Dept of Pathophysiology and Allergy Research

MEDICAL UNIVERSITY OF VIENNA

Emerging Allergens

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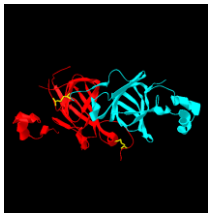
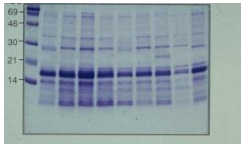
Allergens

- Proteins
- Small mol mass
- Resistant against enzymatic digestion
- Abundancy

These features apply for many proteins - only a minority of those are allergens!!





Allergens

(Food) Allergens belong to a limited number of protein families – sharing structural determinants and displaying sequence similarity - the molecular basis of cross reactivity!

1092 Allergens (IUIS Allergen nomenclature database: www.allergen.org)

(Allergens in ALLFAM Database (http://www.meduniwien.ac.at/allergens/allfam/):
995 Allergens from 186 Protein families
(out of 12.273 Pfam protein families) – 2% of all known domains)

Radauer C, et al J Allergy Clin Immunol 2008, *Hoffmann-Sommergruber & Mills Anal Bioanal Chem 2009*

Protein families of animal food allergens

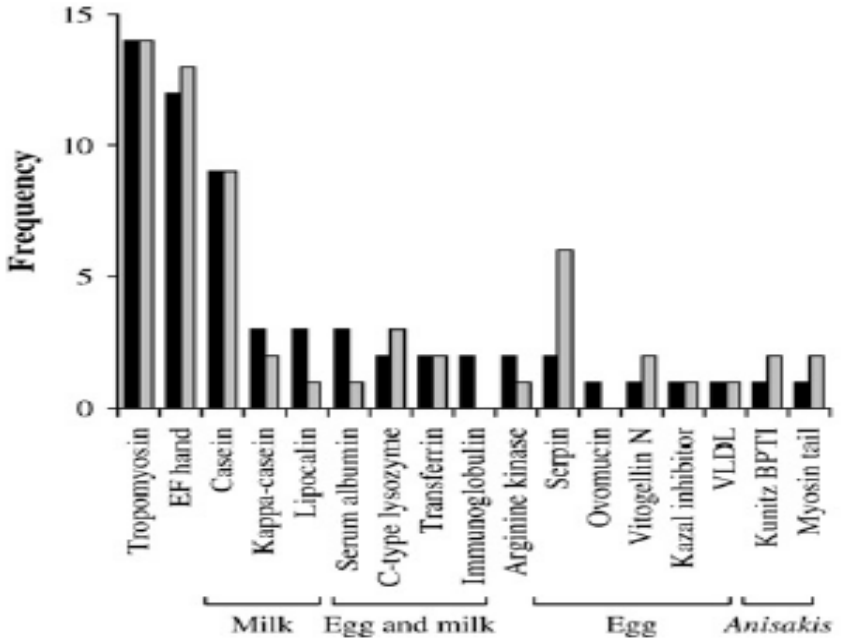
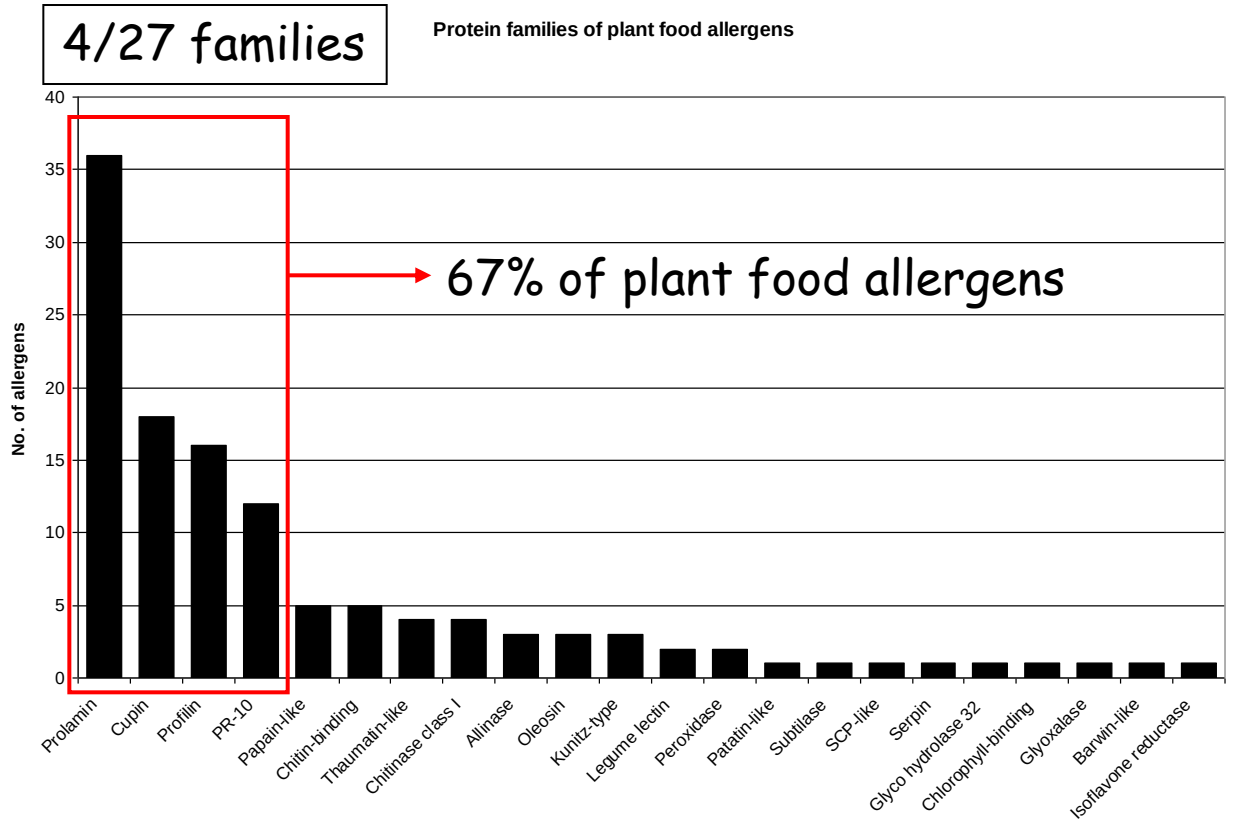


FIG 1. Distribution of animal food allergens into Pfam families. Allergens were from either version 2.1 of the InformAll (www.foodallergens.ifr.ac.uk; *dark bars*) or version 5.0 of FARRP (www.allergenonline.com; *gray bars*) databases. Pfam family names are given apart from very low density lipoprotein (VLDL), arginine kinase, and ovomucin, which represent proteins with multiple different domains. *BPTI*, Bovine pancreatic trypsin inhibitor.

Protein families of plant food allergens



Jenkins et al. J Allergy Clin Immunol 2005

Europrevall

The Prevalence, Cost and Basis of Food Allergy across Europe

- **Allergen library: 53 Allergens**
  - Preparation of natural and recombinant allergens
  - Physicochemical characterisation of natural and recombinant allergens
  - Catalogue of harmonized quality criteria of purified food allergens established
  - Range of analytical methods applied

Sancho et al. Clin Exp Allergy 2010

Europrevall
Physico-chemical Characterization of Allergens

Parameter	Methods
Sequence verification	MALDI-TOF, Q-TOF MS, N-terminal sequencing
Isoforms	2D-PAGE, LC-MS
Folding	CD, FT-IR, NMR
Aggregation	Size exclusion chromatography (SEC)
Purity (proteolysis)	SDS-PAGE, SEC, ES-MS
Biological activity	Lipid binding, enzymatic activity
IgE reactivity (purified nat allergen vs extract; Recombinant vs natural allergen)	ELISA, RAST, CAP, Chip; Immunoblotting (IB), inhibition assay; (human reference sera), Basophil activation test;

Dietary proteins from GM-plants

- Soy
- Corn
- Oilseed rape
- Cotton?

Food Allergen Panels
(for improved diagnosis)

Soy Allergens

- Soy (*Glycine max*): 8 allergens identified and listed in IUIS allergen database.
- Gly m 1 – Hydrophobic protein from soy (7 kDa)
- Gly m 2 – Defensin (8 kDa)
- Gly m 3 – Profilin (14 kDa)
- Gly m 4 – PR 10 (Bet v 1 homologue) (16 kDa)*
- Gly m 5 – Vicillin, beta-conglycinin, (7S)**
- Gly m 6 – Glycinin, (11S)
- Gly m 7 – Seed biotinylated protein (76 kDa)
- Gly m 8 – 2S Albumin***
- Mittag et al. 2004; **Holzhauser et al. 2009; ***Ebisawa et al. 2013;

Soy Allergy

20 Patients with anaphylaxis due to soy ingestion (Salzburg, AT)*

Sera tested by: CAP, CHIP, ELISA

CAP Soy extract: 17/20 negative

CHIP: 14/20 positive to soy allergens

ELISA: 14/20 positive

* Berneder et al. IAA 2013

SAMPLE INFO							Birch	Soybean
Sample ID	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Gly m 5	Gly m 6	Bet v 1	Gly m 4
1	0	0	0	0	0	0	25.9	3.52
2	0	0	0	0	0	0	29.8	0.460
3	0	0	0	0	0	0	7.98	1.57
4	0	0	0	0	0	0	13.9	2.55
5	0	0	0	0	0	0	24.8	4.08
6	0	0	0	0	0	0	12.4	2.11
7	0	0	0	0	0	0	58.4	0.540
8	0	0	0	0	0	0	35.5	0.180
9	0	0	0	0	0	0	9.20	0.070
10	0	0	0	0	0	0	24.9	6.34
11	0	0	0	0	0	0	94.5	13.0
12	0	0	0	0	0	0	50.0	0.160
13	0	0	0	0	0	0	11.3	0.230
15	0	0	0	0	0	0	16.2	0.140
16	0	0	0	0	0	0	24.2	1.07
17	0	0	0	0	0	0	34.5	3.90
18	0	0	0	0	0	0	36.2	1.40
19	0	0	0	0.130	0	0	32.4	1.54
20	26.5	32.3	21.0	37.6	6.04	11.5	0.290	0.120
21	0	0	0	0	0	0	8.40	0.890

Allergen recognition pattern

- 19/ 20 Patients sensitized to Bet v 1 Reactivity to Bet v 1 Homologue, Gly m 4; BP- associated food allergy
- 1/20 sensitized to 7S and 11S globulin (cross reactive to peanut allergens)

Corn allergens

IUIS Database:

Corn (*Zea mays*)

- Zea m 1 – Beta expansin (25 – 35 kDa)
- Zea m 12 – Profilin (14 kDa)
- Zea m 14 – nonspecific lipid transfer protein (8 kDa)*
- Zea m 25 – Thioredoxin

Within Europe food allergic reactions to corn are restricted to certain geographic areas - Italy.

*Pastorello et al. 2000

Oilseed rape

- Oilseed rape (*Brassica napus*)
- Bra n 1 – 2S Albumin (15 kDa)*

Maybe of future interest if protein isolates are used in food production or as a food additive!!

*Puumalainen et al. 2006

Conclusions

- A range of analytical and immunological methods are needed to assess allergenicity of (food) proteins. (reassessment of current methodologies)
- A quantitative outcome is preferred.
- For prevalence of food allergies geographic differences and different dietary habits have to be taken into account!
- Food processing can impact on allergenicity.

Conclusions cont.

- Reevaluation of allergen panels may give further information on clinical relevance.

- Existing knowledge is based on known allergens.