

Do *Plantago lanceolata* Skin Prick Test-Positive Patients Display IgE to Genuine Plantain Pollen Allergens? Investigation of Pollen Allergic Patients from the North-East of France

Teresa Stemeseder^a Carine Metz-Favre^b Frederic de Blay^b Gabrielle Pauli^c
Gabriele Gadermaier^a

^aDepartment of Biosciences, University of Salzburg, Salzburg, Austria; ^bAllergy Division, Chest Diseases Department, Strasbourg University Hospital, Federation of Translational Medicine, EA 3072, University of Strasbourg, Strasbourg, France; ^cMedical University of Strasbourg, Strasbourg, France

Keywords

Plantain pollen allergy · Plantago · Skin prick test · IgE cross-reactivity · Co-sensitization

Abstract

Background: English plantain (*Plantago lanceolata*) is an important weed pollen allergen source triggering allergic symptoms during summer. To elucidate genuine versus cross-reactive sensitization, we investigated IgE reactivity patterns and inhibition capacities of plantain-sensitized patients. **Methods:** Sera of 35 rhinoconjunctivitis patients from the north-east of France with positive skin prick tests (SPT) to *Plantago lanceolata* pollen were tested with clinically relevant allergen sources using ELISA, ImmunoCAP, and immunoblot inhibition. **Results:** The patients were multisensitized with additional reactivity to grass (94.3%), ash (74.3%), birch (71.4%), and mugwort (55.2%) pollen in SPT. Sensitization prevalence to allergen molecules was 34.3% (Pla I 1), 94.3%

(Phl p 1/5), 60.0% (Ole e 1), 65.7% (Bet v 1), 37.1% (profilin), and 40.0% (CCD). In immunoblot, IgE reactivity to plantain pollen was inhibited with relevant pollen extracts and purified rPla I 1. Two sera did not reveal any IgE cross-reactivity, while reactivity to plantain was efficiently inhibited by grass pollen in the sera of 10 patients. The sera from 17 different patients could be inhibited by grass, birch, or ash pollen to varying degrees. Thus, only 37.1% of our patients demonstrated true plantain pollen sensitization, while 62.9% were solely positive due to IgE cross-reactive molecules from other clinically relevant pollen. **Conclusions:** Plantain pollen-sensitized patients are multi-reactors demonstrating varying and complex IgE-reactivity profiles. In vivo and in vitro tests using extracts are typically blurred due to the presence of homologous allergens or CCD in grass, birch, or ash pollen. So far, Pla I 1 represents the only indicative marker allergen for the diagnosis of genuine plantain pollen sensitization.

© 2018 S. Karger AG, Basel

Edited by: O. Palomares, Madrid.

KARGER

© 2018 S. Karger AG, Basel

E-Mail karger@karger.com
www.karger.com/iaa

Dr. Gabriele Gadermaier
Department of Biosciences, University of Salzburg
Hellbrunnerstrasse 34
AT-5020 Salzburg (Austria)
E-Mail Gabriele.Gadermaier@sbg.ac.at

Prof. Gabrielle Pauli
Medical University of Strasbourg
4 Rue Kirschleger
FR-67000 Strasbourg (France)
E-Mail Gaby.Pauli@gmail.com

Introduction

Plantago lanceolata (English plantain) represents the most important allergen source among weeds from the Plantaginaceae family [1–3]. Plantain pollen is usually not included in routine allergy diagnosis, and its clinical relevance may thus be underestimated in some regions [1, 4]. English plantain typically flowers between April and September and shows an overlapping pollination season with grasses [2, 5, 6]. It grows mainly in temperate climate zones and is native in the north-east of France [5, 7–10]. Other *Plantago* spp. are also abundantly present in this climatic region and might potentially play a role in allergic rhinitis while information is so far limited. Other allergenic plants frequently found in this region of France are grasses, ash, birch, oak, and mugwort [1, 10–13]. Several IgE-binding molecules of plantain pollen have been reported by immunoblots using patients' sera and mass spectrometry-based methods [8, 14–16]. In a previous work investigating a Central European cohort, the allergen repertoire of English plantain was shown to be distinct from grasses and other pollen sources [8]. Pla l 1, the major allergen, has been extensively investigated and produced as recombinant protein in *P. pastoris* and *Escherichia coli* [15, 17–19]. The natural allergen is partially glycosylated and migrates as double band between 16 and 20 kDa in gel electrophoresis [15]. The molecule carries a complex N-linked glycan with an alpha 1,3 fucose which was suggested not to constitute a relevant IgE-binding epitope [17].

Pla l 1 is a member of the Ole e 1-like protein family sharing a short consensus sequence but only 28–40% overall sequence identity with other homologous allergens [19]. Relevant pollen allergens from this protein family are Ole e 1 (olive), Fra e 1 (ash), Phl p 11 (timothy grass), and Che a 1 (chenopod) [20, 21]. Recent studies showed a lack of IgE cross-reactivity of Pla l 1 with homologous allergens [19]. However, plantain pollen also harbors the panallergens profilin and polcalcin which are present in other pollen sources [8, 22–31]. Using 2-dimensional immunoblot and mass spectrometry, the presence of fructose biphosphate aldolase (38 kDa), malate dehydrogenase (35 kDa), ATP synthetase (55 kDa), polygalacturonase (43 kDa), and glyceraldehyde-3-phosphate dehydrogenase (39 kDa) have been reported [8, 14].

Patients with a true monosensitization to *Plantago lanceolata* are rarely observed [8, 13]. The vast majority of plantain pollen-sensitized patients demonstrates concomitant sensitizations and allergies to other pollen allergen sources, e.g., grass, birch, ash, or olive pollen [8, 13,

32, 33]. A solid allergy diagnosis enabling discrimination of IgE cross-reactivity or co-sensitization is, however, essential for decision making regarding allergen immunotherapy.

The aim of this study was to analyze sera of 35 patients from the north-east of France with pollinosis symptoms in spring and/or summer and positive skin prick test (SPT) to *Plantago lanceolata*. To elucidate the IgE sensitization profiles, ImmunoCAP, ELISA, immunoblot, and inhibition experiments were performed using the patients' sera. Elucidation of reactivity patterns will contribute to enhanced knowledge regarding plantain allergy diagnosis and as – a consequence – to more accurate indications for consideration in allergen immunotherapy.

Material and Methods

Patients

Sera from 35 patients from 3 different centers in the area of Strasbourg, in the north-east of France, with spring and/or summer pollinosis and positive SPT to *Plantago lanceolata* were included in the study. Further details on the patients are provided in online supplementary Table S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000490004). Ethical permission was obtained from the French Ministère de la Recherche (DC-2010-1095). Written informed consent was received from all individuals.

Skin Prick Tests

SPTs were performed with commercially available extracts of grass (ryegrass, timothy, and Bermuda grass), *Plantago lanceolata* (English plantain), *Fraxinus excelsior* (European ash), *Betula pendula* (silver birch), *Quercus alba* (white oak), and *Artemisia vulgaris* (mugwort) (Stallergenes, Antony, France; ALK-Abello, Hørsholm, Denmark). Skin reactions ≥ 3 mm or $>50\%$ of the histamine-positive control (1/1,000) were considered as positive.

ImmunoCAP Analysis

Specific IgE to allergen components was analyzed in the study centers using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden). Tested allergens were Pla l 1, Phl p 1/5, Ole e 1 as surrogate marker for IgE reactivity to Fra e 1, Bet v 1, and Art v 1 [34]. Additionally, reactivity to panallergens was analyzed using profilin (Phl p 12 or Bet v 2), polcalcin (Phl p 7 or Bet v 4), and CCD (MUXF3 CCD, bromelain). Values ≥ 0.1 kU_A/L were considered positive.

Pollen Extracts and Recombinant Pla l 1

Extracts from pollen of *Plantago lanceolata*, *Phleum pratense*, *Fraxinus excelsior*, *Betula pendula*, *Quercus alba*, and *Artemisia vulgaris* (Allergon AB, Thermo Fisher Scientific, Ängelholm, Sweden) were prepared as 180 mg pollen/mL in dH₂O by rapid agitation for 24 h at 4 °C. Supernatants were taken after centrifugation for 10 min at 14,000 rpm, and protein concentrations were measured using Pierce Coomassie (Bradford) protein assay (Thermo Fisher Scientific, Rockford, IL, USA). Recombinant non-glycosyl-

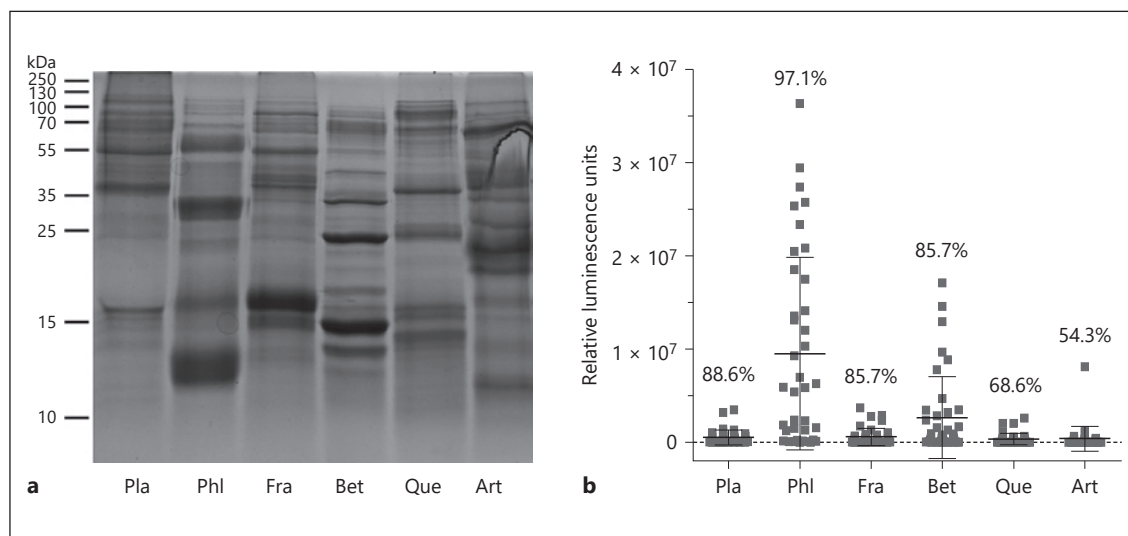


Fig. 1. Gel electrophoresis and IgE reactivity of pollen extracts. **a** 15 μ g aqueous extracts were loaded per lane. **b** IgE reactivity of the patients' sera ($n = 35$) was tested with pollen extracts using ELISA. Horizontal lines with whiskers represent mean $\pm$ standard deviations. Pla, *Plantago lanceolata*; Phl, *Phleum pratense*; Fra, *Fraxinus excelsior*; Bet, *Betula pendula*; Que, *Quercus alba*; Art, *Artemisia vulgaris*.

ated Pla l 1 was expressed as soluble non-tagged protein in *E. coli* and purified using cation exchange and size exclusion chromatography as has been recently described [19].

Patients' ELISA

High-binding ELISA plates (Greiner Bio-One, Frickenhausen, Germany) were coated with 0.25 μ g/well of the respective pollen extract overnight at 4 °C. The plates were washed with 137 mM NaCl, 2.7 mM KCl, 10 mM Tris, pH 7.4, 0.05% v/v Tween 20 (TBST) and blocked with TBST, 0.5% w/v BSA for 3 h at room temperature. The patients' sera were diluted 1:5 in TBST, 0.5% (w/v) BSA, and incubated overnight at 4 °C. After washing with TBST, bound IgE was detected with horseradish peroxidase-conjugated monoclonal anti-human IgE antibodies (Southern Biotech, Birmingham, AL, USA). BM Chemiluminescence ELISA substrate (Roche, Rotkreuz, Switzerland) was used as substrate, and relative luminescence was measured after 3 min of incubation. Values were considered positive when exceeding 5 times standard deviation of blanks of the respective pollen extract. Correlations between IgE reactivity to single extracts in ELISA and results from SPT were calculated as Spearman's rank correlation, and $p < 0.05$ was considered as statistically significant.

Gel Electrophoresis, IgE Immunoblot, and Inhibition Experiments

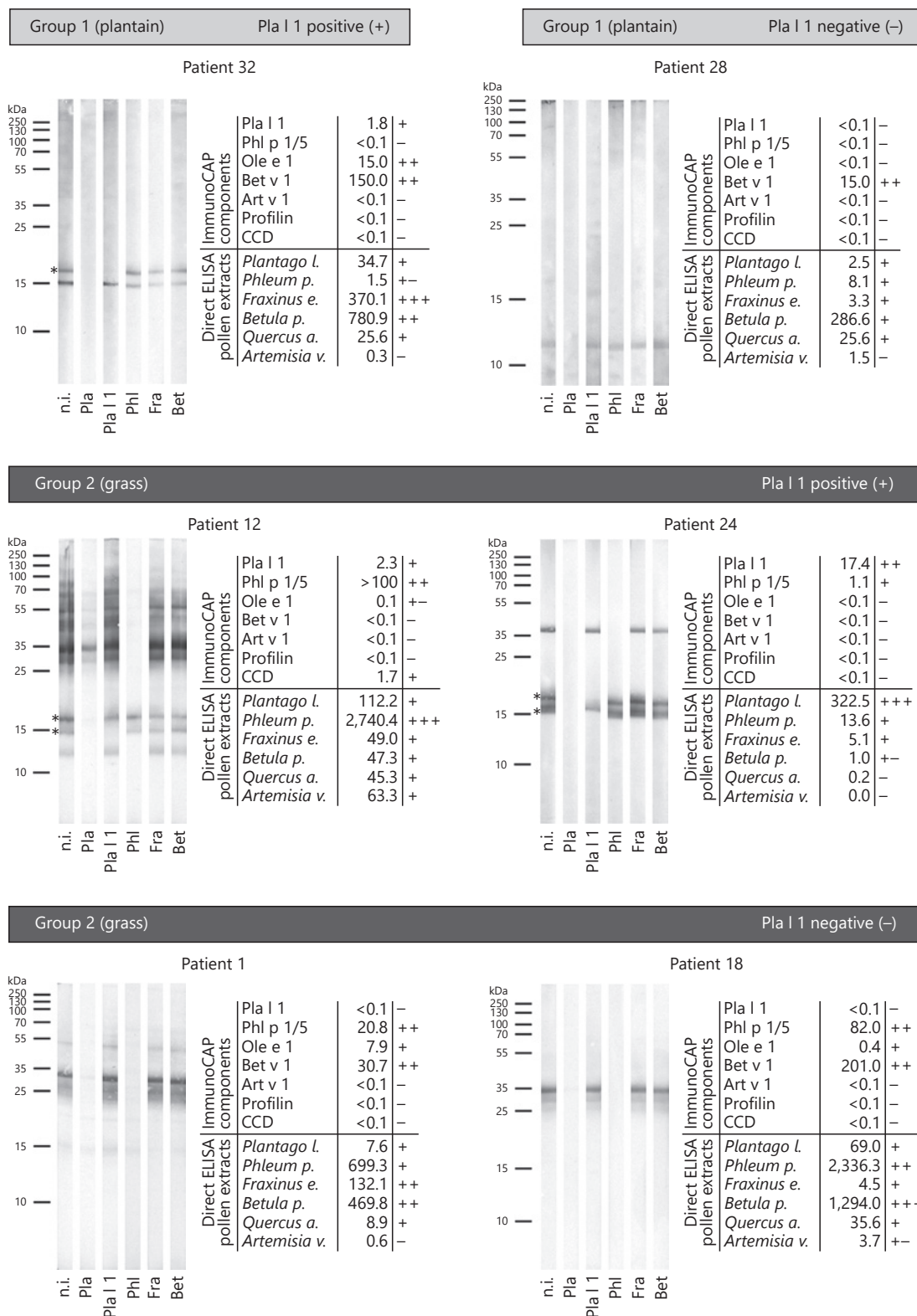
Pollen extracts were analyzed by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using 15% gels and stained with Coomassie Brilliant Blue G-250 (Bio-Rad, Hercules, CA, USA). For immunoblot, plantain pollen extract was precipitated by methanol-chloroform precipitation. 170 μ g protein were separated by SDS-PAGE and subsequently electroblotted on a nitrocellulose mem-

brane (Whatman, Brentford, UK). For immunoblot inhibitions, the patients' sera were preincubated overnight at 4 °C using 150 μ g pollen extracts of English plantain, grass, ash, or birch as well as 20 μ g purified recombinant Pla l 1. The patients' non-inhibited and inhibited sera were incubated with the blotted membrane overnight at 4 °C. Bound IgE was detected with alkaline phosphatase-conjugated monoclonal anti-human IgE antibodies (BD Biosciences, Franklin Lakes, NJ, USA). 5-bromo-4-chloro-3-indolyl phosphate with nitro blue tetrazolium was used as a substrate. Non-atopic human serum and buffer were used as assay controls.

Results

Patients' Symptoms and SPTs

The patients in our cohort suffered from rhinoconjunctivitis mainly in the time between May and July. Additionally, asthmatic symptoms were observed in 54% of the patients, while 34% showed oral symptoms after the consumption of fruits or vegetables. Based on the inclusion criteria, all individuals were reactive to English plantain in SPT. In addition, 94.3% (33/35) of the patients presented a positive SPT to grass, 74.3% (26/35) to ash, 71.4% (25/35) to birch, and 55.2% (16/29) to mugwort pollen extracts. Further demographic information and clinical presentations of our patients are provided in online supplementary Table S1.



(For legend see next page.)

In vitro IgE Reactivity to Pollen Extracts and Allergen Molecules

Aqueous extracts from 6 pollen sources relevant to the north-east region of France were obtained, and protein pattern analyzed by gel electrophoresis (Fig 1a). In general, species-specific protein profiles were observed which demonstrated rather dissimilar protein pattern even within the same botanical families (birch and oak). Prominent protein bands were detected in extracts of grass, ash, and birch, while plantain, oak, and mugwort did not reveal dominant protein bands.

In ELISA, we found a very high sensitization prevalence to grass (97.1%), English plantain (88.6%), ash, and birch pollen extracts (85.7% each), while reactivity against oak (68.6%) and mugwort (54.3%) was less prevailing (Fig 1b). The highest IgE levels were observed against grass and birch pollen allergens, while antibody levels were considerably lower for the other investigated sources. Typically, a multisensitization profile was observed with 30 patients reactive to plantain and grass in combination with another tested extract.

In addition, reactivity to relevant allergen components was tested in ImmunoCAP, and sensitization prevalence was: Pla l 1, 34.3%; Phl p 1/5, 94.3%; Ole e 1, 60.0%; Bet v 1, 65.7%; Art v 1, 17.6%; profilin, 37.1%; and CCD, 40.0%. Polcalcin was negative in 16 tested sera. Individual ImmunoCAP and ELISA results are provided in Figures 2 and 3, and online supplementary Figures S1–S4 for each patient.

Correlation analyses of the SPT results to plantain and IgE levels towards pollen extracts in ELISA (online suppl. Fig. S5) and components in ImmunoCAP (online suppl. Fig. S6) were performed. IgE reactivity with plantain pollen extract correlated highest with SPT results ($r = 0.5811$, $p = 0.0003$). Statistical significance was also found for ash, mugwort, and oak, while there was no relevant correlation with birch and grass pollen. When correlating the SPT results with allergen components, solely IgE reactivity to Pla l 1 was statistically significant ($r = 0.4049$, $p = 0.0175$). Reactivity to profilin ($r = 0.3271$, $p = 0.0590$) and CCD ($r = 0.3228$, $p = 0.0626$) showed weak correlations but were statistically beyond significance.

Immunoblot and Inhibition Experiments

The sera from all patients were tested for IgE reactivity to English plantain in immunoblot and inhibition experiments. Representative examples can be found in Figures 2 and 3, while the blots with residual patients' sera are presented in online supplementary Figures S1–S4. Highly diverse IgE reactivity patterns were observed in our cohort, ranging from 1 to 10 bands detected in immunoblot. The sera reacted to proteins migrating from 12 to 250 kDa as well as less defined high-molecular-weight components.

Immunoblot inhibitions were performed to reveal if sensitization to plantain pollen was genuine or due to IgE cross-reactivity. Therefore, the inhibitory capacity of the clinically relevant pollen sources grass, ash, and birch was investigated (Fig. 2 and 3, online suppl. Fig. S1–S4). For the majority of the patients, the inhibition with plantain extract was complete except for some sera where self-inhibition was not fully achieved but considerably reduced. In order to unequivocally verify IgE reactivity to the major allergen Pla l 1, we performed inhibition experiments with the purified non-glycosylated molecule. Specific inhibition of a protein migrating at approximately 17 kDa was achieved for patients sensitized to Pla l 1 as determined by ImmunoCAP. Patient 25 demonstrated low IgE levels against Pla l 1 which were not detectable in immunoblot experiments, thus incubation with the recombinant molecule did not reveal any difference. In general, IgE reactivity patterns towards plantain proteins other than Pla l 1 were not influenced by inhibition with recombinant purified Pla l 1 verifying the specificity of the assay.

Based on the obtained results, our patients were clustered into 3 different groups according to the inhibitory capacity of the single sera towards plantain pollen in immunoblot (online suppl. Fig. S7). Examples of individual patients from each group are depicted in Figures 2 and 3, while residual results can be found in online supplementary Figures S1–S4. Only 2 sera demonstrated exclusive self-inhibition with plantain extract (group 1, $n = 2$), while IgE reactivity of other sera was impaired by allergens from grass pollen (group 2, $n = 10$). IgE reactivity to plantain extract was cross-inhibited by grass, birch, or ash

Fig. 2. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of group 1 (plantain) and group 2 (grass). Reactivity to Pla l 1 is marked with an asterisk. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla l 1, inhibited with recombinant non-glycosylated Pla l 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excel-*

sior extract; Bet, inhibited with *Betula pendula* extract. ImmunoCAP results are given as kU_A/L . –, <0.1 kU_A/L ; +, $0.1–0.35$ kU_A/L ; ++, >0.35 kU_A/L ; +++, ≥ 15 kU_A/L . ELISA readouts are given as relative luminescence units $\times 10^4$. –, value below 50% of threshold; +, value below threshold; ++, value above threshold; +++, value above 75% of maximum value.

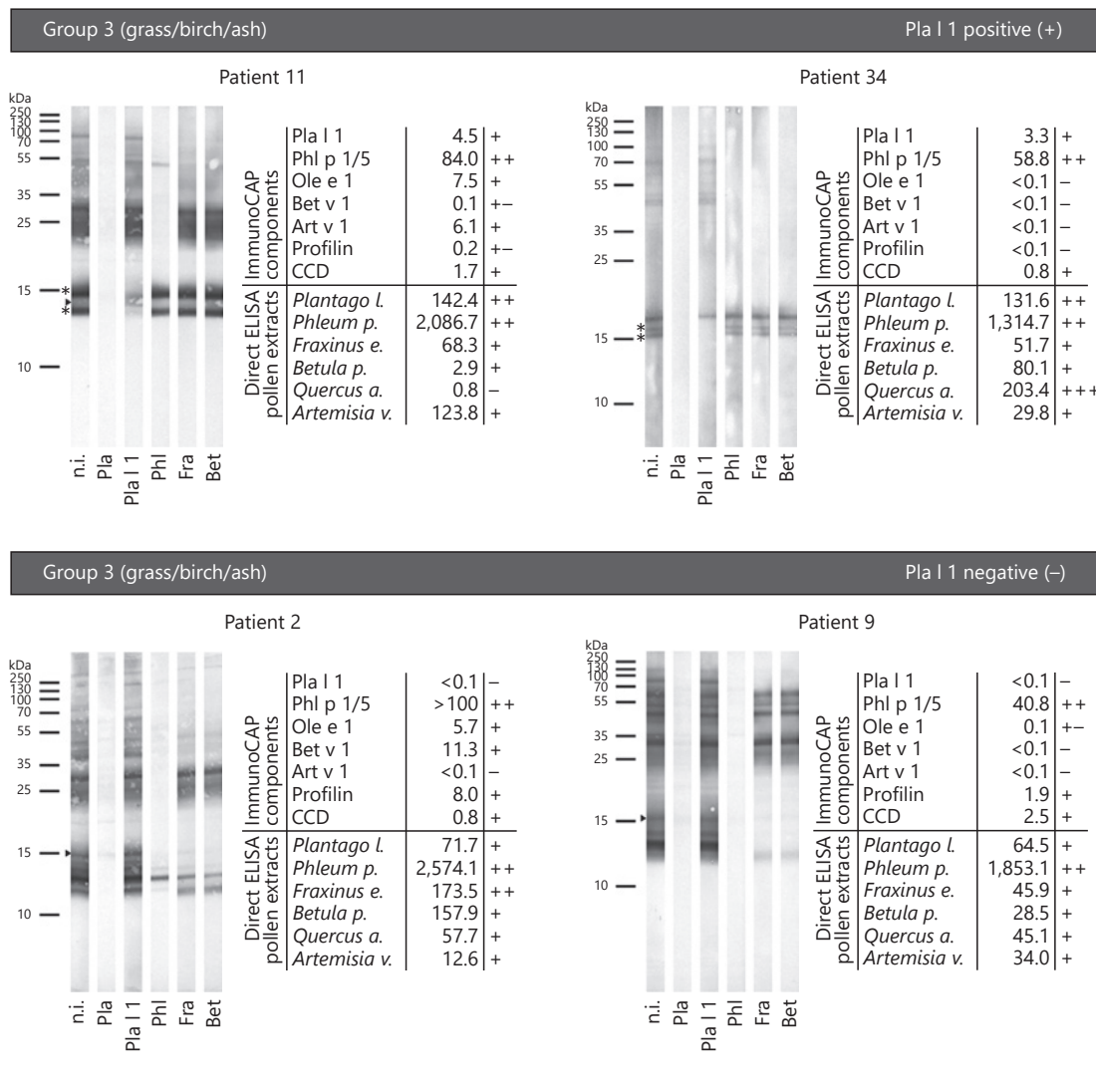


Fig. 3. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of group 3 (grass, birch, or ash). Reactivity to Pla I 1 and profilin is marked with an asterisk and triangle, respectively. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla I 1, inhibited with recombinant non-glycosylated Pla I 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excelsior* extract; Bet, inhibited with *Betula pen-*

dula extract. ImmunocAP results are given as kU_A/L. -, <0.1 kU/L; +-, 0.1–0.35 kU/L; +, >0.35 kU/L; ++, ≥15 kU/L. ELISA readouts are given as relative luminescence units ×10⁴. -, value below 50% of threshold; +-, value below threshold; +, value above threshold; ++, value above 25% of maximum value; +++, value above 75% of maximum value.

pollen in 17 sera (group 3, *n* = 17). Sensitization to Pla I 1 was observed in 50, 40, and 35% of the sera in these respective groups (online suppl. Fig. S7).

Using grass pollen for inhibition, we observed complete inhibition for 15 patients and partial inhibition for 13 different patients. No inhibition was observed in the sera of patients 28 and 32 (group 1). In cases of incom-

plete or no inhibition with grass, the uninhibited reactivity was usually directed against proteins migrating at 12–17 kDa, and to a lesser extent to bands at 40 and 55 kDa, respectively. In addition to grass, pollens of ash and birch were able to further inhibit IgE reactivity to English plantain in the sera of 17 patients. Generally, the degree of inhibition was less compared to that of grass pollen. Most

frequently, reactivity to proteins migrating at 14, 15, 17, 35, 55, and >80 kDa was inhibited, while an IgE-reactive protein at 30 kDa was typically not affected. Notably, identical IgE patterns were observed when using ash or birch pollen for inhibition.

Six sera did not show detectable IgE reactivity to plantain extract in immunoblot (online suppl. Fig. S4). Except for patient 27 who showed low IgE titer towards Pla l 1 (0.26 kU/L), all remaining were negative for Pla l 1 in ImmunoCAP analysis. Four of the immunoblot-negative patients, however, presented weak but positive reactivity in ELISA. A lack of IgE signals in immunoblot in contrast to positive results in SPT and ELISA is a consequence of lower sensitivity of this assay as well as denaturing and reducing conditions during gel electrophoresis.

Discussion

In this study, we analyzed the sera from French patients suffering from allergic symptoms in spring and/or summer who presented positive SPT reactivity to English plantain. All patients were multisensitized to several pollen sources, predominately grass, birch, and ash, which coincides with the high prevalence of those plants in the investigated geographic region [5, 8, 13]. As anticipated, none of the patients reacted solely to English plantain in SPT [13, 32, 35]. A similar picture was observed when investigating in vitro IgE reactivity to 6 different pollen extracts using ELISA. Typically, antibody levels for grass and birch were substantially higher compared to all other tested extracts, which points again at the relevance of these allergen sources in this geographic region. Interestingly, in contrast to all other tested extracts, grass and birch pollen did not correlate with results from SPT with plantain most likely due to the distinct and very dominant major allergens in these pollens.

Notably, only 34.3% of the patients were sensitized to the major English plantain allergen Pla l 1, while 94.3% reacted to the major grass pollen allergens Phl p 1/5. Apart from homologous and cross-reactive molecules, this pattern is frequently observed for plantain-sensitized patients and points at the true co-sensitization to plantain and grass pollen, indicated by genuine reactivity to Pla l 1 and Phl p 1/5, respectively [16]. In this cohort, substantial sensitization was also found for birch and ash pollen, indicated by >60% prevalence of sensitization to the marker allergens Bet v 1 and Ole e 1, respectively. In contrast, only 17.6% were reactive to Art v 1, the major allergen from mugwort pollen, indicating less allergological

relevance in this geographic region. As shown in previous studies, patients reactive to English plantain typically demonstrate a complex multisensitization profile which was additionally corroborated by IgE reactivity to marker allergens from different allergen sources. Regarding the in vivo results of the SPT reactivity to plantain, solely Pla l 1 demonstrated a significant correlation. This result is astonishing as only 34.3% were sensitized to this molecule, further corroborating the high specificity and usability of Pla l 1 as marker allergen for genuine sensitization. In addition, the weak but non-significant correlation of profilin and CCD reactivity potentially indicates involvement in IgE cross-reactivity. However, both molecules are rather weak mediators in histamine release assays or in vivo IgE tests, and the clinical relevance of sensitization is still debated [36–38]. It is, however, interesting to note that profilin and CCD sensitization prevalence differed between the groups. Sensitization to profilin was 0% in group 1, 10% in group 2, and 65% in group 3, while sensitization to CCD was 0% in group 1, 40% in group 2, and 59% in group 3. This suggests that more complex and highly cross-reactive IgE reactivity profiles might at least partially involve reactivity to profilin and CCDs.

As neither SPT nor IgE determination using extracts allows efficient discrimination of IgE co-sensitization and cross-reactivity, we performed inhibition experiments to immunoblotted plantain extract using grass, birch, and ash pollen extracts. In concordance with the Pla l 1 results from ImmunoCAP, we noted IgE reactivity to a double band around 15–17 kDa in immunoblot. The molecule exists as non-glycosylated and glycosylated variant, hence explaining the typical appearance as 2 bands. It has been recently demonstrated that the polypeptide backbone of Pla l 1 does not demonstrate IgE cross-reactivity with other allergens of the Ole e 1-like protein family [15, 17–19]. Consequently, IgE reactivity to the double band was inhibited with recombinant Pla l 1 but not with other pollen, even though they harbor allergenic (olive, ash, grass) as well as non-allergenic (birch) Ole e 1-like molecules. [20, 39, 40]. For 2 patients (No. 12 and 14) only the lower band was inhibited, suggesting additional IgE antibodies directed against the glycan moiety of Pla l 1 [17]. Since reactivity to glycans is not affected by any other tested extract, those IgE antibodies might be specific for the complex N-linked glycan structures of the major plantain pollen allergen [17].

In the sera of 8 patients, pollen extracts but not Pla l 1 were able to inhibit IgE reactivity to a protein migrating around 15 kDa. On a speculative basis, this protein might represent the recently reported calmodulin from plantain

pollen [8, 30]. However, given the fact that polcalcin sensitization seems to be mostly irrelevant in these patients, this could represent another yet unidentified cross-reactive protein. IgE reactivity with a protein migrating around 14 kDa was typically inhibited by all extracts, suggesting the involvement of the recently identified profilin Pla l 2 [8, 31]. This was corroborated by the fact that all patients showing this pattern were positive to profilin in ImmunoCAP. We can, however, not fully exclude overlapping patterns of profilin and other IgE reactive proteins at this molecular weight range. A protein at around 12 kDa was found to be exclusively inhibited by grass extract using the sera of patients 2 and 12, suggesting IgE cross-reactivity due to grass pollen sensitization [41].

Moderate IgE reactivity to a 25-kDa protein was inhibited by grass pollen in the sera of 11 patients. This molecular weight might correspond to English plantain triosephosphate isomerase with a sensitization prevalence of 19.2% [8]. However, the cross-reactive allergen present in grass pollen might also be grass group 5 or a manganese superoxide dismutase [42]. Fifteen patients reacted to a 30-kDa band in English plantain extract which was almost completely inhibited by grass pollen extract. Extensive cross-reactivity between a 30-kDa protein from English plantain and grass pollen was reported before and attributed to Phl p 5 [16]. In accordance with this, all aforementioned patients were also tested positive for Phl p 1/5 in ImmunoCAP. Interestingly, for some patients (5, 12, 15, and 19), inhibition of this protein was more pronounced when using grass pollen extract compared to self-inhibition with English plantain pollen. This fact could be ascribed to stronger antibody affinity to Phl p 5 compared to the English plantain homolog or excess of grass group 5 protein in *Phleum pratense* extract as observed in gel electrophoresis in Figure 1a. In addition, trace amounts of grass pollen in the plantain pollen batch might have caused this observation, explaining a more profound inhibition with, e.g., Phl p 5. A band at 35 kDa was detected by the sera of 12 patients with medium to strong intensity. Earlier reports suggested reactivity to a Pla l 1 dimer [15]; however, since recombinant Pla l 1 was not able to inhibit the signal at 35 kDa, this can be ruled out for our cohort. It is rather suggested to be a grass pollen homolog, potentially involving group 1 or 5 grass pollen allergens as described above [16, 43]. Interestingly, previous proteomic studies did not reveal the presence of such homologs in English plantain pollen extract [8, 14]. Ten patients showed weak IgE reactivity to a band at 40 kDa which might relate to phosphoglycerate kinase or fructose-bisphosphate aldolase [8, 14]. A

similar reactivity was also detected in a previous study involving the sera from English plantain-monosensitized patients [16]. In our study, IgE reactivity was typically abolished with grass pollen extract, suggesting the presence of several highly cross-reactive allergens in the range from 25 to 35 kDa between plantain and grass pollen.

Further, rather weak IgE reactivity against a 55-kDa protein was noted in 9 patients. The molecule was previously identified as ATP synthase subunit beta or adenosylhomocysteinase, and reactivity has been observed before in immunoblot with patients' sera [8, 14–16]. Mostly, reactivity was inhibited by grass pollen extract, while for some sera, ash and birch pollen showed more potent inhibitory capacities. IgE reactivity to a 66-kDa protein was described before [15]. Indeed, some of our sera reacted to a protein migrating at a similar molecular weight, but no clear inhibition patterns were observed. Reactivity to proteins >90 kDa were detected in several patients, and inhibition was achieved by all investigated extracts. We therefore speculate that all these reactivities might be due to cross-reactive molecules potentially involving carbohydrate moieties, as this reactivity pattern was mostly observed in the sera of CCD-positive patients.

In summary, we found highly diverging IgE reactivity against English plantain pollen and diverse patterns of inhibition with other relevant pollen sources. Considering all available information from this study, we identified 13 patients with “true” plantain sensitization which corresponds to 37.1% of the study population. Of those, all except patient 28 were positive to Pla l 1 in ImmunoCAP, reacted to plantain pollen extract in SPT and ELISA, and reported symptoms in May and June. The majority of the patients in this cohort is, however, sensitized to grass pollen and, based on ELISA, 15 out of 35 patients demonstrate a stronger reactivity to this allergen source. Reactivity of only 2 patients' sera to plantain was not altered by inhibiting extracts, corresponding to a correct diagnosis of plantain sensitization. Without using Pla l 1 as marker molecule for genuine sensitization, the majority of the remaining patients would have been diagnosed positive merely as a result of cross-reactive molecules in grass, birch, or ash. Our findings are in agreement with 2 epidemiological studies from Spain investigating reactivity to Pla l 1 in a pollen allergic population which also presented a strong association between plantain and grass pollen sensitization [44, 45]. However, outcomes of this study differ from the data in an Austrian cohort where the vast majority of plantain-sensitized patients was also reactive to Pla l 1 [8]. Thus, IgE profiles upon plantain pol-

len exposure seem to be heterogeneous and depend on investigated patients and/or geographic region.

The frequent multisensitization of plantain-allergic patients leads to complex IgE reactivity profiles. This circumstance does not allow efficient discrimination of co- and cross-sensitization when using plantain pollen extract for diagnosis. Thus, the most reliable diagnostic marker for genuine plantain sensitization to date is Pla 1 1, as it is highly specific for the source and not prone to diagnostic errors due to absent IgE cross-reactivity with homologous molecules [46].

Statement of Ethics

The subjects (or their parents or guardians) have given their written informed consent. The study protocol has been approved by the research institute's Committee on Human Research.

References

- Gadermaier G, Hauser M, Ferreira F: Allergens of weed pollen: an overview on recombinant and natural molecules. *Methods* 2014; 66:55–66.
- Stemeseder T, Hemmer W, Hawranek T, Gadermaier G: Marker allergens of weed pollen – basic considerations and diagnostic benefits in the clinical routine: Part 16 of the Series Molecular Allergology. *Allergo J Int* 2014; 23:274–280.
- Stemeseder T, Klinglmayr E, Moser S, Lufotenegger L, Lang R, Himly M, Oostingh GJ, Zumbach J, Bathke AC, Hawranek T, Gadermaier G: Cross-sectional study on allergic sensitization of Austrian adolescents using molecule-based IgE profiling. *Allergy* 2017; 72:754–763.
- Gadermaier G, Dedic A, Obermeyer G, Frank S, Himly M, Ferreira F: Biology of weed pollen allergens. *Curr Allergy Asthma Rep* 2004;4: 391–400.
- Pauli G, Nardi A, Metz-Favre C, de Blay F: Clinical pertinence of skin tests in pollinosis due to plantain: the value of molecular allergen assays. *Rev Fr Allergol* 2016;56:98–100.
- Scevkova J, Dusicka J, Hrubisko M, Micieta K: Influence of airborne pollen counts and length of pollen season of selected allergenic plants on the concentration of sIgE antibodies on the population of Bratislava, Slovakia. *Ann Agric Environ Med* 2015;22:451–455.
- Holm L, Pancho JV, Herberger JP, Plucknett DL: *A Geographical Atlas of World Weeds*. Toronto, John Wiley and Sons Inc., 1979.
- Gadermaier G, Eichhorn S, Vejvar E, Weillnbock L, Lang R, Briza P, Huber CG, Ferreira F, Hawranek T: *Plantago lanceolata*: an important trigger of summer pollinosis with limited IgE cross-reactivity. *J Allergy Clin Immunol* 2014;134:472–475 e475.
- D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Luccardi G, Popov T, van Cauwenberge P: Allergenic pollen and pollen allergy in Europe. *Allergy* 2007;62: 976–990.
- Bosch-Cano F, Bernard N, Sudre B, Gillet F, Thibaudon M, Richard H, Badot PM, Ruffaldi P: Human exposure to allergenic pollens: a comparison between urban and rural areas. *Environ Res* 2011;111:619–625.
- D'Amato G, Mullins J, Noland N, Spieksma FT, Wachter R: City spore concentrations in the European Economic Community (EEC). VII. Oleaceae (*Fraxinus*, *Ligustrum*, *Olea*). *Clin Allergy* 1988;18:541–547.
- D'Amato G, Spieksma FT, Luccardi G, Jager S, Russo M, Kontou-Fili K, Nikkels H, Wuthrich B, Bonini S: Pollen-related allergy in Europe. *Allergy* 1998;53:567–578.
- Pauli G, Hutt N, Stchetchicova O: Pollinosis to oak, plane tree, plantago and mugwort: myth or reality? *Rev Fr Allergol* 2014;54:557–565.
- Sousa R, Osorio H, Duque L, Ribeiro H, Cruz A, Abreu I: Identification of *Plantago lanceolata* pollen allergens using an immunoproteomic approach. *J Investig Allergol Clin Immunol* 2014;24:177–183.
- Calabozo B, Barber D, Polo F: Purification and characterization of the main allergen of *Plantago lanceolata* pollen, Pla 1 1. *Clin Exp Allergy* 2001;31:322–330.
- Asero R, Mistrello G, Roncarolo D, Casarini M: Detection of allergens in plantain (*Plantago lanceolata*) pollen. *Allergy* 2000;55: 1059–1062.
- Calabozo B, Barber D, Polo F: Studies on the carbohydrate moiety of Pla 1 1 allergen. identification of a major N-glycan and significance for the immunoglobulin E-binding activity. *Clin Exp Allergy* 2002;32:1628–1634.
- Calabozo B, Diaz-Perales A, Salcedo G, Barber D, Polo F: Cloning and expression of biologically active *Plantago lanceolata* pollen allergen Pla 1 1 in the yeast *Pichia pastoris*. *Biochem J* 2003;372:889–896.
- Stemeseder T, Freier R, Wildner S, Fuchs JE, Briza P, Lang R, Batanero E, Lidholm J, Liedl KR, Campo P, Hawranek T, Villalba M, Brandstetter H, Ferreira F, Gadermaier G: Crystal structure of Pla 1 1 reveals both structural similarity and allergenic divergence within the Ole e 1-like protein family. *J Allergy Clin Immunol* 2017;140:277–280.
- Marknell DeWitt A, Niederberger V, Lehtonen P, Spitzauer S, Sperr WR, Valent P, Valenta R, Lidholm J: Molecular and immunological characterization of a novel timothy grass (*Phleum pratense*) pollen allergen, Phl p 11. *Clin Exp Allergy* 2002;32:1329–1340.
- Lombardero M, Obispo T, Calabozo B, Lezaun A, Polo F, Barber D: Cross-reactivity between olive and other species. Role of Ole e 1-related proteins. *Allergy* 2002;57(suppl 71): 29–34.
- Niederberger V, Hayek B, Vrtala S, Laffer S, Twardosz A, Vangelista L, Sperr WR, Valent P, Rumpold H, Kraft D, Ehrenberger K, Valenta R, Spitzauer S: Calcium-dependent immunoglobulin E recognition of the apo- and calcium-bound form of a cross-reactive two EF-hand timothy grass pollen allergen, Phl p 7. *FASEB J* 1999;13:843–856.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

The study was supported by Sparkling Science (www.sparkling-science.at), a program of the Federal Ministry of Science, Research and Economy of Austria (SPA 04/065-Alraune). The funding was assigned to the University of Salzburg. The funders had no role in study design, data collection and analysis, decision to publish, or the preparation of the manuscript.

Author Contributions

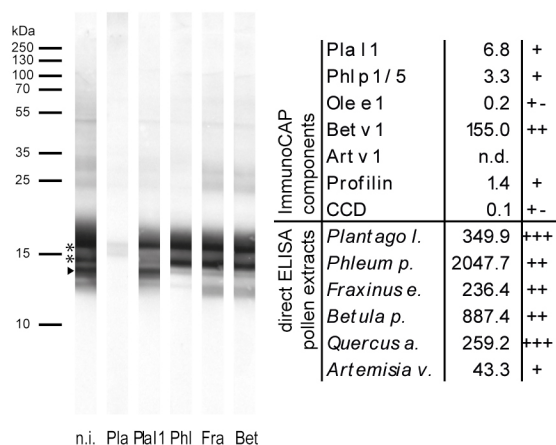
G.G. and G.P. conceived the study. T.S., C.M.-F., and F.d.B. performed the experiments. T.S., G.G., G.P., and C.M.-F. analyzed the data. G.G. and T.S. wrote the manuscript.

- 23 Valenta R, Ball T, Vrtala S, Duchene M, Kraft D, Scheiner O: cDNA cloning and expression of timothy grass (*Phleum pratense*) pollen profilin in *Escherichia coli*: comparison with birch pollen profilin. *Biochem Biophys Res Commun* 1994;199:106–118.
- 24 Niederberger V, Purohit A, Oster JP, Spitzauer S, Valenta R, Pauli G: The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from various plant species. *Clin Exp Allergy* 2002;32:933–941.
- 25 Valenta R, Duchene M, Breitenbach M, Pettenburger K, Koller L, Rumpold H, Scheiner O, Kraft D: A low molecular weight allergen of white birch (*Betula verrucosa*) is highly homologous to human profilin. *Int Arch Allergy Appl Immunol* 1991;94:368–370.
- 26 Engel E, Richter K, Obermeyer G, Briza P, Kungl AJ, Simon B, Auer M, Ebner C, Rheinberger HJ, Breitenbach M, Ferreira F: Immunological and biological properties of Bet v 4, a novel birch pollen allergen with two EF-hand calcium-binding domains. *J Biol Chem* 1997;272:28630–28637.
- 27 Egger C, Focke M, Bircher AJ, Scherer K, Mothes-Luksch N, Horak F, Valenta R: The allergen profile of beech and oak pollen. *Clin Exp Allergy* 2008;38:1688–1696.
- 28 Asturias JA, Arilla MC, Gomez-Bayon N, Martinez J, Martinez A, Palacios R: Cloning and expression of the panallergen profilin and the major allergen (Ole e 1) from olive tree pollen. *J Allergy Clin Immunol* 1997;100:365–372.
- 29 Wopfner N, Willeroidee M, Hebenstreit D, van Ree R, Aalbers M, Briza P, Thalhamer J, Ebner C, Richter K, Ferreira F: Molecular and immunological characterization of profilin from mugwort pollen. *Biol Chem* 2002;383:1779–1789.
- 30 Wopfner N, Gruber P, Wallner M, Briza P, Ebner C, Mari A, Richter K, Vogel L, Ferreira F: Molecular and immunological characterization of novel weed pollen pan-allergens. *Allergy* 2008;63:872–881.
- 31 Moya R, Rubio V, Beitia JM, Carnes J, Lopez-Matas MA: Purification and immunochemical characterization of Pla l 2, the profilin from *Plantago lanceolata*. *Mol Immunol* 2017;83:100–106.
- 32 Nakamaru Y, Maguchi S, Oridate N, Takagi D, Furuta Y, Fukuda S: *Plantago lanceolata* (English plantain) pollinosis in Japan. *Auris Nasus Larynx* 2005;32:251–256.
- 33 Javier F, Emilio F, Montserrat V, Victor S, Purificaci NG: Evolution of the incidence of pollen grains and sensitivity to pollen in the city of Elche (Spain). *Asian Pac J Allergy Immunol* 2015;33:196–202.
- 34 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.
- 35 Couto M, Miranda M: Proposed GA2LEN standardized allergen battery: what about regional sensitization differences? *J Investig Allergol Clin Immunol* 2011;21:491–492.
- 36 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;169:101–107.
- 37 Altmann F: Coping with cross-reactive carbohydrate determinants in allergy diagnosis. *Allergo J Int* 2016;25:98–105.
- 38 Rodriguez Del Rio P, Diaz-Perales A, Sanchez-Garcia S, Escudero C, Ibanez MD, Mendez-Brea P, Barber D: Profilin, a change in the paradigm. *J Investig Allergol Clin Immunol* 2018;28:1–12.
- 39 Marazuela EG, Hajek R, Villalba M, Barber D, Breiteneder H, Rodriguez R, Batanero E: A non-allergenic Ole e 1-like protein from birch pollen as a tool to design hypoallergenic vaccine candidates. *Mol Immunol* 2012;50:83–90.
- 40 Barderas R, Purohit A, Papanikolaou I, Rodriguez R, Pauli G, Villalba M: Cloning, expression, and clinical significance of the major allergen from ash pollen, Fra e 1. *J Allergy Clin Immunol* 2005;115:351–357.
- 41 Ledesma A, Barderas R, Westritschnig K, Quiralte J, Pascual CY, Valenta R, Villalba M, Rodriguez R: A comparative analysis of the cross-reactivity in the polcalcin family including Syr v 3, a new member from lilac pollen. *Allergy* 2006;61:477–484.
- 42 Conti A, Alessio M, Pesca M, Soldarini A, Breda D, Pigatto PD, Burastero SE: Phleum pratense manganese superoxide dismutase identified by proteomic: a new candidate grass allergen. *Ann Allergy Asthma Immunol* 2014;112:261.
- 43 Petersen A, Becker WM, Schlaak M: Epitope analysis of isoforms of the major allergen Phl p V by fingerprinting and microsequencing. *Clin Exp Allergy* 1994;24:250–256.
- 44 Barber D, de la Torre F, Feo F, Florido F, Guardia P, Moreno C, Quiralte J, Lombardero M, Villalba M, Salcedo G, Rodriguez R: Understanding patient sensitization profiles in complex pollen areas: a molecular epidemiological study. *Allergy* 2008;63:1550–1558.
- 45 Barber D, de la Torre F, Lombardero M, Antepará I, Colas C, Davila I, Tabar AI, Vidal C, Villalba M, Salcedo G, Rodriguez R: Component-resolved diagnosis of pollen allergy based on skin testing with profilin, polcalcin and lipid transfer protein pan-allergens. *Clin Exp Allergy* 2009;39:1764–1773.
- 46 Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al: EAACI Molecular Allergy User's Guide. *Pediatr Allergy Immunol* 2016;27(suppl 23):60–66.

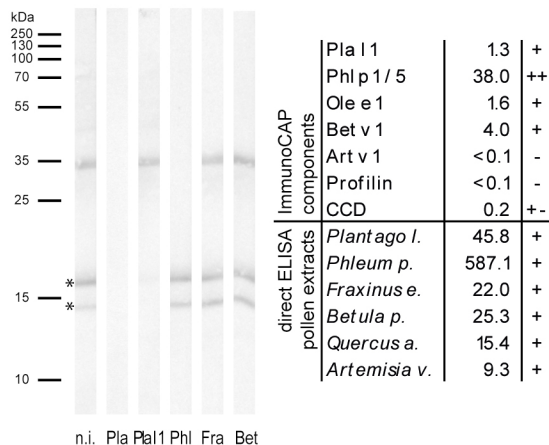
Group 2 (grass)

Pla I 1 POSITIVE (+)

Patient 14



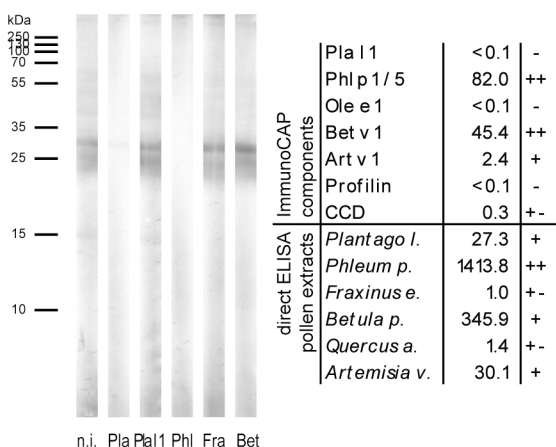
Patient 20



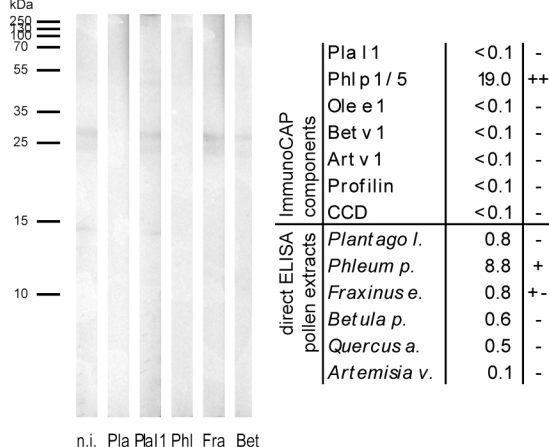
Group 2 (grass)

Pla I 1 NEGATIVE (-)

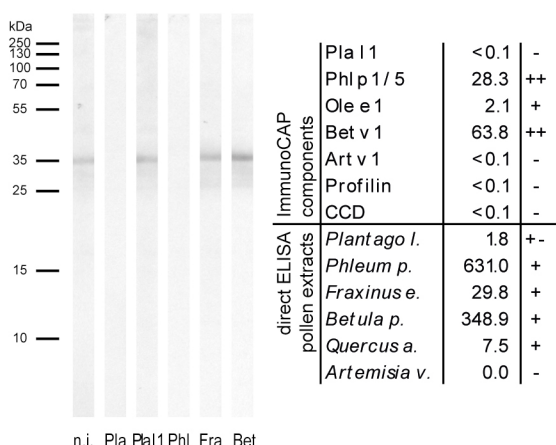
Patient 6



Patient 7



Patient 31



Patient 35

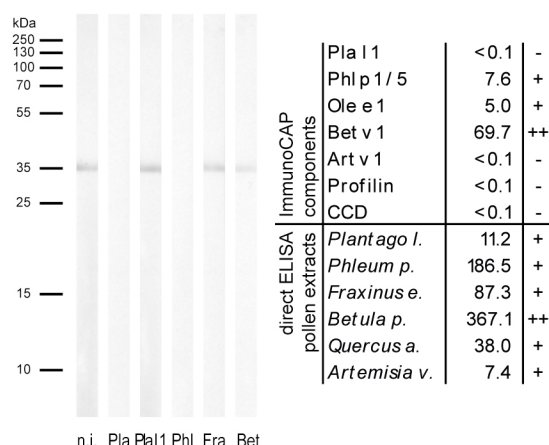
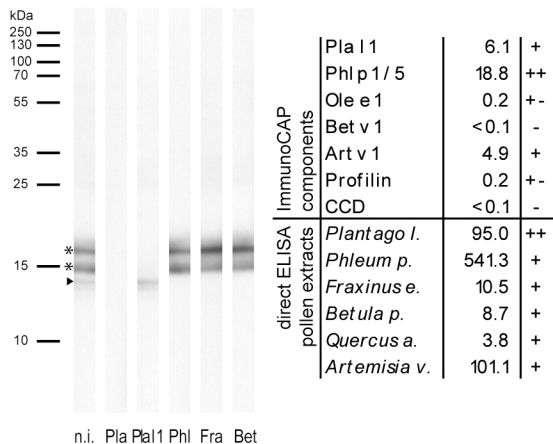
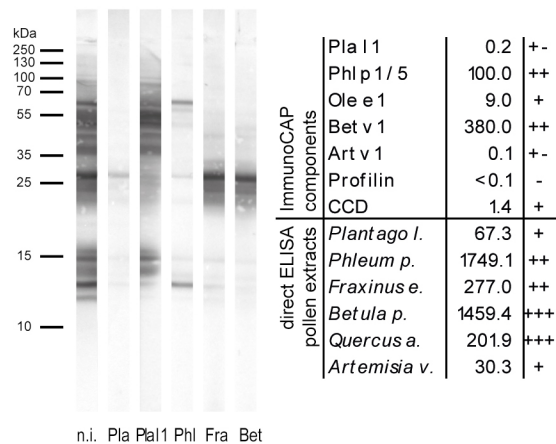


Figure S1. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of group 2 (grass). Reactivity to Pla I 1 and profilin is marked with an asterisk and triangle, respectively. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla I 1, inhibited with recombinant, non-glycosylated Pla I 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excelsior* extract; Bet, inhibited with *Betula pendula* extract. ImmunoCAP results are given as kUA/L, -, <0.1 kUA/L; +-, 0.1-0.35 kUA/L; +, >0.35 kUA/L; ++, ≥15 kUA/L. ELISA readouts are given as relative luminescence units *104. -, value below 50% of threshold; +-, value below threshold; +, value above threshold; ++, value above 25% of maximum value; +++, value above 75% of maximum value; n.d., not determined

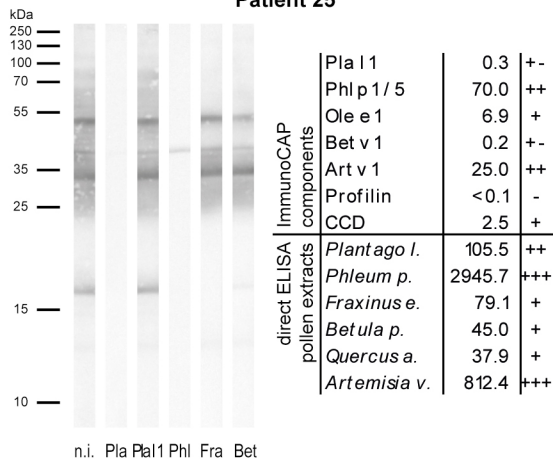
Patient 17



Patient 19



Patient 25



Patient 30

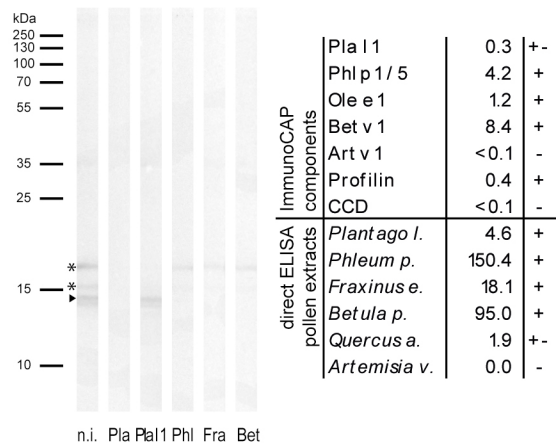


Figure S2. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of group 3 (grass/birch/ash). Reactivity to Pla I 1 and profilin is marked with an asterisk and triangle, respectively. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla I 1, inhibited with recombinant, non-glycosylated Pla I 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excelsior* extract; Bet, inhibited with *Betula pendula* extract. ImmunocAP results are given as KUA/L, -, <0.1 KUA/L; +-, 0.1-0.35 KUA/L; +, >0.35 KUA/L; ++, ≥15 KUA/L. ELISA readouts are given as relative luminescence units *104. -, value below 50% of threshold; +-, value below threshold; +, value above threshold; ++, value above 25% of maximum value; +++, value above 75% of maximum value; n.d., not determined

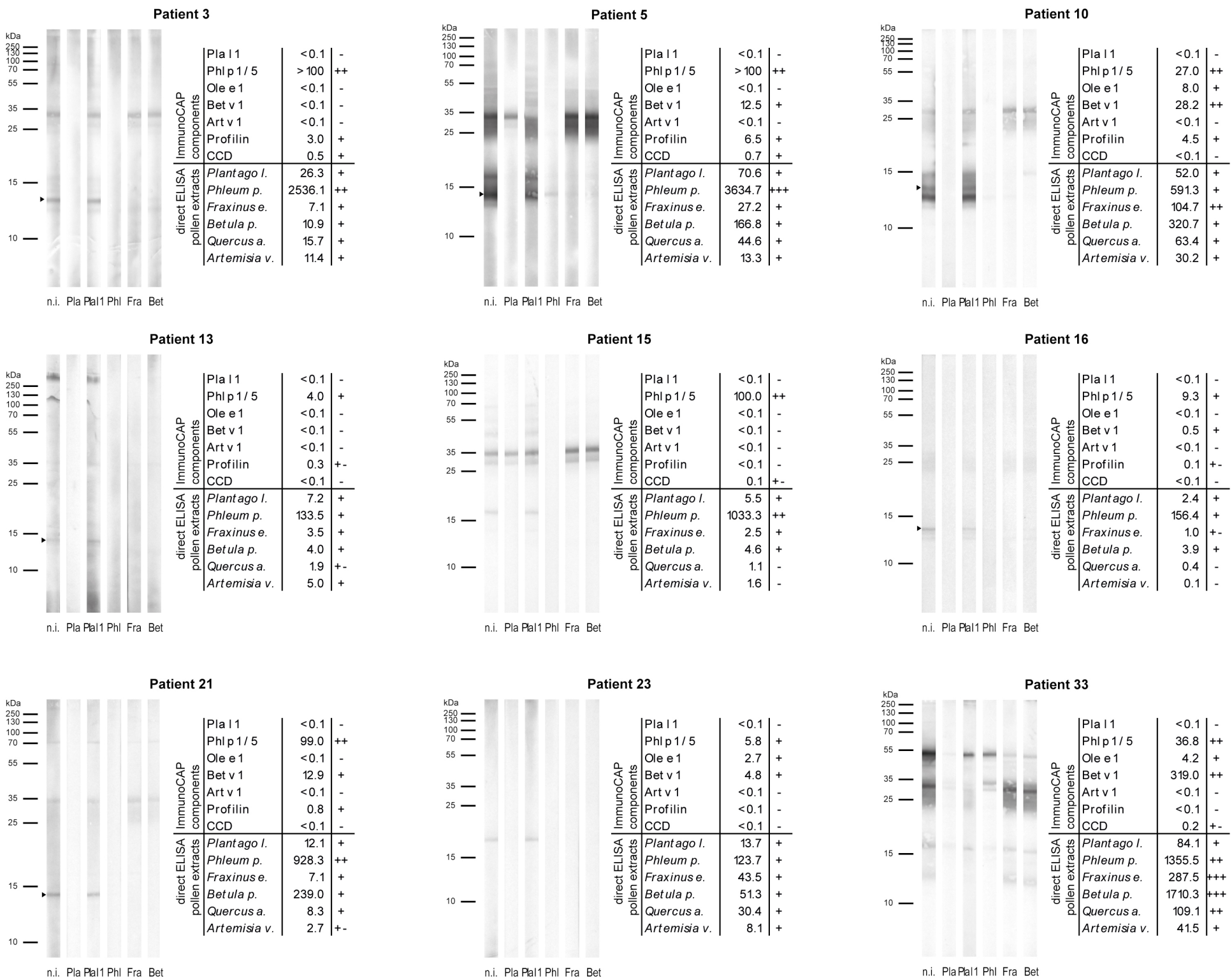
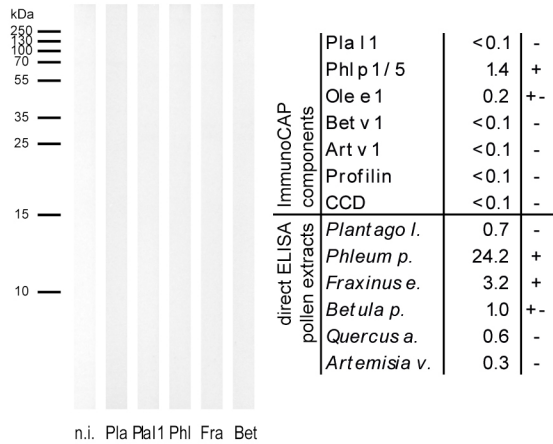


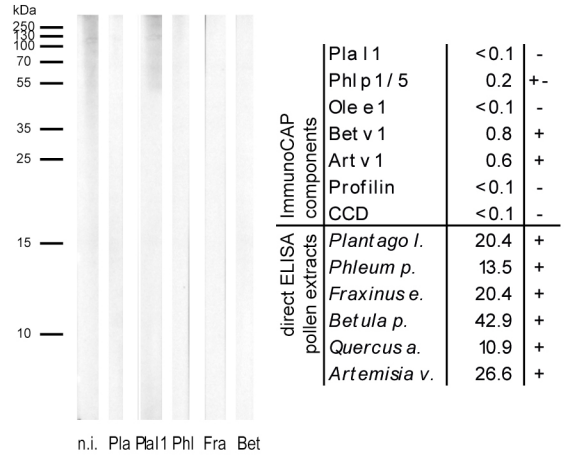
Figure S3. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of group 3 (grass/birch/ash). Reactivity to Pla I 1 and profilin is marked with an asterisk and triangle, respectively. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla I 1, inhibited with recombinant, non-glycosylated Pla I 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excelsior* extract; Bet, inhibited with *Betula pendula* extract. ImmunocAP results are given as kU/L; -, <0.1 kU/L; +-, 0.1-0.35 kU/L; +, >0.35 kU/L; ++, ≥15 kU/L. ELISA readouts are given as relative luminescence units *104. -, value below 50% of threshold; +-, value below threshold; +, value above threshold; ++, value above 25% of maximum value; +++, value above 75% of maximum value; n.d., not determined

no reactivity to plantain extract in immunoblot

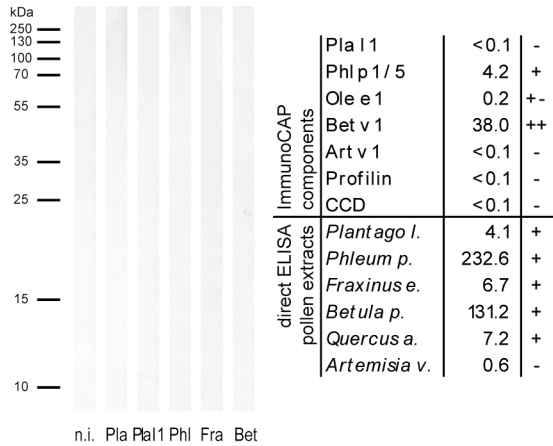
Patient 4



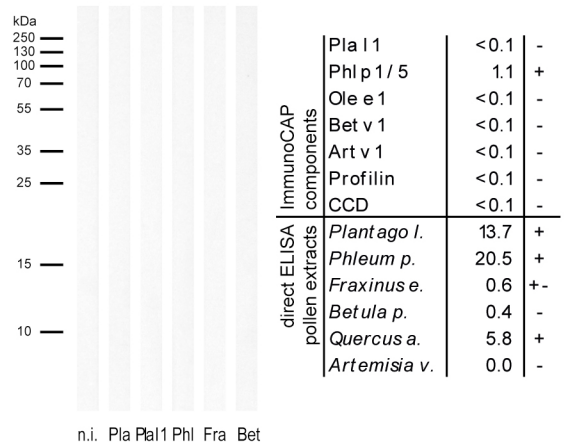
Patient 8



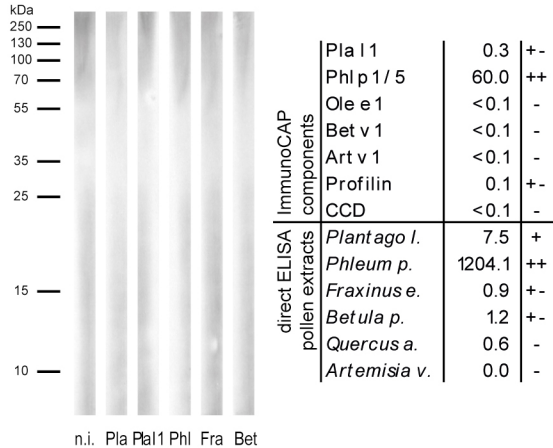
Patient 22



Patient 26



Patient 27



Patient 29

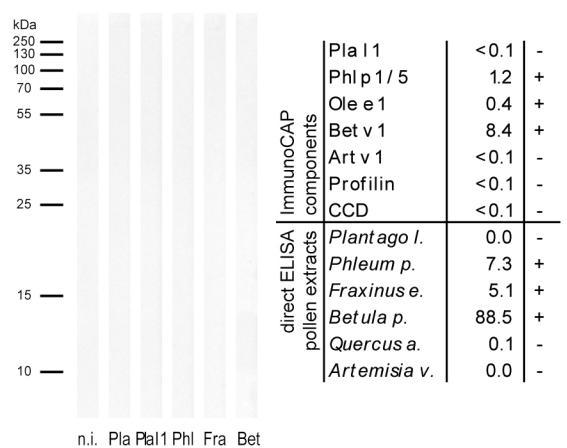


Figure S4. IgE reactivity and inhibition to *Plantago lanceolata* extract in immunoblot of sera not reactive in immunoblot. Immunoblots: n.i., non-inhibited; Pla, inhibited with *Plantago lanceolata* extract; Pla I 1, inhibited with recombinant, non-glycosylated Pla I 1; Phl, inhibited with *Phleum pratense* extract; Fra, inhibited with *Fraxinus excelsior* extract; Bet, inhibited with *Betula pendula* extract. ImmunoCAP results are given as kUa/l, -, <0.1 kUa/l; +-, 0.1-0.35 kUa/l; +, >0.35 kUa/l; ++, ≥15 kUa/l. ELISA readouts are given as relative luminescence units *104. -, value below 50% of threshold; +-, value below threshold; +, value above threshold; ++, value above 25% of maximum value; +++, value above 75% of maximum value; n.d., not determined

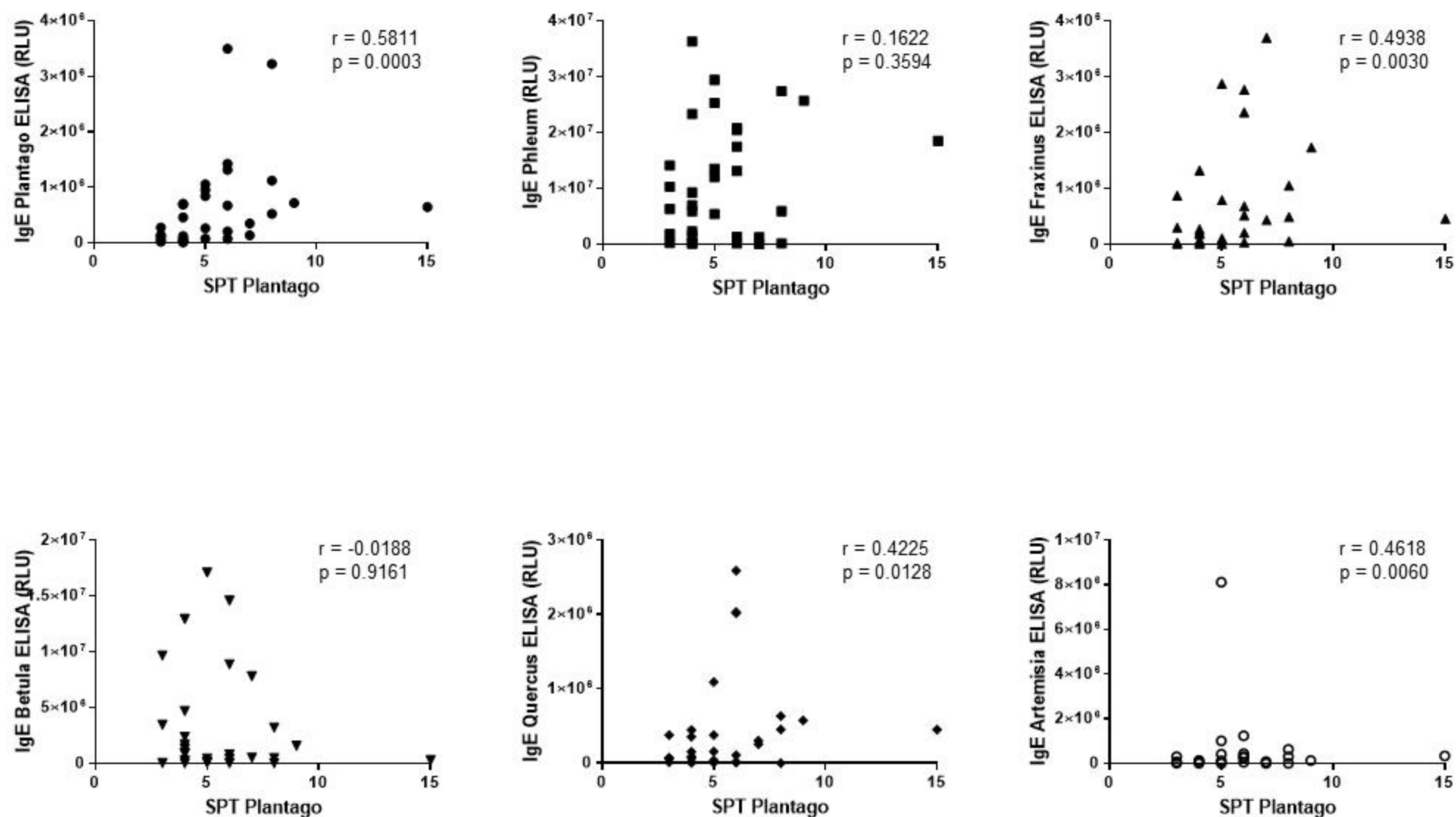


Figure S5: Correlation of *Plantago lanceolata* skin prick test (SPT) results and IgE reactivity to pollen extracts determined in ELISA. RLU, relative luminescence units

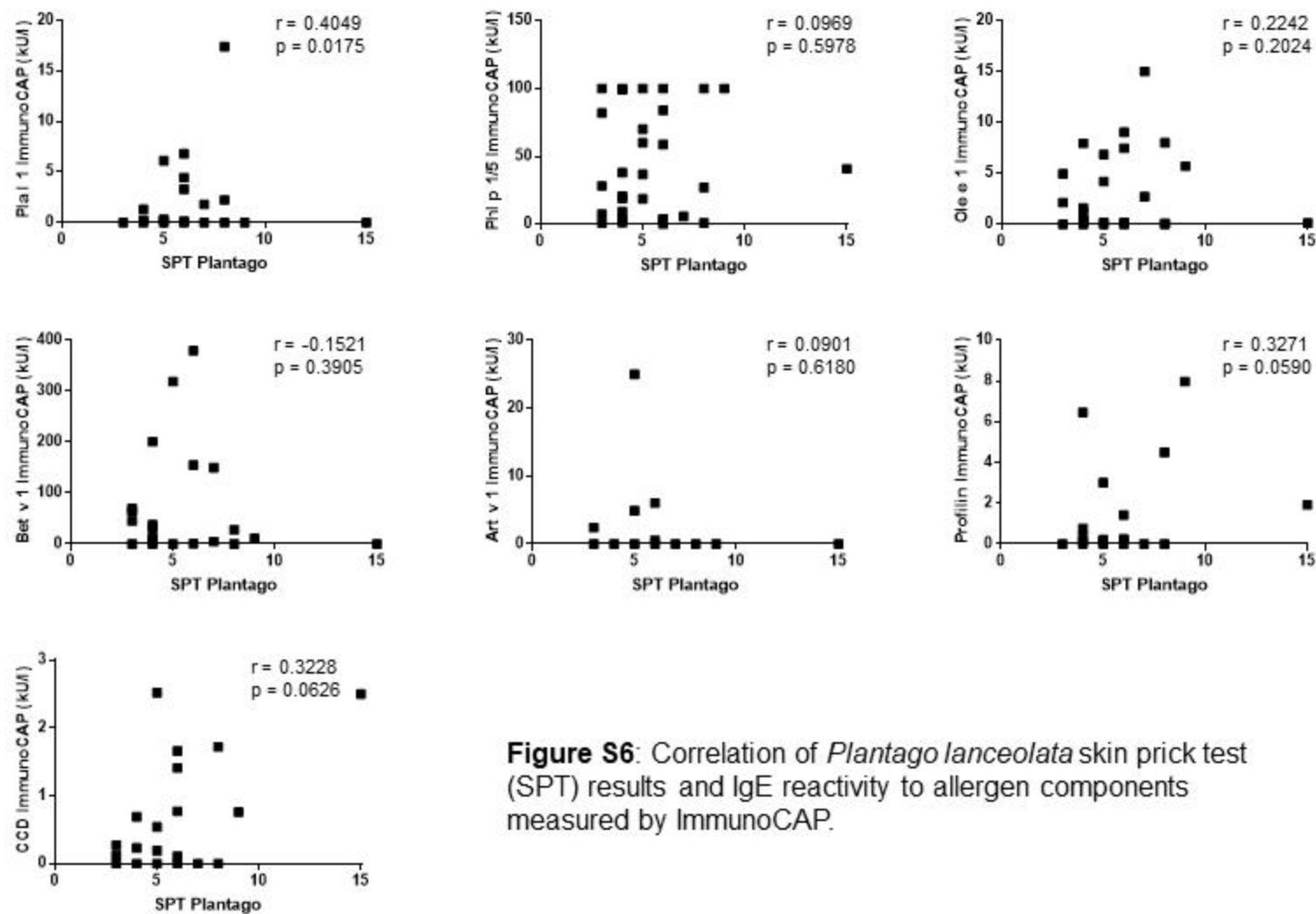


Figure S6: Correlation of *Plantago lanceolata* skin prick test (SPT) results and IgE reactivity to allergen components measured by ImmunoCAP.

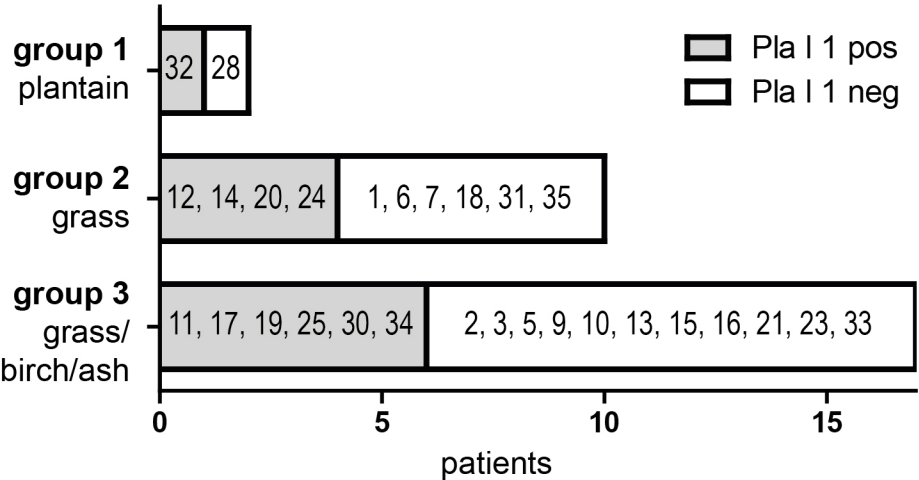


Figure S7: Scheme of patients assigned to 3 inhibition groups. Results from 29 sera (positive signals in immunoblots) were clustered based on their inhibition profile with plantain (group 1), grass (group 2), grass/birch/ash (group 3) pollen. Individual sera are indicated by numbers and sensitization to Pla I 1 is indicated.

Table S1. Demographic data, allergic symptoms and skin prick test results of patients

P #	Age (y)	Gende r	Centre	Symptoms	Months of symptoms	Skin prick test					
						plantain	grass	ash	birch	oak	mugwort
1	54	f	CHU	RC, U	5 - 6	4	8	7	+	n.d.	3
2	33	m	SNCF	RC, OAS	5 - 6	9	9	-	9	n.d.	n.d.
3	14	f	HAG	RC, A, OAS	5 - 7	5	10	5	-	n.d.	3
4	27	f	SNCF	RC, A	4 - 7	4	6	6	-	n.d.	-
5	13	f	HAG	RC, A	5 - 7	4	10	4	5	n.d.	4
6	55	f	CHU	RC,OAS	5 - 7	3	8	-	9	7	9
7	42	f	CHU	RC	5 - 6	4	7	-	-	-	-
8	49	m	SNCF	RC	2 - 7	6	8	-	10	-	12
9	31	m	SNCF	RC, A	4 - 6	15	10	7	5	n.d.	6
10	33	m	SNCF	RC, A, OAS	3 - 9	8	10	10	10	n.d.	8
11	28	m	CHU	RC, A	5 - 6	6	7	6	-	-	10
12	36	m	SNCF	RC, A	5 - 6	8	10	4	-	-	-
13	35	m	CHU	RCA	4 - 8	6	12	5	4	-	4
14	13	m	HAG	RC, OAS	5 - 7	6	8	6	6	n.d.	n.d.
15	7	m	HAG	RC, A	3 - 7	3	4	3	-	n.d.	-
16	46	m	CHU	RC	5 - 6	4	8	5	7	3	3
17	17	m	HAG	RC, A	5 - 7	5	6	2	-	n.d.	8
18	43	m	SNCF	RC, A, OAS	1, 4, 7	4	-	5	4	n.d.	n.d.
19	13	m	HAG	RC, OAS	3 - 6	6	6	-	8	n.d.	-
20	13	m	HAG	RC	2 - 6	4	6	12	4	n.d.	3
21	18	f	HAG	RC, OAS	3 - 7	4	10	4	8	n.d.	n.d.
22	34	f	SNCF	RC, A	2 - 3	4	6	6	5	n.d.	5
23	35	m	SNCF	RC, A	4 - 7	7	10	12	12	n.d.	4
24	31	f	CHU	RC	5 - 7	8	8	-	6	-	-
25	33	m	CHU	RC, A, OAS	5 - 6	5	9	-	-	-	12
26	61	f	CHU	RC	3 - 8	3	6	3	-	n.d.	-
27	16	f	HAG	RC	4 - 7	5	8	-	3	n.d.	-
28	46	f	CHU	RC, OAS	2 - 7	+	+	+	+	n.d.	n.d.
29	40	m	CHU	RC, A	5 - 8	4	6	6	8	4	-
30	14	f	HUS	RC, OAS	3 - 6	4	7	5	6	5	3
31	30	m	SNCF	RC, A	3 - 6	3	10	7	7	n.d.	n.d.
32	55	f	CHU	RC, A, OAS	2 - 3	7	-	10	8	n.d.	-
33	26	m	SNCF	RC, A	2 - 6	5	12	5	12	n.d.	-
34	23	m	CHU	RC	5 - 7	6	10	-	-	-	-
35	28	f	CHU	RC, A, OAS	2 - 7	3	5	10	10	10	-
Ø =		f=15				35/35	33/35	26/35	25/35	5/13	16/29
31.2		m=20				100%	94.3%	74.3%	71.4%	38.5%	55.2%

CHU, University Hospital Strasbourg; SNCF, Outpatient Clinic of the Railway Station Strasbourg; HAG, Hospital of Haguenau; RC, rhinoconjunctivitis; U, contact urticarial; OAS, oral allergy syndrome; A, asthma; skin prick test results are given in mm; +, positive reactivity; -, negative; n.d., not determined.