Marker allergens and panallergens in tree and grass pollen allergy

Part 17 of the Series Molecular Allergology

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Abstract

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 patients reacting with a variety of pollen extracts suspected of polysensitization, CRD allows allergen specific diagnosis regardless of the confounding effect of panallergenic cross-reactivity and administration of tailored, specific immunotherapy.

In this article, allergens indicating specific sensitization to grass and tree pollen are described. 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 pollen major allergen) for olive and other trees including ash from the Oleaceae family, Pla a 1 (major allergen of the London plane tree) for plane trees, Cry j 1 (major allergen of the Japanese cedar), Cup a 1 (major allergen of the Arizona cypress) for cypress trees and Phl p 1 und 5 (Timothy grass major allergens) for sweet grasses including rye. 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 proposed.

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Marker allergens

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 other structurally similar allergenic proteins. Different IgE sensitization profiles can be identified in allergic patients according to reactivity to certain allergens. These allergens are defined as marker allergens [1, 2, 3].

Today, genuine allergic sensitization can be differentiated from cross-reactivity using modern component resolved allergy diagnostic (CRD) [4]. 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.

Allergens, which pinpoint genuine sensitization and may be defined as marker allergens for specific

tree and grass pollen allergies, shall be described in this article.

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 grass pollen allergy with almost 10 % suffering from tree pollen allergy [5, 6]. 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 [7, 8].

Grasses

Most allergenic grasses belong to the botanical family of sweet grasses (Poaceae) mainly found in temperate climate zones. As examples, Timothy grass (*Phleum pratense*), rye grass (*Lolium perenne*), orchard grass (*Dactylis glomerata*), Kentucky blue grass (*Poa pratensis*) belong to the Pooideae subfamily and are closely related. Other grasses, such as Bermuda grass (*Cynodon dactylon*), rice (*Oryza sativa*), common reed (*Phragmites communis*) and Bahia grass (*Paspalum notatum*) belong to the Chloridoideae, Ehrhartoideae, Arundinoideae und Panicoideae subfamilies, respectively, found in hot and tropical climate zones [9, 10, 11, 12]. An overview of the botanical relationship between grasses is shown in **Fig. 1**.

Trees

Unlike grass pollen, allergenic tree pollen originates from different botanical groups of spermatophytes occurring in different geographical regions [13, 14, 15]. The following overview and **Fig. 2** have been compiled according to the principles of phylogenetic classification [16, 17].

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*) and
- the Fagaceae family (oak, *Quercus alba*; common beech, *Fagus sylvatica* and chestnut, *Castanea sativa*).

These trees are mainly found in Northern Europe and in North America [6, 14].

Trees of the family Oleaceae (order Lamiales) are the source of a second important group of cross-reactive allergenic pollen and are an important source of allergens in the Mediterranean region [18]. The olive tree (*Olea europaea*) is the most widely spread species. Other allergenic members of the Oleaceae family are privets (*Ligustrum vul-*

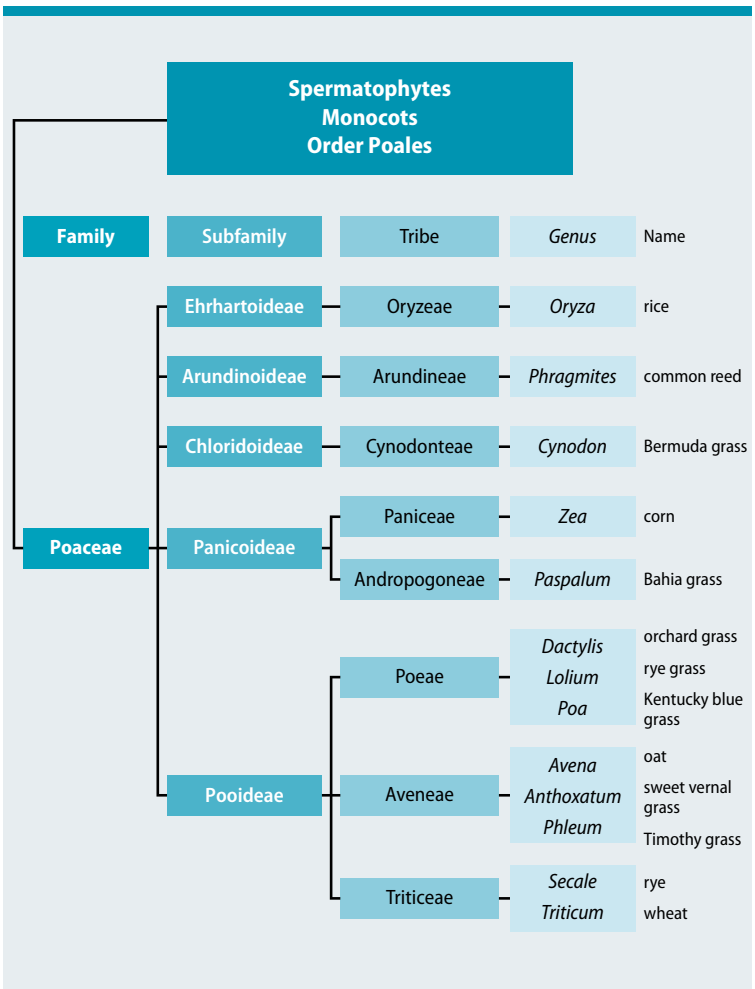

Fig. 1: Phylogenetic botanical relationship between important allergenic grasses. (Adapted from [12])

Abbreviations

CCD	Cross-reactive carbohydrate determinants
cDNA	Complementary deoxyribonucleic acid
CRD	Component resolved diagnostic
Fab	Fragment antigen binding
IgE	Immunoglobulin E
LTP	Lipid transfer protein
nsLTP	Non-specific lipid transfer protein
OAS	Oral allergy syndrome
PR-10	Pathogenesis related proteins

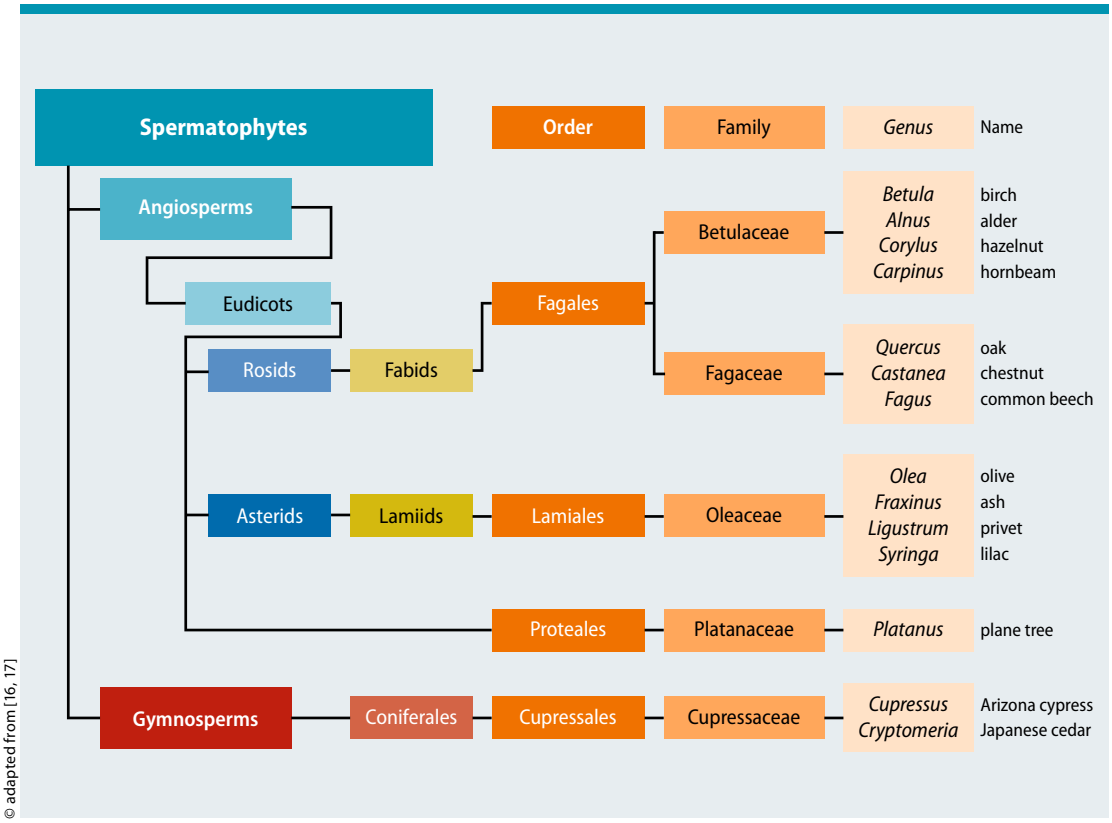

Fig. 2: Phylogenetic botanical relationship between important allergenic trees. (Adapted from [16, 17])

gare), 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.

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*) and the Japanese cedar (*Cryptomeria japonica*) [13, 15, 19].

Important grass pollen allergens

Tab. 1 gives an overview of the most important grass pollen allergens.

Allergens found in all Poaceae grasses

Marker allergen for all sweet grasses – Group 1 (Phl p 1): Group-1 allergens have been isolated and/or cloned from more than twenty Poaceae species [9, 20, 21, 22, 23]. 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 [9, 11, 22, 24]. Most IgE-epitopes of Phl p 1 cluster at the c-terminus [25]. Up to 90 % of all grass pollen allergic patients show IgE-reactivity to group-1 allergens of other grass species [9, 11, 22, 26, 27]. Phl p 1 is the most important group-1 allergen and represents an important cross-reactive major allergen. Cross-reactivity of group-1 allergens has been demonstrated in many studies with natural extracts of different Pooideae species and other Poaceae subfamilies [11, 24, 27]. Purified recombinant Phl p 1 inhibited binding of patient sera to natural extracts of eight different grasses (Timothy grass, *Phleum pratense*; sweet vernal grass, *Anthoxatum odoratum*; oat, *Avena sativa*; Bermuda grass, *Cynodon dactylon*; rye grass, *Lolium perenne*; common reed, *Phragmites communis*; Kentucky blue grass, *Poa pratensis*; rye, *Secale cereale*) inducing an average inhibition of 76 % [26]. Monoclonal antibodies raised against Phl p 1 and defining four distinct epitopes as well as recombinant human Phl p 1-specific IgE-Fabs (fragment antigen binding) recognize and bind to a panel of natural group-1 allergens of different Pooideae grasses [25, 28].

Sequence homologies and cross-reactivity between Phl p 1 and group-1 allergens of tropical and subtropical grasses such as Bermuda grass (*Cynodon dactylon*; 67–70 % sequence identity) or Bahia grass (*Paspalum notatum*) are less pronounced [9, 11, 29]. 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 tropical climate zones, especially with patient sera from tropical climate zones (overview presented in [28]). However, there are indications that these species-specific Ig-E epitopes are not protein epitopes, but carbohydrate epitopes without clinical relevance [30].

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 wide-spread cross-reactivity between group-1 allergens from different grass species.

Group-13: The group-13 grass pollen allergen, a 55 kDa-protein, has also been described in all grasses examined to date [31]. 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 [32].

Allergens found only in Pooideae grasses

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 blue gras (*Poa pratense*) and rye grass (*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. Group-5 allergens are not found in corn (*Zea mays*), Bermuda grass (*Cynodon dactylon*) or rice (*Oryza sativa*), for example [33].

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. 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 [9, 32, 34, 35].

Tab. 1: Important grass pollen allergens

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, sub-familiy 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 foods and latex	Phl p 12	~14
Berberin bridge enzyme glycosylated	clinically reduced relevance	Phl p 4	50–67

Tab. 2: Important grass 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 syndrom	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	Non-specific 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

Most patients display extensive IgE cross-reactivity to the Phl-p-5 isoallergens as well as to different group-5 allergens from Pooideae grasses. [9, 33, 36].

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

Other Pooideae-specific allergens: Group-2/3 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 [9, 37]). Athough patient IgE titers against group-2/3 allergens are often rather low, Phl p 2 shows high allergenic activity in skin tests [32]. 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* [38] and homologues from other plants e. g. olive (Ole e 1), corn (Zea m 13) and tomato, have been identified. Cross-reactivity between homologues from taxonomically unrelated allergen sources is very limited.

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 [26]. Extensive cross-inhibition of patient IgE binding to nine different grass pollen extracts (sweet vernal grass, *Anthoxanthum odoratum*; oat, barley, *Avena sativa*; Bermuda grass, *Cynodon dactylon*; rye grass, *Lolium perenne*; common reed, *Phragmites australis*; Kentucky blue grass, *Poa pratensis*; rye, *Secale cereale*; wheat, *Triticum sativum*; 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) [33]. In a clinical vaccination study involving 64 subjects, patients were sucessfully treated with a mixture of recombinant Phl p1, Phl p2, Phl p 5a+b and Phl p 6 [39]. A proof of principle has thus been established that successful therapy of grass pollen allergy is possible using a combination of distinct grass pollen specific and clinically important allergens.

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

Carbohydrate sensitivity in grass pollen allergic patients

Phl p 1, Phl p 4, Phl p 11 and Phl p 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 [9, 32, 40, 41]. In tropical regions, IgE cross-reactivity is based nearly exclusively on CCD of these glycoproteins [30] 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 [42].

Phl p 4 homologous proteins are found in ambrosia and birch pollen, as well as in peanut, apple, celery and carrot, but clinical significance remains unclear [43].

Important tree pollen allergens

Tab. 2 gives an overview of the most important tree pollen allergens.

Allergens of trees of the order Fagales

Marker allergen for Fagales – Bet v 1: The cDNA (complementary deoxyribonucleic acid) of Bet v 1, the major allergen of birch, was isolated in 1989 [44], 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 [45, 46].

The major allergens of other tree pollen in the order of Fagales from the families of

- Betulaceae (ash, *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 [13, 14, 45, 47]. 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 [48]. 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 soy bean (*Glycine max*, Gly m 4) [14, 15, 49, 50] and are responsible for birch-pollen related oral allergy syndrome [51].

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 [1, 15, 52]. In birch pollen allergic patients, exposure to birch pollen primarily increases Bet v 1-specific IgE without increasing IgE to other birch pollen allergens such as Bet v 2 [53]. Allergy patients in central Africa reacting with natural birch pollen extracts, do not display IgE-antibodies against Bet v 1 but against other birch pollen allergens [54].

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 [55, 56]. 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 [57].

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 the associated oral allergy syndrome.

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 pectin esterase with a clinical significance that has yet to be determined. (For an overview see [13, 14] and **Tab. 2**).

Allergens of trees of the order Lamiales

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 [58]. 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 [59]) such as

- ash tree(*Fraxinus excelsior*, Fra e 1),
- privet (*Ligustrum vulgare*, Lig v 1) and
- 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* (rye grass),
- Phl p 11 from *Phleum pratense*, (timothy grass) and
- Che a 1 from *Chenopodium album* (mercury goosefoot).

There is extensive cross-reactivity between Ole-e-1 homologous allergens of the Oleaceae (Overview in [3]). IgE from sera of two different groups of European patients, one group of which was sensitized to olive pollen the other to 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 [60] showing the existence of specific epitopes for Oleaceae pollen in Ole e 1.

In patients from regions without distribution 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) [61, 62]. 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 [60].

Ole e 1 is the marker allergen for sensitization to olive pollen and is important in this respect in the Mediterranean region. In regions whithout 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 [60].

Other Lamiales-specific allergens: Other specific minor allergens of olive pollen have been described (for an overview see [59] and **Tab. 2**). Ole e 7 is a member of the non-specific lipid-transfer-protein (LTP) family. Sensitization to Ole e 7 is associated with a tendency for severe allergic reactions, however, cross-reactivity with other unspecific LTP-pro-

teins seems to be limited [63]. 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 [64]. Ole e 9 und Ole e 10 are also possibly associated with cross-reactivity to birch, tomatoe, potatoe, bell pepper, banana and latex [65, 66].

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 [67]. 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 [68]. Pla a 1 is used as a marker allergen for plane tree allergy (**Tab. 2**), however, the allergen Pla a 2, a polygalacturonase, may also be important in this respect [68, 69].

Allerges of trees of the order Cupressales

Pollen from trees of the Cupressaceae family (e. g. Arizona cypress, *Cupressus arizonica*; Japanese cedar, *Cryptomeria japonica*) are highly cross-reactive (for an overview see [13, 19]). The prevalence of allergy to different Cupressaceae pollen has increased in central Europe, even though Cupressaceae trees are distributed mainly in the Mediterranean region [70]. 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 be caused by perennial allergens such as from house dust mite [71].

Cry j 1 (from Japanese cedar) is a 40-kDa-protein and was the first Cupressaceae allergen to be described [72]. Together with Cup a 1 from the Arizona cypress [73], it is considered the marker allergen of Cupressales pollen allergy. Both 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 Cry j 1 and Cup a 1.

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 [74].

Polcalcins

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

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

Polcalcins have only been found in the pollen of trees, grasses and weeds. For instance, approximately 10 % of grass pollen allergic patients have specific IgE to Phl p 7, but in sensitized patients, Phl p 7 displays a high allergenic activity [1, 74, 75].

Profilins

Profilin (Bet v 2, 15 kDa) was first identified in birch pollen [77] 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 [1, 78]). 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.

Panallergens: Summary

Cross-inhibition experiments with polcalcins and profilins from different sources have confirmed extensive cross-reactivity of these allergens; the highest IgE-reactivity is observed with the grass pollen allergens Phl p 7 and Phl p 12 [76, 78].

Phl p 7 and Phl p 12 are therefore considered marker allergens for cross-reactivity and the presence of specific IgE to either in patient sera may explain clinical symptoms upon exposure to a range of different allergen sources.

In grass pollen allergy, sensitization to Phl p 7 and 12 is often seen in a late post-clinical phase after sensitization to Phl p 1 and Phl p 5 [79] and may be considered as marker allergens for clinically manifest grass pollen allergy.

Structured approach to clinical routine work (Fig. 3)

Diagnostic tests with marker allergens
- Phl p 1/Phl p 5 (marker for grass pollen),
- Bet v 1 (marker for beech and birch trees, other Fagales trees and the related oral allergy syndrome),
- Ole e 1 (marker for olive trees and other Oleaceae trees including ash),
- Pla a 1 (marker for plane trees),
- 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.

Conclusions for clinical routine work

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

Specific IgE to Bet v 1 characterizes sensitization to Fagales trees (birch, alder, hornbeam, hazelnut, common beech, oak, chestnut) and 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, soy bean) [51].

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 above mentioned marker allergens with specific clinically relevant sensitization profiles was confirmed in clinical studies [80, 81, 82].

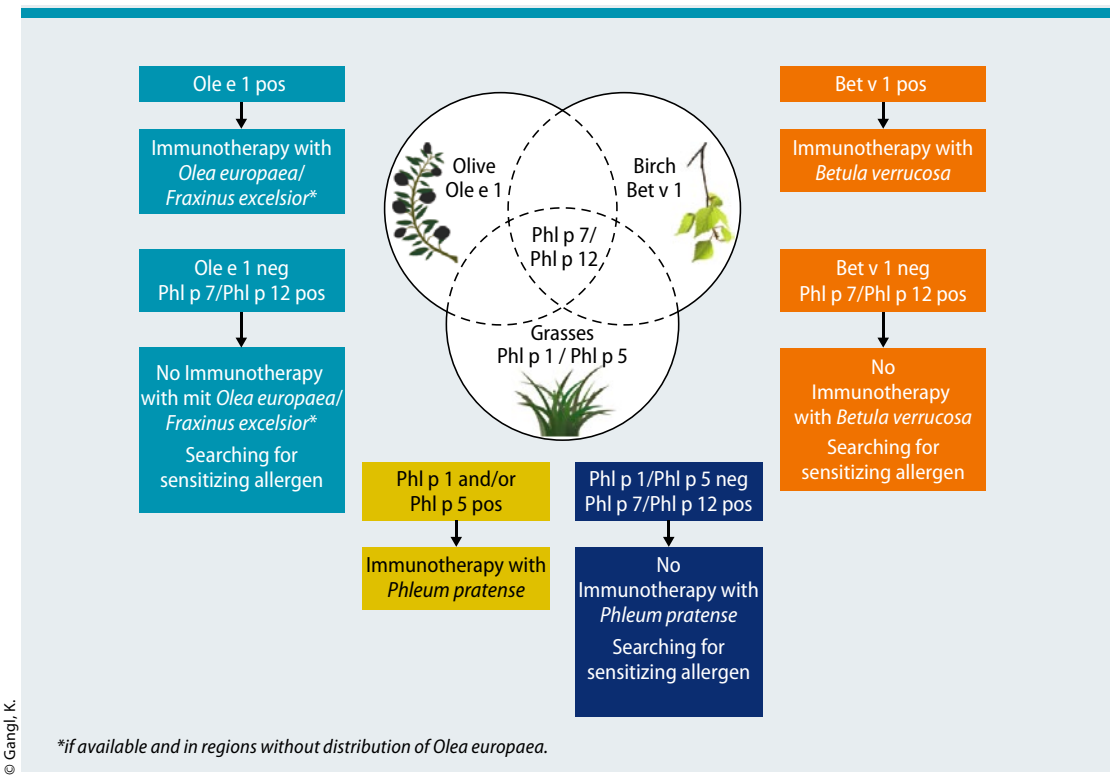

Fig. 3: Proposal for a structured diagnostic procedure in clinical routine work using important marker allergens: Phl p 1, Phl p 5, Phl p 7, Phl p 12, Ole e 1, Fra e 1 and Bet v 1

If no clear-cut sensitization to one of the above-mentioned 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 [83, 84].

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Conflict of interest
The authors declare that there are no conflicts of interest.

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