

# Clinical use of recombinant allergens in personalised allergy diagnosis and management

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Molecular diagnosis of allergy and microarray technology have opened a completely new avenue of insight into sensitization profiles from both the clinical and epidemiological point of view. Molecular diagnosis of food allergy brought a completely new approach based on sensitization to different molecules in frame of one food determining the grade of risk of severe anaphylaxis to the respective food. Nonetheless the usefulness of molecular diagnosis of inhalant allergens and respiratory allergy is also very high. Immediate hypersensitivity to one or more aeroallergens is a risk factor for asthma and allergic rhinitis. Sensitization to these allergens may play a role also in atopic dermatitis. The major sources of allergens in the outdoor air include pollens and fungi, in the indoor air house dust mite and fungi, as well as dander derived from domestic animals and rodents. Sensitization to distinct molecules may represent higher risk for asthma or atopic dermatitis (1-5) and several studies suggest that sensitization to multiple molecules ("molecular spreading") may be associated with higher probability of more severe symptoms of allergy (3-10). While the diagnosis of IgE mediated inhalant allergy is primarily based on clinical history and sensitization demonstrated through an allergen extract prick test and/or measurement of serum specific IgE, this methodology has its limitations. This in vitro and in vivo allergy testing is based on sometimes insufficiently standardized allergen extracts that, owing to the natural variability of the allergen source or manufacturing procedure, can differ in terms of their allergenic content. This issue was already confirmed for grass pollen (11-13), mite (14-16), cat (17), dog (18) and mould (19-22) allergens. An even more important disadvantage of allergenic extracts is that they are incapable of differentiating between primary sensitization and immunological cross-reactivity. Multiple positivities are observed in many patients, but determining whether the sensitization is species-specific or a result of cross-reactivity to proteins with similar protein structures (e.g. profilins, polcalcins, tropomyosins, serum albumins and partially lipocalins) was formerly frequently impossible. Identification of sensitizations and co-sensitizations to species-specific and cross-reacting allergen components may be especially important in decisions concerning specific immunotherapy.

## **POLLEN**

Grasses (Poaceae) are the most common cause of hay fever in Europe, and it has been estimated that more than 40% of allergic patients are sensitized to their pollens. In our study the rate was substantially higher – sensitization to at least one grass-pollen-specific component was observed in about three quarters of patients showing any positivity in the ImmunoCAP ISAC microarray (23).

Grass-pollen sensitized patients in our study were usually co-sensitized to several grass-specific com-



ponents; monosensitization was markedly less frequent. Phl p 1 (beta-expansin) and Phl p 5 (ribonuclease) are generally assumed to be the specific components most commonly involved in grass-pollen allergy, Phl p 1 sensitization being present in cca 90% of all grass-pollen allergic patients. We confirmed general findings that the most frequent targets for IgE binding are Phl p 1, Phl p 4, and Phl p 5 molecules; Phl p 5 sensitization without Phl p 1 sensitization was very exceptional.

In this context, it is necessary to stress again the importance of the allergen content in grass-pollen extracts used for diagnostic and therapeutic purposes. Several studies have focused on this issue and analyzed the composition of some commercially available preparations with respect to their qualitative and quantitative allergen composition. The spectrum of analyzed molecules was limited - one study analyzed only Phl p 5 content (12), other studies involved a wider spectrum of molecules including Phl p 1 and Phl p 4 (11, 13) and considerable heterogeneity of the content of these major allergens was found.

The most frequent sensitization to Betulaceae-derived components was, as expected, to Bet v 1. The sensitization rates to Aln g 1 and Cor a 1.0101 were also rather high, but the vast majority of patients with these latter sensitivities cross-reacted to Bet v 1. Cross-reactivity with plant-food-derived PR-10 proteins was also quite high in these same patients, presenting a pattern already generally known and described. Cross-reacting components ("panallergens") generally do not seem to represent very important pollen sensitization components in Middle Europe, somewhat in contrast to southern countries where the observed sensitization frequency is often higher and the clinical relevance of this sensitization may also be more important. High cross-reactivity of these molecules was described in several studies. In contrast to these findings our group of patients showed certain relationship between sensitization to species-specific molecules and the respective panallergen and cross-reactivity in frame of groups of profilins, polcalcins and LTPs was not complete.

Sensitization to other components from the same pollen source usually precede sensitization to profilins and/or polcalcins (10) and it has been proposed that, at least for grass pollen allergy, panallergens are typically being recognized at the late stage of molecular spreading. Such an assumption is in concordance with our observations of very low rates of mono-sensitizations to profilins and polcalcins.

## **HOUSE DUST MITES (HDM)**

According to several studies, HDM sensitization and allergy is considered to be generally the most frequent among inhalant allergies. In our conditions it holds the fourth position behind grass pollen, birch pollen and cat allergens. Sensitization rate to the most frequent perennial inhalant derived component Fel d 1 and the second most frequent Der f 2 was 1.9x and 2.4x resp. less frequent than sensitization rate to the most frequent pollen derived component Phl p 1 (23), differing from data coming from other regions. This discrepancy may be caused by geographical differences or may be due to the population selected (predominant adult patients, predominant respiratory allergy).

It is known that patients sensitized to mites are not always sensitized to the molecules of group 1 and/or 2, but the number of patients sensitized only to other molecules is generally very low. Mite sensitized patients in our group were usually co-sensitized to several mite specific components; monosensitization was markedly less frequent.

As mentioned before, several studies have analyzed the composition of commercially available HDM preparations with respect to their qualitative and quantitative allergen composition and considerable differences in the allergen content were found (14-16). Group 2 allergens are highly cross-reactive, but as group 1 sensitization could be species specific in some patients and its prevalence is higher in children, an adequate balance of major mite species allergens must be considered in the design of mite allergy vaccines. It is necessary to point out the importance of quantifying at least three major mite components Der f 1, Der p 1 and Der f 2 (or Der p 2). Such information is crucial for effective diagnosis and treatment. Besides these molecules, Der p 23, a new, major house dust mite allergen must be considered to be an important component for allergen specific immunotherapy. The importance of eventual further allergens (so called "mid-tier" allergens – e.g. Der p 5) in this context has yet to be elucidated.

## **FURRY ANIMALS**

Although some previous studies showed that the prevalence of cat allergy is not as high as dust mite allergy, we showed approximately the same global sensitization rate for these both allergen sources.



The explanation for this finding may be that the climate for mites is not optimal in Middle Europe and so the sensitization rate is lower than in other regions. More, the accumulation of cat and dog allergens in workplaces, schools and homes (without an animal), which occurs by passive transfer is a reflection of the overall prevalence of pet ownership in the community which is rather high in our conditions.

The major cat-derived allergen is Fel d 1 (secretoglobin). It is produced in the skin and also in the salivary glands of cats and becomes airborne. The role of Fel d 1 in cat allergy is very dominant, as it has been shown that up to 95% of all cat allergic patients react to Fel d 1 what corresponds to our data.

Lipocalin Fel d 4 has received little attention, because of the dominant role of Fel d 1 in cat allergy. Nevertheless, the importance of Fel d 4 has recently been demonstrated by its cross-reactivity and cross-sensitization with dog, horse, mouse and rat lipocalins. Although a relatively high sensitization rate to Fel d 4 in cat allergic patients was described in other studies, we could not confirm this fact in our patients where the sensitization rate to Fel d 4 was substantially lower and co-sensitization with Fel d 1 was present in the majority of cases.

Although Fel d 1 is most prevalent, co-sensitization with Fel d 4 seems to be more associated with asthma (5). This suggests that sensitization to Fel d 1 may represent original sensitization to cat, and sensitization to Fel d 4 a further evolved immune response with a higher probability of allergic disease. This hypothesis is based on the concept of „molecular spreading“ described for grass-pollen components (10).

Dog lipocalin Can f 1 is secreted from canine sebaceous and salivary glands is found in dog hair, dander, and saliva. It is estimated that 50 – 90% dog allergic patients are sensitized to Can f 1. Despite being a major allergen, Can f 1 alone is not sufficient for diagnosis of dog allergy. Sensitization to Can f 1 is more related to dog symptoms than Can f 5 and Can f 1 is the most important prognostic marker of dog allergy and superior to measurement of IgE levels to dog allergen extract (9). 20 - 33% of dog-sensitized subjects have IgE antibodies to another lipocalin Can f 2, but sensitization to Can f 2 without sensitization to Can f 1 was not observed. We showed that such cases exist but they are very rare.

Expression of the dog prostatic kallikrein Can f 5 is mainly restricted to male animals and the protein is secreted in the urine. Can f 5 has been reported as a major allergen, mainly in a Spanish population where 70% of the subjects were sensitized. It was identified as the major component for dog sensitization in Swedish children as well (5), although only about one tenth of children mono-sensitized to Can f 5 reported symptoms to dog and Can f 5 showed generally a weaker association to dog allergy (9). Can f 5 was the most frequently observed dog-derived molecule sensitization also in our patients. It is necessary to remember that cross-reactivity between Can f 5 and human prostate-specific antigen has been described and that allergy to human seminal plasma, although rare, may be linked to dog allergy via Can f 5 sensitization.

Immunotherapy for dog allergy does not appear as efficacious as immunotherapy in cat allergy. The reason might be associated with the fact, that dog sensitization is complex and involves more molecules in comparison to Fel d 1 clearly dominating in cat allergy. The observed variability of currently available commercial dog extracts regarding their allergen contents likely has a negative influence on both diagnosis and therapy of dog allergy (18).

Severe asthma in children is associated with more frequent sensitization to lipocalins (Can f 2, Fel d 4, Equ c 1) and prostatic kallikrein (Can f 5) (7). This may be at least partially due to molecular polysensitization („molecular spreading“) in more severe patients (10) and our data concerning the frequent co-sensitization to different lipocalins may support this idea.

Serum albumins are minor allergens, and around 15–35% of cat and dog allergic patients are sensitized to Fel d 2 and Can f 3, and around 15–20% of horse allergic patients appear to be sensitized to Equ c 3. Although a high sequence identity reaching above 50% is present, the cross-reactivity between different albumins is variable, which indicates that a significant proportion of albumin-specific IgE is directed towards species-specific epitopes.

Co-sensitization to more animal dander extracts, which is commonly observed, is in our population not explained by cross-reactive serum albumins which are minor sensitizers with a low prevalence compared to other studies. Similarly low frequencies of sensitization to serum albumins as in our study were observed in Swedish children (5).



## MOULDS

Major geographic and age variations in the frequency of sensitization to moulds are seen in different studies and general lack of concordance between positive SPT responses and serum sIgE testing to moulds was described.

The most important allergenic fungi belong to the genera *Alternaria*, *Aspergillus* and *Cladosporium* and *Alternaria* is the most important fungal genus causing respiratory allergies (19). Several studies have also found an association between *Alternaria* sensitization and asthma severity. *Alternaria* belongs to the fungi with both indoor and outdoor occurrence, the latter one showing important seasonal variations in our climate. Alt a 1 is the main allergenic component of *Alternaria alternata*, sensitizing the great majority of patients allergic to *Alternaria* and it is the most important primary sensitizer in mould allergy patients in our region.

The knowledge concerning possible cross-sensitizations among moulds is controversial. Some sources describe that the majority of mould-sensitized patients reacts only to one species, but monosensitizations to other mould species assessed by means of extracts are observed not as frequently what may suggest a cross-reactive potential of these species.

On the other hand it is necessary to bear in mind that fungal extracts consist of a complex mixture of proteins, glycoproteins, polysaccharides, and other substances; these extracts show a considerable variability as a result of interstrain genomic differences, different culture conditions, and variable extraction procedures so that quality of commercial fungal extracts is variable (19-22). The reactivity of such extracts might be even non-specific. Further, different other substances are present in various fungal species and may cause potential cross-reactivity. The sensitization rates to all these potentially cross-reactive molecules (Alt a 6 (enolase), Asp f 1 (ribonuclease), Asp f 3 (peroxisomal protein), Asp f 6 (Mn-SOD) and Cla h 8 (mannitol dehydrogenase) were rather low in our patients to explain the mentioned frequent cross-reactivity detected by extracts.

In our patients, we saw much more frequently mono-sensitizations in frame of mould molecules, the most frequent being mono-sensitization to species specific molecule Alt a 1. The relatively low sensitization rates of *Aspergillus* derived molecules (first of all Asp f 6) compared to *Alternaria* derived molecules does not necessarily reflect a lower sensitization rate to *Aspergillus* because the sensitization rate to *Aspergillus* extract was shown to be considerably higher than the sum of sensitizations to the used molecules. On the other hand, several studies showed considerably lower frequency of sensitization to *Aspergillus* and *Cladosporium* in comparison to *Alternaria* (19) what is in concordance with our findings. The absence of commercially available high quality fungal extracts (22) is a big diagnostic and therapeutic problem in the practice of clinical allergy. It is necessary to incite companies to work on production of such extracts, first of all *Alternaria* extract with defined content of Alt a 1.

## CONCLUSION

Molecular diagnosis of allergy gives a more precise and reliable evaluation for an IgE-based allergy than does an extract-based approach. The geographical differences in sensitization patterns may reflect different ways of sensitization to the same allergen. The sensitization frequency to distinct components may be important in considering specific immunotherapy for allergy to this allergen and for optimizing the composition of therapeutic allergen vaccines used in the respective region.

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