Allergen Data Collection - Update:

Apple *(Malus domestica)*

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Apple *(Malus domestica)*

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Abstract

The prevalence of apple allergy is most frequently associated with birch pollinosis in Northern Europe and North America. 40 to 90 % of birch pollen allergic patients are sensitized to apples. There is evidence for the predominant sensitization route by birch pollen allergens. Apple is known as one of the major foods involved in so-called "Oral Allergy Syndrome", which presents IgE-mediated symptoms occurring mainly at the mucosa of lips, tongue and throat after ingestion of apples and other fruits. Systemic reactions including anaphylaxis occur more frequently in apple allergic patients without related pollinosis.

There are differences in the allergenic potencies of different apple varieties and ripening stages of the fruits. Peels are more allergenic than pulps. The pollen related allergens are unstable to conventional processing of the fruits like canning, pulping or heating, therefore adverse reactions occur almost exclusively after ingestion of fresh fruits. Due to the labile nature of apple allergens diagnostic accuracy is highly dependent on the quality of extracts used in testing procedures.

Up to now four groups of cross-reactive allergens have been recognized in Rosaceae fruits (e.g. apple, apricot, cherry, pear, peach, and plum): 1. Pathogenisis related proteins like the major apple allergen Mal d 1, which are homologue to Bet-v-1 from birch pollen. 2. Glycoproteins in the range of 30-70 kDa, including a 35 kDa allergen cross-reactive to birch pollen and a 60 kDa allergen cross-reactive to mugwort pollen. 3. Actin-regulating profilins of appr. 14 kDa acting as panallergens. 4. Lipid-transfer proteins, which seem to be relevant in a smaller subpopulation of apple allergic individuals without birch pollinosis (9 kDa apple allergen Mal d 3). Lipid-transfer proteins are thought to be potentially stable allergens. Furthermore a thaumatin-homologue allergen, Mal d 2, has been characterized.

The present data collection reviews detailed information on the prevalence and symptoms of apple allergy as well as cross-reactivities, and molecular biological and allergenic properties of the major apple allergens in tabular form.
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Contents

1 Prevalence of Apple Allergy

Prevalence data are based on different diagnostic procedures. While the prevalence of sensitization (sensitivity) can be estimated by SPT, RAST, and immunoblot, a clinical relevant sensitization (allergy) is evaluated by convincing history (anamnesis) or food challenge tests (ideally by DBPCFC).

Country / Subjects	Sensitivity / Allergy to	References
Denmark, Hellerup 101 birch and/or hazelnut allergic patients	apple extract 8% apple peel, fresh 28% (SPT)	Andersen & Lowenstein 1978
France, Paris a) 24 patients with latex and pollen allergy b) 20 patients with latex allergy (no pollen allergy) c) 25 patients with pollen allergy (no latex allergy)	clinical symptoms / SPT a) apple in 4% and 29% b) apple in 0% and 16% c) apple in 48% and 56%	Levy et al. 2000
France, Pierre Benite 580 patients with adverse reactions to food (study period 1984-92)	apple 15% (RAST)	Andre et al. 1994
Finland, Helsinki 73 birch pollen allergic patients	apple 63 % (case history) apple pulp 43 % and apple seed 59% (SPT)	Lahti et al. 1980
Finland, Oulu children with atopic dermatits	apple 0% and 13% in patients 1-3 years and 3-15 years of age (n=14 and 32) (SPT)	Hannuksela 1987
France, Toulouse 142 food allergic children	apple 0.7 % (labial food challenge)	Rance & Dutau 1997
Germany, Berlin 167 pollen and food sensitive patients	apple 93 % and 84 % (SPT and case history)	Jankiewicz et al. 1996
Germany, Frankfurt 80 patients with pollen associated food allergy	apple 41% (SPT, RAST)	Boehncke et al. 1998
Germany, Hamburg 30 patients with pollen associated food allergy	apple 87 % (EAST)	Möller et al. 1997
Italy, Genoa 132 pollen and food sensitive patients	apple 38% (self-reported)	Troise et al. 1992
Italy, Milan 262 fruit and/or vegetable allergic patients	apple 53 % (clinical history)	Ortolani et al. 1988
Italy, Milan 100 fruit and/or vegetable allergic patients	apple 45 % (clinical history)	Ortolani et al. 1989
Japan 171 birch pollen allergic patients	apple 13 % (RAST)	Yamamoto et al. 1995
Netherlands 131 cases of food- induced anaphylaxis (from 1993-1997)	apple 1.5% (survey, reported to the TNO Nutrition and Food Research Institute)	European Commission 1998
Netherlands, Rotterdam 79 tree-pollen allergic patients	apple 65%, 68%, and 44% (SPT, RAST, and case history)	de Groot et al. 1996
Spain, Madrid 29 plant-derived food allergic patients	apple 24% (SPT)	Diez-Gomez et al. 1999
Spain, Salamanca 84 mugwort sensitive patients without other pollen sensitizations	apple 1.2% (RAST)	Garcia-Ortiz et al. 1996

Sweden, Halmstad / Malmö a) 380 birch pollen allergic patients b) 103 patients without birch pollen allergy	a) apple 47% b) apple 6% (questionnaire)	Eriksson et al. 1982
Sweden, Uppsala 128 pollen allergic patients a) birch pollen b) grass / mugwort pollen	a) apple 90% b) apple 46% (SPT, controls = 17% positive)	Dreborg & Foucard 1983
Switzerland, Vaudois 111 patients with pollen- associated food allergy	apple 83 % (RAST)	Bircher et al. 1994
Switzerland, Zurich 402 food allergic adults	apple, kiwi 1.5 % (clinical history, diagnostic tests)	Wüthrich 1993
Switzerland, Zurich 383 food allergic patients (study period 1990-94)	apple 26% (clinical history, diagnostic tests)	Etesamifar & Wüthrich 1998
UK, London 100 patients with food intolerance	apple 1% (repeated challenge)	Lessof et al. 1980
UK, Manchester 90 patients expierenced anaphylactic reactions to foods (study period 1994-1996)	apple 1% (suspected cause of patients' worst reaction)	Pumphrey & Stanworth 1996
USA, Long Beach, CA 137 patients with latex allergy	apple 2 % (convincing history of possible IgE mediated symptoms occurring within 60 min of ingestion)	Kim & Hussain 1999
USA, Ohio 148 respiratory- allergic children with reproduced symptoms after food challenge	apple 2 % (open challenge)	Ogle et al. 1980
USA, Rockville, MD 34 patients allergic to tree pollens	apple 71 % (SDS-PAGE immunoblot)	Hsieh et al. 1995

2 Symptoms of Apple Allergy

Symptoms & Case Reports	References
systemic reactions anaphylaxis (2, 8, 12), exercise-induced anaphylaxis (6) cutaneous symptoms angioedema (1, 5, 13), urticaria (1, 7, 8, 12, 13) gastrointestinal symptoms glottis edema (13), tongue edema (13), itching in mouth (1, 5, 9), itching in throat (1, 9), swelling of lips (9), tingling in mouth (1, 5), vomiting (13), oral allergy syndrome* (3, 4, 7, 8, 10, 11, 12), in general (7, 12) respiratory symptoms allergic rhinitis (1), asthma (5, 7, 13) * symptoms, which could be involved in oral allergy syndrome: local symptoms as intra-oral and lip-irritation, angioedema and systemic symptoms as rhino-conjunctivitis, urticaria-angioedema, asthma, and anaphylaxis (4)	(1) Kremser & Lindemayr 1983 (2) Pigatto et al. 1983 (3) Pastorello et al. 1987 (4) Ortolani et al. 1988 (5) Ortolani et al. 1989 (6) Anibarro et al. 1994 (7) van Ree et al. 1995 (8) Fernandez-Rivas et al. 1997 (9) Möller et al. 1997 (10) Wüthrich 1997 (11) Wüthrich et al. 1997 (12) Fernandez-Rivas & Cuevas 1999 (13) Sánchez-Monge et al. 1999
Percentage of reactions Oral allergy syndrome 82%, with additional systemic symptoms 17%, extra-oral symptoms 18% in 139 apple-allergic patients (1) Onset of symptoms within 5 min after food ingestion in > 50% of patients with oral allergy syndrome and within 30 min in > 90% (1) In 10 apple allergic patients without related pollinosis: 64% anaphylaxis, 27% oral allergy syndrome, and 18% urticaria (2)	(1) Ortolani et al. 1988 (2) Fernandez-Rivas et al. 1997
Threshold for Elicitation of Symptoms Amounts of apple fruit inducing symptoms ranged from 4 g to 32 g (estimated protein content: 12 - 96 mg), lower doses not tested (open challenge, 37 apple allergic patients) (1)	(1) Pastorello et al. 1999

3 Diagnostic Features of Apple Allergy

Parameters / Subjects	Outcome	References
IgE birch pollen allergic patients a) 24 with apple allergy, b) 9 apple tolerant	Apple specific serum IgE (RAST): a) 1.76 +/- 3.39 PRU/mL b) 0.76 +/- 0.44 PRU/mL no significant differences	Pastorello et al. 1987
IgE birch pollen allergic patients: a) responding and b) non-responding to DBPCFC with birch pollen related foods	Apple specific serum IgE (RAST): a) 6.1 kU/L b) 4.0 kU/L (mean values, no significant difference)	Reekers et al. 1999
Histamine Release (HR) 40 birch pollen-allergic patients a) with and b) without fruit allergy	Dose-dependent HR in both groups: apple peel = apple pulp > peach = cherry (to significant higher extent of HR in b) significant increase of basophil sensitivity to birch pollen in group b)	Kleine-Tebbe et al. 1992
IgE and Clinical Relevance patients with clinical apple allergy (a), birch pollen allergy (b), or other allergies (c)	apple specific IgE > 0.35 kU/L in a) 90% (in 85% >0.7 kU/L), b) 44%, and c) 5-10% of patients (RAST)	Bjorksten et al. 1980

SPT, IgE and Clinical Relevance apple-allergic patients	positive reactions in 84% and 3.6% of patients with clinical apple allergy tested with fresh apples and commercial extracts, respectively (SPT) apple specific IgE in 70% of patients with clinical apple allergy (RAST)	Ortolani et al. 1988
a) ***RAST and Clinical Relevance*** **b)** ***SPT and Clinical Relevance*** 44 patients with clinical history of apple allergy	a) RAST (specific IgE > 0.7 kU/L): positive results in 71% positive preditive value 79% negative preditive value 62% b) SPT with commercial extracts and fresh food: positive results in 2.3 % and 81% positive preditive value 100% and 78% negative preditive value 40% and 71%	Ortolani et al. 1989
SPT, Extracts 72 patients with birch pollen associated apple allergy	Positivity in SPT: a) Self-prepared extracts (low temperature method) Granny Smith 91% (n=67) Golden Delicious 87% (n=71) b) 4 commercial allergen extracts: negative in all patients	Vieths et al. 1995a
SPT, Commercial Extracts, Stable Allergens 298 patients with OAS after eating of fruits (Rosaceae) and /or nuts	No positive reaction to commercial apple extract (SPT), 135 positive reactions to fresh apple in Prick-to-Prick test; patients sensitive to stable allergens may be detected with commercial plum or walnut extracts	Asero 1999
Birch Pollen spec. IgE 103 birch pollen-hypersensitive patients free of oral allergy syndrome (at begin of the followed-up study)	Birch pollen specific serum IgE- levels in patients: a) who developed Apiaceae sensitivity 15.5 AU/mL b) who developed apple/hazelnut allergy only 8.5 AU/mL c) who remained free of oral allergy syndrome 5 AU/mL (median values, $P < 0.05$)	Asero 1997
HLA Class II Genes 42 birch pollen allergic patients with and without food allergy	HLA class II DR4 and/or DR7 alleles were present in 42.6% of patients and in 2.4% of controls, no significant differences in the frequency of DPB1 alleles; HLA-DR7 significantly involved in the presentation of apple and pollen allergens	Sénéchal et al. 1999

4 Therapy of Apple Allergy

<table>
<tr><th>Treatment *</th><th>Outcome</th><th>References</th></tr>
<tr><td>Tree Pollen Immunotherapy
72 children with birch pollinosis (age of 6-16 years), prevalence of adverse reactions to apple before immuno-therapy 78%
I. subcutaneous immunotherapy for 3 years with a) birch pollen preparation or b) a mixture of birch, alder, and hazel pollen
II. oral immunotherapy for 10 months with c) birch pollen preparation or d) placebo capsules</td><td>Assessment of food allergy after treatment (self-reported):<table><tr><th></th><th>improved</th><th>unchanged</th><th>worse</th></tr><tr><td>a) (n=19)</td><td>37%</td><td>42%</td><td>21%</td></tr><tr><td>b) (n=20)</td><td>55%</td><td>30%</td><td>15%</td></tr><tr><td>c) (n=14)</td><td>21%</td><td>64%</td><td>14%</td></tr><tr><td>d) (n=14)</td><td>14%</td><td>86%</td><td>0%</td></tr></table>no significant more decrease in birch pollen immunotherapies as compared to placebo oral immunotherapy</td><td>Möller 1989</td></tr>
<tr><td>Birch Pollen Immunotherapy
20 birch pollen allergic patients</td><td>2 or 3 courses of immunotherapy in successive years:
fruit allergy improved in 9 patients
no improvement in 4 patients
fruit allergy developed in 3 patients
At the end of 3 years 16 patients were allergic to fruit, 13 of them to apple
IgE during immunotherapy:
birch and apple specific IgE increased temporary
IgG and IgG4 during immunotherapy:
increase of birch specific Ab only
histamine release:
sensitivity to birch pollen extract decreased significantly during immunotherapy, no significant changes to apple extract</td><td>Herrmann et al. 1995</td></tr>
<tr><td>Birch Pollen Immunotherapy
49 birch pollen allergic patients with apple- induced oral allergy syndrome</td><td>12, 24, or 36 months of pollen- specific immunotherapy:
84 % improved (reported symptoms of OAS)
88 % improved (fresh apple, SPT)
IgE during immunotherapy:
significant decrease in birch pollen- specific IgE levels, apple- specific IgE reduction in 21%, no change in 43%, increase in 38% (RAST)</td><td>Asero 1998</td></tr>
<tr><td>Birch Pollen Immunotherapy
15 patients with birch pollen allergy and associated apple allergy</td><td>two courses of pre-seasonal birch pollen immunotherapy:
in 60% beneficial effects on apple allergy
in 87% improvement of pollen allergy
(immunoblot inhibition corroborated importance of homology from Bet v 1 and Mal d 1)</td><td>Henzgen et al. 1999</td></tr>
<tr><td>Oral Desensitization
1 apple allergic patient
(effectiveness not confirmed by DBPCFC)</td><td>A diluted food extract followed by increased pure food was administered following a standardized protocol, at the beginning pretreatment with oral sodium cromoglycate, length of therapy 3.5 months, after therapy apple was tolerated (maintenance dose: 1 apple twice a week)</td><td>Patriarca et al. 1998</td></tr>
</table>

* Studies may be experimental, unproved, or controversial. Please notice the disclaimer !

5 Composition of Apple

5.1 Distribution of Nutrients (fresh fruit)

Nutrients: Content per 100 g		
Energy 229 kJ (54 kcal)	**Vitamins**	Tyr 5 mg
Water 85.3 g	Carotene 45 µg	Val 12 mg
Protein 0.3 g	Vitamin E 490 µg	
Lipids 0.4 g	Vitamin K 0-5 µg	**Carbohydrates**
Carbohydrate 11.8 g	Vitamin B1 35 µg	Glucose 2210 mg
Organic acids 0.6 g	Vitamin B2 30 µg	Fructose 6040 mg
Fiber 2.3 g	Nicotinamide 300 µg	Sucrose 2470 mg
Minerals 0.3 g	Pantothenic acid 100 µg	Starch 600 mg
	Vitamin B6 45 µg	Sorbit 510 mg
Minerals	Biotin 1-8 µg	
Sodium 3 mg	Folic acid 7 µg	**Lipids**
Potassium 145 mg	Vitamin C 12 mg	Palmitic acid 50 mg
Magnesium 6 mg		Stearic acid 10 mg
Calcium 7 mg	**Amino Acids**	Oleic acid 20 mg
Manganese 65 µg	Arg 8 mg	Linolic acid 100 mg
Iron 480 µg	His 6 mg	Linoleic acid 20 mg
Copper 100 µg	Ile 10 mg	
Zinc 120 µg	Leu 16 mg	**Other**
Phosphorus 12 mg	Lys 15 mg	Malic acid 550 mg
Chloride 2 mg	Met 3 mg	Citric acid 16 mg
Fluoride 7 µg	Phe 9 mg	Oxalic acid 500 µg
Iodine 2 µg	Thr 8 mg	Salicylic acid 310 µg
Selenium 1-6 µg	Trp 2 mg	Purines 3 mg

Reference: Deutsche Forschungsanstalt für Lebensmittelchemie, Garching bei München (ed), **Der kleine "Souci-Fachmann-Kraut" Lebensmitteltabelle für die Praxis**, WVG, Stuttgart 1991

5.2 Proteinfraction

Proteins / Glycoproteins	**Amount**
Mal d 1 (18 kDa allergen)	1-5 mg / 100 g fresh fruit (Golden Delicious) (1) appr. 4.5 mg / 100 g fresh fruit (Golden Delicious) (2)

References: (1) Vieths et al. 1993, (2) Son et al. 1999

6 Allergens of Apple

Proteins / Glycoproteins	Allergen Nomenclature	References
Bet v 1 - homologous Protein [18 kDa]	Mal d 1	Ebner et al. 1991, Vieths et al. 1994, Vanek-Krebitz et al. 1995
Thaumatin-homologous Protein [31 kDa]	Mal d 2	Hsieh et al. 1995
Lipid-transfer Protein [9 kDa]	Mal d 3	Pastorello et al. 1999, Sánchez-Monge et al. 1999
Apple Profilin [14-15 kDa]	Mal d 4*	Vallier et al. 1992, van Ree et al. 1992, Ebner et al. 1995, van Ree et al. 1995
Art v 1 cross-reactive Allergen: 60 kDa		Heiss et al. 1996, Grote et al. 1998
Allergen: 34-35 kDa		Wellhausen et al. 1996
Allergens: >30 kDa (carbohydrate epitopes)		Vieths et al. 1994b
Allergens: 8-16, 18, 35, 50 kDa		Vieths et al. 1992
Allergens: 13, 14, 16, 18, 31 kDa		Hsieh et al. 1995
Allergens: 18 and 67-kDa		Möller et al. 1997

* proposed name not yet listed in WHO/IUIS Allergen Nomenclature

6.1 Sensitization to Apple Allergens

<table>
<tr><th>Country / Subjects</th><th>Sensitivity to</th><th>References</th></tr>
<tr><td>Austria, Vienna
83 birch pollen allergic patients</td><td>double band at 17 and 18 kDa in 97% (SDS-PAGE immunoblot)</td><td>Ebner et al. 1991</td></tr>
<tr><td>Austria, Vienna
20 birch pollen allergic patients</td><td>double band at 17 and 18 kDa in 95%
profilin-homologue 14 kDa allergen in 20 %
(SDS-PAGE immunoblot)</td><td>Ebner et al. 1995</td></tr>
<tr><td>Germany, Berlin
23 (22) birch pollen and apple allergic patients</td><td>Allergens from Golden Delicious:<table><tr><th>Allergens</th><th>mature</th><th>semi-mature</th></tr><tr><td>> 50 kDa</td><td>in 9%</td><td>27%</td></tr><tr><td>50 kDa</td><td>in 13%</td><td>77%</td></tr><tr><td>40-50 kDa</td><td>in 39%</td><td>68%</td></tr><tr><td>35 kDa</td><td>in 4%</td><td>23%</td></tr><tr><td>18 kDa (Mal d 1)</td><td>in 65%</td><td>9%</td></tr><tr><td>16 kDa</td><td>in 26%</td><td>5%</td></tr><tr><td>10-15 kDa</td><td>in 30%</td><td>36%</td></tr></table>(SDS-PAGE / immunoblot)</td><td>Vieths et al. 1993</td></tr>
<tr><td>Germany, Berlin
12 apple and celery allergic patients</td><td>Carbohydrate epitopes on allergens > 30 kDa
(periodate treatment, immunoblot, EAST inhibition)</td><td>Vieths et al. 1994b</td></tr>
<tr><td>Germany, Berlin
33 birch pollen and apple allergic patients</td><td>Mal d 1 in 73 % and 76 % (purified allergen from Golden Delicious and Granny Smith) (SPT)</td><td>Vieths et al. 1995b</td></tr>
<tr><td>Italy, Milan
apple allergic patients
a) with birch pollen allergy
b) without birch pollen allergy</td><td><table><tr><th>Allergen</th><th>a) n = 32</th><th>b) n = 11</th></tr><tr><td>60 kDa</td><td>in 22%</td><td>0%</td></tr><tr><td>43, 51, 84 kDa</td><td>in 34%</td><td>0%</td></tr><tr><td>28 kDa</td><td>in 6%</td><td>0%</td></tr><tr><td>18 kDa</td><td>in 91%</td><td>0%</td></tr><tr><td>15 kDa</td><td>in 50%</td><td>0%</td></tr><tr><td>Mal d 3</td><td>in 28%</td><td>100%</td></tr></table>(SDS-PAGE / immunoblot)</td><td>Pastorello et al. 1999</td></tr>
<tr><td>Spain, Madrid
10 apple and peach allergic patients</td><td>Mal d 3 in 90 % (SDS-PAGE / immunoblot)</td><td>Sánchez-Monge et al. 1999</td></tr>
<tr><td>USA, Rockville, MD
24 tree pollen and apple allergic patients</td><td><table><tr><th>Allergens</th><th>n = 24</th></tr><tr><td>> 38 kDa</td><td>in 58%</td></tr><tr><td>31 kDa</td><td>in 75%</td></tr><tr><td>18 kDa (Mal d 1)</td><td>in 38%</td></tr><tr><td>16 kDa</td><td>in 4%</td></tr><tr><td>14 kDa</td><td>in 25%</td></tr><tr><td>13 kDa</td><td>in 17%</td></tr></table>(SDS-PAGE / immunoblot)</td><td>Hsieh et al. 1995</td></tr>
</table>

6.2 Properties of Bet v 1 - homologous Protein (Mal d 1)

6.2.1 Molecular Biological Properties

Bet-v-1-homologous Protein	References
Allergen Nomenclature Mal d 1	(1) Larsen & Lowenstein 1999
Molecular Mass 18 kDa (1), 17 kDa (2) (SDS-PAGE)	(1) Vieths et al. 1994c, Hsieh et al. 1995 (2) Ebner et al. 1995
Isoelectric Point pI 5.5 (2D-PAGE)	(1) Vieths et al. 1994c
Amino Acid Sequence, mRNA, and cDNA	(1) Vanek-Krebitz et al. 1995 (2) Atkinson et al. 1996 (3) Hoffmann-Sommergruber et al. 1996 (4) Schöning et al. 1996

Mal d 1	**a)**	**b)**	**c)**
SWISS-PROT:	P43211		P43211
GenBank:	X83672	L42952	Z48969
Amino Acids	158	160	158
mRNA	813 bp	778 bp	824 bp
cDNA			

(a) Granny Smith, (b, c) Golden Delicious

Genetic Variants 2 different isoforms from Golden Delicious and Granny Smith. Sequences from other strains (Jamba, Gloster, Royal Gala, Jonagold, and Idared) are identical to one of these isoallergens (1)	(1) Son et al. 1999
recombinant Protein Expression in *Escherichia coli*: rMal d 1 from Golden Delicious (1) rMal d 1 from Granny Smith (2) rMal d 1 isoallergens and mutants from Golden Delicious and Granny Smith (4) Expression in cDNA library: rMal d 1 expressed sequence tags (ESTs) generated from selected clones of cDNA libraries prepared from Fuji apple (Malus domestica Borkh.) (3)	(1) Vanek-Krebitz et al. 1995 (2) Schöning et al. 1996 (3) Sung et al. 1998 (4) Son et al. 1999
Biological Function Bet v 1 family of pathogenisis-related proteins (1)	(1) SWISS-PROT
Sequence Homology Bet v 1, major birch pollen allergen: aa 64.5% identity (1), mRNA 55.6% identity (1) Pollen allergens from Alder (Aln g 1), Hazel (Cor a 1), and Hornbeam (Car b 1): aa 54%, 54%, and 52% identity (2) Stress induced protein from soybean (GMH4): aa 49% identity (2) Pea pathogenesis related protein (PEADRRA): aa 48% identiy (2) 5 Bet v 1 isoforms and 3 Mal d 1 isoforms share 55.6-58.8% aa identity (3)	(1) Vanek-Krebitz et al. 1995 (2) Vieths et al. 1996 (3) Son et al. 1999
Localization Expression of Mal d 1 in fruits, peels, and mature flowers from Fuji apple (Malus domestica Borkh.) (expressed sequence tags (ESTs) from randomly selected clones of cDNA libraries) (1)	(1) Sung et al. 1998

6.2.2 Allergenic Properties

Bet v 1 - homologous Protein	References
Frequency of Sensitization IgE-binding to Mal d 1 in 73 % and 76 % of patients (1)	(1) see 6.1 Sensitization to Apple Allergens
Allergenic Potencies No significant differences in IgE binding potencies between 3 recombinant Mal d 1 isoforms and native Mal d 1 from 7 apple strains, divergent allergenicity of apple strains depends mainly on different expression levels; highest IgE-binding capacity for 2 isoforms from Golden Delicious and Granny Smith (EAST, immunoblot) (1)	(1) Son et al. 1999
IgE-Binding: Critical Amino Acids Strong reduction of IgE binding to 2 mutants S111P (single aa substitution) of recombinant Mal d 1 from Golden Delicious and Granny Smith as compared to non-mutated rMal d 1; only weak reduction of IgE-binding observed for S111C mutants (EAST, immunoblot) (1)	(1) Son et al. 1999
T-Cell Epitopes Specific T-cell proliferation with: Mal d 1 aa 141-155 (synthetic peptide) (1) T-cell proliferation after stimulation with Mal d 1 weaker than with Bet v 1 (1)	(1) Fritsch et al. 1998
Cross-reative T-Cell Epitopes from Bet v 1 Specific proliferation of rMal d 1 induced T-cell clones: Bet v 1 aa 4-18, 13-27, 55-66, 76-90, 109-123, 142-156 (synthetic peptides) (1)	(1) Fritsch et al. 1998
T-Cells / Cytokines 69% of Mal d 1 / Bet v 1 cross-reactive T-cell clones revealed a Th2-like cytokine production pattern (26% Th0- and 5% Th1-like), none of the non-cross-reactive Mal d 1 specific T-cell clones was Th2-like (1)	(1) Fritsch et al. 1998

6.3 Properties of Thaumatin-homologue Protein (Mal d 2)

6.3.1 Molecular Biological Properties

Thaumatin-homologue Protein	References
Allergen Nomenclature Mal d 2	(1) Larsen & Lowenstein 1999
Molecular Mass 31 kDa (SDS-PAGE, 1)	(1) Hsieh et al. 1995
Isoelectric Point	
Amino Acid Sequence N-terminus aa 1-26 (1): AKITFTNNXPNTVWPGILTGFGQKPQ	(1) Hsieh et al. 1995
Sequence Homology Antifungal protein PR5 fragment aa 27-43 (thaumatin-like protein from Arabidopsis thaliana): 46% identity to N-terminal sequence (1) N-terminus of wheat trimatin, oat avematin, and barley thaumatin-like protein: 46% identity to N-terminal sequence (1)	(1) Hsieh et al. 1995

6.3.2 Allergenic Properties

Thaumatin-homologue Protein	References
Frequency of Sensitization IgE-binding to 31-kDa-Allergen in 75% of patients (1)	(1) see 6.1 Sensitization to Apple Allergens

6.4 Properties of Lipid-transfer Protein (Mal d 3)

6.4.1 Molecular Biological Properties

Lipid-transfer Protein	References
Allergen Nomenclature Mal d 3	(1) Larsen & Lowenstein 1999
Molecular Mass SDS-PAGE: 13 kDa (2), 9 kDa (1) MALDI-MS: 9058 Da (2)	(1) Pastorello et al. 1999 (2) Sánchez-Monge et al. 1999
Isoelectric Point pI 7.5 (IEF-PAGE)	(1) Pastorello et al. 1999
Amino Acid Sequence N-terminus: aa 1-31 (2): ITXGQVTSSLAPXIGYVRSGGAVPPAXXNGI aa 1-36 (1): ITCGQVTSSLAPCIGYVRSGGAVPPACCNGIRNING	(1) Pastorello et al. 1999 (2) Sánchez-Monge et al. 1999
Posttranslational Modifications Glycosylation: no detection of carbohydrate moieties in SDS-PAGE with periodic acid-Schiff staining (1)	(1) Pastorello et al. 1999
Biological Function Lipid-transfer proteins are involved in plant defense mechanisms and probably participate in formation of extracellular lipophilic substances (cutin, wax) (1)	(1) Sánchez-Monge et al. 1999
Sequence Homology N-terminus of lipid-transfer proteins from almond, peach, and grape: 87%, 86%, and 61% identity to N-terminal sequence (1) N-terminus of pollen allergens from Parietaria judaica (Par j 1.0102) and Brassica rapa: 32% and 23% identity to N-terminal sequence (1)	(1) Sánchez-Monge et al. 1999

6.4.2 Allergenic Properties

Lipid-transfer Protein	References
Frequency of Sensitization IgE-binding to Mal d 3 in 28-100% of patients (1)	(1) see 6.1 Sensitization to Apple Allergens

6.5 Properties of Apple Profilin

6.5.1 Molecular Biological Properties

Apple Profilin	References
Allergen Nomenclature Mal d 4 (designated)	(1) Scheurer et al. 1999 (GenBank)
Isoallergens and Variants 3 isoforms (1)	(1) Scheurer et al. 1999 (GenBank)
Molecular Mass SDS-PAGE: 15 kDa (1), 14 kDa (2)	(1) Vallier et al. 1992 (2) Ebner et al. 1995
Isoelectric Point	
Amino Acid Sequence,mRNA, and cDNA	(1) Scheurer et al. 1999 (GenBank)

Proteins	GD4-1*	GD4-2*	GD4-5*
SWISS-PROT:			
GenBank:	AF129426	AF129427	AF129428
Amino Acids	131 residues	131 residues	131 residues
mRNA	396 bp (1)	396 bp (1)	396 bp (1)
cDNA			

* 77-80% aa identity (BLAST at NBCI)

Biological Function Profilin family (1)	(1) Scheurer et al. 1999 (GenBank)
Sequence Homology Birch pollen profilin (Bet v 2): aa sequence identity 73-83% (1) Pear profilin (Pyr c 4): aa sequence identity 80-97% (1) Cherry profilin (Pru av 4): aa sequence identy 77-93% (1) Soybean profilin (Gly m 3): 80-86% (1) Latex profilin (Hev b 8): aa sequence identity 81-87% (1)	(1) BLAST at NBCI

6.5.2 Allergenic Properties

Apple Profilin	References
Frequency of Sensitization IgE-binding to profilin in appr. 20% of patients (1)	(1) see 6.1 Sensitization to Apple Allergens

7 Isolation & Preparation

Extract / Purified Allergens	Methods	References
Protein extract	Extraction of apple allergens in an active form, inhibition of reactions with phenolic compounds present in apple, chelators and solid PVPP in extraction medium	Bjorksten et al. 1980
Protein extract	Low temperature extraction method: fresh fruit homogenized in acetone (-40°C), precipitates washed, filtered, lyophylized and water extracted	Vieths et al. 1994c
Protein extract (in vivo and in vitro- test solutions)	Preparation of active extracts: application of inhibitors of cytoplasmic enzymes (phenol oxidases, peroxidases, proteases) during extraction, precipitation in organic solvents (diacetone alcohol) at -20°C and resolubilization in potassium phosphate buffer; Storage of extract solutions at 4°C was most effective with PBS/glycerol and cysteine/sodium citrate/glycerol stabilizing additives	Rudeschko et al. 1995a, 1995b
Protein extract	Peeled apple pulp or powdered whole frozen apple were homogenized with extraction buffer (containing sucrose, polyvinylpyrrolidone, EDTA, and diethyldithiocarbamic acid, pH 9.5-10), filtered, centrifugated and stored at -20°C	Hsieh et al. 1995
Protein extract	Enhanced protein content of apple extracts after anion-exchange chromatography	Martinez et al. 1997
Protein extract (apple peel) (in vivo and in vitro- test solutions)	Fresh peel (Granny Smith) homogenized with potassium phosphate buffer (pH 7.0, containing PVPP, EDTA, diethyldithiocarbamate and sodium azide), centrifuged, dialyzed, lyophylized and resuspended in saline plus phenol	Asero et al. 1999
18-kDa allergen (Mal d 1)	Isolation after modified low-temperature extraction: IEC followed by RP-HPLC	Fahlbusch et al. 1995
18-kDa allergen (Mal d 1)	Isolation after low-temperature extraction method by micropreparative SDS-PAGE followed by electroelution	Vieths et al. 1995b
9-kDa allergen (Mal d 3)	Isolation and purification from raw extract (Bjorksten et al. 1980) by cation exchange chromatography (Resource S column) with sodium citrate buffer (pH 6) applying salt gradient, followed by SEC (Superdex 75 column) with sodium citrate / sodium chloride buffer (pH 6)	Pastorello et al. 1999

8 Cross-Reactivities

Cross-Reacting Allergens	Subjects / Methods	References
Apple birch pollen *	Correlation between birch pollen allergy and apple hypersensitivity (1129 adults with bronchial asthma and/or allergic rhinitis, questionaire)	Eriksson 1978
Apple fruit birch pollen	Apple allergic patients' serum pool: birch pollen inhibited IgE- binding to apple allergens (RAST-inhibition)	Bjorksten et al. 1980
Apple fruit significant associations: apple and cherry, apple and pear, apple and birch pollen *	262 fruit and/or vegetable allergic patients (clinical history, SPT, RAST)	Ortolani et al. 1988

Apple allergen (17 to 18 kDa) birch pollen	32 patients with birch pollen allergy (pooled serum) birch pollen inhibited IgE- binding to 17-18 kDa apple- allergen apple extract did not diminish binding to Bet v 1 (immunblot- inhibition)	Ebner et al. 1991
Apple (15 kDa) celery profilin (15 kDa)	Sera reactive to 15 kDa celery allergen: Inhibition of IgE and celery profilin specific rabbit-mAb binding to 15 kDa apple allergen by celery crude extract and by celery profilin, respectively (immunoblot inhibition)	Vallier et al. 1992
Apple allergens birch pollen	Apple and birch pollen allergic patients (immunoblot inhibition)	Vieths et al. 1992
Apple kiwi fruit	3 kiwi allergic patients (RAST inhibition)	Gall et al. 1994
Apple birch pollen (Bet v 1)	Depletion of birch-pollen extract for Bet v 1 removed approximately 95% of the IgE cross- reactivity between birch pollen and apple extract; Cross-reactive human IgE antibodies reacted with an epitope nonoverlapping with the epitope recognized by a mAb (1 patient, RAST inhibition, two-site RIA)	Akkerdaas et al. 1995
Apple allergens (17 kDa / Mal d 1) birch pollen (Bet v 1)	7 Bet v 1 and Bet v 2 sensitive patients (pooled serum): complete inhibition of IgE- binding by rBet v 1 to 17 kDa apple allergen, no inhibition of binding to 14 kDa allergen (immunoblot inhibition)	Ebner et al. 1995
Apple allergens (14 kDa) birch pollen (Bet v 2 profilin)	7 Bet v 1 and Bet v 2 sensitive patients (pooled serum): complete inhibition of IgE- binding by rBet v 2 to 14 kDa apple allergen, no inhibition of binding to 17 kDa allergen (immunoblot inhibition)	Ebner et al. 1995
Apple allergens (18 kDa / Mal d 1) birch pollen (Bet v 1)	3 birch pollen and apple allergic patients and pooled serum: Allergenic potencies: Bet v 1 > Mal d 1 (EAST inhibition)	Fahlbusch et al. 1995
Apple a) grass pollen (Lolium perenne) b) profilin (Lolium perenne) c) carbohydrate moieties (Lolium perenne)	a) Inhibition of IgE- binding to apple extract by grass pollen (Lolium perenne) in 8 of 9 patients (RAST inhibition) b) Decrease of IgE- binding to apple extract (41%) from anti-profilin- IgE-depleted serum (RAST) c) Inhibition of IgE- binding to apple extract by carbohydrate moieties in 1 patient (proteinase K digested grass pollen extract, RAST inhibition)	van Ree et al. 1995
Apple allergens (18 kDa / Mal d 1) birch pollen (Bet v 1)	2 birch pollen and apple allergic patients and pooled serum: Allergenic potencies: Bet v 1 > Mal d 1 (EAST inhibition, histamine release)	Vieths et al. 1995b
Apple 60 kDa mugwort allergen (Art v 1)	Patients with food and pollen allergy: inhibition of IgE-binding to 60 kDa apple allergen by 60 kDa mugwort allergen in 2 of 3 patients (SDS-PAGE inhibition), 4-36% reduction of IgE-binding to apple proteins by 60 kDa mugwort allergen in 3 patients (RAST inhibition)	Heiss et al. 1996
Apple tomato	Tomato allergic patients (EAST inhibition)	Petersen et al. 1996
Apple pear	Serum pool from 7 birch pollen allergic patients: inhibition of IgE- binding to pear allergens by apple extract from Golden Delicious (EAST inhibition)	Vieths et al. 1996

Apple (34-35 kDa) birch pollen allergen 35 kDa and Bet v 1	Sera from birch pollen allergic patients reactive to 35 kDa allergen: IgE binding to apple extract inhibited by 35 kDa allergen and Bet v 1 of birch pollen (EAST inhibition) 35 kDa allergen from birch pollen inhibited IgE binding to 34-35 kDa apple allergen (immunoblot inhibition)	Wellhausen et al. 1996
Apple a) peach, pear, mugwort pollen b) rye grass profilin *	a) Patients allergic to Rosaceae fruits Allergenic potencies: peach > apple > pear (RAST inhibition) b) Cross-reactivity in patients with apple and pollen allergy, no cross-reactivity to profilin in patients without pollinosis (RAST, histamine release)*	Fernandez-Rivas et al. 1997
Apple (18-kDa / Mal d 1) birch pollen, Bet v 1	7 apple / kiwi allergic patients (EAST inhibition, immunoblot inhibition)	Möller et al. 1997
Apple (67-kDa-Allergen) kiwi allergens (43, 67 kDa)	7 apple / kiwi allergic patients: max. inhibition of IgE-binding to apple extract: kiwi extract 27% (EAST inhibition, immunoblot inhibition)	Möller et al. 1997
Apple Extract allergens rPru a 1 (cherry), rApi g 1 (celery), and rBet v 1 (birch pollen)	0% inhibition of IgE-binding to apple extract by rApi g 1, 90% inhibition by rPru a 1, and 100% inhibition by rBet v 1 (immunoblot inhibition estimated according to band intensities, 4 birch pollen and cherry allergic patients) appr. 50% max. inhibition of IgE-binding to rPru a 1 by rMal d 1, max. inhibition to rApi g 1 by rMal d 1 <20% (EAST inhibition, cherry and/or celery allergic patients)	Scheurer et a. 1999
Apple (18-kDa / Mal d 1) birch pollen, Bet v 1	13 birch pollen and apple sensitive patients 79% of Mal d 1-specific T-cell clones cross-reacted with Bet v 1; 44% Bet v 1-specific T-cell clones cross-reacted with Mal d 1; 6 cross-reactive T-cell epitopes from Bet v 1 Stimulating potencies: Bet v 1 > Mal d 1 (T-Cell proliferation, recombinant allergens)	Fritsch et al. 1998
Apple extract, rMal d1 birch pollen, rBet v 1, rBet v 2 profilin), timothy grass pollen extract (21 patients with clinical relevant allergy to pollen and plant-derived food)	Mixture of rBet v 1 and rBet v 2 inhibited IgE-binding to 10-14 kDa (profilin related) and 17-21 kDa (Bet v 1 related) apple allergens, timothy grass pollen inhibited IgE-binding to 10-14 kDa and 30-100 kDa allergens from apple; only weak inhibition of IgE-binding to Bet v 1 by rMal d 1 (immunoblot inhibition); 92% (4.7-100%) inhibition of IgE-binding to rMal d 1 by mixture of rBet v 1 and rBet v 2 and 96% (23-100%) by mixture of rBet v1, rBet v 2, and timothy pollen extract (52 sera) (RAST inhibition)	Kazemi-Shirazi et al. 2000
Apple pollen birch pollen	Patients with birch pollen allergy and oral allergy to apple fruit; IgE binding inhibitory potency to birch pollen by apple pollen extract 1000-fold lower than homologous inhibition with birch pollen (RAST-inhibition)	Berrens et al. 1990
Apple seeds birch pollen	3 birch pollen-sensitive patients (RAST inhibition)	Lahti et al. 1980
Birch pollen (Bet v 1, Bet v 2 profilin) *	28 patients with oral allergy syndrome after ingestion of apples: 57% sensitive to rBet v 1 20 polysensitized patients with oral allergy syndrome after ingestion of apples: 20% sensitive to rBet v 2 (RAST)*	Rossi et al. 1996

* multiple sensitization (not proven by inhibition-tests)

9 Stability of Apple Allergens

Treatment / Ripening	Effects	References
Apple pulp	Apple-pulp allergens are unstable (skin test)	Lahti et al. 1980
Apple (Ripening) mature and unripe fruits	(1) Stronger IgE binding to allergens of mature Golden Delicious apple (SDS-PAGE immunoblot) (2) Higher relative amounts of 18-kDa allergen (Mal d 1) in mature apples than in unripe apples (Golden Delicious > Boskoop) (SDS-PAGE immunoblot, densitometry, EAST inhibition)	(1) Vieths et al. 1992 (2) Vieths et al. 1993
Apple (Storage, Ripening) a) store-purchased b) storage at 4°C c) different ripening stages of stored apples (controlled atmospheric conditions) d) sprayed to prevent microbial infection	a) Higher relative amounts of 18-kDa allergen (Mal d 1) as compared to fresh apple (Golden Delicious, Granny Smith) b) Increasing amounts of 18-kDa allergen (Mal d 1) after 3 weeks c) No clear correlation between ripening stages and amounts of 31- and 18-kDa allergens, reduction of 16-kDa allergen related to ripening d) Only 16-kDa allergen detected during regulated ripening conditions (see c) (SDS-PAGE immunoblot)	Hsieh et al. 1995
Apple slices (Heat) heating at 175°C for 0.5 h, storage at RT	allergenic activity: heat labile and decreased during storage at RT	Dreborg & Foucard 1983
Apple Extracts (Heat) heat (30 min, 100°C)	Heating of the food reduced allergenic activity, while semipurified protein extracts from apple retained immunoreactivity of the major allergens	Vieths et al. 1998
Apple (Enzymic Hydrolysis) gastric digestion assay	Mal d 1 and Bet v 1 degraded within seconds under physiological conditions	Jensen-Jarolim et al. 1999
Apple Prick Test Solutions 4 commercial prick test solutions	No positive results in SPT with commercial extracts in 72 patients with apple allergy (positivity in SPT with self-prepared extracts up to 91%)	Vieths et al. 1995a
Apple Test Solutions 4 commercial extracts 1 self-prepared peel extract	a) SPT: No reactivity using 3 commercial extracts, 14% sensitivity for 1 commercial extract, 91% sensitivity for peel extract b) RAST: 55% and 9% false negativ results for 1 commercial and for the peel extract, respectively (a) 35 and b) 11 birch- pollen allergic patients with OAS to apple and positive SPT to fresh apple)	Asero et al. 1999
Apple Prick Test Solutions 5 commercial prick test solutions	Only 1 solution showed weak allergenic activity as compared to highly active self-prepared apple extract (EAST)	Hoffman et al. 1999
Apple Extracts (Xenobiotica) spiked with pesticides (chlorpropham, iprodione or thiram)	15 Apple allergic patients: a) SPT: in 40% - 73% inhibitory effect of pesticides (weaker skin reactions), in < 20% stronger skin reactions b) EAST / mediator release: dose- dependent decrease of spec. serum IgE and histamine release by chlorpropham spiked apple extracts, no influence on LTC_4 release (mediator release from basophils, HPLC)	Vieluf et al. 1997

10 Allergen Sources

Reported Adverse Reactions	References
Food / Food additives After ingestion of fresh fruits (1)	(1) see 2 Symptoms of Apple Allergy
Peel vs. Pulp More frequently and more severe adverse reactions after ingestion of the whole fruit (peel and pulp) , 44% of patients tolerated ingestion of apple pulp (18 apple allergic patients)	Fernandez-Rivas & Cuevas 1999

Allergens in Apple Products	Results	References
Apple Allergens tree pollen and apple allergic patients	trace ammounts of active allergens in canned apples (SDS-PAGE immunoblot)	Hsieh et al. 1995
Peel vs. Pulp In vitro allergenicity in apple allergic patients	Peels induced higher SPT, histamine release and RAST results than pulps; higher IgE-binding potency of peel extract than pulp extract in RAST inhibition	Fernandez-Rivas & Cuevas 1999

11 Allergenicity of Different Apple Varieties

Varieties / Subjects	Differences	References
16 Apple strains *(Mal d 1)* patients allergic to birch pollen and apples	Relative amounts of 18-kDa allergen (Mal d 1): great variation in 16 apple strains Allergenicity of strains decreased in the following order: Golden Delicious > Boskoop > Jamba (corresponding to high, low, and very low 18-kDa allergen content) (SDS-PAGE / immunoblot / densitometry, EAST, histamine release, open challenge)	Vieths et al. 1994a
7 Apple strains *(Mal d 1)* patients allergic to birch pollen and apples	Expression levels of Mal d 1 in 100 g of fresh apple: 4.5 mg Golden Delicious, 1.6 mg Granny Smith, 0.7 mg Jona Gold, 0.8 mg Idared, 1.8 mg Gala, 0.5 mg Jamba, and 0.4 mg Gloster (SDS-PAGE coomassie staining)	Son et al. 1999
4 Apple strains *(Mal d 1, Mal d 2)* patients allergic to tree pollen and apples	Relative amounts of 31-kDa allergen (Mal d 2): Golden Delicious and Granny Smith > McIntosh and Red Delicious Relative amounts of 18-kDa allergen (Mal d 1): Golden Delicious > other strains (SDS-PAGE immunoblot)	Hsieh et al. 1995
4 Apple strains *(SPT)* 72 patients with birch pollen associated apple allergy	Positivity in SPT: Granny Smith 91%, Golden Delicious 87%, Boskoop 12%, and Jamba 8% (n=67-72)	Vieths et al. 1995a
2 Apple strains *(SPT)* patients allergic to tree pollen	Granny Smith more positive skin reactions and a better agreement with clinical history than Golden Delicious	de Groot et al. 1996

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Common Abbreveations

2D	two-dimensional
3D	three-dimensional
aa	amino acid(s)
Ab	antibody
Act c 1, 2	nomenclature of kiwi fruit allergens *(Actinidia chinensis)*
Ara h 1-7	nomenclature of peanut allergens *(Arachis hypogaea)*
Bos d 4, 5, 6, 7, 8	nomenclature of cow's milk allergens *(Bos domesticus)*
C	concentration of N,N'-methylenbisacrylamide (crosslinker)
CAST	cellular antigen stimulation test
CCD	cross-reactive carbohydrate determinants
CICBAA	Cercle d'Investigations Cliniques et Biologiques en Allergologie Alimentaire (France)
CIE	crossed immunoelectrophoresis
CNBr	cyanogen bromide
cIEF	capillary isoelectric focussing
CLA	cutaneous lymphocyte antigen
CLIE	crossed line immunoelectrophoresis
CMA	cow's milk allergy
CRIE	crossed radioimmunoelectrophoresis
Cor a 1	nomenclature of hazel pollen allergens *(Corylus avellana)*
Cyn d 1	nomenclature of bermuda grass pollen allergens *(Cynodus dactylus)*
DBPCFC	double-blind, placebo-controlled food challenge
DEAE	diethylaminoethyl (cellulose) (anion exchanger)
DNA	deoxyribonucleic acid
EAST	enzyme allergosorbent test
EC	enzyme classification system
EDTA	ethylenediaminetetraacetic acid, disodium salt
ELISA	enzyme linked immunosorbent assay
EW	egg white
Fuc	fucose
Gad c 1	nomenclature of baltic cod allergen *(Gadus callarias)*
Gal	galactose
Gal d 1, 2, 3, 4	nomenclature of egg white allergens *(Gallus domesticus)*
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gly m 1, 2, 3	nomenclature of soybean allergens *(Glycine max)*
HLA	human leucocyte antigen
HPLC	high performance liquid chromatography
HR	Histamine Release
IEC	ion exchange chromatography
IEF	isoelectric focussing
Ig	immunoglobulin
IL	interleukin
INF-gamma	interferon-gamma

Lol p 1	nomenclature of rye grass allergens *(Lolium perenne)*
LTA4	leukotriene A4
LTB4	leukotriene B4
LTC4	leukotriene C4
LY	lysozyme
Man	mannose
Mal d 1, 2, 3	nomenclature of apple fruit allergens *(Malus domestica)*
MALDI-MS	matrix-assisted laser-induced desorption/ionization mass spectrometry
MAST	multiple allergen sorbent test
MHC	major histocompatibility complex
Mr	molecular mass
NeuNAc	N-acetylneuraminic acid
NMR	nuclear magnetic resonance (spectroscopy)
OA	ovalbumin
OAS	oral allergy syndrome
OM	ovomucoid
Ory s 1	nomenclature of rice allergens *(Oryza sativa)*
OT	ovotransferrin
PAGE	polyacrylamide gel electrophoresis
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
Phl p 1	nomenclature of timothy grass allergens *(Phleum pratense)*
pI	isoelectric point
PCA	passive cutaneous anaphylaxis (test)
PCR	polymerase chain reaction
PVDF	polyvinyliden difluoride
PVPP	polyvinyl polypyrrolidone
RAST	radioallergosorbent test
RBL cells	rat basophil leukaemia cells
RIEP	radioimmunoelectrophoresis
RNA	ribonucleic acid
RT	room temperature
SAFT	skin application food test
SDS	sodium dodecylsulfate
SEC	size exclusion chromatography
SPT	skin prick test
T	total acrylamide concentration
TCC	T-cell clone
TCL	T-cell line
TGF-beta-1	transforming growth factor beta-1
TNF-alpha	tumor necrosis factor alpha
TR	trypsin
Tris	tris-(hydroxymethyl)aminomethane
Xyl	xylose