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Improving Allergy Risk Assessment Strategy for New Food Proteins - *In silico* tools for exploring potential human allergy to novel protein

Key external stakeholders:
Food manufacturers and companies interested in alternative and novel proteins, medics, food scientists and formulation scientists

Practical implications for stakeholders:
Food allergens are almost always proteins, but not all food proteins are allergens. Allergens elicit an IgE response, and then, on subsequent exposures, elicit a clinical response to the same or similar protein. Biochemical characteristics that allow a food protein to survive the extremes of food processing, escape the digestive enzymes of the human gastrointestinal tract, and interact with the immune system can elicit an IgE response and cause an allergic reaction. Bioinformatics can help scientists to develop hypothesis about proteins that may need to be tested further for risks of causing allergy. In silico methodologies and tools like databases and comparison software, play an important role in the assessment of protein allergenicity and allergenicity mechanisms. They can identify whether a novel protein is an existing allergen and/or has the potential to cross react with an existing allergen. They cannot identify whether a novel protein will 'become' an allergen. ImpARAS explored in silico methodologies to help predict human allergy to protein. This was achieved by:

- Building an interdisciplinary, European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity risk assessment strategy. This facilitated international collaboration in the development of more predictive tools to assess allergenicity.
- Assessing a number of physico-chemical features including amino acid searchers on proteins.
- Reviewing current *in silico* tools for assessment of potential human allergenicity to proteins.
- Trialling protein sequences through programmes including FLAPs (ulfh@slv.se), BcePred (http://www.imtech.res.in/raghava/bcepred/), Bpredictor (https://code.google.com/p/my-project-bpredictor/) and Epitopia (http://epitopia.tau.ac.il).
- Looking at the impact of processing technologies including hydrolysis, fermentation, heat-treatment and High Pressure Processing (HPP) treatments on the potential of a protein to act as an allergen.
- ImpARAS identified that fermentation of food proteins reduced the risk of proteins being allergens.

Main results:
To date, most European food allergy initiatives have focused on managing the risks from existing common allergens, whereas the question 'what makes a food protein an allergen?' has received much less attention. An underlying hypothesis of ImpARAS was that allergenicity prediction should not be restricted to analyses of IgE-binding properties and clinical phenomena, as these are 'end-of-pipeline' events, but should also encompass aspects to (intestinal) allergen/protein absorption and initial events related to innate immune functioning that initiate 'allergic inflammation'. The basic question: 'what makes a food protein an allergen?" sums up the purpose of ImpARAS: defining both the intrinsic difference between an innocuous food protein and a potential food allergen and any factors which may modulate this difference in order better to be able to predict the allergenic risk from new or modified food proteins.

Contact Maria Hayes Email: Maria.Hayes@teagasc.ie.

Specific results were:

- The development of an interdisciplinary network that focused on the role and intrinsic properties of the (modified/new) food and food proteins on the development / maintenance of food allergy.
- The Action integrated multi-COST disciplinary knowledge on food (components ~specifically proteins) from different research institutes and countries by bringing together scientists with a broad range of expertise (protein technology, gastro-intestinal physiology, biochemistry, toxicology, immunology, food processing, allergy, risk assessment).
- Novel thinking and new ideas and models were developed to improve the current risk assessment strategy for allergy and its components.
- The Action provided an opportunity to have a broader scope on food allergy, by sharing knowledge and combining research focus between different research institutes and countries. The developed knowledge is applicable to multiple EU and otherwise funded projects.
- The paper "Hayes, M., Rouge, P., Barre, A., Herouet-Guicheney, C., Roggen, E. L. (2015). In Silico Tools for Exploring Potential Human Allergy to Proteins. Drug Discovery Today: Disease Models, 17-18, 3-11" was published.

This Action helped to develop an improved allergy risk assessment strategy for novel proteins by adding more predictive tools to the current risk assessment strategy, and this should help to accelerate the introduction of novel protein (sources) onto the market, and to mitigate the concerns of consumers around novel or genetically modified protein (products). The action also assisted policy makers concerning the safety of novel protein (products).

Opportunity / Benefit:

This project provided information concerning the potential allergenicity of novel proteins to food companies and scientists interesting in developing new products with novel proteins. The methodologies developed will assist food companies in determining the safety of novel protein sources.

Collaborating Institutions:

University of Liege; Medical University of Vienna; Novozymes A/S; University of Warmia and Mazur; DTU; Swiss Institute of Allergy and Asthma; TNO; Nestle Research Center.

Teagasc project team:	Dr. Maria Hayes (Teagasc PI)
External collaborators:	DR. KITTY VERHOECKX, TNO (Coordinator); MR. RENE CREVEL, Unilever, R&D; DR. GABRIEL MAZZUCCHELLI (WP 1 Leader); PROF. KARIN HOFFMANN-SOMMERGRUBER (WP1); DR. ERWIN ROGGEN (WP2 Leader); DR. LIAM O'MAHONY (WP3); DR. BEN REMINGTON, TNO (WP4 Leader); DR. ANNE CONSTABLE (WP4).

1. Project background:

Due to the continuing growth of the world population from 7 billion today to 9 billion in 2050, we will face a shortage of protein sources for human consumption in the near future. For this reason, Horizon 2020 included the topic: "Sustainable European bio-economy; bridging the gap between new technologies and their implementation" within their research program. Food safety assessment is an important requirement before new products can be brought to market. Such assessments include the investigation of microbiological and toxicological hazards as well as the risk of food allergy.

From an industry perspective, there is a need for: a) relatively cheap, easy and reliable tools for screening for allergenicity of new or modified food proteins, b) early risk based decision-making during product development; and, c) an improved risk assessment strategy accepted by regulatory authorities.

The ImpARAS scientific network improved strategies to predict the allergenicity of novel or modified proteins or proteins from novel sources using novel and innovative approaches that were not previously identified. These results were transferred to European food companies to develop safe products, advise food safety authorities on better risk assessment strategies and change public opinion on the safety of novel sustainable food.

2. Questions addressed by the project:

- What methods can be used to screen and identify proteins that may be a risk regarding food allergy?

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- What screening and processing methods can be used to reduce the risk of a novel protein causing an allergic reaction?

3. The experimental studies:

Allergenicity is the potential of any material to cause sensitization and allergic reaction and is frequently associated with the IgE antibody. An existing allergy/allergen is a real and immediate risk. Allergens represent a small fraction of the proteins that humans are routinely exposed to. The reason why these proteins can cause T- and B-cell responses remains largely unanswered. Furthermore, a sensitized individual may respond to proteins that share certain structural features with the protein that elicited the initial immune reaction – a phenomenon known as cross-reactivity. In silico methodologies can identify whether a novel protein is an existing allergen or whether the novel protein has potential to cross-react with an existing allergen. However, they cannot identify whether novel protein will 'become' an allergen. Data produced from the use of in silico methodologies may be used to make a decision about whether additional in vitro and in vivo testing is required, by serum screening, as recommended by Codex Alimentarius Commission (2009). Several in silico methodologies compare amino acid sequences from a novel, trait protein to known food, contact and respiratory allergenic proteins found in allergen databases. This work looked at these databases, looked at sequences novel proteins and determined if certain novel protein could act as allergens if consumed i.e., it predicted the potential of a protein to act as an allergen. In silico methods were developed and applied to novel proteins. These in silico methods are detailed in Figure 1.

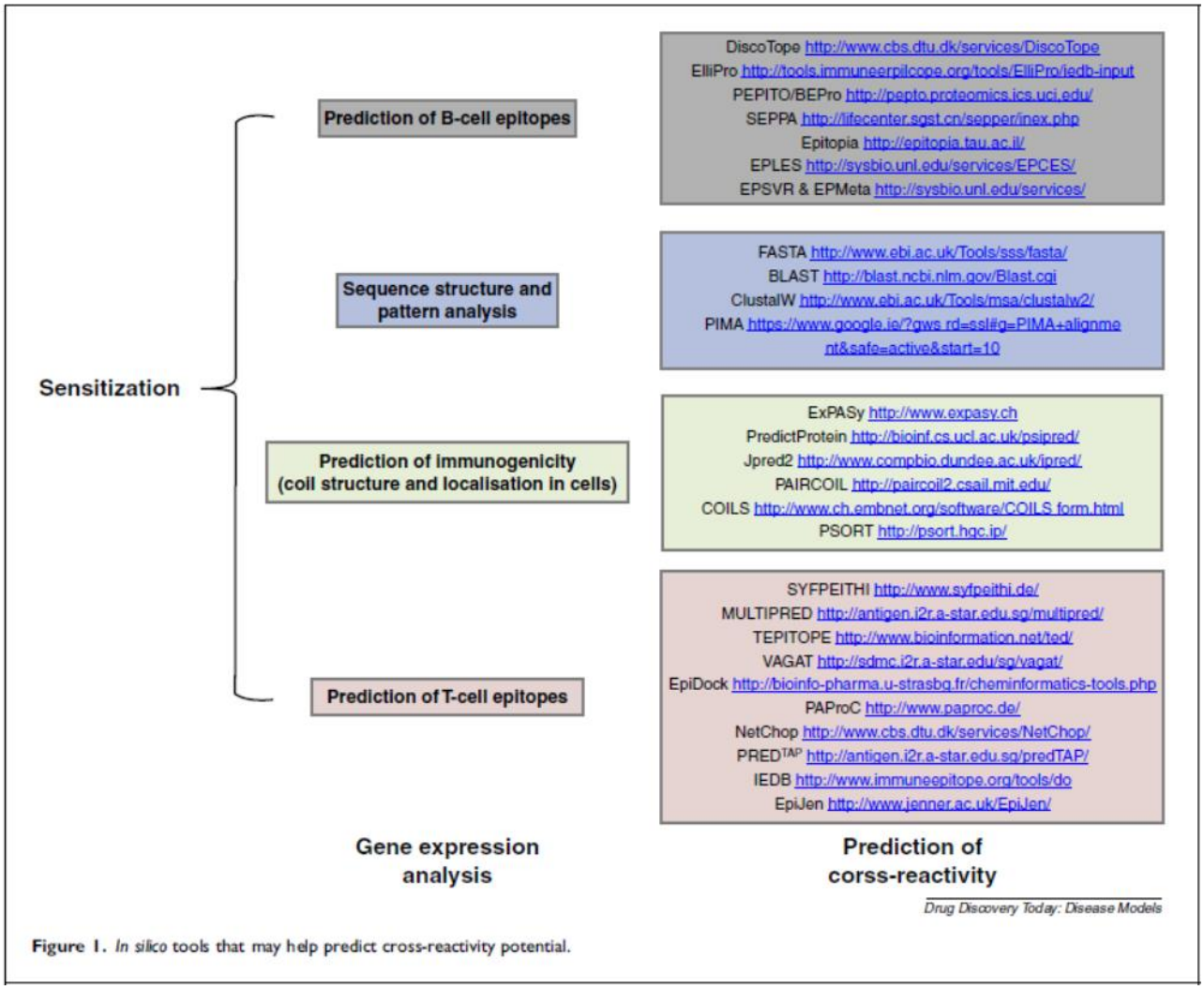

Figure 1. *In silico* tools that may help predict cross-reactivity potential.

Figure 1: In silico tools that may help predict cross-reactivity potential of proteins

4. Main results:

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allergens, whereas the question 'what makes a food protein an allergen?' has received much less attention. An underlying hypothesis of ImpARAS was that allergenicity prediction should not be restricted to analyses of IgE-binding properties and clinical phenomena, as these are 'end-of-pipeline' events, but should also encompass aspects to (intestinal) allergen/protein absorption and initial events related to innate immune functioning that initiate 'allergic inflammation'. The basic question: 'what makes a food protein an allergen?" sums up the purpose of ImpARAS: defining both the intrinsic difference between an innocuous food protein and a potential food allergen and any factors which may modulate this difference in order better to be able to predict the allergenic risk from new or modified food proteins.
Specific results were:

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5. Opportunity/Benefit:

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6. Dissemination:

Several training schools were held during the course of the ImpARAS project at different EU locations including: Vienna (4th-5th June 2018); Liége (29th November 2017); Warsaw training school (September 2016); Serbia training school Belgrade (2015) and Madrid training school (2015).

Main publications:

Hayes, M., Rouge, P., Barre, A., Herouet-Guicheney, C., Roggen, E. L. (2015). In Silico Tools for Exploring Potential Human Allergy to Proteins. Drug Discovery Today: Disease Models, 17-18, 3-11

7. Compiled by: Maria Hayes

Contact Maria Hayes Email: Maria.Hayes@teagasc.ie.