Published in : Digestive and Liver Disease (2025), vol. 57, n°6, pp. 1197-1208
DOI: 10.1016/j.dld.2025.04.015
Status : Postprint (Author's version)

Distribution of epithelial endoplasmic reticulum stress-related proteins in adult and pediatric Crohn's disease: Association with inflammation and fibrosis

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KEYWORDS: Fibrosis; Endoplasmic reticulum stress; Protein disulfide isomerase; Pediatric Crohn's disease

ABSTRACT

Background/Aims: Intestinal strictures in Crohn's disease (CD), driven by fibrosis remain challenging to treat. Current treatments focus on inflammation, but are less effective against fibrosis. Endoplasmic Reticulum Stress-Related Proteins, including Protein disulfide isomerases (PDIs), may contribute to fibrosis; their roles in CD remain unclear. This study investigated the distribution of AGR2, BiP, PDIA6, ERP44 in intestinal epithelium and their association with fibrosis and inflammation in pediatric and adult CD.

Methods: We retrospectively analyzed 224 patients (2009–2023). CD patients with and without strictures, non IBD controls, and ulcerative colitis patients were compared. Immunohistochemistry assessed Endo- plasmic Reticulum Stress-Related protein distribution in epithelium. H&E and Masson's trichrome staining evaluated inflammation and fibrosis. Correlations between protein distribution, inflammation and fibrosis were examined.

Results: AGR2 and BiP were increased in fibro-inflammatory and fibrotic intestinal epithelial tissues, especially in pediatric-onset CD. ERP44 was associated with fibrosis exclusively in pediatric CD. PDIA6 was upregulated in CD compared to non IBD, without fibrosis association. Distinct protein distribution patterns were observed between pediatric and adult CD, and between ileum and colon.

Conclusions: Distinct patterns of AGR2, BiP, PDIA6, and ERP44 in fibrotic and inflammatory intestinal tis- sues suggest potential roles in CD-associated fibrosis, warranting exploration as biomarkers or therapeutic targets.

1. Introduction

Intestinal strictures are a common and debilitating complication of Crohn's disease (CD) in both adult and pediatric populations [1,2]. Managing strictures is challenging due to the lack of antifibrotic drug treatments [3], often leading to surgery, with a high risk of recurrence [4–6]. Although surgery rates decline with immunomodulators and biologics [7–9], these mainly control inflammation without significantly affecting fibrosis progression [10–12].

The pathophysiology of intestinal fibrosis remains poorly understood, partly due to the absence of suitable animal models [13–18]. While chronic inflammation contributes to fibrosis, why some injuries heal while others progress remains unclear. This process involves various cellular types, cytokines, growth factors, and epithelial/endothelial-to-mesenchymal transitions (EMT/EndoMT) [17, 19], promoting fibroblast differentiation into activated myofibroblasts, leading to extracellular matrix (ECM) deposition, and stricture formation [11, 13, 20–22].

Exploring the epithelium's role in fibrosis development is essential, as epithelial cells maintain homeostasis and mucosal barrier integrity, particularly after injury [23]. A pilot proteomic study comparing ileal epithelial proteomes from regions with varying fibrosis and inflammation degree [24], identified increased expression of endoplasmic reticulum stress (ERS)-related proteins, notably some with protein disulfide isomerase (PDI) activity [24]. However, this study was limited to adults, and the mechanisms of fibrosis in children remain largely unexplored. Pediatric-onset CD is often more severe, with earlier complications, yet histopathological differences between pediatric and adult cases remain unclear [25–27].

This study characterizes the distribution of four ERS-related proteins (Anterior Gradient 2 [AGR2], Binding Immunoglobulin Protein [BiP], Protein Disulfide Isomerase Family A Member 6 [PDIA6], and Endoplasmic Reticulum Protein 44 [ERP44]) in ileal and colonic tissues from non IBD and IBD patients with varying inflammation and fibrosis, comparing their distributions between pediatric- and adult-onset CD.

2. Methods

2.1.Patient enrolment

This work was approved by the Ethics Committee of the University Hospital of Liège in 2014 and renewed in 2017 (reference: 2014-156). Additional approvals were obtained from the French Personal Protection Committee (reference: ECH 19/07) and the Medical Ethics Committee of the MontLégia Hospital (reference: 19/02/969).

Patients were retrospectively selected from databases of 4 hospitals between 2009 and 2023: University hospital, Regional Hospital Center and MontLégia Hospital of Liège (Belgium), and

University hospital of Lille (France). We identified pediatric (< 17years) and adult (≥17years) CD patients who underwent intestinal resection. Patients with radiological or endoscopic strictures and corresponding clinical manifestationswere included. Control populations included tissues from adult and pediatric patients without IBD or stricture-free CD (no endoscopic or radiological evidence at sampling). Based on preliminary results, ulcerative colitis (UC) patients were also included to study PDIA6 distribution. Tissues (ileum and colon) were obtained from intestinal resections or biopsies.

For all patients, we collected clinical data: gender, age at sampling and IBD diagnosis, endoscopic and histological findings, disease location and duration, history of strictures if present, treatment history, current treatments and inflammatory markers at sampling [28].

2.2.Scoring of inflammation and fibrosis

Tissues were formalin-fixed, paraffin-embedded (FFPE) [29] and 4 µ¦m sections stained with Hematoxylin & Eosin (H&E), Masson's Trichrome (MT), and Immunohistochemistry (IHC).

Inflammation (I) and fibrosis (F) were scored on H&E sections by an expert gastrointestinal pathologist (N.B.), blinded to clinical characteristics [28]. As no validated histological inflammation score exists in CD, we applied a detailed grading approach (aligned with ECCO key principles) [30]. Lymphoplasmocytic and neutrophils infiltrates were independently scored in the *lamina propria,* to quantify chronic and acute inflammation, respectively (0 = none, 1 = mild, 2 = moderate, 3 = severe) [31–33]. Crypt injuries, edema, granuloma and ulcerations were also reviewed as previously described [28, 34, 35].

Fibrosis was first graded on H&E sections [24], then confirmed on MT-stained sections to highlight collagen accumulation (Staining Kit VWR 1004850001). A four-grade scaling system was used, integrating parameters from previous works [34–38] : F0: no architectural distortion, no ECM deposition or myofibroblast accumulation, F1: ECM and myofibroblast accumulation, preserved layers, increased submucosal thickness, F2: ECM and myofibroblast accumulation with preserved layers, densified ECM network, increased submucosal thickness, F3: massive ECM and myofibroblasts deposition extending into smooth muscle, disrupting layers with trans- mural fibrosis. Fibrosis scoring was applied to resection specimens only, as biopsies were too shallow for accurate fibrosis assessment [13].

2.3. Characterization of immunohistochemical signal of ERS-related proteins

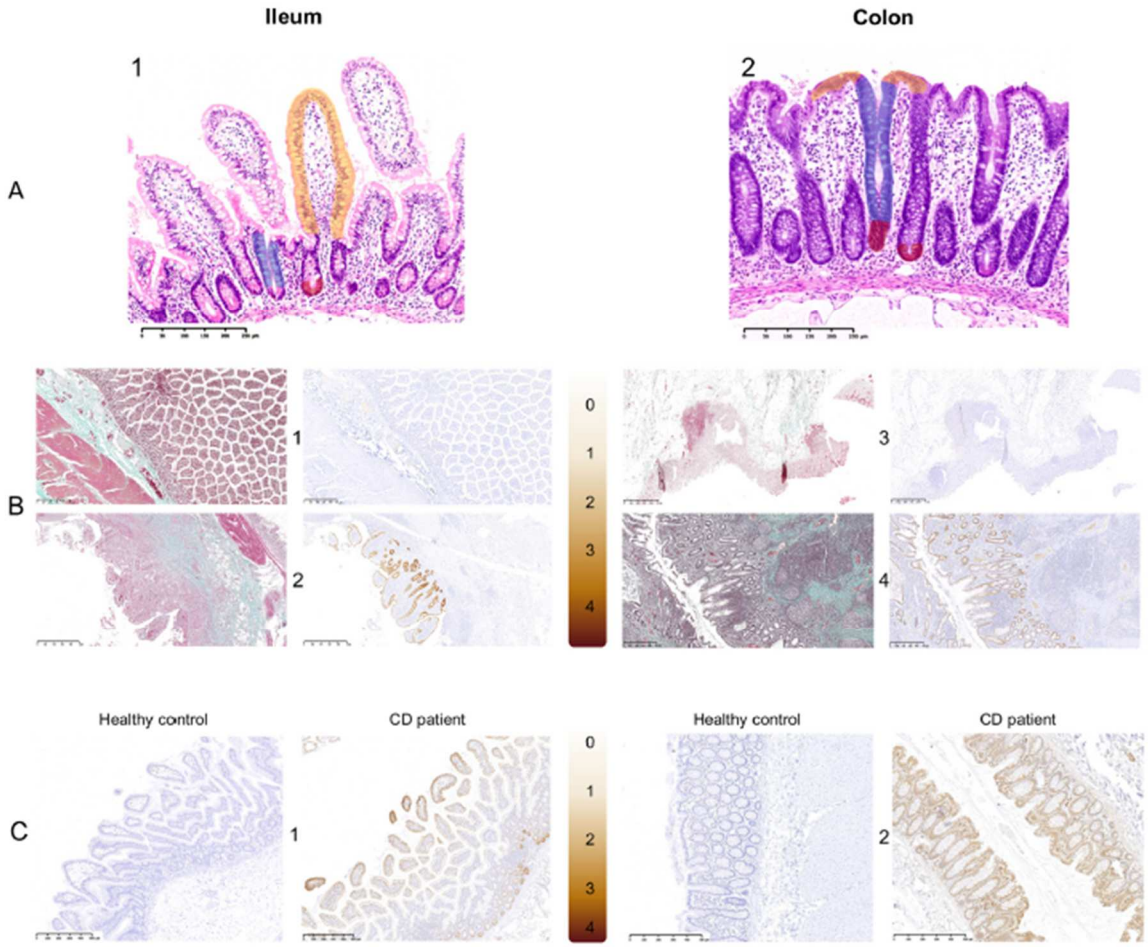
Based on the pilot proteomic study [24], the proteins AGR2, BiP, ERP44 and PDIA6 were selected for IHC characterization. IHC was performed as previously described [24, 39] using commercial antibodies targeting AGR2 (Novus, Rabbit Dako-Agilent, 1:250), BiP (Cell Signaling, Rabbit Dako-Agilent, 1:500), ERP44 (Cell Signal ing, Rabbit Dako-Agilent, 1:1000) and PDIA6 (Sigma, Rabbit Dako-Agilent, 1:2000). Positive and negative controls ensured techical staining reliability between batches. IHC scores were determined by at least two independent observers (EB, M-AM, CM and CS), blinded to clinical information, inflammation or fibrosis scores.

Due to differences in cellular distribution along the crypt epithelium, three zones were characterized in both colon and ileum: 1-surface epithelium (SE), 2-upper and intermediate portion of the crypts, and 3-bottom of crypts, each graded separately (Fig. 1. A). Staining intensity was scored using a semi-quantitative brown shading scale (0 to 4), where 0 = no staining, and 1, 2, 3 and 4 = weak, moderate, strong and very strong staining, respectively, as described by others [39–41] (Fig. 1. B). Immunostaining signals were evaluated manually by two independent observers, reaching a consensus on the final score for each zone. The Intra-class Correlation Coefficient (ICC) was 0.995, indicating excellent reliability of the scoring method [42]. In cases of disagreement, scores were averaged. When several tissue slices (and therefore different blocs) could be obtained from the same patient's gut location and showed identical inflammation and fibrosis grades, the IHC scores were averaged to avoid overfitting. The Bonferroni correction was applied to control for type I error across multiple com- parisons ($n = 12$), adjusting the alpha threshold accordingly.

2.4. Statistics

Inflammation and fibrosis scores were correlated with the IHC scores of each protein across all available ileal and colonic tissues. These correlations were based on IHC score distributions in the following groups: normal tissues (N) (neither inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and/or tissues with both inflammation and fibrosis (IF). Additionally, comparisons of protein distributions were performed between patient groups: pediatric CD *versus* adult-onset CD, CD patients *versus* non IBD controls, and CD *versus* UC.

Figure 1. A. *Representative pictures illustrating the different histological areas studied: 1: Ileum; 2: Colon. Surface epithelium (yellow); crypts (blue); bottom of crypts (red).* ***B.*** *Examples of Masson's Trichrome-stained tissues showing fibrosis grades F1 to F3, alongside IHC staining scores for AGR2 (in brown) in the ileum and colon (1: Ileum, F1 with AGR2 score = 1; 2: Ileum, F3 with AGR2 score = 2.5 to 4; 3: Colon, F1 with AGR2 score = 0.5; 4: Colon, F3 with AGR2 score = 3.5).* ***C.*** *Illustration of PDIA6 distribution (in brown) in normal tissues (without inflammation nor fibrosis) in a healthy control and a CD patient tissues taken in the ileum (1) and colon (2).*



GraphPad Prism (version 10.0.2) was used for graphical illustrations and statistics. IHC scores, inflammation and fibrosis scores were compared between patient groups and tissue groups using ANOVA, Kruskal-Wallis or Tukey's post hoc tests. PDIA6, distribution in CD, UC and non IBD patients were compared using MannWhitney or Welch's *t* -tests. Correlations between inflammation, fibrosis grades and IHC scores, were assessed via Spearman's test. Contingency tables and Fisher's exact

tests evaluated the discriminatory power of the PDIA6 IHC score in differentiating CD, UC, and non IBD controls, using thresholds of < 1 or ≥1.

Results were considered significant after Bonferroni correction for multiple testing. Adjusted significance thresholds were defined as follows: $p < 0.0042$ (*), $p < 0.001$ (**), $p < 0.0001$ (***).

3. Results

3.1. Clinical and sampling data

We included 224 patients and 815 tissue samples in this multicenter study: 119 CD, 31 UC and 74 non IBD controls. Within the CD cohort, 72 patients had a pediatric-onset disease (< 17years). Surgical resection specimens from stenosis and surgical margin (68 patients) and endoscopic biopsies away from stenosis (51 patients), were analyzed. Clinical characteristics (CD, UC and non IBD) and key findings from the comparisons are summarized in Table 1 (Supplementary). Significant clinical differences were observed between pediatric- and adult-onset populations, including treatment at sampling, stricture location and the primary or anastomotic nature of the stenosis.

As multiple tissue slices were available per patient, 4518 tissue slices were analyzed. Table 2 (Supplementary) summarizes the tis- sue samples.

3.2. Comparison of the distributions of ERS-related proteins and association with fibrosis in CD

We conducted a systematic analysis of AGR2, BiP, PDIA6 and ERP44 IHC signal distributions. Illustrations are shown in Supplementary Figure 1.

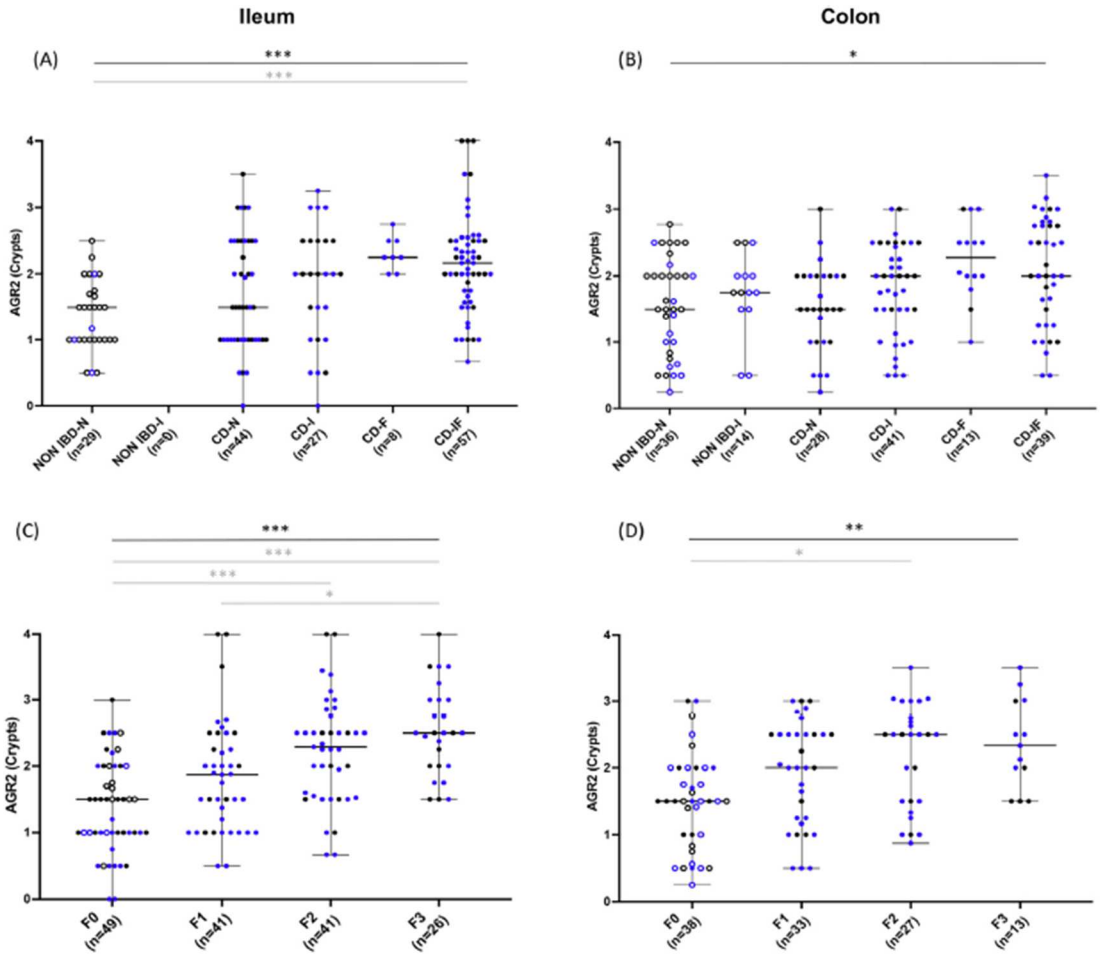
3.2.1. AGR2

AGR2 IHC staining distribution across groups is shown Fig. 2 and Supplementary Figure 2–3. AGR2 was significantly higher in the colonic than ileal surface epithelium, especially in adult controls and normal (no inflammation nor fibrosis) CD samples (Supplementary Figure 2).

In the ileal and colonic crypts of CD patients, AGR2 distribution was increased in the epithelium adjacent to inflammatory and fibrostenosing tissues, compared to non IBD and CD tissues without

inflammation or fibrosis (Fig. 2. A-B). A similar pattern was observed between purely fibrosing tissues (without inflammation) and normal non IBD and CD tissues. Similar differences were also found in the ileal surface epithelium and crypt bottoms in both the ileum and colon (Supplementary Figure 3).

Figure 2. (A) (B) *Distribution of AGR2 IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients.* ***(C) (D)*** *Distribution of AGR2 IHC scores in ileal and colonic crypts according to fibrosis grades.*



• Adult-onset CD.
○ Adult control cases.
• Pediatric-onset CD.
○ Pediatric control cases.
* Statistical significance based on ANOVA test results after Bonferroni correction.
* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

AGR2 IHC staining increased with higher fibrosis grades in ileal and colonic crypts (Fig. 2. C-D). Similar findings were noted in the surface epithelium and crypt bottoms (Supplementary Figure 4).

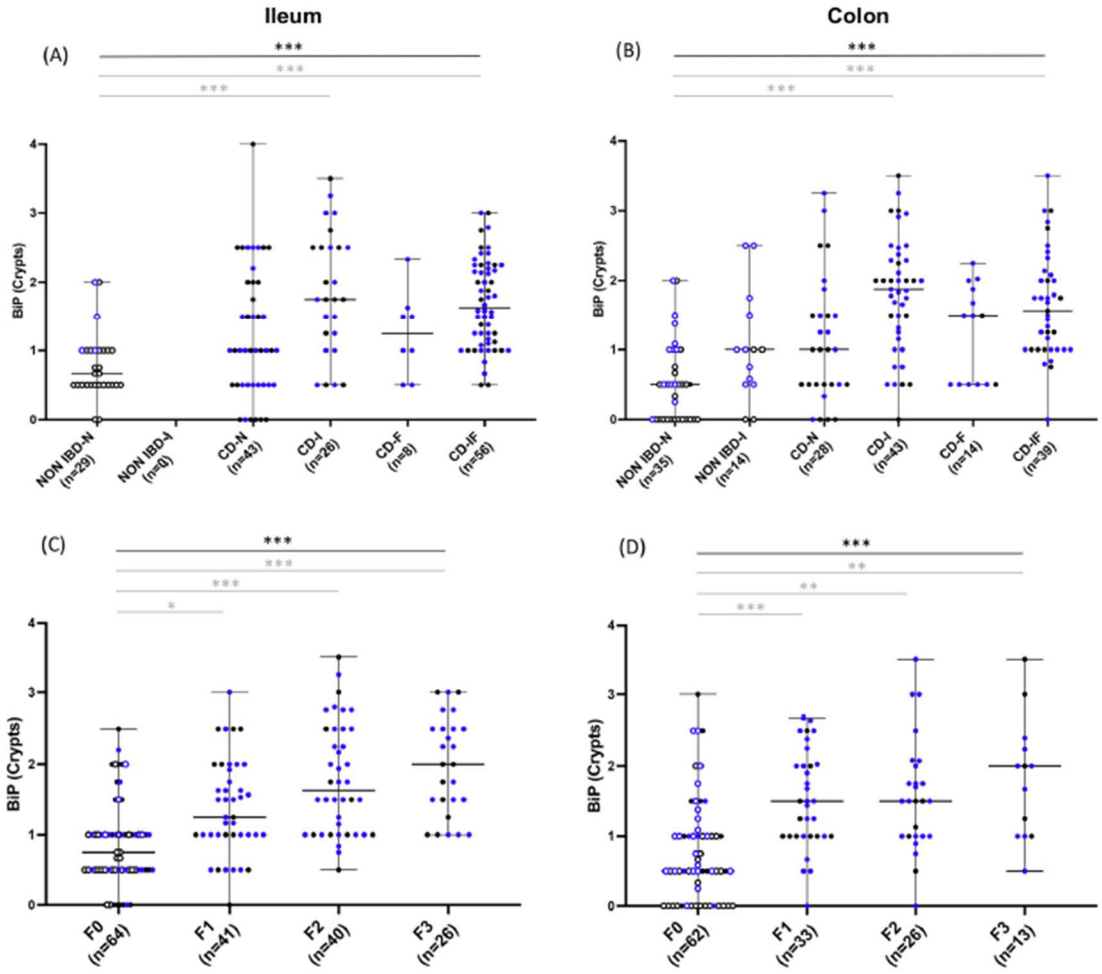
Correlation analyses (Supplementary Figure 5) showed a weak but significant association between AGR2 IHC scores and fibrosis in colonic crypts and crypt bottoms in both adult and pediatric CD cases, but no significant correlation with acute or chronic inflammation. In the ileum, AGR2 IHC scores correlated more strongly with fibrosis than in the colon, particularly in the surface epithelium and crypts. No correlation was found between AGR2 expression and chronic or acute inflammation.

3.2.2. BiP

BiP IHC staining is shown in Supplementary Figure 6 for controls and normal CD samples. BiP distribution was lower in adult colonic tissues compared to ileal tissues and colonic and ileal pediatric tissues.

BiP IHC staining distribution is illustrated in Fig. 3. A-B and Supplementary Figure 7. BiP was significantly increased in the epithelium adjacent to inflammatory and fibrostenosing tissues, compared to non IBD and CD tissues without inflammation or fibrosis. Unlike AGR2, BiP distribution was also elevated in tissues with pure inflammation (without fibrosis), but not in tissues with pure fibrosis.

Figure 3. (A)(B) *Distribution of BiP IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with inflammation and fibrosis (IF) in non IBD cases and CD.* ***(C)(D)*** *Distribution of BiP IHC scores in ileal and colonic crypts according to fibrosis grades.*



• Adult-onset CD.
○ Adult control cases.
• Pediatric-onset CD.
○ Pediatric control cases.
* Statistical significance based on ANOVA test results after Bonferroni correction.
* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

BiP IHC scores was positively associated with fibrosis degree in the ileal and colonic crypts (Fig. 3. C-D). Significant results were also found in the surface epithelium and crypt bottoms (Supplementary Figure 8). Correlation studies revealed a significant association between BiP intensity and inflammation, unlike the other proteins (Supplementary Figure 5 and 9). BiP signal intensity also correlated with fibrosis, showing strong inflammation-fibrosis associations.

3.2.3. PDIA6

The PDIA6 IHC staining distribution is represented in Supplementary Figure 10. In controls and normal CD samples, PDIA6 distribution was lower in adult colonic tissues compared to pediatric colonic tissues. Overall, PDIA6 IHC scores were lower in the colon than in the ileum for both pediatric and adult cases.

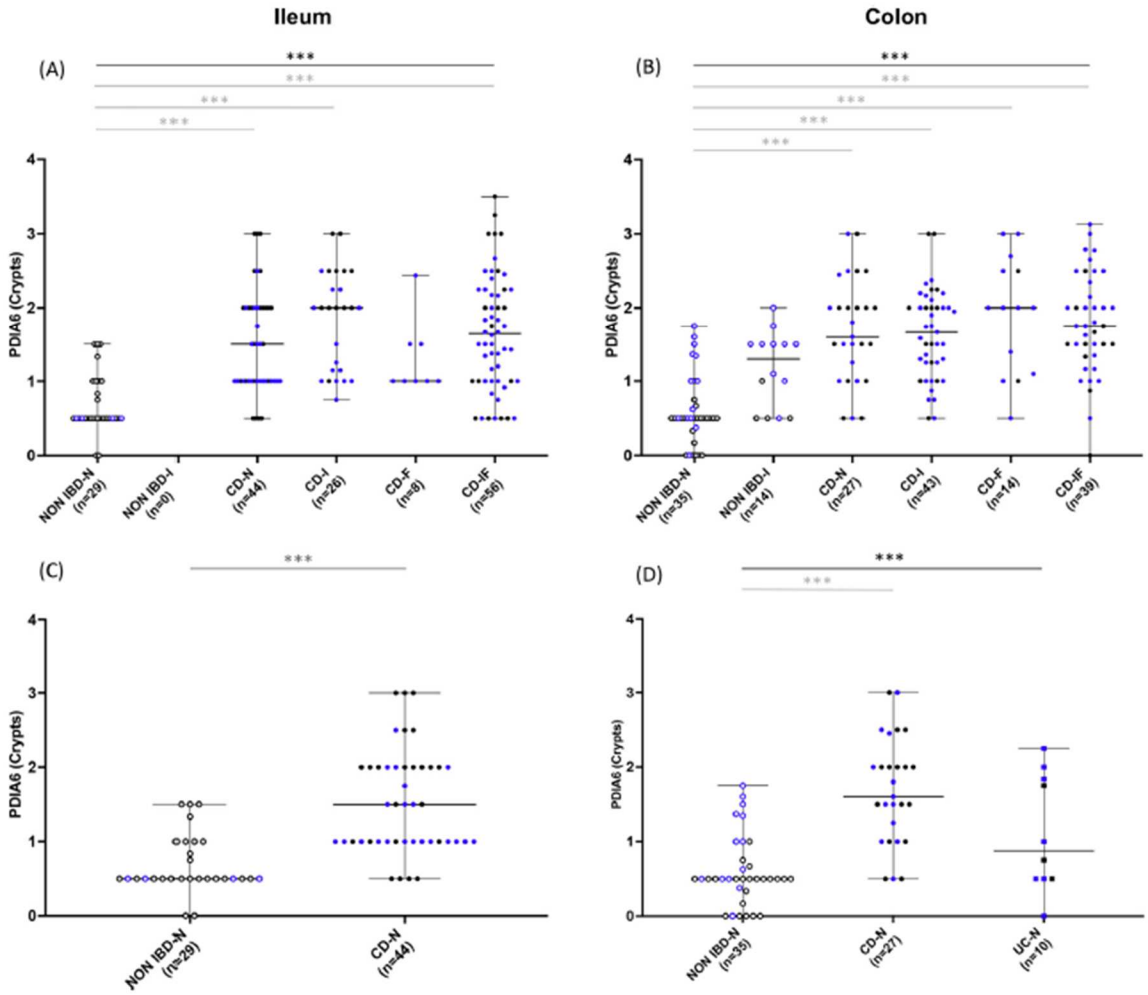
PDIA6 distribution was not increased in fibro-inflamed tissues compared to those without inflammation or fibrosis, in either colon or ileum (Fig. 4. A-B, Supplementary Figure 11). However, PDIA6 distribution was significantly higher in CD tissues compared to non IBD tissues, regardless of location, age of onset or inflammation/fibrosis presence.

Correlation analyses showed no significant association between PDIA6 and inflammation or fibrosis in any population.

3.2.4. ERP44

ERP44 IHC staining is shown in Fig. 5 and Supplementary Figures 12–15. Adult and pediatric-onset populations are presented separately due to significant group differences (see 3.d).

Figure 4. (A)(B) *Distribution of PDIA6 IHC scores in ileal and colonic crypts, including normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients.* ***(C)(D)*** *Distribution of PDIA6 IHC scores in normal ileal and colonic crypts (without inflammation nor fibrosis) based on IBD phenotype (CD or UC) or healthy controls.*



- Adult-onset CD.
- Adult control cases.
- Pediatric-onset CD.
- Pediatric control cases.
- Adult-onset UC.
- Pediatric-onset UC.
- * Statistical significance based on ANOVA test results after Bonferroni correction.
- * Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.
- * Statistical significance based on Mann-Withney test after Bonferroni correction.

Correlation studies showed no significant association between ERP44 and inflammation or fibrosis in either population.

3.3. Differential study of pediatric versus adult-onset cases

3.3.1. AGR2

AGR2 IHC scores were higher in adult than pediatric colonic crypts (Supplementary Figure 2).

In pediatric-onset cases, AGR2 distribution significantly increased with fibrosis grade in both ileal and colonic crypts (Fig. 6. A-B), surface epithelium and crypt bottoms (Supplementary Figure 16.1–4).

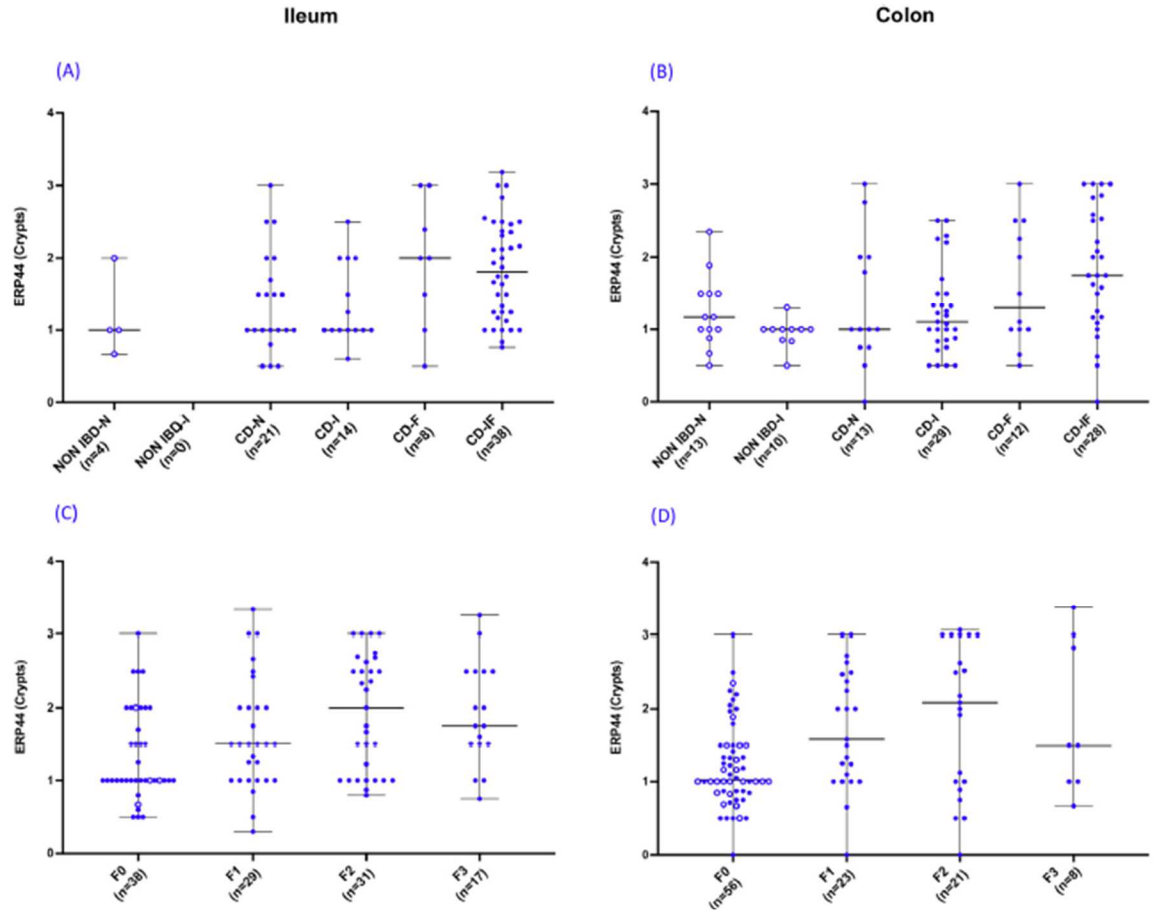
In adults, an increase was observed only in ileal tissues (Fig. 6. C, Supplementary Figure 16.5–6), but not in the colon (Fig. 6. D, Sup- plementary Figure 16.7–8).

3.3.2. BiP

In crypts without inflammation or fibrosis, colonic BiP distribution was lower in adult than in pediatric tissues (Supplementary Figure 6). BiP distribution in the surface epithelium and crypt bottoms were similar in both groups.

In both ileum and colon, BiP distribution according to fibrosis grade were similar in pediatric- and adult-onset CD (Supplementary Figures 17–18).

Figure 5. (A)(B) *Distribution of ERP44 IHC scores in ileal and colonic crypts, including normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and tissues with both inflammation and fibrosis (IF) in pediatric non IBD cases and pediatric-onset CD patients.* ***(C)(D)*** *Distribution of ERP44 IHC scores in ileal and colonic crypts according to fibrosis grades in pediatric-onset cases.*



Pediatric-onset CD.
Pediatric control cases.
* Statistical significance based on ANOVA test results after Bonferroni correction.
* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

3.3.3. PDIA6

PDIA6 distribution was lower in adult colonic tissues compared to pediatric colonic tissues without inflammation or fibrosis, though no difference was seen in the ileum (Supplementary Figure 10).

The difference observed between CD and non-IBD tissues was consistent across both age groups, with no significant variation in results between pediatric and adult populations.

3.3.4. ERP44

In control and normal CD tissues, ERP44 distribution was higher in adult-onset compared to pediatric-onset-cases, with significant differences in the colon (Supplementary Figure 12).

In the ileum, no significant difference was observed between non-inflammatory and inflamed-fibrosing tissues in either adult-onset or pediatric-onset CD, although a trend toward increased expression was noted in the pediatric group (Fig. 5. A, Supplementary Figure 13–14).

In pediatric cases, ERP44 IHC scores were higher in fibroinflammatory CD tissues than in inflammatory non IBD tissues in colonic crypts (significant in the bottom) (Fig. 5. B, Supplementary Figure 13). In adults, ERP44 colonic distribution was more widespread and not influenced by inflammation or fibrosis (Supplementary Figure 14–15).

In pediatric-onset ileal crypts, ERP44 distribution increased with fibrosis grade (Fig. 5 -C.), though no significant change was seen in surface epithelium or crypt bottoms (Supplementary Figure 13.2, 13.6).

In pediatric colonic epithelium, ERP44 distribution increased with fibrosis grade (Fig. 5 -D., Supplementary Figure 13.4 and 13.8).

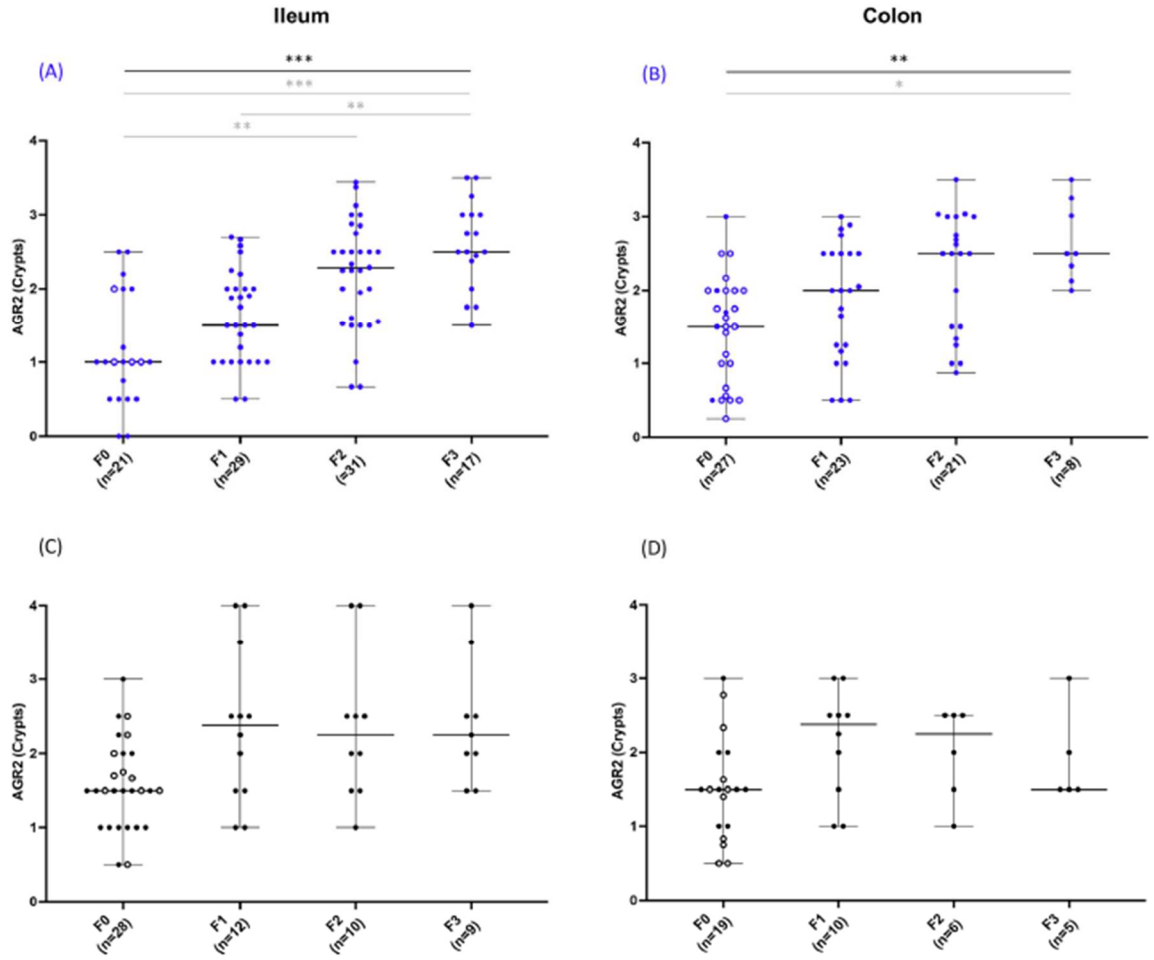
3.4.PDIA6 specific distribution in normal tissues of CD, UC and non IBD controls

In tissues without fibrosis nor inflammation, PDIA6 IHC scores were higher in CD compared to non IBD cases in the ileum, and to UC and non IBD cases in the colon (Fig. 1. C., Fig. 4. C-D, Supplementary Figure 19).

These results were similar when including inflamed and/or fibrotic cases, with no difference between adult and pediatric tissues.

Contingency analyses using a threshold of PDIA6 IHC staining intensity < 1 for normal tissues *vs.* ≥1 from normal CD tissues are provided in Supplementary Figure 20.A-D. Sensitivity, specificity and accuracy were established for each segment and location (Supplementary Figure 20.E).

Fig. 6. (A-B) *Distribution of AGR2 IHC scores according to fibrosis scores in pediatric-onset cases and* ***(C- D)*** *in adult-onset cases in ileal and colonic crypts.* ***The same shape and color coding has been used for all the figures.***



• Adult-onset CD.
○ Adult control cases.
• Pediatric-onset CD.
○ Pediatric control cases.
* Statistical significance based on ANOVA test results after Bonferroni correction.
* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

4. Discussion

We investigated the expression of AGR2, BIP, PDIA6, and ERP44 proteins using IHC on intestinal tissue samples from non IBD tissues, as well as non-inflammatory non-fibrotic, inflammatory, fibro-inflammatory and fibrotic CD tissues, in pediatric and adult- onset cases. The analyses focused on

the surface epithelium and crypts. Our results show significant heterogeneity in the distribution of these proteins, despite their shared chaperone functions. This suggest that although involved in ERS response, their expression patterns differ based on tissue features, disease phenotype and location. The underlying factors driving this heterogeneity - such age, disease stage, or segmental localization - remain incompletely understood.

ERS plays a crucial role in inflammatory and fibrotic diseases in multiple organs [43–45], including the intestine [11, 24, 46– 49]. PDIA6 and ERP44 are members of the PDI family, whereas BiP functions as a chaperone rather than a typical PDI. AGR2, though part of the thioredoxin family and lacking isomerase activity, shares structural traits with PDIs and is often grouped with them [50, 51]. Intestinal epithelial cells, Paneth and goblet cells are all susceptible to ERS due to microbiota exposure [52]. Although the specific roles of these ERS-related proteins remain poorly understood, their expression is influenced by factors like cell type, tissue location, pH, microbial activity [53, 54] and developmental stages [55, 56]. Secretory cells like goblet and Paneth cells require more ER chaperones [57]. Inflammatory and metabolic stress further regulate their expression [53, 58]. The observed differences between adult and pediatric tissues may reflect tissue maturation. Importantly, Eletto et al. showed that ER proteins are not uniformly regulated, with multiple distinct ERS response pathways affecting the PDI expression [59]. However, a full understanding of these differences lies beyond IHC and requires functional validation.

The significant increase of AGR2 in fibro-inflammatory and fibrotic tissues – absent in purely inflammatory ones - suggests its crucial role in CD fibrosis. AGR2 overexpression has been identified in CD and other fibrotic conditions like idiopathic pulmonary fibrosis and fibrolamellar cancers [48, 60, 61]. An increase in cytoplasmic AGR2 has been associated with elevated levels of its extracellular form (eAGR2) and associated with ECM in cancer [62]. Our findings suggest cytoplasmic AGR2 upregulation may also reflect increased eAGR2 release. Indeed, functional assays on epithelial and fibroblast models showed that eAGR2 produced by HT29 epithelial cells was indeed increased along with cytoplasmic AGR2, inducing a paracrine transition of intestinal fibroblasts into myofibroblasts (FMT), as also observed in lung fibroblasts [24, 63]. Furthermore, AGR2's interaction with β-catenin promotes pro-fibrotic gene expression, and its role in fibroblast recruitment supports its

involvement in fibrosis [63, 64]. This is also true in cancer-associated fibroblasts [66], as eAGR2 promotes epithelial proliferation and co tributes to endo-MT and EMT, both implicated in intestinal fibrosis [62, 65, 67–70]. The correlation between AGR2 and fibrosis in both colon and ileum in pediatric cases, but only in the ileum in adults, could be due to higher eAGR2 levels in the colon of pediatric CD patients. However, functional assays and evaluation of local/systemic eAGR2 levels, are beyond the scope of our retrospective IHC characterization and require a dedicated research.

BiP, chaperone involved in the unfolded protein response, was elevated not only in fibrotic but also in purely inflammatory tissues which is expected knowing its role in ERS. The significant correlation between BiP staining intensity and inflammation severity is a relationship not found with the other studied proteins. This observation aligns with studies addressing *HSPA5* (BiP gene) expression in pediatric and adult IBD [71–73]. IL-10 regulates gut inflammation by suppressing BiP expression in intestinal epithelial cells and dysfunction in IL-10 signaling has been associated with chronic inflammation, and very early-onset IBD with higher fibrosis risk [74– 77]. These findings suggest that impaired regulation of ERS, due to IL-10 deficiency, may worsen IBD progression. BiP distribution among tissues in both adult and pediatric tissues probably reflect the inflammation and ER stress degrees rather than an effective direct role in fibrotic process as its role in ER stress is rather restricted to intracellular process and that it is not observed secreted by epithelial cell, on the contrary to AGR2.

PDIA6 and ERP44 interact with AGR2 and BiP, regulating protein folding and ER homeostasis [78–81]. They were increased in the proteome cellular fraction of ileal and colonic ulcer edges [82] and in fibrostenosing CD tissues, as well as in HT-29 cell supernatants after ERS [24]. Higher PDIA6 level in CD compared to non IBD, even in non-affected area, suggests a potential role in early pathogenic mechanism, possibly to stress response or epithelial homeostasis alterations. PDAI6 is more strongly distributed in pediatric cases, and varies between ileum and colon, potentially reflecting higher intestinal proliferation rates in children. Its transcript levels have been shown to vary with development, promoting cell motility and proliferation [83, 84]. Additionally, PDIA6 is upregulated in hypoxic conditions, suggesting a role in highly oxygen-demanding intestinal regions [85, 86]. However, beyond proteomic results in Vieujean *et al*. [24]., no direct evidence between PDIA6 and intestinal fibrosis has been established. Nevertheless, ER stress itself is associated with fibrosis progression [87]. In a murine model, BiP and PDIA6 were correlated with cardiac interstitial fibrosis

[88]. PDIA6 plays a key role in HSC activation and liver fibrosis, potentially serving as a biomarker for cirrhosis and fibrotic liver diseases [89]. Functional inhibition studies in intestinal epithelial models as organoids could help clarify their roles in intestinal fibrosis.

ERP44 IHC signal was higher in normal tissues in adults than in children, in the colon compared to the ileum, and particularly in the surface epithelium and crypt bottoms. ERP44 expression is influenced by endoluminal and intracellular pH [90–92]. The more acidic pH in the colon, compared to the slightly alkaline ileum, may explain the higher ERP44 levels [91, 93]. ERP44 expression is associated with fibro-inflammatory status in both segments only in pediatric-onset CD. However, no difference was found between normal and inflammatory CD tissues, and increased ERP44 levels correlate with the severity of fibrosis, further supporting an association which requires other functional confirmations. Interestingly, studies in renal and cardiac models have suggested that ERP44 modulates fibrotic remodeling by regulating ER homeostasis and redox balance, potentially via attenuation of ER stress and down- stream oxidative and inflammasome pathways [94–96].

Our study's limitations include its retrospective nature and the intrinsic constraints of IHC, though this remains the gold standard for large tissue panels. Our multicenter study is the first multi-center characterization of PDIs in pediatric and adult CD. However, our findings are observational, and further mechanistic validations are required for ERP44, PDAI6 as was performed for AGR2 [24, 62, 63, 70, 97]. Functional studies might help in elucidating the probable complex and interconnected roles of these proteins in fibrosis-related pathways. Additionally, the heterogeneity of the patient cohorts in terms of treatment regimens and disease duration, combined with the limited number of anastomotic strictures, reduces the statistical power for meaningful multivariate adjustments.

Many questions remain about ERS induction and UPR's role in fibrosis. Current treatments remain insufficient to prevent or reverse fibrosis or intestinal strictures. Developing effective drugs require a better understanding of complex interactions between protein produced in response to ER stress and inflammation as BiP, AGR2 and others PDIs, partially described [51, 78, 79, 98–100], but not fully understood. Our findings highlight the complexity of ERS protein networks, whose regulation likely drives distinct cellular outcomes depending on tissue type and disease phenotype. However, given the observational nature of our study and expression variability, these associations require further

validation. Differences between adults and children, as well as between ileal and colonic tissues, suggest distinct physiological and pathophysiological mechanisms which should be explored. Functional studies, including biochemical interaction assays and *in vitro* fibrosis models, are needed to clarify these roles and assess their therapeutic potential.

Author contributions

EB, EL, and M-AM contributed to the conceptualization of the study. Data curation was performed by EB, CS, and M-AM. Formal analysis was conducted by EB, NB, CS, and M-AM. Funding for the project was acquired by EB, EL, and M-AM. The investigation was carried out by EB, CS, NB, CM, FF, and M-AM. Methodology was developed by EB, CS, NB, and M-AM. Resources were provided by EB, DL, HS, SC, PD, and EL. Software-related contributions were made by EB and CS. Supervision was ensured by EL and M-AM. The original draft of the manuscript was written by EB, SV, M-AM, and EL. Authors involved in the review of the manuscript were: NB, HS, DL, PD, EL, SV, and M-AM.

Conflict of interest

None.

Acknowledgements

We thank the people and institutions involved in the providing of human samples (E Leteurtre, C Belkhou, V Deprez, C Fauvet), in the biobanking (S Gofflot, R Thonon, K El Kandoussi, C Shadouh) and in the technical and scientific laboratory assistance (C Hum- blet, T Di Salvo, H Kocadag). We also thank the Epimad group (in particular C Gower-Rousseau, D Turck, A Petillon, H Sarter).

Funding

This work was supported by the University Hospital of Liège (CHU Liège), Uliège internal grants and the Leon Fredericq Foundation (Elvira Eyckelberg Grant - 2018 and 2021).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2025.04.015

References

[1] *Gupta N, Bostrom AG, Kirschner BS, et al. Incidence of stricturing and penetrating complications of Crohn's disease diagnosed in pediatric patients. Inflamm Bowel Dis 2010;16(4):638. doi: 10.1002/IBD.21099*

[2] *Mak JWY, Ng SC. Epidemiology of fibrostenosing inflammatory bowel disease. J Dig Dis 2020;21(6):332–5. doi: 10.1111/1751-2980.12853*

[3] *Rieder F, Latella G, Magro F, et al. European Crohn's and Colitis Organisation Topical Review on Prediction, Diagnosis and Management of fibrostenosing Crohn's Disease. J Crohn's Colitis 2016:873–85. doi: 10.1093/ecco-jcc/jjw055*

[4] *Peyrin-Biroulet L, Loftus EV, Colombel JF, Sandborn WJ. The natural history of adult crohn's disease in population-based cohorts. Am J Gastroenterol 2010;105(2):289–97. doi: 10.1038/ajg.2009.579*

[5] *Boualit M, Salleron J, Turck D, et al. Long-term outcome after first intestinal resection in pediatric-onset Crohn's disease: a population-based study. Inflamm Bowel Dis 2013;19:7–14. doi: 10.10 02/ibd.230 04*

[6] *Splawski JB, Pffefferkorn MD, Schaefer ME, et al. NASPGHAN clinical report on postoperative recurrence in pediatric Crohn disease. J Pediatr Gastroenterol Nutr 2017;65(4):475–86. doi: 10.1097/MPG.0 0 0 0 0 0 0 0 0 0 0 0 01606*

[7] *Ley D, Leroyer A, Dupont C, et al. New therapeutic strategies have changed the natural history of pediatric Crohn's disease: a two-decade population- based study. Clin Gastroenterol Hepatol 2022;20:2588–97. doi: 10.1016/j.cgh. 2022.01.051*

[8] *Peyrin-Biroulet L, Oussalah A, Williet N, Pillot C, Bresler L, Bigard MA. Impact of azathioprine and tumour necrosis factor antagonists on the need for surgery in newly diagnosed Crohn's disease. Gut 2011;60(7):930–6. doi: 10. 1136/GUT.2010.227884*

[9] *Bouhnik Y, Carbonnel F, Laharie D, et al. Efficacy of adalimumab in patients with Crohn's disease and symptomatic small bowel stricture: a multicentre, prospective, observational cohort (CREOLE) study. Gut 2018;67:53–60. doi: 10. 1136/gutjnl- 2016- 312581*

[10] *Rieder F, Fiocchi C, Rogler G. Mechanisms, management, and treatment of fibrosis in patients with inflammatory bowel diseases. Gastroenterology 2017;152(2):340–50. doi: 10.1053/j.gastro.2016.09.047*

[11] *Latella G, Di Gregorio J, Flati V, Rieder F, Lawrance IC. Mechanisms of initiation and progression of intestinal fibrosis in IBD. Scand J Gastroenterol 2015;50(1):53–65. doi: 10.3109/00365521.2014.968863*

[12] *Ma C, Jairath V, Click B, et al. Targeting anti-fibrotic pathways in Crohn's dis- ease – The final frontier? Best Pract Res Clin Gastroenterol 2019. doi: 10.1016/ j.bpg.2019.02.005*

[13] *Rieder F, De Bruyn JR, Pham BT, et al. Results of the 4th Scientific Workshop of the ECCO (Group II): markers of intestinal fibrosis in inflammatory bowel disease. J Crohn's Colitis 2014;8:1166–78. doi: 10.1016/j.crohns.2014.03.009*

[14] *Burke JP, Mulsow JJ, O'Keane C, et al. Fibrogenesis in Crohn's Disease. Am J Gastroenterol 2007;102(2):439–48. doi: 10.1111/j.1572-0241.2006.01010.x*

[15] *Latella G, Rogler G, Bamias G, et al. Results of the 4th scientific workshop of the ECCO (I): pathophysiology of intestinal fibrosis in IBD. J Crohn's Colitis 2014;8:1147–65. doi: 10.1016/j.crohns.2014.03.008*

[16] *Rieder F, Kessler S, Sans M, Fiocchi C. Animal models of intestinal fibrosis: new tools for the understanding of pathogenesis and therapy of hu- man disease. Am J Physiol Gastrointest Liver Physiol 2012;303(7):G786–801. doi: 10.1152/ajpgi.0 0 059.2012*

[17] *Flier SSN, Tanjore H, Kokkotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem 2010;285(26):20202–12. doi: 10.1074/ jbc.M110.102012*

[18] *Latella G, Sferra R , Speca S, Vetuschi A , Gaudio E , Latella G . Can we prevent, reduce or reverse intestinal fibrosis in IBD? Eur Rev Med Pharmacol Sci 2013;17(10):1283–304*

[19] *Rieder F, Kessler SP, West GA, et al. Inflammation-induced endothelial-to- mesenchymal transition: a novel mechanism of intestinal fibrosis. Am J Pathol 2011. doi: 10.1016/j.ajpath.2011.07.042*

[20] *Lawrance IC, Rogler G, Bamias G, et al. Cellular and molecular mediators of intestinal fibrosis. J Crohn's Colitis 2017;11(12):1491–503. doi: 10.1016/j. crohns.2014.09.008*

[21] *Speca S, Giusti I, Rieder F, Latella G. Cellular and molecular mechanisms of intestinal fibrosis. World J Gastroenterol 2012;18(28):3635–61. doi: 10.3748/ wjg.v18.i28.3635*

[22] *Uehara H, Tomoo AE, Ae N, et al. Emergence of fibrocytes showing morphological changes in the inflamed colonic mucosa. Dig Dis Sci 2010;55:253–60. doi: 10.1007/s10620- 009- 0730- 7*

[23] *Mortensen JH, Lindholm M, Langholm LL, et al. The intestinal tissue homeostasis–the role of extracellular matrix remodeling in inflammatory bowel disease. Expert Rev Gastroenterol Hepatol 2019;13(10):977–93. doi: 10. 1080/17474124.2019.1673729*

[24] *Vieujean S, Hu S, Bequet E, et al. Potential role of epithelial endoplasmic reticulum stress and anterior gradient protein 2 homologue in Crohn's Disease fi- brosis. J Crohns Colitis 2021;15(10):1737–50. doi: 10.1093/ECCO-JCC/JJAB061*

[25] *Fumery M, Pariente B, Sarter H, et al. Long-term outcome of pediatric-onset Crohn's disease: a population-based cohort study. Dig Liver Dis 2019;51(4):496–502. doi: 10.1016/j.dld.2018.11.033*

[26] *Lovasz BD, Lakatos L, Horvath A, et al. Evolution of disease phenotype in adult and pediatric onset Crohn's disease in a population-based cohort. World J Gastroenterol 2013;19(14):2217–26. doi: 10.3748/wjg.v19.i14.2217*

[27] *Gower-Rousseau C, Vasseur F, Fumery M, et al. Epidemiology of inflammatory bowel diseases: new insights from a French population-based registry (EPIMAD). Dig Liver Dis 2013;45(2):89–94. doi: 10.1016/j.dld.2012.09.005*

[28] *Magro F, Sabino J, Rosini F, et al. ECCO position on harmonisation of Crohn's Disease mucosal histopathology. J Crohn's Colitis 2022;16(6):876–83. doi: 10. 1093/ecco-jcc/jjac006*

[29] *Magro F, Langner C, Driessen A, et al. European consensus on the histopathology of inflammatory bowel disease - on behalf of the European Society of Pathology (ESP) and the European Crohn's and Colitis Organisation (ECCO). J Crohn's Colitis 2013;7:827–51. doi: 10.1016/j.crohns.2013.06.001*

[30] *Feakins R, Nunes PB, Driessen A, et al. Definitions of histological Abnormalities in Inflammatory bowel Disease: an ECCO position paper. J Crohn's Colitis 2024;18(2):175–91. doi: 10.1093/ecco-jcc/jjad142*

[31] *Geboes K, Desreumaux P, Jouret A, Ectors N, Rutgeerts P, Colombel J-F. Diag- nostic histopathologique de l'activité des maladies inflammatoires chroniques de l'intestin Evaluation de l'effet des traitements médicamenteux. Application des scores histologiques. Gastroenterol Clin Biol. 1999;23:1062–73. http: //www.em-consulte.com/showarticlefile/98102/index.pdf Accessed 19 November 2018*

[32] *Geboes K, Riddell R, Öst A, Jensfelt B, Persson T, Löfberg R. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 20 0 0;47(3):404–9. doi: 10.1136/gut.47.3.404*

[33] *Lang-Schwarz C, Angeloni M, Agaimy A, et al. Validation of the "inflammatory Bowel disease - distribution, Chronicity, activity [IBD-DCA] score" for ulcerative colitis and Crohns´ Disease. J Crohn's Colitis 2021;15(10):1621–30. doi: 10.1093/ecco-jcc/jjab055*

[34] *Rimola J, Planell R, Rodr S, et al. Characterization of inflammation and fibrosis in Crohn's Disease lesions by magnetic resonance imaging. Am J Gastroenterol 2015;110:432–40. doi: 10.1038/ajg.2014.424*

[35] *Chiorean MV, Sandrasegaran K, Saxena R, Maglinte DD, Nakeeb A, Johnson CS. Correlation of CT enteroclysis with surgical pathology in Crohn's disease. Am J Gastroenterol 2007;102:2541–50. doi: 10.1111/j.1572-0241.2007.01537.x*

[36] *Chen W, Lu C, Hirota C, Iacucci M, Ghosh S, Gui X. Smooth muscle hyperplasia/hypertrophy is the most prominent histological change in Crohn's fibrostenosing bowel strictures: a semiquantitative analysis by using a novel histological grading scheme. J Crohn's Colitis 2017;11(1):92–104. doi: 10.1093/ ecco-jcc/jjw126*

[37] *Gordon IO, Bettenworth D, Bokemeyer A, et al. Histopathology scoring systems of stenosis associated with small Bowel Crohn's disease: a systematic review. Gastroenterology 2020;158(1):137–50 e1. doi: 10.1053/j.gastro.2019. 08.033*

[38] *Johnson LA, Luke A, Sauder K, Moons DS, Horowitz JC, Higgins PDR. Intestinal fibrosis is reduced by early elimination of inflammation in a mouse model of IBD: impact of a "top-down" approach to intestinal fibrosis in mice. Inflamm Bowel Dis 2012;18(3):460–71. doi: 10.1002/ibd.21812*

[39] *Quesada-Calvo F, Massot C, Bertrand V, et al. OLFM4, KNG1 and Sec24C identified by proteomics and immunohistochemistry as potential markers of early colorectal cancer stages. Clin Proteom 2014;14:9. doi: 10.1186/ s12014-017-9143-3*

[40] *Zhou M, Gan X li, Ren Y xiang, et al. AGR2 and FOXA1 as prognostic markers in ER-positive breast cancer. BMC Cancer 2023;23(1):1–11. doi: 10.1186/ s12885- 023- 10964- 6*

[41] *Meyerholz DK, Beck AP. Principles and approaches for reproducible scoring of tissue stains in research. Lab Investig 2018;98(7):844–55. doi: 10.1038/ s41374- 018- 0057- 0*

[42] *Liljequist D, Elfving B, Roaldsen KS. Intraclass correlation – A discussion and demonstration of basic features. PLoS One 2019;14(7). doi: 10.1371/journal. pone.0219854*

[43] *Kropski JA, Blackwell TS. Endoplasmic reticulum stress in the pathogenesis of fibrotic disease. J Clin Invest 2018;128(1):64. doi: 10.1172/JCI93560*

[44] *Lenna S, Trojanowska M. The role of endoplasmic reticulum stress and the unfolded protein response in fibrosis. Curr Opin Rheumatol 2012;24(6):663– 8. doi: 10.1097/BOR.0B013E3283588DBB*

[45] *Tanjore H, Lawson WE, Blackwell TS. Endoplasmic reticulum stress as a pro-fibrotic stimulus. Biochim Biophys Acta 2013;1832(7):940–7. doi: 10.1016/J. BBADIS.2012.11.011*

[46] *Kaser A, Blumberg RS. Endoplasmic reticulum stress in the intestinal epithelium and inflammatory bowel disease. Semin Immunol 2009;21(3):156–63. doi: 10.1016/j.smim.2009.01.001*

[47] *Kaser A, Lee AH, Franke A, et al. XBP1 Links ER stress to intestinal inflammation and confers genetic risk for Human inflammatory bowel disease. Cell 2008;134(5):743–56. doi: 10.1016/j.cell.2008.07.021*

[48] *Maurel M, Obacz J, Avril T, et al. Control of anterior GRadient 2 (AGR2) dimerization links endoplasmic reticulum proteostasis to inflammation. EMBO Mol Med 2019;11(6):e10120. doi: 10.15252/emmm.201810120*

[49] *Ma A. Unresolved ER stress inflames the intestine. Cell 2008;134(5):724–5. doi: 10.1016/j.cell.2008.08.023.*

[50] *Appenzeller-Herzog C, Ellgaard L. The human PDI family: versatility packed into a single fold. Biochim Biophys Acta 2008;1783(4):535–48. doi: 10.1016/j. bbamcr.2007.11.010*

[51] *Dudek J, Benedix J, Cappel S, et al. Functions and pathologies of BiP and its interaction partners. Cell Mol Life Sci 2009;66(9):1556–69. doi: 10.1007/ s0 0 018-0 09-8745-y*

[52] *Garg AD, Kaczmarek A, Krysko O, Vandenabeele P, Krysko DV, Agosti- nis P. ER stress-induced inflammation: does it aid or impede disease progression? Trends Mol Med 2012;18(10):589–98. doi: 10.1016/j.molmed.2012. 06.010*

[53] *Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 2005;115(10):2656–64. doi: 10.1172/JCI26373*

[54] *Galligan JJ, Petersen DR. The human protein disulfide isomerase gene family. Hum Genomics 2012;6(1). doi: 10.1186/1479- 7364- 6- 6*

[55] *Wang Y, Yuan T, Wang H, et al. Inhibition of protein disulfide isomerase attenuates osteoclast differentiation and function via the readjustment of cellular redox State in postmenopausal osteoporosis. Inflammation 2024;47(2):626– 48. doi: 10.1007/S10753- 023- 01933- Z/METRICS*

[56] *Chen Y-C, Lu Y-F, Li I-C, Hwang S-PL. Zebrafish Agr2 is required for terminal differentiation of intestinal goblet cells. PLoS ONE 2012;7(4). doi: 10.1371/ journal.pone.0034408*

[57] *Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol 2011;9(5):356–68. doi: 10.1038/ nrmicro2546*

[58] *Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007;8(7):519–29. doi: 10.1038/ nrm2199*

[59] *Eletto D, Maganty A, Eletto D, et al. Limitation of individual folding resources in the ER leads to outcomes distinct from the unfolded protein response. J Cell Sci 2012;125(20):4865–75. doi: 10.1242/jcs.108928*

[60] *Tian Y, Li H, Gao Y, et al. Quantitative proteomic characterization of lung tissue in idiopathic pulmonary fibrosis. Clin Proteomics 2019;16(1):1–11. doi: 10.1186/s12014-019-9226-4*

[61] *Vivekanandan P, Micchelli STL, Torbenson M. Anterior gradient-2 is overexpressed by fibrolamellar carcinomas. Hum Pathol 2009;40(3):293–9. doi: 10. 1016/j.humpath.20 08.08.0 03*

[62] *Fessart D, Domblides C, Avril T, et al. Secretion of protein disulphide isomerase AGR2 confers tumorigenic properties. Elife 2016;5. doi: 10.7554/eLife. 13887.001*

[63] *Merugu SB, Zhou B, Mangukiya HB, et al. Extracellular AGR2 activates neighboring fibroblasts through endocytosis and direct binding to β-catenin that requires AGR2 dimerization and adhesion domains. Biochem Biophys Res Commun 2021;573:86–92. doi: 10.1016/j.bbrc.2021.08.028*

[64] *Zhu Q, Mangukiya HBH, Mashausi DDS, et al. Anterior gradient 2 is induced in cutaneous wound and promotes wound healing through its adhesion domain. FEBS J 2017;284(17):2856–69. doi: 10.1111/febs.14155*

[65] *Wang Z, Hao Y, Lowe AW. The adenocarcinoma-associated antigen, AGR2, promotes tumor growth, cell migration, and cellular transformation. Cancer Res 2008;6 8(2):4 92–7. doi: 10.1158/0008- 5472.CAN- 07- 2930*

[66] *Lai TY, Chiang TC, Lee CY, et al. Unraveling the impact of cancer-associated fibroblasts on hypovascular pancreatic neuroendocrine tumors. Br J Cancer 2024;130(7):1096–108. doi: 10.1038/s41416- 023- 02565- 8*

[67] *Fletcher GC, Patel S, Tyson K, et al. hAG-2 and hAG-3, human homologues of genes involved in differentiation, are associated with oestrogen receptor- positive breast tumours and interact with metastasis gene C4.4a and dystroglycan. Br J Cancer 2003;88(4):579–85. doi: 10.1038/sj.bjc.6600740*

[68] *Ibraghimov-Beskrovnaya O, Ervasti JM, Leveille CJ, Slaughter CA, Sernett SW, Campbell KP. Primary structure of dystrophin-associated glycoproteins linking dystrophin to the extracellular matrix. Nature 1992;355(6362):696–702. doi: 10.1038/355696a0*

[69] *Xu C, Liu Y, Xiao L, et al. The involvement of anterior gradient 2 in the stromal cell-derived factor 1-induced epithelial-mesenchymal transition of glioblastoma. Tumor Biol 2015;37(5):6091–7. doi: 10.1007/ S13277- 015- 4481- 0*

[70] *Mangukiya HB, Negi H, Merugu SB, et al. Paracrine signalling of AGR2 stimulates RhoA function in fibroblasts and modulates cell elongation and migration. Cell Adhes Migr 2019;13(1):332–44. doi: 10.1080/19336918.2019. 1685928*

[71] *Negroni A, Prete E, Vitali R, et al. Endoplasmic reticulum stress and unfolded protein response are involved in paediatric inflammatory bowel disease. Dig Liver Dis 2014;46(9):788–94. doi: 10.1016/j.dld.2014.05.013*

[72] *Stengel ST, Fazio A, Lipinski S, et al. Activating transcription factor 6 mediates inflammatory signals in intestinal epithelial cells upon endoplasmic reticulum stress. Gastroenterology 2020;159(4):1357–74 e10. doi: 10.1053/j.gastro.2020. 06.088*

[73] *Wu W, Zhao Y, Hu T, et al. Endoplasmic reticulum stress is upregulated in inflammatory bowel disease and contributed TLR2 pathway-mediated inflammatory response. Immunopharmacol Immunotoxicol 2024;46(2):192–8. doi: 10.1080/08923973.2023.2298897*

[74] *Shkoda A, Ruiz PA, Daniel H, et al. Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation. Gas- troenterology 2007;132(1):190–207. doi: 10.1053/j.gastro.2006.10.030*

[75] *Tang Z, Huang Z, Yan W, et al. Complications of enterostomy and related risk factor analysis of very early onset inflammatory bowel disease with interleukin-10 signalling deficiency: a single-centre retrospective analysis. BMC Gastroenterol 2020;20(1):1–7. doi: 10.1186/s12876- 020- 1160- 4*

[76] *Lin Z, Wang Z, Hegarty JP, et al. Genetic association and epistatic interaction of the interleukin-10 signaling pathway in pediatric inflammatory bowel disease. World J Gastroenterol 2017;23(27):4 897–909. doi: 10.374 8/wjg.v23.i27. 4897*

[77] *Shim JO, Seo JK. Very early-onset inflammatory bowel disease (IBD) in infancy is a different disease entity from adult-onset IBD; one form of interleukin- 10 receptor mutations. J Hum Genet 2014;59:337–41. doi: 10.1038/jhg. 2014.32*

[78] *Bouchalova P, Sommerova L, Potesil D, et al. Characterization of the AGR2 interactome uncovers new players of protein disulfide isomerase network in cancer cells In brief. Mol Cell Proteomics 2022;21(2):100188. doi: 10.1016/j. mcpro.2021.100188*

[79] *Worfolk JC, Bell S, Simpson LD, et al. Elucidation of the AGR2 interactome in esophageal adenocarcinoma cells identifies a redox-sensitive chaperone hub for the Quality control of MUC-5AC. Antioxidants Redox Signal 2019;31(15):1117–32. doi: 10.1089/ars.2018.7647*

[80] *Anelli T, Alessio M, Bachi A, et al. Thiol-mediated protein retention in the endoplasmic reticulum: the role of ERp44. EMBO J 2003;22(19):5015–22. doi: 10.1093/emboj/cdg491*

[81] *Wang L, Wang X, Wang C. Protein disulfide-isomerase, a folding catalyst and a redox-regulated chaperone. Free Radic Biol Med 2015;83:305–13. doi: 10. 1016/j.freeradbiomed.2015.02.007*

[82] *Pierre N, Salée C, Massot C, et al. Proteomics highlights common and dis- tinct pathophysiological processes associated with ileal and colonic ulcers in Crohn's Disease. J Crohn's Colitis 2019;2020:205–15. doi: 10.1093/ecco-jcc/ jjz130*

[83] *Gao H, Sun B, Fu H, et al. PDIA6 promotes the proliferation of HeLa cells through activating the wnt/ β-catenin signaling pathway. Oncotarget 2016;7(33):53289–98. doi: 10.18632/oncotarget.10795*

[84] *Fernandes SG, Ferreira LGA, Benham AM, Avellar MCW. Epididymal mRNA expression profiles for the protein disulfide isomerase gene family: modulation by development and androgens. Andrology 2024. doi: 10.1111/ANDR. 13700*

[85] *Kiouptsi K, Finger S, Garlapati VS, et al. Hypoxia evokes increased PDI and PDIA6 expression in the infarcted myocardium of ex-germ-free and conventionally raised mice. Biol Open 2019;8(1). doi: 10.1242/bio.038851*

[86] *Vekich J, Belmont P, Thuerauf D, Glembotski CC. Protein disulfide isomerase-associated 6 is an ATF6-inducible ER stress response Protein that protects cardiac myocytes from ischemia/reperfusion-mediated cell death. J Mol Cell Cardiol 2012;53(2):259–67. doi: 10.1016/j.yjmcc.2012.05.005.Protein*

[87] *Li C, Grider JR, Murthy KS, et al. Endoplasmic reticulum stress in subepithelial myofibroblasts increases the TGF- β1 activity that regulates fibrosis in Crohn's disease. Inflamm Bowel Dis 2020;26(6):809–19. doi: 10.1093/ibd/izaa015*

[88] *Souza-Neto FV, Jiménez-González S, Delgado-Valero B, et al. The interplay of mitochondrial oxidative stress and endoplasmic reticulum stress in cardiovascular fibrosis in obese rats. Antioxidants 2021;10(8). doi: 10.3390/ antiox10081274*

[89] *Zhong Y, Sun XX, Zhang P, et al. Identification and localization of xylose-binding proteins as potential biomarkers for liver fibrosis/cirrhosis. Mol Biosyst 2016;12(2):598–605. doi: 10.1039/c5mb00703h*

[90] *Vavassori S, Cortini M, Masui S, et al. A pH-regulated quality control cycle for surveillance of secretory protein assembly. Mol Cell 2013;50(6):783. doi: 10. 1016/J.MOLCEL.2013.04.016*

[91] *Hendershot LM, Feige MJ, Buchner J. Acidification activates ERp44-A molecular litmus test for protein assembly. Mol Cell 2013;50(6):779–81. doi: 10. 1016/j.molcel.2013.06.008*

[92] *Amiri M, Seidler UE, Nikolovska K. The role of pHi in intestinal epithelial proliferation–Transport mechanisms, regulatory pathways, and consequences. Front Cell Dev Biol 2021;9(January):1–7. doi: 10.3389/fcell.2021.618135*

[93] *Yamamura R, Inoue KY, Nishino K, Yamasaki S. Intestinal and fecal pH in human health. Front Microbiomes 2023;2:1–12. doi: 10.3389/frmbi.2023. 1192316*

[94] *Yang M, Liu C, Jiang N, et al. Endoplasmic reticulum homeostasis: a potential target for diabetic nephropathy. Front Endocrinol 2023;14(June):1–9. doi: 10.3389/fendo.2023.1182848*

[95] *Pang X , Zhang Y , Shi X , Li D , Han J . ERp44 depletion exacerbates ER stress and aggravates diabetic nephropathy in db/db mice. Biochem Biophys Res Com- mun 2018;504(4):921–6 .*

[96] *Zhao J, Li J, Li G, Chen M. The role of mitochondria-associated membranes mediated ROS on NLRPP inflammasome in cardiovascular diseases. Front Cardiovasc Med 2022;9(1059576). doi: 10.3389/fcvm.2022.1059576*

[97] *Kantar D, Bachiri K, Villemin C, et al. mAb-based targeting of the eAGR2- fibrinogen interaction as a promising new therapy in Inflammatory Bowel Disease. J Crohn's Colitis 2025;19(Supplement 1):i340. doi: 10.1093/ecco-jcc/ jjae190.0181*

[98] *Meunier L, Usherwood Y-K, Chung KT, Hendershot LM. A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. Mol Biol Cell 2002;13:4456–69. doi: 10.1091/ mbc.E02- 05- 0311*

[99] *Jansen G, Määttänen P, Denisov AY, et al. An interaction map of endoplasmic reticulum chaperones and foldases. Mol Cell Proteomics 2012;11(9):710–23. doi: 10.1074/mcp.M111.016550*

[100] *Ryu J, Park SG, Lee PY, et al. Dimerization of pro-oncogenic protein Anterior gradient 2 is required for the interaction with BiP/GRP78. Biochem Biophys Res Commun 2013;430:610–15. doi: 10.1016/j.bbrc.2012.11.105 .*