

Recognition pattern of kiwi seed storage proteins in kiwifruit allergic children

Caroline Nilsson^{1,2}, Peter Brostedt³, Johanna Hidman³, Jenny van Odijk⁵, Magnus P Borres^{3,4}, Sigrid Sjölander³, Hillevi Englund³

¹Sach's Children's Hospital, Södersjukhuset, Stockholm, Sweden

²Department of Clinical Science and Education, Karolinska Institute, Stockholm, Sweden

³R&D, ImmunoDiagnostics, Thermo Fisher Scientific, Uppsala, Sweden

⁴Department of Women's and Children's health, Uppsala University, Uppsala, Sweden

⁵Respiratory Medicine and Allergology, Sahlgrenska Academy at Göteborg University

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Corresponding author: Hillevi Englund, R&D, ImmunoDiagnostics, Thermo Fisher Scientific, Uppsala, Sweden, Hillevi.Englund@thermofisher.com

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To the Editor:

The consumption of kiwifruits, both green (*Actinidia deliciosa*) and golden (*Actinidia chinensis*) is increasing, and so is the allergy (1, 2). The sweeter golden kiwi is believed to be less allergenic due to its low content of actinidin (Act d 1) which is the major allergen in the pulp of green kiwi (3). The major focus on identifying kiwi allergens has been on allergens from the pulp, however in a European study the combined diagnostic sensitivity of five pulp allergens plus LTP (Act d 10) from seeds was not more than 65% (2). Recently, two novel allergens were found in seeds from green kiwi – Act d 12 and Act d 13 (4). Both are storage proteins, 11S globulin and 2S albumin respectively, and bind IgE in sera from kiwi allergic adults (4, 5). Generally, sensitization to storage proteins is linked to severe allergic food reactions. 2S albumin (Act d 13) was identified as a minor allergen in a population of adult Spanish patients where 18% were sensitized (5). Considering the described differences in symptoms between children and adults, where children on average have more severe reactions (6), Act d 13 could be of greater importance in children. Here, the aim was to, for the first time, investigate the sensitization pattern to seed storage proteins from both green and golden kiwi fruits among children with kiwi allergy.

Five Swedish children (9-17 years of age) being patients at an allergy department in Stockholm, Sweden, were studied. All five reported allergic symptoms within 30 minutes after kiwi ingestion. Four out of five reported systemic allergic symptoms and the fifth patient had oral allergy symptoms (OAS), why all were considered kiwi allergic (Table 1). Also, all patients were allergic to additional foods, i.e. egg, milk, wheat, oats, sesame, peanuts and nuts (Table 1). The children and their caregivers got written information and accepted to donate blood to this case report.

Seed storage proteins from ripe green and golden kiwi were purified and characterized using a two step-approach after extraction. First, proteins were extracted from grinded seeds in a phosphate buffer at neutral pH, where after they were separated by gel filtration (Superdex 75 pg XK 50/100 column,

GE Healthcare) and then further separated with anion (HiScreen Capto Q ImpRes column, GE Healthcare) or cation (HiScreen Capto SP ImpRes column, GE Healthcare) exchange chromatography. Purity of obtained proteins was estimated by SDS-PAGE, analytical gel filtration (Superdex 200 HR 10/30 column, GE Healthcare), and mass spectroscopy (EASY-nLC 1000 interfaced by a Nanospray Flex to an Orbitrap Fusion mass spectrometer, Thermo Scientific).

For quantitative analysis of IgE-levels in serum samples, experimental ImmunoCAP with purified proteins were developed in house (Thermo Fisher Scientific, Uppsala, Sweden) as described previously (7). The cut-off for positive IgE-level was defined as $>0.35 \text{ kU}_A/\text{l}$. The array-based ImmunoCAP ISAC 112 (Thermo Fisher Scientific, Uppsala, Sweden) was used for semi-quantitative component resolved IgE-analysis according to manufacturer's instructions. The cut-off for positive IgE-level was defined as 0.3 ISU.

The biochemical characterization of seed proteins revealed very similar patterns between green and golden kiwi, displaying two dominant peaks – one with high and one with low molecular weight material (Fig 1A). After the second purification step with ion exchange chromatography, four separate protein fractions of different sizes were identified for both green and golden kiwi seeds (Fig 1B, C). Seeds from golden and green kiwi contained equal amounts of respective protein. The mass spectrometry analysis revealed the same results in the corresponding fractions from both green and golden kiwi seeds and allergens from the 11S globulin (in agreement with accession no. gil82469930), 7S globulin (19% protein homology with homolog from *Sesamum indicum* accession no. gil13183177), LTP (in agreement with gil378405189) and 2S albumin (correlation of a few peptides indicated the presence of a 2S albumin) families were identified. Altogether, mass spectrometry data, analytical gel filtration and SDS-PAGE performed under reducing conditions revealed sizes and protein characteristics corresponding to LTP, 2S albumin, 7S globulin and 11S globulin families respectively, for the purified proteins from both green and golden kiwi seeds (Fig 1B-D). The three

allergens 2S albumin, 7S globulin and 11S globulin from golden kiwi seeds are all novel and have not been previously described in the literature. The correlation between IgE-binding to the homologous proteins from green and golden kiwi was strong for the patient sera tested ($r=0.96$, $p<0.0001$) and the IgE concentrations to homologous proteins were equal (Fig 2 and Table 1), implying a strong degree of immunological cross-reactivity.

The sweeter golden kiwi has been suggested as a less allergenic alternative to green kiwi (3, 8). This difference between cultivars could not be confirmed for allergens present in the seeds, as the homologous proteins were present in equal amounts in both seeds. Also, identical IgE binding properties was demonstrated for seed storage proteins from both green and golden kiwi and altogether the results point towards a scenario where patients with reactions associated with sensitization to Act d 12 and Act d 13 are likely to be sensitized to the homologous proteins from golden kiwifruit as well.

Sera from the four children with documented systemic allergic symptoms after kiwi ingestion all displayed IgE-binding to at least two of the three kiwi storage proteins, and to Act d 1 (Table 1). Their sensitization pattern differed, two were mainly sensitized to 11S and 7S globulins, whereas one patient had highest IgE concentration to 2S albumin. Semi-quantitative IgE analysis of the green kiwi pulp proteins Act d 1, Act d 2, Act d 5 and Act d 8 revealed that 4/5 patients were sensitized to the major kiwi allergen Act d 1, but only one to Act d 2 (Table 1). Three out of the five were sensitized to Act d 10 or Act c 10 (LTPs), two to Act d 8 (PR-10 protein) and none had detectable IgE-levels to Act d 5 (Table 1).

Many studies concerning kiwi allergy have focused on kiwi pulp and extracts thereof, as regards to both provocations and diagnostics. However, IgE to pulp allergens have yet only been found in about two thirds of kiwi allergic cases (2), where Act d 1 in particular has been pinpointed as the major

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sensitizer and the allergen related to severe clinical symptoms (2, 3, 9). Contrary, the importance of Act d 1 could not be confirmed in an English cohort (10). In that study, crushed kiwi seeds were added to the extract and a novel major allergen – not being Act d 1 – was suggested (10). Recently, two storage proteins were identified in seeds from green kiwi and described as important allergens in adult Spanish patients (4). Act d 13 was identified as a minor allergen in the population of adult patients where 18% were sensitized. Here, all five children were IgE-positive for at least one of the seed storage proteins from both green and golden kiwi, and 3/5 to the 2S albumins, implying that additional analyses of IgE to both seed and pulp allergens could help reveal a more complete clinical picture regarding kiwi allergy (Table 1). Allergic sensitization to storage proteins has in many studies been found to be associated with severe clinical reactions for several nuts, seeds and legumes, and it is likely that IgE measurements to the storage proteins Act d 12 and Act d 13 could contribute to improved kiwi allergy diagnostics.

One limitation is the small number of investigated individuals in this case report, and another that double blind placebo controlled food challenges (DBPCFC) were not performed on the cases. Kiwi allergy is increasing, but still rare in the Swedish population. The clinical relevance of IgE sensitization to kiwi seeds storage proteins like Act d 12 and Act d 13 is still unknown. All five children in the report were also allergic to peanut and/or tree nuts and legumes, which are foods well known to contain homologous storage proteins. In an earlier study of kiwi allergic children with severe symptoms, more than half of the children also reported peanut and tree nut allergies (6), which could indicate a IgE crossreactivity between kiwi seed storage proteins and nuts that needs to be further investigated to determine its clinical relevance. A number of these children reacted to their first known exposure of kiwi, indicating that they were primarily sensitized to cross reacting allergens from other sources like seeds, peanut and/or tree nuts (6). Although the majority of the patients in this case report were sensitized to the major kiwi allergen Act d 1, their co-sensitization to seed storage proteins could be of importance to understand their kiwi allergy but also to unravel possible cross-reactions to homologous allergens from nuts, seeds and legumes. Serological cross-reaction to storage proteins

from peanuts and tree nuts have been confirmed in *in vitro* settings (5), and the clinical relevance of this remains to be revealed.

Here, three novel seed storage proteins were identified in seeds from golden kiwi fruits and were shown to be of equal importance as the 11S globulin, 7S globulin and 2S albumin proteins in green kiwi. Children with systemic allergy reactions after kiwi ingestion all displayed IgE to the seed storage proteins. However, patient AA with oral allergy symptoms was also sensitized to the kiwi storage proteins, although with lower IgE-levels, why the importance of including IgE-analyses towards e.g. Act d 12 and Act d 13 when diagnosing kiwi allergy needs to be confirmed in a larger study. We conclude that the three new storage proteins we have identified in golden kiwi seeds seem to be as allergenic as 11S globulin, 7S globulin and 2S albumin proteins from green kiwi. Further, as all five children with mild as well as severe symptoms were sensitized to at least one of the three identified storage proteins, the results imply that quantifying IgE to kiwi seed storage proteins could help to elucidate a more complete picture when diagnosing patients. Although, to unravel the clinical relevance of IgE to these proteins and determine if the analyses can help in diagnosis and predicting severity of kiwifruit allergy, further studies including larger cohorts from several geographic areas needs to be performed.

Caroline Nilsson^{1,2}, MD, PhD

Peter Brostedt³, PhD

Johanna Hidman³, MSc

Magnus P Borres^{3,4}, MD, PhD

Jenny van Odijk⁵, PhD

Sigrid Sjölander³, PhD

Hillevi Englund³, PhD

¹*Sachs' Children's Hospital, Södersjukhuset, Stockholm, Sweden*

²*Department of Clinical Science and Education, Karolinska Institute, Stockholm, Sweden*

³*R&D, ImmunoDiagnostics, Thermo Fisher Scientific, Uppsala, Sweden*

⁴*Department of Women's and Children's health, Uppsala University, Uppsala, Sweden*

⁵*Respiratory Medicine and Allergology, Sahlgrenska Academy at Göteborg University*

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Table 1. Demographic and clinical parameters for five children with documented kiwi allergy symptoms. IgE concentrations (kU/l) to the purified storage proteins from green and golden kiwi seeds and to the LTPs (Act d 10 and Act c 10), as well as semi-quantitative IgE concentrations (ISU) for kiwi pulp allergens (Act d 1, 2, 5 and 8), are reported.

Clinical data					Quantitative IgE analyses, kU/l						Semi-quantitative IgE analyses, ISU					
					11S globulin		7S globulin		2S albumin		LTP		Act d 1	Act d 2	Act d 5	Act d 8
Patient	Gender	Age	Kiwi allergy symptoms	Other allergies	Gr	Go	Gr	Go	Gr	Go	Act d 10	Act c 10				
AA	F	17	Itchy mouth	Egg, milk, tree-nuts, banana	0,3	0,3	0,7	0,4	0,8	0,8	0,2	0,3	0	0	0	0
RB	M	16	Dyspnea, edema in mouth and throat, itchy skin	Egg, milk, tree-nuts, peanut	2,4	0,9	0,7	1,1	4	4,2	0,3	0,5	9,7	0	0	0
EN	M	11	Dyspnea, edema in mouth, treated for anaphylaxis at ER	Sesame	0,6	0,4	1,5	0,7	0,1	0,2	0,2	0,4	1,5	1,4	0	3,8
ZF	M	11	Facial edema, general urticaria	Egg, milk, wheat, rye, barley, oat, banana, chicken, turkey, tree-nuts, peanuts	1,9	1,7	2,4	1,4	0,1	0,2	0,1	0,1	9,8	0	0	0,3
ME	F	10	Stomach ache, vomiting, mouth itch	Peanut, seed, legumes	32	24	12	7,2	23	9,9	1,5	1,3	88	0	0,4	1,8

Gr = green kiwi, Go = golden kiwi



