# Allergen Risks from Heritable Genetic Modification in Food Animals

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# FARRP at UNL

- Food Allergen Research and Resource Program.
- **Industrial consortium** ran through Food Science and Technology Dept at UNL.
- >100 member companies.
- Analytical lab (>50,000 samples / year), research, expert guidance, training.
- Currently 3 faculty members, 2 emeriti + 2 adjuncts.

# Food allergy

# Food allergy

- Food allergy affects 3-4% of the adult population of the US. Likely increasing.
- Although only **9 foods are required to be labeled** as allergens (US), **most foods** can cause reactions.
- Specific proteins in foods are known to **sensitize** individuals and **elicit** reactions.
- Type I **IgE**-mediated hypersensitivity.

# Food allergy is not a simple problem

- The involvement of the immune system makes food allergy considerably more challenging issue than most food safety issues :
  - The immune system is *complex.*
  - The immune system is *variable.* Individuals are entirely unique (genetic and environmental factors).

**Allergy comprises hugely complex potential hazards (proteins in foods) with a hugely complex target (immune system).**
**No two allergies are the same.**

# Two major stages of allergy

- **Sensitization** – development of IgE antibodies to a protein or proteins
- **Elicitation** – response to protein(s) to which the individual is sensitized.

**Food ⟶ Sensitization ⟶ Elicitation**

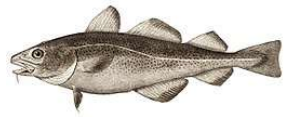
# We barely understand what factors make proteins sensitize

- Exposure to food allergens via **skin or inhalation** is now thought to be a major component of sensitization.
- Early ingestion (4-12 months) is thought to **induce tolerance**.
- Extremely **abundant** proteins in foods tend to be recognized as allergenic
- Were peanut a 'novel food' with no history of consumption, **we could not predict** its status as a major allergen.
- Allergenicity risk assessment is therefore based on **risk of elicitation**.

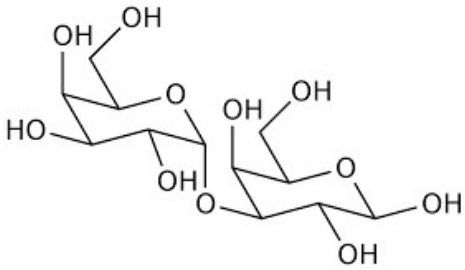
# Animal Food Allergens

# Mammalian meat is not commonly allergenic

- **Milk, Eggs, Fish, Shellfish,** Tree nuts, Peanuts, Wheat, Soybeans, Sesame.
- Humans tend not to be allergic to proteins **similar to those in human bodies**.
- The animal food allergens are mostly:
  - **organs not encountered in human bodies** (eggs)
  - **secreted** (milk), or
  - **taxonomically distant** to humans (**fish** and **shellfish**)



# α-gal syndrome




- **α-gal =** Galactose-alpha-1, 3-galactose
- Initial **sensitization via tick bite**, subsequent **elicitation via meat consumption** (or tick bites).
- **Delayed reaction** (typically 2-6 hours after consumption), Symptoms range from **mild to severe** (anaphylaxis).
- **Proteins modified by α-gal (glycoproteins)** are increasingly implicated in food-elicited reactions.

# Scientific basis for allergen risk assessment in GMOs

# Identification of a Brazil-Nut Allergen in Transgenic Soybeans

**Authors**: Julie A. Nordlee, M.S., Steve L. Taylor, Ph.D., Jeffrey A. Townsend, B.S., Laurie A. Thomas, B.S., and Robert K. Bush, M.D. Author Info & Affiliations

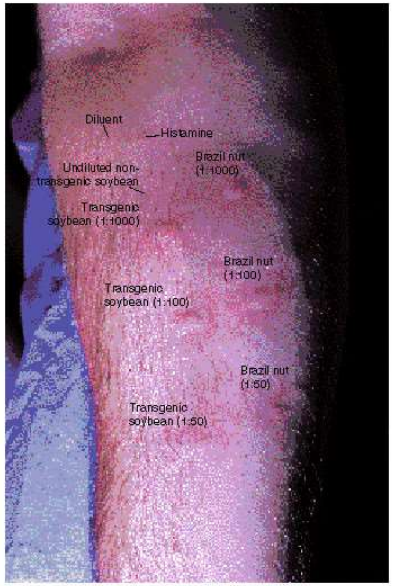
**Published March 14, 1996 | N Engl J Med 1996;334:688-692 | DOI: 10.1056/NEJM199603143341103**

**VOL. 334 NO. 11**

Table 1. Results of Skin-Prick Tests in Three Subjects Allergic to Brazil Nuts.

| Subject No. | Maximal Wheal Diameter/Maximal Flare Diameter (mm) | | | | |
|---|---|---|---|---|---|
| | Diluent | Histamine | Non-transgenic-soybean extract | Transgenic-soybean extract* | Brazil-nut extract* |
| 1 | 0/0 | 6/10 | 2/5 | 15/35 (1:1000) | 10/30 (1:1000) |
| 2 | 1/0 | 7/19 | 2/0 | 9/29 (1:10,000) | 7/26 (1:10,000) |
| 3 | 0/0 | 5/20 | 2/5 | 14/41 (1:1,000,000) | 8/54 (1:1,000,000) |

*The dilutions of the extracts are given in parentheses.




**A gene that encodes an allergenic protein will likely produce an allergen when expressed in a GMO**

# Stability of food allergens to digestion in vitro

James D. Astwood, John N. Leach & Roy L. Fuchs

*Nature Biotechnology* **14**, 1269–1273 (1996) | Cite this article

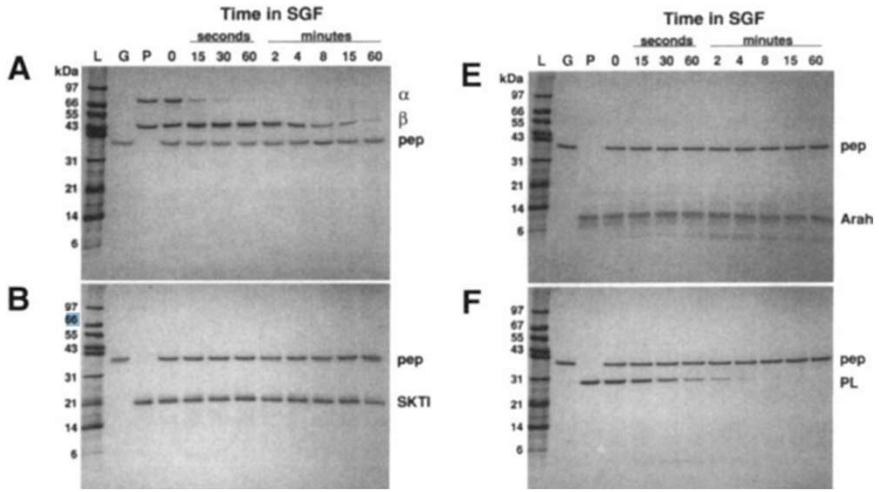



**Table 1. Summary of allergen and protein stability in SGF.**

| Protein | Stability (min) Whole Protein | Fragments |
|---|---|---|
| **Egg allergens** | | |
| Ovalbumin | 60 | — |
| Phosvitin | 60 | — |
| Ovomucoid | 8 | — |
| Conalbumin | 0 | 15 |
| **Milk allergens** | | |
| ß-lactoglobulin | 60 | — |
| Casein | 2 | 15 |
| BSA | 0.5 | 15 |
| **Soybean allergens** | | |
| ß-conglycinin (ß-subunit) | 60 | — |
| SKTI | 60 | — |
| Soy lectin | 15 | — |
| ß-conglycinin (α-subunit) | 2 | 60 |
| Gly m 1 | 0.5 | 8 |
| **Mustard allergens** | | |
| Sin a 1 | 60 | — |
| Bra j IE | 60 | — |
| **Peanut allergens** | | |
| Ara h2 | 60 | — |
| Peanut lectin | 8 | — |
| **Common plant proteins** | | |
| Glycolate reductase (spinach leaf) | 0.25 (15 sec) | — |
| Rubisco LSU (spinach leaf) | 0 (<15 sec) | — |
| Rubisco SSU (spinach leaf) | 0 (<15 sec) | — |
| Lipoxygenase (soybean seed) | 0 (<15 sec) | — |
| PEP carboxylase (corn kernel) | 0 (<15 sec) | — |
| Sucrose synthetase (wheat kernel) | 0 (<15 sec) | — |
| ß-amylase (barley kernel) | 0 (<15 sec) | — |
| Acid phospatase (potato tuber) | 0 (<15 sec) | — |
| Phosphofructokinase (potato tuber) | 0 (<15 sec) | — |

**Resistance to digestion by pepsin is a characteristic of many allergenic proteins**

# Allergen Risk Assessment workflow

- Consider **source** of the transgene
- **Bioinformatic** screening against allergenic proteins
- Resistance to **pepsinolysis**
- **Serum testing** if warranted by above
- **Any scientific evidence** relating to safety will be considered

*CODEX, 2009; Codex Alimentarius Commission, 2003; FAO/WHO, 2001*

Review > Crit Rev Food Sci Nutr. 1996:36 Suppl:S165-86. doi: 10.1080/10408399609527763.

# Assessment of the allergenic potential of foods derived from genetically engineered crop plants

D D Metcalfe [1], J D Astwood, R Townsend, H A Sampson, S L Taylor, R L Fuchs

*"In the end, a balanced judgement of all the available data generated during allergenicity assessment will assure the safety of foods derived from genetically engineered crops."*

**To my knowledge, no proven allergic reaction has occurred as a result of any food being genetically modified**

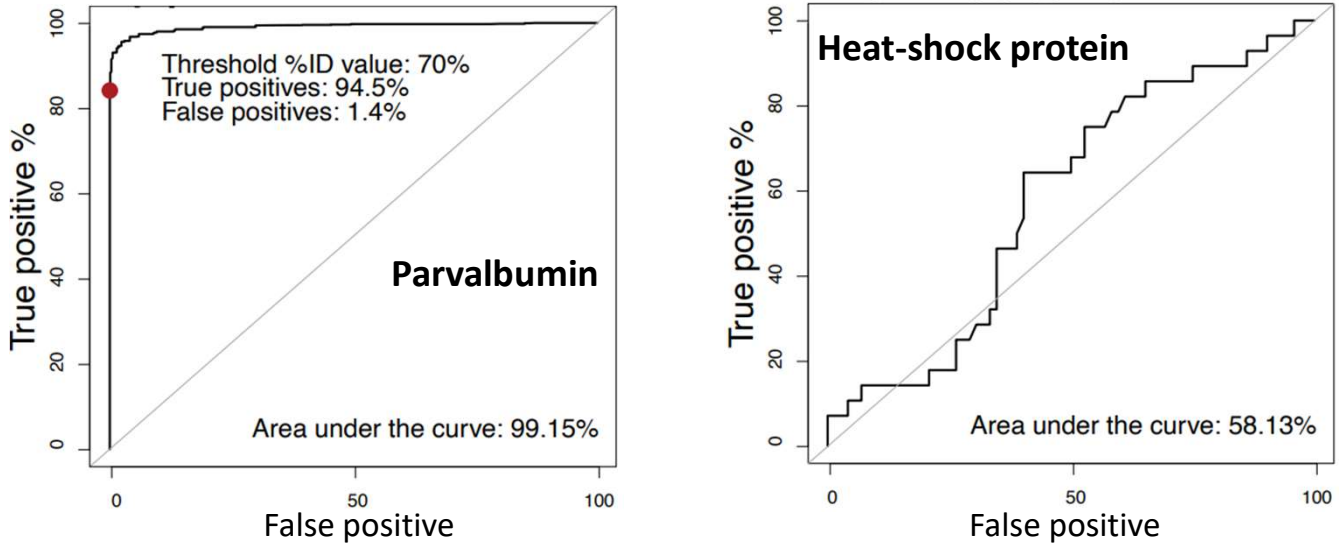
# Sequence Homology (bioinformatic)

- Introducing an allergenic protein is obviously unwise. However, what if a **protein is similar to a known allergen ?**
- **How similar** does a protein have to be to an allergen to make it **allergenic** ?
- FAO/WHO: Primary sequence, **35% Identity** across a sliding 80 residue window.

# Testing the 35% sliding 80-mer

- Little **testing** has occurred. Difficulty in obtaining true allergen / non-allergen sequences.
- Likely **expertise lack** in clinical / food allergy research.
- May be too conservative (Abdelmoteleb *et al*. 2021).

*Food Chem Toxicol 2021 Jan:147:111888*




Pinto NG, Goodman RE, Johnson PE (unpublished data)

# Allergen Sequence Databases

- www.allergenonline.org (FARRP) most frequently used. Others have emerged since AO first release in 2005.
- Researchers submit potential allergens with evidence. **Expert panel reviews**.
- Largely based on **reactivity of proteins with IgE** from clinically relevant human sera. This is suggestive of, but not proof of allergy.
- Some proteins in this and other databases **may not be allergens**. The **degree of allergenicity ('potency')** is not considered either.

food allergy research & resource program farrp

ALLERGEN ONLINE

# Alternatives to current sequence comparisons

- **Multi-feature fusion techniques** (e.g AA composition, dipeptide composition, composition of k-spaced amino acid pairs (Liu *et al*, 2023)
- **Primary** and **tertiary** structure (e.g. AllerCatPro db)
- Primary structure utilizing **machine-learning** techniques (Nedyalkova *et al* 2023)
- Similarity to known **IgE epitopes** (Algpred db).
- Primary sequence utilizing **Restricted Boltzmann Machines** (RBMs) (ALLERDET).
- **Random Forest** approaches including 29 variables derived from sequence and database information (Westerhout *et al*, 2019).

# Testing the pepsinolysis hypothesis

- Recent evidence **does not support resistance to pepsinolysis** as a predictor of allergenicity.
- Many food allergens are susceptible to pepsinolysis. Many non-allergens are resistant.
- Pepsinolysis is still included in most risk assessments, but the **contribution to risk assessment is dubious**.

EFSA J. 2021 Jan; 19(1): e06350.
Published online 2021 Jan 12. doi: 10.2903/j.efsa.2021.6350
PMCID: PMC7801955
PMID: 33473251

Statement on *in vitro* protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants

# Application of other methods for allergen risk assessment in GMOs

# Proteins other than the transgene

- When producing a GMO, **proteins other than the transgene** may change in abundance.
- Especially relevant where **regulatory proteins** are introduced / up-regulated or down-regulated.
- Where the GMO animal is allergenic, this raises the possibility of **changes in the amounts of allergenic proteins**.

# Substantial equivalence in protein abundance

- **Which proteins** have increased in abundance in the GMO?
- **Proteomics** is ideal to describe relative amounts of protein in two similar samples.
- **Known allergens** can be specifically quantified, or **any protein that changes significantly** can be identified depending on the workflow.

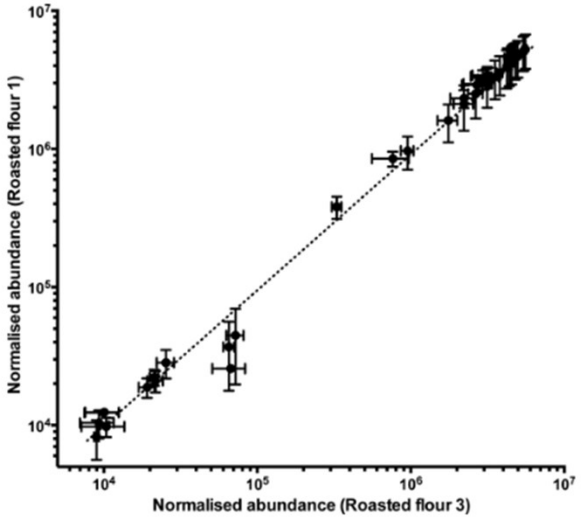

Front Plant Sci. 2013; 4: 41. PMCID: PMC3590489
Published online 2013 Mar 7. Prepublished online 2013 Jan 4. doi: 10.3389/fpls.2013.00041 PMID: 23471542

Proteomic evaluation of genetically modified crops: current status and challenges

Chun Yan Gong and Tai Wang*

# Utility of untargeted proteomics

- Relatively easy to perform as only **relative quantitation** is required.
- Provides extensive information on the **transgene product** itself (e.g. sequence verification, modification, truncation).
- Most **abundant proteins** are identified and quantified.
- Information also useful for **other purposes** (e.g. toxicology, nutrition).



# A note on wild-type comparators

- Usually, we would consider a **wild-type vs GMO** comparison to be suitable for comparing allergen contents.
- However, food organisms of a given type **vary widely** in their protein composition (genetic and environmental).
- The pertinent question is ‘does the GMO **fall outside the normal expected range** in terms of presence of allergenic proteins ?’.

# Conclusions

- Initial assessment of **elicitation potential** draws on initial **taxonomy**, **sequence similarity**, **pepsinolysis**.
- Further risk assessment, if warranted, involves **sera of human allergic individuals** who are sensitized to foods containing sequences similar to the transgene.

# Considerations for the future

- Although **sound in practice**, current regulatory requirements for allergen risk assessment in GMOs could be **updated**.
- The 35% / 80-mer should be **validated**, or changed to a more **discriminatory, validated**, approach.
- Currently no consideration of **self-tolerance** – proteins like those in the human body are unlikely to be allergenic.
- **Allergen databases** – especially including potency and possibly abundance information to facilitate RA.

# Considerations for the future 2

- **Pepsinolysis** is at best poorly predictive of allergenicity. Validate or remove.
- Novel workflows, including proteomics, should be **standardized** to ensure consistency of application.

# Acknowledgements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