

# Recombinant allergen profiles and health-related quality of life in seasonal allergic rhinitis

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## ABSTRACT

We evaluated birch- and timothy-allergic patients for allergen-specific IgE profiles and health-related quality of life (HRQL). We examined 395 patients with seasonal allergic rhinitis against birch or timothy pollen. Sera were analyzed for IgE reactivity to recombinant allergens (Bet v 1, Bet v 2, Bet v 4; Phl p 1/p 5b, Phl p 7, and Phl p 12) and to native pollen extracts (t3 and g6). Subgroup-specific analyses were performed. Patients with cosensitization against structurally unrelated allergens were termed polysensitized. Patients allergic solely to birch or timothy were labeled monosensitized. HRQL was evaluated using an established questionnaire. In patients polysensitized against native birch pollen ( $n = 233$ ) the prevalence of allergens was 86% for Bet v 1, 15% for Bet v 2, and 5% for Bet v 4. Similar for timothy ( $n = 256$ ), the prevalence of allergens was 87% rPhl p 1/p 5b, 5% for rPhl p 7, and 14% for rPhl p 12. Values for birch-monosensitized patients ( $n = 42$ ) were Bet v 1, 100%, and Bet v 2 and Bet v 4, 0%. Values for timothy-monosensitized patients ( $n = 35$ ) were Phl p 1/p 5b, 100%; rPhl p 7, 0%; and rPhl p 12, 3%. No difference in HRQL existed between patients sensitized solely against major versus minor allergens in birch-allergic patients. Polysensitized cohorts showed sensitization profiles comparable with published data. Monosensitized patients showed IgE against major allergens in 100% of cases. Patients sensitized solely against major or minor allergens showed no differences in HRQL.

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**Key words:** Allergen profiles, Bet v 1, Bet v 2/Phl-p 12, birch, clinical study, health-related quality of life, Phl p 1/p 5, recombinant allergens, timothy

In western societies, >25% of the population is affected by type 1 allergies, hence representing one of the major health and socioeconomic problems.<sup>1,2</sup> Especially birch and timothy pollen are widely distributed and among the most common causes for respiratory allergies. To date, diagnosis of type 1 allergies is based on *in vivo* and *in vitro* tests. *In vivo* diagnosis is conducted using allergen extracts for a skin test or other types of allergen-specific provocation. *In vitro* diagnosis is based on the proof of allergen-specific IgE antibodies in the serum. These conventional extracts contain contaminations such as nonallergic components, proteins from other allergen sources, and minor allergens, and, thus, are not suited for the identification of one specific major allergen.<sup>3</sup> Antigen mixes also may increase the rate of false positive reactions because sensitization against widely distributed minor allergens, e.g., profilin or calcium-binding proteins, are common.<sup>4</sup> Advances in molecular biology have shown

the feasibility of recombinantly manufacturing molecules that resemble the natural allergens structurally and immunologically. Recombinant allergens can be produced without any genetic or biological variations and are available in constant quality and quantity.<sup>5–9</sup> The *in vitro* determination of specific IgE antibodies against these recombinant allergens allows for the identification of symptom-inducing antigens divided into major allergens and minor allergens. Allergens from a certain allergen source to which  $\geq 50\%$  of sensitized patients react are designated as major allergens and those to which  $\geq 50\%$  of patients react are designated as minor allergens.<sup>10</sup> Minor allergens occur in many biologically unrelated allergen sources. Recognized by the same IgE antibody, patients appear polysensitized.<sup>11</sup> Profilin is a cytoskeletal protein present in all eukaryotic cells and is an example for a minor allergen.<sup>12</sup> Profilins share a high degree of homology (up to 79%) at the DNA and protein level among different plants, explaining extensive cross-reactivity. Other minor allergens are components of the pollen itself. The calcium-binding protein is abundantly distributed in trees, grass, and weeds, and patients who react against this protein also appear to be polysensitized.<sup>13</sup>

Patients suffering from IgE-mediated allergies of the airways mainly show symptoms such as allergic rhi-

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noconjunctivitis (RCA), allergic asthma, and oral allergy syndrome (OAS).<sup>14,15</sup> Because allergic symptoms have a negative impact on physical, psychological, and social aspects of daily life,<sup>16–18</sup> health-related quality of life (HRQL) gained an increasing importance in clinical assessment over the last years. Valid and sensitive HRQL questionnaires are published for nasal diseases such RCA<sup>19,20</sup> including a variety of items being measured.

In the past, various studies investigated IgE reactivity profiles in birch- and timothy-allergic patients and revealed that sensitization against Bet v 1 decreases from north to south Europe whereas Bet v 2 (profilin) behaves reciprocally. These findings are explained by a low population of birch trees in southern Europe and a higher geographical dominance of certain plants such as grasses that are responsible for sensitization against Bet v 2. However, the number of patients in most studies was too small for a differentiated analysis of subgroups (e.g., monosensitized patients versus polysensitized patients). Furthermore, no correlation with OAS and HRQL was performed in polysensitized patients hampering a clear statement about the impact of sensitization on patients' daily life.

Here, we performed profound subgroup-specific analyses of reactivity profiles in a large cohort of patients and investigated OAS and HRQL. The results of the present study give a detailed prevalence of birch and timothy allergens in south Germany and disclose effects on HRQL. The present data will contribute to the understanding of the distribution and clinical relevance of minor allergens.

## PATIENTS AND METHODS

### Study Design

In this study, we examined 395 individual patients presenting between January 2006 and April 2009 with complaints of RCA due to birch or timothy pollen. All patients underwent routine *in vivo* tests such as skin-prick test and/or allergen-specific nasal provocation and *in vitro* tests such as total IgE and allergen-specific IgEs in serum. Allergen-specific IgE against the following recombinant allergens was measured: major (rBet v 1 and rPhl p 1/p 5b) and minor allergens (rBet v 2, rBet v 4, rPhl p 7 and rPhl p 12). The study was performed in accordance with the Declaration of Helsinki and the study protocol was approved by the local ethics committee. All patients provided written informed consent.

### Clinical History and HRQL Questionnaire

For the evaluation of patients' HRQL, we used a slightly shortened and translated questionnaire as developed by Juniper.<sup>21</sup> The self-translated questionnaire was not validated in the converted form. A standard-

ized clinical history questionnaire included demographic data, living environment, and medical history. A symptom score of 0 (not troubled) to 3 (extremely troubled) or 0 (never) to 3 (always) was used. HRQL questionnaire was answered by the patients during the allergy season and without any influence of antiallergic medication.

### Recombinant Allergens

IgE reactivity to purified natural allergen extracts and recombinant allergens was investigated using a UniCAP 100 system (Phadia, Freiburg, Germany) according to the instructions of the manufacturer. Birch and grass pollen extracts and purified recombinant Bet v 1, Bet v 2, Bet v 4, Phl p 1/p 5b, Phl p 7, and Phl p 12 were purchased from Phadia. The results were expressed in CAP classes of positivity from 0 to 6, where class 0 corresponds to <0.35 kUA/L; class 1, 0.35–0.7 kUA/L; class 2, 0.7–3.5 kUA/L; class 3, 3.5–17.5 kUA/L; class 4, 17.5–50 kUA/L; class 5, 50–100 kUA/L; and class 6, >100 kUA/L. All sera were tested at the allergy laboratory of the Ear, Nose, and Throat Department of the University of Munich using standardized methods.

### Statistical Analysis

OriginPro 7.5 software (OriginLab Corp., Northampton, MA) was used for the statistical evaluation and graphical presentation of the data. The paired *t*-test was used for the comparison in the individual group and ANOVA was used for the comparison of the different groups. Spearman correlation coefficients were used to evaluate whether serum levels of IgE to natural birch and grass pollen extract and the IgE levels to recombinant allergens were correlated. A *p* value of  $\alpha$  of  $\leq 0.05$  was judged significant.

## RESULTS

### Recombinant Allergens

Figure 1 shows IgE reactivity profiles of mono- or polysensitized patients ( $n = 395$ ). Patients were stratified according to their disease history and *in vivo* and *in vitro* tests were stratified as described previously. Patients with cosensitization against structurally unrelated allergens were named polysensitized. Patients allergic solely to birch or timothy were labeled monosensitized. Two hundred thirty-three patients were positive for natural birch pollen (t3); 191 were polysensitized, whereas 41 were monosensitized. Sera of 256 patients showed IgE antibodies for native timothy pollen (g6); 221 were polysensitized and 35 were monosensitized.

*Native Birch Pollen–Polysensitized Patients.* Two hundred thirty-three patients were positive for natural

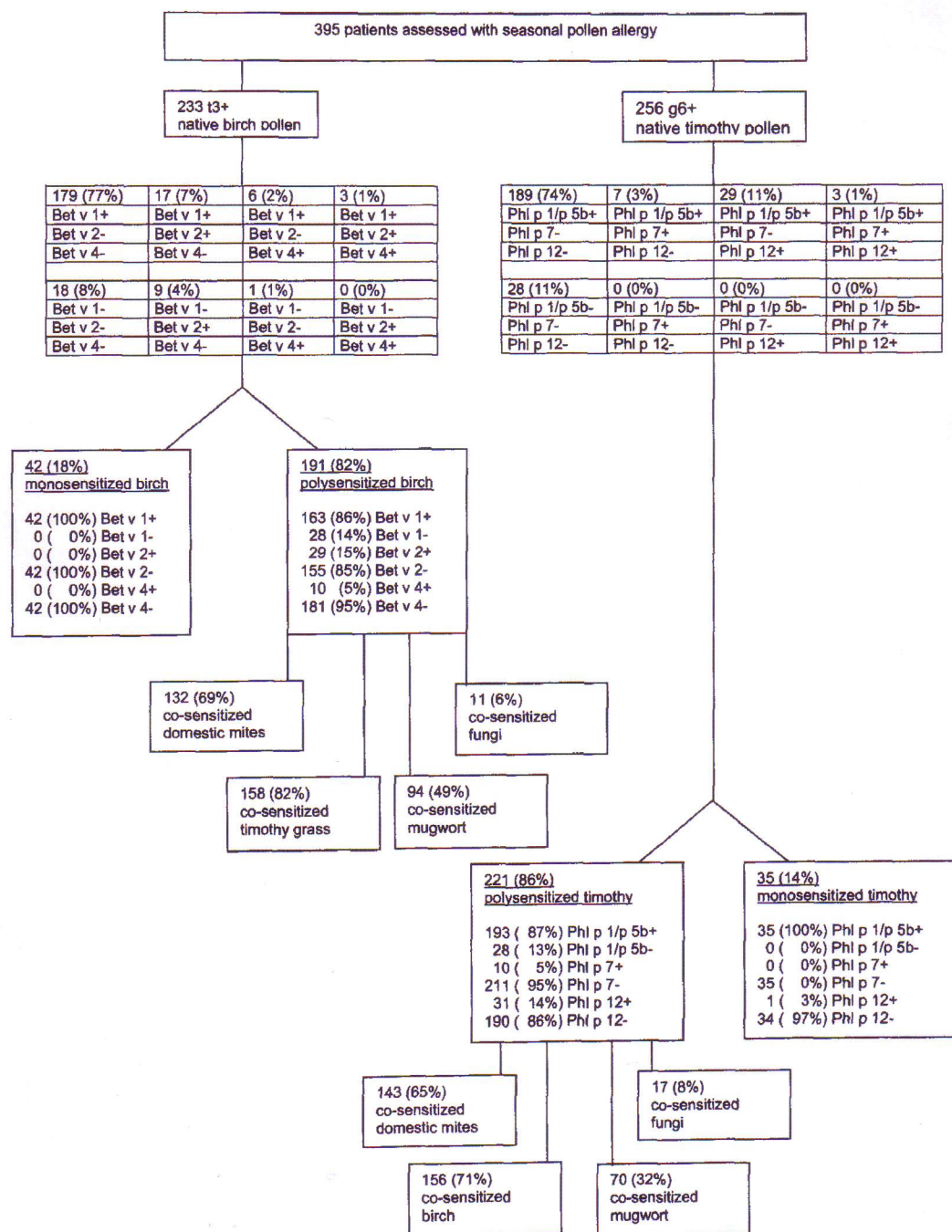


**Figure 1.** IgE different reactivity profiles of mono- or poly-sensitized patients (n = 395).

birch pollen (t3) and revealed a mean CAP class of  $3.4 \pm 1.2$ . Two hundred five (88%) of these patients showed specific IgE against rBet v 1, revealing the same CAP class with t3 in 140 cases (68%). Sixty-six patients differed within one CAP class. The mean CAP class of rBet v 1 was  $3.3 \pm 1.1$ . Table 1 shows significant ( $p < 0.01$ ) positive correlation between the CAP class of t3 and rBet v 1. Twenty-eight (12%) patients showed specific IgE for rBet v 2, 9 patients were within the same CAP class as with t3, 14 patients differed within four CAP classes. The mean CAP class of rBet v 2 was  $2.1 \pm 0.8$ . There was no correlation between levels of t3 and rBet v 2. Sera of 10 patients (4%) contained IgE against rBet v 4, 3 patients were within the same CAP

class, and 7 differed within 2 CAP classes. The mean CAP class of rBet v 4 was  $2.7 \pm 1.7$ . Correspondent to rBet v 2, no correlation was observed between t3 and rBet v 4.

*Birch Pollen-Allergic Patients without Any Cosensitization against Structurally Unrelated Allergens.* We found 42 patients within our cohort solely allergic to birch pollen (labeled "monosensitized"). Cosensitization of these birch-allergic patients was observed against the family of structurally related tree allergens: against alder (39/42, 93%) or hazel (41/42, 98%). There was no patient with specific IgE against alder or hazel, which

**Table 1** Correlations between the CAP class of natural birch and timothy allergen extract (t3 and g6) and recombinant major (Bet v 1 and Phl p 1/p 5b) and minor allergens (Bet v 1, Bet v 4, Phl p 7, and Phl p 12)

| Correlation                                 | <i>r</i> |
|---------------------------------------------|----------|
| t3 <sup>+</sup> /rBet v 1 <sup>+</sup>      | 0.93     |
| t3 <sup>+</sup> /rBet v 2 <sup>+</sup>      | 0.25     |
| t3 <sup>+</sup> /rBet v 4 <sup>+</sup>      | 0.17     |
| G6 <sup>+</sup> /rPhl p 1/p 5b <sup>+</sup> | 0.91     |
| G6 <sup>+</sup> /rPhl p 7                   | 0.26     |
| g6 <sup>+</sup> /rPhl p 12                  | 0.29     |

did not present specific IgE against native (t3) and/or recombinant major (rBet v 1) birch pollen allergen.

In all patients with specific IgE against hazel (*n* = 212), we did not find any patient who did not show specific IgE against t3, rBet v 1, or one of the studied minor allergens. In all 217 patients sensitized against alder, we found two cases with CAP class 1 against alder and no specific IgE against t3, rBet v 1, or one of the studied minor allergens. However, these two patients reported no complaints in association with the alder season but were highly allergic to grass pollen, reflected by patients' history and timothy-specific native and recombinant major IgE (g6 and rPhl p 1/p 5b), CAP class 4.

**Native Grass Pollen–Polysensitized Patients.** Sera of 256 patients showed positive CAP results (mean CAP class,  $3.3 \pm 1.2$ ) for native timothy pollen (g6). Two hundred twenty-eight (89%) patients presented specific IgE against rPhl p 1/p 5b. One hundred thirty-one (57%) of these patients showed the same CAP class with g6; 98 differed within one CAP class. The mean CAP class of rPhl p 1/p 5b was  $3.4 \pm 1.3$ . Correspondent to Bet v 1, there was a significant positive correlation between the CAP class of g6 and Phl p 1/p 5b (Table 1). Ten (4%) patients showed positive results for rPhl p 7, but only one patient was within the same CAP class as with g6, and nine differed within five CAP classes. The mean CAP class of rPhl p 7 was  $2.3 \pm 1.6$  and showed no correlation with g6. Thirty-two patients (13%) revealed specific IgE against rPhl p 12. However, all patients differed within four CAP classes and no correlation with g6 was seen either. The mean CAP class of rPhl p 12 was  $2.1 \pm 0.6$ .

**Grass Pollen–Allergic Patients without Any Cosensitization against Structurally Unrelated Allergens.** We found 35 patients within our cohort who were allergic solely to timothy pollen (labeled "monosensitized"). Cosensitization of these timothy-allergic patients was ob-

**Table 2** The characteristics of all 395 patients

| Characteristics                    |             |
|------------------------------------|-------------|
| Gender                             |             |
| Male                               | 213 (54%)   |
| Female                             | 182 (46%)   |
| Age (yr)                           | $34 \pm 16$ |
| Living surroundings                |             |
| Urban                              | 182 (46%)   |
| Suburban                           | 138 (35%)   |
| Rural                              | 75 (19%)    |
| Smokers                            | 51 (13%)    |
| Self-reported asthmatic complaints | 56 (14%)    |

*Values are number of patients total and percent.*

served against the structurally related ray in 33/35 patients (94%). However, as in the birch pollen patients mentioned previously, there was no patient with specific IgE against ray who did not present specific IgE against timothy (g3) and/or rPhl p 1/p 5b in this subgroup.

In the total cohort of patients with specific IgE against ray (*n* = 238) we found one case with specific IgE against ray CAP class 1 without any specific IgE against g6, rPhl p 1/p 5b, or one of the studied minor allergens. As mentioned previously, this patient did not show any symptoms in association to ray season but was highly allergic to birch pollen, reflected by history and specific IgE against native and recombinant major birch pollen allergen (t3 and rBet v 1).

### Health-Related Quality of Life

To describe the disease-related impact on patient's overall daily life, results from *in vitro* investigations were compared to HRQL-scales. For analysis we used a shortened and translated questionnaire developed and validated by Juniper et al.<sup>21</sup> Characteristics of all 395 patients are given in Table 2.

OAS is a frequently reported symptom of RCA. For analysis of severity and nature of OAS, we studied monosensitized patients only. Patients who were monosensitized toward birch pollen reported to suffer from OAS in 19 of 42 cases (45%). All patients revealed IgE antibodies against the major birch pollen allergen rBet v 1 but tested negative for rBet v 2 and rBet v 4. OAS occurred almost exclusively to Rosaceae, nuts, and carrots. Details and symptom scores are given in Table 3. Similarly, Table 4 shows data of patients who were monosensitized against timothy. Contrary to birch pollen, we did not observe a preferred group of cross-reacting food allergens. Patients allergic to birch were more bothered from OAS than patients suffering from timothy allergy. The symptom score concerning

**Table 3 The number and percentage of patients who were monosensitized to birch pollen suffering from oral allergy syndrome**

| OAS of Patients Monosensitized to Birch Pollen                   | 19 (of 42)  |
|------------------------------------------------------------------|-------------|
| Gender                                                           |             |
| Male                                                             | 11 (58%)    |
| Female                                                           | 7 (42%)     |
| Age (yr)                                                         | 36 ± 17     |
| Bet v 1 <sup>+</sup> /Bet v 2 <sup>-</sup> /Bet v 4 <sup>-</sup> | 19 (100%)   |
| Symptom score                                                    |             |
| Oral itching                                                     | 1.75 ± 1.00 |
| Oral swelling                                                    | 1.13 ± 1.13 |
| Dyspnea                                                          | 0.75 ± 1.18 |
| Skin rash                                                        | 0.60 ± 1.06 |
| Hypotension                                                      | 0.79 ± 1.05 |
| Abdominal pain                                                   | 1.47 ± 1.25 |

Values are number of patients total, percent and symptom score (0 = not troubled to 3 = extremely troubled).

oral itching and oral swelling was significantly ( $p < 0.01$ ) higher among patients allergic to birch pollen.

The clinical relevance of minor allergen sensitization is still not clear to date. To address this question, we plotted HRQL data of two groups of patients sensitized either solely against major or minor allergens. Because this selection resulted in dramatically low numbers of monosensitized birch- and timothy-allergic patients, we plotted mono- and polysensitized patients. For timothy-allergic patients a comparison between major- and minor-sensitized patients was not possible, because sensitization against minor allergens only did not occur. Figures 2 and 3 show data for birch- and timothy-allergic patients. No significant differences were found between these groups.

## DISCUSSION

Besides clinical history, diagnosis of allergy is based on skin tests, provocation tests, and *in vitro* investigations with natural allergen extracts. These are complex mixtures that not only contain relevant allergenic proteins in poorly defined concentrations, but also nonallergenic proteins and carbohydrates.<sup>3</sup> In contrast, recombinant allergens can be thoroughly quantified with respect to protein concentration and IgE reactivity.<sup>22,23</sup> Furthermore, commercial extract potency may vary lot to lot. Having a validated recombinant allergen for testing thus represents a major step forward.

Allergies to pollen of white birch and grass are among the most common type I allergies in Europe.<sup>24</sup> Besides the major birch pollen allergen Bet v 1,<sup>25</sup> other relevant birch pollen allergens are Bet v 2<sup>12</sup> and Bet v 4.<sup>26</sup> For timothy pollen, Phl p 1/p 5b is the major

**Table 4 The number and percentage of patients who were monosensitized to timothy grass pollen with oral allergy syndrome**

| OAS of patients monosensitized to timothy pollen                       | 10 (of 35)  |
|------------------------------------------------------------------------|-------------|
| Gender                                                                 |             |
| Male                                                                   | 5 (50%)     |
| Female                                                                 | 5 (50%)     |
| Age (yr)                                                               | 32 ± 14     |
| Phl p 1/p 5b <sup>+</sup> /Phl p 7 <sup>-</sup> /Phl p 12 <sup>-</sup> | 10 (100%)   |
| Symptom score                                                          |             |
| Oral itching                                                           | 0.43 ± 1.13 |
| Oral swelling                                                          | 0.00 ± 0.00 |
| Dyspnea                                                                | 0.71 ± 0.76 |
| Skin rash                                                              | 0.50 ± 0.84 |
| Hypotension                                                            | 0.50 ± 0.76 |
| Abdominal pain                                                         | 1.00 ± 0.76 |

Values are number of patients total, percent, and symptom score (0 = not troubled to 3 = extremely troubled).

allergen whereas Phl p 7 and Phl p 12 are minor allergens.<sup>27</sup>

Recent studies revealed that IgE reactivity against the major birch allergen Bet v 1 decreases from northern to southern Europe,<sup>28-30</sup> whereas reactivity against the minor allergens (Bet v 2 and Bet v 4) increases. IgE antibodies to Bet v 1 were abundant in patients from Sweden, Finland, Austria (>98%), and France (90%) but they were rarely found in patients from Switzerland (65%) and northern Italy (39-62%).<sup>29</sup> In contrast, Bet v 2 antibodies were rare in Scandinavia (2-12%) while they were common in northern Italy (26-33%). Similar values were found for Bet v 4 because positive patients were rare in Japan (2.5%)<sup>30</sup> and Finland (5%)<sup>28</sup> but were commonly found in Italy (27%).<sup>29</sup>

The prevalence of birch allergens (Bet v 1, 86%; Bet v 2, 15%; Bet v 4, 5%) in southern Germany is in line with results from other European countries of the same latitude and in between the Scandinavian and northern Italian results. In northern Italy birches are rare and other plants such as grasses show a high geographical dominance. Because minor allergens are found in almost every eukaryotic cell, reactivity in southern Europe may be a result of sensitization to other allergens than birch, *e.g.*, timothy pollen, ash, and olive. This thesis is supported by the finding that cross-reactivity appeared higher among patients sensitized against minor allergens (timothy, 91%; mites, 75%) compared with all patients (timothy, 82%; mites, 69%). The present results are in line with former investigations by Shirasaki *et al.*<sup>30</sup> who found a significantly higher frequency of IgE antibodies to mites and grass pollen in Bet v 2-positive patients versus Bet v 2-neg-

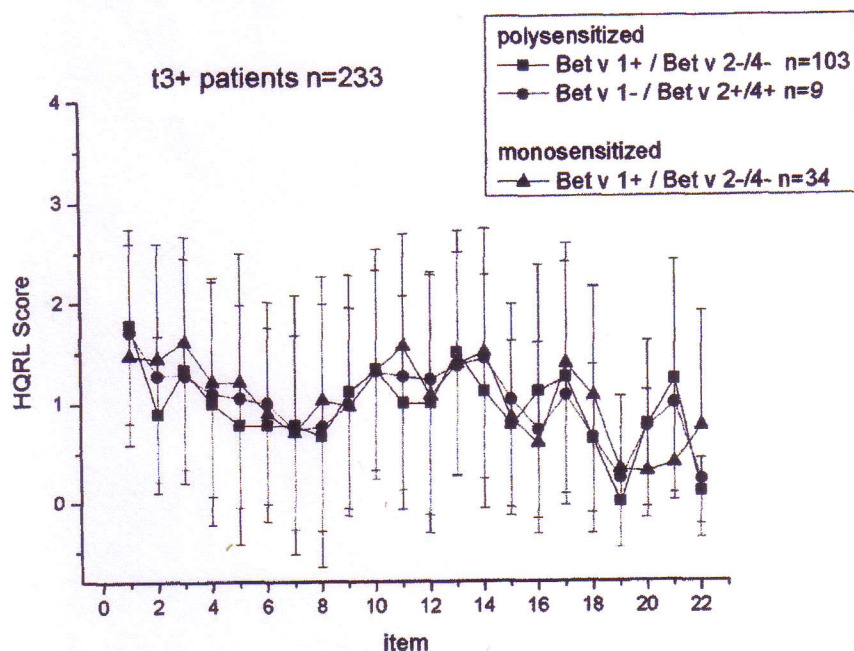


Figure 2. Health-related quality-of-life (HRQL) data of two groups of birch-allergic patients sensitized either solely against major or minor allergens. Because this selection resulted in low numbers of monosensitized patients, we plotted mono- and polysensitized patients.

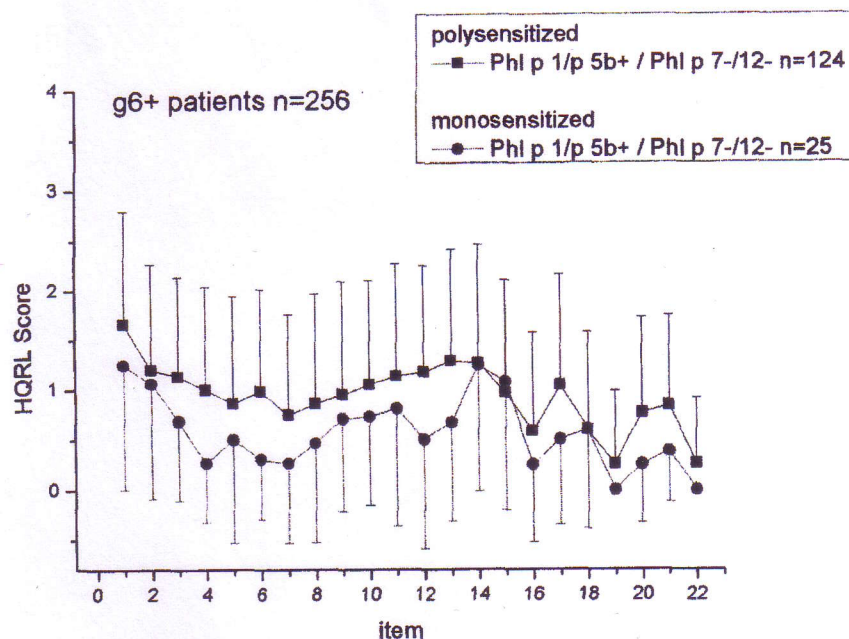


Figure 3. Health-related quality-of-life (HRQL) data of mono- and polysensitized patients allergic solely to major timothy-allergen Phl p1/p5.

ative patients. These authors concluded that because of the wide abundance of profilin, sensitized patients might exhibit relevant reactivity to several allergen sources. However, previous studies investigated polysensitized patients only. A detailed analysis of subgroups regarding monosensitized patients versus polysensitized patients subdivided into major and minor allergens was not conducted so far. For the first time, we investigated a large number of birch- and timothy-allergic patients and performed a detailed subgroup analysis. Compared with polysensitized patients, the results differ among monosensitized cases. All birch-allergic patients showed IgE against Bet v 1, while antibodies against the minor allergens Bet v 2 and Bet v 4 were not detectable. These findings are interesting, because they support the thesis that sensitization

against minor allergens mainly is cross-reactive and increases from mono- to polysensitization.

Grasses are a heterogeneous group of plants providing up to 71 allergens from 15 species distributed among 10 protein families.<sup>31</sup> In the present study, we investigated patients sensitized to native timothy pollen extract and compared the results with IgE reactivity profiles of three timothy recombinant allergens. The prevalence of timothy allergens was 88% for Phl p 1/p 5b and 5% each for Phl p 7 and Phl p 12. These findings are comparable with the results of two previously reported studies. Constantin *et al.*<sup>32</sup> showed a prevalence of 94% for Phl p 1/p 5b, 29% for Phl p 7, and 35% for Phl p 12 among grass pollen-allergic patients in Austria ( $n = 17$ ). In line, Rossi *et al.*<sup>31</sup> showed that patients with grass pollen allergy show a high preva-

lence of sensitization to recombinant allergens such as Phl p 1 (98%) and Phl p 5 (95%) but not to Phl p 7 (8%) and Phl p 12 (41%). Notwithstanding, these studies compared polysensitized patients and a subgroup analysis was not performed either. Comparable with the results of birch allergy, monosensitized patients show antibodies against Phl p 1/p 5b in 100% of the cases. No patient was sensitized against Phl p 7 and one patient only was sensitized against Phl p 12.

Our data challenge the widely applied diagnostic routine of testing related tree or grass allergens. Our results suggest that native, major, and minor allergens of birch and timothy are sufficient to detect all relevant sensitizations. In other words, there is no isolated allergy directed to alder or hazel, respectively, to ray, that does not involve t3, rBet v 1, g6, rPhl p 1/p 5b, profiling, or calcium-binding protein. This might have future therapeutic implications, because specific immunotherapy with rBet v 1 or Phl p 1/p 5b could be sufficient to treat allergic rhinitis caused by all related trees or grasses.

Previous studies<sup>31</sup> reported that the great majority of patients suffering from OAS was sensitized to profilin. In the present study, for analysis of severity and nature of OAS, we investigated monosensitized patients only and could not observe positive reactivity to Bet v 2 or Phl p 12. This contradiction may be explained by the fact that patients were cosensitized to Bet v 1 in previous investigations. Although birch-allergic patients suffered from OAS after eating rosaceae, nuts, and carrots, we did not observe a preferred group of cross-reacting food allergens in patients allergic to timothy. Furthermore, symptoms of OAS were more severe in patients allergic to birch than in patients allergic to timothy. The symptom score concerning oral itching and oral swelling was significantly ( $p < 0.01$ ) higher among patients allergic to birch pollen.

Allergic symptoms interfere with many aspects of daily life. Therefore, determination of subjective perceptions by the implemented questionnaire gives a more realistic description of the impact on patients and is a more accurate tool of measurement than conventional clinical markers. The present data can constitute the basis for the judgment of therapeutic effects and allow the clinician to develop an individual therapeutically based concept. We used a shortened and translated HRQL questionnaire published and validated by Juniper *et al.*<sup>21</sup> The questionnaire, constituted of 22 items, summarized in 6 symptom groups and has been established as a useful instrument for the detection of differences in HRQL.<sup>20</sup> The objective of the present study was to compare HRQL in patients allergic to birch and timothy pollen that revealed different IgE reactivity profiles toward major (rBet v 1 and rPhl p 1/p 5b) and minor allergens (rBet v 2, rBet v 4, rPhl p 7, and rPhl p 12). We performed subgroup analysis and

compared polysensitized versus monosensitized. We could not detect any significant differences in patients suffering from birch allergy against isolated major versus minor allergens. For timothy-allergic patients a comparison between major- and minor-sensitized patients was not possible, because sole sensitization against minor allergens did not occur. The finding in birch-allergic patients points to a relevant role of minor allergen sensitization in seasonal allergic rhinitis. However, HRQL depends on the patient's perception, and severity of symptoms is subjective and influences HRQL directly.<sup>33</sup> Furthermore, numbers of patients in the subgroups were very small and thus the present study has to be considered as a pilot study that deserves replication with larger groups of patients.

The present findings emphasize the importance of incorporating individual HRQL measurements into the clinical evaluation of allergy during initiation and evaluation of therapy outcome because even small changes in HRQL are clinically significant.<sup>34</sup> However, current HRQL questionnaires might be too nonspecific for the discrimination between major and minor allergen sensitization, even though being validated by Juniper,<sup>21</sup> and might thus need revision.

## CONCLUSION

We performed a subgroup-specific analysis of reactivity profiles in a large number of birch- and timothy-allergic patients. Our polysensitized cohorts showed sensitization profiles against major and minor allergens comparable with published data from middle and north Europe. Birch-allergic patients sensitized solely against major or minor allergens showed no differences in HRQL, pointing to a relevant role of minor allergen sensitization in seasonal allergic rhinitis.

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