KEY REGULATOR ELEMENTS IN CELIAC RESPONSE THROUGH WHOLE GENOME COEXPRESSION ANALYSIS

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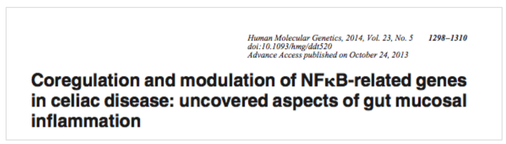
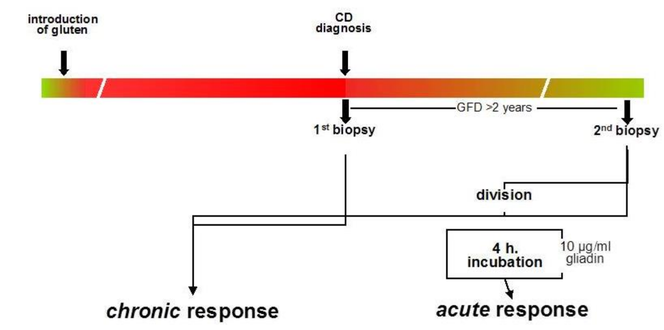
Background:

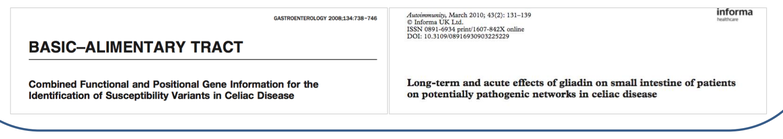
- Celiac disease (CD) is a chronic, immune-mediated gastrointestinal disorder that develops in genetically susceptible individuals in response to ingested gluten.
- The only available treatment for CD is a life-long gluten-free diet (GFD).
- In CD, gliadin provokes a coordinated response and the disruption of coexpression in gene networks.
  - Altered coexpression profile in NFκB pathway.



AIM: to analyze coexpression in a whole genome level under gliadin exposure, and to identify regulatory elements (TF/miRNAs) that could underlie coexpression alterations in the context of CD

Microarray experiment:





Results:

Module construction and regulator discovery

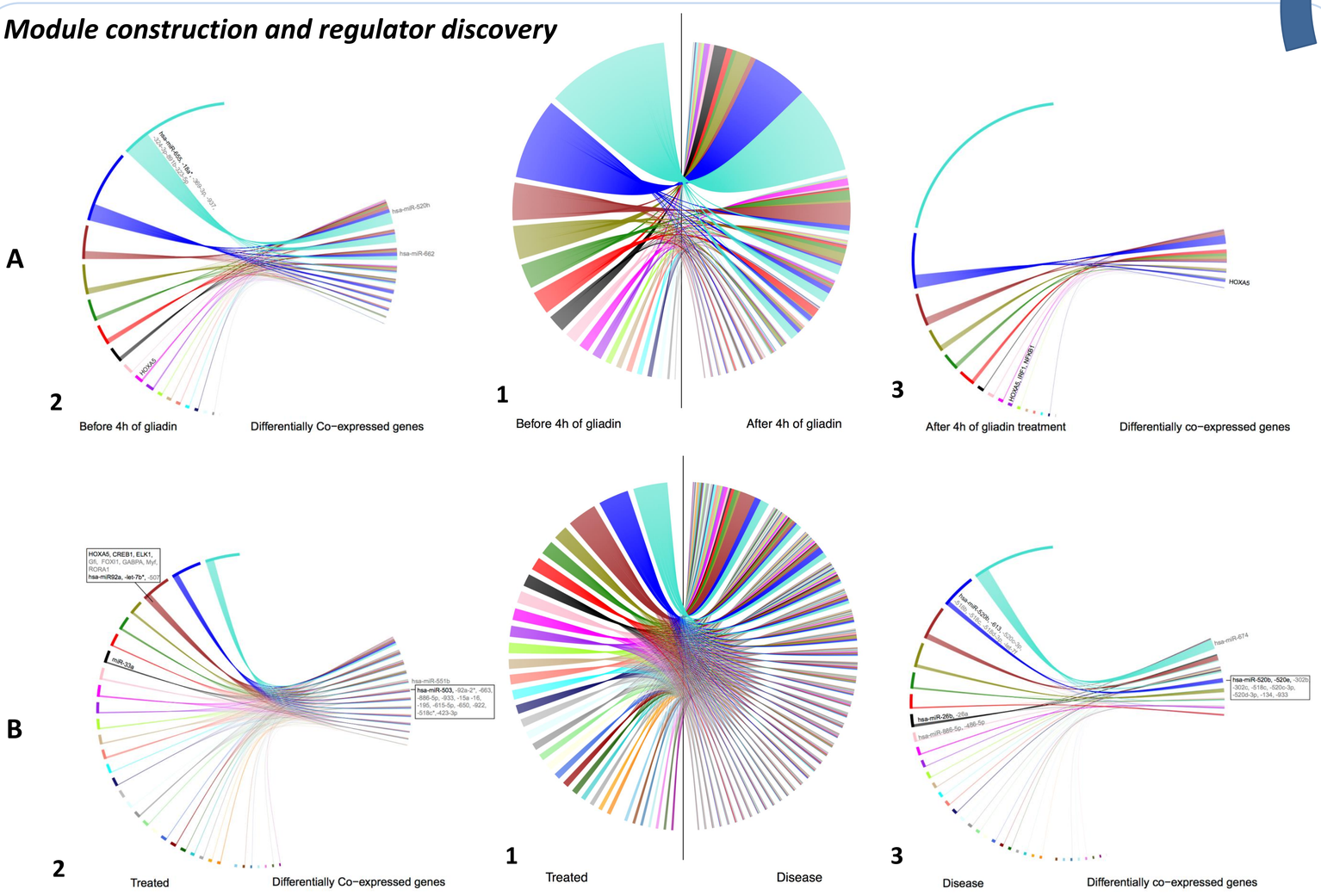


Figure 1. Coexpression modules in **A)** acute and **B)** chronic response to gliadin in CD. **A1)** and **B1)** graphs show a complete reorganization of coexpressed genes when we compared gliadin-free and gliadin-containing conditions. **A2)** and **B2)** represent the transition of genes that abandon a module upon gliadin challenge and create new ones. **A3)** and **B3)** represent genic modules in the gliadin containing conditions but not present in the absence of gliadin. The modules formed from those genes that change coexpression relationships from one condition to the other are also represented. **A2) B2) A3) B3)** Enrichment analysis of regulatory elements in each condition was performed to identify possible regulators of coexpression upon gliadin exposure. Identified regulatory elements are shown in the graphs close to genic modules were they came from, while final candidates selected for further analysis are marked in black.

Validation of candidates

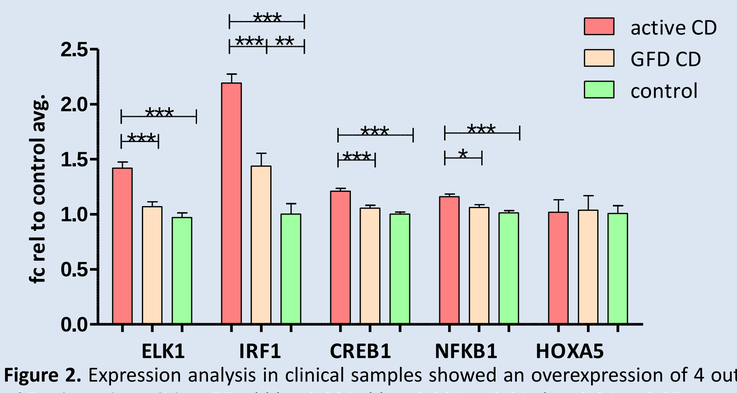


Figure 2. Expression analysis in clinical samples showed an overexpression of 4 out of 5 selected candidate TFs; ***p<0.001, **p= 0.001 to 0.01, *p= 0.01 to 0.05.

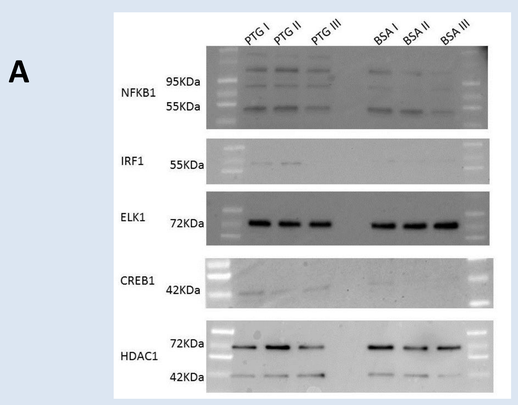
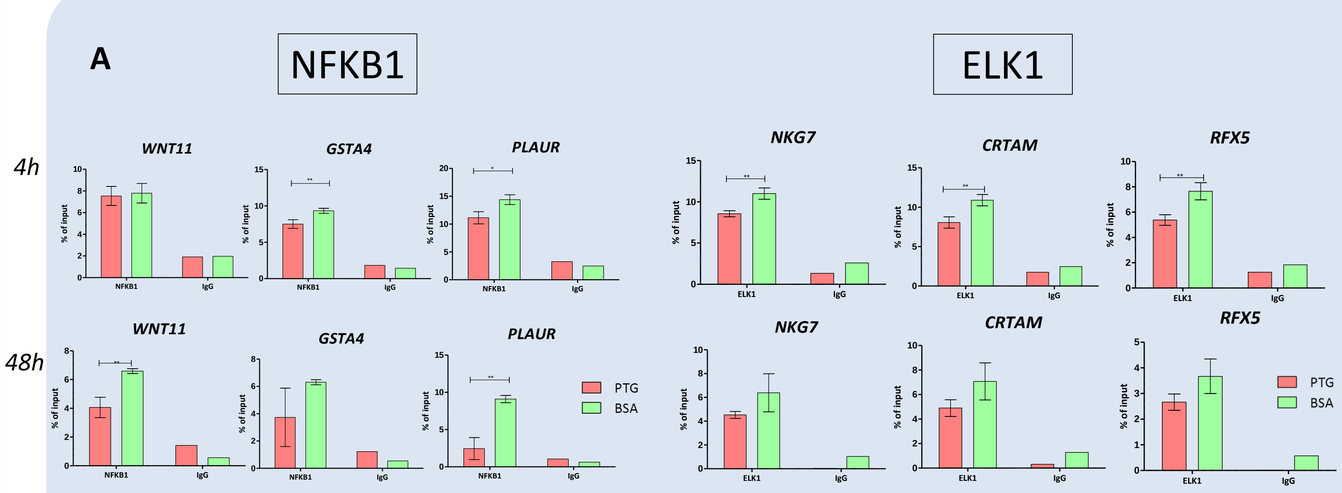
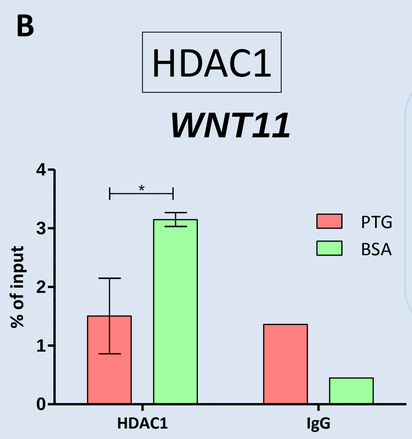


Figure 3. A) IRF1 and CREB1 were overexpressed at a protein level in the nuclear fraction of the C2BBe1 cells after gliadin exposure. **B)** We did not see notable differences in the immunofluoresce assay.



NFKB1 and ELK1 could be guiding HDAC repressors to proinflammatory genes in physiological conditions, and this control could be lost in the presence of the gliadin challenge...



Same pattern in the rest of the target genes:
- *GSTA4*
- *PLAUR*
- *NKG7*
- *CRTAM*
- *RFX5*

Figure 4. A) NFKB1 and ELK1 ChIP in C2BBe1 cells treated with PTG/BSA for 4 and 48 hours showed a decrease in the binding of these TFs to their target genes. **B)** HDAC1 ChIP also showed a decrease in the binding to NFKB1 and ELK1 target genes suggesting a possible HDAC1/TF altered complex in the disease. ***p<0.001, **p= 0.001 to 0.01, *p= 0.01 to 0.05.

Conclusions:

- Gliadin alters coexpression in CD.
- Our pipeline was able to identify regulators that could be relevant to disease.
- Particularly, NFKB1 and ELK1 showed differential expression in patients and altered binding to several targets upon gliadin challenge in C2BBe1 cells.
- NFKB1 and ELK1 could be guiding HDAC repressors to proinflammatory genes in physiological conditions, and this control could be lost in the presence of the gliadin challenge.

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