense*; sweet vernal grass, *Anthoxanthum odoratum*; oat, *Avena sativa*; Bermuda grass, *Cynodon dactylon*; perennial ryegrass, *Lolium perenne*; common reed, *Phragmites communis*; Kentucky bluegrass, *Poa pratensis*; and rye, *Secale cereale*) inducing an average inhibition of 76% (Laffer et al. 1996). Monoclonal antibodies raised against Phl p 1 and defining four distinct epitopes as well as recombinant human Phl p 1-specific IgE-Fabs (antigen-binding

fragments) recognize and bind to a panel of natural group-1 allergens of different Pooideae grasses (Flicker et al. 2006; Duffort et al. 2008).

Sequence homologies and cross-reactivity between Phl p 1 and group-1 allergens of subtropical grasses such as Bermuda grass (*Cynodon dactylon*; 67–70 % sequence identity) or Bahia grass (*Paspalum notatum*) are less pronounced (Andersson and Lidholm 2003; Johansen et al. 2009; Davies 2014; Timbrell et al. 2014). There is no complete cross inhibition between group-1 allergens of grasses originating in temperate climate zones and group-1 allergens of grasses originating in subtropical climate zones, especially with patient sera from subtropical climate zones (overview presented in Davies (2014)). However, there are indications that these species-specific IgE epitopes are not protein epitopes but carbohydrate epitopes without clinical relevance (Cabauatan et al. 2014).

Phl p 1 is the most important marker allergen for genuine sensitization to grasses belonging to all subfamilies of Poaceae for the following reasons:

- Approximately 90 % of grass pollen allergic patient sera contain specific IgE against Phl p 1.
- Group-1 allergens have been found in all Poaceae grasses, but not in other taxonomically unrelated plants.
- There is widespread cross-reactivity between group-1 allergens from different grass species (Niederberger et al. 1998a).

10.3.1.2 Group 13

The group-13 grass pollen allergen, a 55-kDa protein, has also been described in all grasses examined to date (Suck et al. 2000). Although over 50 % of grass pollen allergic patients display IgE reactivity against Phl p 13, it has only little clinical relevance as it showed only low allergenic reactivity in clinical and *in vitro* studies (Westritschnig et al. 2008).

10.3.2 Allergens Found Only in Pooideae Grasses

10.3.2.1 Marker Allergen for Pooideae: Group 5 (Phl p 5)

Group-5 allergens are marker allergens for Pooideae grasses. Homologous allergens have been found in all grasses of the Pooideae subfamily, such as timothy grass (*Phleum pratense*), rye (*Secale cereale*), Kentucky bluegrass (*Poa pratense*), and perennial ryegrass (*Lolium perenne*). Group-5 allergens are not found in grasses belonging to the Panicoideae, Chloridoideae, Ehrhartoideae, or Arundinoideae subfamilies, which are mainly distributed in the southern hemisphere and are highly prevalent in tropical and subtropical climate zones (Davies 2014). Group-5 allergens are not found in corn (*Zea mays*), Bermuda grass (*Cynodon dactylon*), or rice (*Oryza sativa*), for example (Niederberger et al. 1998a).

Phl p 5, one of the best characterized group-5 allergens, is one of several allergens to occur in different isoallergenic forms as Phl p 5a (i.e., Phl p 5.01) and Phl p 5b (i.e., Phl p 5.02). The overall sequence identity between Phl p 5a and Phl p 5b is approximately 65 % but is higher (70–77 %) in important parts of the molecule. Multiple independent IgE epitopes have been identified on both isoforms of Phl p 5 (Levin et al. 2014).

Between 65 and 85 % of grass pollen allergic patients in temperate climate zones display IgE reactivity to group-5 allergens, and the clinical allergenic activity of Phl p 5a is very high (Andersson and Lidholm 2003; Westritschnig et al. 2008; Flicker et al. 2000; Vrtala et al. 1993).

Most patients display extensive IgE cross-reactivity to the Phl p 5 isoallergens as well as to different group-5 allergens from Pooideae grasses (Andersson and Lidholm 2003; Niederberger et al. 1998a; van Ree 2002).

Phl p 5 is therefore an important marker allergen for sensitization to grasses of the Pooideae subfamily.

10.3.2.2 Other Pooideae-Specific Allergens

Group-2/group-3 and group-6 allergens are also only found in the pollen of Pooideae grasses. In some populations more than 50 % of grass pollen allergic patients display IgE reactivity to these molecules, yet the overall rate of patient sensitization is not high enough to give them the status of marker allergens (for an overview, see Andersson and Lidholm 2003; Gangl et al. 2013). Although patient IgE titers against group-2/group-3 allergens are often rather low, Phl p 2 shows high allergenic activity in skin tests (Westritschnig et al. 2008). The allergenic activity of Phl p 6 has not been tested yet in clinical studies.

Group-11 allergens are not very important in the clinic. Although few patients react with these allergens, they have been found in *Phleum pratense* and *Lolium perenne* (Marknell DeWitt et al. 2002), and homologs from other plants, e.g., olive (Ole e 1), corn (Zea m 13), and tomato, have been identified. Cross-reactivity between homologs from taxonomically unrelated allergen sources is very limited.

10.3.3 Allergens from Tropical and Subtropical Grasses

Subtropical grasses of the Panicoideae (e.g., *Paspalum notatum* (Bahia grass), *Sorghum halepense* (Johnson grass), *Imperata cylindrica* (cogon grass), *Cenchrus* sp. (buffel grass)) and Chloridoideae (e.g., *Cynodon dactylon* (Bermuda grass), *Eragrostis* sp. (e.g., Boer love), *Chloris gayana* (Rhodes grass)) subfamilies are abundant in regions adjacent to the equator (Esch 2004; Seidel et al. 2008) and appear to be clinically important for pollen allergy in subtropical regions of Africa, Asia, Central America, and southern parts of the USA (e.g., Florida, Texas) (Prescott and Potter 2001; Liang et al. 2010; Sam et al. 1998; Phillips et al. 1989; Calabria and Dice 2007). Allergens of Panicoideae (Pas n 1 and Pas n 13 of Bahia grass pollen and Sor h 1, Sor h 2, Sor h 13, and Sor h 23 of Johnson grass) and Chloridoideae (Cyn d 1, Cyn d 4, and Cyn d 22) species have potential to serve as diagnostic markers, but studies in relevant patient populations with recombinant allergens devoid of CCD are

needed to determine the clinical relevance (Cabauatan et al. 2014; Davies et al. 2011; Davies 2014; Campbell et al. 2015). Notably, studies from Zimbawe show that tropical grasses are more prevalent than temperate grasses (Westritschnig et al. 2003).

Subjects with and without allergic symptoms from the Philippines contained IgE antibodies against tropical grasses and were mainly sensitized against carbohydrate epitopes which did not induce basophil activation. In this study it is likely that sensitization to tropical grasses is of low clinical relevance, but this needs to be investigated in other populations (Cabauatan et al. 2014). Recombinant Pas n 1 of Bahia grass pollen activated basophils of grass pollen allergic patients indicating clinically relevant sensitization in the Australian population exposed to and allergic to Bahia grass pollen (Davies et al. 2008).

10.3.4 Marker Allergens for Grass Pollen Allergy: Summary

Group-1 and group-5 allergens account for 60–80 % of grass pollen allergic patient IgE in different populations from different geographic areas (Laffer et al. 1996). Extensive cross inhibition of IgE binding of patients to nine different grass pollen extracts (sweet vernal grass, *Anthoxanthum odoratum*; oat, *Avena sativa*; Bermuda grass, *Cynodon dactylon*; perennial ryegrass, *Lolium perenne*; common reed, *Phragmites australis*; Kentucky bluegrass, *Poa pratensis*; rye, *Secale cereale*; wheat, *Triticum sativum*; and corn, *Zea mays*) was achieved with a small panel of purified, recombinant, grass pollen allergens (Phl p 1, Phl p 2, and Phl p 5) and profilin (Bet v 2) (Niederberger et al. 1998a). In a clinical vaccination study involving 64 subjects, patients were successfully treated with a mixture of recombinant Phl p 1, Phl p 2, Phl p 5a + b, and Phl p 6 (Jutel et al. 2005). A proof of principle was thus established that successful therapy of temperate grass pollen allergy in patients from Europe is possible using a combination of distinct grass pollen-specific and clinically important allergens. Recently, a novel recombinant hypoallergenic grass pollen allergy vaccine based on allergen peptides derived from the abovementioned grass pollen allergens has been developed for safe immunotherapy of grass pollen allergy (Focke-Tejkl et al. 2015) and, in clinical trials, has been shown to be hypoallergenic (Niederberger et al. 2015) as well as clinically effective for treatment (Zieglmayer et al. 2016; Cornelius et al. 2016; Gerlich and Glebe 2016).

> Group-1 and group-5 allergens, such as Phl p 1 and Phl p 5, are the most suitable marker allergens for diagnosis of grass pollen allergy in temperate climate zones.

10.3.5 Carbohydrate Sensitivity in Grass Pollen Allergic Patients

Phl p 1, Phl p 4, Phl p 11, Phl p 13 and their subtropical orthologs (e.g., Cyn d 1, Cyn d 4, and Pas n 13) are glycoproteins carrying cross-reactive carbohydrate determinants (CCD). Using CCD-free recombinant allergens in allergen CRD has

the advantage that only functional IgE (i.e., capable of IgE aggregation) directed against protein epitopes is detected. For instance, up to 85 % of grass pollen allergic patients have detectable group-4 allergen-specific IgE. Group-4 allergens are glycoproteins with a molecular weight of 50–67 kDa. However, specific IgE in patient sera is often rather low, and despite *in vitro* reactivity, no clinical reactivity has been described (Andersson and Lidholm 2003; Westritschnig et al. 2008; Niederberger et al. 2001; Zafred et al. 2013). In tropical regions, IgE cross-reactivity is based nearly exclusively on CCD of these glycoproteins (Cabauatan et al. 2014), and in temperate climate zones, the frequency of sensitization seen in patient sera is less than 60 % if recombinant Phl p 4 is used for diagnosis (Tripodi et al. 2012).

Phl p 4 homologous proteins are found in *Ambrosia* sp. and birch pollen, as well as in peanut, apple, celery, and carrot, but clinical significance remains unclear (Grote et al. 2002).

10.4 Important Tree Pollen Allergens

◉ Table 10.2 gives an overview of the most important tree pollen allergens.

10.4.1 Allergens of Trees of the Order Fagales

10.4.1.1 Marker Allergen for Fagales: Bet v 1

The complementary deoxyribonucleic acid (cDNA) of Bet v 1, the major allergen of birch, was isolated in 1989 (Breiteneder et al. 1989), the 17-kDa protein allergen was produced using recombinant gene technology, and IgE reactivity in up to 95 % of birch pollen allergic patients was detected (Valenta et al. 1991b; Menz et al. 1996).

The major allergens of other tree pollen in the order of Fagales from the families of Betulaceae, (alder, *Alnus glutinosa*, Aln g 1; hornbeam, *Carpinus betulus*, Car p 1; hazelnut, *Corylus avellana*, Cor a 1) and Fagaceae (oak, *Quercus alba*, Que a 1; chestnut, *Castanea sativa*, Cas s 1; common beech, *Fagus sylvatica*, Fag s 1) all show pronounced cross-reactivities and sequence homologies within the group and to Bet v 1 (Mothes et al. 2004; Valenta et al. 1991b; Ipsen and Hansen 1991; Marth et al. 2014). Together, they form a group known as pathogenesis-related proteins (PR-10). Recombinant Bet v 1 inhibits IgE reactivity of patient sera with other tree pollen of the Fagales order (Niederberger et al. 1998b). A great number of proteins from different plant foods (nuts, vegetables and spices) display homology and cross-reactivity to Bet v 1, e.g., apple (*Malus domestica*, Mal d 1), hazelnut (*Corylus avellana*, Cor a 1), sweet cherry (*Prunus avium*, Pru av 1), apricot (*Prunus armeniaca*, Pru ar 1), peach (*Prunus persica*, Pru p 1), pear (*Pyrus communis*, Pyr c 1), carrot (*Daucus carota*, Dau c 1), celery (*Apium graveolens*, Api g 1), and soybean (*Glycine max*, Gly m 4) (Mothes et al. 2004; Swoboda et al. 2008; Heiss et al. 1996; Kazemi-Shirazi et al. 2000), and are responsible for birch pollen-related oral allergy syndrome (see Chap. 2).

Table 10.2 Important tree pollen allergens

Pollen	Example	Molecular weight (kDa)	Allergen	Protein
Fagales, e.g., birch	Bet v 1	~17	Marker allergen, major allergen, cross-reactivity with Fagales tree pollen; oral allergy syndrome	PR-10 protein
	Bet v 2	~15	Panallergen, cross-reactivity between plant pollen, plant-derived food, and latex	Profilin
	Bet v 3	~24	Panallergen, cross-reactivity between different plant pollen	Polcalcin family (3 EF-hand)
	Bet v 4	7–9	Panallergen, cross-reactivity between different plant pollen	Polcalcin family (2 EF-hand)
	Bet v 6	~33	Minor allergen	Isoflavone reductase
	Bet v 7	~18	Minor allergen	Cyclophilin
	Bet v 8	~66	–	Pectinesterase
Lamiales, e.g., olive tree	Ole e 1	~16	Marker allergen, major allergen, cross-reactivity between Lamiales tree pollen	Ole-e-1-like protein family, glycosylated
	Ole e 2	15–18	Panallergen, cross-reactivity between plant pollen, plant-derived food, and latex	Profilin
	Ole e 3	~9	Panallergen, cross-reactivity between different plant pollen	Polcalcin family (2 EF-hand)
	Ole e 5	~16	Minor allergen	Superoxide dismutase
	Ole e 6	~6–10	Minor allergen	–
	Ole e 7	~10	Minor allergen, limited cross-reactivity to other nsLTP	Nonspecific lipid transfer protein (nsLTP)
	Ole e 8	~21	Panallergen, cross-reactivity between different plant pollen	Polcalcin family (4 EF-hand)
	Ole e 9	~46	Minor allergen, pollen-fruit-latex syndrome	β-1,8-glucanase
	Ole e 10	~11	Minor allergen, pollen-fruit-latex syndrome	X8-domain protein, glycosyl hydrolase
	Ole e 11	39.4	Minor allergen	Pectin methyl-esterase
Platanaceae, e.g., plane tree	Pla a 1	~18	Marker allergen, major allergen	Invertase inhibitor
	Pla a 2	~43	–	Polygalacturonase
Cupressales, e.g., Arizona cypress, Japanese cedar	Cry j 1/ Cup a 1	41–45	Marker allergen, major allergen	Pectate lyase, glycosylated

Due to the high number of IgE-binding epitopes, Bet v 1 is thought to be the original sensitizing protein in clinically manifest allergy to Fagales pollen or in oral allergy syndrome (Kazemi-Shirazi et al. 2002; Swoboda et al. 2008; Moverare et al. 2002). In birch pollen allergic patients, exposure to birch pollen primarily increased Bet v 1-specific IgE without increasing IgE to other birch pollen allergens such as Bet v 2 (Birkner et al. 1990). Allergy patients in Central Africa reacting with natural birch pollen extracts did not display IgE antibodies against Bet v 1 but against other birch pollen allergens (Westritschnig et al. 2003; Odongo et al. 2015).

Several studies have shown that subcutaneous immunotherapy with birch pollen extract alone is equally effective as therapy with a mixture of different Fagales tree pollen extracts in tree pollen allergic patients (Henzgen et al. 1989; Petersen et al. 1988). Allergy diagnosis (skin test, specific IgE) with recombinant Bet v 1 is as effective in detecting birch pollen allergic patients as diagnosis using natural birch pollen extracts (Tresch et al. 2003).

As a consequence of these in vitro and in vivo data, Bet v 1 represents the marker allergen for sensitization to Fagales tree pollen and indicates the possibility of an associated oral allergy syndrome.

10.4.1.2 Other Fagales-Specific Minor Allergens

Bet v 6 (formerly known as Bet v 5), an isoflavone reductase, is a minor allergen which is cross-reactive with pollen and proteins from several edible plants (fruits, vegetables, and spices); Bet v 7 is a cyclophilin. Both are recognized by less than 20 % of birch pollen allergic patients. Bet v 8 is a pectinesterase with a clinical significance that has yet to be determined (for an overview, see (Mothes et al. 2004; Marth et al. 2014) and Table 10.2).

10.4.2 Allergens of Trees of the Order Lamiales

10.4.2.1 Marker Allergen for Lamiales: Ole e 1

Ole e 1, the most important olive pollen allergen, exists in a non-glycosylated (19 kDa) and a glycosylated (21 kDa) form and is recognized by more than 70 % of olive pollen allergic patients (Villalba et al. 1993). It displays substantial sequence homologies with other members of the Ole-e-1-like protein family. This protein family derives from pollen of other Oleaceae species (for an overview, see Rodriguez et al. 2007) such as:

- Ash (*Fraxinus excelsior*, Fra e 1)
- Privet (*Ligustrum vulgare*, Lig v 1)
- Lilac (*Syringa vulgaris*, Syr v 1)

Moreover this protein family comprises Pla l 1 from plantain (*Plantago lanceolata*, family of Plantaginaceae) as well as allergens from taxonomically unrelated

species such as Lol p 11 from *Lolium perenne* (perennial ryegrass), Phl p 11 from *Phleum pratense* (timothy grass), and Che a 1 from *Chenopodium album* (white goosefoot).

There is extensive cross-reactivity between Ole e 1 homologous allergens of the Oleaceae (Overview in Valenta et al. 2007). IgE from sera of two different groups of European patients either sensitized to olive or ash pollen was inhibited from binding to extracts of different Oleaceae pollen by Ole e 1. Birch pollen, grass pollen, and weed pollen extracts did not inhibit patient IgE binding to Ole e 1 (Palomares et al. 2006) showing the existence of specific epitopes for Oleaceae pollen in Ole e 1.

In patients from regions without local sources of olive pollen such as Austria, Germany, or Northern Italy, specific IgE against Ole e 1 indicates a sensitization to ash pollen (*Fraxinus excelsior*, Fra e 1) (Asero 2011; Niederberger et al. 2002). This is relevant in patients showing clinical symptoms during the birch pollen season, but who are not sensitized to birch or any other member of the Fagales order (Palomares et al. 2006).

Ole e 1 is the marker allergen for sensitization to olive pollen and is important in this respect in the Mediterranean region. In regions without olive pollen, Ole e 1 can be used as a marker allergen for sensitization to ash pollen.

The group-11 grass pollen allergens Phl p 11 and Lol p 11 are members of the Ole-e-1-like protein family due to structural homologies and sequence homologies (e.g., approximately 30 % sequence identity between Ole e 1 and Phl p 11). However, they do not share any IgE epitopes with Ole e 1, and no significant cross-reactivity was detected between Ole e 1 and Phl p 11 or Lol p 11 (Palomares et al. 2006).

10.4.2.2 Other Lamiales-Specific Allergens

Other specific minor allergens of olive pollen have been described (for an overview, see (Rodriguez et al. 2007) and ◉ Table 10.2). Ole e 7 is a member of the nonspecific lipid transfer protein (nsLTP) family. Sensitization to Ole e 7 is associated with a tendency for severe allergic reactions; however, cross-reactivity with other nonspecific LTP seems to be limited (Tordesillas et al. 2011). In some regions of southern Spain, an elevated prevalence of sensitization against Ole e 7 and Ole e 9 was seen, and in some regions, up to 40 % of Ole-e-1-negative allergic patients are sensitized to Ole e 7 (Barber et al. 2007). Ole e 9 und Ole e 10 are also possibly associated with cross-reactivity to birch, tomato, potato, bell pepper, banana, and latex (Palomares et al. 2005; Quiralte et al. 2007).

10.4.3 Allergens of Trees of the Order Proteales

Tree pollen from trees of the Platanaceae family, genus Platanus, comprising about ten species (e.g., London plane tree, *Platanus acerifolia*), are highly cross-reactive and induce severe symptoms in a small number of sensitized patients. In regions with

many plane trees, such as Spain, peaks of allergy symptoms are seen during the wind pollination season (Varela et al. 1997). Pla a 1 from the London plane tree, an invertase inhibitor, is recognized by up to 90% of all plane tree allergic patients and is therefore considered a major allergen of plane trees (Asturias et al. 2002). Pla a 1 is used as a marker allergen for plane tree allergy (◉ Table 10.2); however, the allergen Pla a 2, a polygalacturonase, may also be important in this respect (Asturias et al. 2002, 2006).

10.4.4 Allergens of Trees of the Order Cupressales

Pollen from trees of the Cupressaceae family (e.g., Arizona cypress, *Cupressus arizonica*; Japanese cedar, *Cryptomeria japonica*; mountain cedar, *Juniperus ashei)* are highly cross-reactive (for an overview, see Di Felice et al. 2001; Marth et al. 2014). The prevalence of allergy to different Cupressaceae pollen has increased in Central Europe, even though Cupressaceae trees are distributed mainly in the Mediterranean region (Panzner et al. 2014). It is possible that allergy to Cupressales pollen was underestimated for a long time, because the flowering season is in winter (January to March/April), and clinical symptoms of allergy to Cupressales may have been mistaken for the common cold or thought to have been caused by perennial allergens such as from house dust mite (D'Amato et al. 2007).

Cry j 1 (from Japanese cedar) is a 40-kDa protein and was the first Cupressaceae allergen to be described (Yasueda et al. 1983). Cry j 1 displays high sequence homology and IgE cross-reactivity with other Cupressaceae allergens such as Cup a 1 from the Arizona cypress (Aceituno et al. 2000) and Jun a 1 from the mountain cedar (Midoro-Horiuti et al. 1999). These allergens are glycosylated pectate lyases. Although the major allergen of ragweed (*Ambrosia artemisiifolia*, Amb a 1) is also a pectate lyase, there is only very limited cross-reactivity with the above mentioned allergens (Pichler et al. 2015). Cup a 1 and Cry j 1 are used as marker allergens for Cupressales allergy.

10.5 Panallergens: Markers for Cross-Reactivity

Panallergens are found in grass and tree pollen as well as in many other botanically unrelated plants. They belong either to the polcalcin (calcium-binding allergens carrying two, three, or four binding sites for calcium, so-called EF-hands) or profilin protein families. Amino acid sequences of both protein families are highly conserved regardless of the taxonomical relationship of allergenic plant species leading to extensive immunological cross-reactivity. Therefore, they are considered marker allergens for cross-reactivity in the diagnosis of grass and tree pollen allergy (see Chap. 3).

10.5.1 Polcalcins

Members of the polcalcin protein family (approximately 9-kDa proteins) from tree and grass pollen include:

- 2-EF-hand-proteins Bet v 4, Aln g 4, Ole e 3, Cyn d 7, Phl p 7, Cyn d 7
- 3-EF-hand-protein Bet v 3
- 4-EF-hand-protein Ole e 8

Polcalcins have only been found in the pollen of trees, grasses, and weeds. Almost 10 % of grass pollen allergic patients in temperate regions have specific IgE to Phl p 7, but in sensitized patients, Phl p 7 displays a high allergenic activity (see Chap. 3) (Kazemi-Shirazi et al. 2002; Niederberger et al. 1999).

10.5.2 Profilins

Profilin (Bet v 2, 15 kDa) was first identified in birch pollen (Valenta et al. 1991a) and has since been found in the pollen of many grasses (e.g., Phl p 12, Cyn d 12), trees (e.g., Ole e 2), and weeds, but also in plant-derived food and latex (for an overview. see Kazemi-Shirazi et al. 2002; Radauer et al. 2006). The amount of specific patient IgE varies according to geographical region and allergen source and is found in approximately 10–30 % of pollen allergic patients.

10.5.3 Panallergens: Summary

Cross-inhibition experiments between polcalcins from different sources and between profilins from different sources have confirmed extensive cross-reactivity of allergens within these two protein families; the highest IgE reactivity is observed with the grass pollen allergens, i.e., Phl p 7 for the polcalcins and Phl p 12 for profilins (Tinghino et al. 2002; Radauer et al. 2006).

Phl p 7 and Phl p 12 are considered marker allergens for cross-reactivity, and the presence of specific IgE to either of these allergens in patient sera may explain clinical symptoms upon exposure to a range of different allergen sources. Sensitization to either of these panallergens, Phl p 7 or Phl p 12, may result in apparent polysensitization presenting as multiple positive skin tests to pollen extracts and potential symptoms with unrelated allergen sources.

In patients with grass pollen allergy, sensitizations to Phl p 7 and Phl p 12 are often seen in later stages of allergic disease after sensitization to Phl p 1 and Phl p 5 (Hatzler et al. 2012) and may be considered as markers for clinically manifest grass pollen allergy.

10.6 Conclusions for Clinical Routine Work

Structured Approach to Routine Clinical Work

Diagnostic tests with marker allergens, Phl p 1/Phl p 5 (marker for grass pollen), Bet v 1 (marker for beech and birch trees as well as other Fagales trees), Ole e 1 (marker for olive trees and other Oleaceae trees including ash), Pla a 1 (marker for plane trees), and Cup a 1/Cry j 1 (marker for cypress trees), and with the panallergens (e.g., timothy grass, polcalcin/profilin), Phl p 7/Phl p 12 (indicators for cross-reactivity), establish a patient allergen-specific sensitization profile to tree and grass pollen allergens.

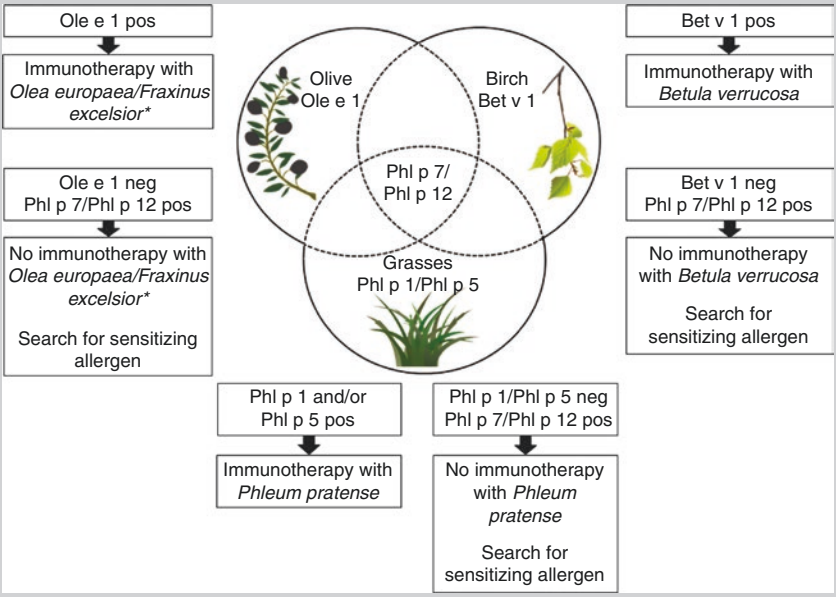

Fig. 10.3 Proposal for a structured diagnostic procedure in clinical routine work using important marker allergens: Phl p 1, Phl p 5, Phl p 7, Phl p 12, Ole e 1, Fra e 1*, and Bet v 1. *if available and in regions without distribution of *Olea europaea*

Genuine sensitization to grass pollen in Europe is reliably diagnosed with a combination of the major grass pollen allergens Phl p 1 and Phl p 5 (◉ Fig. 10.3). If sensitization to Phl p 1 without IgE reactivity to Phl p 5 (and in addition, no reactivity to Phl p 2/Phl p 3 and Phl p 6) is found, this may be due to sensitization to one of the tropical/subtropical grass subfamilies.

Specific IgE to Bet v 1 characterizes sensitization to Fagales trees (birch, alder, hornbeam, hazelnut, common beech, oak, chestnut) and possible related oropharyngeal symptoms (oral allergy syndrome) due to reactions with cross-reactive plant-derived foods (e.g., apple, hazelnut, pear, sweet cherry, peach, carrot, celery, soybean) (see Chap. 2).

Ole e 1 is the major allergen in olive pollen. It displays extensive sequence identity and cross-reactivity with other major allergens of the Oleaceae family such as ash, privet, and lilac. In the Mediterranean region, genuine sensitization to olive pollen is diagnosed with Ole e 1; in more temperate climate zones such as Central Europe, Ole e 1 can be used to prove sensitization to ash pollen and to distinguish it from the clinical symptoms of birch pollen allergy occurring in the same season.

Sensitization to tree pollen of the Platanaceae family is diagnosed with Pla a 1 (possibly including Pla a 2), sensitization to pollen of trees of the Cupressaceae family with Cup a 1/Cry j 1.

Association of the abovementioned marker allergens with specific clinically relevant sensitization profiles was confirmed in clinical studies (Canis et al. 2011; Jahn-Schmid et al. 2003; Twardosz-Kropfmuller et al. 2010; Moreno et al. 2014; Darsow et al. 2014).

If no clear-cut sensitization to one of the abovementioned marker allergens can be detected, the following rules apply:

- Low or no IgE reactivity to genuine marker allergens indicates that a patient is not sensitized to the corresponding allergen source. An allergen extract from this source is not suitable for specific immunotherapy.
- Exclusive sensitization to the panallergens profilin and polcalcin (e.g., Phl p 7 and Phl p 12 from timothy grass pollen) is very rare. This sensitization profile often cannot be attributed to one specific allergen source. Patients therefore are not suited for specific immunotherapy.
- The presence of specific IgE to profilin and/or polcalcin by nature of their cross-reactivity rules out further diagnosis with natural (pollen) extracts, as sensitization to panallergens abolishes analytical specificity (selectivity) of natural extracts.

In these cases, allergy CRD together with a detailed patient history should be used to reach a therapeutic decision. This will ensure that the correct decision for or against specific immunotherapy and its correct composition is taken (Douladiris et al. 2013; Letrán et al. 2013).

Conclusions

Detection of specific IgE using component resolved diagnostics (CRD) identifies the underlying allergen source in suspected cases of tree and grass pollen allergy. Suitable marker allergens can be used to distinguish genuine sensitization to tree or grass pollen from cross-reactivity to pollen panallergens (e.g., profilin and polcalcins) and to overcome the lack of analytical specificity of natural allergen extracts. In allergic patients suspected of polysensitization who react with a variety of pollen extracts, CRD allows specific allergen diagnosis regardless of the confounding effect of panallergenic cross-reactivity and prescription of tailored, specific immunotherapy.

Allergens defined as marker allergens for tree and grass pollen allergy are:

- Bet v 1 (birch pollen major allergen) for birch, beech, and other trees from the Fagales order
- Ole e 1 (olive tree major allergen) for olive and other trees including ash from the Oleaceae family
- Pla a 1 (major allergen of the London plane) for plane trees
- Cry j 1 (major allergen of the Japanese cedar)
- Cup a 1 (major allergen of the Arizona cypress) for cypress trees
- Jun a 1 (major allergen of mountain cedar) for juniper trees
- Phl p 1 (timothy grass group-1 major allergen) for most grasses including rye (*Secale* sp.)
- Phl p 5 (timothy grass group-5 major allergen) for "temperate climate" grasses (*Pooideae*)

Grass and tree pollen allergens with serological and clinical cross-reactivity to a great number of allergen sources are also identified as possible confounding factors in allergen-specific diagnosis with natural extracts. Structured diagnostic procedures for clinical routine work are needed to improve the appropriate selection of allergen sources for AIT.

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