Allergies alimentaires, cherchez le coupable

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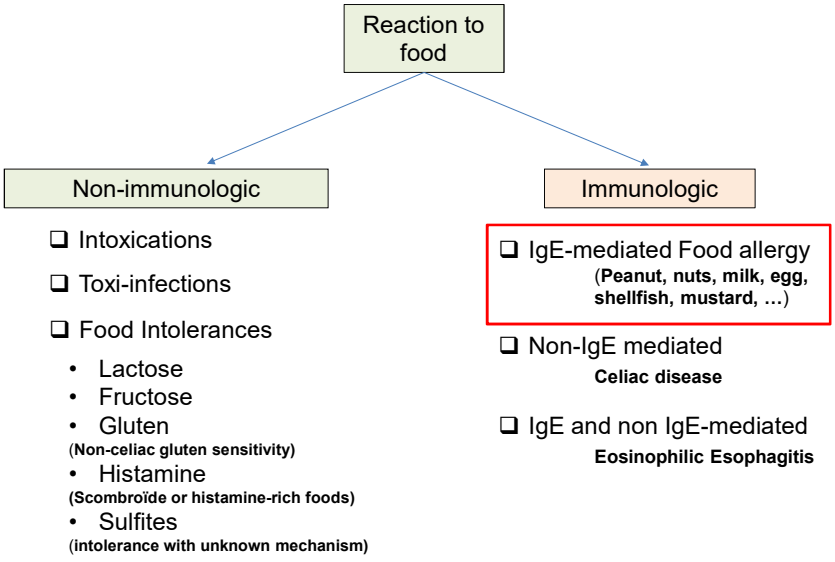
SCAV

Content

- Food allergies and intolerances
- Development of an LC-MS/MS screening method for multiple food allergen
- Application: Food allergen study AAGE21

Undesired reactions to food



Adapted from Maître *et al.*, Revue médicale Suisse, 2014

Legal context

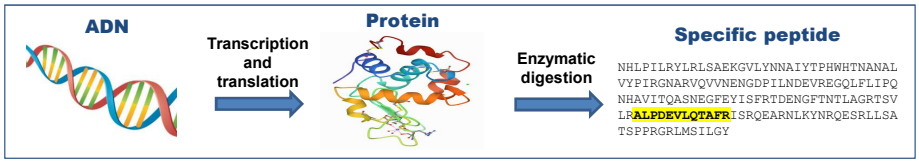
Ordinance on food information

- Ingredients that may cause allergies or other adverse reactions: **Cereals containing gluten, crustaceans, eggs, fish, peanuts, soya, milk, nuts, celery, mustard, sesame, lupines, molluscs and sulfites**

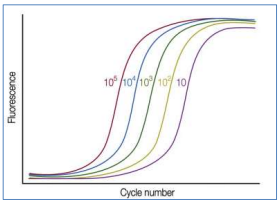
  ➔ "14 majors"

- These ingredients must be **clearly mentioned and highlighted** in the ingredient list on pre-packaged products.
- They must also be mentioned when they unintentionally enter a foodstuff and their content exceeds or may exceed **1 g/kg** for **allergens** of interest (mention: **may contain ...**), except **gluten (200 mg/kg)** and **sulfites (10 mg/kg).**
- For bulk products, information on allergenic ingredients must also be provided, for example orally.

Three strategies for allergen analysis



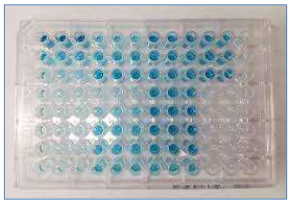
Gene amplification (PCR)



- Highly sensitive
- Multiplex

- ADN quantity ≠ allergen quantity
- Milk, processed foods, etc.

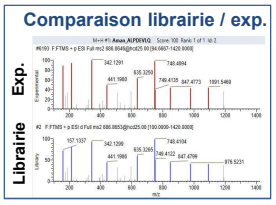
Immunoassay (ELISA)



- Highly sensitive
- Quantitative

- No multiplexing
- Protein denaturation

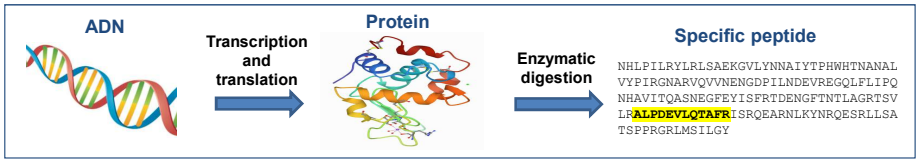
Proteotypic peptide (LC-MS/MS)



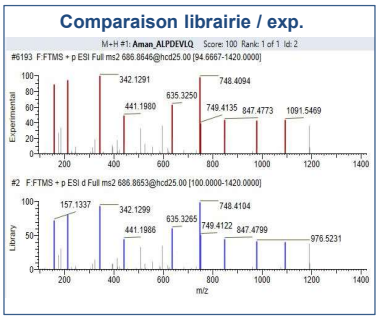
- Multiplex
- Many peptides per allergen

- Not quantitative
- Not very sensitive

In-house method development



- **Extraction of soluble proteins**
- **Reduction / alkylation of cysteins**
- **Digestion with trypsin (overnight)**
- **Desalting on SPE cartridges**
- **LC:** Kinetex C18, 100 x 2.1mm, 2.6µm
  H2O / AcN with 0.1% Formic acid, 0.25 ml/min
- **MS:** Orbitrap Q-Exactive Focus
  PRM mode for MS/MS
  R = 17500 [FWHM]
- **Data treatment / identification criteria:**
  Precursor ion (Δ = 5 ppm)
  3 fragment ions (Δ = 5 ppm)
  RT (Δ = 60s)
  Library search match with syntethic peptide



Selection of proteotypic peptides

Previously described proteins/peptides with similar physicochemical proprieties

No previous data from litterature, or described peptides not observed with extraction procedure

Egg, gluten, almond, pecan nut, mustard, lupine, sesame, molluscs, fishes

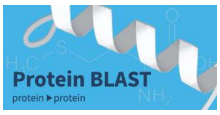
In-house identification of soluble, abundant proteotypic peptides from databases using peptide-spectrum match search tools (Andromeda)

Milk, tree nut, hazelnut, pistachio, brazil nut, cashew, shrimps, cod, soy, peanut

Litterature:

- Ogura *et al.*, Journal of AOAC, 2019
- Planque *et al.*, Journal of Chromatography A, 2017
- Van Vlierberghe *et al.*, Food Chemistry 2019
- Gavage *et al.*, Food Chemistry 2020

Basic Local Alignment Search Tool



Compare potential proteotypic peptide sequence with all sequenced proteins (all species) ➔ ensure specificity to considered allergen

Spiking various food matrixes

- **Ensure specificity in real life samples**
- **Estimate assay sensitivity at legal limit of 1g/kg**

Sensitivity

Spike at 1g/kg with allergen food in various matrices

Matrices: breakfast cereals, pop-corn, spice mixes, baby formula, milk chocolate, protein powder, herbal teas, spirulina food supplements, olive oil, marzipan, sushi, …

	Nuts / Almond	Nuts / Brazil nut	Nuts / Cashew	Nuts / Hazelnut	Nuts / Pecan	Nuts / Pistachio	Nuts / Tree nut
Positive samples	26	28	28	27	28	28	28
True positives tests	24	21	24	24	27	27	24
False positives	**0**	**0**	**0**	**0**	**0**	**0**	**0**
False negatives	2	7	4	3	1	1	4
Sensitivity	**0.92**	**0.75**	**0.86**	**0.89**	**0.96**	**0.96**	**0.86**

Conclusion: Not a sensitivity problem, but a complexity and dynamic range problem

1 g of Hazelnut per kg food

- Hazelnut = 14% protein
- Cor A 9 is a major storage protein : 10% of protein content

14 mg of Hazelnut Cor A 9 allergen

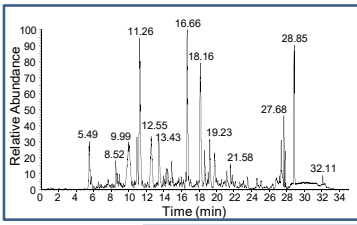
- 59 kDa protein ➔ 1600 Da peptide

380 µg proteotypic peptide

- observed at *z* = 1, 2, 3 and 4

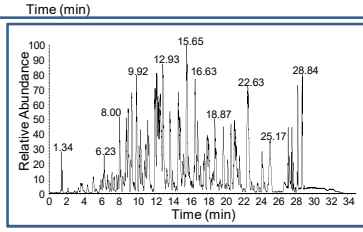
Equivalent of a 300 Da molecule (single-charged) ≈ 40µg

Complexity of the matrix



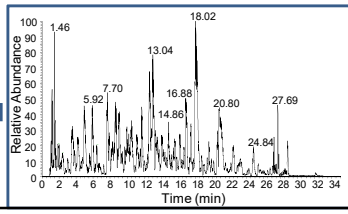
Pop Corn

- Corn



Milk chocolate

- Cacao
- Cow milk



Protein food supplement

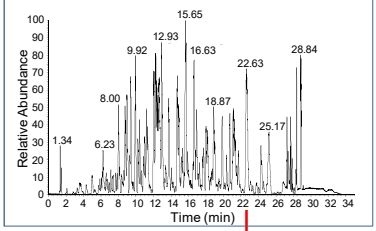
- Wey proteins
- Soy proteins
- Pea proteins
- Chickpea proteins

25 000 proteins / species
1 protein ➔ 20 peptides

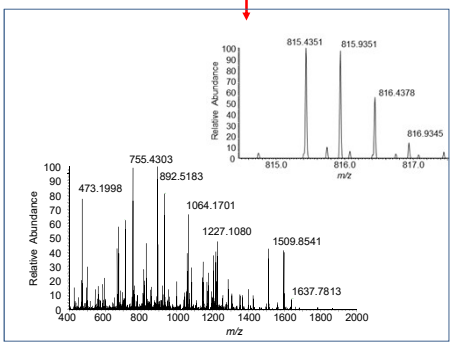
2 * 10^6 peptide species

Specifity - Complexity of the matrix

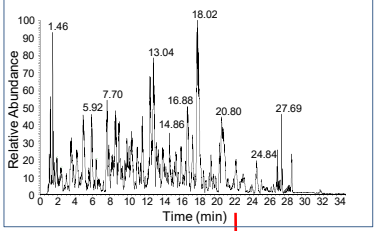
Chocolate



RT = 21.9 min



Protein food supplement



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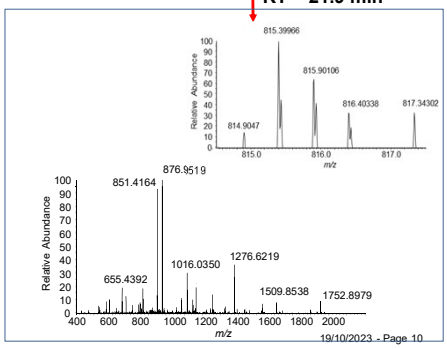
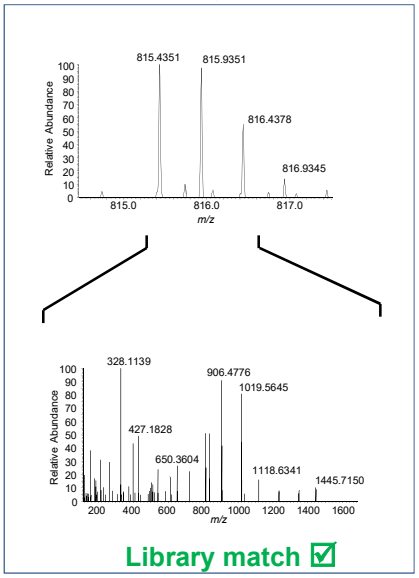


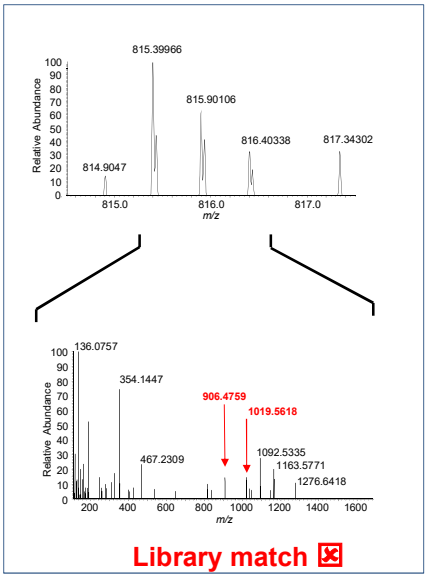
Co-eluting peptides & false negatives

RT = 21.9 min, milk chocolate



Library match ☑

RT = 21.9 min, protein food supplement



Library match ☒

Application on real-life food samples

Analysis of 200 food samples from official food control

- Convenience foods
- Sushis
- Stuffed pasta
- Spice mixes
- Chocolate
- Bread spread

Allergen screening (LC-MS/MS)

- **In >95%, declared allergens were identified**
- **8 cases of undeclared allergen > 1 g/ kg** (quantitative & confirmation analysis by ELISA)
- Trace allergens in the 50 – 100 mg/kg range routinely identified (Quantification by ELISA)
- **No false positive identification**

8 / 200 Non-compliant foods

- Other nut species
- Gluten in spice mixes
- Milk in sushis
- Milk in dark chocolate
- Crustaceans in sauces

➔ **Recall**

Conclusion:

- **Robust method, adapted for official food control**
- **Valid alternative to individual ELISA / PCR assays**

AAGE 21 Study
(Allergies alimentaires à Genève en 2021)

Hôpitaux Universitaires Genève

REPUBLIQUE ET CANTON DE GENEVE

Study goal :

Investigate relationship between nature and **quantity** of food allergen, the **consumed food** and the **severity** of the symptoms

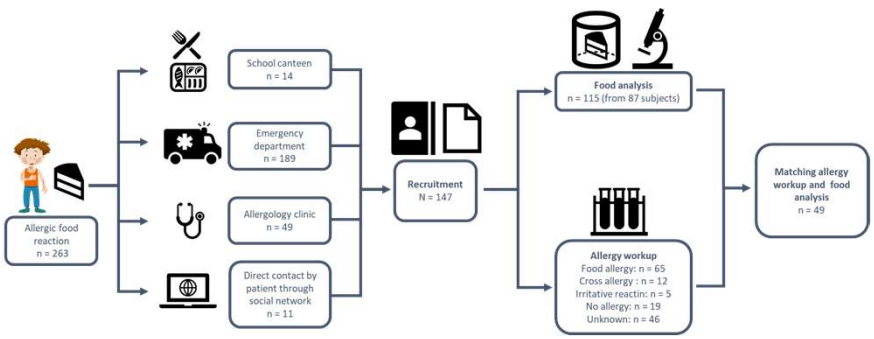
Method :

- Case investigations after emergency and allergological consultations regarding suspected food allergies via a questionnaire
- Collection and analysis of the suspected food, if possible
- Definitive confirmation of food allergy via allergological testing and follow-up

Outcome :

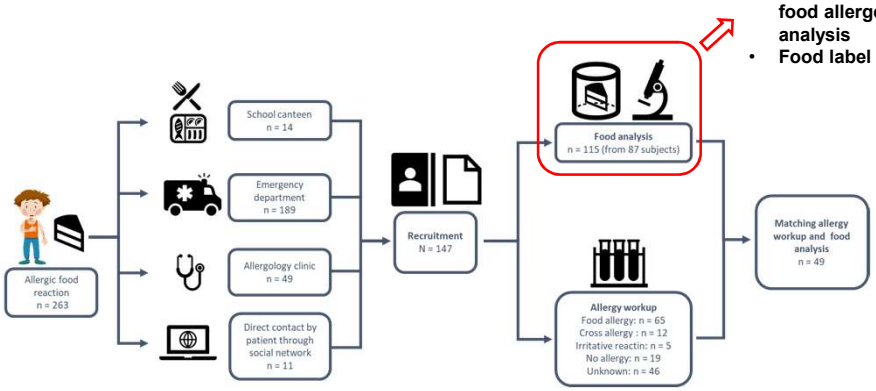
- Link between **allergen quantity** in food **reaction severity**
- Global vision of **cases requiring a medical consultation**
- Study the **circumstances** leading to a food allergy reaction
- **Food legislation sufficient** regarding food allergies?

Study design & recruitment

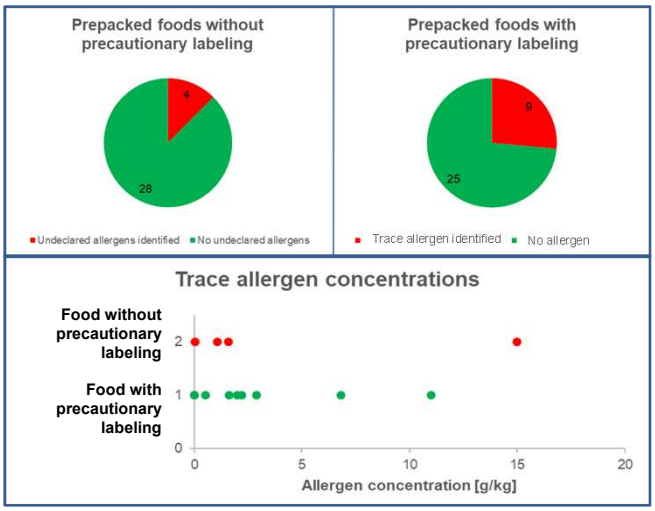


Study design & recruitment

- Quantitative food allergen analysis
- Food label

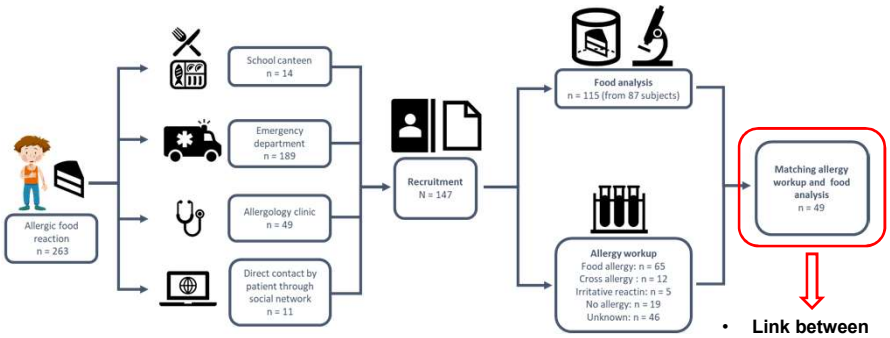


Trace allergen labeling



- For consumers, “traces” is understood as being a very small amount of allergen
- This belief can cause relatively tolerant allergic patients to consume sufficient amounts to trigger a reaction

Study design & recruitment



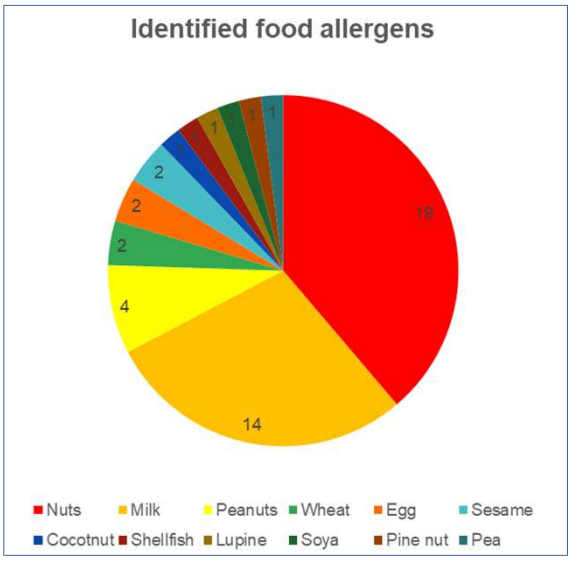
- **Link between food and allergic reaction**
- **Allergen amount**
- **Reasons for adverse reaction**

Food responsible for allergic reactions

All confirmed allergic reactions to food with identified allergen (n = 49)

- 40 pre-packed food
- 6 home-made food
- 3 bulk foods
- 46 children

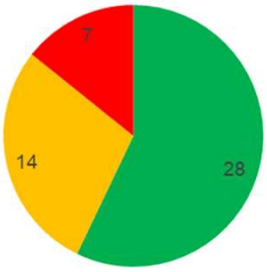
- *Probably biased towards pre-packed food due to food collection method*



Nature & reasons of allergic reactions

7 accidental reactions to undeclared ingredients or traces

- Milk and hazelnuts
- 2 undeclared allergens (vegan chocolate with 2 g/kg milk, sorbet with 9 g/kg milk)
- Label not read in 2 cases
- Trace labeling ignored in 3 cases



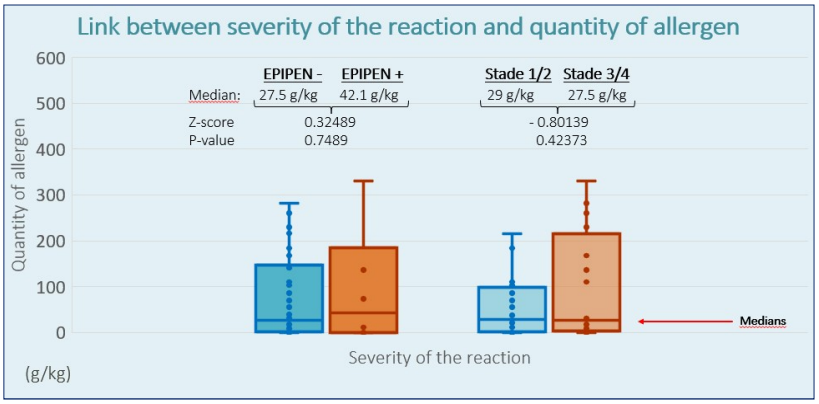
28 First-time reactions

- Patients not aware of food allergy
- Mostly compliant food, allergen is a major ingredient
- 60% Milk and peanuts
- 90% are part of the "14 majors"
- Other allergens: pine nuts, coconut and peas

14 accidental reactions to declared ingredients

- 8 pre-packed foods
- 5 labels not read
- 4 formally compliant labels, but considered as "confusing"
- 7 cases of milk and nuts

Allergen amount *versus* reaction severity



- **Mild reactions**: Grade I or II on the Mueller grading system, (skin or digestive reaction)
- **Severe reactions**: Grade III or IV on the Mueller grading system (dyspnoea, dysarthria, hoarseness, weakness, confusion, and loss of consciousness, anaphylactic choc)
- The use of **Epipen** (intramuscular adrenaline) is also an indicator of severity

Conclusions

- **12 Majors in one shot**
- **Robust, specific, low false negatives**
- **Large variety of food matrices**
- **Limits the parallel use of specific ELISA kits**
- **Suited for official food control**

- **Future work: Quantitative aspects with spiked internal standard protein**

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REPUBLIQUE ET CANTON DE GENEVE

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