STRATEGY FOR SAFETY ASSESSMENT OF INNOVATIVE PROTEINS AND THEIR CAPACITY TO TRIGGER COELIAC DISEASE

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INTRODUCTION

The growth in the world population is accompanied by growing demand for more sustainable food proteins. Before these novel proteins can reach the market, it is necessary to ensure their safety for consumption. One of the most difficult aspects to consider in this assessment is their allergenic potential. Current approaches heavily rely on expert judgement to interpret *a posteriori* any potential findings of the assessment, which can lead to a lack of harmonisation, reproducibility and transparency. On the other hand, an *a priori* approach, based on the development of targeted databases that better integrate the clinical relevance of each entry, is a more fine-tuned strategy to fill the gaps. All dietary proteins have the potential to provoke adverse immune reactions. In this regard, one of the best characterised non-IgE-mediated conditions is Coeliac disease (CD), which is triggered by the gluten proteins. Evaluation of key characteristics of gluten fragments in relation to clinical relevance has been recently reported (Vriz et al. 2021 [1]). Future application of such approaches is likely needed to improve food safety assessment of proteins.

[1] DOI: 10.1016/j.fct.2021.112584

METHODOLOGY

The CD pathogenic pathway involves three key elements required for the disease elicitation that can be used in risk assessment:
- Enzymatic degradation and modification during digestion, affecting the immunogenic properties of gluten;
- Epitope binding to the HLA-DQ receptor of the antigen-presenting cell;
- Activation of pro-inflammatory CD4+ T-cell specific for the HLA-DQ-gluten epitope complex resulting in an inflammatory response.

The investigation may consider 9-mer epitopes relevant for CD, such as those described by Sollid et al. 2020 [2]. The epitopes can be curated and used in relevant *in silico* tools such as models on human digestive enzymes, as well as the HLA-DQ alleles associated with CD development. Furthermore, experimental data from the literature can be used to verify the outcome of the *in silico* tools. Finally, data on T-cell proliferation assays can be also

retrieved from the literature to refine the scenario.

[2] DOI: 10.1007/s00251-019-01141-w

RESULTS

Concerning the first key event associated with CD, *in silico* chymotrypsin digestion has been considered a potential discriminatory tool for the ranking of gluten T-cell epitopes among all digestive enzymes studied. Degradation of different epitopes by chymotrypsin and trypsin may vary, which ultimately might influence the CD elicitation pathway. In this context, the epitopes linked to the CD-associated HLA-DQ2.5 and HLA-DQ8 alleles were classified in the high-risk category to trigger the allergenic response. Furthermore, a positive relationship between the number of prolines and the risk of gluten T-cell epitopes was also identified. With respect to the second key event, HLA-binding data analysis revealed the additional role played by the flanking regions of the 9-mer epitopes, but the findings clearly highlight the need to implement improved and more comprehensive bioinformatics applications to better mimic experimental data. Similarly, in relation to the last key event, the scarcity of data and heterogeneity in the different populations considered in terms of T-cell activation hampered a comprehensive analysis

DISCUSSION

To better improve the safety assessment of proteins, a strategy defining *a priori* the health objectives of the risk assessment should be followed. Such a strategy would require, in a first step, the development of a ranking strategy that describes the specific characteristics for potential cross-reactivity and that is linked to specific harm. In a second step, the best follow-up action needed should be defined for any given hit.
In this respect, the three key events were investigated for their potential to be used in the first step:
- Enzymatic degradation and modification have great potential as a key indicator of relevance since proteins or peptides that are resistant to digestive proteolysis might be more effective at stimulating the immune system.
- Binding affinity to HLA-DQ provides key information as CD4+ T-cells recognise gluten peptides only when presented by the disease-associated HLA-DQ molecules.
- T-cell activation is the area that needs more research, due to the lack of data and limitations in the comparability of studies.
These steps can be considered a premature stage of future artificial intelligence that can be applied to the risk assessment of proteins.