ALERBLOT

RESEARCH

Hunting for new wheat allergens : a 2D Immunoblot and mass spectrometry approach

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Aim of the project :

Wheat is a complex allergenic food containing a lot of different proteins that are difficult to isolate and to identify. Specific molecular IgE detection in wheat allergic patients is limited since only a few molecular allergens are available on the market. Hence this research aimed to develop a diagnostic method linking a patients specific allergenic 2D western blot profile to a particular clinical symptom in wheat allergy. Afterwards, mass spectrometry (LC-MS/MS) was used to identify the specific molecular allergens.

Methods

A total protein extract of wheat seeds was separated on the basis of the isoelectric point and the molecular weight of the proteins. Twenty-five patients presenting positive specific IgE (sIgE) for wheat were classified into 3 different phenotypes: wheat dependent exercise induced anaphylaxis (WDEIA), atopic dermatitis (AD) and pollen rhinitis (PR). Their sera were analyzed by 2D immunoblotting on a standardized wheat seeds extract in order to evaluate their sIgE reactivity against the protein spots. Their sIgE sensitization profiles were compared and protein spots of interest were identified by LC-MS/MS.

Results : Comparison of sensitization profiles related on clinical symptoms and identification of interesting allergens by LC-MS/MS.

1. WDEIA

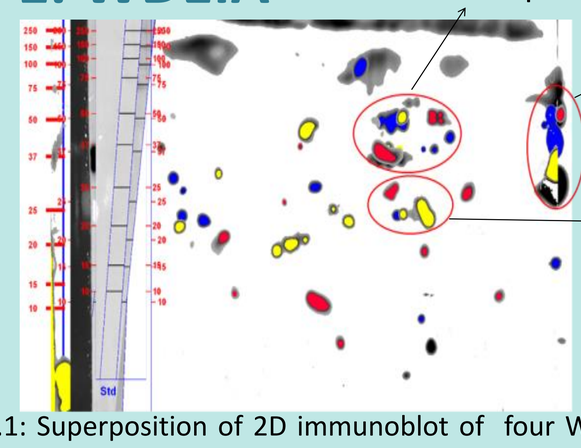


Fig.1: Superposition of 2D immunoblot of four WDEIA patients : case 1 (black), case 2 (blue), case 3 (yellow) and case 4 (red).

2. Pollen rhinitis (PR)

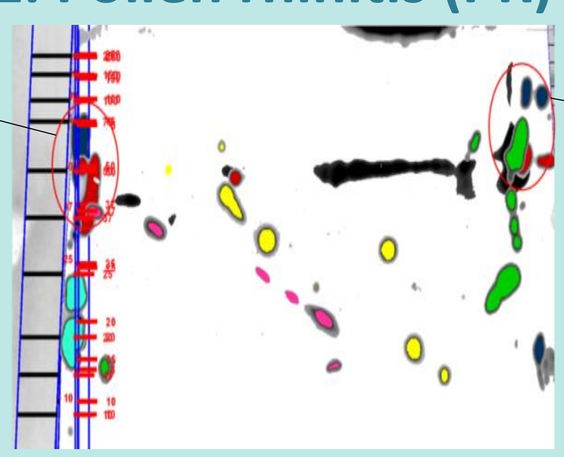


Fig.2: Superposition of 2D immunoblot of seven PR patients : case 15 (pink), case 16 (black), case 17 (green), case 18 (red), case 19 (dark blue), case 20 (yellow) and case 21 (light blue).

3. Atopic dermatitis (AD)

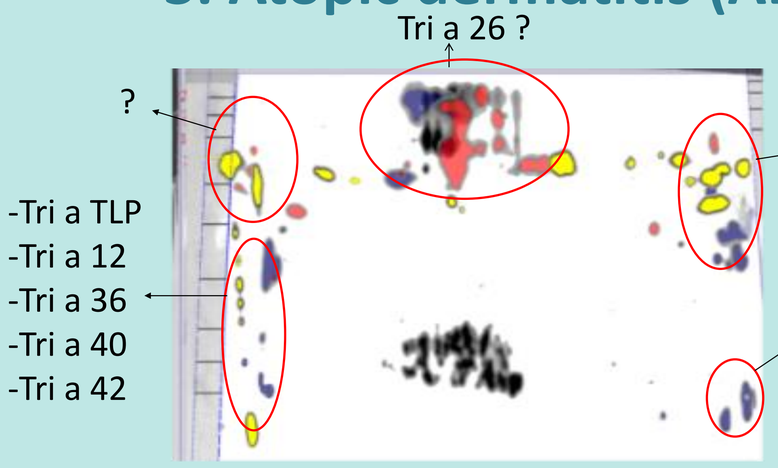
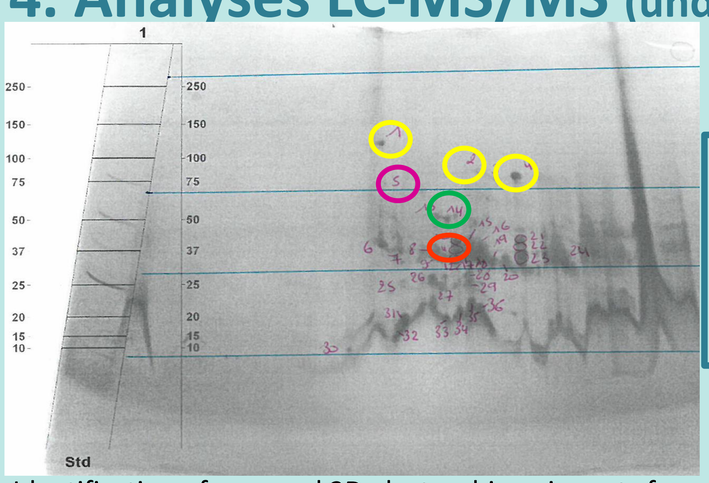


Fig.3: Superposition of 2D immunoblot of four AD patients : case 6 (red), case 7 (yellow), case 9 (blue) and case 11 (black).

4. Analyses LC-MS/MS (under investigation)



Currently identified :
- Spots 1, 2, 4 : tri a 26
- Spot 5 : tri a bA
- Spot 11 : Tri a 34
- Spot 14 : tri a tritin

Fig.4: Identification of a several 2D electrophioresis spots from total wheat proteins extract.

Conclusion :

At this stage, specific sensitization profiles were identified for the 3 phenotype groups (WDEIA, AD, PR). The protein spots of interest detected by sIgE concern one or more allergens. Some wheat allergens were identified by LC-MS/MS. Preliminary results show that Tri a 26 seems to be specific to the AD patient group. All four DA patients have Tria 26 specific IgE. At the end of the study, it will be possible to establish a link between all specific symptomatologies and the responsible allergens newly identified.

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