



Endoplasmic Reticulum stress in bordering epithelium of Crohn's disease patients with intestinal fibrosis

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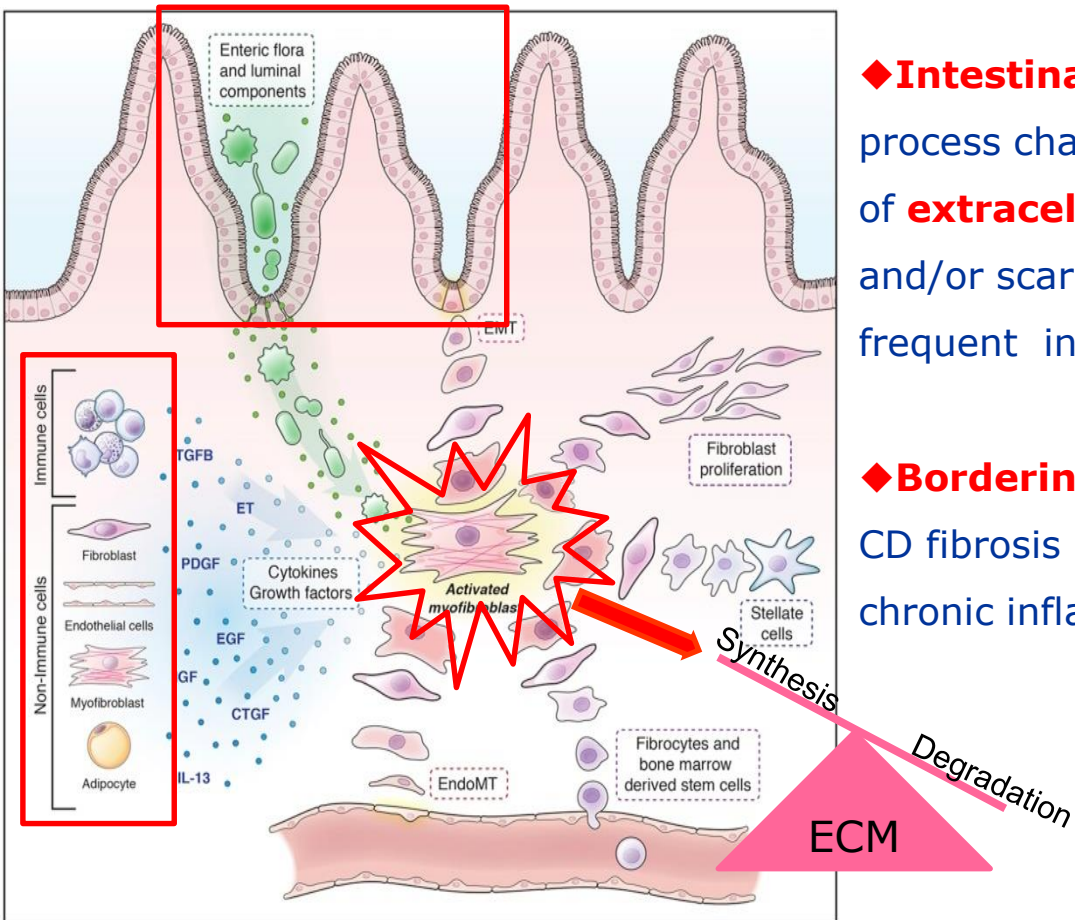




Disclosure of Conflicts of Interest:

Conflict of interest :

No conflicts



◆ **Intestinal fibrosis** is a chronic and progressive process characterized by an excessive accumulation of **extracellular matrix (ECM)** leading to stiffening and/or scarring of the involved tissue, which is a frequent indication for surgery in CD.

◆ **Bordering epithelium** is thought to be involved in CD fibrosis **initiation** as it is the primary site of chronic inflammation and tissue damages.

Bordering epithelium



Components and pathways of intestinal fibrosis

Aim: Comparing the proteomes of bordering epithelial cells isolated in zones adjacent to sub-mucosa showing different degree of inflammation and fibrosis

Patients inclusion and sample selection

CD cases - CHU Liège:

=> Tissue gradation by anatomopathologist

Normal tissue (N)

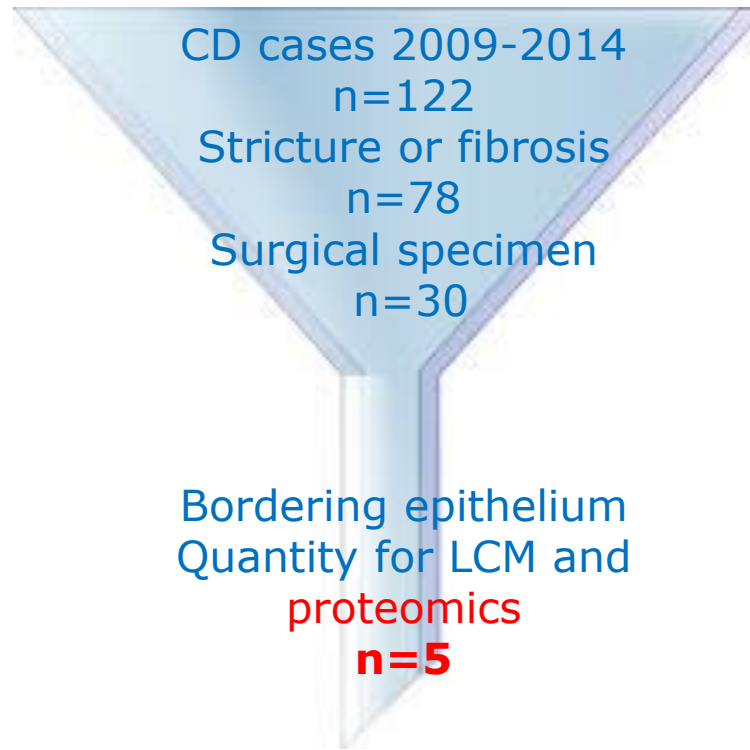
Inflammation (tissue infiltration)

mild (I1) < moderate (I2) < severe (I3)

Fibrosis (ECM remodeling and accumulation)

mild (F1) < moderate (F2) < severe (F3)

=> Masson's trichrome staining (specific for ECM)



CD cases 2009-2014
n=122

Stricture or fibrosis
n=78

Surgical specimen
n=30

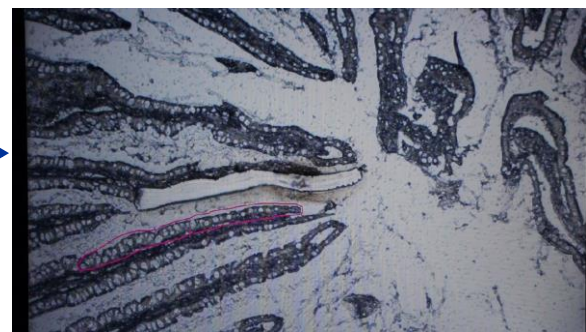
Bordering epithelium
Quantity for LCM and
proteomics
n=5



Experimental workflow



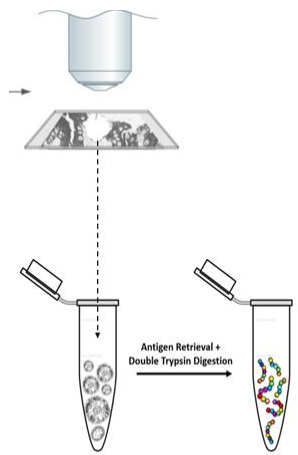
N
I/F1
I/F2-3



20 000 Cells isolated per sample

paired
FFPE
samples
Ileum

Samples processing
protein digestion and peptide extraction
(Longuespée R. et al, Methods (2016))



Label free proteomics



2D nano-UPLC (Waters) coupled to a Hybrid Quadrupole-Orbitrap QExactive-Plus (Thermo)

Data Analysis :

MaxQuant for Protein identification and Label free quantitation (LFQ)

Perseus (VS 1.5.6.0) Differential analysis and Gene Ontology annotations



- **1249 proteins** identified / quantified in at least 50% of the group replicates
- Differential analysis: **226 Proteins with $p < 0.05$** in at least one of the comparisons addressed

N vs IF1
n=90

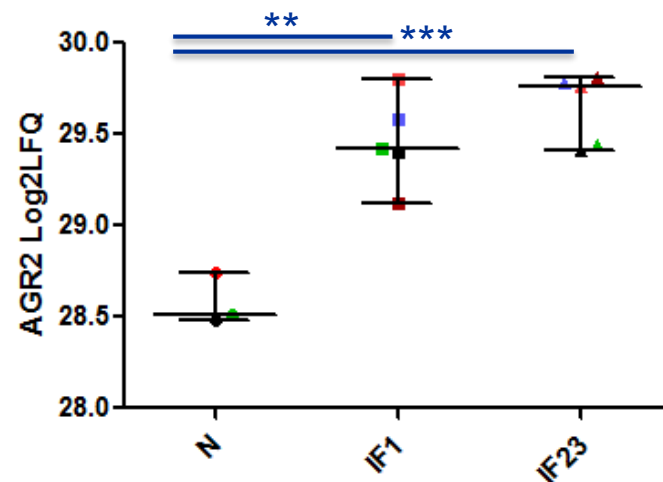
IF1 vs IF23
n=38



N vs IF23
n=140

« best P value »

AGR-2- HUMAN (hAG2 or Gob-4)
Anterior gradient protein 2 homolog

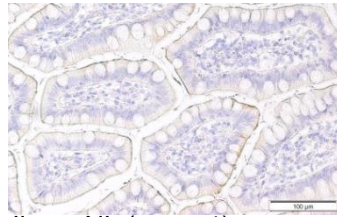


* paired welch's test

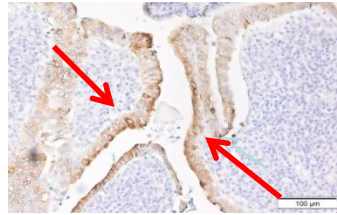
Confirmation by immunohistochemistry of AGR2 distribution (CD patients, n=30)

Global scoring system

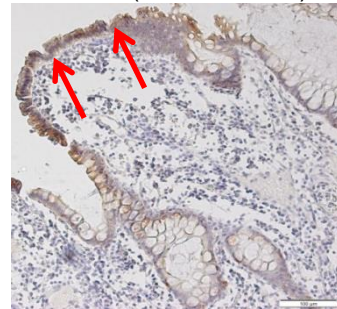
- 0 : no staining
- 1: weak
- 2: medium
- 3: strong
- 4: very strong



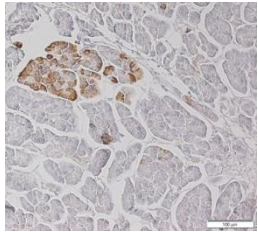
Ileum NL (score 1)



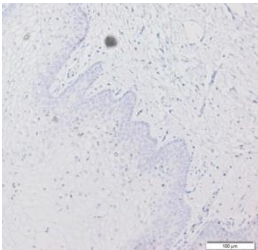
Ileum IF1 (score 2 and 3)



Ileum IF2-3 (score 4)

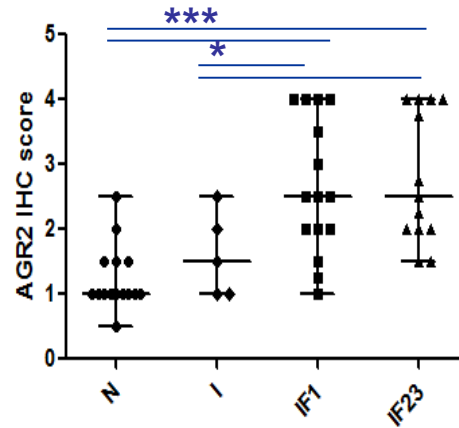


CTL + ADK pancreas

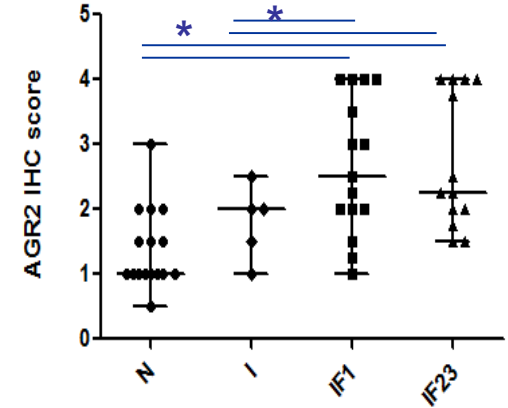


Ab Isotype -CTL esophagus

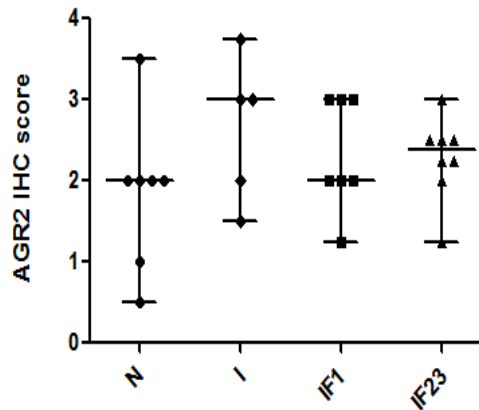
Ileum (bordering epithelium)



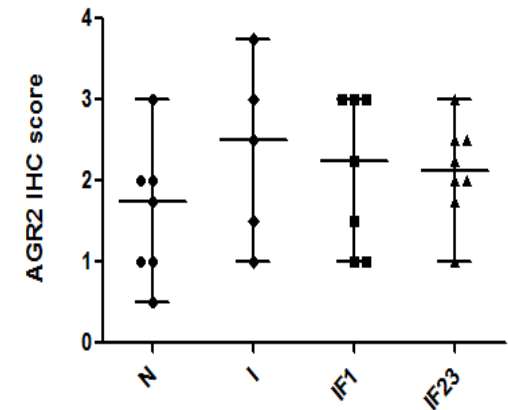
Ileum (crypt epithelium)



Colon (bordering epithelium)



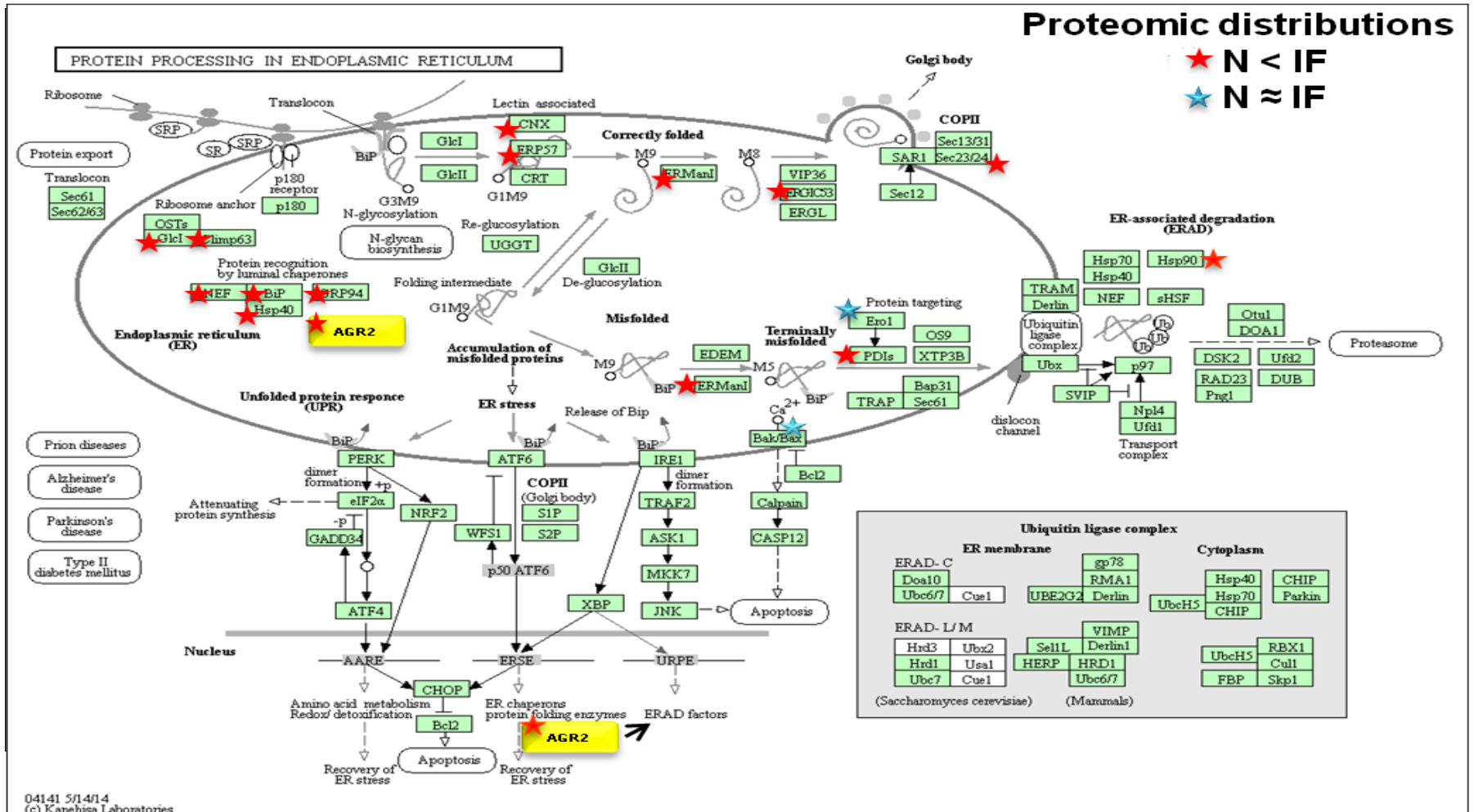
Colon (crypt epithelium)



*Unpaired non parametric test

➔ AGR2 is significantly associated to fibrosis in ileum tissues and not in the colon

- **Chaperone protein** with phosphodissulfide isomerase activity and is involved in **protein folding** and protein maturation control
- Involved in **ER stress** and Unfolded Protein Response
- Down regulated in IBD compared to healthy controls
- **Control EMT** in cancer cells (worse outcome, metastasis, resistance to treatment)





Conclusions and perspectives

Proteomics (n=5 patients, 13 paired samples)

- We could identify many proteins of ER stress/ homeostasis which were increased when fibrosis (associated to stenosis) is present compared to the normal paired tissue
- AGR2 is the most significant one and show a higher proteomic signal at higher fibrosis stages (F2-3)

AGR2 IHC confirmation (n=30 patients, 150 samples)

- AGR2 IHC score: $N \approx I < IF_{1/2-3}$ in ileum , but not in colon

Perspectives:

- IHC confirmation of AGR2 and other ER stress proteins on a larger CD set of patients/tissues
- Study AGR2 involvement in induced ER stress in cell culture model



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Gender (M/F) n: **3/2** 17/13
Age (years), P50 ; range: **32** 40
31-40 17 - 70

Disease Duration, (years), P50 ; range: ; **8.5** 12
4.5-12.5 0 - 46

Surgery, n: **5** 30
Ileocaecectomy, n: **1** 6
Ileocollectomy, n: **3** 17
Coloproctectomy, n: **1** 7

Other concomitant lesions/complications:

stenosis/ stricture n: **3** 16
Fistulae n: **1** 19
Dysplasia n: **0** 0
CD ulcers, n: **5** 28

Previous IBD related surgeries:

0 n: **1** 14
 1 n: **2** 6
 ≥2 n: **2** 10

Surgical specimen for proteomics (n=5) Surgical specimen(n=30)

Treatments before surgery:

none n: **1** 23
Immuno-modulator (stopped before surgery) n: **1** 2
Biologics (stopped before surgery) n: **2** 7
Antimetabolites n: **0** 1
Antibiotherapy n: **2** 11
Analgesic n: **0** 3

Biological data available before surgery

Hb (g/dL) n, P50 ; range: **4/5, 12.2; 9.2- 14.7**
 29/30, 12 ; 9.2 - 15.9

CRP (mg/L) n, P50 ; range: **4/5, 43.95; 1.5-62**
 28/30, 12.5 ; 0.8-261.8

Platelet count (10³/mm³) n, P50 ; range: **4/5, 392; 233-476**
 28/30, 359 ; 203 - 762

Leukocyte count (10³/mm³) n, P50 ; range: **4/5, 7.46; 3.84-9.87**
 29/30, 8.5 ; 2.5- 17.4