

ORIGINAL ARTICLE

Clinical Allergy

Clinical reactivity of celery cultivars in allergic patients: Role of Api g 1

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Summary

Background: Celery (*Apium graveolens* L.) is a vegetable consumed world-wide. Celery stalks and celeriac roots are often ingredients in convenient food products like spice blends and soups.

Objective: In this study, we examined the allergenicity of distinct celeriac cultivars.

Methods: Sixteen celery-allergic patients were identified using a double-blind, placebo-controlled food challenge. Ten different celeriac cultivars were used for skin prick testing in the patients. Two cultivars were further applied for oral food challenges; their protein composition was analysed by immunoblotting, and contents of major allergen Api g 1 were quantified.

Results: From the 10 investigated celeriac cultivars, two cultivars elicited significantly different skin reactivity ("Anita": 5.0 [2.0-12.0] mm vs "Prinz": 7.0 [3.0-9.5] mm; $P = .047$). Moreover, "Anita" induced fewer symptoms after a controlled oral-celeriac challenge in 14 patient ($P < .001$). The protein profiles on 2DE protein gels showed distinct protein patterns and higher protein amounts of Api g 1 in "Prinz" than in "Anita."

Conclusions and Clinical Relevance: Taken together, the data from this study suggest that cultivar Anita is better tolerated in celery-allergic patients than "Prinz." Differences in the protein expression profile between the cultivars, particularly the different content of Api g 1, could cause the different allergenicity.

KEYWORDS

celeriac, celery, DBPCFCs, food allergy, symptom score

1 | INTRODUCTION

Food allergy is a major health problem and has been reported to affect 5%-6% of children and 2%-3% of the adult population.^{1,2} In adults and particularly in Central Europe, food allergy is often caused by a cross-reactivity to the major birch pollen allergen Bet v 1 (*Betula verrucosa*). Bet v 1-homologues cause primarily mild oral allergy symptoms but may also induce severe systemic reactions.³ One common food allergen source that is cross-reactive with Bet v 1 is celery (*Apium graveolens* L.).

The cross-reactivity of Bet v 1 is mediated by Api g 1, which belongs, like Bet v 1, to pathogenesis-related protein family 10 (PR-10).⁴ In hypochloric conditions in the stomach, Api g 1 can acquire features of a complete allergen and sensitize via this route.⁵ To a lesser extent, cross-reactivity can be mediated via Api g 4, a profilin and Bet v 2 homologue.^{6,7} In addition, a cross-reactivity between celery and mugwort (*Artemisia vulgaris* L.) is known, but the responsible cross-reactive allergens have not been completely defined yet. One allergen molecule relevant in the "mugwort-celery-birch-spice-syndrome" is Api g 5, a high-molecular weight glycoprotein. It has been shown that Api g 5-specific IgE antibodies recognize similar structures in mugwort pollen⁶ and spices.⁸ In addition, a highly homologous allergen to Api g 5 has been described in fennel.⁹ Recently, mimotopes for Api g 5 were generated allowing to study cross-reactivity phenomena in the celery-mugwort-birch-spice syndrome.¹⁰ Gadermaier et al¹¹ identified two different lipid transfer proteins (LTPs), Api g 2 and Api g 6. At least Api g 2 seems to be cross-reactive to mugwort LTP (Art v 3).¹² One additional celery allergen was described and confirmed by the International Union of Immunological Societies (IUIS database: www.allergen.org; Table S1).

Celery is a vegetable consumed world-wide⁶ because of its flavour and health benefits. Depending on the variety and the geographical region, celery stalks (*A. graveolens* L. var. *dulce* (Mill.) DC.), smallage leaves (*A. graveolens* L. var. *secalinum* Alef.) or celeriac tubers (*A. graveolens* L. var. *rapaceum* (Mill.) Poir.) are eaten. Nowadays, these plant parts are more often consumed raw, in salads, as a snack or mixed in juice. In particular, celeriac tubers are processed and are the ingredients of many convenient food products, such as spice blends and soups.

Allergic symptoms after celery consumption can range from mild oral reactions to moderate and even severe systemic reactions, including life-threatening anaphylactic shock.¹²⁻¹⁴ Moreover, the fact that celery is often hidden in convenient foods has led to the mandatory declaration of celery in food products (European regulation 1169/2011⁸).

Currently, therapeutic and preventative treatments for subjects suffering food allergies have been proposed.¹³ The use of food with less potential to convey allergies has become a research target. Besides genetically modified plants with reduced allergen content,¹⁴⁻¹⁶ other strategies are followed, which are based on the assumption that particular inherent genetic and environmental factors influence the likelihood of allergic reactions to fruits and vegetables.¹⁷⁻²¹

In this study, we investigated celeriac cultivars recommended by breeders and selected based on their different characteristics by skin prick tests (SPT) and oral food challenges to define a low-allergenic cultivar. By immunoblot analysis and calculation of the Api g 1 allergen concentration, the protein profiles of the cultivars were investigated in more detail.

2 | METHODS

2.1 | Celery-allergic patients

Inclusion criteria for the selection of patients were a positive history of allergic reactions after the ingestion of celery and a positive SPT to celery. All subjects (18-65 years) were recruited in the Allergy-Centre-Charité, Berlin, Germany. The study was approved by the local ethics committee (Charité – Universitätsmedizin Berlin, EK-No.: 1832/Si.258) and was conducted in accordance with the principles of the Declaration of Helsinki. All included subjects gave written informed consent.

2.2 | Plant material

Celeriac (*A. graveolens* L. var. *rapaceum* (Mill.) Poir.) tubers were grown in the field during the summer at the Leibniz Institute of Vegetable and Ornamental Crops, Großbeeren, Germany. The following cultivars were used as follows: Ibis, Kojak, Goliath (Enza Zaden Deutschland GmbH, Dannstadt, Germany), Bergers weiße Kugel, Mars, Monarch, Prinz, Rex (Hild, Samen GmbH (now Nunhems), Marbach, Germany), Anita and Brilliant.

2.3 | In vivo sensitization test

The SPT was performed according to the recommendations of GA²LEN.²² Commercial extracts of common inhalants (ALK-Abelló, Hamburg, Germany) were used. Freshly harvested celeriac tuber material was applied to perform prick-to-prick tests in duplicates. Histamine dihydrochloride (10 mg/mL; ALK-Abelló) and saline solution (pH 7.4; ALK-Abelló) served as positive and negative controls, respectively. The weal diameter was measured as the mean of the largest diameter and the corresponding vertical diameter. The SPT was considered positive when the weal diameter was ≥ 3 mm after 15 minutes in the absence of a reaction to the negative control. Aeroallergen-sensitized subjects without any adverse reactions to celeriac allergens served as controls.

2.4 | Total and specific IgE

Serum samples were stored at $-20^{\circ}\text{C}/-80^{\circ}\text{C}$ until use. The total and specific IgE to celery extract (f85), rApi g 1.01 (f417), rBet v 1 (t215), rBet v 2 (t216), rPrup 3 (f420), nArt v 3 (w233) and cross-reactive carbohydrate determinants (CCD, MUXF3, Ro215) were measured with the ImmunoCAP System (Thermo Fisher Scientific, Uppsala, Sweden) according to the manufacturer's instructions. In

addition, serum samples were tested on a custom-designed allergen-chip ISAC131 (ImmunoCAP ISAC, Thermo Fisher Scientific, Vienna, Austria), where additionally to 112 standard allergens on ISAC112, nApi g 5 (and other 18 allergens non-relevant for this study) were spotted by Thermo Fisher Scientific. The microarray serum test was then performed following the manufacturer's instructions, and results are expressed in ISU (ISAC Standardized Units).

2.5 | Oral-celeriac challenge

A clinically relevant celery allergy and the severity of a reaction to different celeriac cultivars were proven by double-blind, placebo-controlled food challenges (DBPCFCs). DBPCFCs were carried out according to the guideline of the European Academy of Allergy and Clinical Immunology,²³ as described previously.¹⁷ The challenge meal comprised 50 g celeriac blinded with a hypoallergenic infant formula (Nestlé Nutrition GmbH, Frankfurt am Main, Germany), rice flakes (Demeter e.V., Darmstadt, Germany), sugar (Südzucker AG, Zeitz, Germany), cocoa (Krüger GmbH, Bergisch Gladbach, Germany), peppermint syrup (Monin®, Birmensdorf, Switzerland) and a trace of aroma (orange; SHS Gesellschaft für klinische Ernährung GmbH, Heilbronn, Germany). The placebo meal was prepared in a similar manner without celeriac. Challenge meals were prepared shortly before the challenge. Celeriac roots were freshly harvested and delivered twice a week. Between the challenge tests in a given subject, a minimum of 1 day was required.

To compare the severity of symptoms among different celeriac cultivars, we used a standardized symptom scoring procedure as described previously.¹⁷ Briefly, the symptoms are classified into categories based on the affected organs: skin/mucosa, gastrointestinal tract, cardiovascular and respiratory system. Subjective symptoms (eg pruritus, tingle, abdominal pain, throat tightness and headache) are considered to a lesser extent (1 point). Objective symptoms are calculated according to their severity from 2 points (eg urticarial or erythema) to 6 points (eg cardiac arrest). Rhinitis/conjunctivitis is scored with 2 points and add to the respiratory system.

2.6 | Two-dimensional protein gels

Celeriac tubers of the cultivars Anita and Prinz were crushed in a buffer (25 mmol L⁻¹ Tris/HCl pH 7.4, 50 mmol L⁻¹ KCl, 1.5 mmol L⁻¹ EDTA, 2.9 mmol L⁻¹ benzamidine, 2.1 µmol L⁻¹ leupeptin, 1 mmol L⁻¹ phenylmethylsulphonyl fluoride, 1 µmol L⁻¹ pepstatin A) containing 7 mol L⁻¹ guanidine hydrochloride, immediately frozen and stored until use at -80°C. Three biological replicates were used for each cultivar, each containing ten celeriac tubers. Samples were thawed and centrifuged. Proteins were precipitated from the supernatant with ethanol overnight at -20°C. The pellet was resuspended in extraction buffer containing 7 mol L⁻¹ urea, 2 mol L⁻¹ thiourea, 2% carrier ampholytes (pH 2-4; Serva, Heidelberg, Germany) and 70 mmol L⁻¹ DTT. Protein concentrations were determined by Bradford assays (Proteome Factory AG, Berlin, Germany).

2DE was conducted following the protocol by Klose and Kobalz,²⁴ blotted on PVDF membrane and immune stained with pooled serum, as described previously in detail by Welter et al.²¹ Immune-positive spots were in-gel digested with trypsin, and peptides were analysed in a nanoLC-ESI-MS/MS. Proteins were identified using MS/MS ion search of the Mascot search engine (Matrix Science, London, England) and a protein database (National Center for Biotechnology Information (NCBI), Bethesda, USA).

For the Api g 1 spot intensity calculation, the 2DE gels were digitized at a resolution of 150 dpi using a PowerLook 2100XL scanner with a transparency adapter. 2D image analysis and protein spot quantification were performed using the Proteomweaver software 3.1 (Definiens AG, Munich, Germany). The quantification was based on the size and density of the respective protein spot, and the software displays values of single spots for further calculation.

2.7 | Statistics

Statistical analyses were carried out using Statistica (Tulsa, OK, USA) and IBM SPSS Statistics (Chicago, IL, USA). Api g 1 protein quantification data were subjected to one-way analysis of variance (ANOVA) procedures ($P \leq .05$). Clinical allergy tests were evaluated with non-parametric tests, and medians were separated by the Wilcoxon matched pairs test procedure ($P \leq .05$).

3 | RESULTS

3.1 | Identification of subjects with celery allergy

Of 31 eligible subjects, 27 underwent DBPCFCs, and 19 patients developed at least one objective symptom upon celeriac challenge (Figure 1). Sixteen patients were assessed further using SPT with ten different, and oral challenge tests with two celeriac cultivars. The median age of the 16 patients was 29 (20-49) years. Further clinical details and the sensitization profile to selected pollen and food allergens are depicted in Table 1.

3.2 | Identification of cultivars with high and low SPT reactivity

The 16 identified celery-allergic patients underwent SPT with ten different celeriac cultivars (Figure 2A). One patient had to be excluded from SPT analysis due to overlapping weals. The median weal diameter considering all cultivars was 6.0 [2.0-14.5] mm, with a large range of individual differences. A significant difference in SPT reactivity was only determined between the cultivars Anita (5.0 [2.0-12.0] mm) and Prinz (7.0 [3.0-9.5] mm, $P = .047$; Figure 2B).

3.3 | Clinical outcome of celeriac challenge differs among the cultivars

The celeriac cultivars Anita and Prinz were further investigated in the 16 celery-allergic patients by double-blind food challenges (Figure 3).

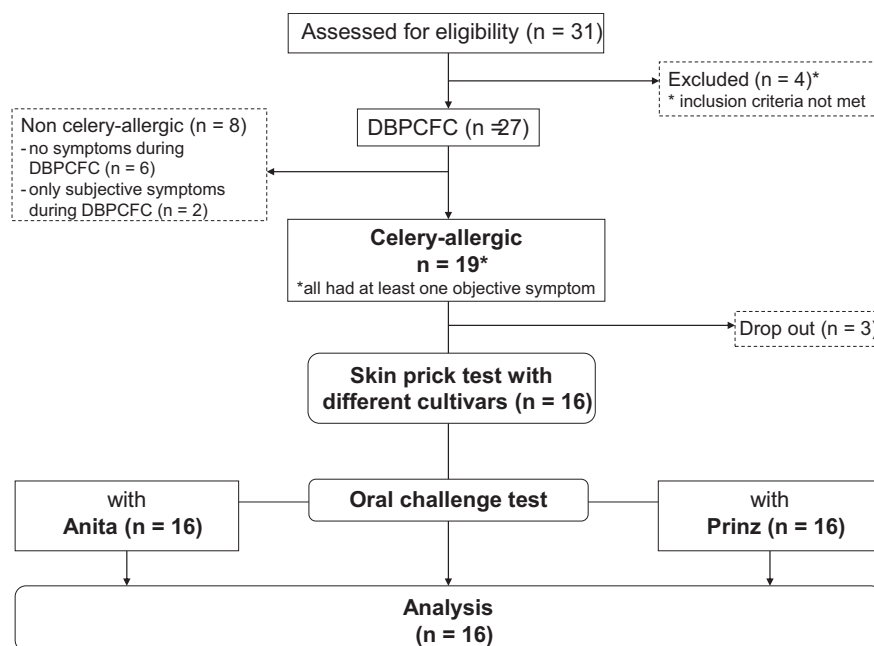


FIGURE 1 Study flow chart consort statement; DBPCFC—double-blind, placebo-controlled food challenge

Our previously published symptom score was used for comparative analysis.¹⁷ Upon food challenge using similar amounts of both celeriac cultivars, “Prinz” induced more symptoms in 14 patients and a significantly higher symptom score than “Anita” (Figure 3A). “Prinz” also gave rise to cardiovascular, respiratory and gastrointestinal symptoms more frequently (Figure 3B). Thus, objective reactions were observed in 63% of the patients after ingestion of the cultivar Prinz. In contrast, we observed significantly less objective reactions after “Anita” ingestion (19%). In three cases, emergency intervention was required after the oral challenge test with the cultivar Prinz.

3.4 | Protein profiles of the cultivars Anita and Prinz

To obtain the molecular basis for the cultivar-dependent differences in the clinical response to celeriac, we analysed the protein profiles of the cultivars Anita and Prinz on 2DE protein gels. The cultivars showed variable protein patterns (Figure 4). To identify responsible allergens, a serum pool of our 16 celery-allergic patients was incubated with the 2DE protein Western blot of “Anita” and “Prinz.” Via mass spectrometry, we could identify the known allergens Api g 1 and Api g 4 from the corresponding 2DE gel (Figure S1). Additionally, four proteins, Enolase 1, Actin, Peptidyl-prolyl cis-trans isomerase, and translationally controlled tumour protein, were identified as novel putative allergens.

The protein spot quantification of the major celery allergen Api g 1 was significantly higher in “Prinz” than in “Anita” (spot intensity: 32.3 ± 3.2 vs 23.2 ± 3.1 , $P = .047$, Figure 4).

4 | DISCUSSION

In this study, we hypothesized that distinct celeriac cultivars display differences in causing allergic symptoms. Indeed, two celeriac

cultivars—“Anita” and “Prinz”—exhibited differences in their allergenic activity assessed by both skin reactivity and clinical severity after a controlled oral-celeriac challenge. Although the protein profiles varied between the two cultivars, a significantly higher amount of the major allergen Api g 1 was identified in the cultivar Prinz and may explain the higher clinical reactivity to this cultivar in cutaneous application and oral allergen challenge.

Apium graveolens L. (wild celery) belongs to the family *Apiaceae*. One popular variety in Europe is celeriac (*A. graveolens* L. var. *rapaceum*), cultivated for its edible roots. In this study, ten different celeriac cultivars frequently sold on the German market were chosen because of their different characteristics. The cultivar Anita is primarily sold in the fresh market but is also storable for a longer time. It is high yielding and resistant to the pathogenic fungus *Septoria apicola*. The cultivar Prinz also has a high market share, but it is especially used for processing and therefore often found in convenient foods. Its edible root is round and smooth. “Prinz” has a white and solid inner quality and a broad resistance to diverse pathogens. It was expected that cultivars with increased resistance/tolerance characteristics against pathogens have a higher allergenic potential, because some defence proteins act also as allergens.^{20,21}

The cultivars Anita and Prinz had significant differences in the median skin reactivity investigated in a defined celery-allergic cohort. To emphasize whether these differences reflect clinical reactivity, we performed oral challenges with both celeriac cultivars. We used our previously published symptom score to evaluate differences in the symptom severity.¹⁷ The application of the symptom score to the outcome of oral challenges with the cultivars Anita and Prinz confirmed the results obtained from the SPT. The celeriac cultivar Anita induced fewer gastrointestinal and cardiovascular symptoms and no respiratory symptoms. Considering the current view on inducing natural tolerance with, for instance, baked milk in milk-allergic and baked egg in egg-allergic subjects,²⁵ a low-allergenic celery cultivar

TABLE 1 Baseline characteristics of the celery-allergic patients (n = 16). For numeric values, the median is shown with [minimum-maximum]

Patient number	Age (y)	Sex	Atopy		Reaction to cooked celery	IgE diagnostic (in kU/L)					slgE (multiplex, in ISU)					Skin prick test (in mm)			Symptom score in points Baseline ^a
			AD	AR		A	Total IgE	slgE (singleplex)				Art v 3	CCD	Api g 5	Birch	Mugwort	Grass		
								Celery	Api g 1	Bet v 1	Bet v 2							Pru p 3	
1	24	f	n	y	n	uk	82	1.0	1.0	15.7	0	0	0	0	9.5	8.0	7.0	7.0	
2	44	m	n	y	n	uk	111	0.7	2.2	13.8	0	0	0	0	6.0	0	0	2.5	
3	27	f	y	y	y	n	1164	5.7	5.6	>100	0	0	0	0	8.5	6.5	7.0	8.5	
4	24	f	y	y	y	uk	547	3.6	2.1	49.1	0	0	0	0	6.0	6.0	6.0	9.0	
5	23	f	n	y	n	n	22	0.5	0.4	4.8	0	0	0	0	6.0	0	3.0	3.0	
6	20	m	n	y	n	n	255	3.1	2.0	14.0	0	0	0	0	5.0	0	3.0	2.5	
7	45	f	y	y	y	y	454	8.4	3.5	16.4	0	0	0	0	7.0	0	7.0	7.0	
8	26	m	y	y	y	n	260	5.6	5.8	66.3	0	0	0	0.7	9.5	3.0	13.0	2.5	
9	38	f	n	y	y	n	46	0.3	0.5	15.0	0	0	0	0	8.5	10.5	5.0	3.0	
10	49	f	n	y	n	n	140	4.2	nd	nd	nd	nd	0.5	9.0	9.0	5.5	6.5		
11	39	f	n	y	n	n	603	0.8	0	0	0	0	0.8	0	3.0	9.0	0	7.5	
12	27	m	n	y	n	y	164	1.5	8.0	41.0	0	0	0	0	12.5	12.5	15.0	5.0	
13	45	f	n	y	y	y	343	0.6	0.9	17.2	0	0	0	0	15.0	10.0	5.5	11.0	
14	30	f	n	y	y	n	677	11.3	5.4	95.0	0	0	0	0.7	12.0	0	13.0	4.5	
15	25	f	n	y	n	y	141	1.8	4.1	29.0	0	0	0	0	9.5	0	8.0	4.5	
16	30	m	y	n	n	n	5000	15.1	0.5	0.4	6.0	0.3	1.2	8.8	7.0	4.0	7.0	3.5	
Median	29						258	2.5	2.1	16.4	0	0	0	0	9	9	7	4.8	
Min-max	20-49						22-5000	0.3-15.1	0.0-8.0	0.0-100	0.0-6.0	0.0-0.3	0.0-1.2	0.0-8.8	3-15	3-13	3-15	2.5-12.0	

f, female; m, male; AD, atopic dermatitis; AR, allergic rhinitis; A, Asthma; n, no; y, yes; uk, unknown; nd, not determined; CCD, cross-reactive carbohydrate determinant; ISU, ISAC Standardized Units.

^aBaseline celiac challenge was performed with a celiac challenge bought in a conventional supermarket.

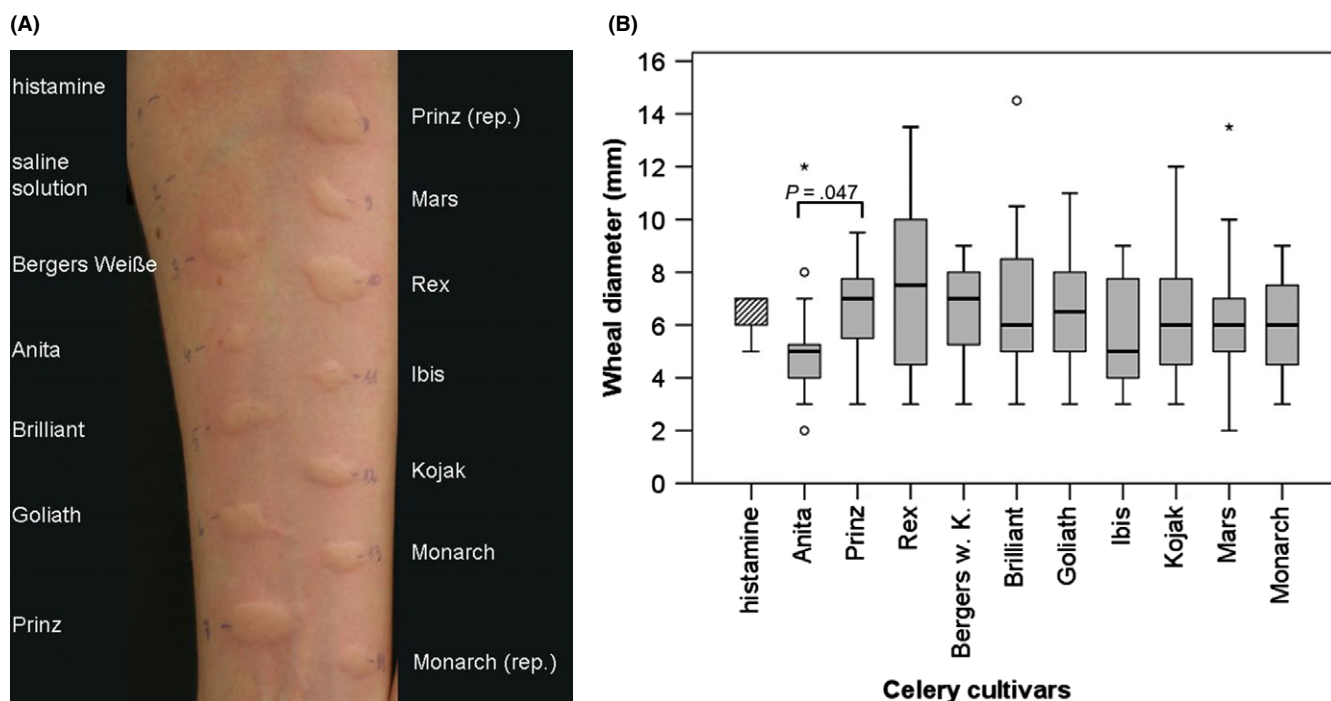


FIGURE 2 Skin prick test (SPT) reactivity differs between cultivars. A, Example picture of SPT with 10 different cultivars performed as prick-to-prick on the surface of the interior forearms. B, Results for 15 of 16 celery-allergic patients are shown; in one patient, the weals merged and could not be measured. The median is depicted as a black line. Histamine dichloride (10 mg/mL) was used as a positive control. Wheal diameters differ significantly between Anita and Prinz (Wilcoxon matched pairs test, $P < .05$, $n = 15$). Outliers are shown as dots and asterisks

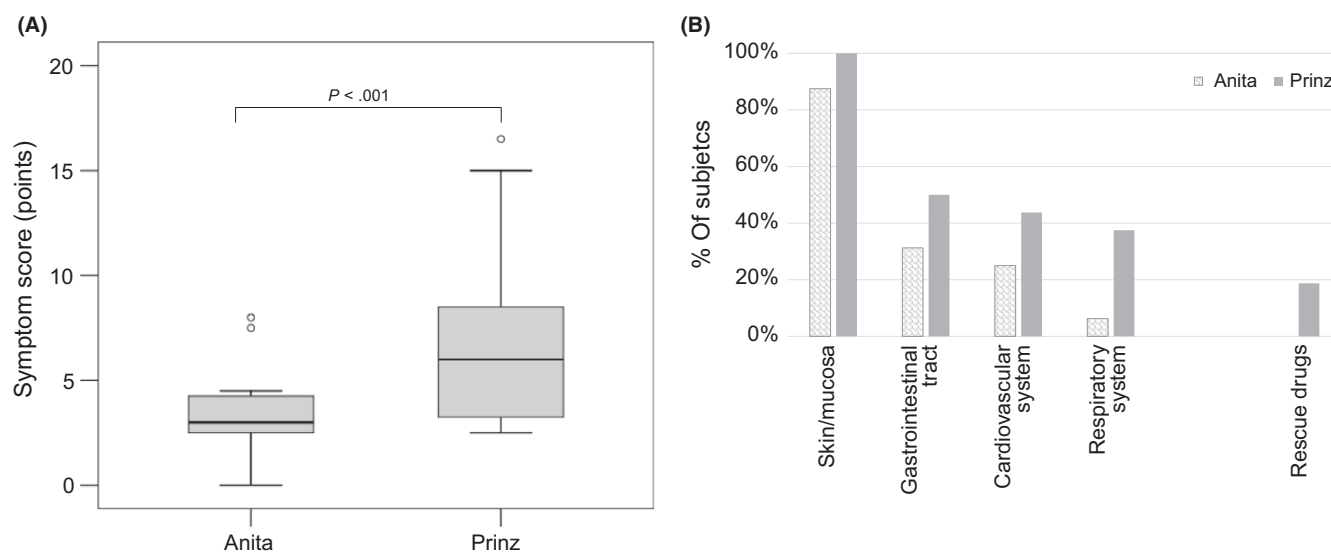


FIGURE 3 Outcome of the oral food challenge with the cultivars Anita and Prinz. A, Symptoms after celery challenge were evaluated according to a standardized symptom scoring.¹⁷ The median is depicted as a black line, and differences between the cultivars Anita and Prinz are statistically significant (Wilcoxon matched pairs test, $P < .001$, $n = 16$). B, The allergic symptoms that occurred after celery consumption are summarized and shown by percentage. *Skin/mucosa*: angioedema, pruritus, mucosal swelling, generalized erythema, increase of SCORAD score (>10 points), urticaria; *gastrointestinal tract*: nausea, stomach ache, diarrhoea, abdominal pain/cramp; *cardiovascular system*: drop/rise in blood pressure ($>/< 20$ mm Hg), reduced general health condition, dizziness, cool sweating, headache; *respiratory system*: decreased lung function, dyspnoea, throat tightness, lump in one's throat, rhinitis

might be useful to promote natural tolerance in celery-allergic individuals.

To study the molecular basis for the different clinical impact of the cultivars Anita and Prinz, 2DE protein gels were performed and

revealed cultivar-specific protein expression profiles. Their quantification showed substantial differences in the Api g 1 protein content: higher amounts of Api g 1 were contained in "Prinz" compared with "Anita," supporting the results from the SPT and oral challenge test.

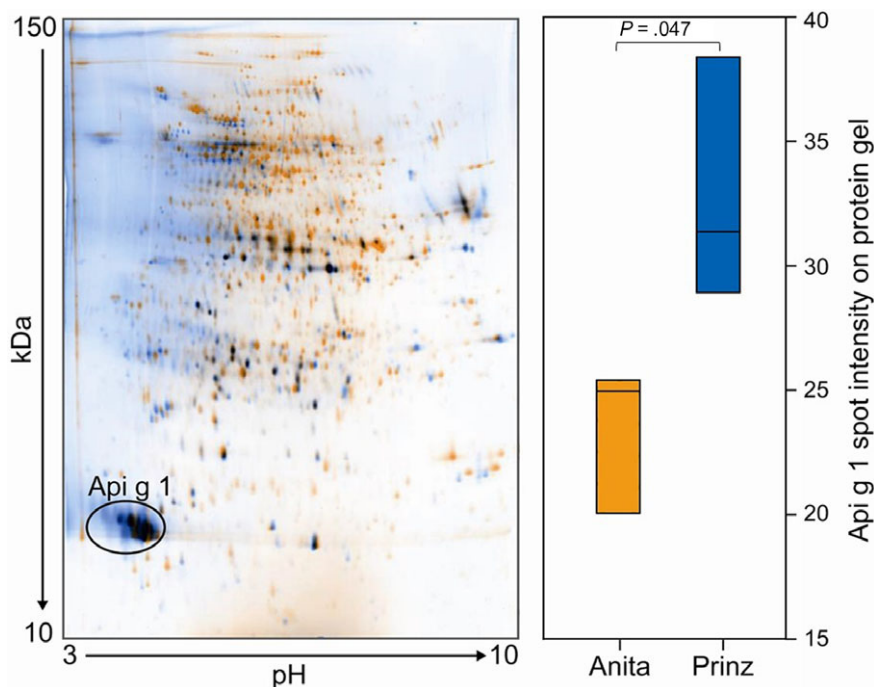


FIGURE 4 Protein profile comparison on 2DE gels with the two cultivars Prinz (blue) and Anita (orange). The overlay of the 2DE images and spot intensity calculation of Api g 1 was performed with Proteome Weaver software. The protein spot (Api g 1) was identified via GC-MS. Differences in the spot intensity of Api g 1 between the protein gels of Anita and Prinz were statistically significant (one-way ANOVA. $P < .05$, $n = 3$)

Api g 1 is a PR-10 protein and the major celery allergen in Central Europe.²⁶ The primary sensitization towards Api g 1 is caused by the major allergen Bet v 1 from birch pollen.²⁷ In our cohort, all patients were sensitized to birch pollen (Table 1). Additionally, for all subjects except one (no. 11) Bet v 1 and Api g 1-specific IgE were detectable. PR-10 proteins might be expressed differently depending on their location in the plant. The gene expression of PR-10 proteins in tomato leaves was strongly induced under pathogen attack (PepMV), which also occurred in the fruit, while the time kinetic differed.²⁰ Such different expressions of PR-10 proteins were also previously described in different apple cultivars²⁸ or horticultural strain of bell peppers²⁹ and are in line with our results.

The content and turnover of phenolic compounds can additionally affect the allergenicity of fruits and vegetables.^{30,31} The total phenol content was similar between the investigated cultivars Anita and Prinz (Folin-Ciocalteu method,³² data not shown). However, not the total content but the specific composition of phenolic compounds might be the reason for differences in the characteristics of different celery cultivars.³² Similar results regarding the phenolic composition were presented for peanuts and apples.^{30,31} Thus, future studies should consider the phenolic profile of celeriac cultivars.

The second known allergen identified on the 2DE immunoblots in the cultivars Anita and Prinz was the profilin Api g 4. However, profilins may not be clinically relevant in our cohort as sIgE to Bet v 2, one representative of the profilin family, was negative in almost all subjects (Table 1). Therefore, differences in the profilin expression most likely have no impact on the clinical allergenic activity of the cultivars. For one subject (no. 11), we could not detect IgE sensitization to any of the recombinant allergens measured. The total IgE was elevated with 603 kU/L and sIgE to celery extract, and CCDs

were low but detectable (0.8 kU/L each). This subject showed different symptom scores between "Anita" (2.5 points) and "Prinz" (5.5 points). However, the relevant allergen could not be identified. One subject (no. 16) showed sIgE to Bet v 2, CCD, LTPs and Api g 5. Taking into account that this subject suffered from atopic dermatitis, had a high total IgE level, and no history pointing to profilin, LTP or Api g 5 sensitization (eg no. 16 tolerated heated celeriac), the clinical relevance of these sensitizations is questionable.

Other well-known celery allergens like Api g 2 and Api g 6, both LTPs, were not identified on the 2DE immunoblots. Api g 2 is mainly present in celery stalks¹² and Api g 6 in celeriac roots (tubers). LTPs are known to cause primarily severe systemic reactions and are relevant allergens in Southern Europe.³³ Thus, it is very likely that our celery-allergic patients are not sensitized to these allergens.

Cultivar differences may also be caused by allelic diversity of loci in genes encoding proteins with allergic potential.³⁴ Such differences have been already observed in tomatoes¹⁷ where they seem to play a larger role than abiotic or biotic environmental factors.^{18,20} For breeding hypoallergenic cultivars, it will be essential to identify allelic variety and to test the allergenicity of the encoded allergens showing different amino acid sequences. Additionally, we must bear in mind that new allergens are continuously being identified, as shown for tomatoes.²¹

In this study, positive protein spots of putative novel celery allergens (Enolase 1, Actin, Peptidyl-prolyl cis-trans isomerase, translationally controlled tumour protein) were additionally identified. All these proteins have a highly conserved structure and are known allergens but have not been described as celery allergens until now.

Enolase is involved in glucose metabolism.³⁵ It is well-known as an inhalant allergen in pollen³⁵ and is present on the surfaces of different fungal species.³⁶ Actin has just been identified as a major

allergen of the carpet clam (*Paphia textile*).³⁷ Peptidyl-prolyl cis-trans isomerase, also known as cyclophilin, is a known allergen from birch (Bet v 7)³⁸ and has recently been described as a new tomato allergen.²¹ Translationally controlled tumour protein has been described as a minor allergen in *Alternaria alternata*.³⁹

However, the activity of these potentially new celery allergen candidates needs to be confirmed in further studies.

5 | CONCLUSION

Taken together, this study shows clinical differences among celeriac cultivars. As a possible reason for these observations, lower Api g 1 expression was identified in the cultivar with lower clinical activity ("Anita") in comparison with the cultivar with higher clinical activity ("Prinz"), as measured by SPT and DBPCFCs.

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CONFLICT OF INTEREST

PZ received speaker fees and research grants from and is employee of Thermo Fisher Scientific. All other authors declare that there is no conflict of interest regarding the investigation described in the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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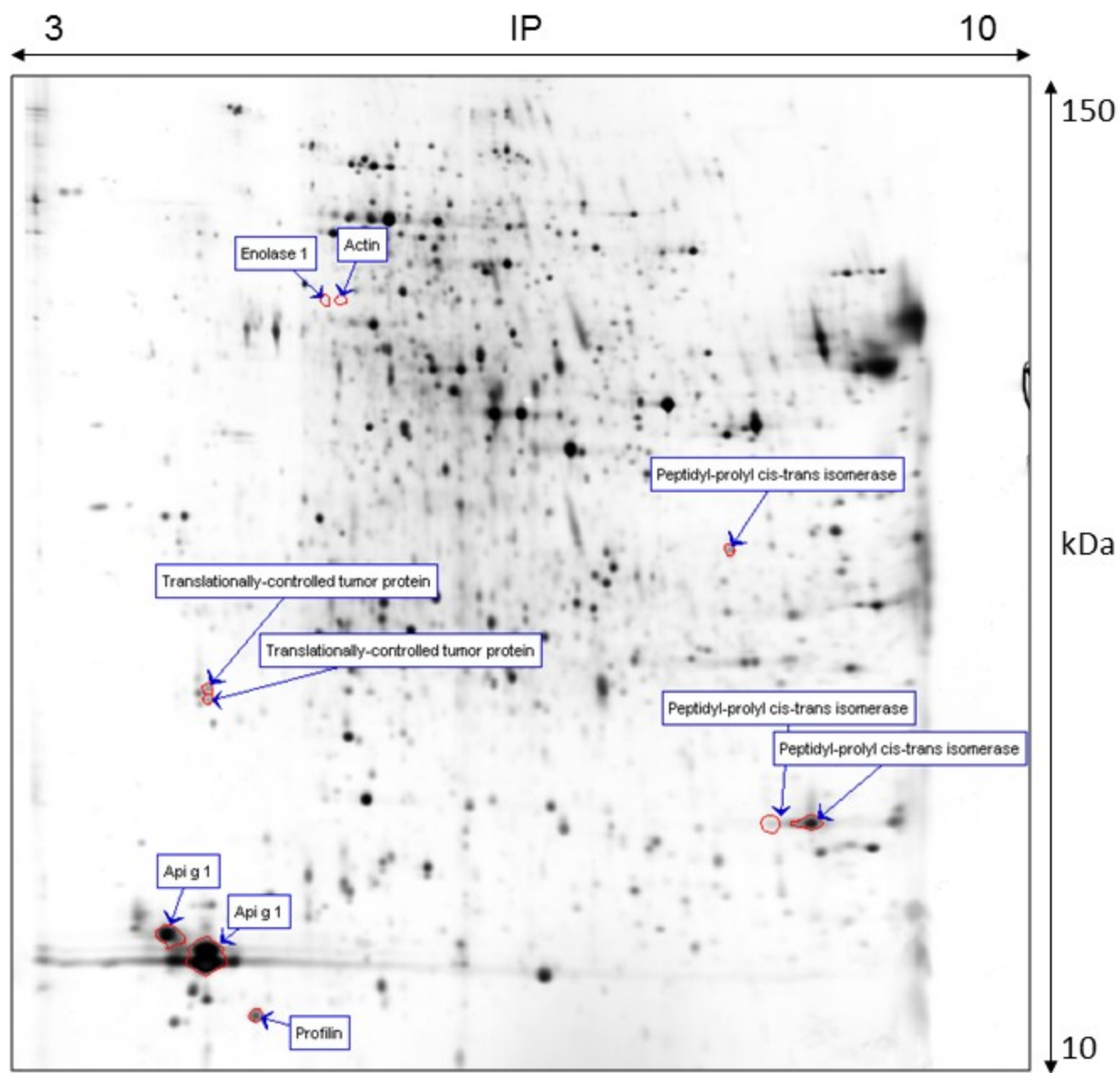


Table S1: Known and confirmed celery allergens according to the allergen nomenclature, International Union of Immunological Societies (IUIS).

Allergen	Biochemical name	Molecular weight
Api g 1	PR-10 protein	15 kDa
Api g 2	nsLTP, type 1	9 kDa
Api g 3	Chlorophyll-binding protein	28 kDa
Api g 4	Profilin	14 kDa
Api g 5	FAD-containing oxidase (Glycoprotein)	58 kDa
Api g 6	nsLTP, type 2	7 kDa

PR – pathogenesis-related, nsLTP – non-specific lipid transfer protein, FAD – Flavinadenin dinucleotide;