

Allergo J Int (2017) 26:301–310
DOI 10.1007/s40629-017-0033-z



The benefit of molecular diagnostics in allergic rhinitis

Sven Becker · Moritz Gröger · Thilo Jakob · Ludger Klimek

Received: 21 February 2017 / Accepted: 24 April 2017 / Published online: 18 September 2017
© Springer Medizin Verlag GmbH 2017

Abstract

Background Molecular component-resolved diagnostics has established itself in recent years as an integral part of the routine diagnosis of allergic rhinitis.

Methods A systematic literature review was performed.

Results By using allergen components appropriately, it is possible to identify IgE sensitizations to specific marker allergens and to crossreactive allergens and this way explain polysensitizations obtained in extract-based diagnostics. The detection of individual molecular sensitization patterns enables better classification of the clinical symptoms, thus enhancing the possibility of individual, personalized treatment planning. This implies greater diagnostic reliability for specific immunotherapy, which should in turn result in a better treatment response—despite there being no scientific evidence of this as yet.

Discussion In spite of the immense expansion of our knowledge in the field of component-resolved diagnostics, the clinical relevance of molecular findings still needs to be consistently assessed on the basis

of the individual patient's symptoms. This paper is intended to provide a guide to—and highlight possible courses of action when—making appropriate use of individual components in the diagnosis of allergic rhinitis.

Keywords Molecular allergology · Component-resolved diagnostics · Allergic rhinoconjunctivitis · Bet v 1 · Phl p 1 · Der p 1

Abbreviations

CCD	Cross-reactive carbohydrate determinants
EIA	Enzyme immunoassay
FABER	Patient-friendly allergen nano-bead array
FEIA	Fluorescence enzyme immunoassay
IgE	Immunoglobulin E
ISAC	Immuno solid-phase allergen chip
IUIS	International Union of Immunological Societies
MeDALL	Mechanisms of the development of allergy
nsLTP	Non-specific lipid transfer protein
sIgG	Specific immunoglobulin G
sIgE	Specific immunoglobulin E
SIT	Specific immunotherapy
WHO	World Health Organization

Introduction

Since the major birch allergen Bet v 1 was first cloned in 1988, the molecular diagnosis of allergies has established itself as an integral part of the routine diagnostic work-up for allergic rhinitis, food allergies, and allergic asthma [1]. Furthermore, it also plays an important role in Hymenoptera venom allergy [2]. Whereas in the past only allergen extracts were used for diagnostic purposes (prick tests, intracutaneous tests, determination of specific immunoglobulin E [IgE], and provocation testing with extracts), specific

S. Becker (✉)
Department of Otorhinolaryngology, Head and Neck Surgery, University Medical Center of Johannes Gutenberg University, Langenbeckstr. 1, 55131 Mainz, Germany
sven.becker@unimedizin-mainz.de

M. Gröger
Department of Otorhinolaryngology, Head and Neck Surgery, University of Munich, Munich, Germany

T. Jakob
Department of Dermatology and Allergology, University Medical Center Gießen (UKGM), Justus-Liebig-University, Gießen, Germany

L. Klimek
Center for Rhinology and Allergology, Wiesbaden, Germany

allergen components for the relevant allergen sources are available today for serological testing [3, 4]. Proteins or glycoproteins that have been recombinantly produced or purified from the allergen source represent the actual allergy-eliciting substances. Allergen components to which more than 50% of patients are sensitized are defined as major allergens, while minor allergens have a sensitization rate of <10%. Allergen components that fall into the 10%–50% range are referred to as “intermediary allergens” [5]. In addition to the allergen components specific to an allergen source, allergen extracts also contain other allergen components, which, for instance, have remained highly conserved during the course of evolution and can be found in a large number of plants and which are able to mimic polysensitizations (profilins, polcalcins) in extract-based diagnostics. Therefore, by using allergen components appropriately, new aspects of the diagnosis and management of allergic rhinitis emerge in terms of: (1) the identification of the eliciting allergen source, (2) the understanding of cross-reactivity, (3) the clarification of complex sensitization patterns, (4) greater accuracy when prescribing SIT. Moreover, it is possible to formulate a risk profile for the occurrence of anaphylactic reactions in food allergies.

Not only these aspects, but also the fact that the number of allergen extracts commercially available for skin testing is likely to continue decreasing in the future, highlight the growing importance of laboratory diagnostics using allergen components [6].

This paper is intended to provide a guide to—and highlight possible courses of action when—making appropriate use of individual components in the diagnosis of allergic rhinitis.

Allergen nomenclature

The system for naming allergen components was developed by a subcommittee on allergen nomenclature (Allergen Nomenclature Sub-committee, www.allergen.org) under the auspices of the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS) [7–9]. The name of an allergen component consists of the first three letters of the allergen source's Latin name, followed by the first letter of its species name. For example, allergens in the birch pollen (*Betula verrucosa*) extract are indicated by the stem “Bet v”.

The allergen components are then numbered with Arabic numbers in the order of their discovery, i.e., “Bet v 1”. If allergens that are homologous in terms of structure and immunological behavior are found in other species, e.g., in hazelnut pollen or alder pollen, numbering is guided by the Arabic number of the allergen first described, assuming this has not already been assigned, e.g., “Cor a 1” (from *Corylus avellana*) or “Aln g 1” (from *Alnus glutinosa*), but “Ara h 8” (from *Arachis hypogaea*) in peanut. Allergens of this kind

are assigned to groups: one talks about Group I tree pollen allergens [8].

Group I allergens from other allergen sources may have completely different characteristics. For example, in terms of their structure, Group I grass allergens differ entirely from Group I tree pollen allergens.

The nomenclature takes into account isoallergens and allergen variants, allergen-encoding genes, messenger RNA (mRNA), and complementary DNA (cDNA), as well as allergenic peptides of recombinant or synthetic origin, in both original and modified form [10]. Isoallergens and their variants are given a four-digit numerical code after the major allergen number. The first two numbers (01–99) denote isoallergens with >67% sequence identity. The third and fourth numbers (01–99) denote variants of an isoallergen with >90% sequence identity, e.g., Bet v 1.0101 and Bet v 1.0103 [11]. Data on new allergens or related molecules are carefully checked before these are named and included in the official list of allergens (www.allergen.org).

The identification and characterization of allergen components is a dynamic process. The most exhaustive round-up of these components and their characteristics can be found in the Allergome database (www.allergome.org), which is updated weekly.

Reactions to cross-reactive proteins

Profilins

Profilin is present in all eukaryotic cells, where it forms part of the cytoskeleton. It has been present, highly conserved, over the course of evolution in virtually all plants and can also be found with a structure homology of over 75% in evolutionarily widely distinct organisms (see Table 1). It is for this reason that profilin is referred to as a panallergen [12]. Although profilin sensitizations rarely cause symptoms, they present considerable problems in diagnostic testing with allergen extracts, since they may mimic polysensitizations [13]. Furthermore, profilins are also found in plant foods.

Polcalcins

Polcalcins are calcium-binding proteins that control the calcium level in plants. In this way, they influence plant germination and growth. The approximately 40 polcalcins identified to date exhibit high structural homology (77%) and are thus able to mimic polysensitizations when only allergen extracts are used (see Table 2). As with profilins, sensitization to polcalcins rarely causes symptoms [13].

Sensitization to profilin, as well as to polcalcin, generally follows sensitization to a major allergen from the major allergen sources (primarily grasses, birch, mugwort, ragweed) [14]. Thus, it makes sense during the diagnostic work-up to investigate, alongside

Table 1 Profilins from various allergen sources. For diagnostic purposes, one need only test for the profilin—alongside the major allergens—of the leading allergen extract in the skin prick test

Botanical order	Allergen source	Allergen component
Fagales	Birch (<i>Betula verrucosa</i>)	Bet v 2
	Hazel (<i>Corylus avellana</i>)	Cor a 2
	Alder (<i>Alnus glutinosa</i>)	Aln g 2
	Oak (<i>Quercus alba</i>)	Que a 2
	Beech (<i>Fagus sylvatica</i>)	Fag s 2
Gramineae	Timothy grass (<i>Phleum pratense</i>)	Phl p 12
Asteraceae	Mugwort (<i>Artemisia vulgaris</i>)	Art v 4
	Ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 8
Urticaceae	Wall pellitory (<i>Parietaria judaica</i>)	Par j 3
Oleacea	Olive (<i>Olea europaea</i>)	Ole e 2
	Ash (<i>Fraxinus excelsior</i>)	Fra e 2

Table 2 Polcalcins in various allergen sources. As with profilin, one need only test for the polcalcin—alongside the major allergens—of the leading allergen extract in the skin prick test

Botanical order	Allergen source	Allergen component
Fagales	Birch (<i>Betula verrucosa</i>)	Bet v 4
	Alder (<i>Alnus glutinosa</i>)	Aln g 4
	Oak (<i>Quercus alba</i>)	Que a 4
	Beech (<i>Fagus sylvatica</i>)	Fag s 4
Gramineae	Timothy grass (<i>Phleum pratense</i>)	Phl p 7
Asteraceae	Mugwort (<i>Artemisia vulgaris</i>)	Art v 5
	Ragweed (<i>Ambrosia artemisiifolia</i>)	Amb a 9/Amb a 10
Urticaceae	Wall pellitory (<i>Parietaria judaica</i>)	Par j 4
Oleacea	Olive (<i>Olea europaea</i>)	Ole e 3/Ole e 8
	Ash (<i>Fraxinus excelsior</i>)	Fra e 3

the major allergen, the profilin and/or polcalcin in the main allergen extract in the skin prick test. It is not necessary to determine profilin and polcalcin from several allergen sources.

Cross-reactive carbohydrate determinants

In addition to the above-mentioned cross-reactivity due to the structure homology of profilins and polcalcins, one also encounters other cross-reactive confounders in in vitro and in vivo diagnostics. Numerous allergen extracts contain N-glycans with α 1,3-linked fucose. These carbohydrate structures not found in humans can induce IgE and may be the cause of strong cross-reactivity between proteins that are structurally wholly dissimilar. These are referred to as cross-reactive carbohydrate determinants, or CCDs. Approximately 10%–20% of all atopic individuals exhibit CCD-specific IgE antibodies. Although sensitizations have no clinical relevance, they are regularly responsible for false positive findings.

This has a particularly hampering effect in the investigation of potentially life-threatening forms of allergy, such as peanut allergy or latex allergy. Likewise in insect venom allergies, it is estimated that CCDs mimic double sensitization to bee venom and wasp venom in 25% of all patients. The use of CCD-free allergen components offers an inestimable advantage over tests with allergen extracts. If suitable single molecules are not available for tests, simple commer-

cial screening tests for suitable glycan sources (e.g., bromelain, CCD-MUXF3) can at least provide valuable information on whether anti-CCD IgE antibodies of this kind are present in serum.

Investigations for CCD antibodies in the case of allergic rhinitis are beneficial if polysensitization that cannot be explained either by the relevant major allergens or by profilin and polcalcin is present.

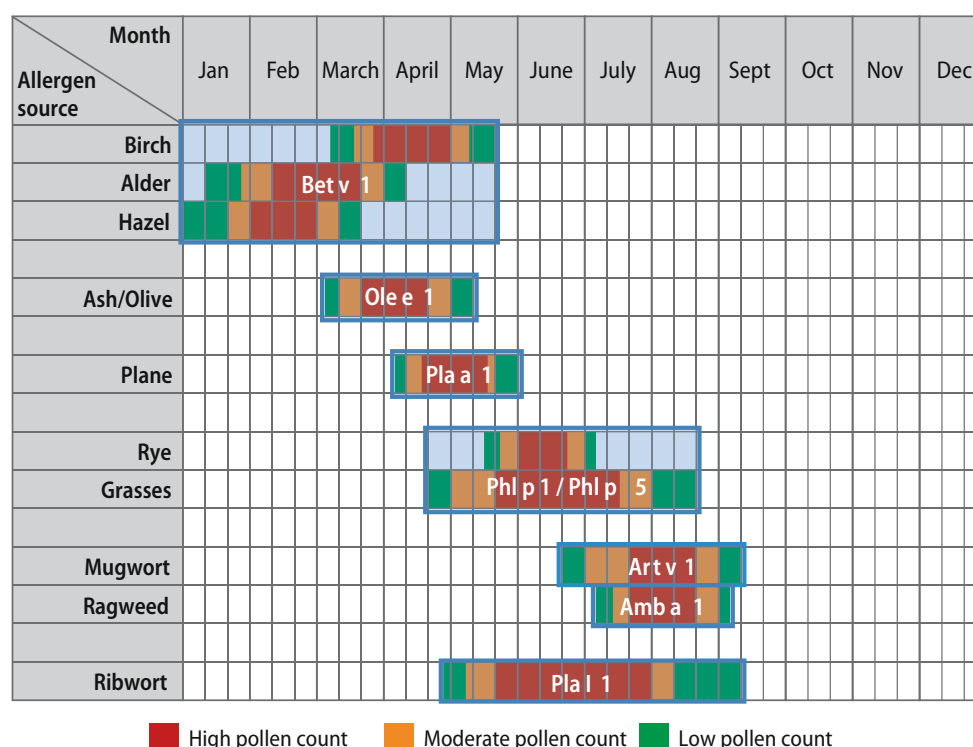
Reactions to grasses

Grasses are found all over the world. In temperate climates such as Europe, one finds almost exclusively grasses from the family Poaceae (grasses), which exhibit strong cross-reactivity. In this context, timothy grass (*Phleum pratense*) represents the most important grass species from an allergological perspective. With a sensitization prevalence of around 20% in children and adults in Germany, timothy grass is the allergen source with the highest sensitization rate [15, 16].

To date, 13 allergen components from *Phleum pratense* have been described. The detection of specific immunoglobulin E (sIgE) to Phl p 1 and/or Phl p 5 demonstrates true sensitization to grasses with a high degree of certainty [17, 18]. Both allergen components have sensitization rates of up to 95% in the grass pollen-sensitized group and are therefore referred to as marker allergens for grass pollen sensitization (see Table 3 and Fig. 1).

Table 3 Marker allergens and cross reactive allergens in important pollen allergen sources

Allergen source	Marker allergen	Cross-reactive allergens	
		Profilin	Polcalcin
Birch pollen Hazel pollen Alder pollen	Bet v 1	Bet v 2	Bet v 4
Grass pollen	Phl p 1 Phl p 5	Phl p 12	Phl p 7
Ash pollen Olive pollen	Ole e 1	Ole e 2	Ole e 3
Mugwort pollen	Art v 1	Art v 4	Art v 5
Ragweed pollen	Amb a 1	Amb a 8	Amb a 9/Amb a 10
Wall pellitory pollen	Par j 2	Par j 3	Par j 4

Fig. 1 Molecular pollen allergen calendar for important allergen sources and their respective marker allergens

Rye also belongs to the grasses. Therefore, sensitization to rye can also be sufficiently well detected by means of marker allergen determination [18].

In the case of suspected grass allergy, molecular diagnostics needs to answer the following questions:

1. Is true sensitization to the marker allergens Phl p 1 and/or Phl p 5 present?
2. Is additional sensitization to profilin/polcalcin (Phl p 12/Phl p 7) present?
3. In the case of testing negative to the marker allergens and testing positive for profilin and/or polcalcin: What is the underlying allergen source responsible for “false” sensitization to the grass extract?

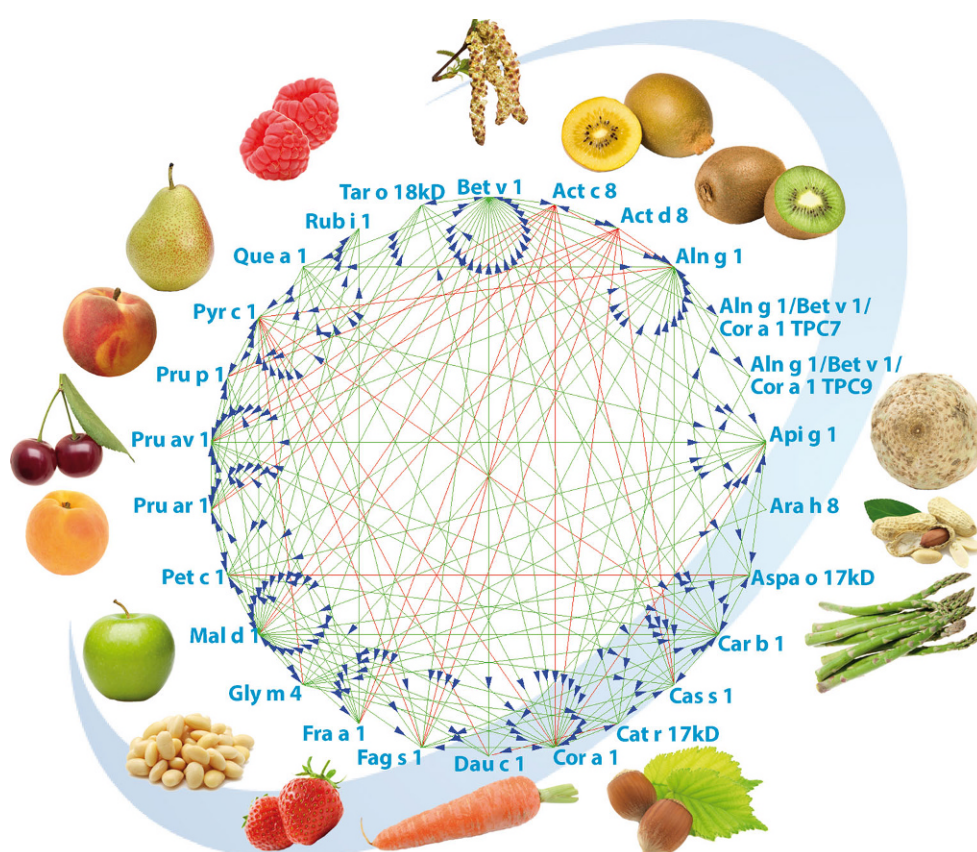
SIT is beneficial in those patients that test positive to the marker allergens and exhibit corresponding symptoms. In the case of positive detection of sIgE to profilin and/or polcalcin and negative testing for marker allergens, SIT should not be performed. In such cases,

the search for the sensitizing major allergen takes priority.

Reactions to tree pollen

In addition to grasses, tree pollens are the most important allergen sources in the European region. With a prevalence of around 19%, birch is the second most frequent cause of sensitizations to aeroallergens in the German population [15]. Other relevant early-blooming trees include hazel and alder, with a sensitization prevalence of around 16% in Germany. Birch, hazel, and alder belong to the botanical order Fagales and, within this, to the birch family (Betulaceae). The chestnut (*Castanea sativa*), common beech (*Fagus sylvatica*), and white oak (*Quercus alba*) also belong to the order Fagales, but to the beech family (Fagaceae).

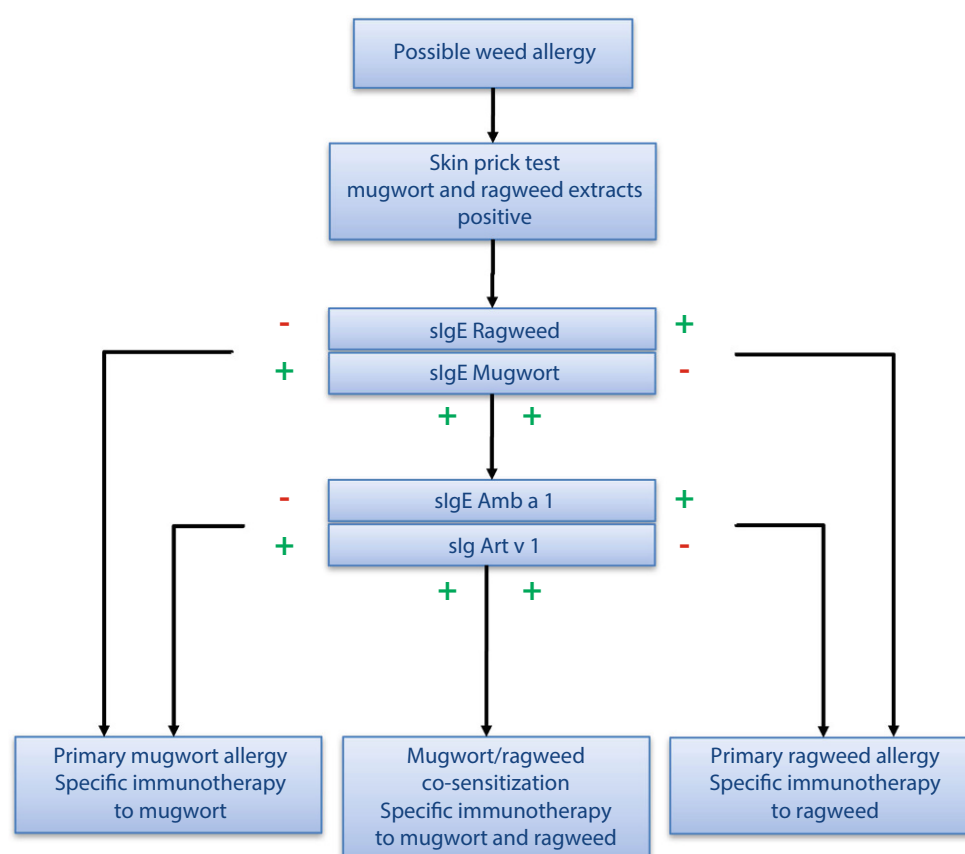
All trees in the order Fagales have a major allergen protein from the group of PR-10 proteins, of which



With almost the same flowering period, and in contrast to birch, the ash tree (*Fraxinus excelsior*) from the family of olive trees is primarily of relevance in Central Europe. In southern Europe, on the other hand, the olive (*Olea europaea*) is of clinical relevance. The marker allergen for the olive family is Ole 1, which has high structural homology with the major allergens of other trees in the family, such as ash (Fra e 1) (see Table 3). Testing for Ole e 1 is sufficient to prove sensitization to ash or other trees in the family [21].

SIT in tree pollen-related rhinoconjunctivitis is beneficial if sIgE to the marker allergen Bet v 1 and/or Ole e 1 is found, not, however, in the case of sole sensitization to profilin and/or polcalcin [18, 22]. As in grass allergies, a search for the sensitizing major allergen is recommended in the case of the latter. If tests for Bet v 1 and Ole e 1 are positive, nasal provocation testing with ash should be performed. Where this is negative, and in the case of relevant symptoms, SIT to birch is indicated; if provocation testing is positive, further nasal provocation testing with birch should be carried out in order to exclude an allergy to both allergen sources. If provocation testing is once again positive, SIT to birch and ash is indicated.

Fig. 3 A possible decision-making algorithm for suspected weed allergy to mugwort and/or ragweed. Modified according to [24]



Reactions to weeds

With a sensitization prevalence of around 9% in the German population, mugwort (*Artemisia vulgaris*) is the third most common cause of pollen-mediated type I allergy in Germany [15]. In addition to the major allergen Art v 1, the non-specific lipid transfer protein (nsLTP) Art v 3 is commercially available for allergy diagnostics. The latter allergen component is able to account for cross-reactions to nsLTPs in foods. Although profilin (Art v 4) and polcalcins (Art v 5) are also found in mugwort pollen, they are not commercially available specifically for mugwort.

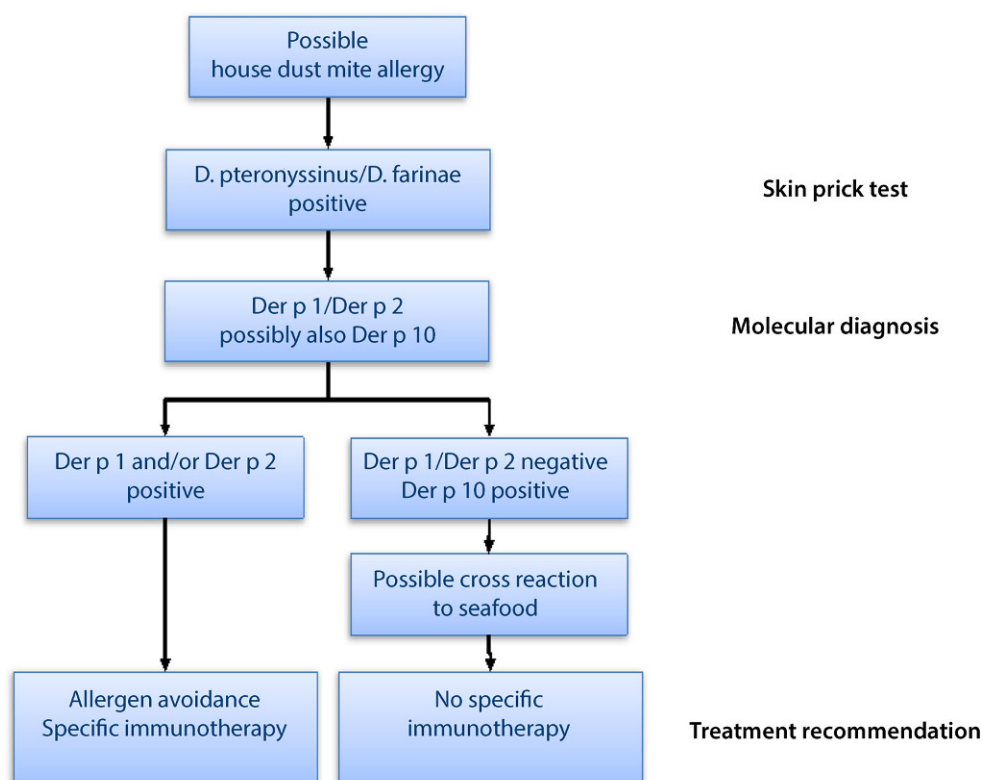
Differentiating the underlying genuine sensitization can be challenging when using allergen extracts in regions where ragweed (*Ambrosia artemisiifolia*) grows [23]. Both plants belong to the aster family (Asteraceae). Whereas ragweed with its major allergen Amb a 1 (profilin Amb a 8, polcalcins Amb a 9 and Amb a 10) represents a highly relevant allergen source in large swaths of North America, it is less common in Central Europe. Nevertheless, high sensitization rates are seen particularly in patients with co-sensitization to mugwort, caused in part by cross reactions between Amb a 1 and Art v 6 (not commercially available as yet). Using the relevant major allergens in diagnostics, however, can be beneficial in many cases to identify the true sensitization (see Fig. 3).

Sensitizations to other herbs, such as ribwort (*Plantago lanceolata*) from the family of Plantaginaceae, or wall pellitory (*Parietalis officinalis*) from the family of Urticaceae, are rare and exhibit few cross-reactions with one another. In terms of marker allergens, Pla l 1, a protein similar to Ole e 1, is available for ribwort, while Par j 2, a nsLTP, is available for wall pellitory.

Reactions to house dust mites

Of all the perennial allergen sources, house dust mites are the most important [25]. Whilst *Dermatophagoides pteronyssinus* is widespread in Europe, *Dermatophagoides farinae* predominate in the US [26]. If present in large numbers, they cause rhinoconjunctivitis and are frequently responsible for the onset of allergic bronchial asthma [27]. More than 20 different *Dermatophagoides pteronyssinus* allergen components, with varying prevalences in terms of sensitization in Europe, have been described to date [28, 29]. Der p 1, Der p 2, Der p 10 (tropomyosin) and, since May 2017, Der p 23 are commercially available, the first two and Der p 23 being classified as major allergens. The majority of treatment solutions (allergen extracts) available for SIT are standardized on Der p 1 and Der p 2 [30]. As mentioned above, sensitizations to major allergens are subject to considerable regional variation and may be absent in a significant number

Fig. 4 A possible decision-making algorithm for suspected house dust mite allergy. Modified according to [24]. In the case of negative testing for the major allergens Der p 1 and Der p 2, SIT should be discussed critically with the patient



of cases, despite positive reactions to mite extracts being detected [28, 29].

Der p 23 represents an important new major allergen [31]. In addition to having prevalence rates comparable to the major allergens described above, Der p 23 appears to also have a predictive value for the onset of allergic bronchial asthma in childhood [32].

In contrast to the extract-based diagnostics used to date, allergen component-resolved testing (Der p 1 and Der p 2) permits a better estimation of whether or not SIT will be beneficial in an individual patient (see Fig. 4). Since not all patients exhibit sensitization to at least one of the two major allergens contained in the treatment solutions, SIT should be discussed critically and possibly even discarded if sensitization to Der p 1 and Der p 2 is absent [33].

In the case of intolerance reactions to seafood (e. g., shrimps, octopus, mussels), determining Der p 10 may reveal possible cross reactions between tropomyosin from house dust mites and seafood [34, 35].

Reactions to fur-bearing animals (dog, cat, and horse)

In addition to the house dust mite, fur-bearing animals (primarily the cat) represent yet another important source of indoor allergens [36]. Due to their small size, cat allergens reach the deep airways more frequently, where they can elicit not only bronchial hyperresponsiveness but also bronchial asthma.

Allergen components are found in saliva, urine, and animal dander and are easily transported on clothing to other areas of daily life (school, workplace, public buildings). At present, Fel d 2 and Fel d 4, in addition to Fel d 1, are available for the molecular diagnosis of cat allergy. The allergen component Fel d 1 is the major cat allergen and is found in the animal's saliva as well as on its skin. From a biochemical perspective, the protein belongs to the uteroglobins. The minor allergen Fel d 2 (serum albumin) exhibits strong cross-reactions to canine (Can f 3) and equine (Equ c 3) serum albumin. Lipocalins are also cross-reactive with one another (Fel d 4, Equ c 1, Can f 6) [37]. Sensitizations to major allergens from the respective animals (Fel d 1, Can f 1, Can f 2, Can f 5, Equ c 1) indicate specific sensitization. In the case of negative testing to the respective major allergens and positive detection of antibodies to lipocalin or serum albumin, one should consider cross-reactivity or co-sensitization to other animal species and continue to seek for the genuine sensitization [38].

Established test methods and new trends in molecular diagnostics

Fluorescence enzyme immunoassay (FEIA)—more rarely enzyme immunoassay (EIA)—determinations are common today. Test methods to detect specific IgE to particular allergen components do not differ in this context from extract-based methods. Allergen components are added (so-called spiking) to some

allergen extracts, e.g., latex extracts, to improve test sensitivity [39]. As of 2017, more than 50 allergen components are available for the molecular diagnosis of allergic rhinitis. Other components will be added to elucidate the patient's sensitization profile. This requires a sound knowledge of local prevalences of various sensitizations, as well as relevant marker allergens and cross reactions, and can prove time-consuming in the first instance.

Singleplex assays are available for the detection of specific antibodies to a particular allergen component, as well as allergen microarrays for simultaneous testing with multiple allergen components [40, 41]. The latter test method is particularly helpful if patients are polysensitized and exhibit other allergies, e.g., to food, in addition to allergic rhinitis.

The first standardized allergen microarray has been available for several years and makes it possible to measure specific IgE antibodies to, at present, 112 different allergen components from 51 allergen sources (ImmunoCAP ISAC-Chip, Immuno Solid-phase Allergen Chip). A mere 20 µl serum or plasma is sufficient to this end, thereby making for a gentler procedure, e.g., when testing young children [42].

Further developments to the ISAC chip, such as the MeDALL-chip (mechanisms of the development of allergy), enable the detection of sIgE and sIgG to more than 170 allergen components and are already being used for scientific purposes [32, 43].

The most recent developments, such as the FABER chip (patient friendly allergen nano-bead array), make it possible to detect sIgE to 122 allergen components and a further 122 allergen extracts in 100 µl patient serum (www.caam-allergy.com/en/faber). However, larger studies on this chip have not been published as yet.

Allergen components in therapeutic solutions for SIT

Specific allergen components not only play a role in the diagnostic context, but also in the production of therapeutic solutions for SIT. Immunotherapy is primarily beneficial in the case of sensitization to major allergens [22, 44]. The extracts used for SIT are standardized first and foremost on the relevant major allergens. This is intended to guarantee that consistent quantities of the major components are present in the therapeutic solution, thereby ensuring particularly good immunomodulation in terms of tolerance induction [45]. This is all the more important when one considers that the content of intermediate and minor allergens differs greatly not only between batches of a product, but also between different products from the various manufacturers [46, 47].

The advantages of molecular component-resolved diagnostics vs. testing with whole extracts

Molecular diagnostics using allergen components enables targeted testing for relevant sensitizations to allergy-eliciting proteins [48]. Measuring sIgE to defined, relevant single molecules makes it possible to eliminate diagnostic confounders (e.g., IgE reactivity to panallergens or CCDs), thereby enabling greater analytical and diagnostic sensitivity and precision [49].

By revealing more complex individual sensitization patterns, it becomes easier to classify clinical symptoms and enables one to differentiate primary (genuine) sensitizations and cross reactions. Assuming all relevant technical requirements are fulfilled, it is possible to achieve greater sensitivity and specificity. This paves the way to a personalized and stratified diagnosis of allergic rhinitis [50].

The greater the diagnostic precision, the more SIT can be tailored individually and precisely to the patient. Initial studies show that the use of molecular diagnostics modifies prescribing behavior in relation to SIT in children with allergic rhinitis [51]. The extensive diagnostic procedure involved here will, in some cases, generate higher costs that will need to be taken into account from a regulatory perspective. On the other hand, one can expect improved diagnostics in the medium to long term to lead to improved treatment response and thus also to a better cost-benefit ratio. Studies on this effect are not available as yet.

What does remain problematic is the fact that, to date, not all relevant allergens are available for molecular diagnosis, the production of correctly folded recombinant molecules is at times challenging, and polymorphisms are sometimes difficult to reproduce [52]. In addition, molecular diagnostics remains merely a method to detect sensitization. Whether this sensitization is relevant in terms of an allergy, not even allergen component-resolved diagnostics is able to prove—to do this, further evaluation on the basis of patient history or provocation testing is required.

Conflict of interest M. Gröger has received speakers honoraria and sponsorship for further education from Thermo Fisher Scientific/Phadia. T. Jakob has received research support by ALK-Abelló, Allergy Therapeutics/Bencard, Allergopharma, Cosmetics Europe, Novartis and Thermo Fisher Scientific; speakers honoraria from ALK-Abelló, Allergy Therapeutics/Bencard, Allergopharma, Leti, Novartis and consulting fees from ALK Abelló, Allergy Therapeutics/Bencard, Allergopharma, Leti, and Novartis. L. Klimek has received research support, fees for consulting and/or expert testimony, fees for lectures and/or educational presentations and/or fees for board membership from ALK-Abelló, Allergopharma, Bencard, Bionorica, Biomay, Boehringer Ingelheim, Cytos, Circassia, Euroimmun, Dr. Focke, HAL, GSK, Leti, Lofarma, MEDA/Mylan, Novartis, Stallergenes, Thermofisher und Roxall. S. Becker declares that he has no competing interests.

References

- Breiteneder H, Hassfeld W, Pettenburger K, Jarolim E, Breitenbach M, Rumpold H, et al. Isolation and characterization of messenger RNA from male inflorescences and pollen of the white birch (*Betula verrucosa*). *Int Arch Allergy Appl Immunol*. 1988;87:19–24.
- Jakob T, Rafei-Shamsabadi D, Spillner E, Müller S. Diagnostics in hymenoptera venomallergy: current concepts and developments with special focus on molecular allergy diagnostics. *Allergo J Int*. 2017;26:93–105.
- Jakob T, Forstenlechner P, Matricardi P, Kleine-Tebbe J. Molecular allergy diagnostics using multiplex assays: methodological and practical considerations for use in research and clinical routine: part 21 of the series molecular allergology. *Allergo J Int*. 2015;24:320–32.
- Kleine-Tebbe J, Jakob T. Molecular allergy diagnostics using IgE singleplex determinations: methodological and practical considerations for use in clinical routine: part 18 of the series molecular allergology. *Allergo J Int*. 2015;24:185–97.
- Klimek L, Pfaar O, Rietschel E. (editors) *Allergien bei Kindern und Jugendlichen. Grundlagen und klinische Praxis*, 1st ed. Stuttgart: Schattauer; 2013.
- Klimek L, Werfel T, Vogelberg C, Jung K. Authorised allergen products for intracutaneous testing may no longer be available in Germany: allergy textbooks have to be rewritten. *Allergo J Int*. 2015;24:84–93.
- Marsh DG, Goodfriend L, King TP, Lowenstein H, Platts-Mills TA. Allergen nomenclature. *Bull World Health Organ*. 1986;64:767–74.
- King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W. Allergen nomenclature. WHO/IUIS allergen nomenclature subcommittee. *Int Arch Allergy Immunol*. 1994;105:224–33.
- King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W. Allergen nomenclature. *Allergy*. 1995;50:765–74.
- Kleine-Tebbe J, Ollert M, Jakob T. Molekulare Allergologie – Teil 1. Nomenklatur, Proteinfamilien, Datenbanken und potenzieller Nutzen. *Allergo J*. 2010;19:390–4.
- Radauer C, Nandy A, Ferreira F, Goodman RE, Larsen JN, Lidholm J, et al. Update of the WHO/IUIS allergen nomenclature database based on analysis of allergen sequences. *Allergy*. 2014;69:413–9.
- Valenta R, Duchene M, Ebner C, Valent P, Sillaber C, Deviller P, et al. Profilins constitute a novel family of functional plant pan-allergens. *J Exp Med*. 1992;175:377–85.
- San Nicolo M, Braun T, Eder K, Berghaus A, Groger M. Clinical relevance of IgE to profilin and/or polcalcin in pollen-sensitized patients. *Int Arch Allergy Immunol*. 2016;169:101–7.
- Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, et al. Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy*. 2008;63:1550–8.
- Haftenberger M, Laußmann D, Ellert U, Kalcklösch M, Langen U, Schlaud M, Schmitz R, Thamm M. Prevalence of sensitisation to aeroallergens and food allergens: results of the German health interview and examination survey for adults (DEGS1). *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 2013;56:687–97.
- Schmitz R, Ellert U, Kalcklösch M, Dahm S, Thamm M. Patterns of sensitization to inhalant and food allergens – findings from the German health interview and examination survey for children and adolescents. *Int Arch Allergy Immunol*. 2013;162:263–70.
- Canis M, Gröger M, Becker S, Klemens C, Kramer MF. Recombinant allergen profiles and health-related quality of life in seasonal allergic rhinitis. *Allergy Asthma Proc*. 2010;31:219–26.
- Canis M, Gröger M, Becker S, Klemens C, Kramer MF. Recombinant marker allergens in diagnosis of patients with allergic rhinoconjunctivitis to tree and grass pollens. *Am J Rhinol Allergy*. 2011;25:36–9.
- <http://www.allergome.org>. Status: 20 January 2010.
- Asam C, Hofer H, Wolf M, Aglas L, Wallner M. Tree pollen allergens – an update from a molecular perspective. *Allergy*. 2015;70:1201–11.
- Imhof K, Probst E, Seifert B, Regenass S, Schmid-Grendelmeier P. Ash pollen allergy: reliable detection of sensitization on the basis of IgE to Ole e 1. *Allergo J Int*. 2014;23:78–83.
- Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy*. 1999;29:896–904.
- Canis M, Becker S, Gröger M, Kramer MF. IgE reactivity patterns in patients with allergic rhinoconjunctivitis to ragweed and mugwort pollens. *Am J Rhinol Allergy*. 2012;26:31–5.
- Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27(Suppl 23):1–250.
- Raulf M, Bergmann KC, Kull S, Sander I, Hilger C, Brüning T, et al. Mites and other indoor allergens – from exposure to sensitization and treatment. *Allergo J Int*. 2015;24:68–80.
- Fernández-Caldas E. Mite species of allergologic importance in Europe. *Allergy*. 1997;52:383–7.
- Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol*. 1998;102:558–62.
- Becker S, Kramer MF, Havel M, Welz C, Markmann S, Gröger M. IgE reactivity profiles among house dust mite allergic patients in Bavaria. *Eur Arch Otorhinolaryngol*. 2013;270:3177–82.
- Weghofer M, Thomas WR, Kronqvist M, Mari A, Purohit A, Pauli G, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest*. 2008;38:959–65.
- Brunetto B, Tinghino R, Braschi MC, Antonicelli L, Pini C, Iacovacci P. Characterization and comparison of commercially available mite extracts for in vivo diagnosis. *Allergy*. 2010;65:184–90.
- Weghofer M, Grote M, Resch Y, Casset A, Kneidinger M, Kopec J, et al. Identification of Der p 23, a peritrophin-like protein, as a new major Dermatophagoides pteronyssinus allergen associated with the peritrophic matrix of mite fecal pellets. *J Immunol*. 2013;190:3059–67.
- Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. *J Allergy Clin Immunol*. 2017;139:541–9.
- Becker S, Schleiderer T, Kramer MF, Haack M, Vrtala S, Resch Y, et al. Real-life study for the diagnosis of house dust mite allergy – the value of recombinant allergen-based IgE serology. *Int Arch Allergy Immunol*. 2016;170:132–7.
- Becker S, Gröger M, Canis M, Pfrogner E, Kramer MF. Tropomyosin sensitization in house dust mite allergic patients. *Eur Arch Otorhinolaryngol*. 2012;269:1291–6.
- Wittman AM, Akkerdaas JH, van Leeuwen J, van der Zee JS, Aalberse RC. Identification of a cross-reactive allergen

- (presumably tropomyosin) in shrimp, mite and insects. *Int Arch Allergy Immunol.* 1994;105:56–61.
36. Konradsen JR, Fujisawa T, van Hage M, Hedlin G, Hilger C, Kleine-Tebbe J, et al. Allergy to furry animals: new insights, diagnostic approaches, and challenges. *J Allergy Clin Immunol.* 2015;135:616–25.
 37. Eder K, Becker S, San Nicoló M, Berghaus A, Gröger M. Usefulness of component resolved analysis of cat allergy in routine clinical practice. *Allergy Asthma Clin Immunol.* 2016;12:58.
 38. Asarnoj A, Hamsten C, Wadén K, Lupinek C, Andersson N, Kull I, et al. Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: a BAMSE/MeDALL study. *J Allergy Clin Immunol.* 2016;137:813–821.e7.
 39. Huss-Marp J, Raulf M, Jakob T. Spiking with recombinant allergens to improve allergen extracts: benefits and limitations for the use in routine diagnostics: part 19 of the series molecular allergology. *Allergo J Int.* 2015;24:236–43.
 40. Klimek L, Vetter D, von Bernus L, Thorn C. Microarray technique for component resolved diagnosis (CRD) in type-I allergies. An innovative technology at the border between research tool and routine diagnostics. *HNO.* 2011;59:988–93.
 41. Huss-Marp J, Gutermuth J, Schäffner I, Darsow U, Pfab F, Brockow K, et al. Comparison of molecular and extract-based allergy diagnostics with multiplex and singleplex analysis. *Allergo J Int.* 2015;24:46–53.
 42. Ott H, Baron JM, Heise R, Ocklenburg C, Stanzel S, Merk HF, Niggemann B, Beyer K. Clinical usefulness of microarray-based IgE detection in children with suspected food allergy. *Allergy.* 2008;63:1521–8.
 43. Skrindo I, Lupinek C, Valenta R, Hovland V, Pahr S, Baar A, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol.* 2015;26:239–46.
 44. Valenta R, Kraft D. From allergen structure to new forms of allergen-specific immunotherapy. *Curr Opin Immunol.* 2002;14:718–27.
 45. Klimek L, Willers J, Schendzielorz P, Kündig TM, Senti G. Immunotherapy of allergic rhinitis without allergens? : new options for immunomodulation by vaccination with virus-like particles and CpG motifs. *HNO.* 2013;61:826–33.
 46. Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy.* 2008;38:1400–8.
 47. Focke M, Marth K, Valenta R. Molecular composition and biological activity of commercial birch pollen allergen extracts. *Eur J Clin Invest.* 2009;39:429–36.
 48. Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA.* 2004;101(Suppl2):14677–82.
 49. Niederberger V, Valenta R. Molecular approaches for new vaccines against allergy. *Expert Rev Vaccines.* 2006;5:103–10.
 50. Chaker AM, Klimek L. Individualized, personalized and stratified medicine: a challenge for allergology in ENT? *HNO.* 2015;63:334–42.
 51. Stringari G, Tripodi S, Caffarelli C, Dondi A, Asero R, Di Rienzo Businco A, et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. *J Allergy Clin Immunol.* 2014;134:75–81.
 52. Ott H, Fölster-Holst R, Merk HF, Baron JM. Allergen microarrays: a novel tool for high-resolution IgE profiling in adults with atopic dermatitis. *Eur J Dermatol.* 2010;20:54–61.