Draft Book of Abstracts and Bios

Allergenicity assessment, Virtual meeting on MS Teams, 15-16 June 2021

Chair: Javier Moreno (Institute of Food Science Research (CIAL, CSIC-UAM), Madrid, Spain)

Abstracts

Clinical relevance of food allergens

Thomas Eiwegger (The University of Toronto, Canada)

Allergenicity risk assessment

Claire Mills (University of Manchester, United Kingdom)

There are two facets to assessing the allergenic potential of food.

(1) Assessing the risks posed to the existing food allergic population: Technologies, such as transgenesis and gene editing, have the potential to change the potency of allergenic foods to elicit allergic reactions through intended or unintended changes in the levels of allergens. Transgenesis can also be used to express an allergen from one source in another food source, representing a "hidden" allergen. Thus, it is critical to ascertain whether a newly expressed protein is sourced from an allergenic food. Lastly, homology between newly expressed or novel proteins and existing allergens provide potentially cross-reactive IgE epitopes. These are amenable to bioinformatic analysis which allows the potential for a newly expressed protein to trigger a reaction in the existing allergic population to be assessed with a high degree of assurance.

(2) Assessing the potential for a newly expressed protein, an innovative protein, or a novel food protein to initiate the production of an IgE response in a naïve individual: The risk assessment process for de novo sensitisation is uncertain because our knowledge of the mechanism(s) of sensitisation is incomplete and effective animal models for predicting allergenic potential of proteins are lacking. Susceptibility to in vitro digestion, including the pepsin-resistance test, provides an indication of the potential for a new protein to reach the gut mucosa in an immunologically active form. However, uncertainties remain over the interpretation of such test results for allergenicity risk assessment. Cell-based models may provide an alternative but are unvalidated and it is unclear how read-outs may be used in the risk assessment process. More effective post-market surveillance could also provide valuable information as to the effectiveness of current approaches in preventing new allergens finding their way into the marketplace.

ImpARAS, EU Cost Action

Kitty Verhoeckx (Medical University, Utrecht, The Netherlands)

The growing world population and increased pressure on agricultural resources are driving a shortage of dietary protein sources. As a result, the industry is developing more sustainable novel food sources such as insects, algae and duckweed and new processing techniques. Consumer exposure to these

novel or processed proteins, could cause new food allergies, exacerbating a public health issue which is already affecting an estimated 20 million Europeans directly. Introduction of novel foods should not add to the burden of allergy and this calls for a reliable, harmonised, evidence-based, and validated allergenicity assessment strategy. COST Action ImpARAS, a four-year networking project, identified and characterised gaps in the current allergy risk assessment strategy, and proposed new ideas and plans for improving this strategy. This presentation reports on the lessons learned from the ImpARAS network and suggestions for future research, not on the research itself. To enable the safe introduction of novel and more sustainable food protein sources, while protecting humans from food allergy, a multidisciplinary approach is needed based on novel methodologies, harmonized decision-making criteria, a clear ranking of existing known allergenic proteins (weakly to strongly allergenic), and improved understanding of what determines the relative allergenic potency of proteins.

COMFA, EU Cost Action

Daniel Munblit (Imperial College, London, United Kingdom)

Food allergy has a major quality of life impact on affected citizens and their careers. The adverse impact of food allergy on quality of life is similar to other chronic diseases such as asthma, arthritis and type 1 diabetes mellitus and effects are greater with increasing age. Chronicity, the increase in prevalence and the potential to lead to life-threatening reactions, make food allergy a significant burden on the European Community. The European Academy of Allergy and Clinical Immunology (EAACI) have stated "Governments and the general public are expected to face increasing direct and indirect costs, due to its major effects on lifestyle and quality of life. Unfortunately, a high number of unmet needs remain to be resolved because of gaps in current scientific knowledge in pathophysiology, preventive measures, standardization and patient care".

The Core Outcome Measures for Food Allergy (COMFA) consortium aims to provide a solid knowledge base taking advantage of existing links, in part created through previous EU funding, to establish multidisciplinary Network of scientists, clinicians, patient representatives and industry required to address this challenge in a cohesive, pan-European manner. COMFA is focused on the development of a core outcome set for food allergy. Crucial parameters for outcome measure includes pathway includes domain definition (that is what to measure), specific measurement instrument (that is how to measure that particular outcome/domain). Core Outcome Set will allow trialists to design their trial outcomes with confidence, reduce the risks for industry in new product development, support regulators in assessing and authorising products, and allow individuals affected by food allergy to understand the relative benefits or harms of different treatment options.

In silico approaches for Allergenicity (IgE and non IgE)

Ivan Dimitrov (Medical University of Sofia, Bulgaria)

Existing methods for in silico assessment of the allergenicity of an unknown protein are based on the comparison of its structure with the structure of known allergens and the search for similarity. All approaches have one common drawback - they are limited by the available information. The manifestation of protein allergenicity occurs in two phases - sensitization and elicitation. In practice, the protein is considered an allergen after the manifestation of an allergic reaction to it. Therefore, all the methods are based on the information obtained in the second phase. In this presentation we will discuss the main approaches for in silico assessment of protein allergenicity, the challenges of modern in silico methods and possible solutions to overcome the existing limitations of these methods.

% identity analysis

Rick Goodman (University of Nebraska, Lincoln, USA)

Bioinformatics evaluations for potential risks of IgE mediated cross-reactivity or allergy and of glutens that can impact patients with celiac disease. The CODEX guidelines looking for homology of >35% identity over 80 amino acids is far too conservative for food safety as many proteins are conserved in evolution across broad taxonomic space. We need to classify protein types and establish scientifically justified criteria that does not capture multiple false positive matches which we found searching predicted proteomes of 23 diverse species including humans compared to www.AllergenOnline.org. The data shows specific protein types that are highly conserved including lipid transfer proteins, vicilins, glycinins, and some enzymes (Abdelmoteleb et al., Food and Chem Toxicol 2021). Without modification, many new foods and modifications that are beneficial for food security will be delayed or lost due to requirements for unnecessary demands for serum IgE tests using many donors with diverse allergic specificities. For risks of celiac disease, gluten peptides from wheat, barley, rye and oats that cause T cell proliferation or toxicity should include predicted native and deamidated glutamine forms as shown in our celiac database. Other members of *Pooideae* likely have multiple gluten proteins. An example will be provided from a wild grass relative of wheat. Comparisons of sequences by BLASTP to the NCBI protein database can provide useful information to sort out irrelevant matches. Predictions by SVM, predictions of epitopes by artificial intelligence and similar methods have not been proven to be predictive and should not be performed.

3D structure analysis

Sebastian Maurer-Stroh (Bioinformatics Institute (BII), Singapore)

Safety assessment of proteins for allergenic potential is an important task ranging from new food trends, industrial enzymes to everyday consumer products. Building on data sets and expert knowledge gained by many groups, we previously developed AllerCatPro, a computational workflow considering the sequence and 3D structure of proteins for a comprehensive assessment of the allergenic potential of proteins. Using a benchmark set of known allergens and likely non-allergens sharing the same structural fold, we showed that the 3D epitope similarity method increased accuracy of classification by 2-fold compared to the classical linear window approach. An adjusted hexamer hit approach led to a 6-fold reduction of false positives. We have since been working on several extensions, these include:

1) update and annotation of the underlying data set

2) increased cross-links in the output (adding protein family views beyond the best hit)

3) a structure-guided approach to detect Celiac disease peptides and

4) inclusion of explicit knowledge of measured epitopes from IEDB both in a linear and 3D context for selected families.

The latter has been used in a project comparing insect proteins with known shellfish allergens to fine tune and derive family-specific thresholds to better separate known allergens from likely non-allergens.

Random forest analysis

Tanya Krone (TNO, The Netherlands)

Alternative and sustainable protein sources (e.g., algae, duckweed, insects) are required to produce (future) foods. However, introduction of new food sources to the market requires a thorough risk

assessment of nutritional, microbial and toxicological risks and potential allergic Current Codex (2009) and EFSA (2010; 2017) guidance indicates that sequence identity to known allergens is acceptable for predicting the cross-reactive potential of novel proteins and resistance to pepsin digestion and glycosylation status is used for evaluating de novo allergenicity potential. Other physicochemical and biochemical protein properties, however, are not used in the current weight-of-evidence approach. In this study, we have used the Random Forest algorithm for developing an in silico model that yields a prediction of the allergenic potential of a protein based on its physicochemical and biochemical properties. Results show a robust model performance with a sensitivity, specificity, and accuracy each greater than ≥85%. As the model only requires the protein sequence for calculations, it can be easily incorporated into the existing risk assessment approach.

In vitro approaches for Allergenicity (IgE and non IgE)

Daniel Lozano-Ojalvo (Medical School, New York City, USA)

Before introducing proteins from new or alternative dietary sources into the market, a compressive risk assessment, including allergic sensitization, should be carried out in order to ensure their safety. A working group from the European COST Action ImpARAS focused on *in vitro* methods to predict sensitization applied the adverse outcome pathway (AOP) concept to structure the current mechanistic understanding of the molecular and cellular pathways evidenced to drive food allergies [1]. This AOP framework offered the biological context to identify *in vitro* approaches that reflect the molecular initiating and key events driving immune sensitization to food antigens. As a part of our work in the COST Action ImpARAS, we have clustered, structured, and discussed the existing *in vitro* models currently available, which have been previously used for allergenic food proteins, as well as the major read-outs, strengths, and limitations of these approaches [2].

The application of the AOP framework offers the opportunity to anchor existing testing methods to specific building blocks of the AOP for food sensitization. In general, *in vitro* methods evaluating mechanisms involved in the innate immune response are easier to address than assays addressing the adaptive immune response due to the low precursor frequency of allergen-specific T and B cells. Novel *ex vivo* culture strategies may have the potential to become useful tools for investigating the sensitizing potential of food proteins. When applied in the context of an integrated testing strategy, the *in vitro* methods identified by our working group may reduce, if not replace, current animal testing approaches. (References: 1. van Bilsen et al., 2017. Clin. Transl. Allergy, 7:13; 2. Lozano-Ojalvo et al., 2019. Trends Food Sci. Technol., 85: 307).

Protein stability

Joana Costa (REQUIMTE-LAQV/Faculty of Pharmacy University of Porto, Porto, Portugal)

Are physicochemical properties shaping the allergenic potency of plant and animal allergens [1,2]? Literature data regarding this topic is vast and presents a huge variability, which increases the complexity of the analysis. Allergen abundance, protein structure and presence of post-translational modifications, glycation and aggregation phenomena, as well as stability towards heat, light/radiation, pressure and chemical/enzymatic hydrolysis are examples of properties that may shape protein allergenicity.

Extensive analysis of the available literature shows that each parameter has variable effects, depending on its nature and the allergen. For example, glycosylation and phosphorylation are common but not universal traits of animal allergens. Protein aggregation that contributes to increased allergenicity of plant allergens like 2S albumins, while for legumins and cereal prolamins, the same phenomenon leads to a decrease. High ordered molecular structures can favour allergenicity, but conformational

loss and exposure of hidden epitopes can also have a comparable effect. Likewise, molecular stability linked to structural resistance to heat and proteolysis are common features promoting plant protein allergenicity, but it fails to explain the potency of some unstable allergens. Still, there are key knowledge gaps on how physicochemical properties modulate protein allergenicity from animal/plant origin [1,2].

At this stage, a systematic approach to link the physicochemical properties with clinical plant/animal allergenicity in real-life scenarios is still missing, which is prompting the need for exhaustive and standardised biomolecular studies validated in clinical context to unravel causal interactions between physicochemical properties and the basis of protein allergenicity.

References:
Costa J et al. (2021) https://doi.org/10.1007/s12016-020-08826-1
Costa J et al. (2020) https://doi.org/10.1007/s12016-020-08810-9

Protein digestion

Alan Mackie (University of Leeds, United Kingdom)

The primary objective of the assessment of novel proteins is to evaluate whether they are safe to consume, including potential allergenicity. As part of a suite of assessments, the *in vitro* digestion of protein has been seen as a useful exercise. Thus, in line with the guidance offered by the EFSA GMO Panel we are using an early phase and a late phase gastric simulation as well as a simulation of the infant gastric compartment, all followed by intestinal phases. These digestion scenarios were used with a panel of 10 proteins from plant and animal origin that were proteins with distinct allergenic potential. The results from the SDS-PAGE and densitometry show significant and mainly expected differences between the different digestion scenarios. The milk proteins were fully digested in the intestinal phase but the BLG was largely resistant to pepsin. In contrast, the egg proteins showed significant persistence except under late phase conditions. For the plant proteins, KTI and ConA were largely resistant to all conditions whereas LIP and AP were only resistant to infant conditions. Similarly, Ara h 1 showed some resistance to infant gastric conditions. The LC-MS analysis of peptides was able to highlight a number of clusters where differences were seen between the digestion scenarios and these could in some cases be mapped onto the primary sequence and where relevant compared with known allergenic epitopes. Under the different digestion scenarios, we were able to show significant differences in the persistence of peptides larger than 9 amino acids and significant overlap of abundant peptides from early phase intestinal digestion and known epitopes for a number of proteins. Although, linking these differences to immunological responses (epitope mapping) still seems to be quite challenging, there are clear differences between scenarios and strong potential for improved risk assessment.

Binding affinity in celiac disease

Frits Koning (University of Leiden, The Netherlands)

I will briefly discuss how uncontrolled immune reactions to the gluten and gluten-like proteins in wheat, barley and rye cause the intestinal inflammation that is characteristic of celiac disease. I will next discuss the properties of the gluten fragments that trigger these immune reactions and how this knowledge can be used to identify sequences in other proteins that may be harmful to celiac disease patients as well. Finally, I will discuss the relevance of this for allergenicity assessment.

In vivo approaches for allergenicity (IgE and non IgE)

Katrine Bogh (Technical university of Denmark, Kgs. Lyngby, Denmark)

In this lecture an overview of in vivo models for IgE and non-IgE mediated food allergy will be provided. Animal models of IgE mediated allergy will be discussed and an overview of the different parameters that requires consideration when designing predictive animal models will be presented. Examples on the impact of the parameters on the predictive capacity of the animal experiments will be given in relation to i) the proteins, such as dose-response relationship and processing, ii) the host, such disease status, iii) the environment, such as diet, and iv) the experimental design, such route of administration, use of adjuvant and end-point analyses.

Allergenicity assessment of new foods is a difficult task, and yet no animal models have been validated for such purpose. This lecture will give three examples of attempts to develop predictive animal models. Finally, this lecture will provide an example of how animal models can be used in the evaluation of de novo sensitisation as well as of cross-reactive capacity.

Allergenicity assessment of GMOs – CropLife Europe

Andre Silvanovich, (Monsanto, St Louis, Missouri, USA)

CLE/CLI (Crop Life Europe/Crop Life International) member companies have extensive experience assessing allergenicity of novel food proteins using a variety of tools and methods. Perhaps less recognized, CLE/CLI member companies have in the past and continue to this day to evaluate those tools and methods with the goal of improving their reliability. This presentation will highlight key CLE/CLI member company scientific contributions over the past 25 years in areas such as bioinformatics, in vitro digestive fate, and the overall assessment strategy of novel food protein allergenicity assessment.

Bios

F. **Javier Moreno** is working at the Instituto de Investigación en Ciencias de la Alimentación (CIAL) belonging to Spanish National Research Council (CSIC). Co-author of more than 130 peer-reviewed papers (Food Science & Technology, Nutrition or Biotechnology fields). Main areas of research include food biochemistry and food technology. For full list of publications: www.orcid.org/0000-0002-7637-9542. Currently, Chair of the EFSA "GMO Applications Food-Feed" Working Group and member of GMO Panel.

Dr **Thomas Eiwegger** is a pediatrician, pediatric pneumologist and pediatric allergist at the level of an Assoc Prof at Depts of Paediatrics and Immunology at University of Toronto and Karl Landsteiner University of Health Sciences, Krems. He works as clinician scientist at the Hospital for Sick Children, Toronto. May 2021, he was appointed chair of the Dept of Pediatrics, University Hospital St. Pölten, Austria.

Prof. **Claire Mills** currently holds a chair in Molecular Allergology and is based in the Manchester Institute of Biotechnology and part of the University's Respiratory and Allergy Research team, which is based at the Wythenshawe Hospital, part of the Manchester University NHS Foundation Trust. She led the EU integrated projects iFAAM and EuroPrevall and now coordinates the European Food Safety Authority Project ThrAll and the UK Food Standards Agency project PAFA. Professor Mills' personal research interests are focused on structure-function relationships in food proteins particularly with regards what makes some proteins, and not others, become allergens, including the effects of the food matrix and processing on resistance of food proteins to digestion and the role this plays in determining the allergenicity of foods.

Prof. **Kitty Verhoeckx** received her bachelor's degree in Analytical Chemistry in 1992. In 1991 she started her career at the Netherlands Organisation for Applied Scientific Research TNO, where she had various research positions and was involved in various research topics (pesticides, hormones and animal pharmaceuticals, analytics (targeted and untargeted proteomics, metabolomics), systems biology, bioactive lipids etc. In November 2005 she gained her PhD degree at the University of Leiden on the topic:" Systems biology based studies on anti-inflammatory compounds". Since January 2011, Kitty Verhoeckx is involved in food allergy projects (predicting allergenicity of novel proteins for instance insects, effect of protein digestion, transport, and processing on allergenicity and biomarkers for food allergy to improve diagnosis). She was a management committee member of the COST Action INFOGEST (food digestion), participated in the ILSI Europe task force 'Allergenicity and processing' and was chair of the COST Action ImpARAS, a European network to improve the current risk assessment strategy of novel proteins. She was also a member of the EFSA focus group on food allergy. Currently, Kitty Verhoeckx is working as assistant professor at the dermatology and allergology department of the University Medical Center Utrecht and is management committee member of COMFA, a European network for the development of a core outcome set for measuring the effectiveness of immunotherapy for food allergy and a member of EFSA focus group on digestion in allergenicity and protein safety assessment.

Prof. **Daniel Munblit** is a professor at Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia and Honorary Senior Lecturer at National Heart and Lung Institute, Faculty of Medicine, Imperial College London, London, United Kingdom.

Prof. **Ivan Dimitrov** is an associate professor at the Medical University in Sofia, Bulgaria. He has a PhD in Physical Chemistry and a master's degree in Chemical Engineering. His research activities are in the field of immunoinformatic and drug design and their application to assess the immunogenicity of proteins. He is one of the creators of two of the existing web tools for predicting protein allergenicity: AllerTOP and AllergenFP, as well as tools for in silico prediction of antigenicity (Vaxijen) and immunogenicity (Epitop and EpiDock).

Prof. **Rick Goodman** is a Research Professor in the Food Allergy Research and Resource Program. Manager of www.AllergenOnline.org database that includes a Celiac Disease peptide database. Chair of the WHO/IUIS Allergen Nomenclature Sub-Committee. He holds a PhD from the Ohio State University in Dairy Science (1990), post-doctoral work at Cornell and the University of Michigan, Pulmonary. He worked at Monsanto from 1997-2004 testing the potential allergen risk of genetically engineered crops. In 2004 he joined UNL to start the AllergenOnline database. He guides graduate student research on allergen identification and food safety evaluations and evaluate potential risks of allergic cross-reactivity for companies developing new foods or biotechnology products.

Sebastian Maurer-Stroh obtained his PhD degree from the University of Vienna, Austria, before carrying out research at the VIB Switch laboratory in Brussels, Belgium, under a Marie Curie fellowship. He joined A*STAR's Bioinformatics Institute (BII) in 2007 where he is leading a group of experts in protein sequence analysis and was appointed Executive Director of the institute in 2021. He also spearheads infectious disease outbreak analysis as expert for national and global virus surveillance with GISAID and industry collaborations with local SMEs and large multinationals on enzyme AI sequence analysis and prediction of allergenicity potential of proteins.

Tanja Krone, PhD, completed her studies and PhD at the University of Groningen. She works as a statistician for TNO since 2016 on a wide variety of topics working with multidisciplinary teams in different research areas, with a strong focus on health-related topics such as allergenicity, exposure and mental health. Her statistical expertise focuses on Bayesian analysis and time series analyses, but she has a wide interest and experience in other statistical areas too, such as multilevel analysis, dimension reduction, random forest and research design.

Dr. **Daniel Lozano-Ojalvo** is an Instructor in the Precision Immunology Institute at the Icahn School of Medicine at Mount Sinai and a member of Jaffe Food Allergy Institute (New York, USA). He graduated with a degree in Veterinary Medicine and went on to pursue his Master's degree in Health Science Research in 2012 (University of Extremadura, Spain). In 2017, he obtained his PhD degree granted by the Autonomous University of Madrid (Spain), which was awarded with a diploma of excellence. During his PhD at the Instituto de Investigación en Ciencias de la Alimentación (Spanish Council for Scientific Research, CSIC, Spain) his studies were focused on peptide-based immunotherapy for the prevention and treatment of egg allergy. In 2018, Dr. Lozano-Ojalvo joined Cecilia Berin's laboratory at Mount Sinai Hospital. Currently, his main area of research is in studying the evolution, heterogeneity, and function of effector and regulatory allergen-specific T cells in allergic patients.

Dr **Joana Costa** has a PhD in Pharmaceutical Sciences from Faculty of Pharmacy University of Porto (FFUP). Currently, she is a Researcher at REQUIMTE-LAQV/FFUP with special focus on food allergen studies, participating in several international/national projects on food allergens, food authentication, plant food supplements and GMO analysis. She is author/co-author in more than 66 publications in international peer-reviewed journals, 6 book chapters and the co-principal researcher of a FCT project "Allergen risk assessment of foods: structural and immunogenic changes induced by novel processing technologies and alternative protein sources".

Prof. **Alan Mackie** is a researcher whose research focuses on the link between food structure and the kinetics of nutrient release. Using a combination of *in vitro* and *in vivo* studies his team have shown that thermal processing, emulsification, and enzymatic cross-linking can all be used to alter the rate and patterns of protein digestion. In 2016, he moved to University of Leeds to continue these studies in the School of Food Science and Nutrition. There his team have been focussing on upper gastrointestinal tract digestion and mucosal interactions. He has over 200 peer-reviewed publications with an h-index of 68 (Google Scholar).

Prof. **Frits Koning** is a staff member in the department of Immunology (150 fte) of the Leiden University Medical Centre since 1993. He is the chairman of the scientific advisory board of the LUMC and served for 10 years as the CEO of the Dutch Celiac Disease Consortium (CDC) in which immunologists, geneticists, food specialists and medical doctors collaborate with industrial partners to improve the quality of life of patients with celiac disease. He is well recognized for his contributions to the field of immune mediated disorders, celiac disease in particular. Through his work it is now well-established which gluten fragments are disease causative and how they are recognized by disease-related T cells, providing a molecular basis for the genetic association between HLA-DQ and celiac disease. In his most recent work he uses high dimensional flow cytometry to unravel the involvement of the innate and adaptive immune system in Inflammatory Bowel Diseases.

Dr. **Katrine Lindholm Bøgh** is a Senior Researcher and heads the Research Group for Food Allergy at the National Food Institute at the Technical University of Denmark, Denmark. She holds a PhD degree from the Technical University of Denmark in the interface between biochemistry and allergology. Her research focus is development of new and improved strategies to diagnose, predict, prevent, manage, and treat food allergies. Research is focused on determining which properties of food proteins contribute to allergy development, and which properties contribute to tolerance development, as well as the underlying immune mechanisms with the use of animal models.

Prior to joining the Regulatory Science Group at Monsanto in 1999 **Andre Silvanovich** received a Ph.D. in Cell and Developmental Biology from the University of Minnesota. He has held several individual contributor and management roles in the Legacy Monsanto Regulatory Science organization including leading teams responsible for conducting the Protein Characterization and Safety assessments, Immunoassay Development and Protein Expression Analysis, Production Bioinformatics and Next Generation sequencing. Recently, Andre was promoted to Senior Science Fellow and appointed Senior Data Scientist in the Regulatory Data Science & Digital Transformation organization at Bayer. Andre authored greater than 500 technical documents related to bioinformatic assessment of protein allergenicity and toxicity and has developed numerous software tools that are used to support bioinformatic evaluation of legacy Monsanto and Bayer Crop Science GM products. He is currently a co-chair of Health and Environmental Sciences Institute Proteins Allergens Toxins and Bioinformatics committee and has chaired Molecular Characterization Team and currently chairs the Allergy Experts Team in Crop Life International.