Natural history and characterization of intestinal fibrosis in pediatric Crohn's Disease

Emeline BEQUET

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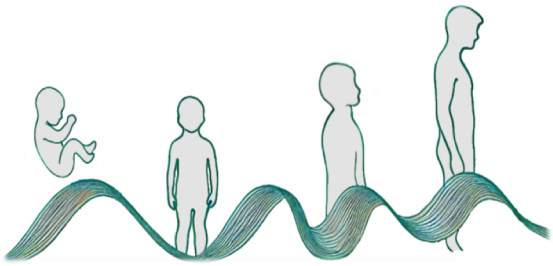
Doctor in Medical Sciences



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Chercher la vérité suppose d'avoir le courage d'être incertain.
Simone Weil

À toutes celles et ceux qui doutent, avancent quand même, et finissent par se trouver en chemin.

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À vous tous, du fond du cœur : MERCI !

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LIST OF ABBREVIATIONS

α-SMA	Alpha-smooth muscle actin
ACE	Angiotensin converting enzyme
ADAM17	A disintegrin and metalloproteinase domain 17
AGR2	Anterior gradient protein 2 homolog
ARPC1B	Actin related protein 2/3 complex subunit 1B
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
ATF6f	Activating transcription factor 6 fragment
ATG16L1	Autophagy related 16-like 1
bFGF	basic Fibroblast growth factor
BiP	Binding-immunoglobulin protein
BTK	Bruton tyrosine kinase
CARD15	Caspase recruitment domain-containing protein 15
CD	Crohn's disease
CHOP	C/EBP homologous protein
CI	Confidence interval
COL7A1	Collagen type VII alpha 1 chain
CTE	Computed tomography enterography
CTGF	Connective tissue growth factor
CTLA4	Cytotoxic T-lymphocyte associated protein 4
CRP	C-reactive protein
CVID	Common variable immune deficiency
CYBB	Cytochrome b-245 beta chain
DC	Dendritic cell
DDA	Disulfide bond disrupting agent
DNA	Deoxyribonucleic acid
DR4	Death receptor 4
DR5	Death receptor 5
ECM	Extracellular matrix
ECCO	European Crohn's and Colitis Organization
EGF	Epidermal growth factor
EIC	Extraintestinal comorbidity
EIM	Extraintestinal manifestation
eIF-2α	Eukaryotic translation initiation factor 2α
EMT	Epithelial-to-mesenchymal transition
EndoMT	Endothelial-to-mesenchymal transition
EO-CD	Early-onset Crohn's disease
EO-IBD	Early-onset inflammatory bowel disease
EO-UC	Early-onset ulcerative colitis
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
ERP44	Endoplasmic reticulum resident protein 44
ERS	Endoplasmic reticulum stress
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology and Nutrition
ESR	Erythrocyte sedimentation rate
ET	Endothelin
ETS	E transformation specific
FAP	Fibroblast activation protein
FFPE	Formalin-fixed paraffin-embedded
FMT	Fecal microbiota transplantation

FOXP	Forkhead box protein
FTS	Fused toes homolog
GADD34	Growth arrest and DNA damage-inducible protein
GH	Growth hormone
GI	Gastrointestinal
GRP78	Glucose-regulated protein 78
GRP94	Glucose-regulated protein 94
GWAS	Genome-wide association studies
H&E	Hematoxylin and eosin
HSPA5	Heat shock protein family A member 5
IBD	Inflammatory bowel disease
IBDU	Unclassified inflammatory bowel disease
ICOS	Inducible T-cell co-stimulator
IEC	Intestinal epithelial cell
IFN-γ	Interferon γ
IGF	Insulin-like growth factor
IHC	Immunohistochemistry
IKK	Ikappa B kinase
IL	Interleukin
IL10RA/B	Interleukin 10 receptor α/β subunit
ILC	Innate lymphoid cell
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
IQR	Interquartile range
IRE1	Inositol-requiring enzyme 1
JNK	c-Jun N-terminal kinase
LEF-1	Enhancer-binding factor/T-cell factor 1
LRBA	Lipopolysaccharide-responsive and beige like anchor
MFGE8	Milk fat globule-EGF factor 8
MMP	Matrix metalloproteinase
MRE	Magnetic resonance enterography
MRI	Magnetic resonance imaging
miR	microRNA
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stem cell
MT	Masson's Trichrome
MUC1	Mucin 1
MUC2	Mucin 2
NCF1	Neutrophil cytosolic factor 1
NEMO	NF kappa B essential modulator
NF-κB	Nuclear factor-kappa B
NGS	Next-generation sequencing
NKT cell	Natural killer T cell
NLR	NOD-like receptor
NOD2	Nucleotide-binding oligomerization domain 2
OB-Cadherin	Osteoblast cadherin
PAMP	Pathogen-associated molecular pattern
PCDAI	Pediatric Crohn's Disease Activity Index
PDGF	Platelet-derived growth factor
PDI	Protein disulfide isomerase
PDIA1	Protein disulfide-isomerase A1
PDIA6	Protein disulfide-isomerase A6
PDO	Patient-derived organoids

PERK	Protein kinase related endoplasmic reticulum
PGE2	Prostaglandin E2
PIBD	Pediatric inflammatory bowel disease
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PRR	Pattern recognition receptor
PSC	Primary sclerosing cholangitis
RAG1	Recombination activating 1
RAG2	Recombination activating 2
RNA	Ribonucleic acid
ROS	Reactive oxygen species
S1P	Sphingosine-1-phosphate
SMCE	Small bowel capsule endoscopy
scRNAseq	Single-cell RNA sequencing
SH2D1A	SH2 domain containing 1A
SKIVL2	Super killer viralicidic 2-like
SMC	Smooth muscle cell
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 3
STXBP2	Syntaxin binding protein 2
TGF-β	Transforming growth factor beta
TGPS	Targeted genome panel sequencing
TIMPs	Tissue inhibitors of metalloproteinases
TNF-α	Tumor necrosis factor alpha
TL1A	Tumor necrosis factor-like cytokine 1A
TLR	Toll-like receptor
TRAF2	TNF receptor-associated factor 2
Treg	Regulatory T cell
TRIM22	Tripartite motif containing 22
TTC37A	Tetratricopeptide repeat domain 37A
TTC7A	Tetratricopeptide repeat domain 7A
UC	Ulcerative colitis
UPR	Unfolded protein response
US	Ultrasound
VEO-CD	Very early-onset Crohn's disease
VEO-IBD	Very early-onset inflammatory bowel disease
VEO-UC	Very early-onset ulcerative colitis
WES	Whole exome sequencing
(s)XBP1	(spliced) X-box binding protein 1
XIAP	X-linked inhibitor of apoptosis protein
ZO-1	Zona occludens 1

INTRODUCTION

1. INTRODUCTION

1.1. CROHN'S DISEASE: OVERVIEW AND PATHOGENESIS

1.1.1. Definition and classification

Inflammatory bowel diseases (IBD), which comprise Crohn's disease (CD) and ulcerative colitis (UC), are multifactorial chronic disorders that require lifelong care and may relapse in case of inadequate treatment or loss of response. These conditions are characterized by excessive activation of both the innate and adaptive immune systems, which results in persistent inflammation of the gastrointestinal tract. It is generally accepted that IBD arise from a complex interplay of genetic susceptibility, environmental factors, and alterations in the gut microbiota, leading to a dysregulated immune response[1–4].

While both CD and UC share similar clinical features – such as abdominal pain, diarrhea, and weight loss – they differ in inflammation patterns. CD can affect any part of the digestive tract, from the mouth to the anus and involves patchy, transmural inflammation[5–7], extending through the entire thickness of the intestinal wall, which can lead to complications such as strictures, fistulas, and abscesses[8]. UC, on the other hand, is restricted to the colon and rectum, with continuous inflammation limited to the mucosal layer[9].

Both diseases belong to the IBD spectrum and are distinguished from each other based on their anatomical distribution and the nature of inflammation. However, some researchers suggest that IBD may be better understood as a phenotypic continuum, with UC and ileal CD representing opposite ends of the spectrum, while intermediate forms occupy the space in between[10]. Indeed, a subset of patients exhibits overlapping features of both diseases, referred to as unclassified IBD (IBDU)[11] where clear differentiation between CD and UC remains challenging.

In CD, the Vienna classification was introduced in 2000 as the first attempt to categorize different clinical phenotypes of the disease[12]. This system was later refined by the Montreal classification in 2005, which provides a more detailed description of the extent and behavior of CD[13] (Table 1). The Montreal classification also introduced a system for classifying UC, offering a more comprehensive approach to understanding both conditions. This classification was further modified in 2011 to accommodate the pediatric population[14] (see section 1.2.3.).

Table 1. Summary of revised 'Montreal classification' of Crohn's disease (adapted from Silverberg et al.[13])

Age at diagnosis (A)	
A1: 16 years or younger	
A2: 17-40 y	
A3: over 40 y	
Location (L)	**Upper GI modifier (L4)**
L1: Terminal ileum	L1 + L4: Terminal ileum + Upper GI
L2: Colon	L2 + L4: Colon + Upper GI
L3: Ileocolonic	L3 + L4: Ileocolonic + Upper GI
L4: Upper GI	
Behavior (B)	**Perianal disease modifier (p)**
B1: Non stricturing non penetrating	B1p: Non stricturing, non penetrating + perianal
B2: Stricturing	B2p: Stricturing + perianal
B3: Penetrating	B3p: Penetrating + perianal

Although the current classification has demonstrated good performance in terms of inter-observer agreement[15], it is suboptimal and should be revised in the future, for integrating the clinical data with other information based on genetic and molecular data, ultimately enhancing the understanding of IBD pathogenesis and improving patient care[16].

1.1.2. Epidemiology

The incidence and prevalence of IBD (Figure 1), especially CD, have been rising globally, especially in industrialized nations, causing a growing public health burden[17–19]. These rates vary significantly across geographic regions due to multiple factors, including differences in dietary habits, environmental exposures, genetics, and healthcare systems. Until recently, industrialized countries tended to report higher rates of IBD in the last decades, whereas in some developing regions, the prevalence remained lower. However, the incidence is now rising in these areas due to changes in lifestyle and increasing urbanization[1,20,21]. The incidence of both CD and UC has risen significantly over time, with differences observed across age groups. In recent years, the most pronounced increase occurred in individuals under 17 years of age. This rise in pediatric IBD cases is particularly strong for those between 6 and 17 years old[19].

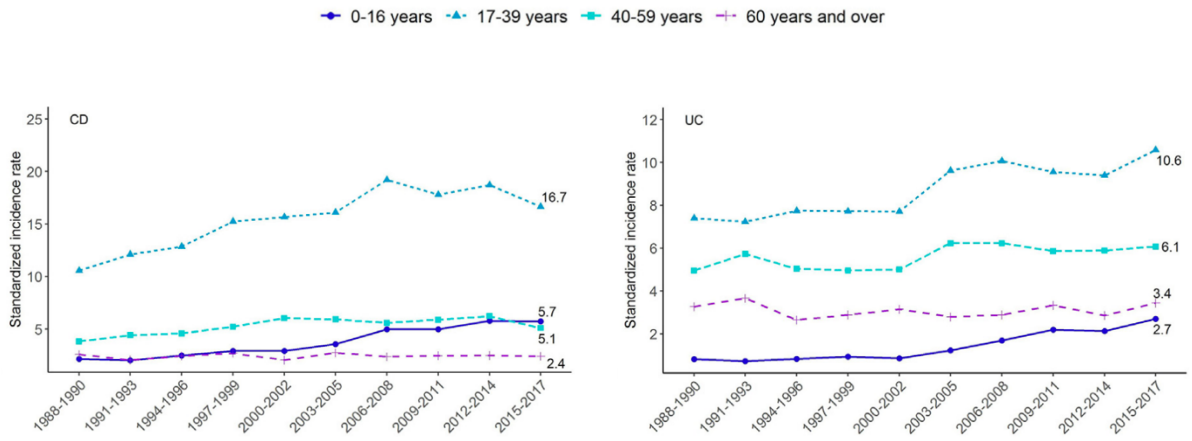

Figure 1. Changes over time in the standardized incidence rates for CD (n = 13,445) and UC n = 8803) in Northern France from 1988 to 2017 by age group (adapted from Sarter et al.[19])

Currently, a relative stabilization of incidence in western industrialized countries is observed, while incidence is rising in developing countries, in parallel to changes in lifestyle and increasing urbanization[22]. Kaplan provided an interesting view of the global epidemiological trends in the burden of IBD[23]. In developing countries, IBD is in the 'Emergence stage', characterized by the reporting of only sporadic cases. Newly industrialized nations are experiencing the 'Acceleration in Incidence stage', marked by a sharp incidence increase, although the overall prevalence remains relatively low. In contrast, Western countries have entered the 'Compounding Prevalence stage', where incidence has stabilized but prevalence continues to rise significantly. This upward trend in prevalence will eventually plateau as these countries move into the 'Prevalence Equilibrium stage', where the ageing IBD population and stable incidence rates balance each other out (Figure 2).

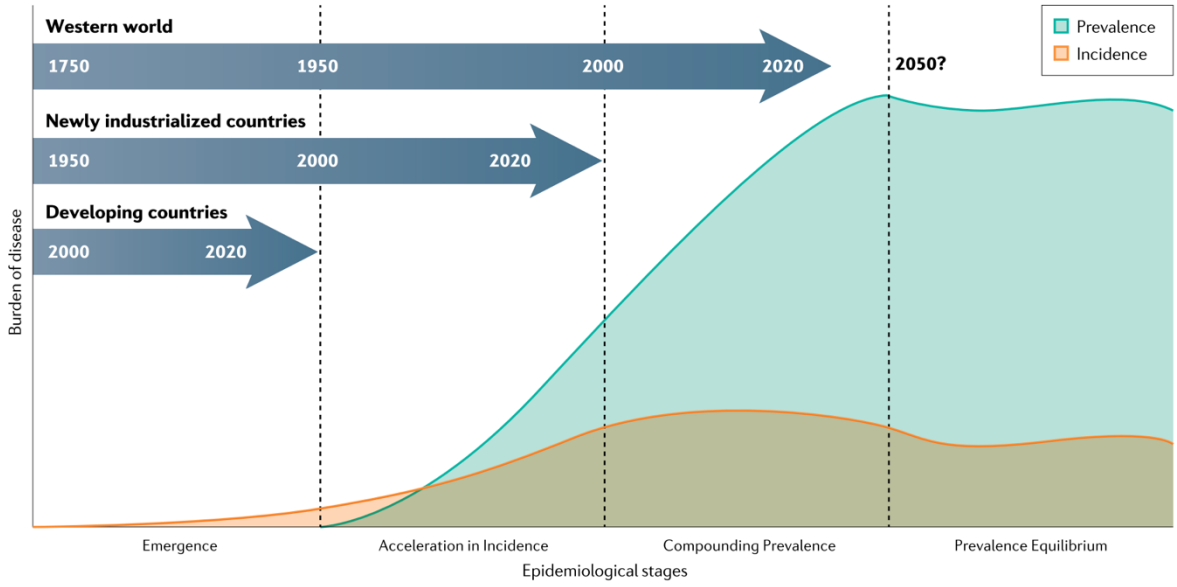

Figure 2. Four epidemiological stages of IBD evolution. The changing pattern in the incidence (orange) and prevalence (blue) of IBD across the four the stages of IBD evolution[23].

Projections for IBD prevalence in industrialized countries are dramatic, including in Belgium, where it is estimated that almost 120,000 people will be affected by IBD by 2030[23], with a similar trend expected in the pediatric population[24]. *A fortiori* in pediatric patients, this increase has significant implications for the healthcare system due to the chronic nature of IBD, the need for long-term management, and the potential for severe complications occurrence such as fibrosis and strictures[25–28]. Addressing these challenges, particularly given the increasing incidence of disease onset in pediatric populations[29,30], is essential for improving outcomes and advancing therapeutic strategies.

1.1.3. Pathophysiological mechanisms

The pathophysiology of CD remains partially unclear but likely results from a complex interplay between environmental and microbial factors, leading to an inappropriate immune response and loss of integrity of the epithelial barrier in genetically predisposed individuals, as illustrated by Ramos and Papadakis[31] (Figure 3).

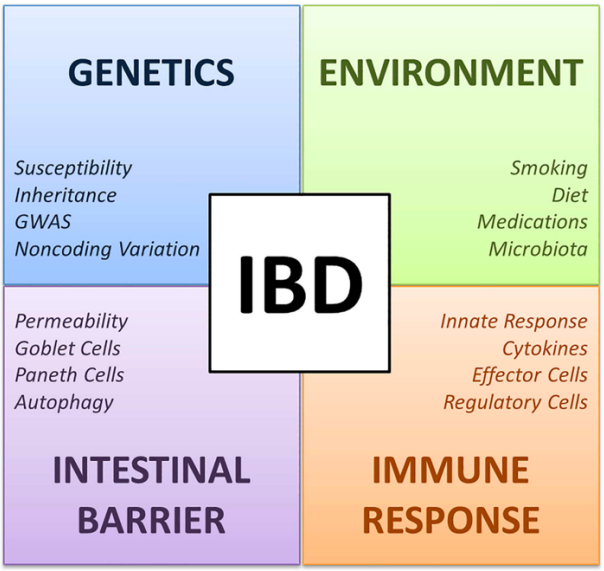

Figure 3. Mechanisms involved in the pathogenesis of IBD[31]

GWAS, Genome-wide association studies; IBD, inflammatory bowel diseases.

1.1.3.1. Genetics

Genetic factors play a central role in the development of IBD, for both CD and UC. Twin studies have provided strong evidence of a genetic predisposition to IBD, with monozygotic twins showing significantly higher concordance rates for both CD and UC than dizygotic twins, suggesting a strong genetic influence[32]. These studies also helped to apprehend and control for the impact of environmental and epigenetic factors, enhancing our understanding of the genetic basis of IBD.

However, most of the current understanding of IBD genetics has come from genome-wide association studies (GWAS), which have identified over 240 loci associated with disease susceptibility[33–35], highlighting genes involved in immune response, epithelial integrity and cellular stress, suggesting diverse molecular pathways involved in IBD pathogenesis[36,37]. These studies indicate that IBD is a polygenic disease, driven by multiple common genetic polymorphisms. Key genes such as *NOD2*/*CARD15*, *ATG16L1*, and *IL-10* have been identified, with mutations linked to immune dysregulation and impaired autophagy, which predispose individuals to chronic inflammation and fibrosis in CD[38]. In addition to polygenic forms of IBD, monogenic forms have been described, primarily affecting infants and young children[39] (see section 1.2.2).

However, the findings from GWAS explain only a modest fraction (20-25%) of the heritability of IBD, indicating that other genetic factors remain unidentified. The search for rare genetic variants with larger effect sizes, which may contribute to the "missing heritability", is a promising direction for future research.

In addition to genetic factors, epigenetic mechanisms are believed to play a crucial role in IBD pathogenesis. Epigenetics refers to heritable changes in gene expression that do not involve changes in the DNA sequence. Environmental factors, such as diet and microbiome composition, can influence IBD risk through epigenetic modifications like DNA methylation, histone modifications, and the regulation of non-coding RNAs[40]. These changes can affect immune responses, cell differentiation, and gene expression, contributing to the chronic inflammation and fibrosis.

While the genetic insights gained so far have significantly advanced our understanding of CD and UC, they have only modestly improved our ability to predict the course of the disease, especially its complications. The most widely replicated genetic association is between fibrostenosing ileal CD and *NOD2*/*CARD15* variants, but other associations have been less consistently demonstrated and require validation in larger cohorts[38,41].

The rapid advancements in genomic analysis, particularly with next-generation sequencing (NGS), have significantly enhanced our understanding of the human genome and its connection to disease. However, to truly harness the potential of the vast genomic data being generated, greater collaborations among scientists are necessary to translate these insights into clinically actionable results. Moving forward, research into rare variants, epigenetic modifications, and gene-environment interactions will be critical to uncover the full genetic landscape of IBD, ultimately enabling the development of more targeted and personalized therapeutic strategies[42].

1.1.3.2. Environment and microbiota

Beyond genetics, growing evidence points to the environment and the gut microbiota as key players in IBD pathogenesis. Emerging epidemiological trends, particularly in developing countries undergoing industrialization, suggest that environmental factors may significantly influence the onset of intestinal inflammation in genetically predisposed individuals[31]. Smoking, exposure to diets rich in saturated fatty acids and ultra-processed food have been

reported to increase the risk of CD[43–46]. In a large review of meta-analyses, Piovani *et al.* further underscored the importance of environmental factors in IBD pathogenesis. Based on evidence from 71 environmental variables, nine factors were identified as being associated with increased IBD risk, while seven factors were found to reduce it. Key positive associations with IBD include smoking (especially in CD), urban living (for both CD and UC), appendicectomy and tonsillectomy (both linked to CD) and the use of antibiotics and oral contraceptives (associated with IBD)[1,47]. Factors such as physical activity, breastfeeding, high levels of vitamin D and folate were shown to reduce the risk of IBD, while tea consumption and H. pylori infection also offering some protective benefits, particularly for UC[1]. Stress is increasingly recognized as another environmental factor that can exacerbate IBD by activating the gut-brain axis, influencing gut motility, immune functions, and the microbiota composition. Such changes can increase gut permeability and promote inflammation, contributing to disease progression. Moreover, stress has been shown to alter the gut microbiota, potentially shifting the balance towards more pathogenic or pro-inflammatory microbial populations, which may further compromise the intestinal barrier and immune responses[35]. While meta-analyses provide valuable insights into the associations between environmental factors and IBD, the methodological quality varied considerably, and many associations were derived from retrospective studies. As a result, it remains unclear whether these associations reflect true causative effects of environmental exposures or are influenced by recall bias[1].

The role of the intestinal microbiota in the pathogenesis of IBD has garnered increasing attention, as it plays a key role in both maintaining gut health and mediating disease[48]. The composition of the gut microbiota is influenced by a combination of environmental factors, such as diet and antibiotic exposure, as well as host genetics[49,50]. Studies comparing the gut microbiota of IBD patients with and without antibiotic exposure have shown that antibiotics may worsen dysbiosis, which in turn amplifies inflammation[51]. Approximately one-third of the fecal bacterial taxa are heritable, highlighting the interaction between genetic predisposition and microbial composition[50]. In IBD, the gut microbiota is characterized by reduced diversity, which may predispose individuals to colonization by pathogenic microbes or pathobionts and loss of beneficial microbes[48,52]. A decrease of anti-inflammatory bacteria, such as *Faecalibacterium prausnitzii*, coupled with an overgrowth of pro-inflammatory bacteria, has been reported[53–55]. Along with alterations in bacterial diversity, changes in fungal and viral diversity have also been observed in IBD[56]. Other studies showed that the uninflamed mucosa of IBD patients already exhibits a distinct microbiota compared to healthy individuals,

suggesting that dysbiosis could either drive inflammation or result from the altered immune response in IBD[56]. Figure 4 illustrates the interplay between the microbiota and immune response in the context of IBD, highlighting the balance between intestinal homeostasis, microbial diversity, and immune regulation, and how disruptions of this equilibrium can contribute to disease progression[57].

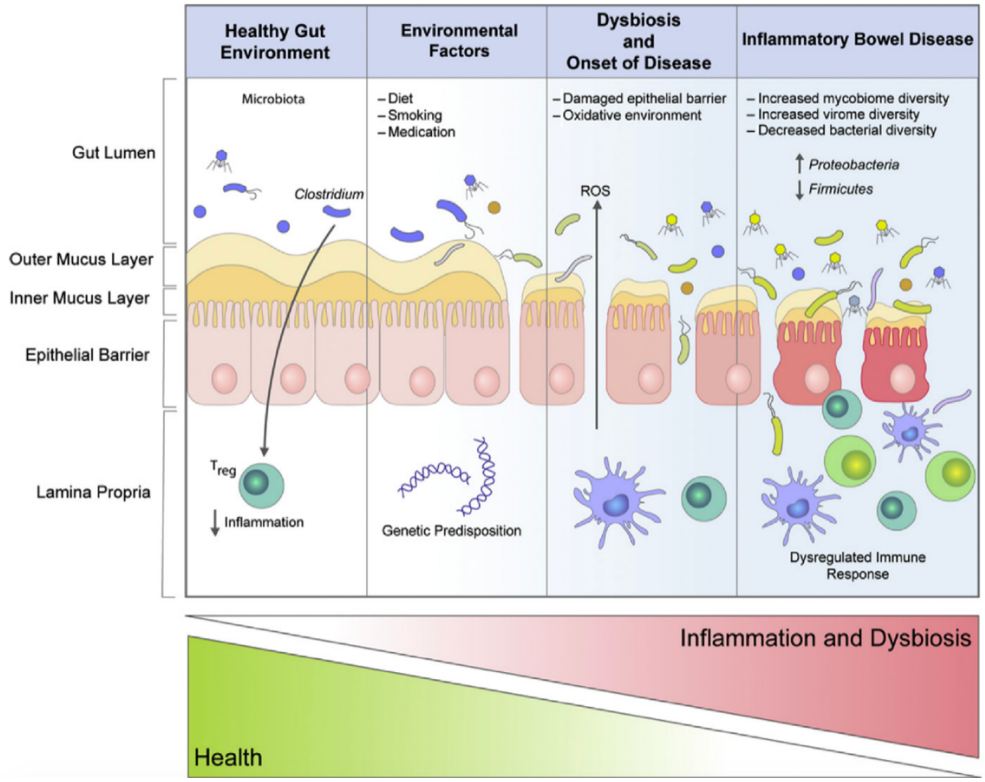

Figure 4. The microbiota and immune response in IBD[57]

ROS, reactive oxygen species; Treg, Regulatory T cell.

It has been hypothesized that dysbiosis in CD and UC may differ in terms of onset and underlying mechanisms: UC could be linked to an environmental imbalance that leads to a sudden dysbiosis at any point in life, whereas CD might involve an early-life interaction between the microbiome and immune system, with events potentially programming early dysbiosis[58]. Moreover, the microbiome's composition can change over a person's lifetime, with influences beginning at conception and continuing throughout adulthood[49,59], with a window of opportunity for microbiota modulation from birth to adolescence[60] (Figure 5).

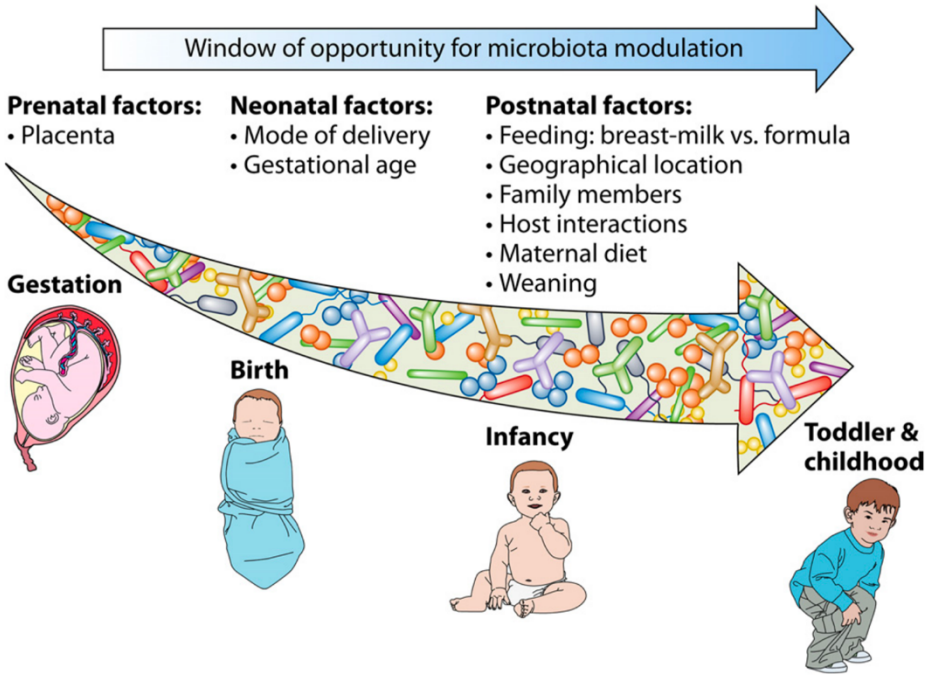

Figure 5. Window of opportunity for microbiota modulation from gestation to childhood. The schematic representation shows a list of prenatal, neonatal, and postnatal factors that contribute to the bacterial gut composition in infants[60].

Interestingly, fecal microbiota transplantation (FMT) has emerged as a potential therapeutic strategy to modify the gut microbiota in IBD patients. However, clinical outcomes remain inconsistent. While some trials have demonstrated selective and transient benefits, particularly in UC, others have reported no significant effect[61]. The success of FMT appears to be strongly dependent on both the donor and recipient, as well as the administration protocol used. Despite these challenges, FMT remains a promising but unpredictable approach, highlighting the complexity of microbiome-based therapies for IBD[56,59].

1.1.3.3. Innate and adaptive immune response

The innate and adaptive immune responses are essential for maintaining intestinal homeostasis and protecting against infections. The intestinal immune system is in constant interaction with the gut microbiota, which plays a critical role in shaping immune responses[62]. In healthy individuals, a balanced relationship between the gut microbiota and the mucosal immune system helps establish immune tolerance, preventing unnecessary inflammatory reactions

against the commensal microbiota. However, in IBD patients, this balance is disrupted, leading to an inappropriate immune activation against the microbiota and environmental antigens, resulting in chronic inflammation.

Innate immunity plays a central role in the early response to microbial and dietary antigens in the gut[63]. The mucus layer and epithelial barrier form the initial defense against bacterial invasion. Paneth cells, along with other epithelial cells, secrete antimicrobial peptides that help restrict bacterial growth and penetration. Epithelial cells, stromal cells, and innate immune cells, such as macrophages and dendritic cells (DCs), can detect invading bacteria through both extracellular and intracellular pattern recognition receptors (PRRs). These receptors, including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), are crucial components that identify pathogen-associated molecular patterns (PAMPs) found on pathogens as well as commensal bacteria, triggering a cascade of immune responses[64,65] (Figure 6).

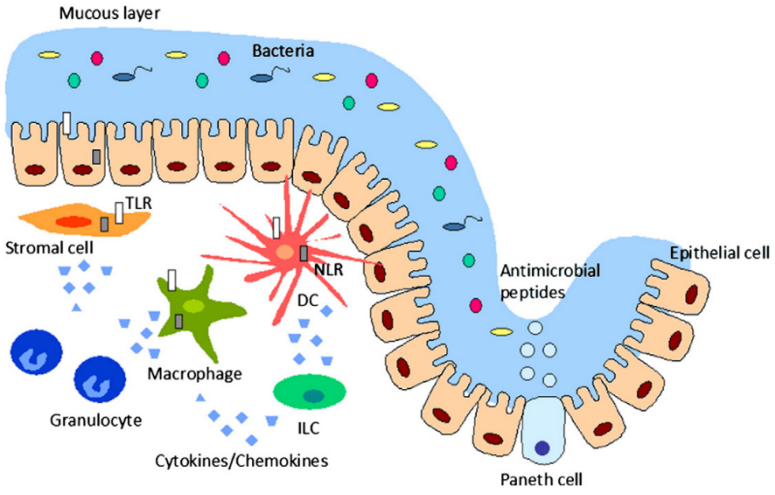

Figure 6. Innate responses in the gut[64]

DC, dendritic cell; ILC, innate lymphoid cell; NLR, Nod-like receptor; TLR, Toll-like receptor.

In IBD, the recognition of microbial antigens through PRRs is often exaggerated, resulting in the excessive production of pro-inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α), Interleukin 1β (IL-1β) and Interleukin 6 (IL-6), and the recruitment of inflammatory

cells, such as neutrophils and macrophages[65], thereby perpetuating the vicious cycle of inflammation and causing damage to the intestinal epithelium[66].

The adaptive immune response, involving T cells, is also critical in the development and progression of IBD. In healthy individuals, the immune system maintains a delicate balance between regulatory T cells (Tregs) and other immune cell subsets, such as Th17 cells and innate lymphoid cells (ILC1). Tregs help to suppress excessive immune responses, promoting tolerance and preventing unnecessary inflammation, whereas Th17 cells and ILC1 contribute to immune defense against pathogens. This reciprocal balance between immune cells and the cytokines they secrete ensures that the intestine remains in a state of homeostasis (Figure 7)[67].

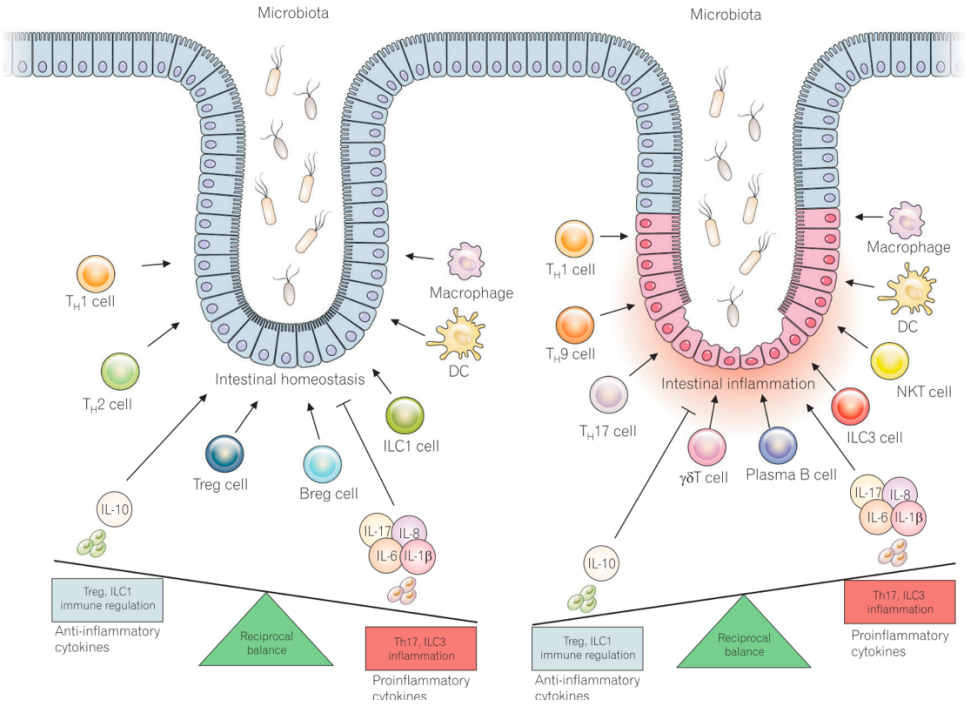

Figure 7. Adaptive immune response – Reciprocal balance for intestinal immune homeostasis and inflammation[67]

DC, dendritic cell; IL, interleukin; ILC, innate lymphoid cell; NKT cell, natural killer T cell; Treg, regulatory T cell.

However, in IBD patients, this balance is disrupted. In CD, the immune response is typically Th1/Th17-dominant, characterized by the secretion of pro-inflammatory cytokines such as

Interferon γ (IFN-γ) (from Th1 cells) and IL-17 (from Th17 cells). In UC, Th2 responses (characterized by IL-4, IL-5, and IL-13) are prominent, although recent evidence suggests that a mixed Th1/Th2 response contributes to the chronic nature of the disease. The involvement of Th17 cells, particularly in the mucosal immune response, is now increasingly recognized in both CD and UC, although their exact roles may vary depending on the site of inflammation and the microbiota composition[68,69].

1.1.3.4. Intestinal barrier

The intestinal epithelial barrier, which separates the gut lumen from the underlying immune and connective tissues, as well as the vascular and nervous networks, is crucial for preventing the translocation of microbes and antigens. This barrier is composed of a monolayer of tightly joined epithelial cells – primarily enterocytes, goblet cells and endocrine cells – which regulate permeability to pathogens and selectively absorb nutrients[70].

Enterocytes, which are the predominant epithelial cells, form tight junctions that prevent the leakage of luminal contents into the underlying tissue, while goblet cells secrete mucus, which forms a thick protective layer that traps pathogens and facilitates their clearance[71]. Additionally, goblet cells release antimicrobial molecules, including trefoil factors and mucins, which contribute to the defense against harmful microbes. Paneth cells, located within the crypts of the small intestine, produce additional antimicrobial peptides, such as defensins which protect against both Gram-positive and Gram-negative bacteria[70,72].

The physical barrier is further supported by mucus layers in the large intestine, where goblet cells secrete MUC2, forming a two-layered structure: an inner, bacteria-free layer and an outer layer inhabited by microbiota. This structure, along with antimicrobial molecules like IgA and defensins, protects the mucosal surface from microbial invasion. In the small intestine, antimicrobial peptides such as the Reg3 family also contribute to maintaining the barrier by preventing bacterial colonization[72].

The intestinal epithelium is also supported by a complex network of resident immune cells, such as dendritic cells and macrophages, which help monitor and modulate immune responses. The immune cells' contribution to barrier dysfunction in IBD is also evident in the dysregulated activity of macrophages and dendritic cells. In healthy individuals, these cells help maintain

immune tolerance to commensal microbiota and facilitate repair of the epithelial layer. In IBD, they become chronically activated and release inflammatory cytokines, further impairing the epithelial barrier[73]. Notably, the activation of the NF-κB (Nuclear factor-kappa B) signaling pathway in both immune and epithelial cells plays a crucial role in maintaining inflammation and barrier dysfunction[71,74]. Moreover, key genetic mutations, including those affecting tight junction proteins and immune-related molecules (e.g., *NOD2*/*CARD15*, *ATG16L1*), contribute to the disruption of the epithelial barrier and increased intestinal permeability[70,75,76].

Restoring the intestinal barrier and modulating the inflammatory response have emerged as critical therapeutic strategies in IBD. Targeting specific signaling pathways, may help improve epithelial barrier function by reducing inflammation and promoting epithelial repair. Furthermore, understanding the complex interactions between epithelial cells, immune cells, and the microbiome is essential for developing more targeted treatments that can restore intestinal homeostasis and reduce the inflammatory burden of IBD[62].

1.1.3.5. Endoplasmic reticulum stress in IBD

Endoplasmic reticulum (ER) stress is a cellular response triggered by the accumulation of misfolded or unfolded proteins in the ER lumen. This accumulation further disrupts normal protein folding, leading to adaptive mechanisms known as the unfolded protein response (UPR), which aim to restore ER homeostasis[77]. The UPR is initiated in eukaryotic cells by three major sensor proteins located in the ER membrane: inositol-requiring enzyme 1 (IRE1), PKR-like ER kinase (PERK), and activating transcription factor 6 (ATF6). These sensors are activated when misfolded proteins accumulate, leading to their dissociation from BiP (Binding immunoglobulin protein, also known as 78-kDa Glucose regulated protein – GRP78 – or Heat shock protein family A member 5 – HSPA5), a chaperone protein that normally binds and inactivates these sensors[78–81]. BiP plays a crucial role in maintaining the homeostasis of the ER by ensuring that the sensors remain inactive under normal conditions[82]. When unfolded proteins are present, BiP is sequestered away from the sensors to assist in protein folding, allowing IRE1, PERK, and ATF6 to become activated. Once activated, IRE1, PERK, and ATF6 initiate a series of signaling cascades that promote the expression of genes involved in protein folding, such as chaperones, and genes that aid in the degradation of misfolded proteins via the ER-associated degradation (ERAD) pathway[77,80,83,84]. Each of these three branches of the UPR has

a distinct molecular mechanism and targets specific pathways to restore ER homeostasis (Figure 8)[85]:

- IRE1 is the most evolutionarily conserved of the UPR sensors. Upon activation, IRE1 undergoes autophosphorylation and oligomerization, leading to the activation of its endoribonuclease activity. This cleaves the messenger RNA (mRNA) of X-box binding protein 1 (*XBP1*), resulting in a spliced form (*sXBP1*), which acts as a potent transcription factor. *sXBP1* activates the expression of genes involved in protein folding, such as *HSPA5*, and proteins related to the ERAD pathway[77]. Additionally, IRE1 can also promote the degradation of certain mRNAs through a process which serves to reduce the overall protein load entering the ER, alleviating ER stress[78].

- PERK, upon sensing ER stress, phosphorylates the eukaryotic translation initiation factor 2α (eIF2α), which reduces global protein translation, by decreasing the nascent protein load entering the ER and alleviating ER stress[79,86]. However, this also leads to the selective translation of certain mRNAs, including that of Activating Transcription Factor 4 (*ATF4*), a key transcription factor that activates the expression of genes involved in amino acid metabolism, redox regulation, and apoptosis. ATF4 also induces the expression of CHOP (C/EBP homologous protein), which is associated with pro-apoptotic signals if the stress persists, helping to trigger cell death in severely stressed cells[77,86].

- ATF6 is a type II transmembrane protein that, upon ER stress, is transported from the ER to the Golgi apparatus. In the Golgi, ATF6 is cleaved by site-1 and site-2 proteases, producing a cytosolic fragment (ATF6f) that functions as a transcription factor. ATF6f activates genes involved in protein folding, including chaperones like BiP, as well as genes involved in the ERAD pathway. This pathway also enhances the overall protein-folding capacity of the ER, helping to clear misfolded proteins and restore homeostasis[80].

Beyond initiating apoptosis, only activated when adaptive responses fail, the primary roles encoded by UPR-associated genes are therefore threefold:

1. to reduce the buildup of unfolded proteins;
2. to enhance the ERAD pathway and clear misfolded proteins;
3. to boost the protein-folding capacity of the ER by upregulating chaperones and folding enzymes, such as protein disulfide isomerases (PDIs), including the ER-resident chaperone Anterior Gradient Protein 2 (AGR2)[87].

Collectively, these mechanisms coordinate a complex response to ER stress by balancing adaptive processes (like protein folding and degradation) with cell survival. While the UPR aims to alleviate the ER's burden by increasing its capacity to fold proteins and eliminate misfolded ones, prolonged stress that cannot be resolved ultimately triggers apoptosis to prevent the survival of damaged cells[77–79,88–90].

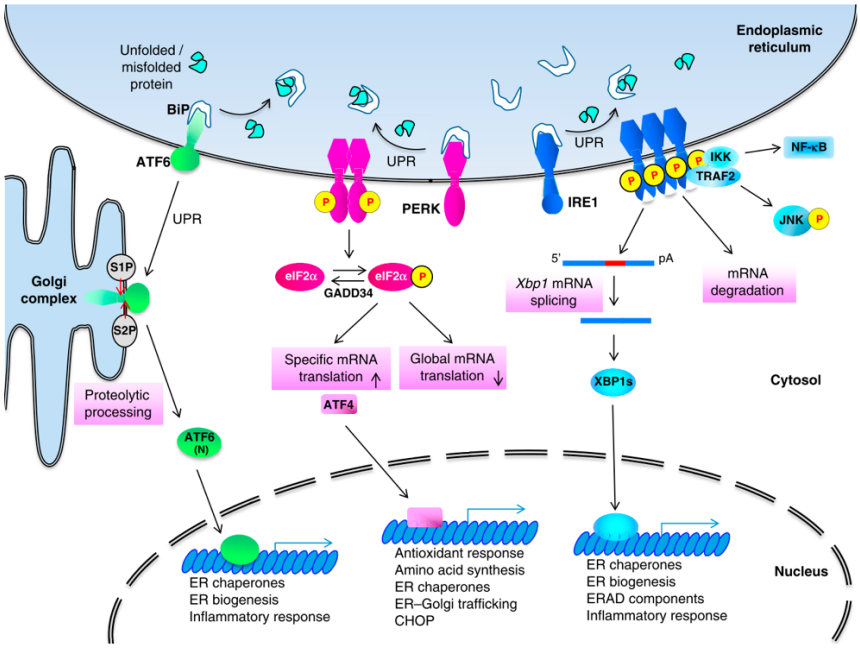

Figure 8. The unfolded protein response in mammals[85]

ATF, Activating transcription factor; BiP, binding-immunoglobulin protein; CHOP, C/EBP homologous protein; eIF2α, eukaryotic translation initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; GADD34, growth arrest and DNA damage-inducible protein; IKK, IkappaB kinase; IRE1, Inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; mRNA, messenger ribonucleic acid; NF-κB, Nuclear factor-kappa B; PERK, PKR-like ER kinase; S1P, sphingosine-1-phosphate; S2P, sphingosine-2-phosphate; *TRAF2*, TNF receptor-associated factor 2; UPR, unfolded protein response; XBP1, X-box binding protein 1.

Chronic ER stress and dysfunctional UPR signaling have been linked to various diseases, including neurodegenerative disorders and inflammatory diseases[77]. In IBD, particularly in the

intestinal epithelial cells (IECs), persistent ER stress impair protective functions of the UPR[91], contributing to both chronic inflammation and epithelial cell apoptosis[92,93]. IECs are particularly vulnerable to chronic ER stress due to their constant exposure to inflammatory cytokines, microbial products, and oxidative stress. This persistent stress can overwhelm the protective functions of the UPR, leading to epithelial barrier dysfunction, increased permeability, and exacerbation of inflammation[94,95].

Genetic studies have highlighted the role of polymorphisms in *XBP1* in predisposing individuals to IBD. Mutations in *XBP1* impair the ability of IECs, including Paneth cells, to effectively respond to ER stress, compromising their function in producing antimicrobial peptides such as defensins, which are critical for maintaining intestinal homeostasis[91,94,96]. Similarly, polymorphisms in *NOD2*/*CARD15*, a gene essential for the innate immune system by recognizing bacterial components and associated with CD[97,98], have been associated with impaired Paneth cell function, further exacerbating ER stress and reducing defensin secretion. Mutations in *ATG16L1*, a key autophagy-related gene and a known risk factor for CD, also lead to Paneth cell dysfunction by impairing their ability to handle ER stress and maintain cellular homeostasis[99]. This disruption in Paneth cells' antimicrobial defense contributes to an altered microbiota and increased inflammation[100]. Goblet cells are also affected by ER stress in IBD, leading to lower mucus production, resulting in a thinner mucus layer, which compromises the physical barrier protecting the epithelium from luminal bacteria[92]. The proper folding of MUC2 glycoprotein, the main component of the mucus, critically depends on AGR2 which ensures the maturation of mucins in secretory cells. Dysregulation of AGR2 or its interactants not only exacerbates ER stress and impairs goblet cell function but also diminishes mucin production[101]. For instance, *Agr2*−/− mice produce significantly less intestinal mucin and develop spontaneous ileal and colonic inflammation[102]. Consequently, the combined dysfunction of Paneth and goblet cells due to ER stress amplifies epithelial barrier defects and chronic inflammation, reinforcing the vicious cycle.

In IBD, the activation of key UPR sensors such as IRE1, PERK, and ATF6 is dysregulated, contributing to both chronic inflammation and epithelial cell apoptosis[92,93]. Furthermore, genetic predisposition to a defective UPR has been identified in IBD patients, underscoring the role of chronic ER stress in epithelial cells[91,96]. This chronic ER stress and defective UPR in epithelial cells lead to impaired protein folding, the secretion of pro-inflammatory cytokines, and compromised mucosal barrier integrity, which collectively contribute to the pathogenesis

of IBD, and probably in different ways in the ileum and colon[103,104]. Therefore, while the UPR is crucial for protecting cells from stress, its maladaptive activation in IBD can drive tissue damage and chronic inflammation, reinforcing the disease cycle[105]. Other stress pathways, such as autophagy and oxidative stress, have also been implicated in IBD pathogenesis, though they are not the focus of this work[106,107].

1.1.4. Diagnostic considerations

The diagnosis of IBD is classically based on a combination of clinical, biological, imaging, endoscopic and histological evidence[108].

CD should be suspected in patients, especially younger ones, who experience chronic abdominal pain and diarrhea, along with symptoms such as blood in the stool, unexplained weight loss, fever, or the presence of perianal lesions. Physical examination, a tenderness or a palpable mass in the affected regions could be found, and signs of bowel obstruction might also be present, as well as perianal lesions[109]. CD patients can also experience two types of extraintestinal manifestations (EIMs). The first group is associated with the activity of intestinal lesions, including conditions such as peripheral arthritis, erythema nodosum, episcleritis, and oral aphthae. The second group occurs independently of intestinal lesion activity and includes complications like pyoderma gangrenosum, uveitis, sacroiliac arthritis, ankylosing spondylitis, and primary sclerosing cholangitis (PSC)[109,110].

When a CD is suspected, a complete biochemical assessment is essential, including full blood count (to search thrombocytosis, anemia and leukocytosis), serum inflammatory marker (C-reactive protein – CRP), electrolytes, liver enzymes and stool samples to exclude a microbial origin[108]. Fecal calprotectin is a calcium-binding protein primarily derived from neutrophils found at abnormally high levels in the stools of IBD patients[111,112]. Compelling studies and increasing clinical experience suggest its growing importance in the diagnosis and management of IBD[113].Although useful, these biological markers are not very specific individually and must be integrated into an overall clinical approach[114].

Multimodal imaging, including ultrasound (US), computed tomography enterography (CTE), and magnetic resonance imaging (MRI) is crucial for assessing the presence, severity and disease extent especially in cases where clinical and endoscopic evaluations are limited, for

both adults and children[115]. The guidelines recommend a systematic small bowel assessment at diagnosis, using US, CTE and MRI in small bowel CD[108,109]. Ultrasonography has demonstrated excellent diagnostic accuracy for detecting both new-onset and established CD and is a cost-effective option[116]. A comparative study of intestinal US and MRI in CD patients found that both methods had similar diagnostic accuracy, although US was slightly less effective in defining the extent of the disease. Moreover, access to this technology is often confined to specialist centers with dedicated expertise[117]. In parallel, Fiorino *et al.* have demonstrated that MR and CT are equally accurate in assessing disease activity and bowel damage in CD, but MR may be superior to CT in detecting intestinal strictures and ileal wall enhancement[118]. The use of MRI, while extremely useful, is limited by its accessibility, which can delay timely diagnosis and treatment.

For suspected CD, ileocolonoscopy with a minimum of two biopsies from inflamed areas is essential for diagnosis[119]. Additional biopsies from uninflamed regions and all colonic segments, including the rectum, can further assist in the diagnostic process. Characteristic endoscopic features of CD include discontinuous lesions, aphthous ulcers, strictures, fistulae, and perianal involvement[108]. Upper gastrointestinal endoscopy is advised if a diagnosis remains uncertain after lower gastrointestinal (GI) endoscopy or if patients present with upper GI symptoms[109]. When both ileocolonoscopy and upper GI endoscopy fail to confirm the diagnosis, capsule endoscopy can be considered to detect small intestinal lesions that might have been missed by conventional imaging. Small bowel capsule endoscopy (SBCE) has demonstrated superiority over other imaging modalities in detecting small bowel involvement in CD[108]. It may be particularly useful in patients with suspected small intestinal lesions not visible through other examinations[109]. However, in cases of known or suspected strictures, a patency capsule should be used beforehand to ensure safe passage of the device.

Histological examination of endoscopic biopsies or resection specimens is crucial for diagnosing suspected IBD and distinguishing UC, CD, and other forms of colitis[109,120]. The optimal number of sections to examine in routine practice is not clearly defined, with studies suggesting a minimum of two biopsies from the inflamed regions and additional biopsies from uninflamed regions or two from at least five sites along the colon, including the rectum, and the terminal ileum[108,121]. In colonic biopsies, focal discontinuous chronic inflammation (increase cellularity – lymphocytes and plasma cells – in the *lamina propria*), focal crypt irregularity (distortion, crypts branching and shortening), and granulomas (collection of epithelioid

histiocytes, in the *lamina propria*, with multinucleate giant cells) are widely recognized microscopic features for diagnosing CD in colonic endoscopic biopsies[122]. These same features, along with irregular villous architecture, are used to assess biopsy samples from the ileum[121]. Acute inflammation is histologically defined by the presence of acute inflammatory cells, specifically neutrophils, in the *lamina propria* and/or within the surface epithelium, crypt epithelium, or crypt lumens. Acute inflammation can be graded using a validated scoring system. Cryptitis is defined as the presence of at least one neutrophil in the crypt epithelium and a crypt abscess, as the presence of neutrophils in the crypt lumen[122]. Figure 9 provides illustrations of typical features observed in CD.

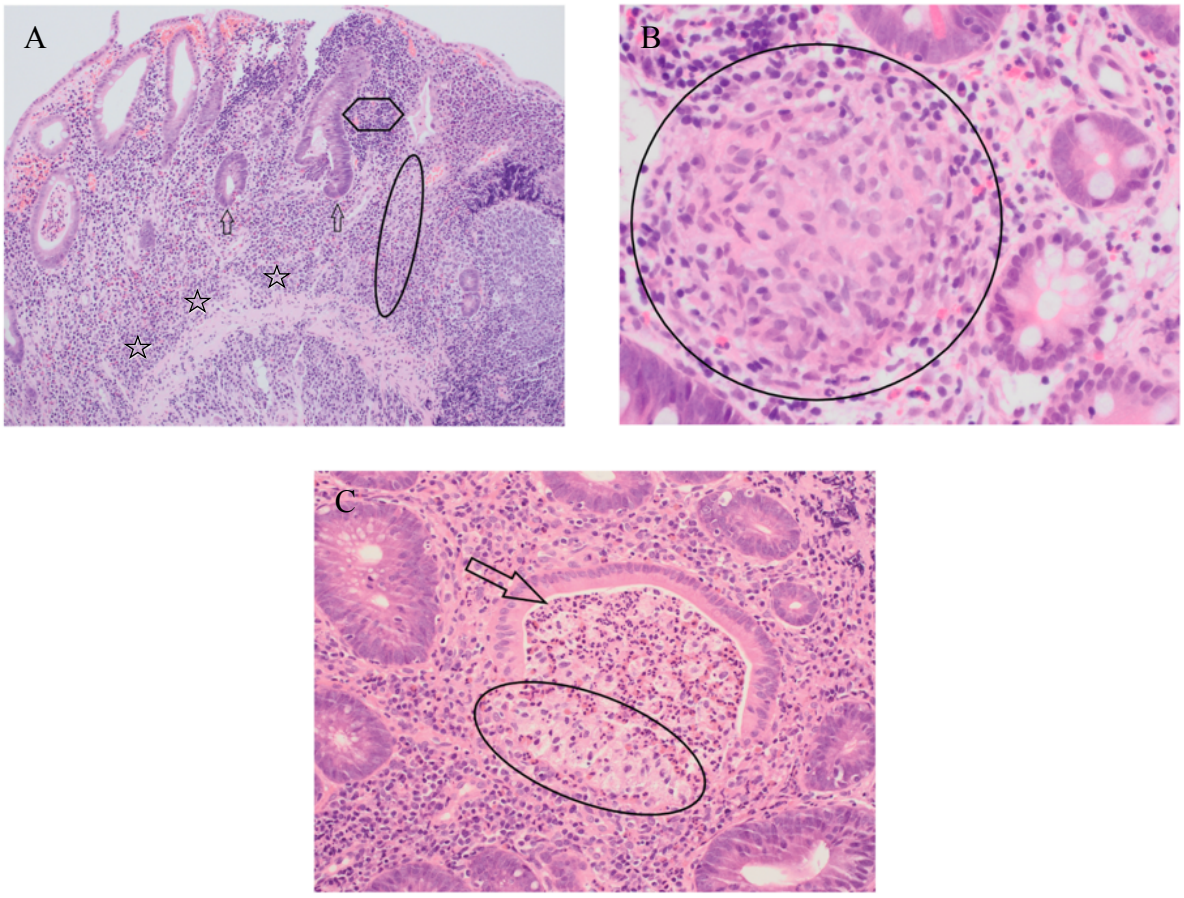

Figure 9. Illustrative examples of the histological lesions observed in CD (adapted from Feakins et al., 2024[122]). (A) Extensive crypt distortion (with crypt branching and irregularity); crypt atrophy, with shortening (failure to reach the muscularis mucosae; (ellipse) and wider spacing (hexagon); severe epithelial mucin depletion (arrows); diffuse basal plasmocytosis (stars); (B) Granuloma (at least five histiocytes without necrosis); (C). Crypt abscess (arrow) and cryptolytic granuloma (circle)

1.2. PEDIATRIC CROHN'S DISEASE: A SPECIFIC ENTITY

1.2.1. Epidemiology of pediatric inflammatory bowel disease

The global incidence of pediatric inflammatory bowel disease (PIBD) is steadily rising, with significant variations across different regions[30,123]. According to various studies, 10 to 25% of incident cases of IBD will occur during childhood or adolescence[27]. Systematic reviews show a temporal evolution in incidence trends, from an increase reported in 77% of pediatric studies between 1950 and 2009 to 84% between 2010 and 2020[30,124]. The incidence of PIBD – and particularly for CD – has surged over recent decades[19], especially in countries with historically low rates[30] (Figure 10 and Figure 11). However, some evidence suggests that incidence may be beginning to level off in certain regions[30]. Two Canadian studies indicate that the largest increase in incidence occurs in young children (0–9 years), with for example an annual rise of up to 6.5% for PIBD and 3.3% for CD[27,29]. This contradicts other studies, which report a stagnation or decrease in very early-onset IBD (VEO-IBD), while showing an increase among children older than 10 years[125–127]. The relative incidences of pediatric-onset CD and UC are constant over time, with a higher proportion of CD compared to UC, as in adults[30,128].

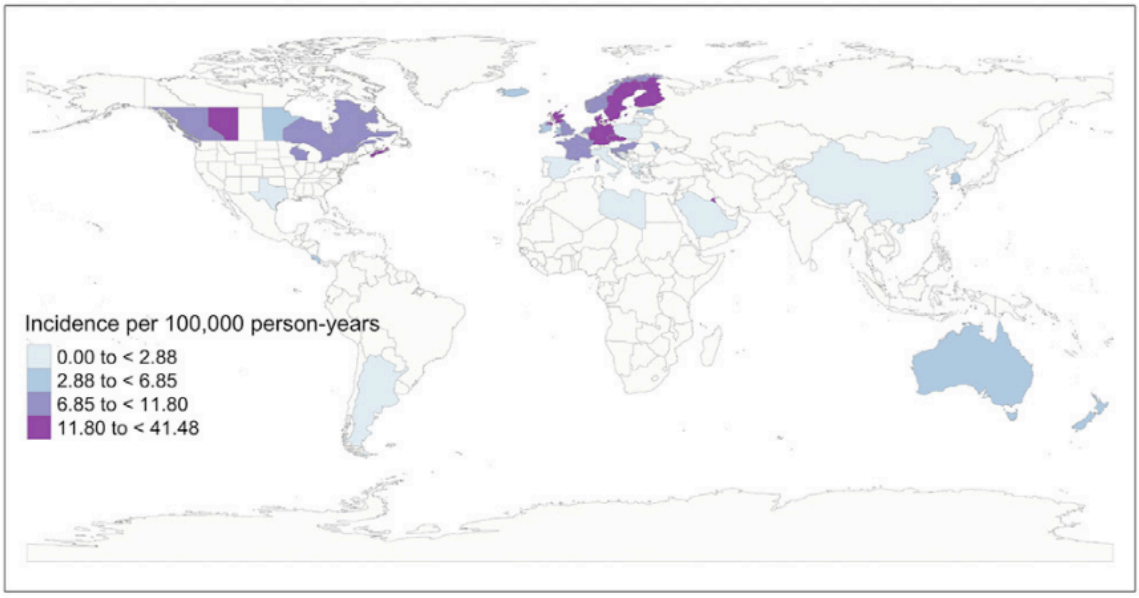

Figure 10. Global incidence of pediatric-onset IBD – Systematic review 2010–2020[30]

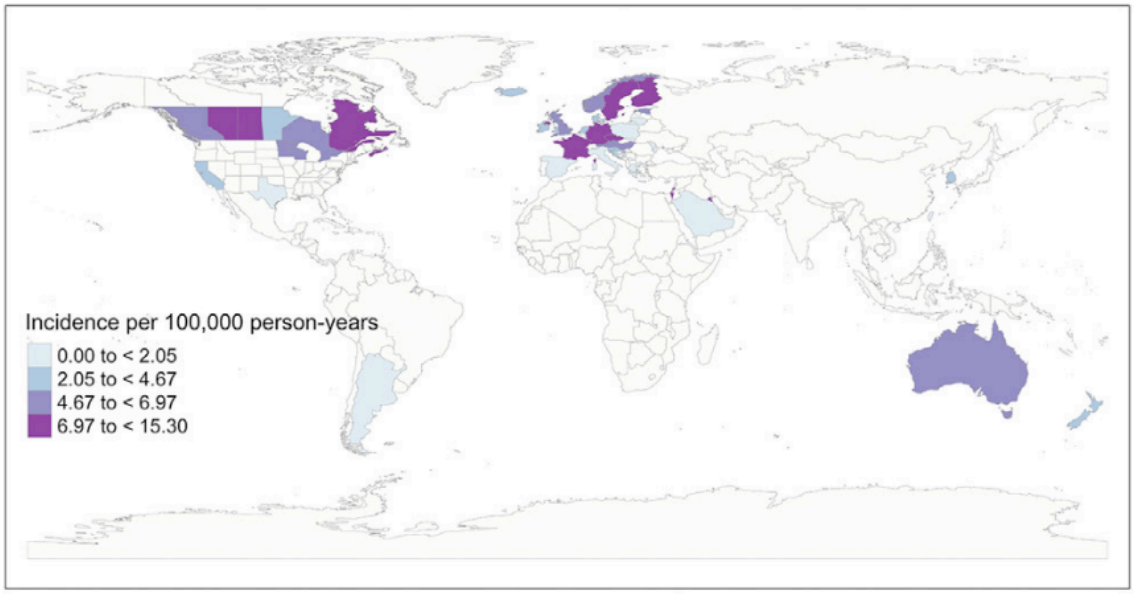

Figure 11. Global incidence of pediatric-onset CD – Systematic review 2010–2020[30]

The current prevalence of PIBD in Western Europe is 58.9 to 66.3 per 100,000. Recent studies consistently report a rising prevalence of both pediatric and adult IBD[30,129]. An analysis of two large American claim databases revealed an overall increase of PIBD prevalence of 133% from 2007 to 2016, with CD being twice as prevalent as UC[18]. The prevalence of VEO-IBD varies by geographical region, with an increase reported in Canada[130] and Israel[131]. In areas where UC was once predominant, the CD ratio among adults has been shifting toward parity. Environmental factors, such as Westernization and its effects on the microbiome, likely play a key role in this change[30,128]. However, a global understanding remains incomplete due to the lack of worldwide data. Kaplan's four-step model of evolution[23] could potentially be applied to PIBD in the near future, although further population-based studies are needed to better understand how PIBD may evolve.

While a male predominance is well documented in PIBD, studies show that the sex ratio shifts after puberty, with a slight female predominance emerging in adolescence and adulthood. This transition mirrors patterns observed in other immune-mediated diseases. Despite this shift, the rate of increase in IBD incidence appears similar between sexes[29,132,133].

With the global rise in IBD incidence and prevalence among both adults and children, the overall burden on healthcare systems is expected to be heavier in the coming years. This is

compounded by the physical, psychological, social, and economic challenges faced by patients living with a lifelong disease. In the future, issues such as accessibility, affordability, disparities in healthcare resources, and the high cost of biologic therapies may place additional strain on healthcare systems and widen the gap in care quality across the world[25,26,134–136].

1.2.2. Genetic and environmental features

Genetic and environmental factors both play a crucial role in the development of pediatric CD. On the genetic side, having a first-degree relative with IBD is the strongest known risk factor for developing the disease, with Ruban *et al.* reporting a positive family history in 25.2% of PIBD cases[137]. Studies have highlighted the significant contribution of susceptibility loci, such as mutations in *NOD2*/*CARD15*, which are particularly associated with early-onset forms of CD[138,139]. GWAS have identified genetic loci specifically associated with pediatric-onset IBD, highlighting genetic distinctions between early- and adult-onset IBD and their shared pathogenetic mechanisms[140–142]. Common genetic risk variants for IBD are observed in some patients who exhibit classical forms of the disease. However, pathogenic rare variants, often associated with monogenic disorders following a Mendelian inheritance pattern, are found in only a small subset of IBD patients. These common risk variants may influence the impact of pathogenic rare variants[39] (Figure 12).

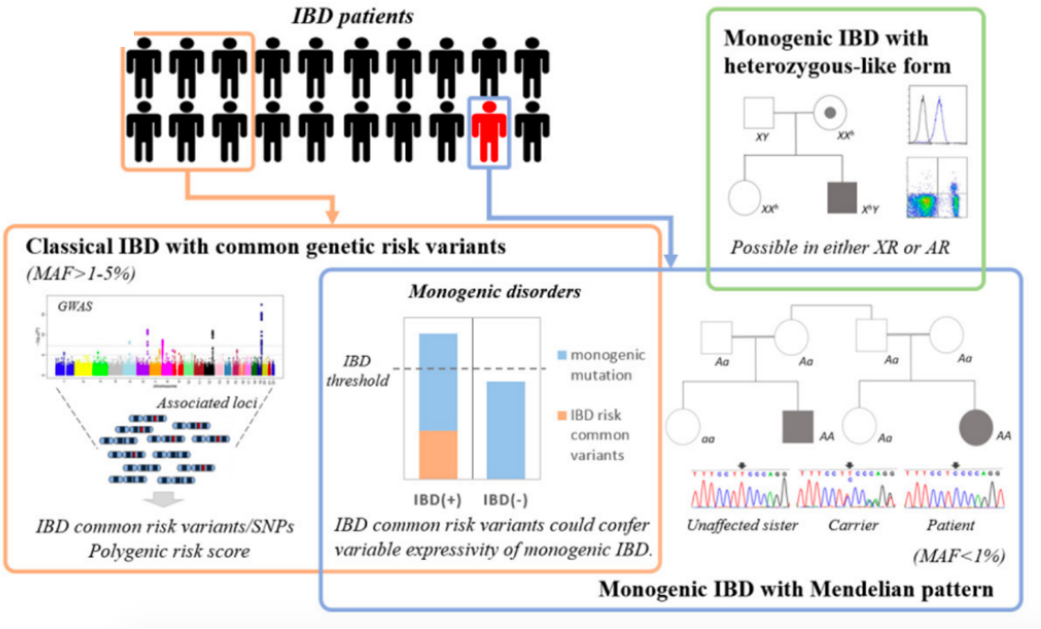

Figure 12. Interaction of common and rare genetic variants in IBD pathogenesis[39]

Although rare, monogenic disorders can mimic CD, particularly in very early-onset cases, where severe disease or onset in infancy should prompt an investigation for single-gene defects[142]. While most studies on monogenic IBD have concentrated on children diagnosed before the age of six (VEO-IBD), recent research has revealed a significant prevalence of monogenic IBD even in older children (aged over 6 years) and adults[39,143] (Figure 13).

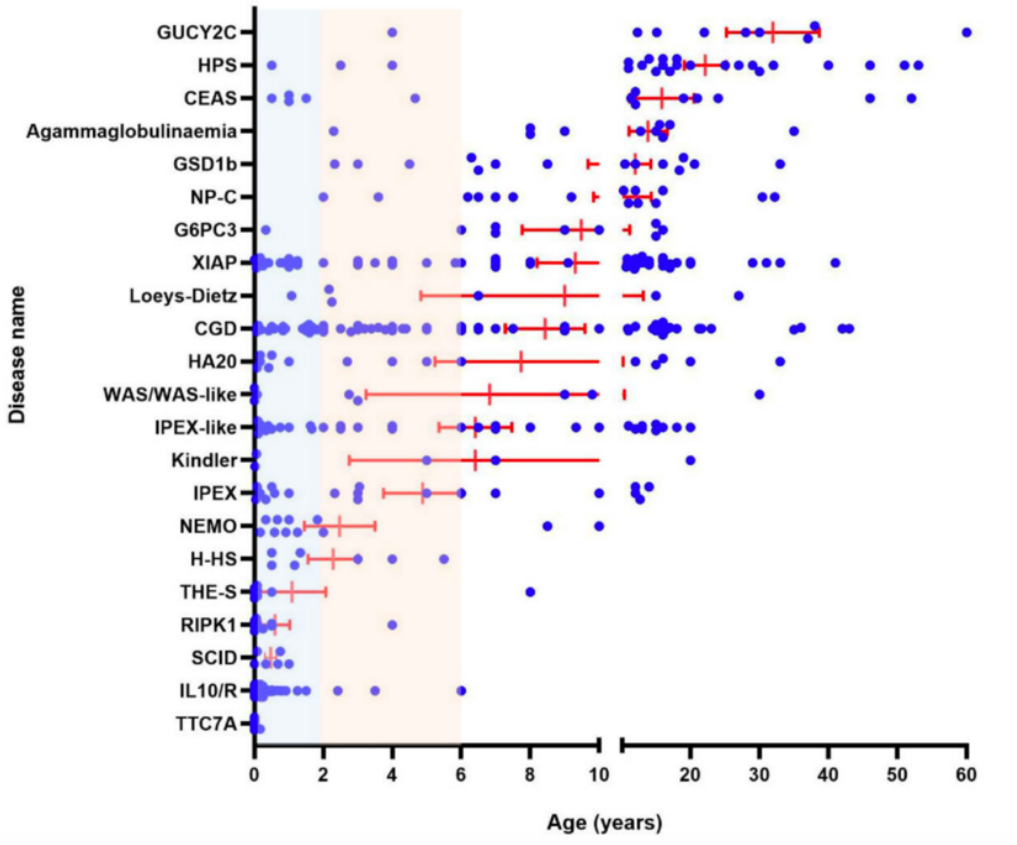

Figure 13. Age distribution of IBD onset categorized by the associated monogenic disorder. The blue-shaded area represents ages 0–1.9 years, while the red-shaded area corresponds to ages 2–5.9 years. Each blue dot represents an individual patient, with vertical red lines indicating the mean and horizontal lines representing the standard error[143].

In this systematic review of monogenic IBD cases, 750 patients were identified across 303 articles published between 2000 and 2020. The genes most commonly associated with monogenic IBD were *IL10RA/B*, *XIAP*, *CYBB*, *LRBA* and *TTC7A*. Most cases were diagnosed before the age of six, with significant differences in genetic disorders across age groups. Several phenotypic features have been associated with monogenic IBD, including extraintestinal comorbidities (EICs) such as infections, dermatologic conditions, tumors and autoimmune manifestations. Specific endoscopic and histological features may also prompt consideration of monogenic IBD[142] (Table 2).

Table 2. Phenotypic features of monogenic IBD (adapted from Uhlig et al.[142])

Phenotypic features		Exemplar disorder and gene defect
Infection	Recurrent typical or single/recurrent atypical infections in patients without immunosuppressive therapy	Primary immunodeficiency, chronic granulomatous disease
Immune activation with and without infection	Hemophagocytic lymphohistiocytosis	*XIAP* and *STXBP2*
Autoimmune features	Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX or IPEX-like) syndrome	FOXP, *LRBA*, *CTLA4*, *STAT3*, and *STAT1*.
Dermatological features	Oral leukoplakia Ectodermal dysplasia with dysplastic nails and conical teeth	Telomeropathies, NF kappa B essential modulator (*NEMO*), *IKBKG* defects
Tumors	Woolly hair with trichorrhexis nodosa B cell lymphomas Gastric adenocarcinomas	Trichoenterohepatic syndrome because of *TTC37A* or *SKIVL2* IL-10 signaling defects *CTLA4* and *LRBA*
Intestine	Multiple intestinal atresia	*TTC7A*
Endoscopic and histology features	Complex perianal fistulizing disease accompanying luminal inflammation, especially if manifest in the first year of life Intestinal epithelial cell apoptosis Tissue eosinophilia Enteropathy with villous flattening, similar to celiac disease Germinal cell hypoplasia Granulomas and pigmented macrophages	IL10, IL10RA, and IL10RB, *TGFB1*, or *XIAP*. *TTC7A*, *LRBA*, *XIAP*, *SH2D1A*, *ARPC1B*, or COL7A1, IPEX, IPEX-like syndrome or WAS IPEX, or IPEX-like syndrome and CVID Defect in humoral immunity such as in ICOS or BTK deficiency Chronic granulomatous disease

ARPC1B, Actin Related Protein 2/3 Complex Subunit 1B; BTK, Bruton tyrosine kinase; COL7A, Collagen Type VII alpha 1 Chain; CTLA4, Cytotoxic T-lymphocyte associated protein 4; CVID, Common variable immune deficiency; FOXP, Forkhead box protein; ICOS, Inducible T-Cell Co-Stimulator; IL-10, Interleukin 10; IPEX, Immune dysregulation, polyendocrinopathy, enteropathy X-linked; *LRBA,* Lipopolysaccharide-Responsive and Beige Like Anchor; *NEMO*, NF kappa B essential modulator; *SH2D1A*, SH2 domain containing 1A; *SKIVL2*, Super killer viralicidic 2-like; STAT1, Signal Transducer And Activator Of Transcription 1; STAT3, Signal Transducer And Activator Of Transcription 3; STXBP2, Syntaxin Binding Protein 2; TGFB1, transforming growth factor beta 1; *TTC37A*, Tetratricopeptide Repeat Domain 37A; *TTC7A*, Tetratricopeptide Repeat Domain 7A; *XIAP*, X-linked inhibitor of apoptosis protein.

Next-generation DNA sequencing (NGS), whole exome sequencing (WES) or targeted genome panel sequencing (TGPS) are increasingly recommended in routine clinical practice for diagnosing monogenic causes of IBD, particularly within a multidisciplinary care setting.

However, genetic testing is not universally recommended for all IBD patients and should instead be guided by specific criteria, such as age onset (infantile, very early-onset, pediatric or young adult IBD), family history, significant comorbidities, or EIMs. These analyses not only aid in diagnosis but also provide insights into disease prognosis, progression and tailored treatment strategies[39,142,144].

The management of monogenic IBD often includes interventions such as surgery, biologics, or more specific treatments with hematopoietic stem cell transplantation, highlighting the importance of early diagnosis and a better understanding of these rare conditions for effective management[143]. Such disorders highlight the diversity of genetic contributions and underscore the importance of genetic testing in specific contexts, as these cases often do not respond to conventional IBD treatments and may require alternative therapeutic options[142,143,145,146].

The increasing incidence of pediatric CD over the past decades underscores the growing impact of environmental factors on disease onset and progression. Early-life exposures, including breastfeeding, diet, and maternal smoking, have been implicated in this rise. Although breastfeeding has been associated with a protective effect against complicated disease phenotypes, this relationship is not consistently observed, with some studies reporting conflicting results[20,147–149]. Maternal smoking during pregnancy has been shown to increase the risk of IBD[20]. Through a mouse model of postnatal growth restriction, Ley *et al.* demonstrated that nutrition during early life impacts intestinal maturation and gut health in later life. Specifically, postnatal growth restriction altered the intestinal barrier in pups at weaning, increasing intestinal permeability and altering gut bacterial colonization[150]. Additionally, Guo *et al.* conducted the first prospective analysis of early-life diet, revealing that high dietary quality at 1 year of age is associated with a lower risk of developing IBD. High consumption of fish and vegetables was associated with a reduced risk of IBD, whereas a high intake of sugar-sweetened beverages was associated with an increased risk[46]. Environmental factors also influence the microbiome, which interacts with genetic predispositions to modulate intestinal inflammation[150]. For instance, *in utero* exposure to antibiotics, especially during the third trimester, has been associated with an increased risk of IBD, likely due to impaired microbiota implantation in the infant[60,151]. Beyond influencing IBD development, early-life environmental factors also affect disease phenotypes and course in pediatric CD[148].

These findings emphasize the need for a multidisciplinary approach to better understand and manage pediatric CD, integrating genetic data with modifiable environmental factors.

1.2.3. Phenotype of pediatric Crohn's disease

Pediatric CD often presents with a different and more severe phenotype compared to adult-onset cases, characterized by extensive disease involvement, a higher prevalence of penetrating and stricturing complications, and significant systemic manifestations such as growth failure[152–155].

Accurately classifying disease phenotype is crucial for understanding genotype-phenotype correlations and choosing appropriate therapeutic options. While the Montreal Classification of IBD has been widely used[13], it presents limitations in capturing the dynamic features of pediatric disease, such as changes in disease location, behavior over time, and growth failure. To address these gaps, an international group of PIBD experts modified the Montreal criteria, resulting in the Paris Classification[14], which better reflects the specific characteristics of pediatric disease by incorporating age at diagnosis, disease location, disease behavior, and the presence of growth failure (Table 3).

Table 3. Montreal and Paris Classifications for Crohn's disease (adapted from Levine et al.[14])

	Montreal	Paris
Age at diagnosis	A1: below 17 y A2: 17-40 y A3: above 40 y	A1a: 0-<10 y A1b: 10-<17 y A2: 17-40 y A3: >40 y
Location	L1: terminal ileal ± limited cecal disease L2: colonic L3: ileocolonic L4: isolated upper disease*	L1: distal 1/3 ileum ± limited cecal disease L2: colonic L3: ileocolonic L4a: upper disease proximal to ligament of Treitz* L4b: upper disease distal to ligament or Treitz and proximal to distal 1/3 ileum
Behavior	B1: non stricturing non penetrating B2: stricturing B3: penetrating p: perianal disease	B1: non stricturing non penetrating B2: stricturing B3: penetrating B2B3: both penetrating and stricturing disease, either at the same or different times p: perianal disease
Growth	NA	G0: No evidence of growth delay G1: Growth delay

*In both the Montreal and Paris Classification systems L4 and L4a/L4b may coexist with L1, L2, L3, respectively.
B1 – Non stricturing, nonpenetrating disease: uncomplicated inflammatory disease without evidence of stricturing or penetrating disease.
B2 – Stricturing disease: the occurrence of constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical examination combined with prestenotic dilation and/or obstructive signs or symptoms but without evidence of penetrating disease.
B3 – Penetrating disease: the occurrence of bowel perforation, intraabdominal fistulas, inflammatory masses and/or abscesses at any time in the course of the disease, and not secondary postoperative intra-abdominal complication (excludes isolated perianal or rectovaginal fistulae).
B2B3 – Stricturing and penetrating disease: the presence of both B2 and B3 phenotypes in the same patient, either at the same moment in time, or separately over a period of time.
NA, non-applicable.

The Montreal Classification defines age at diagnosis broadly, grouping all pediatric cases into a single category (A1, <17 years)[13]. However, early-onset and very early-onset IBD (<10 years according to this classification) exhibit distinct phenotypic, genetic, and immunologic profiles compared to those of older children and adolescents[14,156,157]. The Paris Classification refines age categories into A1a (<10 years), A1b (10-<17 years), A2 (17-40 years), and A3 (>40 years), enabling a more precise characterization of age-related differences in disease phenotype[156–158]. The Paris Classification refines disease location by distinguishing proximal (L4a) and distal (L4b) small bowel involvement, which is particularly relevant in pediatrics due to differences in management and severity. Unlike the broad L4 category in the Montreal Classification, this distinction clarifies disease distribution, as significant small bowel involvement is associated

with poorer outcomes, such as growth failure and stricturing disease, whereas the impact of gastro-duodenal involvement remains unclear[156,159,160]. In terms of disease behavior, the Paris Classification allows simultaneous classification as B2B3 for patients with both stricturing and penetrating disease, addressing the overlapping behaviors often seen in pediatric CD[161,162]. Finally, it introduces growth failure (G1/G0) as a key feature, acknowledging its significance as a hallmark of pediatric CD and the systemic effects of chronic inflammation[163,164].

Disease location in pediatric CD varies with age of onset, with younger children more frequently presenting with isolated colonic disease, whereas older children show a higher prevalence of ileocolonic and upper gastrointestinal involvement[133,165]. Children therefore more often present with bloody stools[133,166]. Histological examination frequently reveals upper gastrointestinal lesions, reflecting the extensive and pan-enteric nature of pediatric CD compared to adults[161]. The progression of disease location over time, such as ileocolonic and upper gastrointestinal involvement, has been reported in some studies, although this finding is not consistent across all reports[162,167].

At diagnosis, most pediatric CD cases present with an inflammatory phenotype (B1)[19,167,168]. However, progression to more complicated phenotypes – stricturing (B2), penetrating (B3), or both (B2B3) – is frequently observed (Figure 14)[162,165,167,169,170]. Nevertheless, behavioral classification studies show variability in phenotype distribution at diagnosis, highlighting challenges in distinguishing inflammatory from fibrostenotic stenoses in pediatric populations.

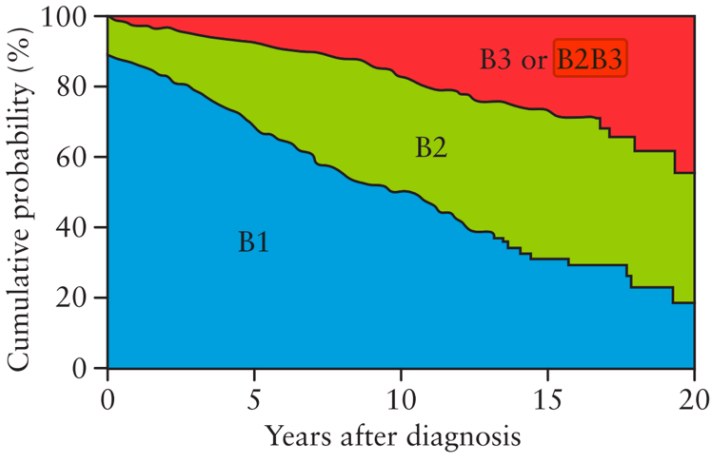

Figure 14. Cumulative probability of inflammatory (B1), stricturing (B2), and penetrating (B3) or structuring and penetrating (B2B3) behavior in 594 patients with pediatric-onset CD[170]

Complicated behavior increases with disease duration[161,162], and is influenced by age at onset and disease location, with older children more likely to develop stricturing disease, possibly due to a higher prevalence of ileal involvement[171]. However, the impact of age on disease behavior remains debated, whereas disease duration has a predictive value[172].

Perianal disease is commonly linked to intraabdominal penetrating complications, similar to adult findings, although the mechanisms of penetrating disease in pediatrics may differ[165].

Pediatric CD often leads to growth failure, with many children presenting with a height z-score below the expected range at diagnosis, as inflammation negatively impacting growth velocity[163,173]. Malnutrition, due to reduced intake, nutrient malabsorption, and increased metabolic demands from inflammation, exacerbates growth delay. Delayed diagnosis, male gender, and jejunal involvement are also associated with more severe growth impairment[174,175]. Pubertal delay is common in pediatric CD, driven by inflammatory cytokines like TNF-α and IL-1, which disrupt the hormonal signals needed for puberty[176,177]. This delay, along with vitamin D deficiency, can impact bone health, leading to osteoporosis[178].The mechanisms underlying growth and pubertal delays are summarized in Figure 15, where factors such as inflammation, nutrient deficiencies, and hormonal disruptions are shown to contribute to these outcomes.

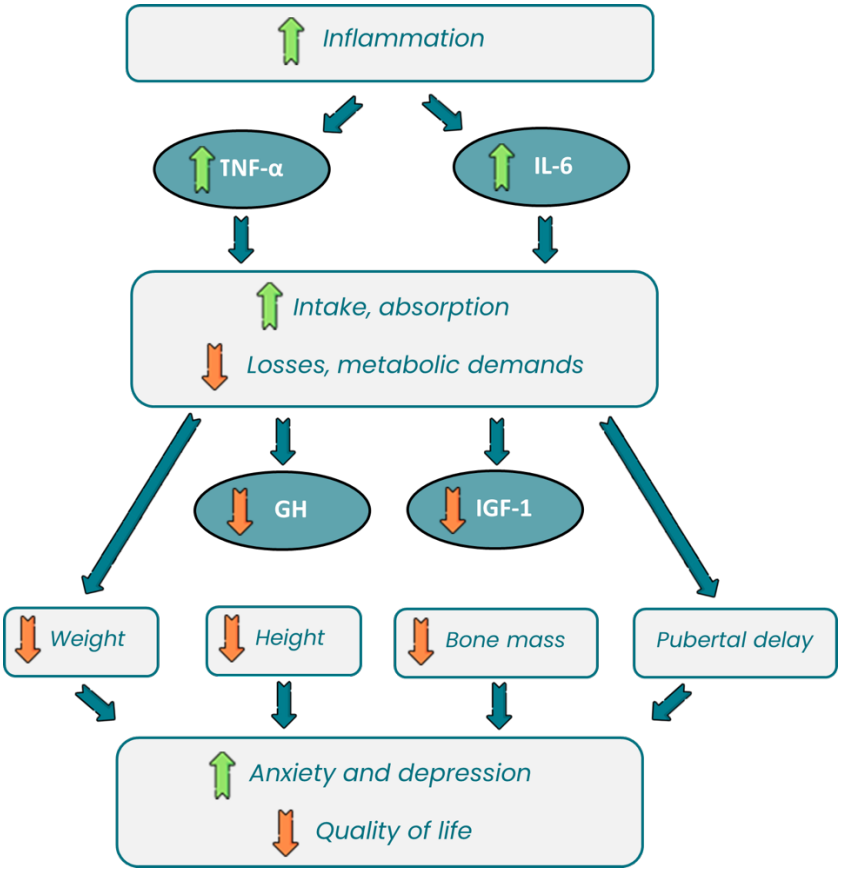

Figure 15. Overview of mechanisms in PIBD-related growth delay: schematic representation of factors that impact growth delay and its consequences (adapted from Wong et al.[179])

GH, growth hormone; IGF-1, insulin-like growth factor 1; IL-6, Interleukin 6; TNF-α, Tumor necrosis factor alpha.

A common misconception is that effectively treating intestinal inflammation adequately address EIMs in most IBD patients. As in adults, while conditions such as peripheral arthritis, oral aphthous ulcers, episcleritis, and erythema nodosum are often linked to active intestinal inflammation and may improve with standard therapy targeting the gut, other manifestations like anterior uveitis, ankylosing spondylitis, and primary sclerosing cholangitis typically occur independently of intestinal disease activity[109,110]. Moreover, the occurrence of EIMs is linked to a more aggressive pediatric CD progression[180].

Adolescents with IBD experience significantly higher levels of fatigue compared to their healthy peers. Fatigue is influenced by multiple factors, including active disease, heightened anxiety or depression, and strained family dynamics. Notably, even in remission, fatigue often

persists and continues to significantly impact a child's quality of life, making it one of the most distressing and challenging symptoms to manage[181,182].

Psychological factors, particularly anxiety and depression, play a crucial role in this context. A systematic review of 28 studies involving 8107 participants (mean age: 14.3 years) revealed pooled prevalence rates of 16.4% for anxiety symptoms, and 4.2% for anxiety disorders, as well as 15.0% for depressive symptoms and 3.4% for depressive disorders. These rates appear lower than those reported in adult IBD populations. However, variations in assessment tools and cut-off values limit the comparability of findings, and the results should be interpreted cautiously. Importantly, studies with a higher proportion of active disease reported increased rates of depressive symptoms, underscoring the potential impact of disease activity on mental health. Standardized and cross-culturally validated tools are necessary to provide more accurate prevalence estimates and guide interventions aimed at fostering resilience in PIBD patients[183].

Absenteeism, related to frequent medical appointments and hospitalizations, poses a challenge for children with IBD, particularly those with refractory disease. A French cohort of 104 children and adolescents with IBD missed 4.8% of possible school days, with gastrointestinal symptoms accounting for about one-third of these absences and medical visits or procedures for roughly one-quarter[184]. Nevertheless, children with IBD tend to achieve higher levels of education and have higher rates of employment than the general population[19]. Those who present prolonged active disease or mental health issues at diagnosis are more likely to experience academic difficulties[185–187].

1.2.4. Disease course

Pediatric CD often exhibits an aggressive course, with a high risk of stricturing (B2) or penetrating (B3) complications occurring in about 30-50% of patients within 5-10 years of diagnosis[135,169,188], especially in groups of older children (≥ 6 years)[171]. Extensive ileocolonic disease (L3), upper tract involvement (L4), and EIMs at diagnosis are associated with worse outcomes[189]. Early treatment strategies also impact outcomes; corticosteroid-based management has been associated with more unfavorable disease progression, such as a higher risk of complications and surgical intervention, compared to immunomodulators or anti-TNF therapies[162]. Optimized therapeutic approaches, including the early use of immunosuppressants

and anti-TNF agents, could contribute to reducing surgical rates and improving long-term outcomes[167,190].

In the follow-up study of Fumery *et al.*, 43% of patients with pediatric-onset CD underwent at least one intestinal resection, at the end of the follow-up, highlighting the significant risk of surgery over the course of the disease. Additionally, the incidence of cancer was higher than expected, with a crude cancer rate of 1.1%[169].

Studies such as those conducted by the Porto IBD Working Group of the European Society for Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the RISK study funded by the Crohn's and Colitis Foundation of America, aim to identify more precise predictive tools for PIBD. In the meantime, several factors have been associated with poorer outcomes in children with CD, including:

- deep colonic ulcerations on endoscopy;
- persistent severe disease unresponsive to adequate induction therapy;
- extensive (pan-enteric) disease;
- significant growth retardation (height Z-score below -2.5);
- severe osteoporosis;
- stricturing (B2) or penetrating (B3) disease behavior at diagnosis;
- severe perianal disease[190].

Clinical, laboratory and endoscopic parameters often fail to predict long-term disease progression[191,192]. Advances in prediction models, such as PREDICT-EPIMAD, which integrate clinical, serological, and genetic data, are promising for identifying patients at risk of complicated diseases[193].

1.2.5. Diagnostic specificities

Patients with CD often present with symptoms such as abdominal pain, diarrhea, and weight loss. However, in children and young people, these classic symptoms are less consistently observed, making the diagnosis more challenging. In fact, only about 25% of children eventually diagnosed with CD demonstrate this triad at presentation[160,194]. For instance, some children may present only with lethargy and mild abdominal pain or, more commonly, delayed

growth[195]. Beyond the classic symptoms of CD, growth retardation and delayed puberty are particularly frequent in pediatric cases (observed in approximately one-third of children with CD)[196]. Therefore, it is crucial to systematically assess these aspects during clinical examinations and through close monitoring of height history and growth charts[197].

Biomarkers play a crucial role in diagnosing pediatric CD, with distinct considerations compared to adults. Blood tests often reveal anemia, elevated inflammatory markers (such as CRP and Erythrocyte Sedimentation Rate – ESR), thrombocytosis, and hypoalbuminemia. However, the absence of these abnormalities does not rule out CD, particularly in children, where presentation may be atypical[123]. Fecal calprotectin correlates strongly with intestinal inflammation. It has a high sensitivity in diagnosing IBD in children, although age-related variations in calprotectin levels must be considered[194]. Comparative studies have shown slightly higher diagnostic accuracy in adults, with a sensitivity of 93% and specificity of 96% compared to children and adolescents, who exhibit respectively 92% and 76%[198]. The difference in specificity between adults and pediatric populations underscores the importance of adapting diagnostic thresholds based on age to improve accuracy and reduce unnecessary interventions. For example, elevated fecal calprotectin is more common in infants under one year. It emphasizes the importance of symptom-based thresholds and tailored diagnostic approaches in pediatrics[199,200]. Serological markers such as ASCA have limited screening value for pediatric CD but may help predict disease phenotype and surgical risk[123,201].

Pediatric IBD imaging has unique considerations compared to adults. Radioprotection is crucial, favoring non-ionizing modalities like US and magnetic resonance enterography (MRE)[197,202,203]. US offers high diagnostic performance, especially in specialized pediatric centers, providing detailed assessment of bowel inflammation, vascularization, and complications like strictures or fistulas[116,204]. MRE, the modality of choice for small bowel exploration, enables transmural and extraluminal evaluation without radiation, making it crucial for monitoring disease extent and activity[203,205]. Although CTE and MRE similarly detect active inflammation, Quencer *et al.* demonstrated MRE is significantly more sensitive for mural fibrosis and – without the use of ionizing radiation – should be the preferred imaging modality for non-acute cases[206]. However, it may require general anesthesia, depending on the patient's age. For the future, transabdominal and transperineal US are emerging tools for perianal disease and mucosal inflammation, offering accuracy comparable to endoscopy with greater patient acceptance[207]. Diffusion-weighted MRI demonstrates comparable diagnostic performance to

contrast-enhanced MRI in children with known or suspected IBD, making it a viable alternative for assessing bowel abnormalities[208].

In PIBD, endoscopy should be conducted by a specialized pediatric gastroenterologist under general anesthesia or deep sedation in a child-appropriate environment[194,203]. In CD, the disease distribution differs from adults, with more colonic involvement and less ileitis, as well as a higher frequency of epithelioid-cell granulomas, particularly during the disease course. Additionally, upper gastrointestinal tract involvement is more common in children, emphasizing the importance of comprehensive biopsy sampling for accurate diagnosis, including from endoscopically normal areas[121,209]. Ileocolonoscopy, accompanied by multiple biopsies, is the most critical diagnostic tool, as incomplete colonoscopy or rectosigmoidoscopy alone is insufficient. Even in expert settings, terminal ileum intubation fails in approximately 10% of cases[203]. As in adults, reliable diagnosis requires biopsies from at least six locations, including the rectum and ileum, with a minimum of two samples per site[210]. Upper GI endoscopy is advised for all pediatric cases, as it detects macroscopic abnormalities in 35-60% of CD patients, microscopic ones in nearly 100% and granulomas in 25-61% of cases[121,211–214]. Esophagogastroduodenoscopy is especially beneficial for diagnosing CD in patients presenting with nonspecific pancolitis or other ambiguous findings[203,215].

A delay in diagnosing pediatric CD is a significant concern due to the impact on disease outcomes and patient well-being. Research indicates that the time from symptom onset to diagnosis often exceeds five months for CD, longer than for other IBD subtypes[216–218]. Atypical symptoms, such as isolated abdominal pain, frequently contribute to these delays, which typically occur before referral to specialized care[217,219]. Prolonged diagnostic delays are associated with worse outcomes, including a higher likelihood of complications like strictures and fistulas and impaired growth[220]. It highlights the urgent need to improve awareness and streamline diagnostic processes, ensuring timely identification and management of pediatric CD[217,220].

Unclassified IBD accounts for approximately 7% of PIBD cases at diagnosis, a frequency similar to adults, contrary to previous perceptions that it is more common in children[11,221]. In pediatric cohorts, the rate of diagnostic change is significantly higher, with 50% of IBDU cases being reclassified, predominantly into UC (32.7%) or CD (17%)[221,222]. Moreover, the frequency of IBDU is higher in younger children, particularly in VEO-IBD, reflecting increased diagnostic challenges in this population[168].

1.2.6. Very early-onset inflammatory bowel disease

Very early-onset IBD refers to IBD diagnosed in children under the age of six, and is characterized by a more severe and refractory disease course compared to older children and adults[145,223]. Regarding classification, VEO-IBD is typically diagnosed in children between two and six years of age, with cases diagnosed within the first two years categorized as infantile-onset IBD[224]. Children with VEO-IBD often display a unique phenotype and tend to experience a more aggressive form of the disease. The early onset and the intensity of the disease suggest a stronger genetic contribution, with monogenic defects being identified in some cases. These defects often involve genes related to primary immunodeficiencies and intestinal barrier functions, indicating a need for targeted therapies[145,223,225].

The increasing incidence of VEO-IBD calls for improved awareness and prompt recognition, as early intervention can improve outcomes[130,226]. VEO-IBD constitutes approximately 4 to 15% of PIBD cases, with rare instances of diagnosis occurring in the first year of life[130,171,227]. Its incidence ranges from 0.2 to 3.6 per 100,000 person-years globally, with heterogeneous trends over time likely due to small case numbers and regional variations. The prevalence of VEO-IBD, reported in a few studies, ranges from 1.9 to 5.8 cases per 100,000[30]. Given the genetic influence, it is not surprising that familial IBD is more common in VEO-IBD compared to later-onset pediatric IBD[228]. Several epidemiologic studies have reported a higher prevalence of VEO-IBD in males (possibly due to X-linked inheritance in monogenic IBD), though it is not clear whether this trend applies to both CD and UC, as many studies lack gender-specific data for each condition[133,229,230].

Up to one-third of VEO-IBD cases are now recognized as monogenic, linked to mutations in genes associated with primary immunodeficiencies[231]. The identified genetic disorders and functional immune pathways involved include intestinal epithelial barrier function, phagocyte-mediated bacterial killing, hyper- or auto-inflammatory pathways, and abnormalities in the development and function of the adaptive immune system[232–239]. Defects in intestinal barrier integrity, such as mutations in *ADAM17* and *TTC7A*, disrupt epithelial defense, leading to chronic inflammation[240–242]. Impaired bacterial recognition and clearance, as seen in Chronic Granulomatous Disease, result from mutations in genes like *CYBB* and *NCF1*, causing intestinal inflammation in up to 40% of cases[243–245]. Loss-of-function mutations in the *IL-10/IL-10R* pathway, which regulates inflammation, are strongly associated with severe early-onset IBD and respond well to hematopoietic stem cell transplantation[225,246]. Additionally, defects in

regulatory T cells (e.g., *FOXP3* mutations in IPEX syndrome) and adaptive immune development (e.g., *RAG1/RAG2* mutations) contribute to severe intestinal manifestations[223,247,248]. Lastly, mutations in genes like *XIAP* and *TRIM22* are linked to autoinflammatory disorders and severe VEO-IBD phenotypes, including colonic and perianal disease[223,249,250]. Patients with monogenic VEO-IBD have higher morbidity compared to those with non-monogenic VEO-IBD or other forms of PIBD. While the age of onset alone does not reliably predict disease severity or therapy response, the specific causative genetic defect plays a crucial role in determining individual prognosis[251,252]. Since GWAS for PIBD primarily involve adolescent-aged individuals, their relevance to patients with VEO-IBD may be restricted. A more tailored approach targeting these unique patients would provide a valuable complement to GWAS[253]. Advances in NGS, including WES, have improved the identification of genetic variants and facilitated more accurate diagnoses[254].

The increasing incidence of VEO-IBD reported in few studies could further suggest the involvement of environmental factors[130]. Particularly relevant is the development of the gut microbiome during the first three years of life, which coincides with the onset of VEO-IBD, suggesting that microbial exposures could contribute to disease initiation[255]. The interaction between the developing immune system and the gut microbiome during this period is vital for health and disruptions in this balance can lead to disease[256].

The VEO-IBD's phenotype in this group is diverse: while some children experience relatively mild disease, others develop more extensive and severe forms of IBD than older-onset cases, including those seen in adults[134,233,257]. Children with VEO-IBD most frequently present with a colonic phenotype, such as pancolitis, at the time of diagnosis[166,168,223,233,257,258]. However, the extent and location of the disease can evolve over time, making it difficult to distinguish between UC and CD. Consequently, the diagnosis of IBDU is more common in VEO-IBD compared to older-onset IBD[171,229,257,259,260], and it is even more pronounced in infantile IBD[261]. In CD, Aloi *et al.* and De Bie *et al.* confirmed that disease location was strongly influenced by the age of onset, with younger children more likely to have isolated colonic disease, while older children more frequently show involvement of the terminal ileum[165,257] (Figure 16).

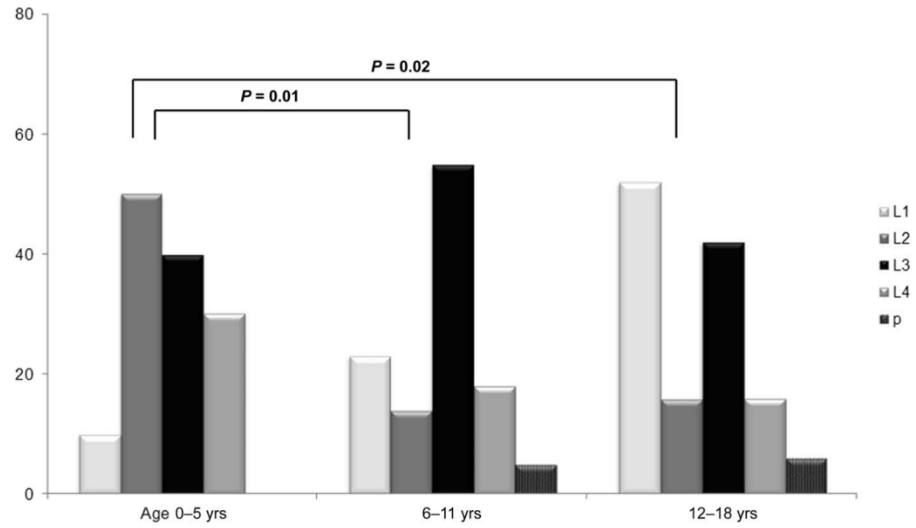

Figure 16. Disease location at diagnosis according to Paris classification in very early-onset CD (VEO-CD, 0–5 years) and early-onset CD (6–11 and 12–18 years). L1: ileum, L2: colon; L3: ileocolonic; L4: upper gastrointestinal; p: perianal disease[257].

Moreover, monogenic IBD often presents a CD-like phenotype, characterized by predominant colonic involvement, frequent perianal disease and features such as pseudo-polyps and nodules, while upper gastrointestinal involvement is less common. In contrast, non-monogenic IBD more frequently involves the upper gastrointestinal tract, with specific genetic defects further influencing disease presentation[262]. A recent systematic review revealed that only one-third of patients with monogenic IBD have a history of EICs before the onset of IBD, but three-quarters develop at least one EIC during the disease course, with atypical infections, dermatological abnormalities, and autoimmune conditions being the most commonly reported[143].

Histologically, VEO-IBD often presents with nonspecific intestinal inflammation that shares features of both CD and UC, with four distinct patterns:

- CD-like pattern: features discontinuous inflammation with granulomas, crypt abscesses, and deep ulcerations in the colon, alongside villous atrophy and chronic enterocolitis in the small bowel.
- UC-like pattern: characterized by continuous inflammation with cryptitis, Paneth cell metaplasia, ulcerations, and significant architectural distortion or atrophy in the colon.
- Enterocolitis-like pattern: involves extensive villous atrophy, widespread leukocytic and eosinophilic infiltrates, mucosal friability and susceptibility to CMV colitis.

- Apoptotic pattern: defined by severe glandular atrophy, gland dropout, and extensive apoptosis, with distinctive "exploding crypts"[239].

These patterns highlight the histological diversity of VEO-IBD. In CD, studies have shown that microscopic gastritis is more prevalent in EO cases compared to very early-onset CD (VEO-CD). Additionally, while a majority of VEO-CD patients exhibit chronic colitis, a proportion of early-onset CD (EO-CD) patients may present without chronic inflammation in the colon or ileum. Eosinophils are also found more frequently in EO-IBD patients than in VEO-IBD patients, indicating differences in immune cell involvement across age groups[168].

The propensity for complications in VEO-IBD remains debated, with some studies reporting a higher prevalence of stricturing complications, while others suggest no significant difference compared to IBD with later-onset[134,171,257]. Collen *et al.* reported that patients with monogenic IBD were more likely to present with stricturing and penetrating disease as well as EIMs[263]. Guz-Mark *et al.* also demonstrated that pediatric patients with monogenic disease have lower response rates to induction therapies and higher rates of surgical intervention[261].

A coordinated and comprehensive approach to VEO-IBD enables more precise and effective care. The genetic foundations of VEO-IBD offer an opportunity to refine therapeutic decisions, moving beyond trial-and-error methods. Treatments that are successful for patients with known causal genetic variants can also be applied to those without an identified genetic cause. While only a small proportion of VEO-IBD patients have a monogenic cause, these individuals often benefit from targeted therapies. Traditional immunological studies have limited value, but genetic sequencing can help identify monogenic causes in many cases[264].

1.3. STRICTURING COMPLICATIONS IN ADULT AND PEDIATRIC CROHN'S DISEASE

1.3.1. General considerations

The progression of CD, in both adult and pediatric populations, is characterized by the development of complications, including strictures, fistulas, abscesses, and an increased risk of intestinal cancer over time (Figure 17)[265].

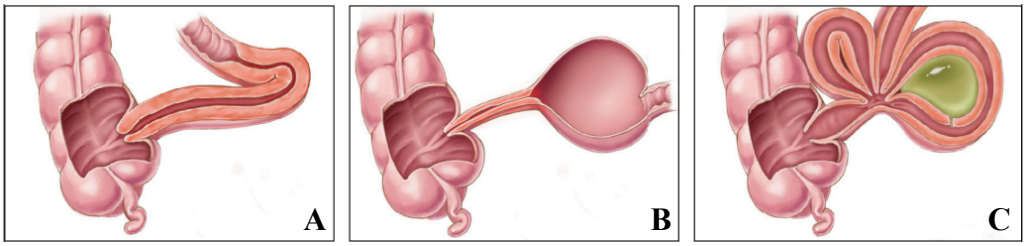

Figure 17. Complications of CD (adapted from Torres et al. 2017[265]). (A) Inflammatory and fibrotic mural thickening of the distal ileum without upstream dilatation; (B) Inflammatory and fibrotic mural thickening of the distal ileum with upstream dilatation suggesting a stricture; (C) Deep and transmural fistula and formation of an abscess.

Louis *et al.*[266] and Cosnes *et al.*[8] independently showed that although most patients with CD initially present with a non-stricturing, non-penetrating phenotype at diagnosis, the disease frequently progresses over time toward stricturing or penetrating complications. Pediatric-onset CD is often associated with a more aggressive disease phenotype, characterized by rapid progression to penetrating or stricturing complications[135,162,169]. Patients with early-onset disease are also more likely to require earlier surgical interventions than adults[171,267].

A stricture is defined as a segment of the bowel with persistent narrowing of the lumen, often accompanied by proximal bowel dilatation and varying degrees of intestinal obstruction. In CD, strictures are associated with reduced quality of life, an increased risk of penetrating complications, and may necessitate emergency surgical resection, which is frequently associated with poor outcomes[268]. Strictures result from chronic, sometimes subclinical transmural inflammation that triggers an excessive wound-healing response. This process leads to fibrosis, driven by the accumulation of activated myofibroblasts producing excessive

extracellular matrix (ECM). Over time, this progressive remodeling causes structural bowel damage, leading to a gradual loss of intestinal function and increased disability. The extent of cumulative bowel damage can be measured using the Lémann index (Figure 18)[265,269].

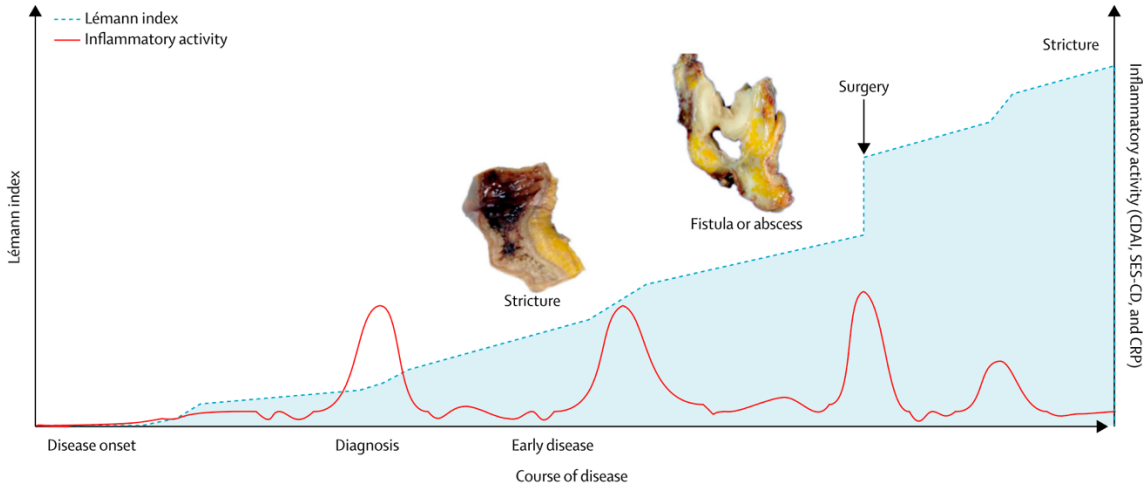

Figure 18. Progression of intestinal damage and inflammatory activity in a hypothetical CD patient[265]

Studies suggest that the cumulative risk of developing strictures increases with disease duration, affecting up to 50% of patients after 20 years[162,265,270,271]. Lovasz *et al.* demonstrated that the risk of progressing to a complicated disease phenotype is similar between pediatric- and adult-onset CD, with probabilities of 49.7% and 61.3% in children and 55.1% and 62.4% in adults after 5 and 10 years of follow-up, respectively, as shown in Figure 19. No significant difference was observed in the time to progression from a non-stricturing, non-penetrating phenotype (B1) to a stricturing (B2) or penetrating (B3) phenotype between pediatric and adult-onset patients[188].

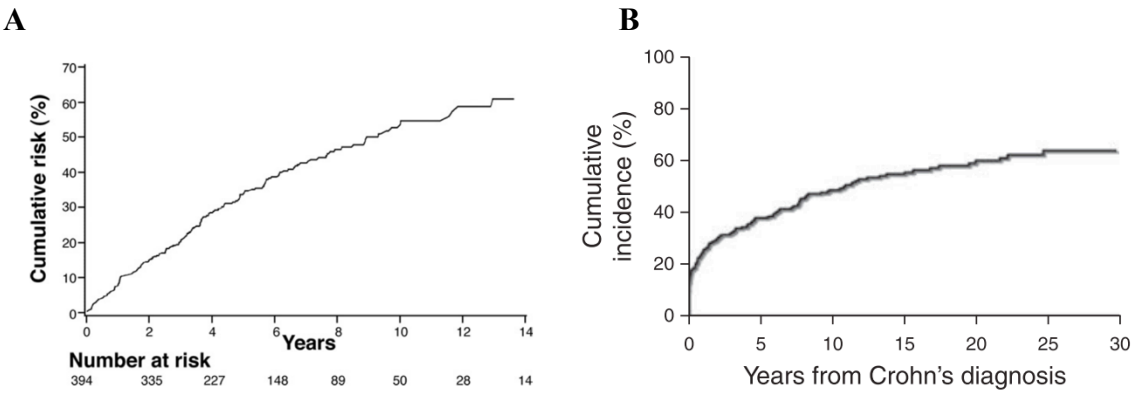

Figure 19. Cumulative risk and incidence of intestinal resection in pediatric (A) and adult-onset (B) CD (adapted from Vernier-Massouille et al. 2008[162] and Peyrin-Biroulet et al. 2010[271])

1.3.2. Evaluation

The most common locations for *de novo* strictures are the ileum and the ileocolonic region, together accounting for 40-60% of all cases. However, strictures can occur anywhere along the gastrointestinal tract, including the upper GI tract and rectum[270,272]. Ileal disease, perianal disease, age below 40 years at diagnosis, and deep mucosal ulcerations are risk factors for the development of strictures and are associated with a higher need for surgery[272–276]. In the pediatric population, female sex, disease behavior, and the presence of perianal disease at diagnosis have been associated with an increased the likelihood of requiring surgery[277]. A positive family history of IBD is also linked to a higher risk of stricturing phenotypes (11.3% *vs.* 2.8%, $p = 0.052$)[137].

In advanced intestinal fibrosis, patients may develop symptoms of obstruction, prompting further diagnostic evaluation. Various imaging modalities are used, with barium small bowel follow-through assessing the extent of narrowing and endoscopy detecting severe luminal narrowing, although the latter is unable to evaluate transmural disease.

Transabdominal US is a non-invasive, widely available, and radiation-free technique for assessing intestinal strictures in CD, making it particularly safe for young and pregnant patients. It detects pathological changes in the small and large bowel, such as bowel wall thickening (>4 mm), luminal narrowing, and upstream distension, although its accuracy heavily depends on the operator[117,276]. However, CD often involves skip lesions and multiple intestinal segments,

with strictures ranging from a few centimeters to over 20 cm in length. This complexity can make it challenging to accurately assess the relationship between the strictured segment, adjacent bowel loops, and surrounding structures[276]. This is why cross-sectional imaging techniques like CTE and MRE are now often preferred in both adult and pediatric patients, although challenges remain in distinguishing abnormalities due to inflammation or fibrosis, and strictures from motility issues or underdistension (Figure 20)[108,115,265,272,278]. Fibrosis notably arises as a complication of intestinal inflammation. Assessing the relative contributions of inflammation, fibrosis, and smooth muscle hypertrophy in dominant strictures has been a major focus of imaging research, as the accuracy of fibrosis measurement decreases when inflammation is present. A universally accepted clinical or histological scoring system for fibrosis associated with strictures has not yet been established[115]. However, Rimola *et al*. have demonstrated that MRI can differentiate varying degrees of fibrosis and inflammation in CD bowel lesions: by assessing gadolinium enhancement over time, they could identify segments with a significant fibrotic component, regardless of inflammation levels[278]. Other studies have shown that pre-stenotic upstream small bowel dilatation greater than 3 cm is significantly associated with confluent transmural fibrosis[268]. Emerging techniques, such as T2 relaxometry and magnetization transfer imaging, also show promise as non-invasive tools for accurately assessing both intestinal fibrosis and inflammation[117].

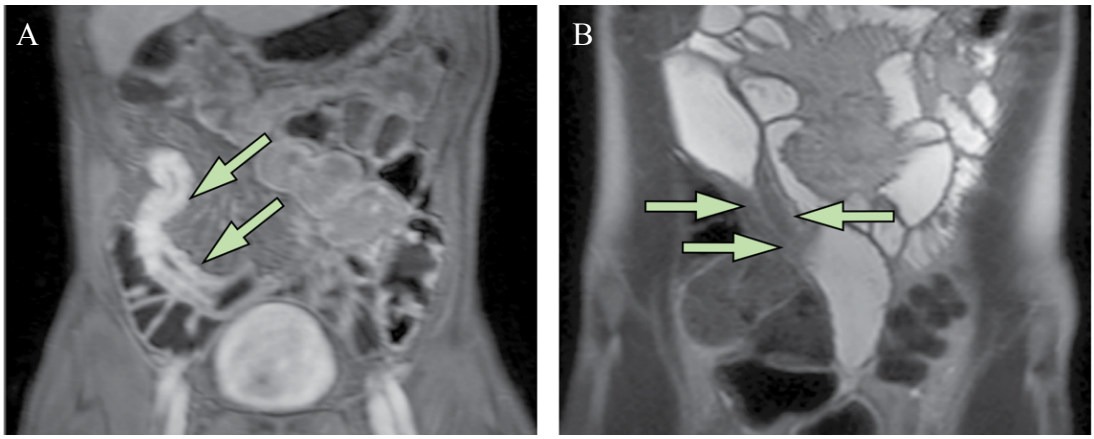

Figure 20. MRE illustrations (adapted from Torres et al.[265]). (A) T1-weighted MRE imaging with fat saturation after injection of gadolinium chelates shows mural thickening and enhancement in the distal ileum (arrows) in a patient with active CD. (B) T2-weighted MRE imaging shows a narrowed luminal segment with thickened wall and upstream dilatation (arrows), suggesting the presence of a stricture.

Ileocolonoscopy with biopsies remains the gold standard for diagnosing and monitoring CD, but its invasive nature and cost limit its use as a screening tool. Although biomarkers like CRP and fecal calprotectin serve as non-invasive indicators of inflammation[279], no reliable non-invasive biomarker for fibrosis currently exists. Thus, in patients with suspected strictures, endoscopy provides essential visual and histological evaluations to assess disease activity, lesion severity, mucosal status, and to rule out conditions such as malignancies[276,279], which are more frequent in patients with colonic strictures[108,280].

Histological evaluation of intestinal fibrosis in CD remains challenging due to several limitations. Endoscopic biopsies, while accessible during procedures, are often inadequate for assessing fibrosis due to their limited depth and susceptibility to sampling errors[281]. Although surrogate markers such as staining for myofibroblasts (alpha-smooth muscle actin [α-SMA]), tenascin, and collagen subtypes have been proposed, these methods do not reliably capture deeper tissue layer fibrosis. Additionally, no validated or widely accepted histopathological scoring system exists to grade fibrosis severity. Many studies rely on their own semiquantitative parameters, which lack standardization, making comparisons across studies difficult[272].

Histological specimens from strictured intestines in CD often reveal thickening across all bowel wall layers, characterized by dense collagen deposition and mesenchymal cell expansion. Notably, an increased proportion of collagen types III and V, associated with healing and scar contraction, contributes to stricture formation[282]. Beyond fibrosis, smooth muscle hyperplasia and hypertrophy could play a predominant role in the development of the stricturing phenotype in CD-associated fibrostenosis[283,284]. In 2020, a systematic review highlighted the limitations of existing histologic scoring systems for assessing fibrosis and inflammation in small bowel CD strictures. These systems lack standardized development methods, show inconsistent definitions of fibrosis, and often overlook critical components like muscular hypertrophy[285]. The lack of formal validation and reliability testing for existing scoring systems highlights a significant gap in the evaluation of fibrosis. This underscores the need for a comprehensive and reliable histopathology index to assess fibromuscular stenoses[272,285]. Recently, a consensus effort specifically addressing small bowel strictures in CD has proposed refined histological criteria[286]. According to this framework, strictures are defined by increased thickness of the bowel wall compared to adjacent healthy segments, submucosal or mural fibrosis, muscularization of the submucosa, and thickening of the *muscularis mucosae*. Importantly, both naïve and anastomotic strictures can be characterized by these microscopic features. While

none of the current histological scoring systems has been deemed appropriate for use in clinical trials, these consensus criteria represent a key step toward standardizing gross and histopathological assessment in small bowel CD[286]. They also provide a foundation for developing validated scoring tools suitable for prospective trials. However, it is important to note that these definitions have so far been proposed only for the small intestine, and their extrapolation to colonic disease remains to be formally evaluated.

1.3.3. Management

Currently, surgery is an essential component in the management of strictures in CD, either for specific indications or in cases where medical or endoscopic treatments fail[276]. In adults, endoscopic balloon dilatation is a recognized treatment for symptomatic CD-associated strictures, with studies reporting both short- and long-term efficacy and safety[287]. The IBD Porto Group study has shown that endoscopic balloon dilatation is both effective and safe for pediatric patients with stricturing CD, with the majority avoiding surgery over 12 months and experiencing significant clinical improvement[288]. However, the overall risk of requiring surgery remains significant. Vernier-Massouille *et al.* reported cumulative surgical probabilities in pediatric patients of 7%, 20% and 34% at 1, 3, and 5 years, respectively[162].

The primary goal of surgical intervention is to remove or bypass diseased segments while preserving as much bowel as possible to reduce the risk of short bowel syndrome. Early surgical resection is recommended for symptomatic patients with localized ileocecal fibrostenosing CD, especially when endoscopic balloon dilatation is not feasible or strictures exceed 5 cm[289,290]. Early surgery may prevent complications, such as fistulization and obstruction, and is associated with longer clinical remission and reduced need for repeated surgery compared to prolonged medical treatment[289].

In addition to potential early and late postoperative complications[271], a major challenge of surgery in stricturing CD is the high rate of post-surgical recurrence, reaching up to 55% in adult-onset CD and 77% in pediatric-onset CD after 10 years[271,291], often necessitating a second resection[292]. Smoking is the strongest and most widely accepted risk factor for postoperative recurrence after resection or stricturoplasty in fibrostenosing CD[289]. Female gender, isolated ileal disease or ileocecal disease, and the presence of perianal fistulas were also associated with

a higher risk of postoperative recurrence[271]. Recent studies have identified the microbiota signature, particularly the presence of *Ruminococcus spp*., as a marker for the development of fibrostenosing CD, with a specific association found in pediatric patients[293]. *NOD2*/*CARD15* variants are associated with an increased risk of stricturing CD and early surgical recurrence[172,294]. However, since these variants are also linked to ileal disease, it is challenging to disentangle their direct contribution to stricture formation from the influence of ileal disease location. The relationship between *NOD2*/*CARD15* genotype and the development of stricturing patterns in CD remains unclear, as studies have yielded conflicting results. Some studies have found no association between this genotype and disease progression, regardless of whether patients carried the variant or exhibited different genotypic expressions[275]. Endoscopic recurrence often precedes clinical symptoms and serves as a strong predictor of the risk for future surgeries. Early postoperative surveillance, combined with prophylactic treatment – as anti-TNF therapy – has proven more effective in preventing complications and reducing the need for repeated surgery than treating recurrence[295].

While recent studies suggest that the use of immunomodulators and biologics has contributed to the decline in the need for surgery[167,296–298], the progression from inflammatory to complicated phenotypes remains unchanged, and surgery continues to be a frequent necessity in CD[169,299,300]. Anti-TNF therapy has been shown to reduce the need for surgery in newly diagnosed patients, whereas azathioprine demonstrates only modest efficacy in lowering long-term surgical risk[296]. However, non-pharmacological factors may also contribute to this trend, including shifts in care from surgeons to gastroenterologists, earlier diagnosis, more frequent endoscopic monitoring, a focus on achieving mucosal healing, and the adoption of standardized practice guidelines[169]. Currently, medical treatments primarily control inflammation and do not specifically target fibrosis, which underscores the importance of developing targeted antifibrotic strategies to improve long-term outcomes and potentially avoid surgical resection.

1.3.4. Intestinal fibrosis

1.3.4.1. General overview

Fibrosis is a physiological repair process in which ECM is deposited in connective tissue to restore tissue integrity after injury. While crucial for wound healing, persistent, uncontrolled, or repetitive activation due to inflammatory damage leads to pathological fibrosis, resulting in

tissue architectural distortion and potential functional loss, with clinical impact depending on the affected organ (Figure 21)[301]. In CD, intestinal fibrosis underlies the development of fibrostenotic complications and remains a major cause of surgery. Despite its clinical relevance, the mechanisms driving intestinal fibrosis in IBD are not yet fully understood, partly due to the lack of representative animal models[281,282,302,303].

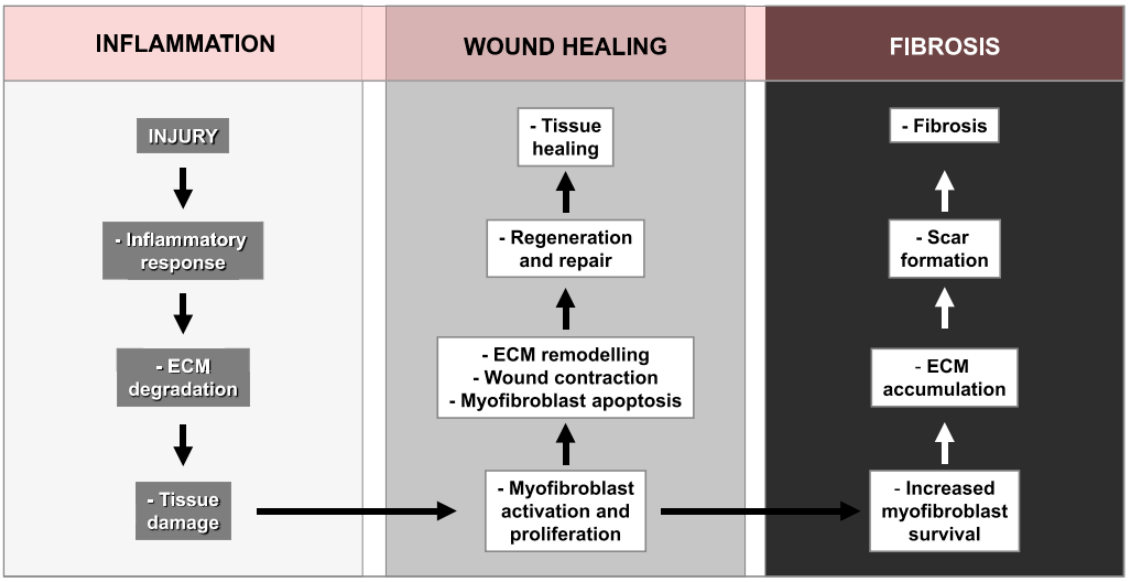

Figure 21. Schematic representation of fundamental processes occurring after an injury[301]

ECM, Extracellular matrix.

In CD, fibrogenesis involves a wide range of cellular players, including immune cells (monocytes, neutrophils, mast cells, eosinophils and basophils) releasing pro-inflammatory and pro-fibrotic mediators, and mesenchymal cell populations (fibroblasts, myofibroblasts and smooth muscle cells [SMCs]) which originate from mesenchymal stem cells (MSCs) and contribute to ECM deposition[304]. These cells are highly plastic, transitioning between phenotypes under inflammatory conditions, with fibroblasts differentiating into myofibroblasts in response to transforming growth factor β1 (TGF-β1) and myofibroblasts transdifferentiating into SMCs[305,306]. Other contributors include pericytes, which surround endothelial cells and differentiate into fibroblasts under inflammatory stimuli, fibrocytes and bone marrow-derived stem cells, which migrate from the bloodstream to inflamed sites, and stellate cells[304,307,308].

Moreover, pivotal biological events like epithelial-to-mesenchymal (EMT) and endothelial-to-mesenchymal transitions (EndoMT) further expand the pool of mesenchymal cells by converting epithelial and endothelial cells into cells with mesenchymal features[290,309–312].

The deposition of ECM is also modulated by a host of soluble factors produced by immune cells during inflammation (notably TGF-β) which activates mesenchymal cells and also plays immunoregulatory roles, thereby complicating its therapeutic targeting[290,305,313,314]. Other profibrotic molecules, including activins, connective tissue growth factor, various cytokines, and components of the renin-angiotensin system, together with factors such as endothelin and microbial signals like flagellin, may drive both inflammation-dependent and -independent fibrogenesis[305,307,315]. A summary of the key profibrogenic and antifibrogenic mediators is provided in Table 4.

Table 4. Examples for profibrogenic and antifibrogenic mediators in gut fibrosis (adapted from Latella et al. 2013 and Rieder et al. 2024)[305,311]

Profibrogenic mediators	Antifibrogenic mediators
• TGF-β1 • TNF-α • IL-1, IL-4, IL-6, IL-11, IL-13, IL-17, IL-33, IL-34, IL-36 • TL1A • CTGF, PDGF, EGF, bFGF • ET-1 • ACE • IGF-1 • Cadherin 11 • Flagellin • ROS • Altered ECM composition (e.g. hyaluronan) • Increased ECM Stiffness • …	• IFN-α • IFN-γ • PGE2 • MMPs • IL-7, IL-10, IL-12 • Altered ECM composition (e.g. MFGE8) • …

ACE, Angiotensin converting enzyme; bFGF, basic fibroblast growth factor; CTGF, connective tissue growth factor; ECM, extracellular matrix; EGF, epidermal growth factor; ET, endothelin; IFN-γ, Interferon gamma; IGF-1, insulin-like growth factor 1; IL, interleukin; MFGE8, milk fat globule-EGF factor 8; MMP, matrix metalloproteinase; PDGF, platelet derived growth factor; PGE2, prostaglandins; ROS, reactive oxygen species; TGF-β1, transforming growth factor-beta 1; TL1A, tumor necrosis factor-like cytokine 1A.

All these mechanisms drive fibroblast differentiation into activated myofibroblasts, resulting in excessive ECM production, tissue remodeling, and eventually the formation of strictures in CD[281,304,306,316,317] (Figure 22).

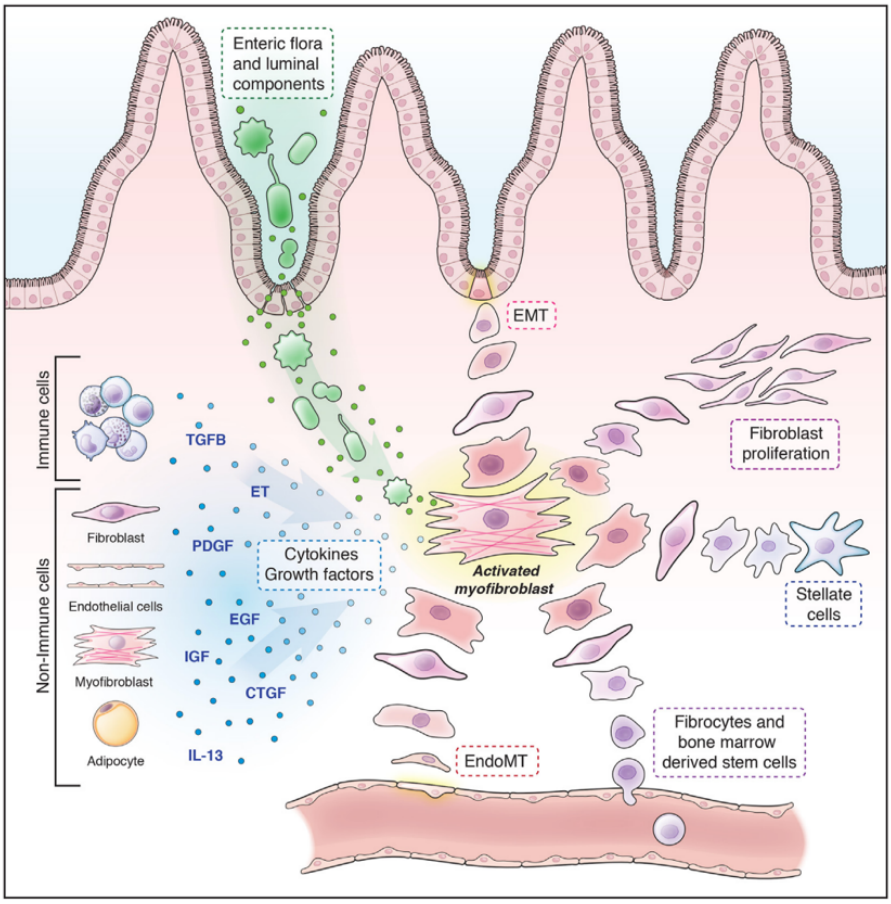

Figure 22. Pathophysiology of intestinal fibrosis: Soluble factors (red) and different origins of mesenchymal cells (blue)[290]

CTGF: connective tissue growth factor; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; EndoMT: endothelial-to-mesenchymal transition; ET: endothelins; IGF, Insulin-like growth factor; IL-13, Interleukin 13; PDGF: platelet-derived growth factor; TGF-β, transforming growth factor beta.

Recent findings, including these from single-cell RNA sequencing, have challenged the traditional view that myofibroblasts are the sole source of ECM by revealing fibroblast heterogeneity and emphasizing the role of *muscularis propria* thickening as a predominant factor contributing to luminal obstruction[283,318–322]. Single-cell analyses have transformed the understanding of fibrotic diseases by revealing the transcriptional diversity of mesenchymal

and other cell types involved in fibrosis across various organs[323]. In IBD, distinct subsets of fibroblasts have been identified, with some showing increased activity and numbers in strictured tissues compared to non-strictured ones. Fibroblasts continue to emerge as central players in stricturing CD, acting as both signal senders and receivers, although their precise distribution within the intestinal layers remains to be determined[305,324].

1.3.4.2. Inflammatory and immune drivers

Although potent anti-inflammatory therapies can reduce acute inflammation, they do not prevent the progression of fibrosis once initiated[325]. Acute inflammatory events, leading to epithelial apoptosis and necrosis, activate mechanisms of tissue repair that, when sustained, result to deleterious exacerbated ECM accumulation[314]; the reasons why certain injuries resolve while others progress to fibrosis remain unclear.

Chronic inflammatory damage initiates a cascade of cellular and molecular events driving ECM deposition[326]. Pro-inflammatory cytokines such as TNF-α, IL-13, IL-17, and TL1A activate fibroblasts and myofibroblasts, while additional immune signals promote the EndoMT and EMT[309,327]. Ongoing inflammation sustains fibrosis by maintaining these mesenchymal conversions and reinforcing cytokine-driven activation of fibroblasts and myofibroblasts[309,328]. Moreover, eosinophil activation contributes to the inflammatory milieu through degranulation and cytokine release, although its precise role in fibrogenesis remains to be fully elucidated[329].

Importantly, the removal of inflammatory stimuli does not necessarily halt the fibrotic process, which appears to become self-perpetuating once established[304–306,311,330]. This underscores the need for antifibrotic strategies beyond inflammation control.

1.3.4.3. Microbial, oxidative and structural influences

In addition to inflammation, non-inflammatory factors such as genetic predisposition, mechanical stress, and dysregulated cellular signaling significantly contribute to the progression of intestinal fibrosis[284,331]. The gut microbiota and redox imbalances have emerged as key determinants in the development of intestinal fibrosis.

Dysbiosis can directly affect fibroblast function, with specific bacterial taxa differentially associated with fibrotic changes in various gut segments[314,331]. For example, beneficial microbes like *Faecalibacterium prausnitzii* have been shown to be negatively associated with fibrosis in mice[327,331]. Dysbiosis can lead to elevated levels of TGF-β1 in intestinal tissues, thereby promoting collagen deposition and fibrosis, a process that can be mitigated by antibiotic treatment or inhibition of TGF-β signaling[314].

In parallel, an imbalance between reactive oxygen/nitrogen species production and antioxidant defenses activates multiple signaling pathways and transcription factors that drive fibrogenesis by enhancing the production of profibrotic mediators and impeding ECM degradation[314,331,332].

Moreover, emerging evidence suggests that short-chain fatty acid-producing bacteria, in concert with lactic acid bacteria, may exert anti-fibrotic effects through the induction of Peroxisome proliferator-activated receptor gamma (PPAR-γ), representing a promising target for therapeutic intervention[333].

Another hallmark of fibrotic remodeling is the imbalance between matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]). In normal tissue homeostasis, ECM undergoes continuous remodeling by MMPs, which can be activated by antifibrotic mediators and are regulated by TIMPs[302,334]. In intestinal fibrosis, this delicate balance is lost: MMP expression decreases while TIMPs are overexpressed, resulting in excessive ECM deposition[335].

Figure 23 provides an overview of the sequence leading from tissue injury to fibrosis[311].

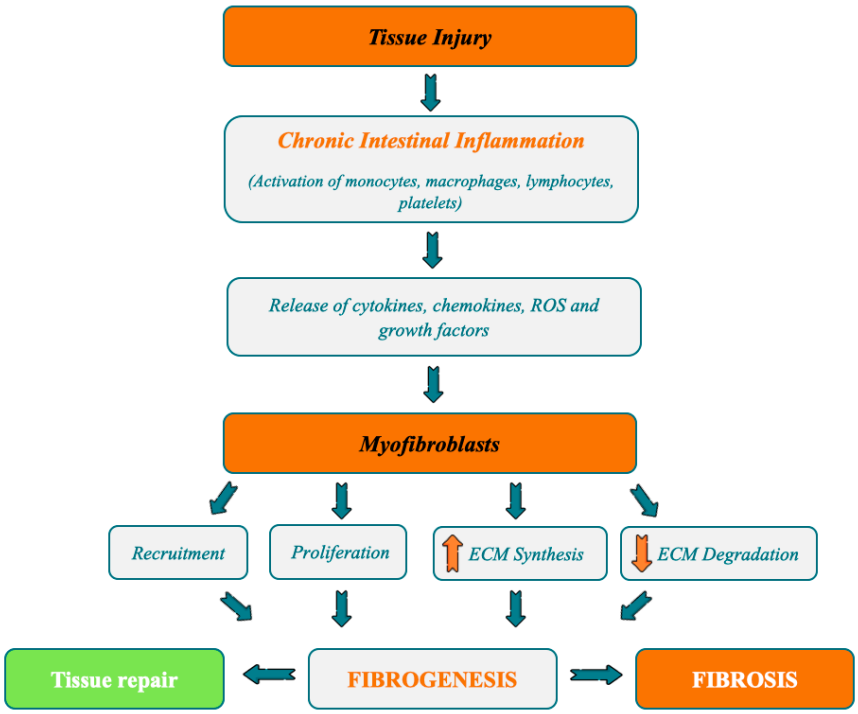

Figure 23. Sequence of events occurring from tissue injury to fibrosis (adapted from Latella et al. 2013)[311]

ECM, Extracellular matrix; ROS, reactive oxygen species.

Recent studies also have implicated creeping fat in promoting *muscularis propria* thickening in CD through the release of long-chain free fatty acids, which drive smooth muscle hyperplasia[336]. Additionally, fibroblast subsets within creeping fat display inflammatory and fibrotic signatures, further implicating mesenteric adipose tissue in tissue remodeling[337].

1.3.4.4. Role of the endoplasmic reticulum stress

As mentioned above, chronic ER stress and impaired UPR in intestinal epithelial cells contribute to IBD by compromising barrier function and promoting inflammation and cell death[92–95,338,339]. Evidence from other organ systems underscores the role of ER stress in driving fibrotic remodeling[338,340]. For instance, in the kidney, prolonged ER stress coupled with oxidative stress, shifts normal fibroblasts toward a fibrotic phenotype[341]. Similarly, in obesity-related cardiovascular fibrosis in rats, mitochondrial oxidative stress and ER stress activation lead to elevated TGF-β1 levels and ECM accumulation[342]. In the lung, ER stress due to

misfolded surfactant proteins predisposes alveolar epithelial cells to apoptosis and EMT, thereby amplifying fibrogenesis following additional injurious stimuli[343]. The inhibition of the ER stress-induced IRE1α signaling pathway has been shown to block TGF-β-induced myofibroblast activation *in vitro*, attenuate fibrosis *in vivo*, and reverse the fibrotic phenotype of activated myofibroblasts in animal models of skin and liver fibrosis[344].

Translating these insights to the intestine, recent studies reveal that ER stress in both subepithelial myofibroblasts and intestinal epithelial cells plays a direct role in fibrotic progression. Li *et al.* demonstrated that ER stress in subepithelial myofibroblasts enhances TGF-β1 activation through increased GRP78 (BiP) binding and subsequent transcriptional upregulation via ATF6α and XBP1, thereby promoting collagen deposition[345]. Similarly, Vieujean *et al.* showed that ER stress in intestinal epithelial cells leads to the intracellular accumulation of AGR2 and BiP and subsequent secretion of this ERS-related protein – eAGR2 – which, through paracrine signaling, induce fibroblast-to-myofibroblast differentiation[346]. Currently these observations are replicated using organoids derived from IBD patients[347].

Collectively, these studies suggest that, as in other organs, ER stress within the intestinal microenvironment not only exacerbates inflammation but also directly contributes to fibrogenesis, highlighting its potential as a therapeutic target to prevent fibrostenotic complications in IBD[348].

ER stress plays a dual role in fibrosis, acting both as a driver of apoptosis in IECs and as a pro-fibrotic signal. In IECs, prolonged ER stress leads to cell death and barrier dysfunction, while in fibroblasts and myofibroblasts, ER stress promotes cell survival and ECM production through adaptive UPR mechanisms[348]. This imbalance suggests that therapeutic strategies targeting ER stress must be cell-type specific. For instance, selective inhibition of ER stress in fibroblasts may reduce collagen deposition, while preserving ER stress responses in epithelial cells could help restore barrier integrity and prevent apoptosis-driven remodeling[338,348]. Furthermore, AGR2 represents an attractive therapeutic target due to its dual function in epithelial homeostasis and fibrogenesis. Strategies aimed at modulating AGR2 secretion could potentially prevent EMT and fibroblast activation, while preserving its role in tight junction maintenance and ER proteostasis[349–353].

1.3.4.5. Role of the intestinal epithelium

Previous studies highlight the crucial role of the intestinal epithelium in the progression of fibrosis, demonstrating that ER stress in IECs drives fibrosis through both paracrine signaling and direct EMT[310,346,348]. The intestinal epithelium is highly susceptible to ER stress due to its high protein synthesis demands and constant exposure to luminal antigens[343,346].

When ER homeostasis is disrupted, IECs undergo significant changes that contribute to fibrosis, including the secretion of pro-fibrotic cytokines, increased apoptosis leading to loss of epithelial integrity and the induction of EMT. EMT is a critical process in which epithelial cells progressively lose their polarity and cell-cell adhesion properties, acquire mesenchymal features such as migratory capacity and, upon differentiation into fibroblasts, gain the ability to produce ECM[310,354,355]. This process is mediated by transcription factors, which repress epithelial markers and upregulate mesenchymal markers such as α-SMA and vimentin[332] (Figure 24). The result is the transformation of IECs into a fibroblast-like phenotype, contributing directly to the pool of myofibroblasts responsible for excessive collagen deposition and fibrosis. TGF-β1 is a well-known inducer of EMT and plays a central role in intestinal fibrosis[333]. In CD, TGF-β1-mediated EMT has been demonstrated in intestinal tissues, with lineage-tracing studies confirming that IECs transition into myofibroblast-like cells during fibrotic progression[310]. Moreover, this process is exacerbated by chronic inflammation, which sustains TGF-β1 signaling through the recruitment of immune cells and the release of additional pro-fibrotic mediators[333].

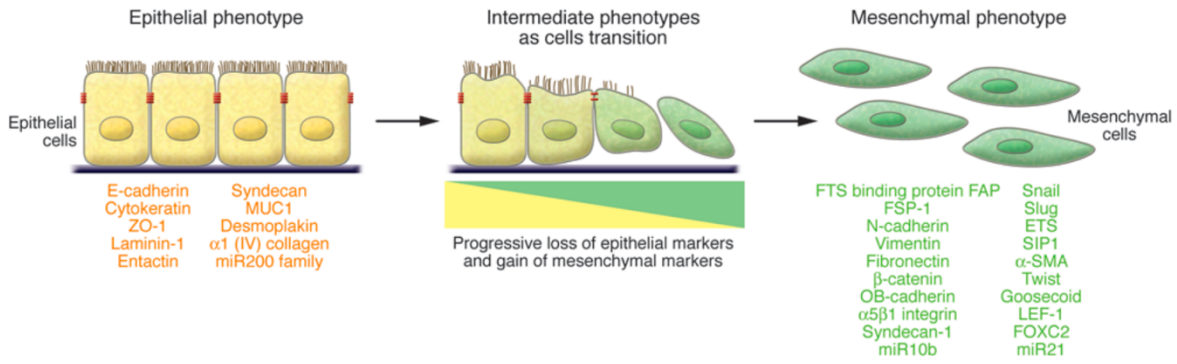

Figure 24. Epithelial-to-mesenchymal transition[355]. The epithelial and mesenchymal cell markers commonly used by EMT researchers are listed. Colocalization of these two sets of distinct markers defines an intermediate phenotype of EMT, indicating cells that have passed only partly through an EMT.

α-SMA, alpha-smooth muscle actin; ETS, E transformation specific; FAP, fibroblast activation protein; FOXC2, forkhead box C2; FTS, Fused toes homolog; LEF-1, enhancer-binding factor/T-cell factor 1; miR, micro RNA; *MUC1*, mucin 1; OB-Cadherin, osteoblast cadherin; *ZO-1*, zona occludens 1.

Recent studies have also highlighted the involvement of ERS-related proteins in EMT and fibrosis. AGR2, highly expressed in IECs, is secreted into the extracellular space under ER stress conditions, where it modulates EMT by interacting with TGF-β/Smad signaling[349,356]. AGR2 has been shown to suppress epithelial markers (E-cadherin) while enhancing mesenchymal markers (vimentin, α-SMA), thereby promoting the transition toward a fibrotic phenotype[356]. Furthermore, secreted AGR2 (eAGR2) can act in a paracrine manner on surrounding fibroblasts, accelerating myofibroblast activation and ECM production[346].

In CD, epithelial barrier dysfunction is also a hallmark of disease progression. IECs maintain mucosal integrity through tight junction proteins, which are notably regulated by AGR2. Under certain conditions, AGR2 expression is reduced, leading to tight junction disassembly and increased permeability[357]. This loss of barrier function allows for luminal antigens and microbiota-derived factors to penetrate deeper layers of the intestine, activating TGF-β1-driven fibrotic pathways[333]. *AGR2*-deficient mice exhibit severe ileitis, increased ER stress markers, and a compromised mucosal barrier integrity, underscoring its role in epithelial homeostasis and fibrosis prevention[350]. In humans, however, AGR2 expression data in UC are conflicting: while some studies report decreased expression compared to controls, others have shown increased levels during both active disease and remission[358,359]. Taken together, these findings

suggest that impaired regulation – not simply loss – of AGR2 may contribute to epithelial dysfunction, highlighting the complexity of its role in intestinal inflammation and fibrogenesis.

In summary, the intestinal epithelium actively contributes to fibrosis through EMT, paracrine signaling, and barrier dysfunction, with ER stress playing a pivotal role in these processes. TGF-β1-driven EMT transforms IECs into a pro-fibrotic phenotype, while ER stress-induced AGR2 secretion enhancing fibroblast migration and activation. Simultaneously, ER stress-induced apoptosis and tight junction disruption weaken the epithelial barrier, sustaining chronic inflammation and fibrosis. Understanding these interconnected mechanisms, targeting epithelial stress responses and mesenchymal transitions, particularly AGR2 and TGF-β1, may offer therapeutic benefits in preventing fibrostenotic complications in CD.

SCIENTIFIC RATIONALE FOR STUDYING PEDIATRIC CROHN'S DISEASE AND INTESTINAL FIBROSIS, AND OBJECTIVES OF THIS THESIS

2. SCIENTIFIC RATIONALE FOR STUDYING PEDIATRIC CROHN'S DISEASE AND INTESTINAL FIBROSIS, AND OBJECTIVES OF THIS THESIS

Understanding the distinct course of pediatric IBD remains a key scientific challenge. Despite growing evidence of unique clinical, pathological, and genetic features in PIBD, particularly VEO-IBD[18,30,123,134,233,257], the progression of disease phenotypes – especially the development of intestinal fibrosis – remains poorly understood. The heterogeneity in disease behavior and fibrosis onset across different age groups raises unanswered questions about the roles of age, genetics, and environment in shaping disease trajectory.

While pediatric CD is often considered more aggressive than adult-onset forms, data on disease progression remain inconsistent. Some studies report a high rate of complications, particularly in older children, with a significant proportion developing stricturing or penetrating disease within a few years of diagnosis[135,169,171,188]. However, other reports suggest that pediatric patients may follow a disease course similar to that of adults[188]. These discrepancies highlight the need for a better understanding of how the disease evolves in younger patients.

In parallel, the characterization of intestinal fibrosis in pediatric CD remains insufficient. Despite its clinical impact, particularly in children[28,193], the mechanisms leading to fibrosis and potential age-related differences compared to adults are still unclear. A better understanding of these mechanisms could help explain the observed discrepancies in disease progression and support the identification of early therapeutic targets in the future.

Thus, the objectives of this work are:

1. to assess the evolution of incidence rates and disease phenotypes of VEO-IBD and EO-IBD over the last decades;
2. to assess the progression of disease phenotypes over the disease course in this population of EO-IBD and VEO-IBD;
3. to describe the expression of ERS-related proteins in intestinal tissues of pediatric and adult CD patients and compare normal tissues with inflammatory and fibrotic areas.

By combining epidemiological analysis with a pathological study focusing on fibrosis, this work aims to improve our understanding of disease heterogeneity in pediatric CD and to explore the association of specific proteins with different stages of fibrotic progression in tissues. While

the first two objectives focus exclusively on the pediatric population, the third incorporates a comparative approach to highlight molecular differences between pediatric and adult tissues.

INCIDENCE AND PHENOTYPE AT DIAGNOSIS OF VERY EARLY-ONSET COMPARED WITH LATER-ONSET PEDIATRIC INFLAMMATORY BOWEL DISEASE: A POPULATION-BASED STUDY (1988–2011)

3. INCIDENCE AND PHENOTYPE AT DIAGNOSIS OF VERY EARLY-ONSET COMPARED WITH LATER-ONSET PEDIATRIC INFLAMMATORY BOWEL DISEASE: A POPULATION-BASED STUDY (1988–2011)

Publication in Journal of Crohn's and Colitis, 2017, 519-526[126]
doi:10.1093/ecco-jcc/jjw194
Refer to Appendix 1 for the journal article

3.1. ABSTRACT

Background and Aims: Very early-onset inflammatory bowel disease (VEO-IBD) is a form of IBD that is distinct from that of children with an older onset. We compared changes over time in the incidence and phenotype at diagnosis between two groups according to age at IBD diagnosis: VEO-IBD diagnosed before the age of 6 years, and early-onset IBD (EO-IBD) diagnosed between 6 and 16 years of age.

Methods: Data were obtained from a cohort enrolled in a prospective French population-based registry from 1988 to 2011.

Results: Among the 1412 pediatric cases (< 17 years), 42 (3%) were VEO-IBD. In the VEO-IBD group, the incidence remained stable over the study period. In contrast, the incidence of EOIBD increased from $4.4/10^5$ in 1988–1990 to 9.5/105 in 2009–2011 (+116%; $p< 10^{-4}$). Crohn's disease (CD) was the most common IBD, regardless of age, but ulcerative colitis (UC) and unclassified IBD were more common in VEO-IBD cases (40% *vs.* 26%; $p= 0.04$). VEO-IBD diagnosis was most often performed in hospital (69% *vs.* 43%; $p< 10^{-3}$). Rectal bleeding and mucous stools were more common in patients with VEO-IBD, whereas weight loss and abdominal pain were more frequent in those with EO-IBD. Regarding CD, isolated colonic disease was more common in the VEO-IBD group (39% *vs.* 14%; $p= 0.003$).

Conclusions: In this large population-based cohort, the incidence of VEO-IBD was low and stable from 1988 to 2011, with a specific clinical presentation. These results suggest a probable genetic origin for VEO-IBD, whereas the increase in EO-IBD might be linked to environmental factors.

3.2. INTRODUCTION

Inflammatory bowel diseases (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), are multifactorial chronic disorders evolving with a relapsing and remitting course. It is generally accepted that genetic susceptibility, environmental factors, and changes in the gut microbiota cause excessive innate and adaptive immune responses[360–362]. Pediatric-onset IBD represents 8-25% of cases of IBD[363,364]. The incidence of pediatric-onset IBD is increasing, especially in industrialized countries, and children are now being diagnosed at a younger age[124,259]. A small number of monogenic mutations[225,365–367] have been identified in children with IBD diagnosis at a very young age, but genome-wide association studies failed to detect large differences between adult-onset and pediatric-onset disease[140,141]. Several studies reported different disease phenotypes in children with a diagnosis of IBD before 10 years of age compared with children aged over 10 years[134,257,364], adolescents, or adults, leading to the Paris modification of the IBD Montreal classification, differentiating children with a diagnosis made before 10 years of age (A1a) from those with a diagnosis at 10-17 years (A1b)[14]. Thus, age at diagnosis is important clinically and it appears that very early-onset IBD (VEO-IBD) (age < 6 years at diagnosis) might be a distinct form. The phenotype of children with VEO-IBD is loosely defined but is usually considered as being more severe than when diagnosed later in life[171,368]. However, most published studies have not been population based but covered patients followed in referral centers, and the incidence and natural history of VEO-IBD are still poorly understood.

In this population-based study covering 1988–2011, we compared changes over time in the incidence and phenotype at diagnosis between VEO-IBD and early-onset (EO)-IBD (diagnosis at 6-16 years).

3.3. PATIENTS AND METHODS

3.3.1. Patient population and EPIMAD methodology

The study population included all children prospectively recorded in the EPIMAD registry with a diagnosis of definite or probable CD, UC, or unclassified inflammatory bowel disease (IBDU), diagnosed before 17 years of age from January 1988 to December 2011, according to validated and published diagnostic criteria[132,364,368–371]. The study population was divided into two groups according to age at diagnosis; VEO-IBD was defined as IBD diagnosed before 6 years of age, and EO-IBD was defined as IBD diagnosed between 6 and 16 years of age. The cut-off of 6 years was chosen based on previous studies[134,171,257,259].

The EPIMAD Registry is a prospective population-based study recording all cases of IBD documented since 1988 in Northern France (Figure 25). This study area includes 5 864 508 inhabitants, representing 9.3% of the total French population, and is divided into four administrative areas. The population distribution for those aged under 17 years is as follows: Nord, 593 837; Pas-de-Calais, 332 228; Somme, 115 969; and Seine-Maritime, 270 107; with a total of 1 312 141 children (2011 national population census data from the National Institute of Statistics and Economic Studies — INSEE 2011) (http://www.insee.fr/en/).

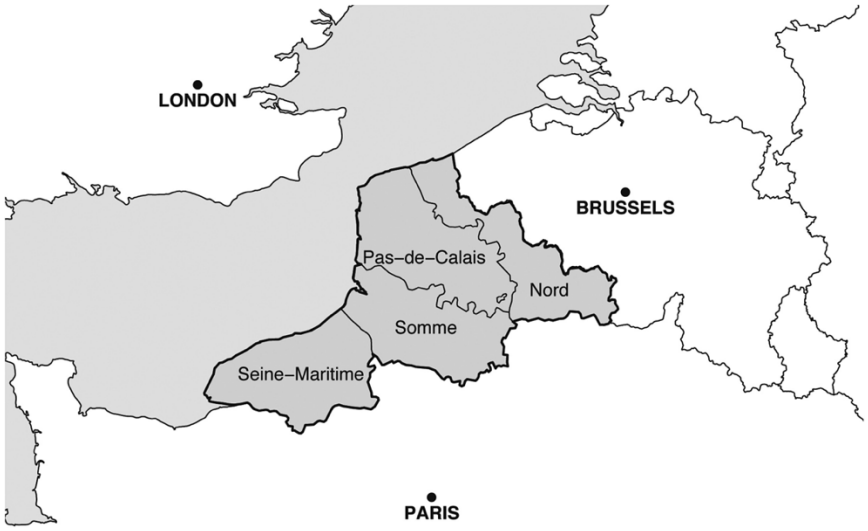

Figure 25. Map of France showing the study area of the EPIMAD Registry, which includes the Nord, Pas-de-Calais, Somme, and Seine-Maritime (Northern France)

The methodology of the EPIMAD Registry has been described in detail[132,364,368–371]. Briefly, data from all patients newly diagnosed with IBD are collected from all adult (n= 254) and pediatric (n= 15) gastroenterologists (GEs) practicing in the private and public sectors in these regions of France. Only residents of the studied areas at the time of diagnosis are included. Each GE reports all patients consulting for the first time with clinical symptoms compatible with IBD; he/she is contacted by phone at least three times a year by an interviewer who visits the GE's office and collects data from medical charts on a standardized questionnaire for each new case (Appendix 2). The data collected include age at diagnosis, gender, interval between the onset of symptoms and diagnosis, and clinical, radiological, endoscopic and histological findings at the time of diagnosis. Information on the management of each diagnosis is also recorded. The final diagnosis of IBD is established by two expert gastroenterologists and recorded as definite, probable, or possible CD or UC, according to previously published criteria[369]. Only definite and probable cases are considered for further analyses. Cases for which the diagnosis of IBD is probable, but without conclusive argument for differentiating CD from UC, are classified as IBDU.

Approval was obtained from the Ethics Committee of Lille University and Hospital, and this study followed the regulations and instructions set up by the *Comité National des Registres* (approval numbers 97 107 and 983 792).

3.3.2. Additional data collected for the present study

Data were extracted from the medical records of adult and pediatric GEs, and were collected in standardized questionnaires. Sociodemographic and clinical characteristics at diagnosis were collected: age, gender, family history of IBD defined as any case of IBD in at least one family member of the first or second degree), time between onset of symptoms and diagnosis, symptoms, disease phenotype, and EIMs (defined as joint, skin, ocular, or hepato-biliary manifestations). IBD location and its phenotype at diagnosis were defined according to the Paris classification as described by Levine *et al.*[14].

For CD, the location and phenotype were defined as follows: Pure small bowel involvement (L1); pure colonic involvement (L2); or ileocolonic involvement (L3; L1 with cecal involvement was considered as L3); upper gastrointestinal disease (L4 that could be associated

with L1, L2, or L3); L4a (upper disease proximal to the ligament of Treitz); and L4b (upper disease distal to the ligament of Treitz and proximal to the distal one-third of the ileum were grouped as L4). CD phenotypes were classified as follows: inflammatory (non-stricturing and non-penetrating, B1); stricturing (B2); or penetrating (B3) disease. B2 and B3 behaviors were pooled and defined as 'complicated behavior'. The 'p' index could be added to the B1, B2, or B3 classes when concomitant perianal disease was present (including abscesses and/or fistulae).

For UC, the location was defined as follows: proctitis defined as involvement limited to the rectum (E1); left-sided colitis defined as involvement limited to the colorectum below the splenic flexure (E2); extensive colitis defined as involvement of the colorectum above the splenic flexure and below the hepatic flexure (E3); or pancolitis defined as involvement above the hepatic flexure (E4).

To assess the further evolution of IBD phenotype over time adequately, only patients who had a complete bowel investigation (small and large bowel for CD and total colonoscopy for UC) were considered.

3.3.3. Statistical analysis

Incidence rates were computed as the number of incident cases (i.e. new diagnoses) divided by the population at risk. To identify any possible changes in the incidence of IBD, we divided the 24-year study into eight equal 3-year periods: 1988–1990, 1991–1993, 1994–1996, 1997–1999, 2000–2002, 2003–2005, 2006–2008, and 2009–2011. The mean annual incidence rates were calculated for each 3-year period and for the entire study period, and are presented with their 95% confidence intervals (CIs). Incidence rates were determined in the overall population and in subgroups according to age categories (< 6 or 6-16 years) and gender. For each of the four administrative areas, population data by age and gender were obtained using yearly estimations of population obtained from INSEE, and based on a mixed procedure exhaustive census before 2004 and random sampling after 2004. Temporal trends in incidences over time were tested by means of log-linear Poisson regression analyses taking overdispersion and person-years at risk into account (introduced as an offset variable after log transformation).

Qualitative variables were expressed as frequencies and percentages and 95% CIs. For comparing qualitative variables between age groups, we used chi-square or Fisher's exact test

according to the number of expected events. Analyses were performed with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical significance was accepted at $p \leq 0.05$.

3.4. RESULTS

3.4.1. Incidence

From 1988 to 2011, 1412 children with a diagnosis of IBD before the age of 17 years were included in the EPIMAD registry (8% of all IBD cases). Among them, 42 (3% of all pediatric IBD cases) were diagnosed before the age of 6 (VEO-IBD), with six children (14% of those with VEO-IBD) before the age of 1 year and 13 children (31% of those with VEO-IBD) aged 2 years or younger. A total of 1370 IBD cases were diagnosed between the ages of 6 and 16 years and were considered as EO-IBD; 52% of the patients were male, with no significant difference between the two age groups. In the VEO-IBD group, the incidence of IBD over the entire study period (1988–2011) was $0.40/10^5$ (95% confidence interval: 0.30-0.50) including $0.25/10^5$ for CD (0.10-0.30), $0.12/10^5$ for UC (0.06-0.20), and $0.03/10^5$ for IBDU (0.00-0.06). In the EO-IBD group, the incidence of IBD was $6.4/10^5$ (95% CI 6.0-6.7), including $4.7/10^5$ for CD (4.4-5.0), $1.5/10^5$ for UC (1.4-1.7), and $0.2/10^5$ for IBDU (0.1-0.3) during the same period. The overall incidence of pediatric-onset IBD increased from $3.0/10^5$ in 1988–1990 to $6.3/10^5$ in 2009–2011 (+ 110%; $p< 10^{-4}$ by Poisson regression). In the VEO-IBD group, the incidence remained stable (not significant; $p= 0.14$ by Poisson regression) during the whole period, whereas the incidence of EO-IBD increased from 4.4 to $9.5/10^5$ (+ 116%; $p< 10^{-4}$ by Poisson regression) during the same period (Figure 26).

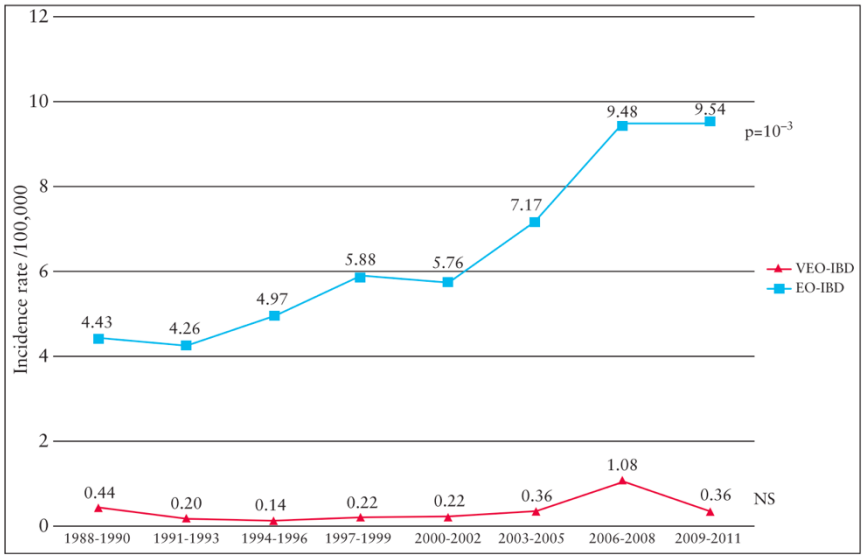

Figure 26. Incidence of very early-onset (< 6 years) inflammatory bowel disease (VEO-IBD) and early-onset (6-16 years) inflammatory bowel disease (EO-IBD), indicated by 3-year consecutive periods from 1988 to 2011 in Northern France

The increasing incidence in the EO-IBD group was noteworthy for cases of early-onset UC (EO-UC) and EO-CD, whereas the incidences of very early-onset UC (VEO-UC) and VEO-CD remained stable during the study period (Figure 27).

Figure 27. Incidence of very early-onset (< 6 years) Crohn's disease (VEO-CD) and early-onset (6-16 years) Crohn's disease (EO-CD), and very early-onset ulcerative colitis (VEO-UC) and early-onset (6-16 years) ulcerative colitis (EO-UC), indicated by 3-year consecutive periods from 1988 to 2011 in Northern France

3.4.2. IBD classification at diagnosis

In the VEO-IBD group, 60% had CD (n= 25), 33% had UC (n= 14), and 7% had IBDU (n= 3). In the EO-IBD group, 74% had CD (n= 1,007), 24% had UC (n= 329), and 2% had IBDU (n= 34). The distribution of cases according to diagnosis was significantly different (p= 0.04) between the two age groups, with UC and IBDU more frequent in the VEO-IBD group than in the EO-IBD group (40% *vs.* 26%) and CD more common in the EO-IBD group than in the VEO-IBD group (74% *vs.* 60%) (Figure 28).

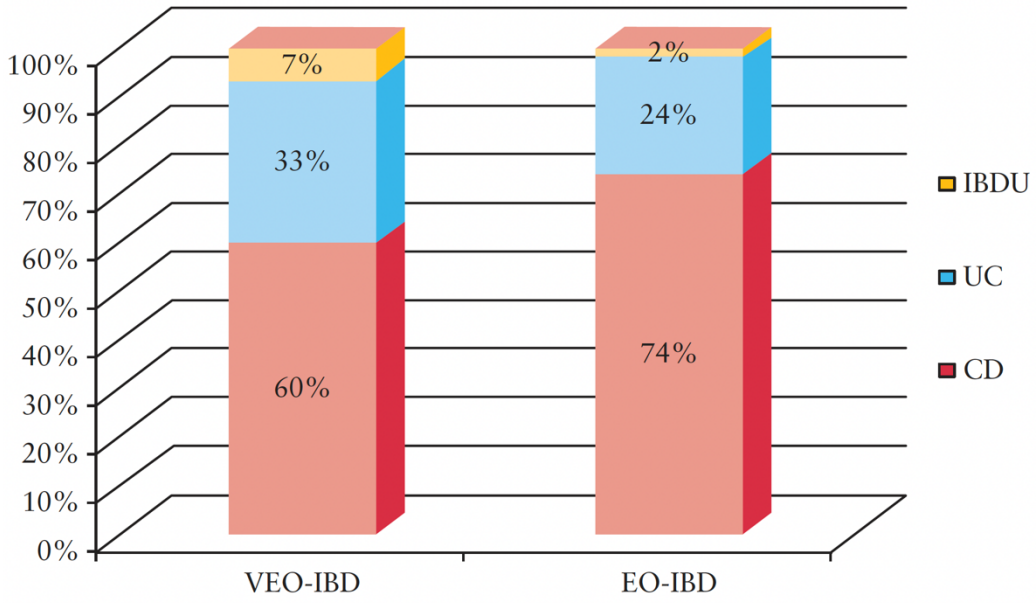

Figure 28. Distribution of inflammatory bowel disease (IBD): Crohn's disease (CD), ulcerative colitis (UC), and inflammatory bowel disease unclassified (IBDU) in very early-onset (< 6 years) IBD (VEO-IBD) (n= 42) and in early-onset (6-16 years) IBD (EO-IBD) (n= 1370), issued through the population-based EPIMAD Registry between 1988 and 2011

3.4.3. IBD phenotype at diagnosis

In cases of CD, isolated colonic disease (L2) was significantly more frequent in the VEO-IBD group (n= 9; 39%) than in the EO-IBD group (n= 128; 14%; p= 0.003; Table 5). Involvement of the proximal gastrointestinal tract (L4) was similar in the two age groups (32%, n= 8 in the VEO-IBD group and 35%, n= 355 in the EO-IBD group; p= 0.74). At diagnosis, there was no significant difference between the two age groups regarding the rates of complicated forms of CD; stricturing lesions (B2) or penetrating lesions (B3) were 13% (n= 3) in the VEO-IBD group and 22% (n= 208) in the EO-IBD group (p= 0.26), respectively. Anoperineal disease was present in 8% (n= 2) of the VEO-IBD group and 6% (n= 59) in the EO-IBD group (p= 0.66).

UC location at diagnosis was not different between the two age groups (p= 0.138). Regarding the location at diagnosis in the VEO-CD group or the VEO-UC group, no significant difference was found between children aged 2 years or younger (31% of the VEO-IBD group) and children aged 3-6 years.

3.4.4. Initial IBD clinical presentation

IBD diagnosis was more often performed in hospital in the VEO-IBD than in the EO-IBD group (69% *vs.* 43%; $p< 10^{-3}$). There was no significant difference in the prevalence of a family history of IBD between the two age groups. The time between the onset of symptoms and IBD diagnosis was not influenced by age at diagnosis in our cohort in any type of IBD. The initial clinical presentation was different according to age groups (Table 5).

Table 5. Comparison of socio-demographic characteristics, clinical presentation, disease phenotype, and location at diagnosis between VEO-IBD (< 6 years) (n= 42) and EO-IBD (6-16 years) (n= 1370)

Variables *N* [%]	VEO-IBD [< 6 years]	EO-IBD [6–16 years]	*p*-Value
All IBD [*N* = 1472]	42 [3%]	1370 [97%]	
Crohn's disease	25 [60%]	1007 [74%]	
Ulcerative colitis	14 [33%]	329 [24%]	0.05
IBD unclassified	3 [7%]	34 [2%]	
Male gender	22 [52%]	708 [52%]	0.93
Diagnosis in a hospital setting	29 [69%]	583 [43%]	< 0.001
Time between onset of symptoms and IBD diagnosis > 6 months	11 [27%]	407 [30%]	0.67
IBD family history	4 [10%]	210 [15%]	0.30
Diarrhoea	32 [76%]	899 [66%]	0.15
Rectal bleeding	34 [81%]	624 [46%]	< 0.0001
Mucous stools	17 [40%]	281 [21%]	0.002
Abdominal pain	18 [43%]	1013 [74%]	< 0.0001
Weight loss	9 [21%]	670 [49%]	< 0.001
EIMs	7 [17%]	231 [17%]	0.97
Crohn's disease [*N* = 1032]	25 [60%]	1007 [74%]	0.04
Diagnosis in a hospital setting	17 [68%]	455 [45%]	0.02
Rectal bleeding	17 [68%]	303 [30%]	< 0.0001
Pure colonic location [L2[a]]	9 [39%]	128 [14%]	0.003
Abdominal pain	12 [48%]	809 [74%]	< 0.0001
Weight loss	5 [20%]	566[56%]	< 0.001
EIMs	5 [20%]	208 [21%]	0.936
Ulcerative colitis [*N* = 343]	14 [33%]	329 [24%]	0.04
Diagnosis in a hospital setting	10 [71%]	112 [34%]	< 0.001
Abdominal pain	3 [21%]	180 [55%]	< 0.05
EIMs	1 [7%]	19 [6%]	0.576
Ulcerative proctitis [E1[a]]	1 [19%]	92 [30%]	0.138
Left-sided UC [E2]	N = 4 [36%]	N = 79 [26%]	
Extensive UC [E3]	N = 3 [27%]	N = 30 [10%]	
Pancolitis [E4]	N = 3 [27%]	N = 102 [33%]	

*According to the Paris Classification[14]
EIMs, extraintestinal manifestations; EO, early-onset; IBD, inflammatory bowel disease; VEO, very early-onset.

3.4.5. All IBD patients (n=1412)

Rectal bleeding and mucous stools were significantly more frequent in the VEO-IBD group than in the EO-IBD group (81% *vs.* 46%; $p< 10^{-4}$ and 40% *vs.* 21%; $p= 0.002$, respectively), whereas weight loss and abdominal pain were less common (21% *vs.* 49%; $p< 10^{-3}$ and 43% *vs.* 74%; $p< 10^{-4}$, respectively). There was no difference in the frequency of diarrhea or EIMs between the two age groups.

Diagnostic procedures (gastroscopy, total colonoscopy, ileoscopy, CT, and MRI scans) were performed as frequently in the VEO-IBD group as in the EO-IBD group.

3.4.6. CD patients (n= 1032)

CD diagnosis was more often performed at hospital in the VEO-CD group than in the EO-CD group (68% *vs.* 45%; $p= 0.02$). Only rectal bleeding was significantly more frequent in the VEO-CD group (68% *vs.* 30%; $p< 10^{-4}$) whereas weight loss and abdominal pain were less common in the VEO-CD group (20% *vs.* 56% and 48% *vs.* 80%, respectively; $p< 10^{-3}$).

3.4.7. UC patients (n= 343)

UC diagnosis was more often performed in hospital in the VEO-UC group than in the EO-UC group (71% *vs.* 34%; $p< 10^{-2}$). Only abdominal pain was less common in the VEO-UC group (21% *vs.* 55%; $p= 0.01$).

3.5. DISCUSSION

This population-based prospective study, conducted in a large pediatric cohort (n= 1412) over a 24-year period, showed that the incidence of EO-IBD increased by 116% in Northern France from 1988 to 2011 whereas the incidence of VEO-IBD remained stable during the same period. CD was the most common IBD in the two age groups, with a more frequent isolated colonic location in the VEO-IBD group. UC and IBDU were more common in the VEO-IBD group than in the EO-IBD group. The diagnosis of VEO-IBD was most often performed in hospital. Rectal bleeding and mucous stools were more frequent at diagnosis in the VEO-IBD group, reflecting a colonic location, whereas weight loss and abdominal pain were the most frequent clinical symptoms in the EO-IBD group.

Previous epidemiological data have shown a dramatic increase in pediatric-onset IBD worldwide[29,372–376]. We also found that the incidence of EO-IBD in our cohort, but not that of VEO-IBD, has been rising continuously since 1988. It is generally accepted that the influence of genetics in the pathogenesis of IBD is higher in children with VEO-IBD. It is unlikely that genetic factors have changed over a period of 24 years, as opposed to environmental factors. This could explain the increased incidence of IBD in those aged 6-16 years and the stability in the incidence of VEO-IBD. Table 6 shows the prevalence of VEO-IBD (with a diagnosis before the age of 5 or 6 years, depending on the series) reported since 2002[160,227,229,259,376,377]. Studies by Sawczenko *et al.* in 2003[160] and Benchimol *et al.* in 2014[134] showed a proportion of VEO-IBD similar to that in our population, namely 4% and 5%, respectively. This proportion was lower than the 6.5-15% reported previously. This wide range in the prevalence of VEO-IBD is probably associated with the study population, with higher prevalence rates being reported in studies from referral centers. In contrast to the stable incidence of VEO-IBD over time in our work, a Canadian study showed that the increased incidence of IBD from 1994 to 2009 was higher in these with VEO-IBD (< 6 years) (+ 7.4% average yearly change) than in those aged 10-16 years with IBD (+ 2.2% average yearly change)[134].

Our study was performed through a population-based registry, whereas Benchimol *et al.* applied a diagnosis algorithm through a health administrative database[134]. In addition, our study was focused on a specific region in France, a narrower area than that studied by Benchimol, which might have influenced the results. Therefore, our conclusions should be interpreted with caution.

Table 6. Comparison of prevalence of very early-onset (<6years) inflammatory bowel disease, Crohn's disease (CD), ulcerative colitis (UC), and IBD unclassified (IBDU) in the literature

Reference	Year	Country	Period	Method of data collection	Number of patients[a]	VEO-IBD[b] (%)	VEO-CD[c] (%)	VEO-UC[c] (%)	VEO-IBDU[c] (%)
Bequet	2016	France	1988–2011	General population	1412	3	60	33	7
Benchimol[134]	2014	Canada	1994–2002	Health administrative database	7143	5	33	56	11
Aloi[257]	2014	Italy	2009–2013	Hospital	506	11	18	59	22
Paul[377,d]	2006	USA	1995–2000	Hospital	413	10	N1	66	NA
Heyman[259]	2005	USA	2000–2002	Hospital	1370	15	36	40	24
Griffiths[227,d]	2004	Canada	1980–1999	Hospital	861	6,5	36	64	NA
Sawczenko[160,d]	2003	UK & Ireland	1998–1999	Monitoring Register[e]	739	4	31	38	12
Mamula[229,d]	2002	USA	1977–2000	Hospital	82 (< 5 years)	-	33	44	23

NA, not available

[a] Number of patients with pediatric-onset IBD [< 16 or 17 years]

[b] Percentage of VEO-IBD among pediatric-onset IBD

[c] Proportion of CD, UC, and IBDU in VEO-IBD

[d] In these studies, VEO-IBD is defined by a diagnosis before the age of 5 years.

[e] British Pediatric Surveillance Unit [BPSU], British Society of Gastroenterology Research Unit [BSGRU], and Pediatric Register Inflammatory Bowel Disease [PRIB]

VEO-CD, very early-onset Crohn's disease; VEO-IBD, very early-onset inflammatory bowel disease; VEO-IBDU, VEO-IBD, very early-onset unclassified inflammatory bowel disease; VEO-UC, very early-onset ulcerative colitis.

Although most represented in both age groups, CD was significantly more common in the EO-IBD group than in the VEO-IBD group; UC was significantly more common in the VEO-IBD group than in EO-IBD group in our study. However, CD represented 60% of IBD cases in our VEO-IBD group, unlike previous studies that reported a predominance of UC over CD in VEO-IBD[134,229,257,377]. This could have arisen from the diagnostic criteria used, as well as from specific environmental factors and lifestyle in the study area[147,378]. Complete bowel investigations were obtained as often in the VEO-IBD group as in the EO-IBD group and the diagnostic criteria (definite or probable IBD cases) did not change over time. Thus, the risk of misdiagnosing patients seems to have been low.

In children with VEO-IBD, the diagnosis was most often done at hospital. This was probably because of the lack of expertise and equipment – particularly endoscopy – for diagnosing IBD in very young children in an extra-hospital environment, as well as higher parental anxiety levels concerning the age of the child, leading parents to consult hospitals. Moreover, the time between the onset of symptoms and diagnosis was not delayed in the VEO-IBD group, whatever the type of IBD.

As previously reported[134,171,257–259,377], the initial presentation was different according to age group, with mucous bloody stools significantly more frequent in cases of VEO-IBD, probably because of the higher rate of isolated colonic disease in those with CD and a higher proportion of UC compared with older children. As noted by Gupta *et al.*[171], weight loss and abdominal pain were significantly more common in the EO-IBD group and this was also the case in our study. This was likely linked to a higher proportion of CD in this age group and the difficulties in expressing abdominal pain among very young children. Gupta *et al.*[171] also reported that the rates of EIMs at diagnosis of IBD were similar in both age groups. In those with CD, the rate of complicated behaviors (B2 or B3) at diagnosis was similar in both age groups in our study, which contrasts with Gupta *et al.*'s finding of a higher rate of complicated behaviors in those with IBD aged 6-16 years[171]. Upper gastrointestinal location (L4) and anoperineal lesions were found to be similar in both age groups. The rate of L4 among those with VEO-CD (32%) was similar to that reported by Aloi *et al.*[257] but much higher than that found by Heyman *et al.* (5%)[259]. In those with UC, the rates of ulcerative proctitis (E1) and extensive colonic involvement (E3/E4) were similar in both age groups. In the literature, VEO-UC has been studied less than VEO-CD, which currently limits the detection of significant phenotypic differences between the two age groups.

In our study, age at diagnosis of IBD was not linked to the presence of a positive family history, which contrasts with findings from referral centers[229,259,379] where it has been shown that severe cases of IBD, that are more often followed in referral centers, are more often associated with a IBD family history[380]. The differences between studies could also be explained by the ethnic variability in the study areas. For example, some North American cities included in previous studies have a large Jewish population, in which there is a known genetic susceptibility to IBD[227]. Some recent studies identified novel gene variants associated with all cases of IBD but also in those with VEO-IBD, and sequencing exomes could be a new diagnostic tool to identify variants in genes that could contribute to the pathogenesis of VEO-IBD[145,381–384].

Our study had some strengths and limitations. It was a large population-based study, had a long duration, used validated and published diagnostic criteria, and had a high-level data collection (96.5%)[369]. However, because of the small number of patients with VEO-IBD, the results should be interpreted with caution.

3.6. CONCLUSION

Our large pediatric-onset population-based study over a 24-year period showed stability in the incidence of VEO-IBD, with a classification into UC and IBDU more frequent than in those aged 6-16 years with IBD. The diagnosis of VEO-IBD was most often done at hospital and the initial presentation with colorectal symptoms was associated with a more frequent isolated colonic involvement in those with CD. Further longitudinal studies, especially genetic, are needed to increase the understanding of the pathogenesis of IBD and to help predict the subsequent course of these rare diseases in very young children and improve treatment strategies.

PHENOTYPIC EVOLUTION TOWARD A STRICTURING BEHAVIOUR IN PEDIATRIC CROHN'S DISEASE: DATA FROM THE EPIMAD COHORT *INSPIRED*

4. PHENOTYPIC EVOLUTION TOWARD A STRICTURING BEHAVIOUR IN PEDIATRIC CROHN'S DISEASE: DATA FROM THE EPIMAD COHORT *INSPIRED*

Short communication - Unpublished additional data

4.1. INTRODUCTION

Crohn's disease (CD) in children is characterized by a dynamic disease course, with phenotypic progression often occurring over time. In particular, the development of stricturing complications is a critical concern due to its impact on treatment strategy and long-term outcomes. Longitudinal studies have shown that the likelihood of developing a stricturing phenotype increases steadily over the disease course, with up to half of patients affected after two decades of follow-up[162,265,270,271]. This progression appears to occur at a similar rate in both pediatric and adult-onset CD, without major differences observed in the time to transition from a non-complicated (B1) to a stricturing (B2) or penetrating (B3) phenotype. Notably, the interval between diagnosis and the development of complications did not differ significantly by age at onset, suggesting that early-onset disease does not necessarily imply a faster progression but remains equally prone to structural damage over time[188].

However, pediatric CD is a heterogeneous condition, and very early-onset IBD (VEO-IBD), defined as diagnosis before age 6, represents a distinct subgroup accounting for 4 to 15% of pediatric cases[130,171,227]. While some children have milder forms, others develop severe and extensive disease, often with early complications. VEO-IBD may include monogenic forms, which can mimic classic CD but follow different biological pathways[231]. In this context, comparing early-onset and very early-onset disease might help clarify whether and how age at onset might influence disease progression. Our 2017 study found no difference in complication rates at diagnosis, underscoring the importance of long-term follow-up to capture divergent trajectories[126]. However, other studies have reported higher rates of complicated phenotypes in older children compared to those with VEO-IBD, highlighting inconsistencies in the literature[134,171,233,257]. Understanding these patterns is key for improving risk prediction and optimizing care in pediatric CD.

4.2. METHODS

Between 2018 and 2020, the EPIMAD group conducted a retrospective review of medical records for patients diagnosed with pediatric IBD between 1988 and 2011 to study the evolution of phenotypes and the disease course, considering the changes in therapeutic strategies over time *(Inspired* Cohort)[385]. Data were collected systematically at each patient visit, from diagnosis to the last follow-up, using a standardized reporting form. This included information on visit dates, EIMs, comorbidities, radiologic and endoscopic findings, treatments, hospitalizations, and surgeries. All data were subsequently reviewed by two expert gastroenterologists. Disease location and behavior were classified at each visit using the Paris classification. Patients were followed from diagnosis until the end of data collection (December 31, 2013), the date of loss to follow-up, or the date of death[167]. Disease behavior was assessed at diagnosis and over time and a chi-square test was used to evaluate whether phenotypic evolution over time was statistically significant. Kaplan-Meier analysis was used to estimate the cumulative probability of progression from a non-stricturing (B1) to a stricturing (B2) CD phenotype.

4.3. RESULTS

The initial cohort of the EPIMAD study included 1412 pediatric IBD cases, with 1032 diagnosed as CD. Due to loss to follow-up and errors in date records, the final cohort 1988–2011 reviewed between 2018 and 2020 consisted of 1007 CD patients. The median follow-up for these CD patients was 8.8 years [IQR, 4.6-14.2]. At diagnosis, 827 patients had a non-stricturing, non-penetrating phenotype (B1), while 145 presented with stricturing disease (B2) and 32 with penetrating disease (B3) (3 missing values). Over the follow-up period, 33.4% (276/827) B1 patients progressed to a stricturing B2 phenotype, highlighting the dynamic nature of pediatric CD and the propensity for disease progression toward a more severe form (Figure 29). Among them, 273/811 (33.7%) EO-CD patients and 3/16 (18.8%) VEO-CD patients progressed from B1 to B2, when the population was stratified by age group.

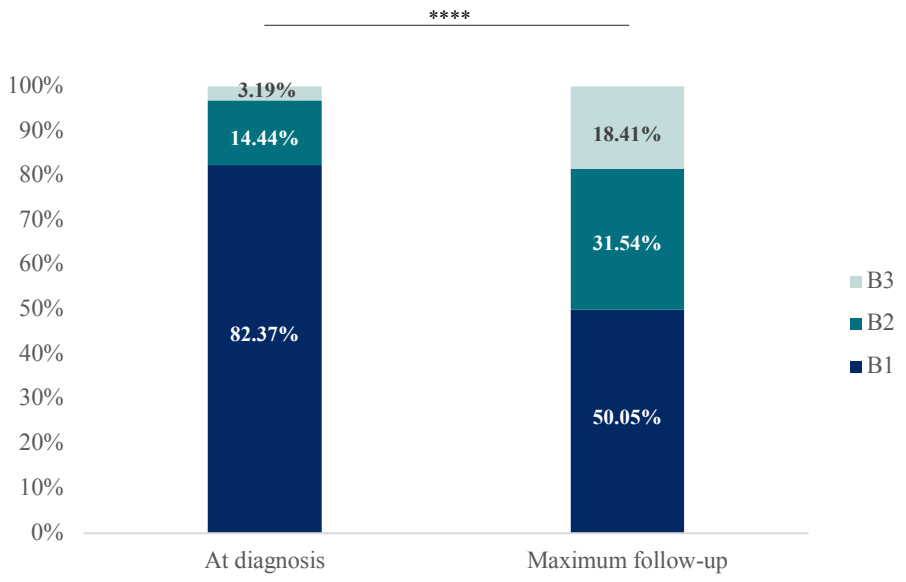

Figure 29. Phenotype progression of pediatric CD patients (cohort 1988–2011) from diagnosis to maximum follow-up (**=*p*<0.0001 - Chi-square test)**

The risk of progression to a stricturing phenotype was estimated using the Kaplan-Meier method to calculate the cumulative probability over time. Among the 827 patients initially diagnosed with a B1 phenotype, the cumulative probability of progressing to a stricturing B2 phenotype was 5.9% after one year of follow-up (95% CI, 4.3-7.6). This risk increased to 24.1%

after five years of follow-up (95% CI, 20.9-27.2) (Figure 30). When the population was stratified by age group, no progression was observed at one year among the 16 VEO-CD patients (0%, 95% CI, 0-0), with a five-year progression probability of 6.7% (95% CI, 0-18.5). In contrast, patients diagnosed after 6 years (n=811) showed a cumulative probability of 6.1% at one year (95% CI, 4.4-7.7) and 24.4% at five years (95% CI, 21.2-27.6) (log-rank p= 0.2).

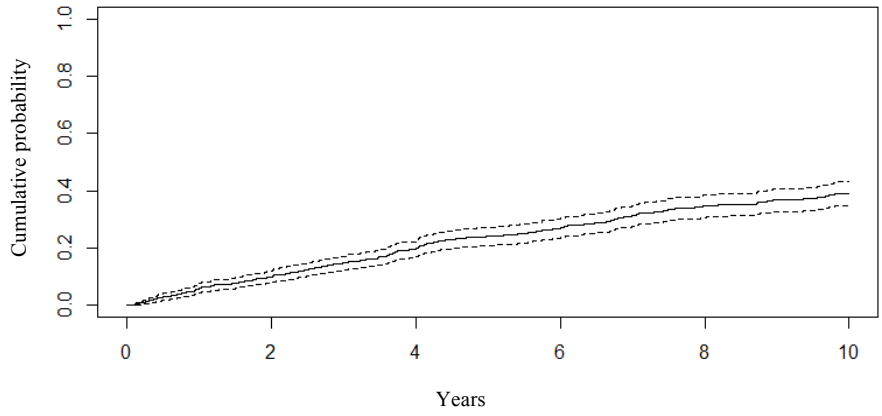

Figure 30. Kaplan-Meier curve depicting the cumulative probability of progression from B1 to B2 behavior over time in our pediatric CD cohort. The solid line represents the estimated cumulative probability of progression, and the dashed lines indicate the 95% confidence interval.

4.4. DISCUSSION

These results highlight the value of large-scale cohort studies in capturing phenotypic evolution and the substantial risk of progression to a stricturing phenotype over time, underscoring the need for long-term monitoring to better understand the disease's natural history and guide therapeutic decisions. In our cohort, nearly one out of three patients with an initial B1 phenotype progressed to B2 over a median follow-up of 8.8 years. These findings are consistent with previous pediatric studies, which report that 30-50% of children with CD develop stricturing or penetrating complications within 10 to 20 years of diagnosis[135,162,386].

While we have not included adult patients in this work, previous literature suggests that disease progression follows a comparable course in adult-onset CD, with similar timelines for the transition from inflammatory to complicated phenotypes[8,266].These parallels suggest that the mechanisms driving fibrostenotic complications may be shared across the different age groups considered, although the long-term impact of age at diagnosis remains an area for further investigation.

Interestingly, the lower cumulative probability of progression in VEO-CD compared to EO-CD may reflect a slower or biologically distinct progression pathway. VEO-IBD is known to be a heterogeneous entity, sometimes involving monogenic disorders that can mimic CD but following different immune and fibrotic mechanisms[146,235]. Although the difference between groups did not reach statistical significance, the small number of VEO-IBD cases limits the power to detect subtle differences. Further studies with larger early-age cohorts are warranted to clarify age-related phenotypic progression patterns.

Taken together, these findings highlight the importance of early identification of patients at risk of fibrostenotic complications. Given that fibrosis tends to be irreversible and a leading cause of recurrent surgery in CD, anticipating its development is crucial. Future work should aim to better characterize the biological and clinical factors associated with fibrotic progression in pediatric CD, including age of onset, genetic background, immune dysfunction, and microbial influences. In particular, prospective studies integrating clinical data with molecular biomarkers could support the development of predictive tools and targeted early interventions.

4.5. CONCLUSION

These data confirm the progressive nature of pediatric CD, with a significant proportion of children initially presenting with an inflammatory phenotype developing stricturing complications over time. Although the risk appears lower in VEO cases, disease progression remains an important concern across all pediatric age groups. These results underscore the need for sustained, individualized follow-up and reinforce the value of large-scale prospective cohorts to inform precision medicine approaches in PIBD.

DISTRIBUTION OF EPITHELIAL ENDOPLASMIC RETICULUM STRESS-RELATED PROTEINS IN ADULT AND PEDIATRIC CROHN'S DISEASE: ASSOCIATION WITH INFLAMMATION AND FIBROSIS

5. DISTRIBUTION OF EPITHELIAL ENDOPLASMIC RETICULUM STRESS-RELATED PROTEINS IN ADULT AND PEDIATRIC CROHN'S DISEASE: ASSOCIATION WITH INFLAMMATION AND FIBROSIS

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Refer to Appendix 3 for the journal article

5.1. ABSTRACT

Background and Aims: Intestinal strictures, a debilitating complication of Crohn's disease (CD) in both pediatric and adult populations, are largely driven by fibrosis. Current treatments focus on inflammation, but are less effective against fibrosis. Endoplasmic reticulum stress (ERS)-related proteins, including protein disulfide isomerases, may be involved in fibrosis; however, their roles in CD remain unclear. This study aimed to evaluate the distribution of ERS-related proteins (AGR2, BiP, PDIA6, ERP44) in the intestinal epithelium of pediatric and adult CD patients and potential associations with fibrosis and inflammation.

Methods: We retrospectively analyzed patients from four hospitals (2009–2023). CD patients with strictures were compared with CD patients without strictures, non IBD controls, and ulcerative colitis patients. Intestinal tissues were analyzed using immunohistochemistry to assess ERS-related protein distributions. Inflammation and fibrosis were evaluated using H&E and Masson's trichrome staining. Correlations between protein distribution, inflammation and fibrosis were examined.

Results: We analyzed 224 patients and 815 intestinal samples. AGR2 and BiP were significantly increased in the epithelium of fibro-inflammatory and fibrotic intestinal tissues, especially in pediatric-onset CD. PDIA6 was upregulated in CD compared to non IBD without any correlation with fibrosis. ERP44 was associated with fibrosis exclusively in pediatric CD. Significant differences in protein distributions were observed between pediatric- and adult-onset CD, as well as between ileum and colon.

Conclusions: The distinct patterns of AGR2, BiP, PDIA6, and ERP44 in fibrotic and inflammatory intestinal tissues suggest their potential roles in CD-associated fibrosis, and should be explored as biomarkers or therapeutic targets for managing fibrosis in CD.

5.2. INTRODUCTION

Intestinal strictures are a common and debilitating complication of CD in both adult and pediatric populations[28,270]. Managing strictures is particularly challenging due to the lack of drug treatments that effectively target the initiation and progression of fibrosis[289], often leading to the need for surgery, accompanied by a high risk of fibrosis recurrence[271,292,295]. While recent works suggest that the need for surgery is decreasing with the use of immunomodulators and biologics[167,296,297], these medical treatments mainly control inflammation and do not specifically target fibrosis, nor have demonstrated significant effects on its progression[290,316,388].

The pathophysiology of intestinal fibrosis remains complex and poorly understood, partly due to the lack of suitable animal models replicating key features of the human disease[281,282,302,303,310,311]. Fibrosis in CD generally results from chronic inflammatory damage, but the factors that determine why some injuries heal and others progress to fibrosis are unclear. This process involves several cell types, cytokines, and growth factors, as well as key biological processes like epithelial- and endothelial-to-mesenchymal transitions (EMT and EndoMT)[309,310]. These mechanisms allow the differentiation of fibroblasts into activated myofibroblasts, leading to excessive extracellular matrix (ECM) secretion and deposition with tissue remodeling, ultimately resulting in stricture formation[281,304,306,316,317].

Exploring the role of the epithelium in fibrosis development is essential, as epithelial cells are key in maintaining homeostasis and the integrity of the mucosal barrier, particularly following injury[389]. To gain a deeper understanding of the epithelium's role in the development of intestinal fibrosis, a pilot proteomic study was conducted comparing the proteomes of ileal epithelial cells isolated from regions with varying degrees of fibrosis, taken from inflammatory fibro-stenosing resection specimens[346]. The findings revealed increased expression of several proteins in fibrotic regions, including those involved in ER stress, notably proteins with PDI activity[346]. However, this study was limited to adult patients whereas the mechanisms of fibrosis in children remain largely unexplored. Current literature suggests that pediatric-onset CD tends to be more severe, with complications arising earlier, yet little is known about the differences or similarities in the underlying histopathological mechanisms between pediatric and adult cases[169,188,370]. Therefore, this study aims to characterize the distributions of four specific endoplasmic reticulum stress (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]) within the intestinal epithelium. The analysis was

conducted in both ileal and colonic tissues across different conditions (non IBD and IBD patients, with tissue samples presenting a varying degree of inflammation and fibrosis), comparing the protein distribution between pediatric- and adult-onset CD.

5.3. METHODS

5.3.1. Patient enrolment

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

Patients were selected retrospectively through the databases of 4 hospitals from 2009 to 2023: University hospital of Liège (Belgium), Regional Hospital Center Citadelle of Liège (Belgium), University hospital of Lille (France) and MontLégia Hospital of Liège (Belgium). We first searched for pediatric (<17years) and adult (≥17years) CD patients who underwent intestinal resection and for which tissue material was available in the participating hospital biobanks. We selected patients who had radiological or endoscopic evidence of strictures with corresponding clinical manifestations. Control populations were selected to include tissues from adult and pediatric patients without IBD or with CD, but stricture-free (neither endoscopic nor radiological evidence) at the time and site of sampling. Based on preliminary results, we also included tissues from patients with UC to specifically study PDIA6 distribution. All ileal and colonic tissues were obtained from intestinal resections or endoscopic biopsies.

For all patients, we collected clinical data including gender, age at the time of sampling, age at IBD diagnosis, endoscopic and histological findings, disease location and duration, history of stricturing disease if applicable, treatment history, current treatments and the presence of biological inflammatory markers at the time of sampling[390].

5.3.2. Scoring inflammation and fibrosis

All tissues from intestinal resections or endoscopic biopsies were formalin-fixed, paraffin-embedded (FFPE)[121] and 4µm-thick slices were used for each block for hematoxylin & eosin (H&E) or Masson's Trichrome (MT) staining as well as for Immunohistochemistry (IHC).

Inflammation (I) and fibrosis (F) were scored on H&E sections with an expert gastrointestinal pathologist (N.B.) who was blinded to the clinical characteristics[390]. As no validated histological inflammation score exists for CD, we applied a detailed grading approach (aligned with European Crohn's and Colitis Organization [ECCO] key principles)[122]. Lymphoplasmocytic and neutrophils infiltrates were independently scored in the *lamina propria,* and these scores were used to quantify chronic and acute inflammation, respectively (0 = none, 1 = mild, 2 = moderate, 3 = severe)[391–393]. Crypt injuries (cryptitis and crypt abscesses), presence of edema, granulomas, and ulcerations were reviewed as previously described[278,390,394].

Intestinal fibrosis was graded initially on H&E sections[346], with confirmation on Masson's Trichrome-stained sections to highlight collagen accumulation (Staining Kit VWR 1004850001). A four grade scaling system was used, integrating parameters from previous works[278,283,285,325,394], as follows:

- F0: no architectural distortion, no ECM deposition or myofibroblast accumulation;
- F1: ECM and myofibroblast accumulation, preserved layers and increased submucosal thickness;
- F2: ECM and myofibroblast accumulation with preserved layers, densified ECM network, and increased submucosal thickness;
- F3: massive ECM and myofibroblasts deposition extending into the smooth muscle, with disruption of normal layers and evidence of transmural fibrosis.

Fibrosis scoring was exclusively applied to resection specimens, as biopsies were too limited in depth to provide accurate assessment[281].

5.3.3. Characterization of immunohistochemical signal of ERS-related proteins

Based on the findings of the pilot proteomic study[346], the proteins AGR2, BiP, ERP44 and PDIA6 were selected for further IHC characterization. IHC was performed as previously described[346,395] using commercial antibodies targeting AGR2 (Novus, Rabbit Dako-Agilent, 1:250), BiP (Cell Signaling, Rabbit Dako-Agilent, 1:500), ERP44 (Cell Signaling, Rabbit Dako-Agilent, 1:1000) and PDIA6 (Sigma, Rabbit Dako-Agilent, 1:2000). Positive and

negative controls were used to ensure technical staining reliability between batches. IHC scores were determined by at least two independent observers (EB, M-AM, CM and CS), using the same method, without prior knowledge of the clinical information, inflammation or fibrosis scores.

Due to the different cellular compositions and distributions along the crypt epithelium, three distinct zones were characterized in both the colon and the ileum, each graded separately (Figure 31):

1. Surface epithelium (SE),
2. Upper and intermediate portion of the crypts,
3. Bottom of crypts.

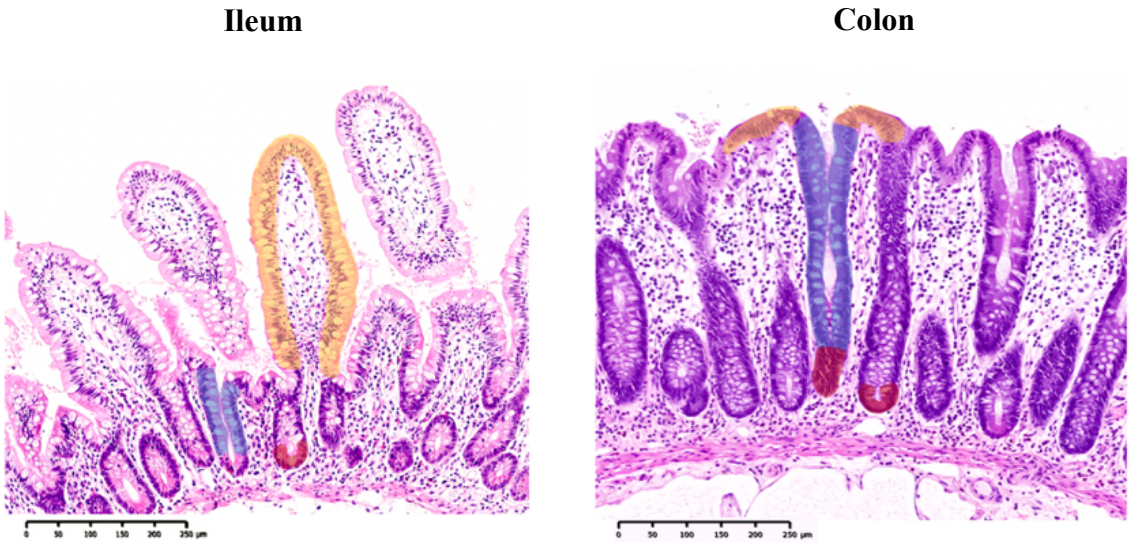

Figure 31. Representative pictures illustrating the different histological areas studied: ileum and colon: surface epithelium (yellow); crypts (blue); bottom of crypts (red)

The distribution of the four ERS-related proteins was evaluated using a semi-quantitative brown staining scale (from 0 to 4), where 0 = no staining and 1, 2, 3 and 4 = weak, moderate, strong, and very strong staining, respectively, as previously described by others[395,396] (examples in Figure 32). Immunostaining signals were evaluated manually by two independent observers, reaching a consensus on the final score for each zone. The intraclass correlation coefficient (ICC) was 0.995, indicating excellent reproducibility of the scoring method. In cases of

disagreement, scores were averaged. When several tissue slices (and therefore different blocks) were obtained from the same patient's gut location and showed identical inflammation and fibrosis grades, the IHC scores were averaged to avoid data overfitting. Bonferroni correction was applied to control for type I error across multiple comparisons (n= 12), adjusting the alpha threshold accordingly.

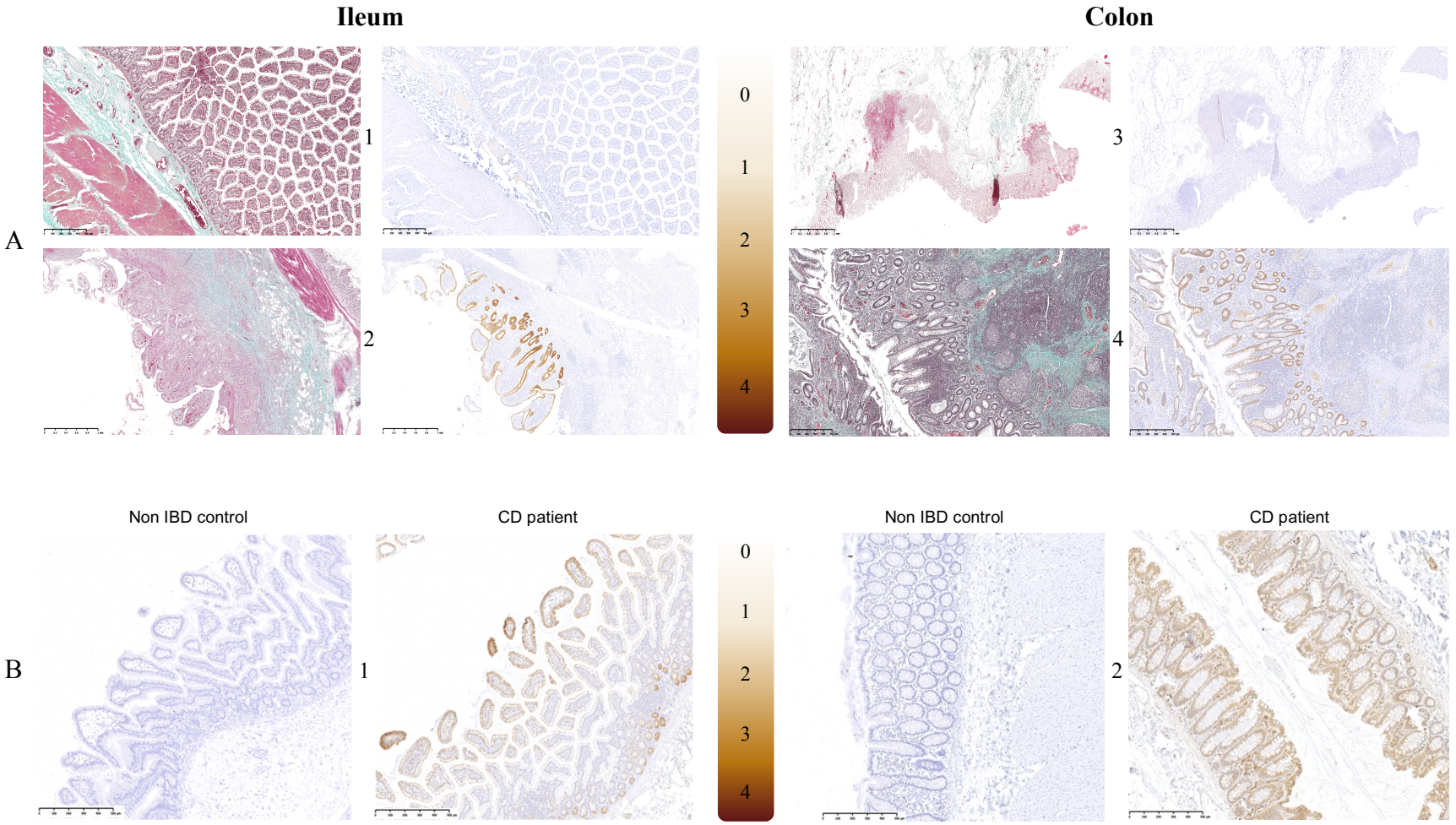

Figure 32. (A) Examples of Masson's Trichrome-stained tissues showing fibrosis grades F1 to F3, alongside IHC staining scores for AGR2 (in brown) in the ileum (1: F1 with AGR2 score= 1; 2: F3 with AGR2 score= 2.5 to 4) and colon (3: F1 with AGR2 score=0.5; 4: F3 with AGR2 score=3.5). (B) Illustration of PDIA6 distribution (in brown) in normal tissues (without inflammation nor fibrosis) in a CD patient and in a non IBD control taken in the ileum (1) and colon (2)

5.3.4. Statistics

Inflammation and fibrosis scores were established and 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.

GraphPad Prism (version 10.0.2) was used for statistical analyses and graphical illustrations. ERS-related proteins IHC scores, as well as 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 was compared using Mann-Whitney or Welch's t-tests. Correlations between inflammation, fibrosis grades and IHC scores, were assessed via Spearman's correlation test. Contingency tables and Fisher's exact tests were applied to evaluate 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: $p< 0.0042$ (*), $p< 0.001$ (**), $p< 0.0001$ (***).

5.4. RESULTS

5.4.1. Clinical and sampling data

We included 224 patients and 815 tissue samples in this multicenter study, grouped as follows: 119 CD, 31 UC, and 74 non IBD controls. Within the CD cohort, 72 patients had a pediatric-onset disease (<17years). For CD, surgical resection specimens from the stenosis and surgical margin of 68 patients, and endoscopic biopsies taken away from stenoses for 51 patients, were analyzed. The clinical characteristics of the cases (CD, UC, and non IBD) and key findings from the comparisons are summarized in Table 7. 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.

Table 7. Clinical characteristics of patients. (A) CD patients; (B) non IBD control cases; (C) UC patients

(A) Crohn's disease cases (n=119)	Pediatric-onset cases (n=72)	Adult-onset cases (n=47)
Characteristics of CD patients without any argument indicating stricture	**32**	**19**
Male gender, n (%)	21 (65.6)	8 (42.1)
Age at CD diagnosis– median (range) – *years**	13.1 (6.9-16.9)	27.63 (20.1-64.8)
Ileal/Ileo-colic/colic disease, n (%)*	2 (6.3)/ 24 (75)/ 6 (18.7)	6 (32.6)/ 9 (47.4)/ 2 (10.5) 2 NA
Treatment at the time of sampling, n (%)		
• Anti-TNF (infliximab or adalimumab)*	3 (9.7)	7 (36.8)
• Steroids (topical or systemic)	5 (16.1)	1 (5.3)
• Immunomodulators (azathioprine, methotrexate, purinethol)	1 (3.2)	4 (21.1)
• 5-Aminosalicylic acid	3 (9.7)	2 (10.5)
• Antibiotics	5 (16.1)	0 (0)
• Vedolizumab	0 (0)	0 (0)
• Ustekinumab	0 (0)	0 (0)
Time between CD diagnosis and sampling – median (range) – *years**	0 (0-6.9)	10,3 (0-39.9)
Characteristics of patients with stenosis (resection tissues)	**40 (and 4 recurrence)**	**28 (and 1 recurrence)**
Male gender, n (%)	19 (47.5)	15 (53.6)
Age at CD diagnosis– median (range) – *years**	12.1 (7.2-15.9)	24,8 (17.1-65.2)
Age at stenosis diagnosis– median (range) – *years**	15.2 (8-29)	34.8 (17.5-65.8)
Age at surgery – median (range) – *years**	15.9 (8-36.6)	35.7 (18.8-70.8)
Surgery in childhood (<17 years), n (%)	31 (70.5)	Non-applicable
Time between		
• CD and stenosis diagnoses– median (range) – *years**	2.5 (0-13)	7.61 (0-39.8)
• CD diagnosis and surgery – median (range) – *years**	4.2 (0.1-20.4)	7.78 (0-46.4)
• Stenosis diagnosis and surgery– median (range) – *years*	0.2 (0-7.5)	0.26 (0-9.2)
Characteristics of stenoses, n (%)		
• Ileal/Ileo-colic/Colic*	16 (35.6)/ 25 (55.6)/ 4 (8.9)	19 (67.9)/ 4 (14.3)/ 5 (17.9)
• Primary/Anastomotic*	41 (93.3)/ 3 (6.7)	22 (75.9)/ 7(24.1)
Treatment at the time of sampling, n (%)		
• Anti-TNF (infliximab or adalimumab)	15 (37.5)	6 (21.4)
• Steroids (topical or systemic)*	13 (32.3)	2 (7.1)
• Immunomodulators (azathioprine, methotrexate, purinethol)*	25 (62.5)	4 (14.3)
• 5-Aminosalicylic acid*	7 (17.5)	0 (0)
• Antibiotics*	4 (10.0)	14 (50)
• Vedolizumab	1 (2.5)	0 (0)
• Ustekinumab	1 (2.5)	0 (0)

(B) Non IBD control cases (n=74)	**Pediatric cases (*n=26*)**	**Adult cases (*n=48*)**
Male gender, n (%)	15 (57.7)	23 (47.9)
Age at time of collection – median (range) - *years*	6.6 (0-15.4)	66.43 (32.1-91.8)
Sampling origins		
Diagnostic biopsies (non-IBD), n (%)	**14 (53.9)**	**1 (2.1)**
Resection margins, n (%)	**12 (46.1)**	**47 (97.9)**
Hirschsprung's disease	6 (23.1)	
Small bowel atresia	1 (3.8)	
Intestinal duplication	2 (7.7)	
Meckel's diverticulum	1 (3.8)	
Anorectal malformation	1 (3.8)	1 (2.1)
Small bowel perforation	1 (3.8)	
Colorectal adenocarcinoma		18 (37.5)
Diverticular disease		16 (33.3)
Stoma closure		4 (8.3)
Colic volvulus		2 (4.2)
Polyposis		2 (4.2)
Extra-digestive tumor (resection of need)		1 (2.1)
Small bowel resection («Candy-cane Syndrome»)		1 (2.1)
Ischemic colitis		1 (2.1)
Refractory infectious colitis		1 (2.1)

(C) Ulcerative colitis cases (n=31)	**Pediatric-onset cases (*n=23*)**	**Adult-onset cases (*n=8*)**
Male gender, n (%)	13 (56.5)	7 (87.5)
Age at UC diagnosis– median (range) – *years**	11.2 (1.3-15.2)	27.8 (18.9-49.4)
Age at time of collection – median (min-max) – *years**	11.9 (4-42.1)	39.7 (27.7-63.6)
Treatment at the time of sampling, n (%)		
• Anti-TNF (infliximab or adalimumab)	1 (4.4)	1 (12.5)
• Steroids (topical or systemic)	7 (30.4)	2 (25)
• Immunomodulators (azathioprine, methotrexate, purinethol)*	6 (26.1)	2 (25)
• 5-Aminosalicylic acid	12 (52.2)	2 (25)
• Antibiotics*	5 (21.7)	0 (0)
• Vedolizumab*	0 (0)	2 (25)
Sampling origins		
Patients with surgical resection tissues (n)	**2**	**8**
Patients with biopsy tissues (n)	**21**	**0**

* Statistical significance
NA, not available

As multiple tissue slices were available per patient, 4518 tissue slices have been analyzed. Table 8 provides a summary of the tissue samples included.

Table 8. Origin and number of tissue samples analyzed (hematoxylin-eosin and Masson's Trichrome staining, and immunohistochemistry using anti-AGR2, anti-BiP, anti-ERP44, and anti-PDIA6 antibodies)

		Tissues from pediatric-onset cases	Tissues from adult-onset cases	Total number of slices
CD patients	**Ileum**	168	99	1602
	Colon	234	106	2040
Non IBD controls	**Ileum**	6	31	222
	Colon	45	33	468
UC patients	**Colon**	63	30	186
Total number of tissue slices		**516**	**299**	**4518**

5.4.2. Comparison of the distribution of ERS-related proteins and association with fibrosis in CD

We conducted a systematic analysis of AGR2, BiP, PDIA6 and ERP44 IHC signal distributions. Pathological illustrations are shown in Figure 33 and Figure 34.

Ileum

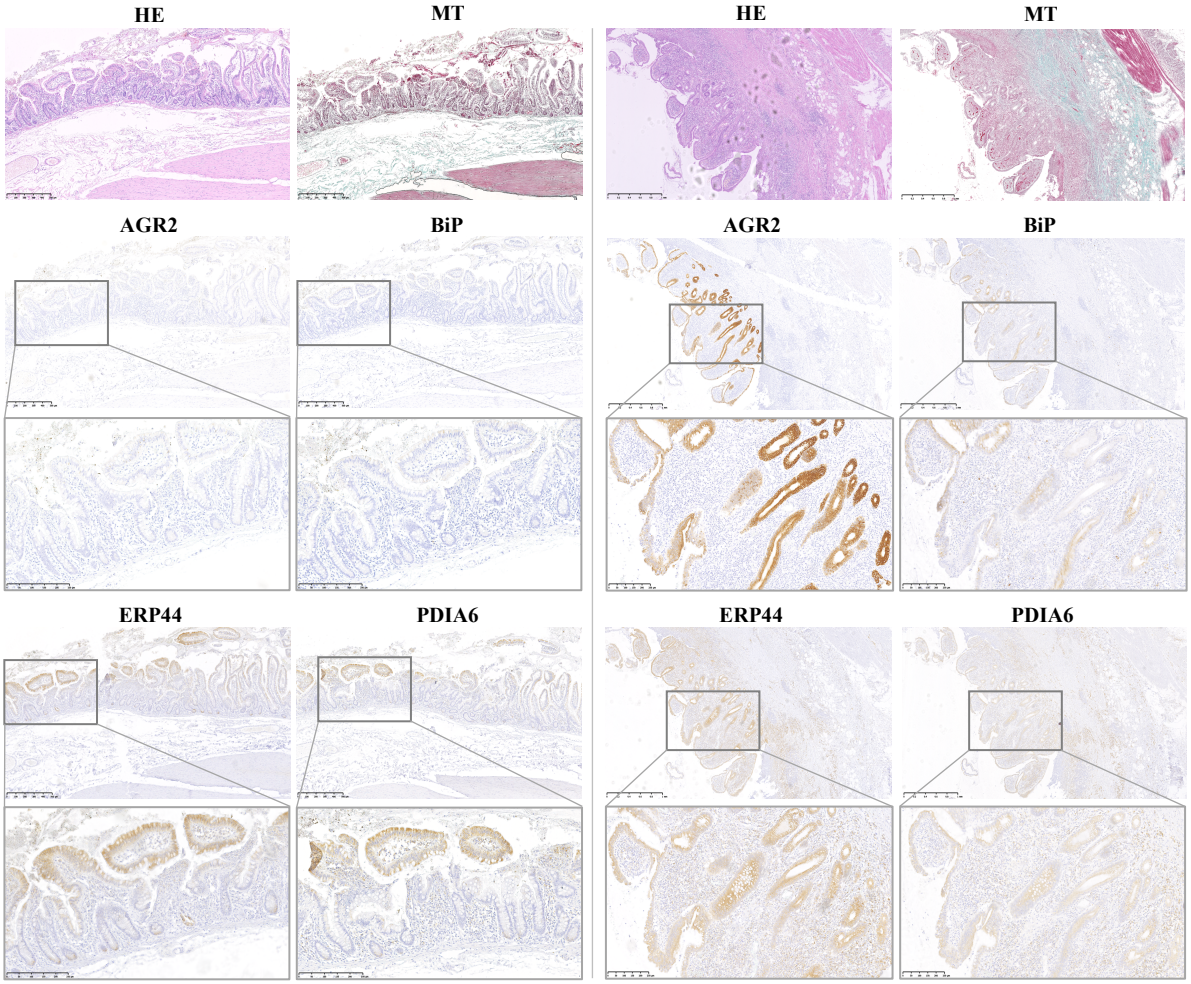

Figure 33. Representative pictures illustrating the histopathology of tissues after hematoxylin-eosin (H&E) and Masson's Trichrome (MT) staining, and the distribution of the 4 proteins detected by immunohistochemistry in the ileum (CD patient with no inflammation nor fibrosis on the left; CD patient with chronic and acute inflammation (IC and IA)= 3 and fibrosis= F2 on the right)

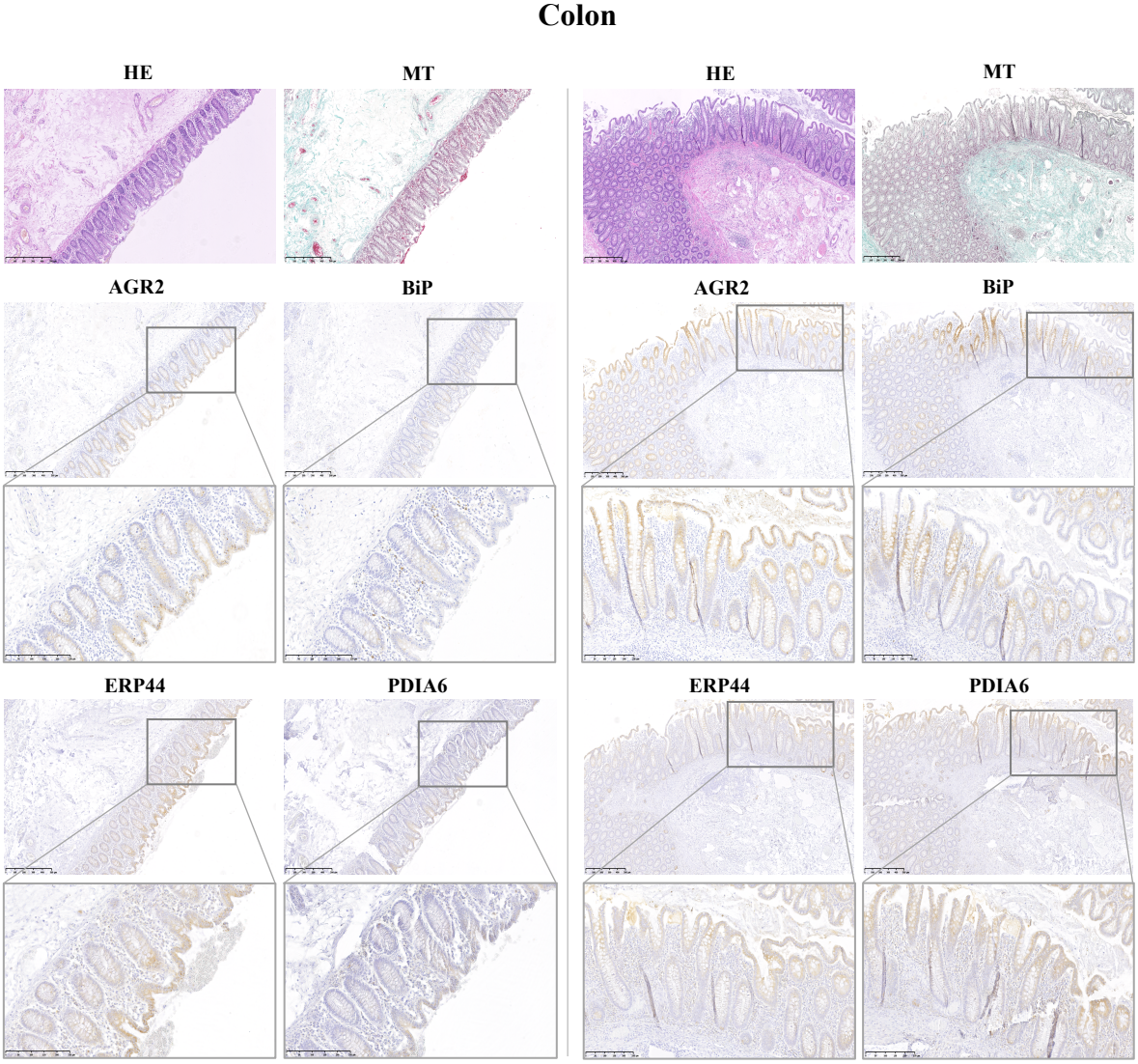

Figure 34. Representative pictures illustrating the histopathology of tissues after hematoxylin-eosin (HE) and Masson's Trichrome (MT) staining, and the distribution of the 4 proteins detected by immunohistochemistry in the colon (CD patient with no inflammation nor fibrosis on the left; CD patient with chronic and acute inflammation (IC and IA)= 3 and fibrosis= F2 on the right)

5.4.2.1. AGR2

The AGR2 IHC staining distribution across groups is shown in Figures 35, 36, 37 and 38.

As shown in Figure 35, AGR2 was significantly higher in the colonic than ileal surface epithelium, especially in adult controls and normal (no inflammation nor fibrosis) CD samples, with a statistically significant difference.

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 (Figure 36.A-B). A similar distribution 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 bottom of the crypts in both the ileum and colon (Figure 37).

AGR2 IHC staining increased with higher fibrosis grades in the crypts of both ileum and colon (Figure 36.C-D). Similar findings were noted in the surface epithelium and the bottom of the crypts (Figure 38).

Correlation analyses showed a weak but significant association between AGR2 IHC scores and fibrosis in colonic crypts and bottom of crypts 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 (Figure 39).

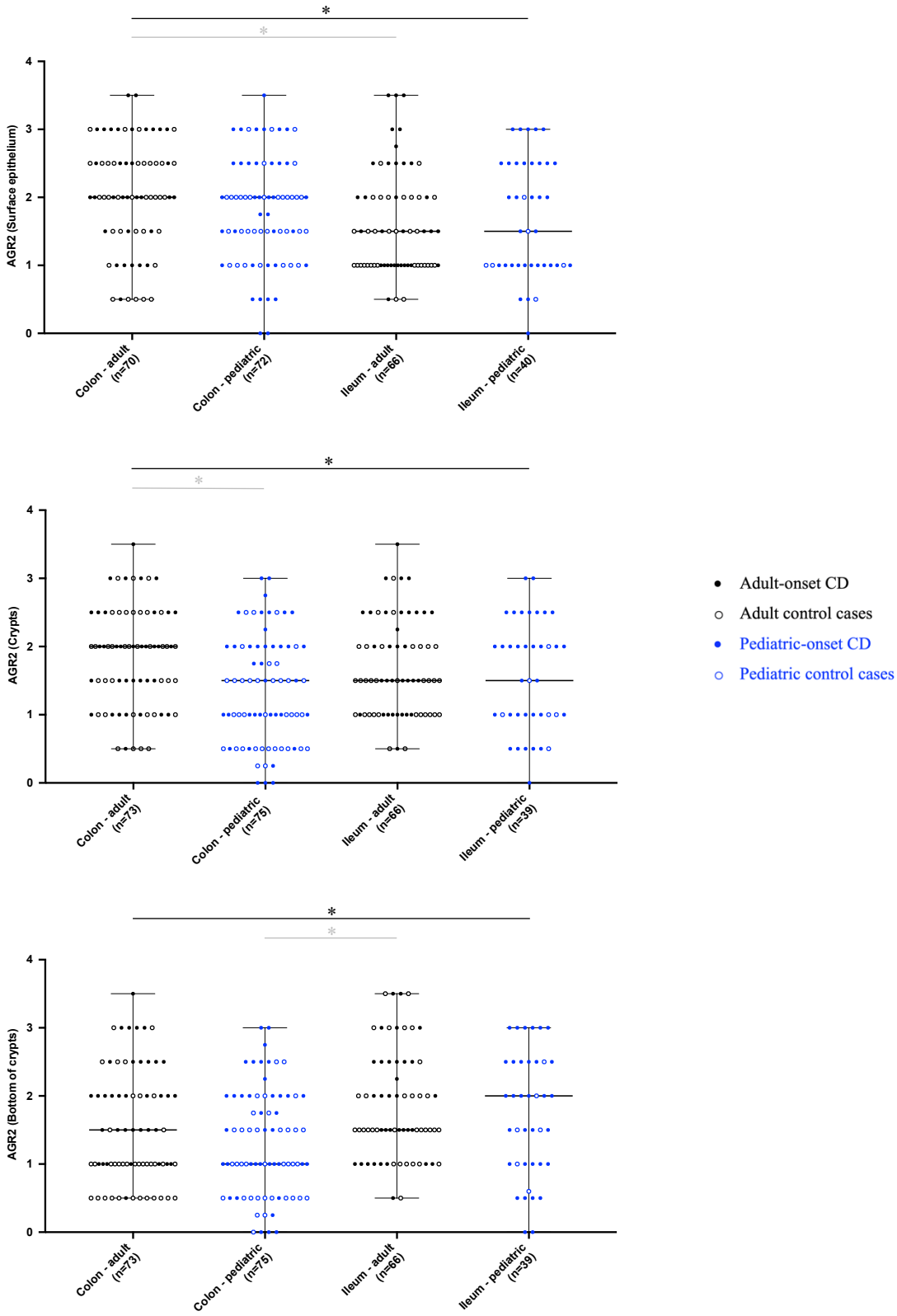

Figure 35. Distribution of AGR2 IHC scores in normal tissues from control cases, adult-onset CD patients and pediatric-onset CD

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

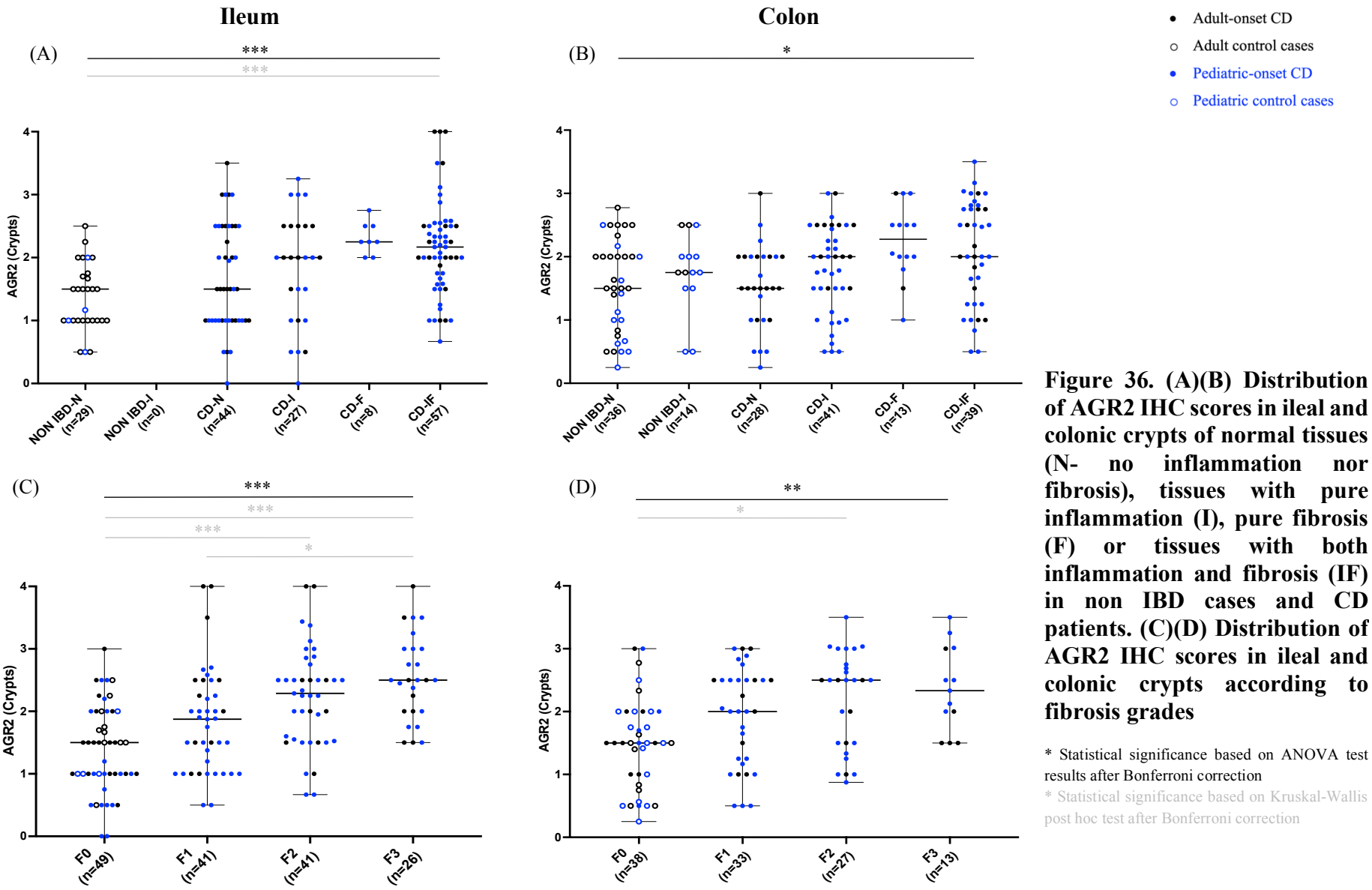

Figure 36. (A)(B) Distribution of AGR2 IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients. (C)(D) Distribution of AGR2 IHC scores in ileal and colonic crypts according to fibrosis grades

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

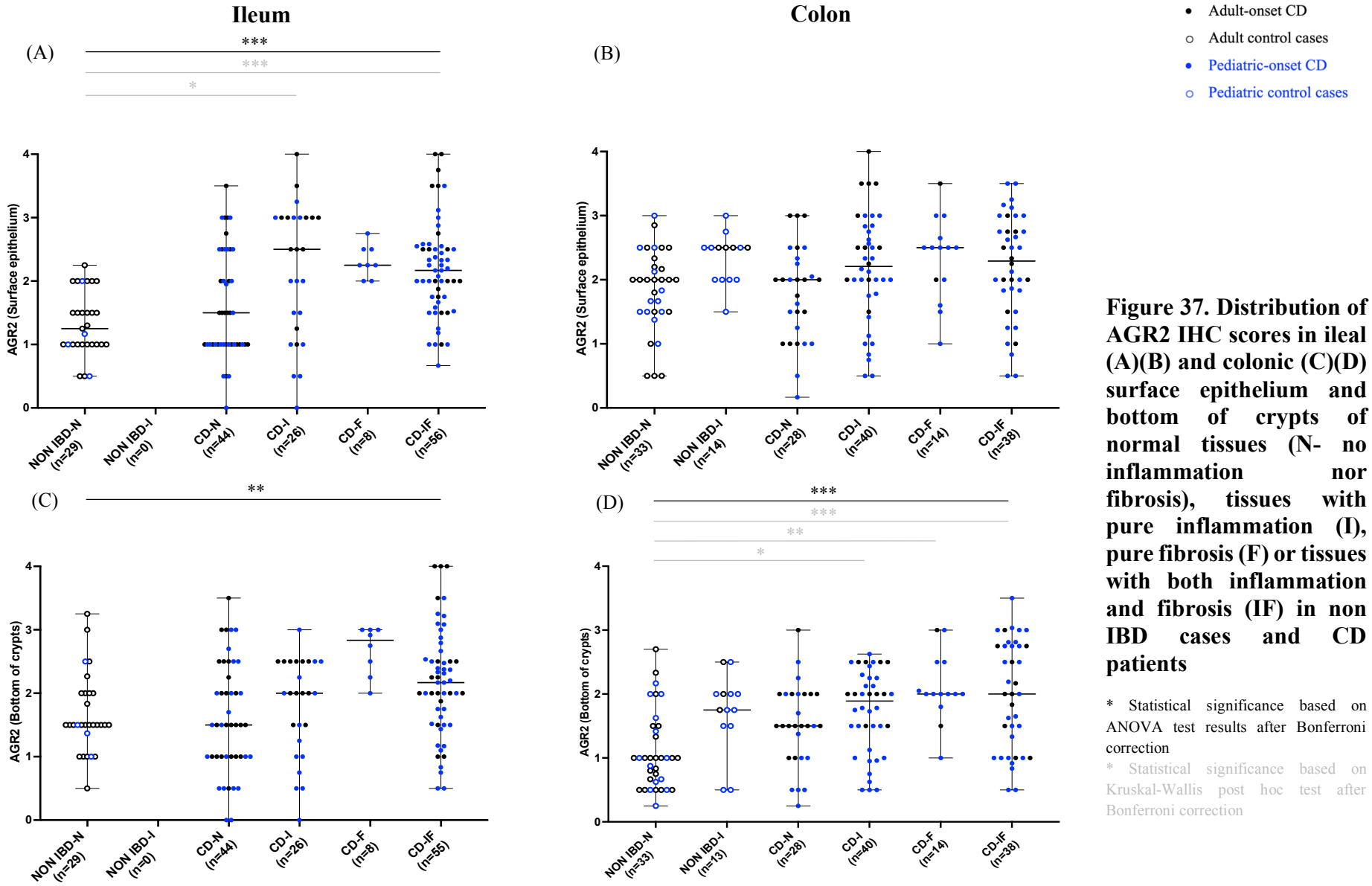

Figure 37. Distribution of AGR2 IHC scores in ileal (A)(B) and colonic (C)(D) surface epithelium and bottom of crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

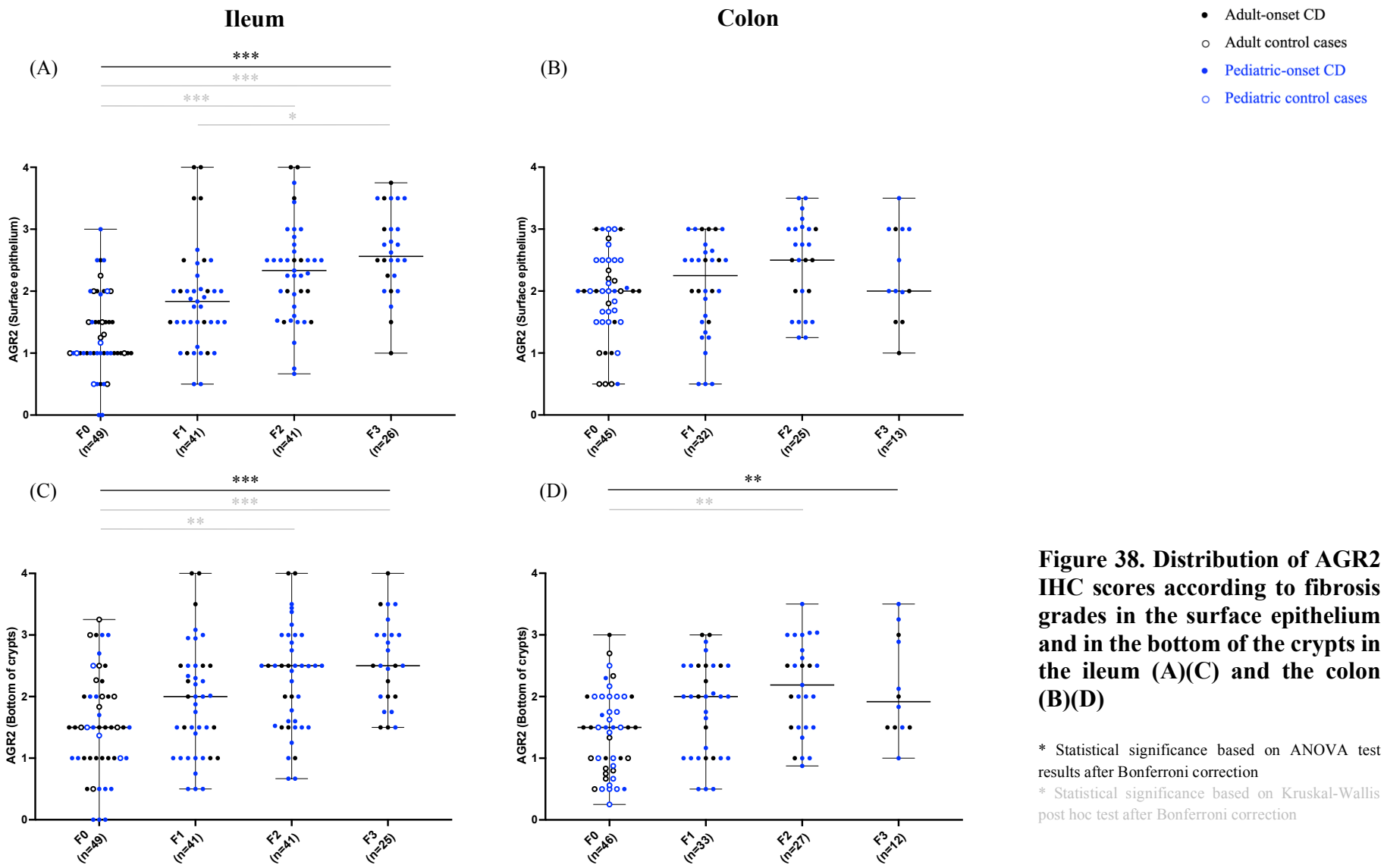

Figure 38. Distribution of AGR2 IHC scores according to fibrosis grades in the surface epithelium and in the bottom of the crypts in the ileum (A)(C) and the colon (B)(D)

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

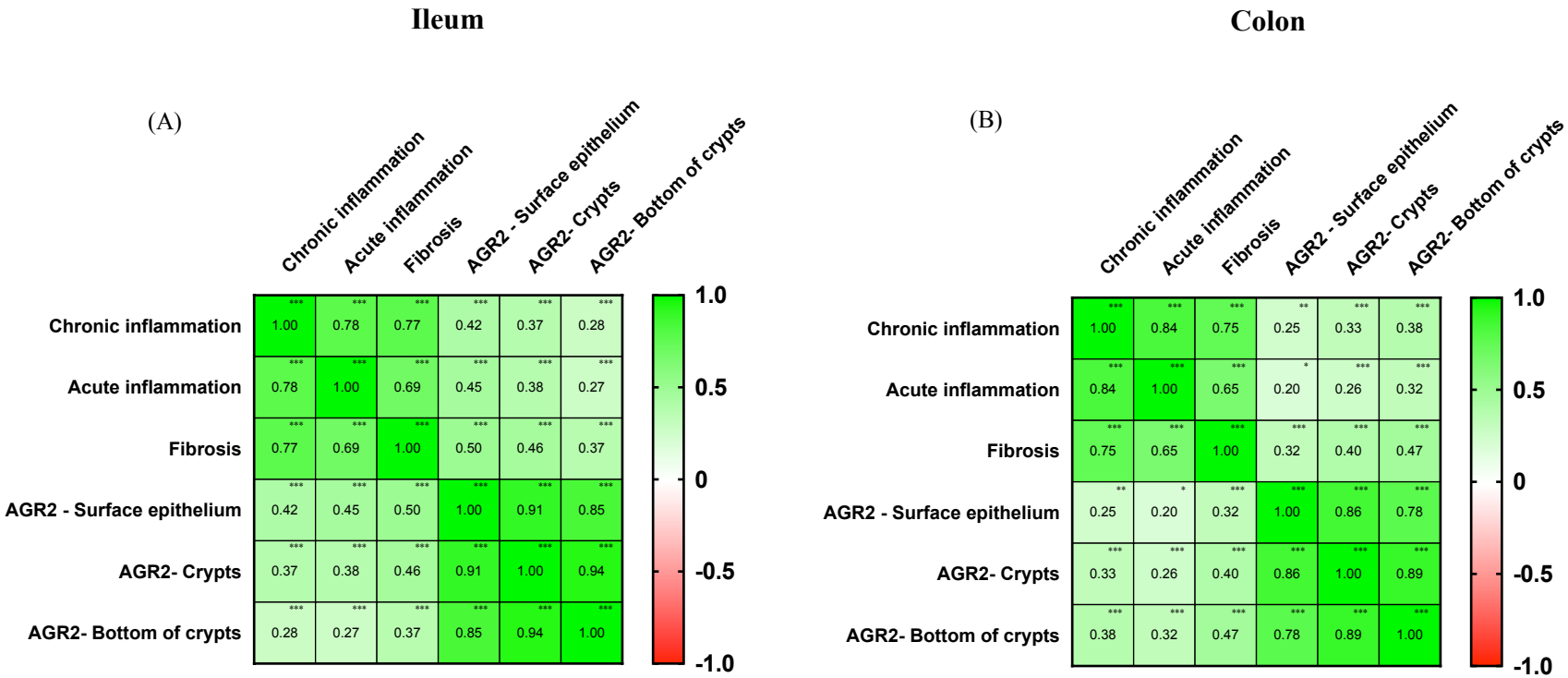

Figure 39. Heatmap of correlation results. Spearman correlation coefficient (r) between the IHC scores of AGR2 and the grades of fibrosis in the ileum (A) and colon (B) of pediatric and adult cases

All *r* coefficients present a significative *p-value* (*= $p<0.0042$, **= $p<0.001$, ***= $p<0.0001$ after Bonferroni correction).

5.4.2.2. BiP

The distribution of BiP IHC staining is shown in Figure 40 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 shown in Figure 41.A-B and Figure 42. 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.

BiP IHC scores was positively associated with fibrosis degree in the ileal and colonic crypts (Figure 41.C-D). Significant results were also found in the surface epithelium and bottom of crypts (Figure 43).

Correlation studies revealed a significant association between BiP staining intensity and inflammation, unlike the other three proteins (Figure 44). BiP signal intensity also correlated with fibrosis, showing strong inflammation-fibrosis association.

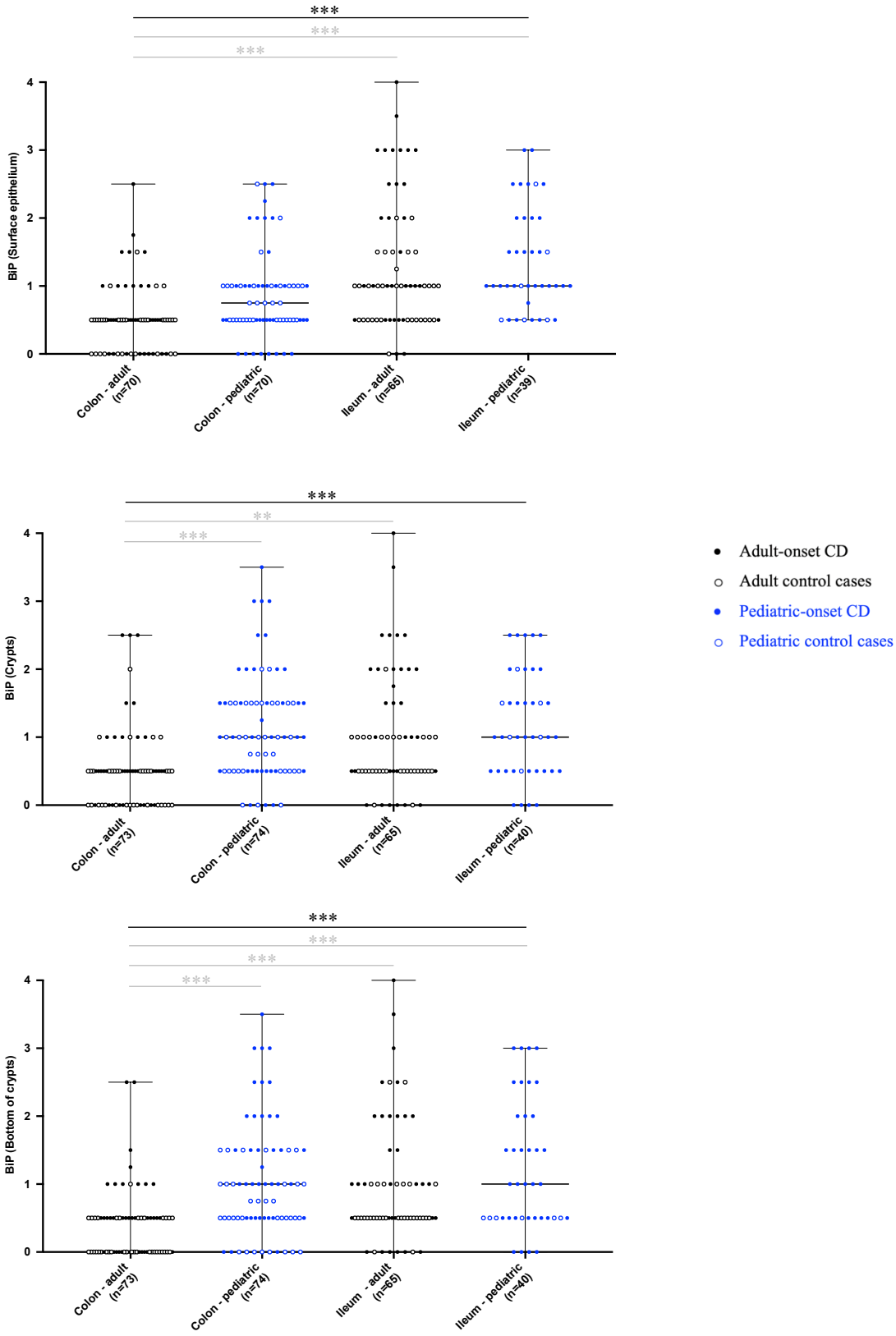

Figure 40. Distribution of BiP IHC scores in normal tissues from control cases, adult and pediatric CD patients

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

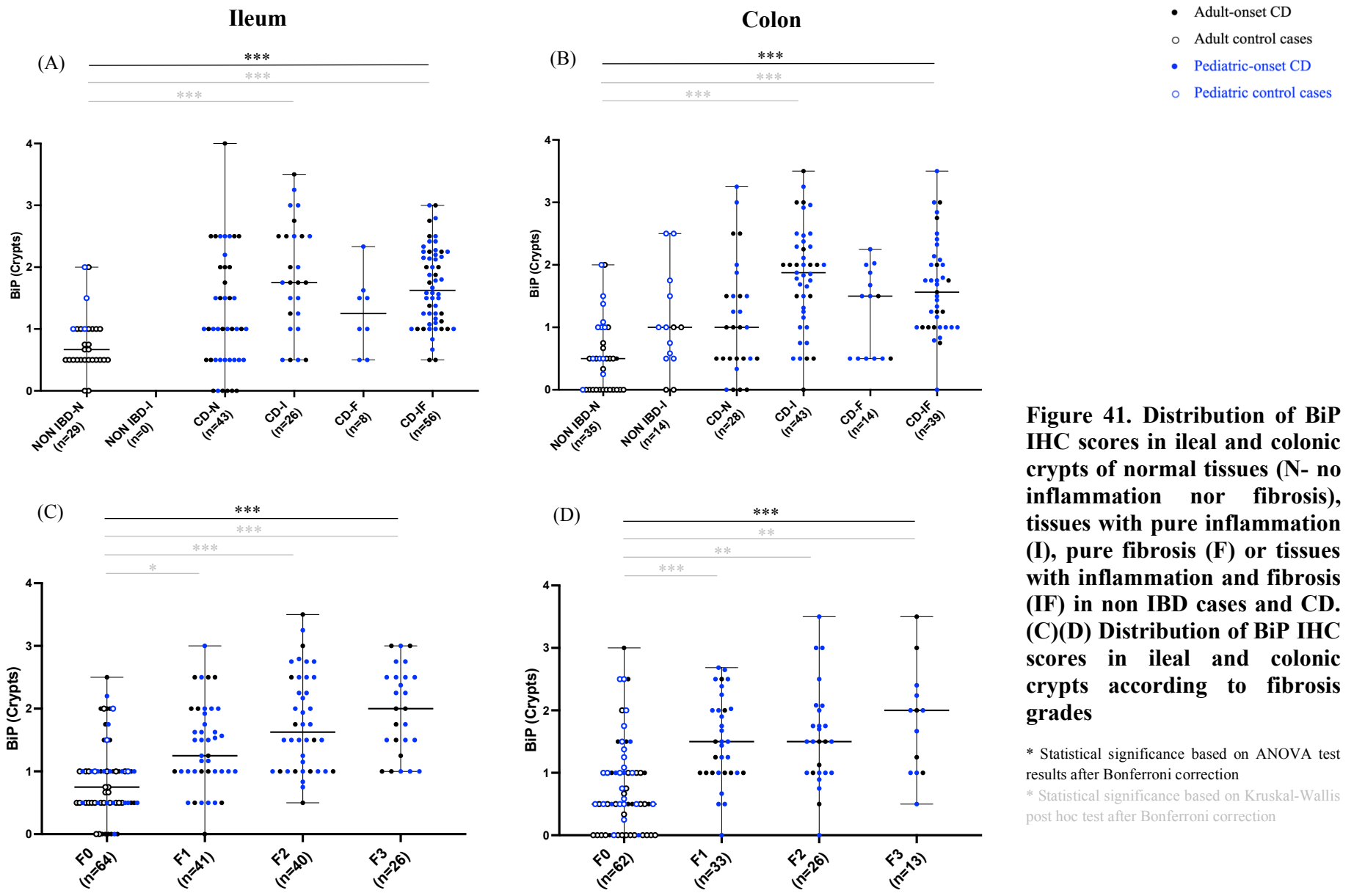

Figure 41. Distribution of BiP IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with inflammation and fibrosis (IF) in non IBD cases and CD. (C)(D) Distribution of BiP IHC scores in ileal and colonic crypts according to fibrosis grades

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

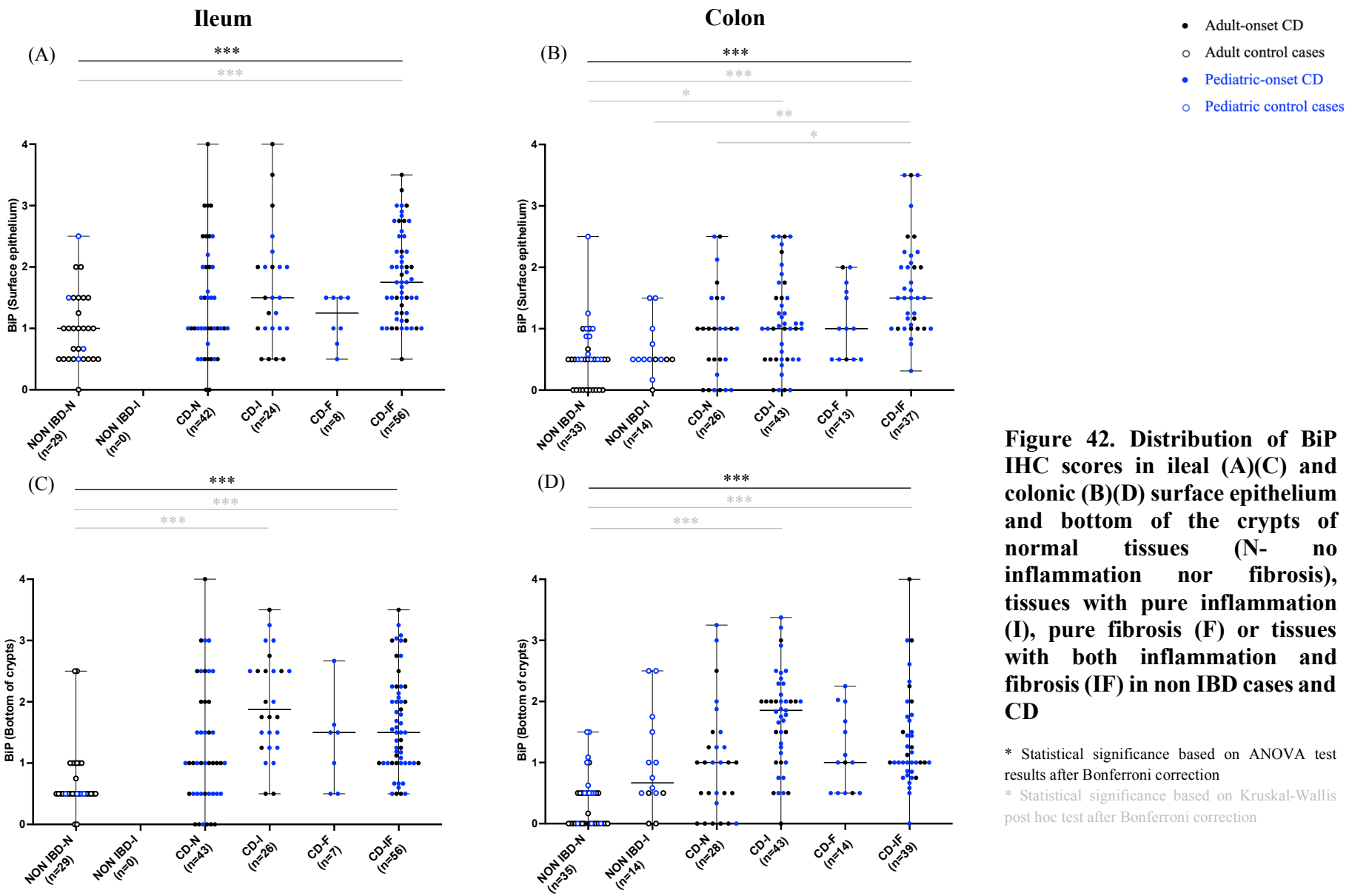

Figure 42. Distribution of BiP IHC scores in ileal (A)(C) and colonic (B)(D) surface epithelium and bottom of the crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

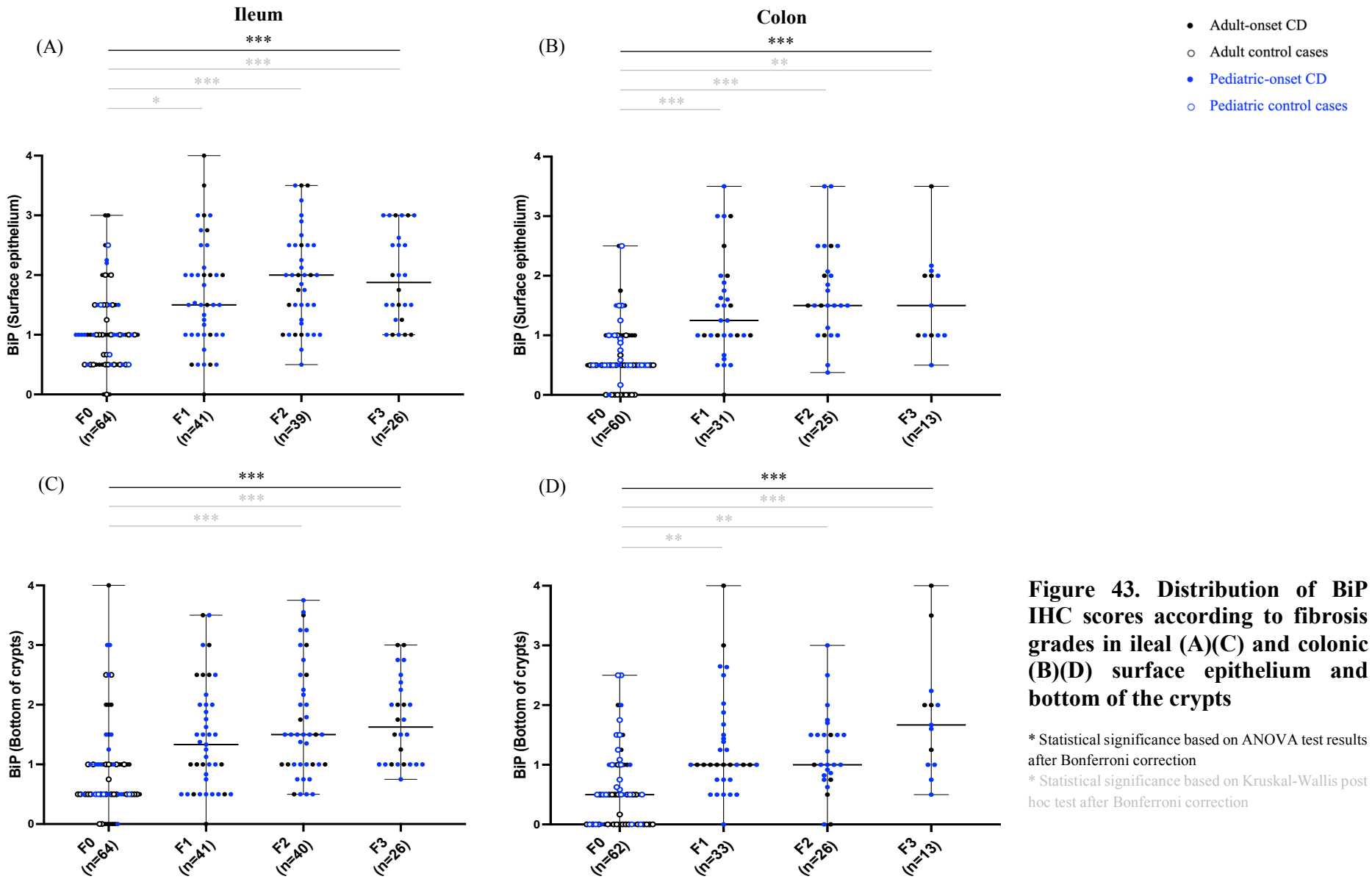

Figure 43. Distribution of BiP IHC scores according to fibrosis grades in ileal (A)(C) and colonic (B)(D) surface epithelium and bottom of the crypts

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

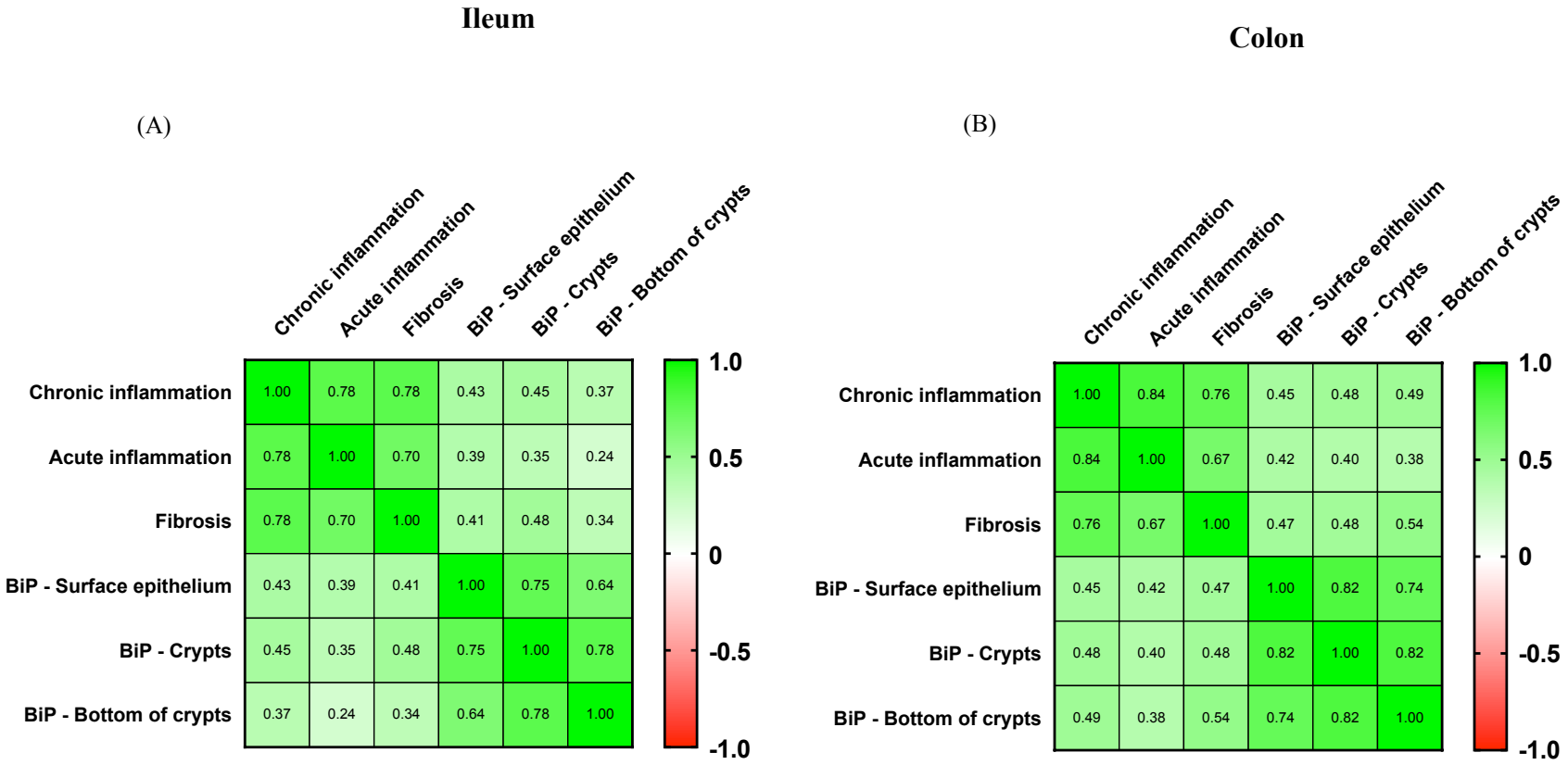

Figure 44. Heatmap of correlation results. Spearman correlation coefficient (r) between the IHC scores of BiP and the grades of fibrosis in the ileum (A) and colon (B) of pediatric and adult cases

All r coefficients communicated present a $p<0.0001$ (***) after Bonferroni correction.

5.4.2.3. PDIA6

The PDIA6 IHC staining distribution is represented in Figure 45 for 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 (Figure 46 and Figure 47). However, PDIA6 distribution was significantly higher in CD tissues compared to non IBD tissues, regardless of location, age of onset or inflammation/fibrosis presence (see details point 3.4.5.).

Correlation analyses showed no significant association between PDIA6 and inflammation or fibrosis in any population (not shown).

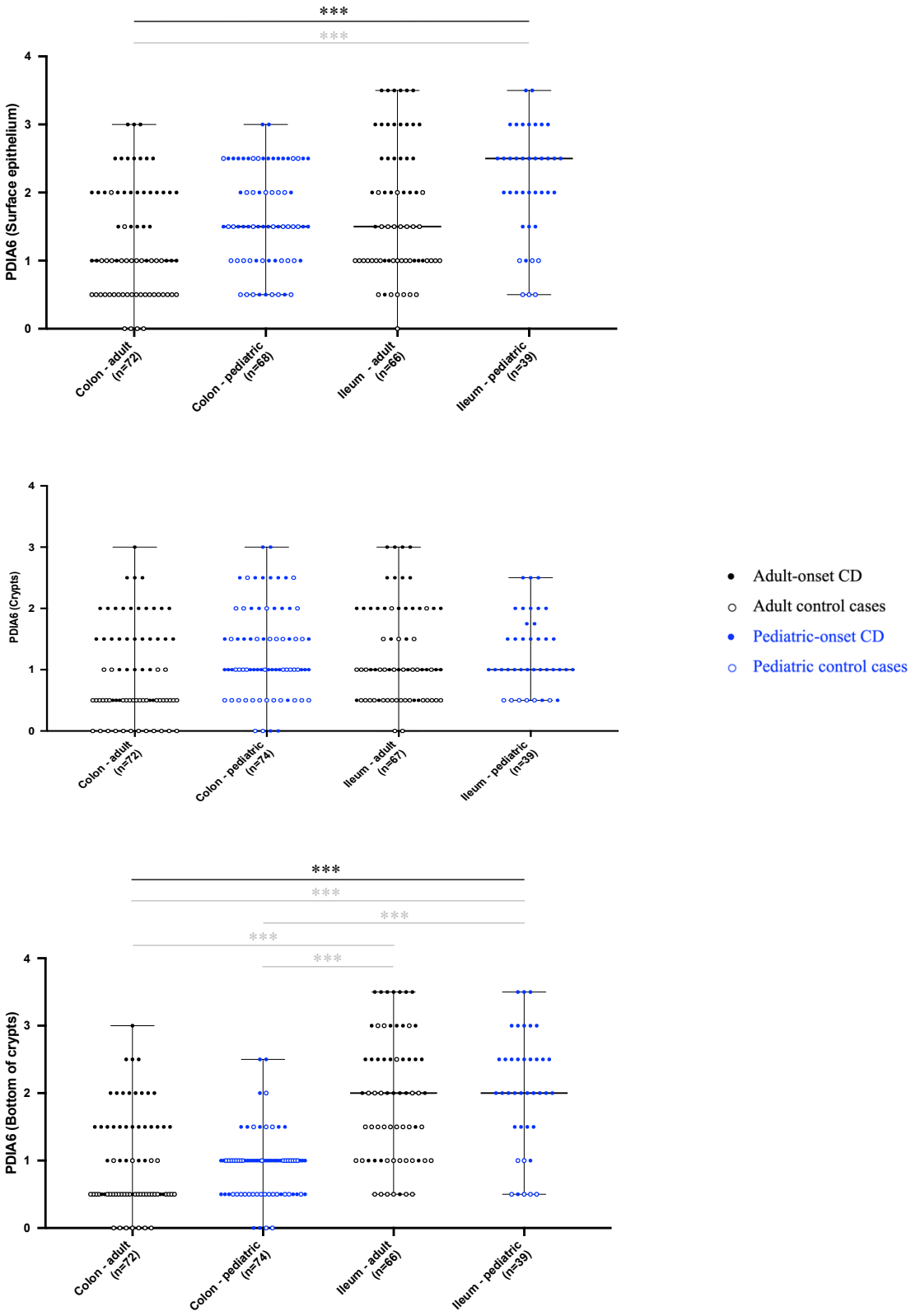

Figure 45. Distribution of PDIA6 IHC scores in normal tissues from control cases, adult and pediatric CD patients

** Statistical significance based on ANOVA test results after Bonferroni correction*

** Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction*

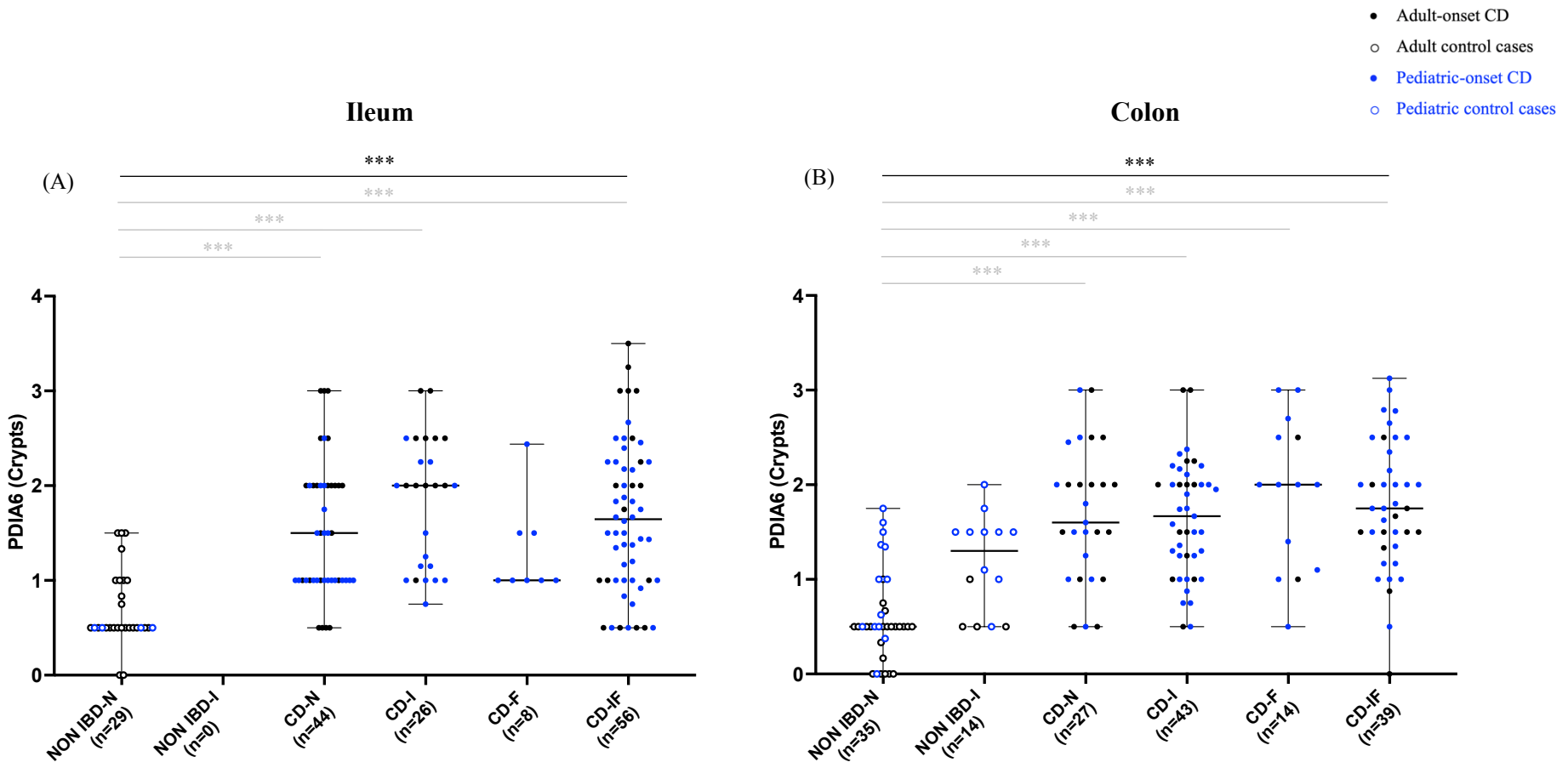

Figure 46. Distribution of PDIA6 IHC scores in ileal (A) and colonic (B) crypts, including normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients

** Statistical significance based on ANOVA test results after Bonferroni correction*

** Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction*

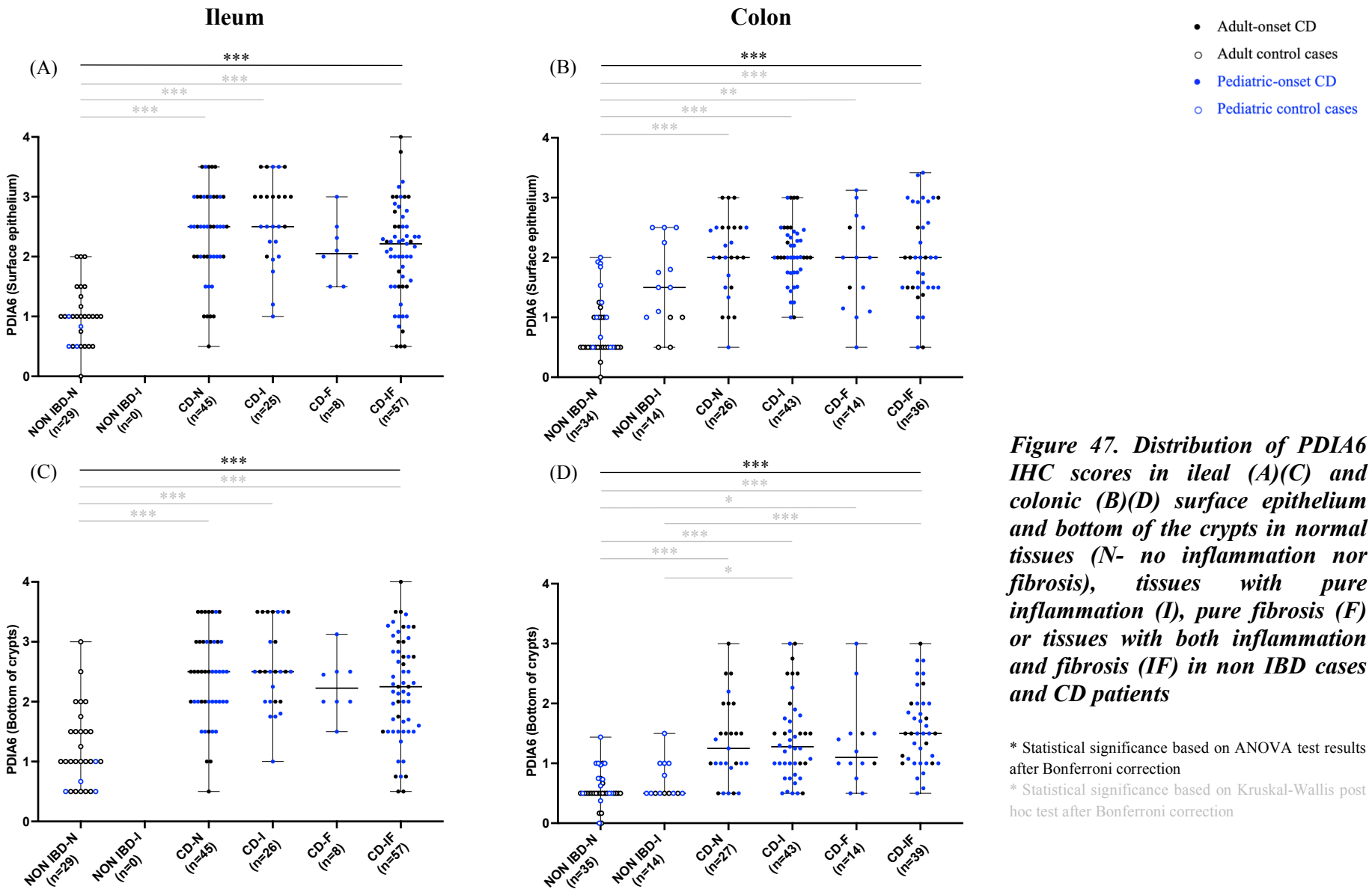

Figure 47. Distribution of PDIA6 IHC scores in ileal (A)(C) and colonic (B)(D) surface epithelium and bottom of the crypts in normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

5.4.2.4. ERP44

The distribution of ERP44 IHC staining is represented in Figure 48 for controls and normal CD samples. In control and normal CD tissues, ERP44 distribution was higher in adult-onset compared to pediatric-onset-cases, with significant differences in the colon.

Due to significant group differences, ERP44 IHC staining in pediatric and adult-onset CD are presented separately in point 5.4.3.4.

Correlation studies showed no significant association between ERP44 and inflammation or fibrosis in either population (not shown).

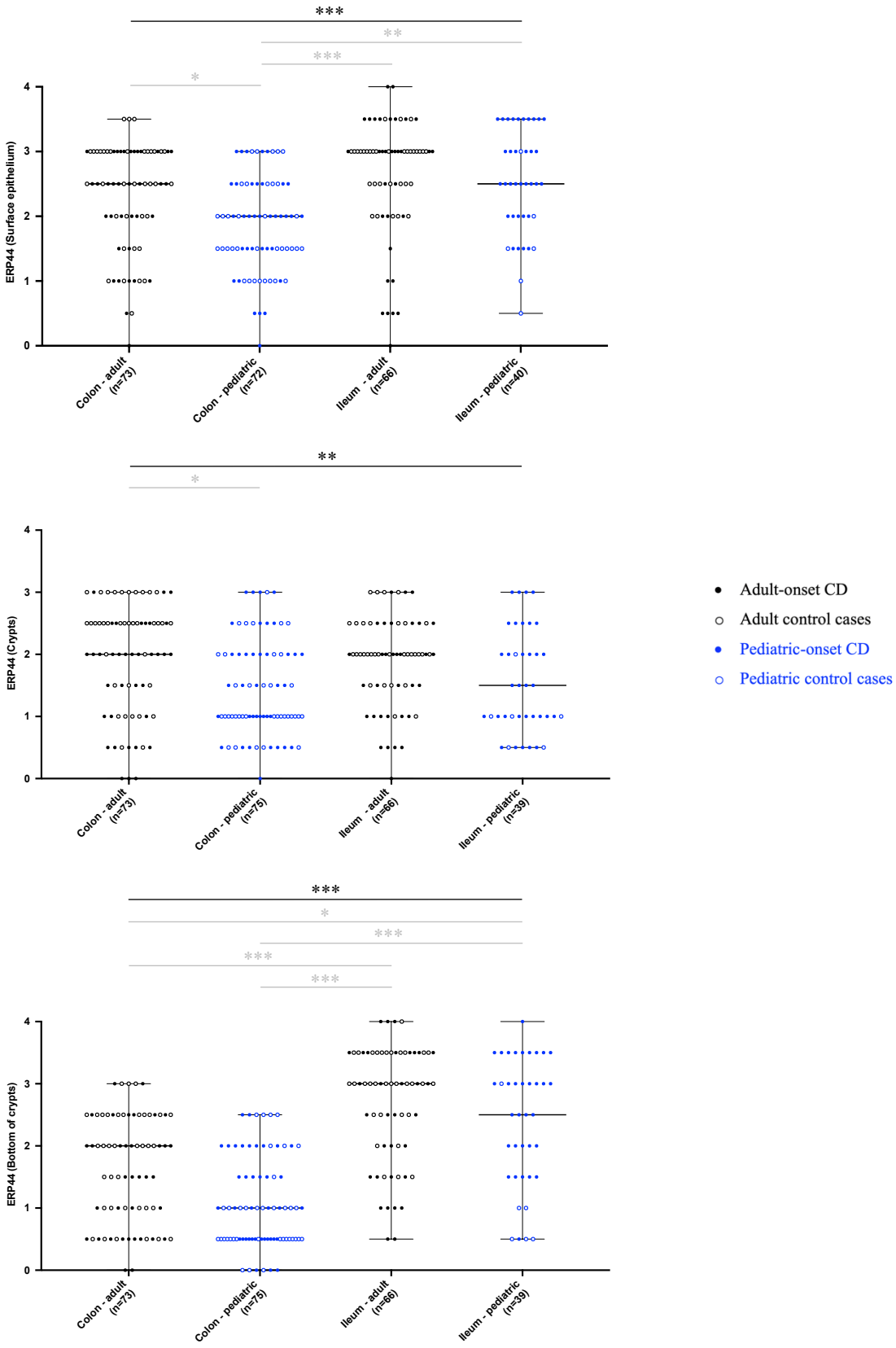

Figure 48. Distribution of ERP44 IHC scores in normal tissues from control cases, adult and pediatric CD patients

** Statistical significance based on ANOVA test results after Bonferroni correction*

** Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction*

5.4.3. Differential study of pediatric *versus* adult-onset cases

5.4.3.1. AGR2

As illustrated in Figure 35, the AGR2 IHC scores were higher in adult than pediatric colonic crypts.

In pediatric-onset cases, AGR2 distribution significantly increased with fibrosis grade in both ileal and colonic crypts (Figure 49.A-B), as well as in the surface epithelium and bottom of crypts (Figure 50.A-D).

In adult cases, an increase was observed only in ileal tissues, but not in the colon (Figure 49.C-D and Figure 50.E-H).

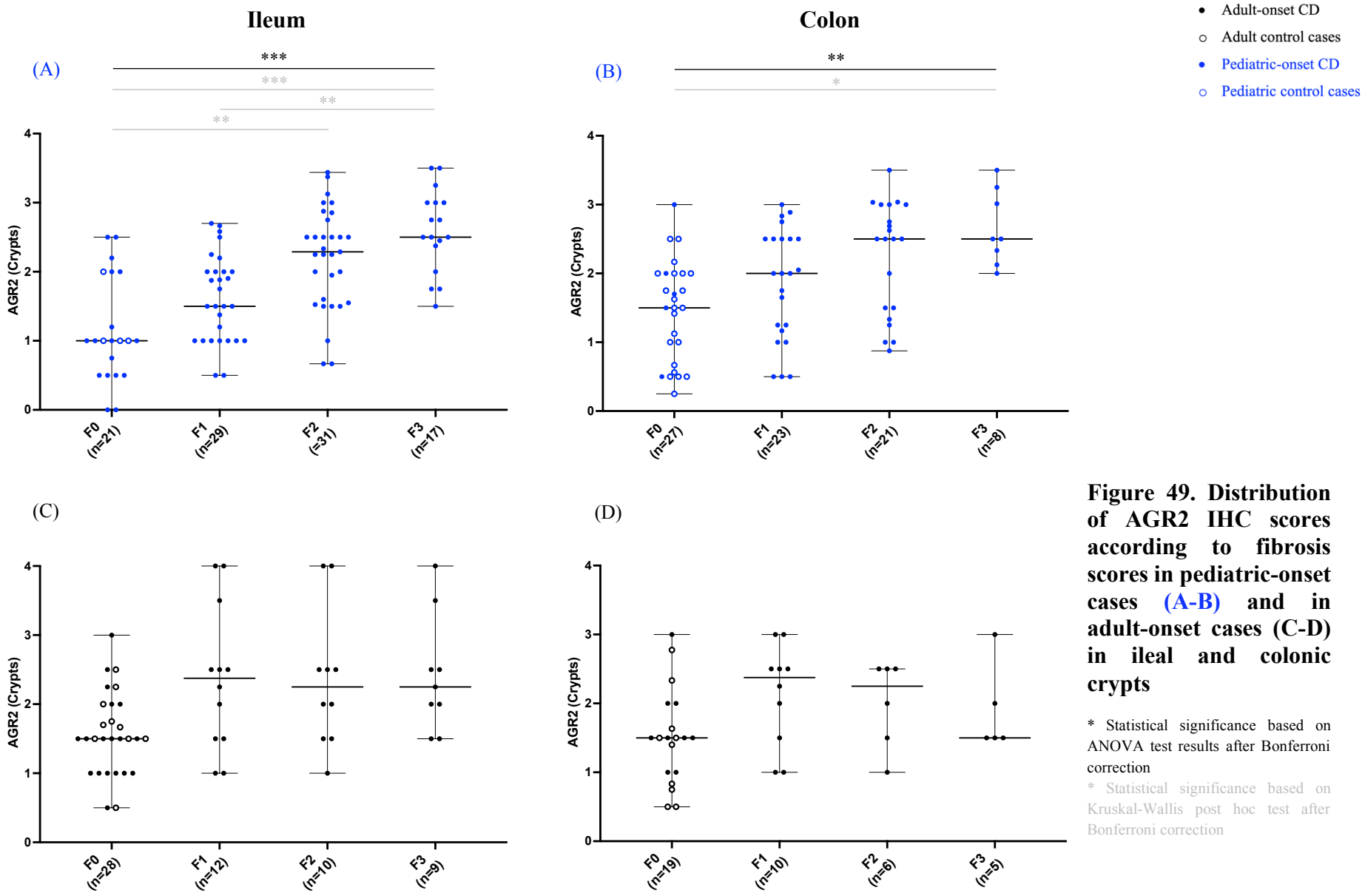

Figure 49. Distribution of AGR2 IHC scores according to fibrosis scores in pediatric-onset cases (A-B) and in adult-onset cases (C-D) in ileal and colonic crypts

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

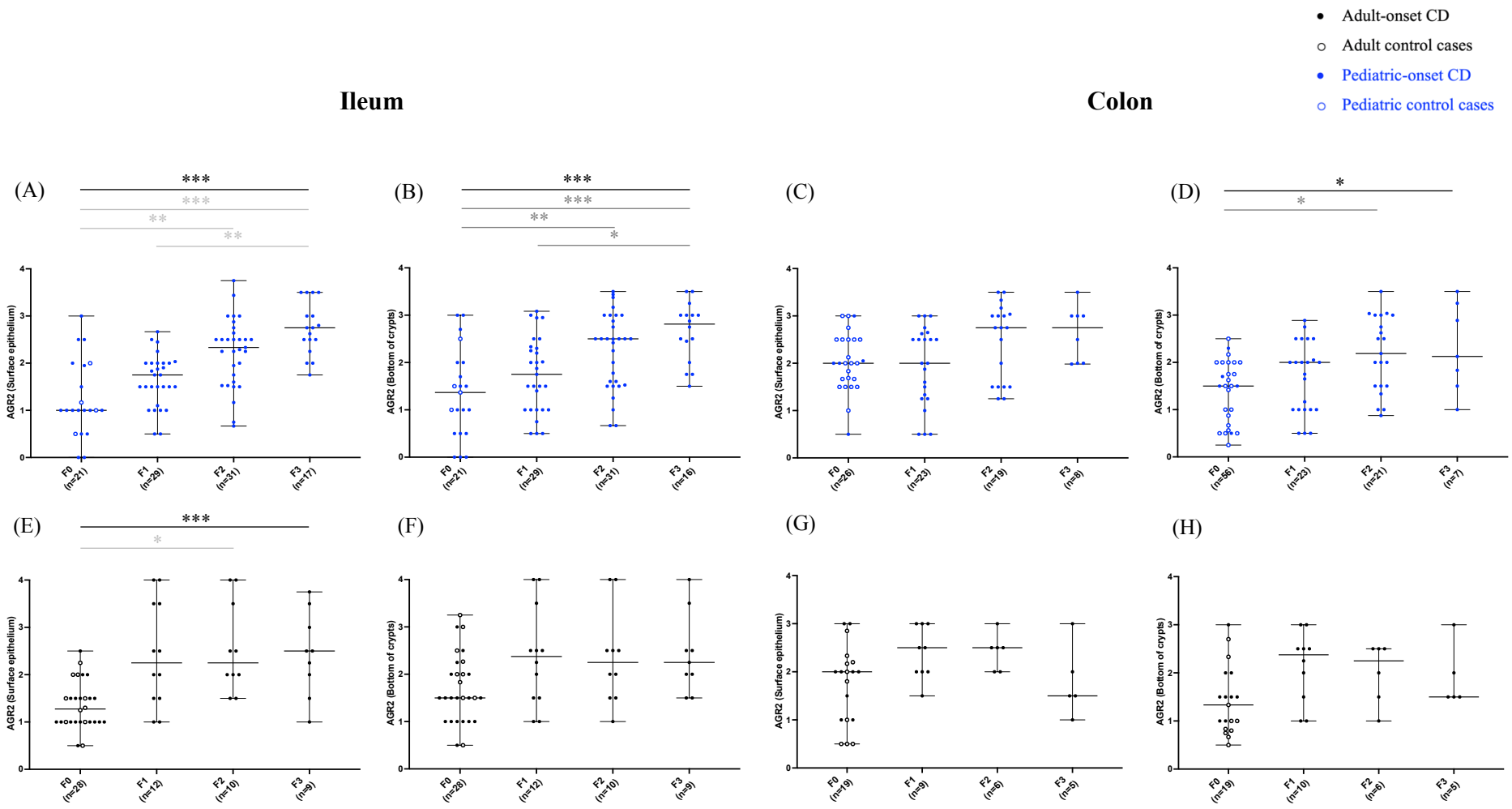

Figure 50. Distribution of AGR2 IHC scores according to fibrosis grades in pediatric-onset (A-D) and in adult-onset (E-H) cases in ileal and colonic surface epithelium and bottom of the crypts

* 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-Whitney test after Bonferroni correction

5.4.3.2. BiP

In crypts without inflammation or fibrosis (Figure 40), colonic BiP distribution was lower in adult tissues than in pediatric ones. BiP distribution in the surface epithelium and bottom of crypts were similar in both groups.

In both ileum and colon, BiP distribution according to fibrosis grade were similar in pediatric-onset and adult-onset CD. The observations are similar at the level of the crypts as well as in the surface epithelium and the bottom of the crypts (Figure 51 and Figure 52).

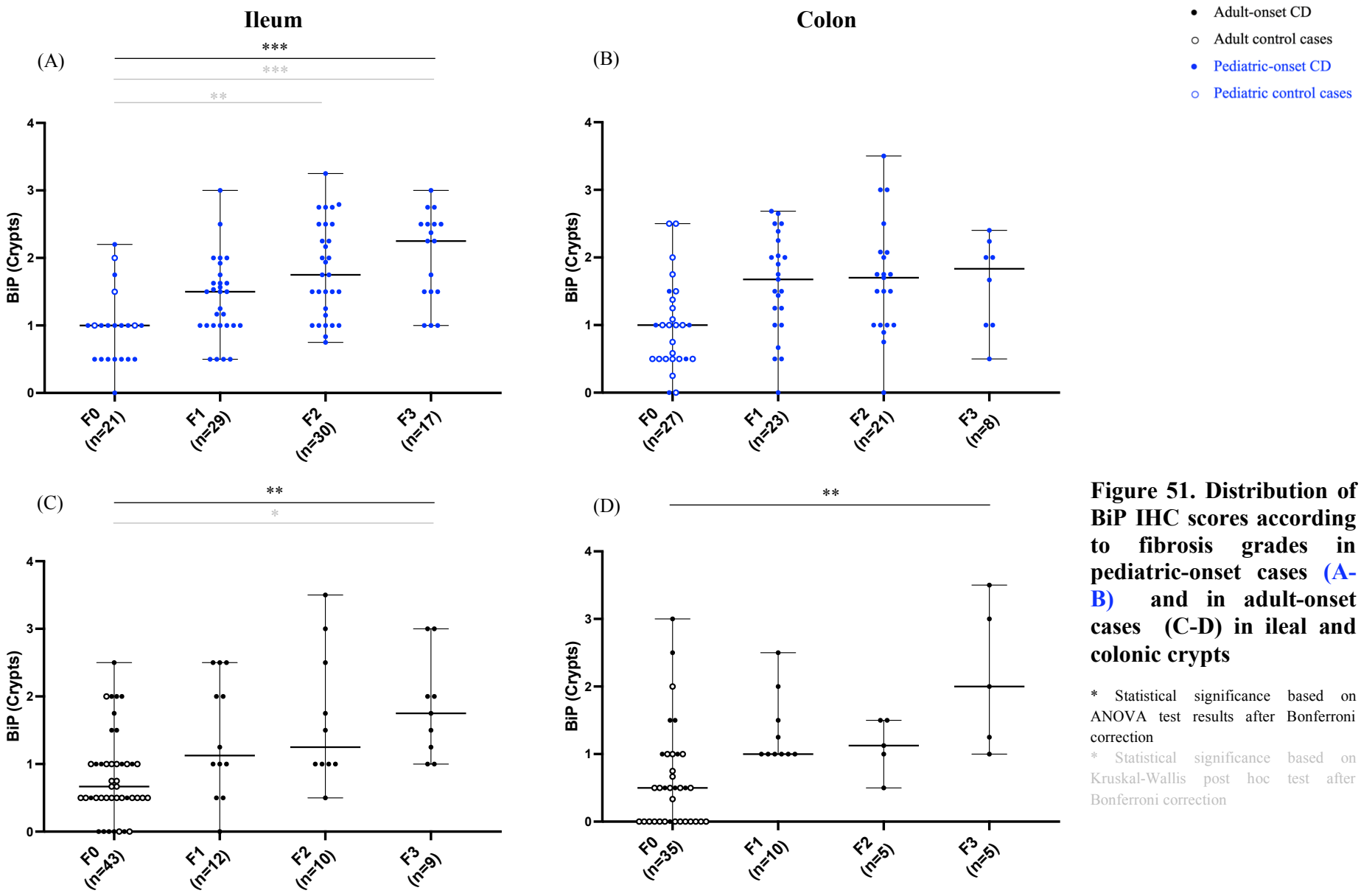

Figure 51. Distribution of BiP IHC scores according to fibrosis grades in pediatric-onset cases (A-B) and in adult-onset cases (C-D) in ileal and colonic crypts

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

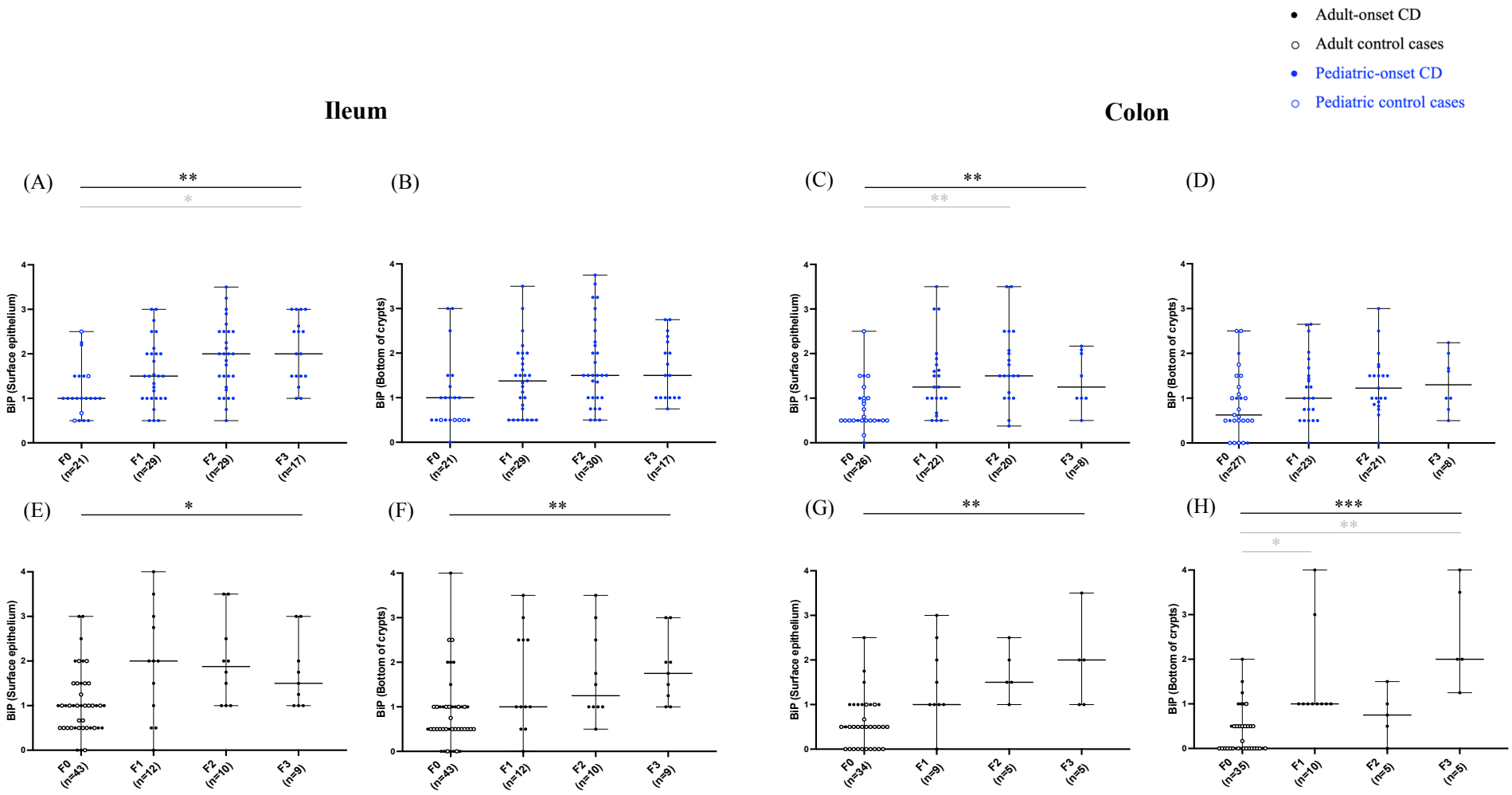

Figure 52. Distribution of BiP IHC scores according to fibrosis scores in pediatric-onset (A-D) and in adult-onset (E-H) cases in ileal and colonic surface epithelium and bottom of the crypts

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

5.4.3.3. PDIA6

As shown in Figure 45, 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.

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.

5.4.3.4. 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 (Figure 48).

Due to significant group differences, ERP44 IHC staining in pediatric and adult-onset CD are presented separately in this section.

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 (Figure 53 and Figure 54). In the colon of pediatric cases, ERP44 IHC scores were higher in fibro-inflammatory CD tissues compared to inflammatory non IBD tissues in the crypts (significant in the bottom) (Figure 53). In adult cases, ERP44 colonic distribution was more widespread and not associated with inflammation or fibrosis (Figure 54).

In pediatric ileal crypts, ERP44 distribution significantly increased with higher fibrosis grade; however, no significant changes were observed in the surface epithelium or bottom of crypts (Figure 55). In pediatric colonic epithelium, ERP44 distribution increased with fibrosis grade (Figure 55). In adult cases, no significative association with fibrosis was observed (Figure 56).

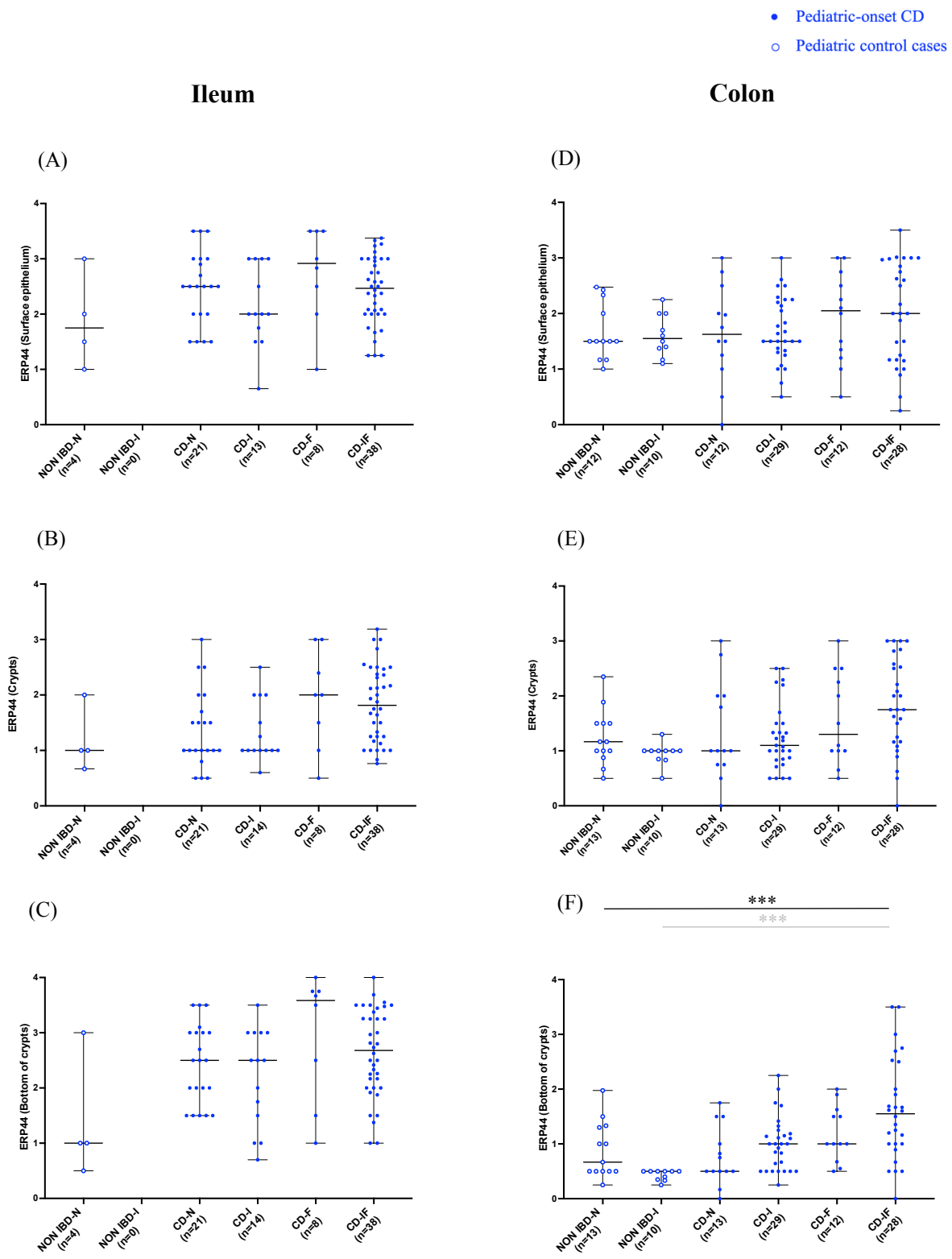

Figure 53. Distribution of ERP44 IHC scores in ileal (A-C) and colonic (D-F) surface epithelium, crypts and bottom of the crypts in normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation in pediatric patients

* Statistical significance based on ANOVA test results after Bonferroni correction

* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction

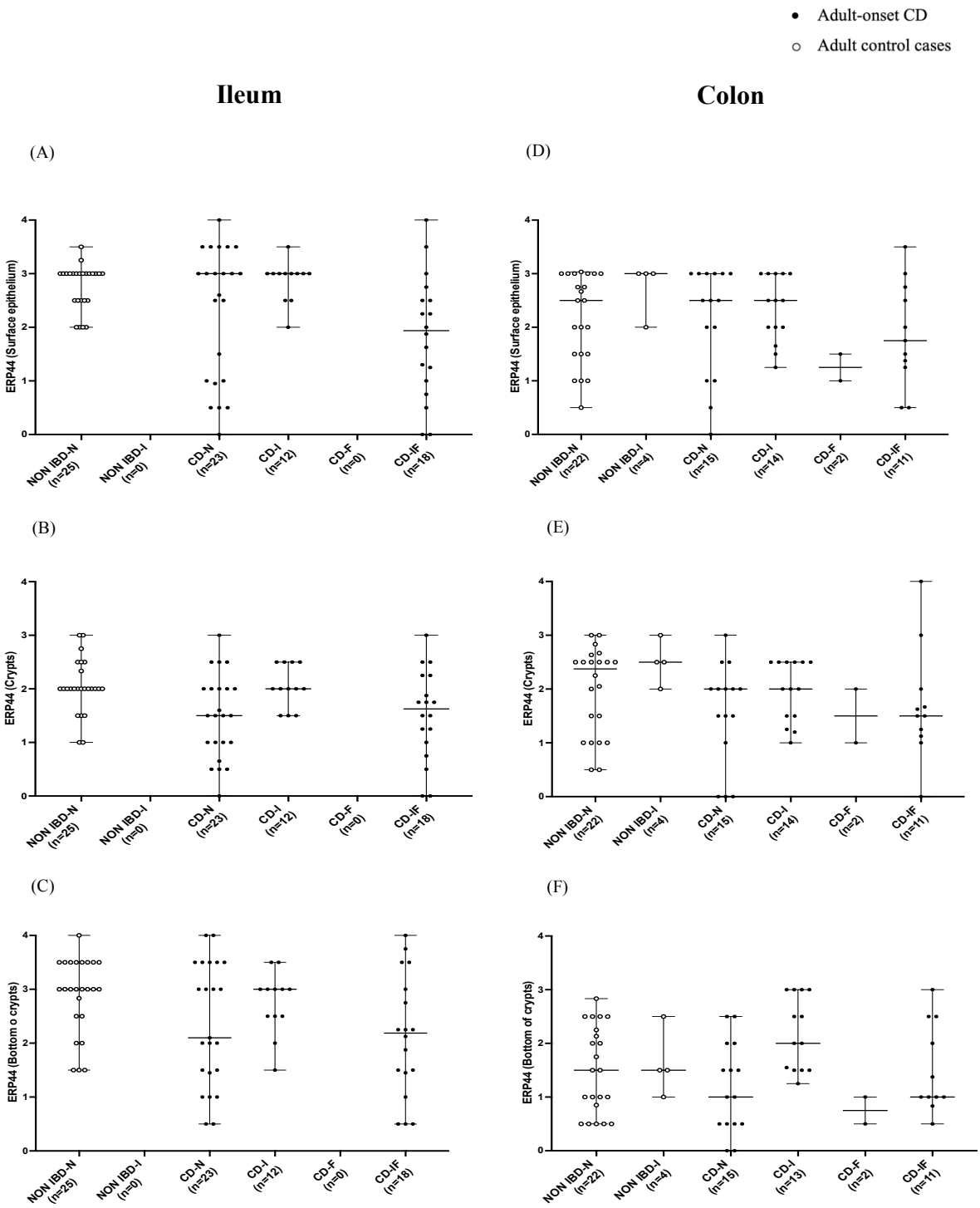

Figure 54. Distribution of ERP44 IHC scores in ileal (A-C) and colonic (D-F) surface epithelium, crypts and bottom of the crypts in normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation in adult patients

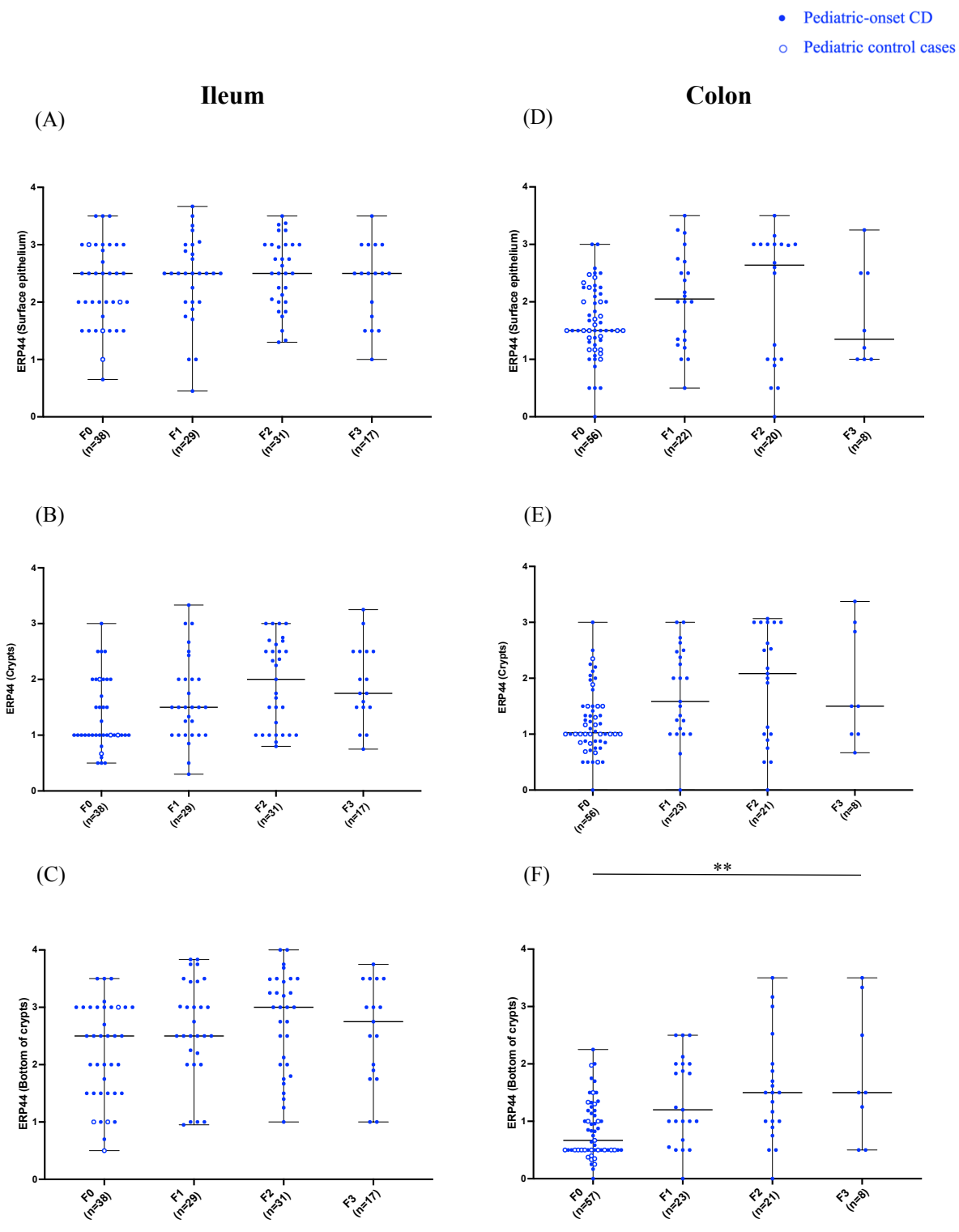

Figure 55. Distribution of ERP44 IHC scores in ileal (A-C) and colonic (D-F) tissues according to fibrosis grades in pediatric-onset cases

* Statistical significance based on ANOVA test results after Bonferroni correction

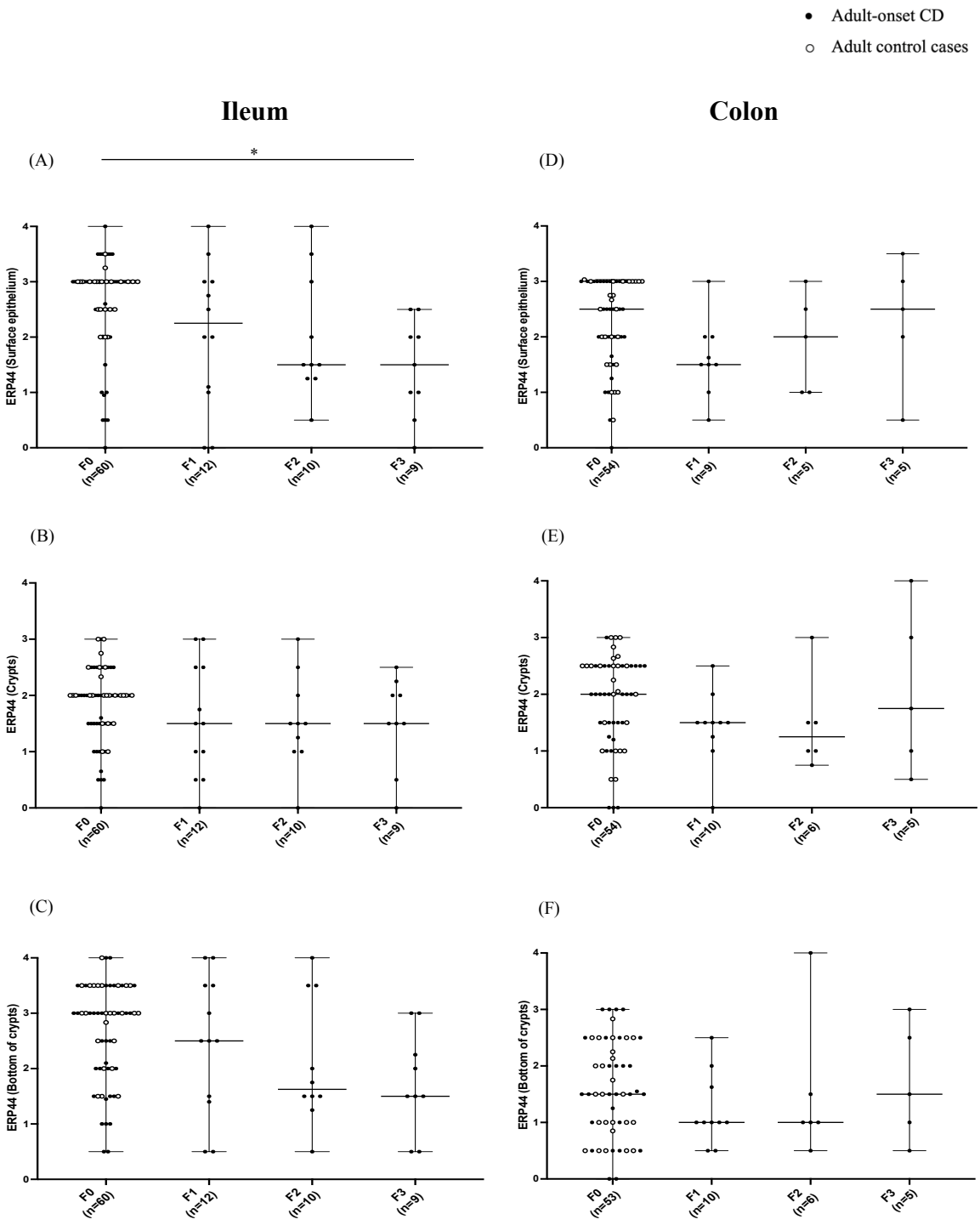

Figure 56. Distribution of ERP44 IHC scores in ileal (A-C) and colonic (D-F) tissues according to fibrosis grades in adult-onset cases

* Statistical significance based on ANOVA test results after Bonferroni correction

5.4.4. 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 (Figure 57 and Figure 58).

These results were similar when including inflamed and/or fibrotic cases, with no difference was observed 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 Figure 59. Sensitivity, specificity and accuracy using this threshold were established for each segment and segment location (Table 9).

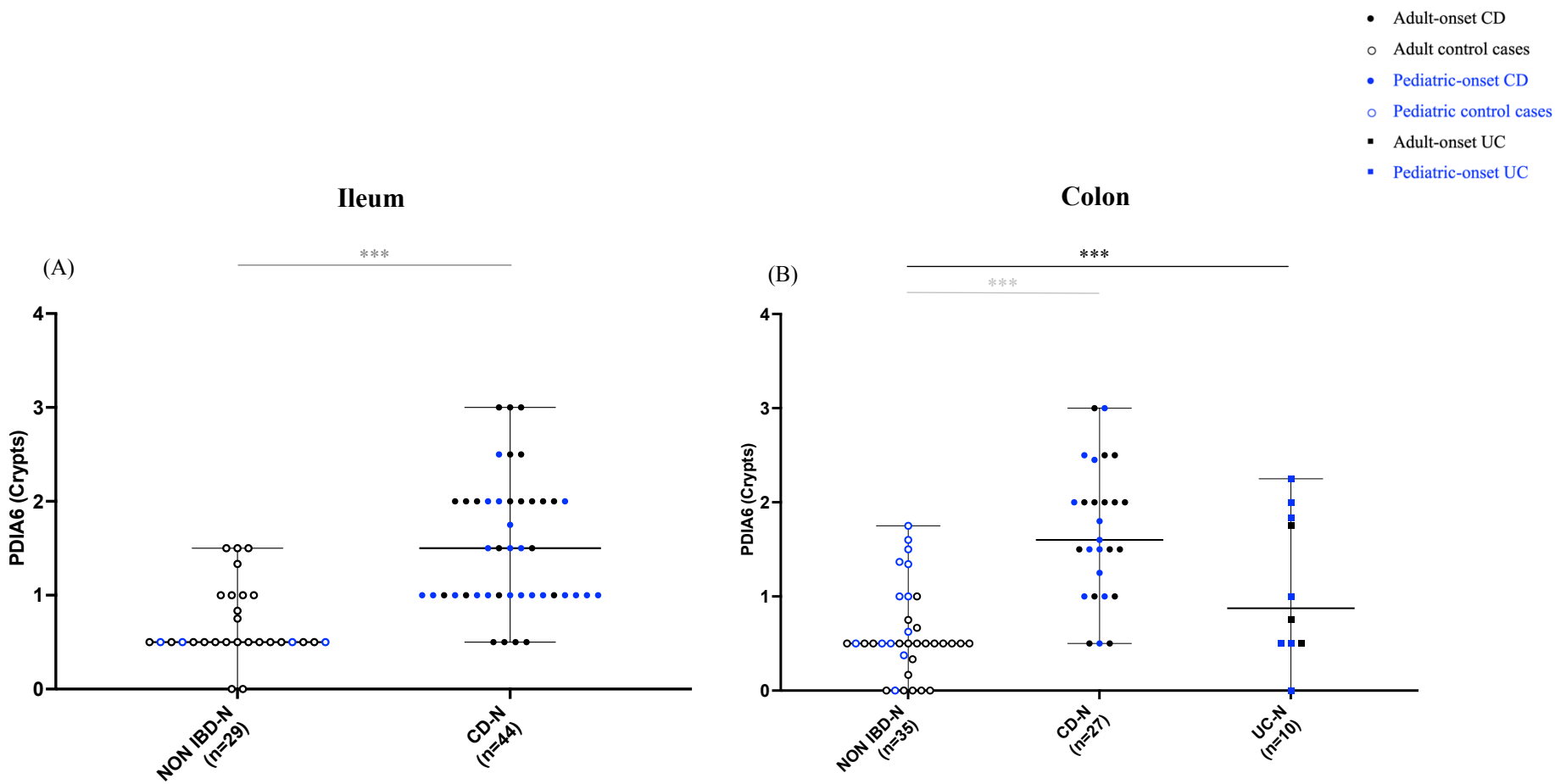

Figure 57. Distribution of PDIA6 IHC scores in normal ileal and colonic crypts (N-without inflammation nor fibrosis) based on IBD phenotype (CD or UC) or healthy controls

* 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-Whitney test after Bonferroni correction

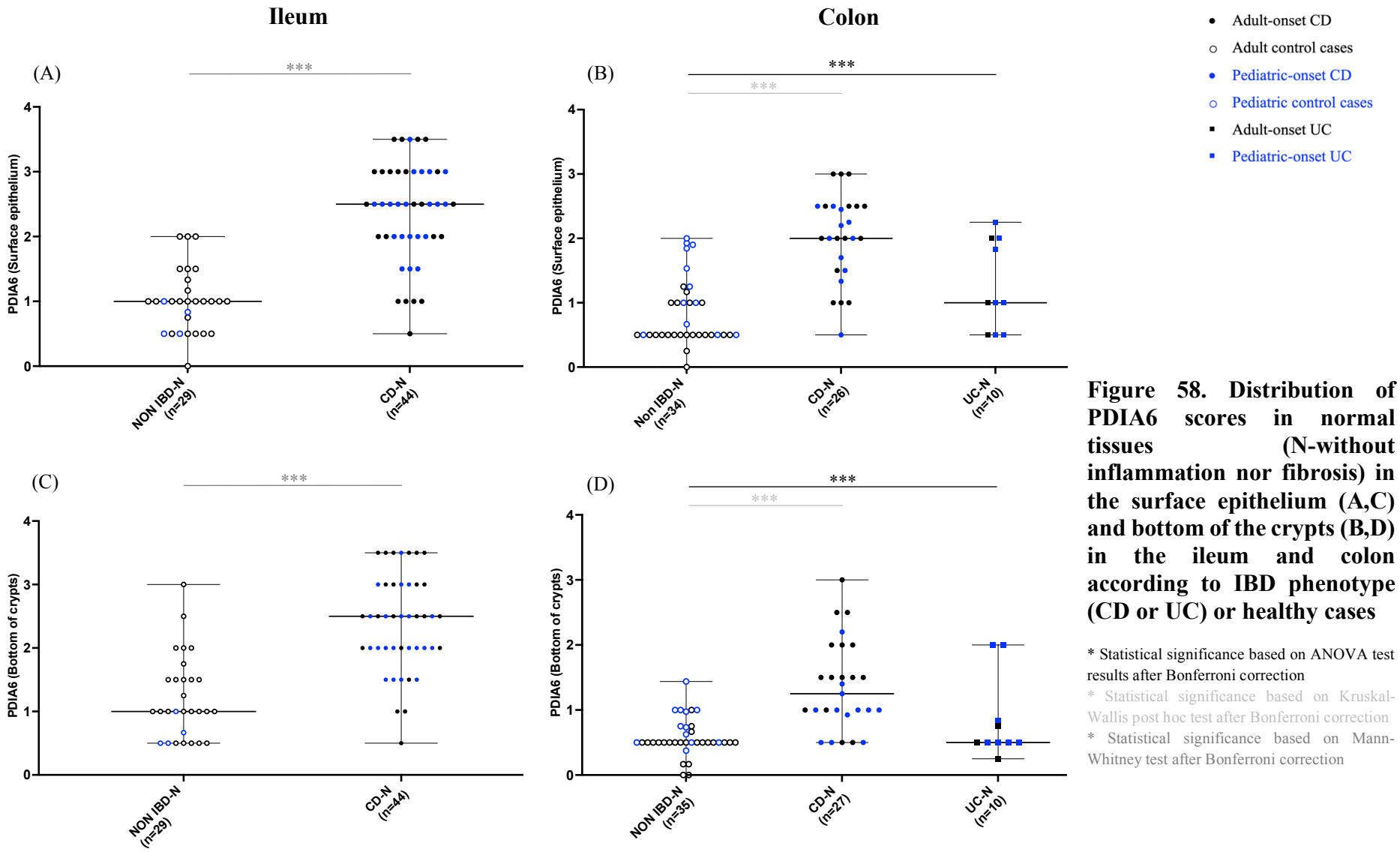

Figure 58. Distribution of PDIA6 scores in normal tissues (N-without inflammation nor fibrosis) in the surface epithelium (A,C) and bottom of the crypts (B,D) in the ileum and colon according to IBD phenotype (CD or UC) or healthy cases

* 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-Whitney test after Bonferroni correction

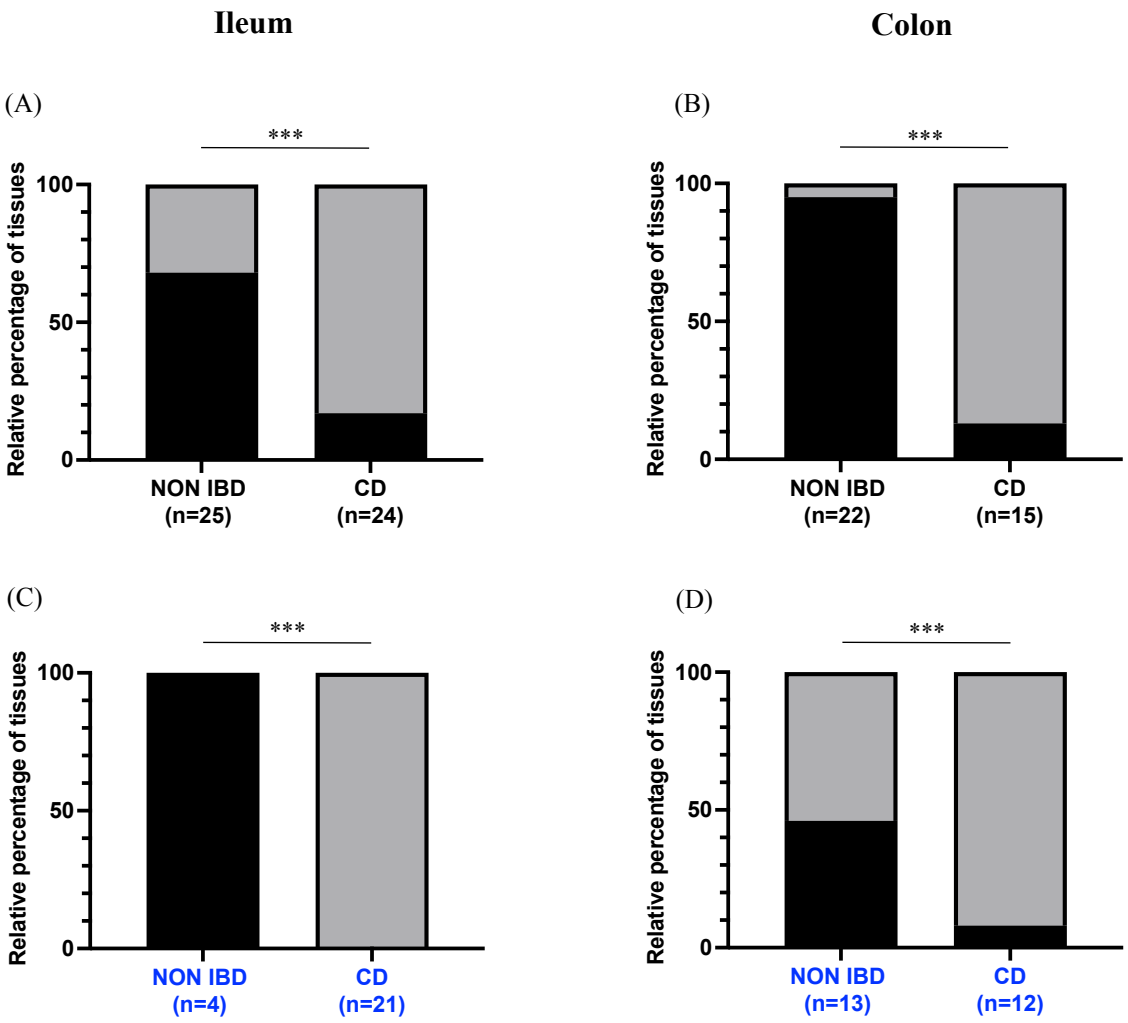

Figure 59. Contingency table result with relative percentage of tissues with PDIA6 score <1 and ≥1 in non IBD and CD in adults (A)(B) and pediatric-onset (C)(D) cases

PDIA6 <1 **PDIA6 ≥1**

* Statistical significance based on Fischer's exact test

Table 9. Summary of sensitivity, specificity and accuracy of PDIA6 IHC score= 1 (with negative for CD when "< 1" and positive for CD when "≥ 1") as a discriminant.

		Tissues from pediatric-onset cases			Tissues from adult-onset cases		
		Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Ileum	Surface epithelium	100	75	96	96.3	28	63.5
	Crypts	100	100	100	83.3	68	75.5
	Bottom of crypts	100	75	96	96.3	20	59.6
Colon	Surface epithelium	90.9	33.3	60.9	100	72.7	83.8
	Crypts	91.7	46.2	68	86.7	95.5	91.9
	Bottom of crypts	66.7	69.2	66.7	86.7	95.7	92.1

5.5. 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 variability indicates that while these proteins are involved in the same global cellular process (ER stress response), their expression patterns differ based on tissue features, disease phenotype, and location. However, the underlying factors driving this heterogeneity - whether patient age, disease stage, or segmental localization - remain incompletely understood.

Despite various proposed histopathological indices, no scoring system for evaluating fibrosis in CD has gained widespread consensus or consistent application[272,285,397,398]. This highlights a major gap in the field. Recently, consensus criteria for small bowel CD have been introduced to standardize histopathological assessment, identifying key features such as submucosal or transmural fibrosis, muscularization of the submucosa, and thickening of the *muscularis mucosa* compared to adjacent normal tissue[286]. While promising, these criteria remain limited to small bowel disease and have yet to be integrated into validated tools for clinical trials. One of the key advances needed in intestinal fibrosis research is the development of a validated histopathological scoring system that accounts for both inflammatory and fibrotic components and is applicable across all intestinal segments, to ensure comparability of findings across studies[399].

ER stress plays a crucial role in inflammatory and fibrotic diseases in multiple organs[338,340,343], including the intestine[91,96,316,346,349,400]. PDIA6 and ERP44 are members of the PDI family, whereas BiP functions as a chaperone rather than a typical PDI. Although AGR2 belongs to the thioredoxin family, it does not possess isomerase activity[401,402]. However, it shares structural features with PDIs, and is classified as a PDI family member based on sequence homology. These proteins are known to regulate ER homeostasis, oxidative protein folding, and the UPR, processes deeply implicated in fibrotic progression[101,403]. Intestinal epithelial cells, Paneth, and goblet cells are all susceptible to ER stress notably due to microbiota exposure[105]. Although the specific roles of the ERS-related proteins remain poorly understood, their expression is influenced by factors like cell type, tissue location, pH, microbial activity[79,404] and

developmental stages[405,406]. For example, goblet and Paneth cells require more ER chaperones due to secretory functions[100]. Inflammatory pathways and metabolic stress also regulate the expression of these proteins[79,407]. 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 ERS-related proteins expressions[408]. However, a full understanding of these differences goes beyond this IHC characterization and requires validation in functional models.

The significant increase in AGR2 distribution in fibro-inflammatory and fibrotic tissues suggests its crucial role in CD fibrosis. This increase is not observed in purely inflammatory tissues, suggesting that AGR2 is more strongly linked to fibrosis than to inflammation. AGR2 has been identified as a key regulator of IRE1β signaling in goblet cells, where it prevents excessive UPR activation by disrupting IRE1β oligomerization. Loss of AGR2 leads to spontaneous IRE1β activation, suggesting that dysregulated AGR2 function may impact ER stress responses and fibrosis development[409]. AGR2 overexpression has been identified in CD and other fibrotic conditions like idiopathic pulmonary fibrosis and fibrolamellar cancers[349,410,411]. An increase in cytoplasmic AGR2 has been associated with the increased release of its extracellular form (eAGR2) and associated with ECM in cancer[89]. Our findings suggest that the increase in cytoplasmic AGR2 may also be an indicator of increased eAGR2. Functional assays showed that, under ER stress, HT29 epithelial cells increased eAGR2 along with cytoplasmic AGR2, inducing a paracrine transition of intestinal fibroblasts into myofibroblasts, a process also observed in lung fibroblasts[346,353]. Furthermore, AGR2's interaction with β-catenin promotes pro-fibrotic gene expression, and its role in fibroblast recruitment supports its involvement in fibrosis[353]. This is also true in cancer-associated fibroblasts[412], as eAGR2 promotes epithelial proliferation and contributes to endo-MT and EMT, both implicated in intestinal fibrosis[89,413–416]. The correlation between AGR2 and fibrosis in both the 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 and systemic eAGR2 levels, are beyond the scope of our IHC characterization on FFPE tissues and require dedicated investigation. For example, recent studies have shown that AGR2 can reflux into the cytosol under stress, where it interacts with and inhibits p53, enhancing cell survival in cancer models[417]. This mechanism could sustain fibroblast survival in fibrosis. Moreover, extracellular AGR2 has been successfully targeted *in vivo* using antibody-based approaches, leading to reduced fibrosis in preclinical models[346,351].

BiP, chaperone involved in the UPR, was elevated not only in fibrotic but also in purely inflammatory tissues which is expected knowing its role of chaperone in ER stress and UPR. It has been associated with inflammatory and fibrotic diseases when its function is disrupted[346,348]. 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[418–420]. 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[93,421–423]. These findings suggest that impaired regulation of ER stress, due to IL-10 deficiency, may worsen IBD progression. BiP distribution in both adult and pediatric tissues probably reflects the degree of inflammation and ER stress, rather than a direct involvement in fibrotic process, as its role in ER stress is primarily restricted to intracellular functions and it is not secreted by epithelial cells, unlike AGR2. Nevertheless, BiP's dissociation from UPR sensors (IRE1, PERK, ATF6) has been shown to trigger prolonged ER stress and drive fibrosis in hepatic models[424], raising the possibility of similar pathways contributing to intestinal fibrosis.

PDIA6 and ERP44, both interact with AGR2 and BiP, regulating protein folding and ER homeostasis[403,425–427]. They were found to be increased in the protein lysates of ileal and colonic biopsies taken from ulcer edges[428], and in the epithelium of fibrostenosing CD tissues, as well as in the supernatant of HT-29 cells after ER stress[346]. In our data, PDIA6 levels primarily distinguished CD from non IBD tissues, with no direct association observed with fibrosis severity. Higher PDIA6 levels in CD compared to non IBD, even in non-affected area, suggest a potential role in early pathogenic mechanism in CD, possibly to stress responses or epithelial homeostasis alterations. PDIA6 is more strongly distributed in pediatric cases, and varies between ileum and colon, potentially reflecting higher intestinal cell proliferation rates in children. Its transcript levels have been shown to vary with development, promoting cell motility and proliferation[429,430]. Additionally, PDIA6 is upregulated in hypoxic conditions, suggesting a role in highly oxygen-demanding intestinal regions[431,432]. PDIA6 has also been shown to downregulate IRE1-mediated UPR activation, an essential process for maintaining mucin homeostasis[433,434], thereby indirectly influencing fibroblast activation. However, aside from proteomic observations in Vieujean *et al*.[346], no direct link between PDIA6 and intestinal fibrosis has been established. Although ER stress is known to contribute with fibrosis progression[345], direct evidence involving PDIA6 remains limited. In other organs, BiP and

PDIA6 levels correlate with interstitial fibrosis, as reported in a murine cardiac model[342]. PDIA6 plays a key role in hepatic stellate cells activation and liver fibrosis, potentially serving as a biomarker for cirrhosis and fibrotic liver diseases[435]. Importantly, ERP44 and AGR2 have been detected outside the ER in fibrotic conditions[346,417], suggesting potential non-canonical extracellular roles that may contribute to matrix remodeling. The precise contribution of PDIA6 outside the ER and its potential specific role in fibrosis remain unclear and require further investigation. Ongoing studies in our laboratory are addressing these aspects using *in vitro* culture models[90,346].

In contrast, 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 bottom of crypts. ERP44 distribution 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. ERP44, a redox-sensitive chaperone involved in disulfide bond formation and ER stress regulation, plays a crucial role in maintaining protein homeostasis, which is increasingly implicated in both fibrosis and cancer[436,437]. ERP44 expression is influenced by endoluminal and intracellular pH[438–440]. The more acidic pH in the colon, compared to the slightly alkaline ileum, may explain the higher colonic ERP44 levels[439,441]. 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 downstream oxidative and inflammasome pathways[442–444]. Recent mechanistic insights suggest that ERP44 promotes selective ER retention of misfolded glycoproteins during stress, while PDIA6 facilitates their recovery[445], indicating a functional complementarity which may influence the balance between adaptive wound healing responses and uncontrolled pathological fibrosis. Additionally, ERP44 and AGR2 interact with death receptors DR4/DR5 in a breast cancer model, suppressing apoptotic signaling[437]. Given their role in stress response and secretory pathways, dysregulation of ERP44 and eAGR2 could promote fibroblast activation and tissue stiffening.

Our study's limitations include its retrospective nature, and the immunohistochemistry techniques albeit the gold standard for protein characterization in large sets of tissues. Our multicenter study represents the only characterization of ERS-related proteins in relation to fibrosis and inflammation in pediatric and adult tissues. However, our findings are

observational, and further mechanistic validations are required for ERP44 and PDIA6, as has been performed for AGR2[89,346,351,353,416]. Functional studies might help in elucidating the probable complex and interconnected roles of these proteins in fibrosis-related pathways. Additionally, the heterogeneity of patient cohorts in terms of treatment regimens and disease duration, combined with the limited number of anastomotic stenoses, reduces the statistical power for robust multivariate adjustments.

5.6. CONCLUSION

Many questions remain about ER stress induction and UPR's role in intestinal CD fibrosis. Current IBD treatments remain insufficient to prevent or reverse fibrosis or intestinal strictures. Developing effective drugs require a better understanding of complex interactions between proteins produced in response to ER stress and inflammation, as BiP, AGR2 and others ERS-related proteins, partially described[402,403,425,446–448], 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 potentials.

GENERAL DISCUSSION AND PERSPECTIVES

6. GENERAL DISCUSSION AND PERSPECTIVES

6.1. PEDIATRIC IBD EPIDEMIOLOGY AND HETEROGENEITY

Pediatric IBD is a heterogeneous condition, primarily comprising CD and UC, and occasionally classified as indeterminate colitis (IBDU), with further variation in clinical phenotypes and age at diagnosis[449]. Such heterogeneity makes it difficult to tailor treatment and predict disease outcomes. Studying if and how age at onset might shape disease trajectory should provide new insights into distinct pathophysiological mechanisms – particularly in VEO-IBD compared to EO-IBD.

PIBD is increasing globally, especially in regions with historically low rates[30,450]. Our results confirm a rising incidence of EO-IBD, contrasting with the stable incidence of VEO-IBD[126]. This divergence may reflect age-dependent exposure to environmental risk factors in EO-IBD, *versus* a stronger genetic predisposition in VEO-IBD. Interestingly, adult-onset IBD incidence in Western countries appears to have plateaued, suggesting different dynamics within the different age groups[25]. Multiple factors may explain the international variability in reported incidence rates, including population-level differences in genetic susceptibility, environmental exposures, access to diagnostic technologies, and improvements in healthcare infrastructures. Methodological discrepancies between surveillance systems further contribute to artificially widening the observed gap between Western countries and newly industrialized regions[25]. Moreover, the global rise in PIBD may partly reflect improved recognition that young children can develop IBD, greater access to specialized care, and enhanced diagnostic capabilities, particularly in regions transitioning from emergence to higher-prevalence phases. While most countries are still in the acceleration phase, some Western regions may be approaching a plateau, suggesting that PIBD could follow a similar epidemiological trajectory to adult-onset disease in the coming years[30].

Though many epidemiological studies exist, they often focus on selected cohorts from specialized centers or administrative health data, limiting their ability to reflect the actual incidence and prevalence of IBD in the entire population[130]. To address this limitation, general population-based registries, like EPIMAD in Northern France, are essential for accurately estimating disease incidence, burden, and natural history[25]. In Belgium, there is currently no such registry, although the temporary BELCRO registry, initiated in 2008, offers extensive data

on newly diagnosed pediatric CD cases and disease activity[451–453]. Unfortunately, BELCRO is no longer operational, hindering further insights into disease progression in Belgium.

Our study, along with others, indicates a continued rise in the overall incidence of PIBD, despite stable rates in VEO-IBD – an observation that contrasts with findings from other cohorts[126,134,224]. These discrepancies underscore the importance of establishing harmonized, long-term, and multicentric surveillance systems to better understand age-specific patterns and disease dynamics. Methodological biases – such as under-reporting, heterogeneity in cohort definitions, or variations in registry coverage – may partly explain the conflicting epidemiological data and highlight the urgency of creating robust and standardized national or European PIBD registries[130,134].

6.2. PHENOTYPE DIVERGENCES ACROSS AGE GROUPS

Although children share certain similarities with adult-onset disease, important differences in both clinical presentation and disease course exist[155,219]. Our findings reaffirm that VEO-IBD and EO-IBD differ in clinical presentation and progression, raising important questions about the underlying disease mechanisms. VEO-IBD is more frequently associated with colonic disease, rectal bleeding, and perianal involvement, and includes a subset of monogenic forms – notably involving mutations in the IL-10 signaling pathway (e.g., IL10RA) – that are linked to severe, refractory disease[251,252,423,454]. However, VEO-IBD are not uniformly aggressive. While monogenic forms exhibit high morbidity and often require targeted therapeutic approaches, non-monogenic cases may follow a milder trajectory[251,252]. EO-IBD, and especially EO-CD, more commonly involves the ileum and is associated with a more severe ileal involvement and higher risk of progression to stricturing or penetrating complications[168,172,455].

These divergences may reflect fundamental differences in gut and immune system maturation. VEO-IBD often arises in a context of immature intestinal immunity and epithelial barrier development, along with early-life microbiota establishment. These features may predispose to distinct immunological responses, including heightened susceptibility to dysregulated inflammation or fibrosis in a genetically vulnerable host. Animal models support this view: early-life nutritional stress, such as postnatal growth restriction, impairs intestinal barrier formation and alters microbiota composition, resulting in increased permeability and mucosal inflammation[150]. Microbiota imbalances may normalize over time; however, the early insult leads to long-term immune dysregulation and heightened susceptibility to colitis in adulthood, highlighting the concept of early programming of intestinal immunity[150,456–458]. In older children and adults, the maturation of gut-associated lymphoid tissues (e.g., Peyer's patches) and cumulative environmental exposures may contribute to a greater propensity for ileal disease and fibrostenotic progression[156]. Moreover, the high prevalence of adherent-invasive *Escherichia coli* in ileal CD represents another potential microbiota-driven mechanism[43].

Current phenotype classifications, such as the Paris classification, are primarily based on clinical symptoms and radiologic or endoscopic findings at diagnosis[14]. Fibrostenotic progression (B2 phenotype) is typically assessed in a symptom-driven manner, meaning that silent or subclinical progression may go undetected. Imaging modalities such as MRI or intestinal US are often used to assess disease status, but typically in response to clinical deterioration rather than through systematic, routine monitoring. Their availability and

interpretation remain variable, especially in younger patients. As a result, the true prevalence and timing of B2 progression may be underestimated, particularly in the absence of systematic, symptom-independent follow-up.

Disease management approaches differ between pediatric and adult populations, as pediatric gastroenterologists tend to prioritize growth, nutritional status, and the need for comprehensive imaging, whereas adult specialists may follow different diagnostic and therapeutic algorithms[459]. A recent systematic review reveals the increasing focus on early detection and intervention, particularly in pediatric patients. Specifically, the prompt and aggressive use of biological medications in the early stages of PIBD plays a key role in achieving disease remission and supporting growth[398]. However, the overall risk of progression to surgery remains significant, both in adult and pediatric cohorts.

6.3. STRICTURING PROGRESSION IN PEDIATRIC AND ADULT CD

The progression toward a stricturing phenotype appears to be influenced by multiple factors, including age at diagnosis, disease location, and underlying molecular mechanisms. The stricturing phenotype (B2) is particularly problematic due to its association with mechanical complications such as intestinal obstruction, upstream inflammation, and fistula formation. In fact, most fistulas originate in or around stenotic segments, highlighting the central pathogenic role of fibrotic remodeling. Notably, emerging evidence suggests that the pathophysiological mechanisms driving fistulizing and stricturing phenotypes may partially overlap, involving shared pathways such as chronic inflammation, EMT, and imbalances between MMPs and their TIMPs[460]. Moreover, current treatments, including biologics, remain largely ineffective in halting or reversing fibrosis once established, which often leads to the need for surgical resection[289,296,305]. As such, the B2 phenotype is not only associated with increased morbidity but also with a diminished quality of life and a higher risk of surgical intervention.

Genotype-phenotype studies have identified associations between NOD2 variants and the development of fibrostenotic CD, particularly in the small bowel[294,461]. Patients carrying *NOD2* mutations tend to exhibit increased fibrosis and more complicated disease courses. However, animal models with *NOD2* knockdown or human-equivalent variants do not spontaneously develop fibrosis, suggesting that additional environmental or immune factors are required. *TLR* variants, especially TLR4, are also associated with an increased risk of fibrostenotic disease[461]. TLRs function as microbial sensors, triggering pro-inflammatory and pro-fibrotic signaling pathways. In CD, *TLR* overexpression may contribute to abnormal immune responses against commensal bacteria. Notably, patients with heightened antibody responses to microbial peptides (e.g., *Anti-Saccharomyces cerevisiae* antibodies) tend to develop fibrostenotic complications earlier, pointing toward a defective innate immune tolerance that may drive fibrogenesis[302,461].

Our results suggest a slower progression toward stricturing complications in VEO-IBD patients compared to those diagnosed after the age of 6. Data on pediatric CD progression have yielded somewhat contradictory conclusions. While some studies report that older children often experience a more aggressive disease course with a higher risk of stricturing or penetrating complications due to increased ileal involvement, other research indicates that the risk of disease progression in pediatric patients is comparable to that in adults[135,169,188,462]. These observations support the notion that not all pediatric CD cases share the same tendency toward

fibrostenotic evolution. They also underscore the complexity of disease progression, suggesting that factors such as age at diagnosis, disease location, and underlying genetic or molecular mechanisms may differentially influence long-term outcomes. Ouahed *et al.* further reviewed genes implicated in epithelial function and immune regulation in PIBD, particularly in monogenic forms, highlighting how these pathways may underlie the distinct clinical severity observed in early-onset disease[454]. It also suggests that specific genetic and inflammatory profiles could serve as predictive markers for stricturing complications, reinforcing the idea that pediatric CD represents a distinct entity compared to adult-onset disease. In parallel, recent epigenetic findings reveal that specific methylation profiles detectable at the time of surgery can predict CD recurrence within three years, in both adult and pediatric cohorts[463]. This underscores the potential of epigenetic profiling as a prognostic tool, providing valuable insights into long-term disease progression and therapeutic responsiveness.

Emerging diagnostic tools, such as genetic testing and endoscopic evaluations, are crucial in enhancing the management of PIBD[398]. However, current diagnostic practices may fail to adequately capture subclinical or "silent" progression, particularly in children. According to the ESPGHAN-ECCO consensus led by Van Rheenen *et al.*, clinical disease activity scores such as the Pediatric Crohn's Disease Activity Index (PCDAI) do not reliably reflect mucosal healing, as up to half of patients in clinical remission may still exhibit endoscopic inflammation [464]. They established that it was key to identify patients at high risk of a complicated disease course at the earliest opportunity, in order to reduce long-term bowel damage. The MINI (Mucosal Inflammation Noninvasive) index, which combines clinical symptoms with inflammatory markers (fecal calprotectin, CRP and ESR) has demonstrated good accuracy in predicting mucosal healing in pediatric CD cohorts[465]. But still, in the absence of systematic imaging protocol, these indices may not adequately capture transmural or fibrostenotic progression. The lack of clear recommendations regarding the timing and frequency of follow-up imaging – particularly in asymptomatic patients – constitutes a major gap. As a result, many cases of silent progression may go undetected until complications arise.

While cross-sectional imaging techniques such as MRI and intestinal US can detect bowel wall thickening or prestenotic dilatation, they fall short in quantifying fibrotic burden itself, limiting their value in monitoring response to antifibrotic therapies[305]. Novel imaging sequences, including T2 relaxometry and magnetization transfer, are currently being explored for their potential in non-invasively and accurately differentiate inflammation from fibrosis, which

remains a major unmet need in clinical practice[117]. Moreover, no imaging-based gold standard currently exists to stratify patients by fibrosis severity. Moreover, retrospective study designs, often reliant on symptom-driven imaging, introduce bias and hamper time-dependent phenotypic analyses.

To overcome these limitations, prospective longitudinal studies with standardized imaging at predefined intervals are needed to better capture disease evolution and allow early risk stratification. Furthermore, developing composite clinical scores incorporating genetic, serologic, and imaging markers would support early risk stratification and timely therapeutic escalation, particularly in patients predisposed to fibrostenotic complications[190,193]. Until then, routine assessments and proactive monitoring strategies will remain essential to limit the silent progression to B2 phenotypes, especially in pediatric-onset CD. In parallel, identifying non-invasive biomarkers that correlate with fibrotic remodeling is needed to complement imaging approaches and enable early detection and risk monitoring in routine clinical practice. This need represents a key objective of ongoing translational efforts, including our work.

6.4. FIBROSIS IN CD: FROM MECHANISMS TO MOLECULAR TARGET

Given that fibrosis is a major complication of CD in both adult and pediatric patients, a deeper understanding of its pathogenesis is essential for the identification of novel biomarkers and therapeutic targets which stay unmet clinical needs.

Intestinal fibrosis in IBD arises from a multifactorial and dynamic process involving immune and non-immune cell populations, mesenchymal remodeling, and continuous exposure to luminal stimuli such as microbial products and environmental factors. Mesenchymal cells, including fibroblasts and myofibroblasts, play a central role in shaping tissue architecture and driving fibrotic responses, influenced by cytokines, growth factors, and cellular senescence[305]. Recent single-cell analyses have highlighted the cellular heterogeneity of fibrotic niches and revealed interactions between mesenchymal and immune compartments that promote chronic remodeling[319]. While inflammation is a known driver of fibrosis, accumulating evidence suggests that fibrogenesis can persist independently once triggered, indicating a shift to inflammation-independent mechanisms early in disease progression[305,330]. In parallel, components such as creeping fat and smooth muscle hyperplasia have been linked to stricture formation, and the gut microbiota – especially during early life – has emerged as a key modulator of fibrotic processes through its effects on barrier function, immune tone, and metabolic outputs[336,466–468].

Among these complex pathways, increasing attention has been given to ER stress and its downstream signaling as a critical mediator of fibroblast activation and intestinal matrix remodeling[346,347]. Our immunohistochemical analyses demonstrated that ER stress-related proteins are differentially expressed across tissue types and age groups. AGR2 showed the strongest association with fibrotic changes, BiP was linked to both inflammation and fibrosis, and ERP44 was more strongly associated with fibrosis in younger patients. PDIA6 consistently upregulated in CD – even in non-affected regions – shows higher immunohistochemical signals in pediatric patients, suggesting it may act as an early pathogenic factor tied to elevated epithelial proliferation and altered homeostasis in the gut. These findings underscore the age- and tissue-specific variability of ER stress responses in CD and suggest that ERS-related proteins may play a context-dependent role in fibrogenesis. Supporting this view, Vanhove *et al.* developed an *ex vivo* intestinal epithelial cell culture system from patient biopsies and demonstrated that individuals carrying higher numbers of ER stress and autophagy genetic risk

alleles showed amplified epithelial ER stress responses, as measured by BiP induction. Their study highlights how intrinsic epithelial susceptibility to ER stress can vary between patients and could be quantified to support personalized stratification[469]. Altogether, these findings reinforce the relevance of ER stress pathways in disease progression and fibrosis, and suggest their potential clinical utility for patient stratification and the development of targeted therapies.

ER stress-related proteins could be notably implicated in fibrotic progression through their roles in ER homeostasis, protein folding, and UPR regulation[101,403]. AGR2, a key modulator of IRE1β signaling, and BiP, a central UPR chaperone, have been associated with fibrosis when dysregulated[409,433,437]. PDIA6 contributes to UPR modulation and may influence fibroblast activation[433,434]. In a breast cancer model, ERP44 and AGR2 have been shown to interact with death receptors (DR4/5) and regulate apoptosis[437], with potential relevance in fibroblast persistence. Notably, they can be secreted under stress, acquiring extracellular functions that support fibrosis[346]. Targeting eAGR2 has already shown antifibrotic effects in preclinical models[346,351], and manipulating ER stress pathways offers promising avenues to mitigate fibrostenotic complications, as shown in liver and skin models[344]. Disrupting ERP44 and AGR2 interactions with DR4/DR5 using small molecules, such as disulfide bond-disrupting agents (DDA), could thus represent a novel therapeutic strategy to induce myofibroblast apoptosis and mitigate fibrotic progression, potentially through a paracrine mechanism. Supporting this hypothesis, prolonged ER stress has been associated with a relocalization of ER stress-related proteins such as AGR2, ERP44, and PDIA6 outside the ER, where they may acquire potential alternative functions that might contribute to fibrosis[346,417]. Specifically, AGR2 has been shown to reflux from the ER to the cytosol, where it interacts with and inhibits wild-type p53, thereby enhancing cell survival in cancer models[417].

In this context, patient-derived organoids (PDOs) and co-culture models of epithelial cells and fibroblasts provide valuable tools to explore the paracrine effects of secreted ERS-related proteins, including their impact on EMT (autocrine) and myofibroblast activation (paracrine)[470,471]. Organoids, derived from different gastrointestinal segments, retain genetic background and epigenetic properties and can be stored in biobanks for IBD research. They could serve as versatile models to study epithelial response to stress or cell-to-cell interactions and paracrine effects. Moreover, the generation of patient-derived organoids from CD patients is feasible and provides a valuable model to study epithelial pathology, although it remains

technically demanding. Indeed, organoids from CD patients often show slower initial growth, increased risk of contamination, and reduced viability at early passages[466,472].

At the same time, there is a growing interest in identifying reliable biomarkers that can predict or assess fibrosis. Several biomarkers have been explored, including extracellular matrix components, serological biomarkers, and gene variants. However, none have yet demonstrated sufficient specificity to distinguish stricturing disease from other inflammatory manifestations[305,397]. A recent study investigated the potential of PRO-C11 and PRO-C16 (collagen pro-peptides linked to type XI and type XVI collagen synthesis), biomarkers for diagnosing intestinal fibrosis in pediatric CD patients. Both biomarkers showed significant elevation in serum from patients with intestinal fibrosis when compared to healthy controls or those without fibrosis, correlating with MRE-based fibrosis severity scores. The results suggest that PRO-C11 and PRO-C16 could serve as non-invasive biomarkers for monitoring fibrosis and stenosis in pediatric CD[473]. Moreover, candidate biomarkers such as serum microRNAs, anti-microbial antibodies and circulating fibrocytes count might show potential for predicting a disabling disease course, though their use in clinical applications remains inconclusive[474]. Our observation indicates that PDIA6 is upregulated in CD tissues, even in areas without inflammation or fibrosis, suggesting a potential role as an early marker of epithelial stress. Although PDIA6 has been detected extracellularly *in vitro* under ER stress conditions[346], there is currently no evidence supporting its active secretion or role in circulation. Therefore, while its detection in serum is promising[475], its relevance as a circulating biomarker for clinical use remains to be fully established and requires further investigation in different pathological situation (in IBD, non IBD controls and other fibrotic pathologies).

Despite the lack of current discriminating fibrosis specific biomarkers and the persistence of grey areas in the pathophysiology of intestinal fibrosis, preclinical studies have shown promising results on antifibrotic therapies, targeting key signaling pathways like TGF-β and PDGF, as well as agents modulating fibroblast activity[467,476]. Recent findings indicate that epithelial cells exposed to inflammatory or ER stress conditions secrete higher levels of eAGR2, which directly stimulate fibroblasts to proliferate and upregulate fibrotic markers like α-SMA and collagen. Additionally, neutralizing eAGR2 with targeted antibodies in murine colitis models significantly reduces fibroblast activation, suggesting that AGR2 is a key driver of fibroblast-to-myofibroblast transition[351,477]. These results prove that targeting eAGR2 for improving fibrosis-related inflammation in CD could be effective.

Moreover, several pathways have been identified to reverse EMT, including mTOR inhibition, TGF-β receptor and fibroblast growth factor 1 signaling inhibition, with mesenchymal-to-epithelial transition, showing promise in experimental models of renal, pulmonary, and liver fibrosis[478]. Additionally, PPAR-γ has emerged as a key regulator with both anti-inflammatory and antifibrotic properties, modulating cytokine production (IL-4, IL-5, IL-6) and interfering with profibrotic mediators such as PDGF and TGF-β. Preclinical and early clinical data suggest that PPAR-γ agonists can attenuate fibrotic progression in intestinal fibrosis models, making them an attractive therapeutic track for CD-related fibrosis[479,480]. Other promising candidates include tranilast, which has shown anti-TGF-β and anti-EMT properties in experimental models and may delay symptomatic progression of intestinal strictures in CD; spironolactone, which inhibits TGF-β-induced profibrotic gene expression in colonic myofibroblasts and intestinal organoids; and pirfenidone, which reduces collagen deposition and TGF-β/MMP-9 expression in murine models of intestinal fibrosis[471,480].

Some potential therapies are under clinical investigation for intestinal fibrosis, such as TNF-like 1A (TL1A) inhibitors which reduce Th1/Th17 responses while enhancing Treg activity, as these have demonstrated anti-fibrotic effects in preclinical models[481–483]. Inhibitors of integrin αvβ3 such as cilengitide have also been shown to reduce TGF-β1-induced collagen production[477,484]. Other promising agents such as the ALK5 inhibitor, which targets TGF-β signaling, has demonstrated efficacy in lung, liver and renal fibrosis[485–487], and is currently being evaluated in a Phase 2a trial for fibrostenotic CD. Additionally, ROCK inhibitors such as AMA0825 have been shown to reverse established intestinal fibrosis in mouse models and CD biopsies by limiting myofibroblast accumulation and restoring autophagy[488].

Knowing that the gut microbiota strongly influences both inflammation and fibrosis in IBD, targeting the microbiota through probiotics, prebiotics, or fecal microbiota transplantation might offer a promising strategy to modulate the fibrogenesis or reduce the progression of CD[466–468]. Restoring a healthier microbiome could not only serve as a preventive strategy but also represent a novel therapeutic target for managing active inflammation in PIBD and avoid complication as fibrosis. Given the established link between creeping fat and muscle thickening[336], targeting creeping fat could also represent a future strategy for managing stricturing CD.

Another approach to keep patients away from the knife involves locally injecting MSCs into short CD strictures to leverage their anti-inflammatory and anti-fibrotic properties[480]. In a phase

I-II pilot trial, MSC injections were well tolerated, with half of the patients experiencing complete or partial resolution of the stricture at 12 weeks and some maintaining benefits at 48 weeks[489]. While further research is needed, these results suggest that MSC local injection could be a promising option for treating CD strictures.

An integrated 'omics' approach – encompassing transcriptomics, proteomics, and metabolomics – could also provide a powerful framework for illuminating the link between ER stress and fibrotic pathways, including the specific roles of each protein studied is this thesis. As Rieder *et al.* suggest, deploying these multiomics tools in carefully characterized patient populations, spatially mapping the key cells and pathways, and drawing parallels to other fibrotic disorders could lead to the creation of a high-resolution gut 'omics' atlas. Such a resource would be invaluable for both the IBD research community and industry, particularly in the context of personalized medicine, by enabling the development of tailored therapeutic strategies that address patient-specific disease drivers[305,490].

6.5. LIMITATIONS

This thesis benefits from the robust design of the studies, each addressing complementary facets of pediatric CD. First, the use of a large, population-based registry provided a comprehensive and long-term view of incidence and phenotypic characteristics, bolstering the reliability and generalizability of the epidemiological findings. The study of disease course further highlighted the progressive nature of pediatric CD, with nearly one-third of children initially diagnosed with an inflammatory phenotype evolving toward a stricturing behavior over time. These findings emphasize the value of longitudinal follow-up and the need for early risk stratification. Ultimately, leveraging a multicenter design and immunohistochemical analyses to examine the distribution of ERS-related proteins in both pediatric and adult samples provided a novel molecular perspective on intestinal fibrosis and its association with ERS proteins. The complementarity between large-scale epidemiological data and molecular-level investigations thus provides complementary documentation of disease progression, aiming at a better characterization and understanding of the fibrosis process in CD. Nonetheless, several limitations must be acknowledged. Patient selection across multiple centers could introduce variability in clinical practice, while regional differences may affect the consistency of data collection and interpretation. In addition, the rarity of very early-onset IBD constrains statistical power and limits definitive conclusions for this subgroup. Finally, although IHC revealed significant associations between the expression of certain ERS-related proteins and fibrosis, it does not fully clarify causality, necessitating further functional studies to unravel the precise molecular mechanisms. Despite these constraints, the observations remain robust and underscore key directions for future research and improved clinical management of pediatric CD.

6.6. CONCLUSIONS

Pediatric CD presents a formidable clinical challenge, notably due to the risk of intestinal fibrosis, which can severely impair quality of life. This thesis aimed to address three key objectives: (i) to assess the evolution of incidence and phenotypic presentation in VEO-IBD and EO-IBD, (ii) to examine the progression of disease behavior over time in these pediatric subgroups, and (iii) to characterize ER stress-related proteins in inflamed and fibrotic intestinal tissues from pediatric and adult CD patients.

Our population-based study revealed a stable and low incidence of VEO-IBD over time, contrasting with a marked increase in EO-IBD cases, and confirmed distinct clinical patterns based on age at onset. We also demonstrated the progressive nature of pediatric CD, with approximately one-third of patients initially presenting with an inflammatory phenotype subsequently developing stricturing complications – though this risk appeared lower in VEO cases. Histopathological analyses identified AGR2 as the protein most strongly associated with fibro-inflammatory remodeling, particularly in pediatric-onset CD, while PDIA6 was consistently upregulated in CD tissues, even outside fibrotic or inflamed areas, suggesting a broader or earlier role in epithelial stress responses.

By integrating clinical and molecular perspectives, this work advances our understanding of the fibrotic evolution of pediatric CD, particularly in relation to age at onset and epithelial stress-associated mechanisms. Moving forward, a two-pronged strategy appears essential: large-scale epidemiological surveillance to monitor disease progression, combined with molecular exploration to identify and validate fibrosis-related biomarkers. Such integrated efforts could help identify therapeutic targets and support more precise, individualized strategies. Stratifying patients based on clinical and molecular features may be more informative than broad comparisons across age groups, and longitudinal follow-up can offer critical insights into how fibrotic risk evolves over time. To achieve these goals, long-term cohort studies must be sustained, with systematic collection of clinical, histological, and biological samples enabling molecular analyses. Moreover, interdisciplinary collaborations – bridging pediatric gastroenterology, immunology, genetics, molecular biology, biomedical engineering, and bioinformatics – will be key to developing novel diagnostic tools and anti-fibrotic treatments.

In this perspective, the findings presented in this thesis underscore the relevance of an integrated approach, combining clinical observation, experimental research and molecular profiling. Such

integration could enhance our understanding of disease mechanisms in pediatric Crohn's disease and ultimately support the development of earlier and more effective therapeutic interventions.

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7. REFERENCES

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APPENDICES

8. APPENDICES

8.1. JOURNAL ARTICLE 1: INCIDENCE AND PHENOTYPE AT DIAGNOSIS OF VERY EARLY-ONSET COMPARED WITH LATER-ONSET PEDIATRIC INFLAMMATORY BOWEL DISEASE: A POPULATION-BASED STUDY (1988-2011)

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Incidence and Phenotype at Diagnosis of Very-early-onset Compared with Later-onset Paediatric Inflammatory Bowel Disease: A Population-based Study [1988–2011]

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Abstract

Background and Aims: Very-early-onset inflammatory bowel disease [VEO-IBD] is a form of IBD that is distinct from that of children with an older onset. We compared changes over time in the incidence and phenotype at diagnosis between two groups according to age at IBD diagnosis: VEO-IBD diagnosed before the age of 6 years, and early-onset IBD [EO-IBD] diagnosed between 6 and 16 years of age.

Methods: Data were obtained from a cohort enrolled in a prospective French population-based registry from 1988 to 2011.

Results: Among the 1412 paediatric cases [< 17 years], 42 [3%] were VEO-IBD. In the VEO-IBD group, the incidence remained stable over the study period. In contrast, the incidence of EO-IBD increased from 4.4/10^5 in 1988–1990 to 9.5/10^5 in 2009–2011 [+116%; $p < 10^{-4}$]. Crohn's disease

[CD] was the most common IBD, regardless of age, but ulcerative colitis [UC] and unclassified IBD were more common in VEO-IBD cases [40% *vs* 26%; $p = 0.04$]. VEO-IBD diagnosis was most often performed in hospital [69% *vs* 43%; $p < 10^{-3}$]. Rectal bleeding and mucous stools were more common in patients with VEO-IBD, whereas weight loss and abdominal pain were more frequent in those with EO-IBD. Regarding CD, isolated colonic disease was more common in the VEO-IBD group [39% *vs* 14%; $p = 0.003$].

Conclusions: In this large population-based cohort, the incidence of VEO-IBD was low and stable from 1988 to 2011, with a specific clinical presentation. These results suggest a probable genetic origin for VEO-IBD, whereas the increase in EO-IBD might be linked to environmental factors.

Key Words: Inflammatory bowel disease; paediatric; very-early-onset; incidence; clinical presentation

1. Introduction

Inflammatory bowel diseases [IBDs], comprising Crohn's disease [CD] and ulcerative colitis [UC], are multifactorial chronic disorders evolving with a relapsing and remitting course. It is generally accepted that genetic susceptibility, environmental factors, and changes in the gut microbiota cause excessive innate and adaptive immune responses.[1–3] Paediatric-onset IBD represents 8–25% of cases of IBD.[4,5] The incidence of paediatric-onset IBD is increasing, especially in industrialised countries, and children are now being diagnosed at a younger age.[6,7] A small number of monogenic mutations[8–11] have been identified in children with IBD diagnosis at a very young age, but genome-wide association studies failed to detect large differences between adult-onset and paediatric-onset disease.[12,13] Several studies reported different disease phenotypes in children with a diagnosis of IBD before 10 years of age compared with children aged over 10 years,[5,14,15] adolescents, or adults, leading to the Paris modification of the IBD Montreal classification, differentiating children with a diagnosis made before 10 years of age [A1a] from those with a diagnosis at 10–17 years [A1b].[16] Thus, age at diagnosis is important clinically and it appears that very-early-onset IBD [VEO-IBD] [age < 6 years at diagnosis] might be a distinct form. The phenotype of children with VEO-IBD is loosely defined but is usually considered as being more severe than when diagnosed later in life.[17–18] However, most published studies have not been population based but covered patients followed in referral centres, and the incidence and natural history of VEO-IBD are still poorly understood.

In this population-based study covering 1988–2011, we compared changes over time in the incidence and phenotype at diagnosis between VEO-IBD and early-onset [EO]-IBD [diagnosis at 6–16 years].

2. Patients and Methods

2.1. Patient population and EPIMAD methodology

The study population included all children prospectively recorded in the EPIMAD registry with a diagnosis of definite or probable CD, UC, or unclassified inflammatory bowel disease [IBDU], diagnosed before 17 years of age from January 1988 to December 2011, according to validated and published diagnostic criteria.[5,18–22] The study population was divided into two groups according to age at diagnosis; VEO-IBD was defined as IBD diagnosed before 6 years of age, and EO-IBD was defined as IBD diagnosed between 6 and 16 years of age. The cut-off of 6 years was chosen based on previous studies.[7,14,15,17]

The EPIMAD Registry is a prospective population-based study recording all cases of IBD documented since 1988 in Northern France [Figure 1]. This study area includes 5 864 508 inhabitants, representing 9.3% of the total French population, and is divided into four administrative areas. The population distribution for those aged under 17 years is as follows: Nord, 593 837; Pas-de-Calais, 332 228; Somme, 115 969; and Seine-Maritime, 270 107; with a total of 1 312 141 children [2011 national population census data from the National Institute of Statistics and Economic Studies — INSEE 2011] [http://www.insee.fr/en/]

The methodology of the EPIMAD Registry has been described in detail.[5,18–22] Briefly, data from all patients newly diagnosed with IBD are collected from all adult [N = 254] and paediatric [N = 15] gastroenterologists [GEs] practising in the private and public sectors in these regions of France. Only residents of the studied areas at the time of diagnosis are included. Each GE reports all patients consulting for the first time with clinical symptoms compatible with IBD; he/she is contacted by phone at least three times a year by an interviewer who visits the GE's office and collects data from medical charts on a standardized questionnaire for each new case. The data collected include age at diagnosis, gender, interval between the onset of symptoms and diagnosis, and clinical, radiological, endoscopic and histological findings at the time of diagnosis. Information on the management of each diagnosis is also recorded. The final diagnosis of IBD is established by two expert gastroenterologists and recorded as definite, probable, or possible CD or UC, according to previously published criteria.[20] Only definite and probable cases are considered for further analyses. Cases for which the diagnosis of IBD is probable, but without conclusive argument for differentiating CD from UC, are classified as IBDU.

Approval was obtained from the Ethics Committee of Lille University and Hospital, and this study followed the regulations and instructions set up by the Comité National des Registres [approval numbers 97 107 and 983 792].

2.2. Additional data collected for the present study

Data were extracted from the medical records of adult and paediatric GEs, and were collected in standardised questionnaires. Sociodemographic and clinical characteristics at diagnosis were collected: age, gender, family history of IBD [defined as any case of IBD in at least one family member of the first or second degree], time between onset of symptoms and diagnosis, symptoms, disease phenotype, and extraintestinal manifestations [EIMs] [defined as joint, skin, ocular, or hepato-biliary manifestations]. IBD location and its phenotype at diagnosis were defined according to the Paris classification as described by Levine *et al.*[16] as follows.

[i] Pure small bowel involvement [L1]; pure colonic involvement [L2]; or ileocolonic involvement [L3; L1 with caecal involvement was considered as L3]; upper gastrointestinal disease [L4 that could be associated

Figure 1. Map of France showing the study area of the EPIMAD Registry, which includes the Nord, Pas-de-Calais, Somme, and Seine-Maritime [Northern France].

with L1, L2, or L3]; L4a [upper disease proximal to the ligament of Treitz]; and L4b [upper disease distal to the ligament of Treitz and proximal to the distal one-third of the ileum were grouped as L4]. CD phenotypes were classified as follows: inflammatory [non-stricturing and non-penetrating, B1]; stricturing [B2]; or penetrating [B3] disease. B2 and B3 behaviours were pooled and defined as 'complicated behaviour'. The 'p' index could be added to the B1, B2, or B3 classes when concomitant perianal disease was present [including abscesses and/or fistulae].

[ii] For UC, the location was defined as follows: proctitis defined as involvement limited to the rectum [E1]; left-sided colitis defined as involvement limited to the colorectum below the splenic flexure [E2]; extensive colitis defined as involvement of the colorectum above the splenic flexure and below the hepatic flexure [E3]; or pancolitis defined as involvement above the hepatic flexure [E4]. To assess the evolution of IBD phenotype over time adequately, only patients who had a complete bowel investigation [small and large bowel for CD and total colonoscopy for UC] were considered. The rate of complete bowel investigations did not change over time

2.3. Statistical analysis

Incidence rates were computed as the number of incident cases [i.e. new diagnoses] divided by the population at risk. To identify any possible changes in the incidence of IBD, we divided the 24-year study into eight equal 3-year periods: 1988–1990, 1991–1993, 1994–1996, 1997–1999, 2000–2002, 2003–2005, 2006–2008, and 2009–2011]. The mean annual incidence rates were calculated for each 3-year period and for the entire study period, and are presented with their 95% confidence intervals [CIs]. Incidence rates were determined in the overall population and in subgroups according to age categories [< 6 or 6–16 years] and gender. For each of the four administrative areas, population data by age and gender were obtained using yearly estimations of population obtained from INSEE, and based on a mixed procedure exhaustive census before 2004 and random sampling after 2004. Temporal trends in incidences over time were tested by means of log-linear Poisson regression analyses taking overdispersion and person-years at risk into account [introduced as an offset variable after log transformation].

Qualitative variables were expressed as frequencies and percentages and 95% CIs. For comparing qualitative variables between age groups, we used chi-square or Fisher's exact test according to the number of expected events. Analyses were performed with SAS software version 9.4 [SAS Institute Inc., Cary, NC, USA]. Statistical significance was accepted at $p \leq 0.05$.

3. Results

3.1. Incidence

From 1988 to 2011, 1412 children with a diagnosis of IBD before the age of 17 years were included in the EPIMAD registry [8% of all IBD cases]. Among them, 42 [3% of all paediatric IBD cases] were diagnosed before the age of 6 [VEO-IBD], with six children [14% of those with VEO-IBD] before the age of 1 year and 13 children [31% of those with VEO-IBD] aged 2 years or younger. A total of 1370 IBD cases were diagnosed between the ages of 6 and 16 years and were considered as EO-IBD; 52% of the patients were male, with no significant difference between the two age groups.

In the VEO-IBD group, the incidence of IBD over the entire study period [1988–2011] was $0.40/10^5$ [95% confidence interval: 0.30–0.50] including $0.25/10^5$ for CD [0.10–0.30], $0.12/10^5$ for UC [0.06–0.20], and $0.03/10^5$ for IBDU [0.00–0.06]. In the EO-IBD group, the incidence of IBD was $6.4/10^5$ [95% CI 6.0–6.7], including $4.7/10^5$ for CD [4.4–5.0], $1.5/10^5$ for UC [1.4–1.7], and $0.2/10^5$ for IBDU [0.1–0.3] during the same period.

The overall incidence of paediatric-onset IBD increased from $3.0/10^5$ in 1988–1990 to $6.3/10^5$ in 2009–2011 [+ 110%; $p < 10^{-4}$ by Poisson regression]. In the VEO-IBD group, the incidence remained stable [not significant; $p = 0.14$ by Poisson regression] during the whole period, whereas the incidence of EO-IBD increased from 4.4 to $9.5/10^5$ [+ 116%; $p < 10^{-4}$ by Poisson regression] during the same period [Figure 2]. The increasing incidence in the EO-IBD group was noteworthy for cases of EO-UC and EO-CD, whereas the incidences of VEO-UC and VEO-CD remained stable during the study period [Figure 3].

3.2. IBD classification at diagnosis

In the VEO-IBD group, 60% had CD [$N = 25$], 33% had UC [$N = 14$], and 7% had IBDU [$N = 3$]. In the EO-IBD group, 74% had CD [$N = 1,007$], 24% had UC [$N = 329$], and 2% had IBDU [$N = 34$]. The distribution of cases according to diagnosis was significantly different [$p = 0.04$] between the two age groups, with UC and IBDU more frequent in the VEO-IBD group than in the EO-IBD

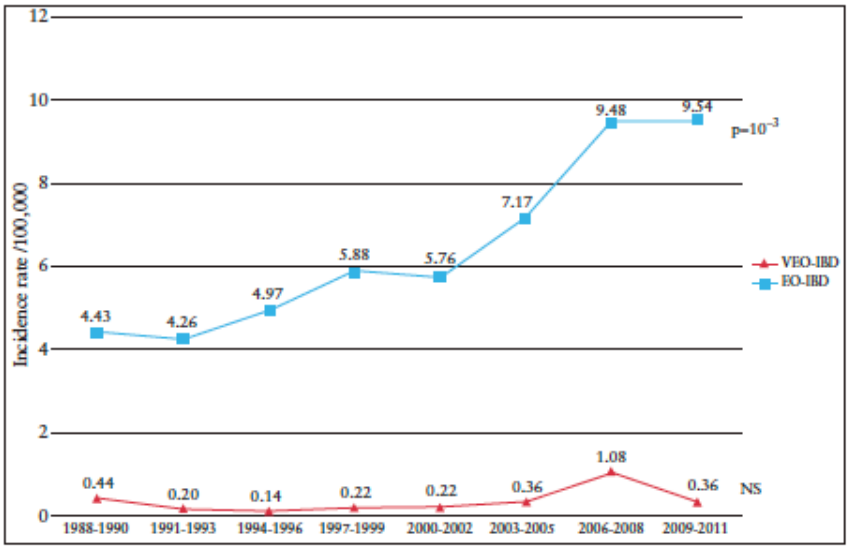

Figure 2. Incidence of very-early-onset [< 6 years] inflammatory bowel disease [VEO-IBD] and early-onset [6–16 years] inflammatory bowel disease [EO-IBD], indicated by 3-year consecutive periods from 1988 to 2011 in Northern France.

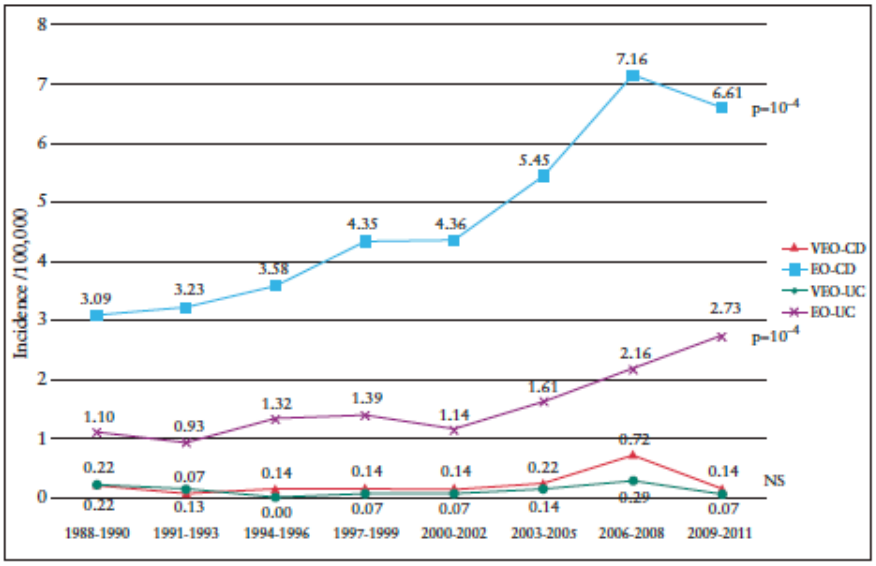

Figure 3. Incidence of very-early-onset [< 6 years] Crohn's disease [VEO-CD] and early-onset [6–16 years] Crohn's disease [EO-CD], and very-early-onset ulcerative colitis [VEO-UC] and early-onset [6–16 years] ulcerative colitis [EO-UC], indicated by 3-year consecutive periods from 1988 to 2011 in Northern France.

group [40% *vs* 26%] and CD more common in the EO-IBD group than in the VEO-IBD group [74% *vs* 60%; Figure 4].

3.3. IBD phenotype at diagnosis

In cases of CD, isolated colonic disease [L2] was significantly more frequent in the VEO-IBD group [N = 9; 39%] than in the EO-IBD group [N = 128; 14%; p = 0.003; Table 1]. Involvement of the proximal gastrointestinal tract [L4] was similar in the two age groups [32%, N = 8 in the VEO-IBD group and 35%, N = 355 in the EO-IBD group; p = 0.74]. At diagnosis, there was no significant difference between the two age groups regarding the rates of complicated forms of CD; stricturing lesions [B2] or penetrating lesions [B3] were 13% [N = 3] in the VEO-IBD group and 22% [N = 208] in the EO-IBD group [p = 0.26], respectively. Anoperineal disease was present in 8% [N = 2] of the VEO-IBD group and 6% [N = 59] in the EO-IBD group [p = 0.66].

UC location at diagnosis was not different between the two age groups [p = 0.138]. Regarding the location at diagnosis in the VEO-CD group or the VEO-UC group, no significant difference was found between children aged 2 years or younger [31% of the VEO-IBD group] and children aged 3–6 years.

3.4. Initial IBD clinical presentation

IBD diagnosis was more often performed in hospital in the VEO-IBD than in the EO-IBD group [69% *vs* 43%; $p < 10^{-3}$]. There was no significant difference in the prevalence of a family history of IBD between the two age groups. The time between the onset of symptoms and IBD diagnosis was not influenced by age at diagnosis in our cohort in any type of IBD. The initial clinical presentation was different according to age groups [Table 1].

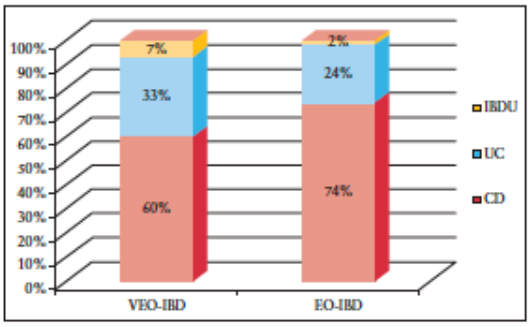

Figure 4. Distribution of inflammatory bowel disease [IBD]: Crohn's disease [CD], ulcerative colitis [UC], and inflammatory bowel disease unclassified [IBDU] in very-early-onset [< 6 years] IBD [*N* = 42] and in early-onset [6–16 years] -IBD [*N* = 1370], issued through the population-based EPIMAD Registry between 1988 and 2011.

3.4.1. All IBD patients [*N* = 1412]

Rectal bleeding and mucous stools were significantly more frequent in the VEO-IBD group than in the EO-IBD group [81% *vs* 46%; $p < 10^{-4}$ and 40% *vs* 21%; $p = 0.002$, respectively], whereas weight loss and abdominal pain were less common [21% *vs* 49%; $p < 10^{-3}$ and 43% *vs* 74%; $p < 10^{-4}$, respectively]. There were no differences in the frequency of diarrhoea or EIMs between the two age groups. Diagnostic procedures [gastroscopy, total colonoscopy, ileoscopy, CT, and MRI scans] were performed as frequently in the VEO-IBD group as in the EO-IBD group.

3.4.2. CD patients [*N* = 1032]

CD diagnosis was more often performed in hospital in the VEO-CD group than in the EO-CD group [68% *vs* 45%; $p = 0.02$]. Only rectal bleeding was significantly more frequent in the VEO-CD group [68% *vs* 30%; $p < 10^{-4}$] whereas weight loss and abdominal pain were less common in the VEO-CD group [20% *vs* 56% and 48% *vs* 80%, respectively; $p < 10^{-3}$].

3.4.3. UC patients [*N* = 343]

UC diagnosis was more often performed in hospital in the VEO-UC group than in the EO-UC group [71% *vs* 34%; $p < 10^{-2}$]. Only

Table 1. Comparison of socio-demographic characteristics, clinical presentation, disease phenotype, and location at diagnosis between VEO-IBD [< 6 years] [*N* = 42] and EO-IBD [6–16 years] [*N* = 1370].

Variables *N* [%]	VEO-IBD [< 6 years]	EO-IBD [6–16 years]	*p*-Value
All IBD [*N* = 1472]	42 [3%]	1370 [97%]	
Crohn's disease	25 [60%]	1007 [74%]	
Ulcerative colitis	14 [33%]	329 [24%]	0.05
IBD unclassified	3 [7%]	34 [2%]	
Male gender	22 [52%]	708 [52%]	0.93
Diagnosis in a hospital setting	29 [69%]	583 [43%]	< 0.001
Time between onset of symptoms and IBD diagnosis > 6 months	11 [27%]	407 [30%]	0.67
IBD family history	4 [10%]	210 [15%]	0.30
Diarrhoea	32 [76%]	899 [66%]	0.15
Rectal bleeding	34 [81%]	624 [46%]	< 0.0001
Mucous stools	17 [40%]	281 [21%]	0.002
Abdominal pain	18 [43%]	1013 [74%]	< 0.0001
Weight loss	9 [21%]	670 [49%]	< 0.001
EIMs	7 [17%]	231 [17%]	0.97
Crohn's disease [*N* = 1032]	25 [60%]	1007 [74%]	0.04
Diagnosis in a hospital setting	17 [68%]	455 [45%]	0.02
Rectal bleeding	17 [68%]	303 [30%]	< 0.0001
Pure colonic location [L2[a]]	9 [39%]	128 [14%]	0.003
Abdominal pain	12 [48%]	809 [74%]	< 0.0001
Weight loss	5 [20%]	566[56%]	< 0.001
EIMs	5 [20%]	208 [21%]	0.936
Ulcerative colitis [*N* = 343]	14 [33%]	329 [24%]	0.04
Diagnosis in a hospital setting	10 [71%]	112 [34%]	< 0.001
Abdominal pain	3 [21%]	180 [55%]	< 0.05
EIMs	1 [7%]	19 [6%]	0.576
Ulcerative proctitis [E1[a]]	1 [19%]	92 [30%]	0.138
Left-sided UC [E2]	N = 4 [36%]	N = 79 [26%]	
Extensive UC [E3]	N = 3 [27%]	N = 30 [10%]	
Pancolitis [E4]	N = 3 [27%]	N = 102 [33%]	

[a]According to the Paris Classification.
VEO, very-early-onset; IBD, inflammatory bowel disease; EO, early-onset; EIMs, extraintestinal manifestations.

Table 2. Comparison of prevalence of very-early-onset (< 6 years) inflammatory bowel disease [VEO-IBD], Crohn's disease [CD], ulcerative colitis [UC], and IBD unclassified [IBDU] in the literature.

Reference	Year	Country	Period	Method of data collection	Number of patients[a]	VEO-IBD[b] [%]	VEO-CD[c] [%]	VEO-UC[c] [%]	VEO-IBDU[c] [%]
Bequet	2016	France	1988–2011	General population	1412	3	60	33	7
Benchimol[14]	2014	Canada	1994–2002	Health administrative database	7143	5	33	56	11
Aloi[15]	2014	Italy	2009–2013	Hospital	506	11	44	48	7
Paul[29]	2006	USA	1995–2000	Hospital	413	10	NA	66	NA
Heyman[7]	2005	USA	2000–2002	Hospital	1370	15	36	40	24
Griffiths[30]	2004	Canada	1980–1999	Hospital	861	6,5	36	64	NA
Sawczenko[31]	2003	UK & Ireland	1998–1999	Monitoring register[d]	739	4	31	38	12
Mamula[32]	2002	USA	1977–2000	Hospital	82 [< 5 years]	-	33	44	23

NA, not available.
[a]Number of patients with paediatric-onset IBD [< 16 or 17 years].
[b]Percentage of VEO-IBD among paediatric-onset IBD.
[c]Proportion of CD, UC, and IBDU in VEO-IBD.
[d]In these studies, VEO-IBD is defined by a diagnosis before the age of 5 years.
[e]British Paediatric Surveillance Unit [BPSU], British Society of Gastroenterology Research Unit [BSGRU], and Paediatric Register Inflammatory Bowel Disease [PRIB].

abdominal pain was less common in the VEO-UC group [21% *vs* 55%; $p = 0.01$].

4. Discussion

This population-based prospective study, conducted in a large paediatric cohort [$N = 1412$] over a 24-year period, showed that the incidence of EO-IBD increased by 116% in Northern France from 1988 to 2011 whereas the incidence of VEO-IBD remained stable during the same period. CD was the most common IBD in the two age groups, with a more frequent isolated colonic location in the VEO-IBD group. UC and IBDU were more common in the VEO-IBD group than in the EO-IBD group. The diagnosis of VEO-IBD was most often performed in hospital. Rectal bleeding and mucous stools were more frequent at diagnosis in the VEO-IBD group, reflecting a colonic location, whereas weight loss and abdominal pain were the most frequent clinical symptoms in the EO-IBD group.

Previous epidemiological data have shown a dramatic increase in paediatric-onset IBD worldwide.[23–28] We also found that the incidence of EO-IBD in our cohort, but not that of VEO-IBD, has been rising continuously since 1988. It is generally accepted that the influence of genetics in the pathogenesis of IBD is higher in children with VEO-IBD. It is unlikely that genetic factors have changed over a period of 24 years, as opposed to environmental factors. This could explain the increased incidence of IBD in those aged 6–16 years and the stability in the incidence of VEO-IBD. Table 2 shows the prevalence of VEO-IBD [with a diagnosis before the age of 5 or 6 years, depending on the series] reported since 2002.[7,28–32] Studies by Sawczenko *et al.* in 2003[31] and Benchimol *et al.* in 2014[14] showed a proportion of VEO-IBD similar to that in our population, namely 4% and 5%, respectively. This proportion was lower than the 6.5–15% reported previously. This wide range in the prevalence of VEO-IBD is probably associated with the study population, with higher prevalence rates being reported in studies from referral centres. In contrast to the stable incidence of VEO-IBD over time in our study, a Canadian study[14] showed that the increased incidence of IBD from 1994 to 2009 was higher in those with VEO-IBD [< 6 years] [+ 7.4% average yearly change] than in those aged 10–16 years with IBD [+ 2.2% average yearly change].

Our study was performed through a population-based registry, whereas Benchimol *et al.*[14] applied a diagnosis algorithm through a health administrative database. In addition, our study was focused on a specific region in France, a narrower area than that studied by Benchimol, which might have influenced the results. Therefore, our conclusions should be interpreted with caution.

Although most represented in both age groups, CD was significantly more common in the EO-IBD group than in the VEO-IBD group; UC was significantly more common in the VEO-IBD group than in EO-IBD group in our study. However, CD represented 60% of IBD cases in our VEO-IBD group, unlike previous studies that reported a predominance of UC over CD in VEO-IBD.[14,15,29,32] This could have arisen from the diagnostic criteria used, as well as from specific environmental factors and lifestyle in the study area.[33,34] Complete bowel investigations were obtained as often in the VEO-IBD group as in the EO-IBD group and the diagnostic criteria [definite or probable IBD cases] did not change over time. Thus, the risk of misdiagnosing patients seems to have been low.

In children with VEO-IBD, the diagnosis was most often done in a hospital setting. This was probably because of the lack of expertise and equipment—particularly endoscopy—for diagnosing IBD in very young children in an extra-hospital environment, as well as higher parental anxiety levels concerning the age of the child, leading parents to consult

hospitals. Moreover, the time between the onset of symptoms and diagnosis was not delayed in the VEO-IBD group, whatever the type of IBD.

As previously reported,[7,14,15,17,29,35] the initial presentation was different according to age group, with mucous bloody stools significantly more frequent in cases of VEO-IBD, probably because of the higher rate of isolated colonic disease in those with CD and a higher proportion of UC compared with older children. As noted by Gupta *et al.*,[17] weight loss and abdominal pain were significantly more common in the EO-IBD group and this was also the case in our study. This was likely linked to a higher proportion of CD in this age group and the difficulties in expressing abdominal pain among very young children. Gupta *et al.*[17] also reported that the rates of EIMs at diagnosis of IBD were similar in both age groups. In those with CD, the rate of complicated behaviours [B2 or B3] was similar in both age groups in our study, which contrasts with Gupta *et al.*'s finding of a higher rate of complicated behaviours in those with IBD aged 6–16 years/[17] Upper gastrointestinal location [L4] and anoperineal lesions were found to be similar in both age groups. The rate of L4 among those with VEO-CD [32%] was similar to that reported by Aloi *et al.*[15] but much higher than that found by Heyman *et al.* [5%].[7] In those with UC, the rates of ulcerative proctitis [E1] and extensive colonic involvement [E3/E4] were similar in both age groups. In the literature, VEO-UC has been studied less than VEO-CD, which currently limits the detection of significant phenotypic differences between the two age groups.

In our study, age at diagnosis of IBD was not linked to the presence of a positive family history, which contrasts with findings from referral centres[7,32,36] where it has been shown that severe cases of IBD that are more often followed in referral centres are more often associated with a family history of IBD.[37] The differences between studies could also be explained by the ethnic variability in the study areas. For example, some North American cities included in previous studies have a large Jewish population, in which there is a known genetic susceptibility to IBD.[20] Some recent studies identified novel gene variants associated with all cases of IBD but also in those with VEO-IBD, and sequencing exomes could be a new diagnostic tool to identify variants in genes that could contribute to the pathogenesis of VEO-IBD.[39-42]

Our study had some strengths and limitations. It was a large population-based study, had a long duration, used validated and published diagnostic criteria, and had a high-level data collection [96.5%].[20] However, because of the small number of patients with VEO-IBD, the results should be interpreted with caution.

In conclusion, our large paediatric-onset population-based study over a 24-year period showed stability in the incidence of VEO-IBD, with a classification into UC and IBDU more frequent than in those aged 6–16 years with IBD. The diagnosis of VEO-IBD was most often done in a hospital setting and the initial presentation with colorectal symptoms was associated with a more frequent isolated colonic involvement in those with CD. Further longitudinal studies, especially genetic, are needed to increase the understanding of the pathogenesis of IBD and to help predict the subsequent course of these rare diseases in very young children and improve treatment strategies.

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Conflict of Interest

None.

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Author Contributions

EB: concept and study design, acquisition and interpretation of data; drafting the manuscript. HS: data management, interpretation of data, statistical analysis. MF: initiation of the study, interpretation of data; drafting and critical revision of the manuscript. FV: initiation of study, interpretation of data. LA-D: interpretation of data; drafting and critical revision of the manuscript. BP: interpretation of data; drafting and critical revision of the manuscript. DL: : interpretation of data; drafting and critical revision of the manuscript. CS: interpretation of data; drafting and critical revision of the manuscript. HC: drafting and critical revision of the manuscript. J-EL: drafting and critical revision of the manuscript. LP-B: interpretation of data; drafting and critical revision of the manuscript. GS: interpretation of data; drafting and critical revision of the manuscript. DT: concept and study design; acquisition of data, interpretation of data; drafting and critical revision of the manuscript. CG_R: concept and study design; acquisition of data, interpretation of data; drafting and critical revision of the manuscript.

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8.2. EPIMAD SURVEY

GENERALITES

N° d'enregistrement /__/__/__/__/__/__/

Sexe : 1. Masculin /__/
2. Féminin

Date de naissance /__/__/__/__/__/__/__/__/

Code INSEE patient /__/__/__/__/__/__/__/__/

Lieu de naissance (en clair) _____

1. France 5. Asie /__/
2. Europe du Nord 6. Afrique Noire
3. Europe du Sud 7. Amérique du Nord
4. Afrique du Nord 8. Autre. Code INSEE /__/__/__/__/__/

Catégories socioprofessionnelles (CSP) _____

0. Inconnu
1. Agriculteurs exploitants
2. Artisans, commerçants, chef d'entreprise
3. Cadres, professeurs
4. Techniciens, professions intermédiaires
5. Employés
6. Ouvriers 7. Retraités
8. Inactifs
9. Autres /__/

Nom du Gastro-Entérologue + N°_____ /__/__/__/

Ville du Gastro-Entérologue _____

Nom du Gastro-Entérologue de suivi + N°_____ /__/__/__/

Source de première prise en charge

1. Secteur privé (cabinet de ville ou clinique)
2. Secteur public ou PSPH ou autre système de soins (CSM, SNCF…)
5. Chirurgie /__/

DIAGNOSTIC CLINIQUE

Date du diagnostic évoqué par le GE /__/__/__/__/__/__/__/__/

Délai entre premiers symptômes/diagnostic évoqué (en mois. <6sem.=1). /__/__/__/

Décès à la première poussée 1. Oui 2. Non 0. Inconnu /__/
Si oui date : /__/__/__/__/__/__/__/__/

Antécédents personnels :

1. Abcès ou fistule anale ou fissure 1. Oui 2. Non 0. Inconnu /__/
2. Tuberculose 1. Oui documentée
2. Oui non documentée /__/
3. Non
3. Appendicectomie 1. Oui 2. Non 0. Inconnu /__/
Si oui date : /__/__/__/__/__/__/__/__/
4. Tabac 1. Fumeur actuel
2. Non Fumeur /__/
3. Ancien fumeur
0. Inconnu
5. Prise de médicaments dans le mois précédant le début des symptômes

1. Oui - Antibiotiques - Ticlid
2. Non - AINS - Corticoïdes /__/
0. Inconnu - Anticoagulants - Autres : _____
- Veinotoniques

ANTECEDENTS FAMILIAUX :

BIOLOGIE

• Coproculture

0. Non réalisée ou inconnu
1. Réalisée, normale
2. Réalisée, positive à l'un ou plusieurs de ces germes suivants : /__/

Cocher : . d'amibiase ? . de staphylocoque doré ?
. de salmonelles ? . de clostridium ?
. de shigelles ?. de campylobacter ?
. de yersinia ? . autre, lequel ? : _____

EXAMENS MORPHOLOGIQUES :

1. Réalisée et normale
3. Réalisée et anormale (remplir fiche)
5. Non réalisée
0. Inconnu

- Coloscopie totale /__/
- Endoscopie basse /__/
- Iléoscopie /__/
- Radio colique /__/
- Radio du grêle /__/
- Fibro OGD /__/
- Echographie /__/
- Enteroscanner ou IRM ou EnteroIRM /__/
- Vidéocapsule /__/

HISTOLOGIE :

A. Biopsies : localisation : _____

1. Oui
2. Non
8. Non réalisée
0. Inconnu

- Granulome digestif /__/
- Granulome anal /__/
- Muqueuse normale /__/
- Muqueuse inflammatoire /__/
- Conserv. mucosecrétion /__/
- Infiltrat inflamm. spécifique /__/
- Infiltrat inflamm. non spécifique /__/
- Perte de substance /__/
- Abcès cryptique /__/

B. Pièces d'exérèse : localisation _____

1. Oui
2. Non
8. Non réalisée
0. Inconnu

- Granulome /__/
- Fissure /__/
- Fistule /__/
- Nodule lymphoïde /__/
- Mucosecrétion conservée (colon) /__/
- Sclérose /__/

0. Inconnu /__/

Synthèse des lésions anatomiques :

1. Oui
2. Non
0. Inconnu

- Lésion(s) ano-périnéale(s) /__/
- Lésion(s) du rectum (macroscopique) /__/ • Rectum (micro) /__/
- Lésion(s) du sigmoïde /__/
- Lésion(s) du colon gauche /__/
- Lésion(s) du colon transverse /__/
- Lésion(s) du colon droit /__/
- Pancolite /__/ • Colon (micro) /__/
- Lésion(s) de l'iléon /__/
- Lésion(s) du jéjunum /__/
- Lésion(s) du duodénum /__/ • Grêle (micro) /__/
- Autres lésions /__/ • Autres lésions (micro) /__/

Si oui, préciser : _____

- Type de la Maladie de Crohn /__/
  1. Inflammatoire
  2. Sténosant
  3. Pénétrant

- Lésions(s) segmentaire(s) suspendue(s) sur un même organe /__/
- Lésions bi ou plurifocales sur un même organe : /__/
- Lésion(s) de plus de 15cm
  - sur le grêle /__/
  - sur le colon /__/
- Fistule /__/
- Récidive après résection /__/

DIAGNOSTIC FINAL DU GE :

1. RCH certaine
2. RCH probable
3. RCH possible
4. Crohn certain
5. Crohn probable
6. Crohn possible
7. Proctite certaine
8. Proctite probable
9. Proctite possible
10. Colite chronique inclassable
11. Colite aigüe non spécifique
12. Autre diagnostic
13. Colite aigüe /__/__/
14 Cas inclassé

	Rectum	Sigmoïde colon G	Colon Transverse et droit	Caecum	Iléon
A) COLOSCOPIE					
Disparition du réseau vasculaire					
Aspect granité					
Sang et pus à la surface de la muqueuse					
Intervalle de muqueuse saine					
Pseudomembranes					
Pseudopolypes					
Ulcération cicatrisée					
Erythème franc					
Œdème franc					
Ulcération aphtoïde					
Ulcération superficielle					
Ulcération profonde					
Ulcération non précisée					
Sténose non ulcérée					
Sténose ulcérée					
Diverticules					
B) LAVEMENT OPAQUE					
Ulcérations					
Fistule					
Sténoses					
Dilatation					
Double contour					
Raccourcissement (aspect tubulé, rétracté, microrectie)					
Diverticule					

RADIOGRAPHIE DU GRELE	**Duodénum**	**Jéjunum**	**Iléon**
Ulcération			
Sténose			
Dilatation			
Epaississement des plis			
Fistule			

DIAGNOSTIC D'INCIDENCE:

1. RCH certaine	8. Proctite probable
2. RCH probable	9. Proctite possible
3. RCH possible	10. Colite chronique inclassable
4. Crohn certain	11. Colite aigüe non spécifique
5. Crohn probable	12. Autre diagnostic
6. Crohn possible	13. Colite aigüe
7. Proctite certaine	14 Cas inclassé

- Epimad 1 /__/__/
- Epimad 2 /__/__/
- DIAG. FINAL 1 /__/__/

DIAGNOSTIC FINAL DE SUIVI:

- DIAG. FINAL 2 /__/__/ • Date DIAG FIN 2 /__/__/__/__/__/__/__/__/
- DIAG. FINAL 3 /__/__/ • Date DIAG FIN 3 /__/__/__/__/__/__/__/__/
- DIAG. FINAL 4 /__/__/ • Date DIAG FIN 4 /__/__/__/__/__/__/__/__/
- DIAG. FINAL 5 /__/__/ • Date DIAG FIN 5 /__/__/__/__/__/__/__/__/

DONNEES DE SUIVI :

- Date des dernières nouvelles : /__/__/__/__/__/__/__/__/
  (avec ou sans changement diagnostique)
- Décès (au cours du suivi) 1. Oui
  2. Non /__/
  Si décès, date du décès /__/__/__/__/__/__/__/__/
- Dossier exceptionnel : /__/
  Si oui, présentation majoritairement extradigestive ? /__/

Cocher : . Forme clinique /__/ . Association d'autres maladies ? /__/
. Forme Familiale /__/ précisez : _____
. Forme Conjugale /__/ . Association familiale d'autres maladies? /__/

Commentaires : _____
_____
_____

_____

N° d'enregistrement /__/__/__/__/__/__/

Nom _____

Nom de jeune fille _____ Prénom _____

8.3. JOURNAL ARTICLE 2: DISTRIBUTION OF EPITHELIAL ENDOPLASMIC RETICULUM STRESS-RELATED PROTEINS IN ADULT AND PEDIATRIC CROHN'S DISEASE: ASSOCIATION WITH INFLAMMATION AND FIBROSIS.

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Alimentary Tract

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

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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 Endoplasmic 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 tissues 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 anti-fibrotic 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 myofi-

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broblasts, 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 4μm 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 transmural 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 Signaling, 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 Intraclass 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 comparisons ($n = 12$), 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.

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 Mann-Whitney 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 discrimi-

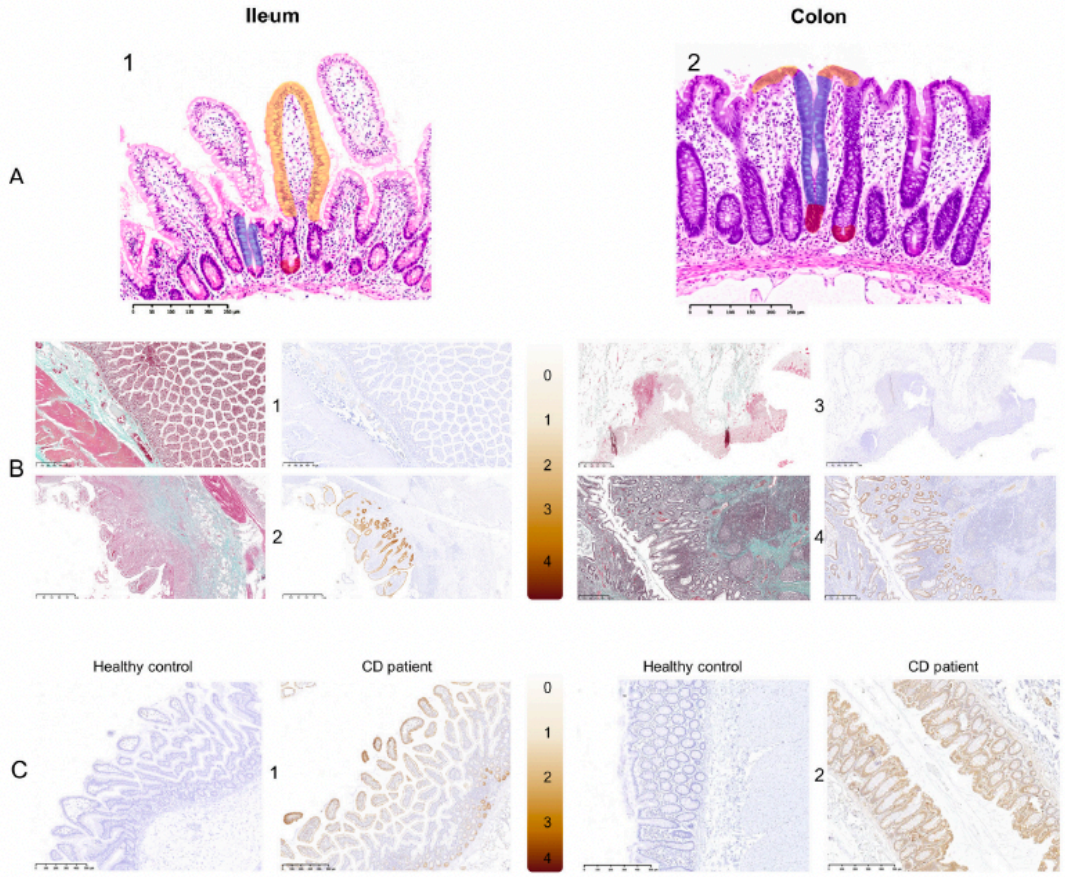

Fig. 1. A. Representative pictures illustrating the different histological areas studied: 1: Ileum; 2: Colon. Surface epithelium (yellow); crypts (blue); bottom of crypts (red). **B.** Examples of Masson's Trichrome-stained tissues showing fibrosis grades F1 to F3, alongside IHC staining scores for AGR2 (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).

natory 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 tissue 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. 2A-B). A similar pattern was

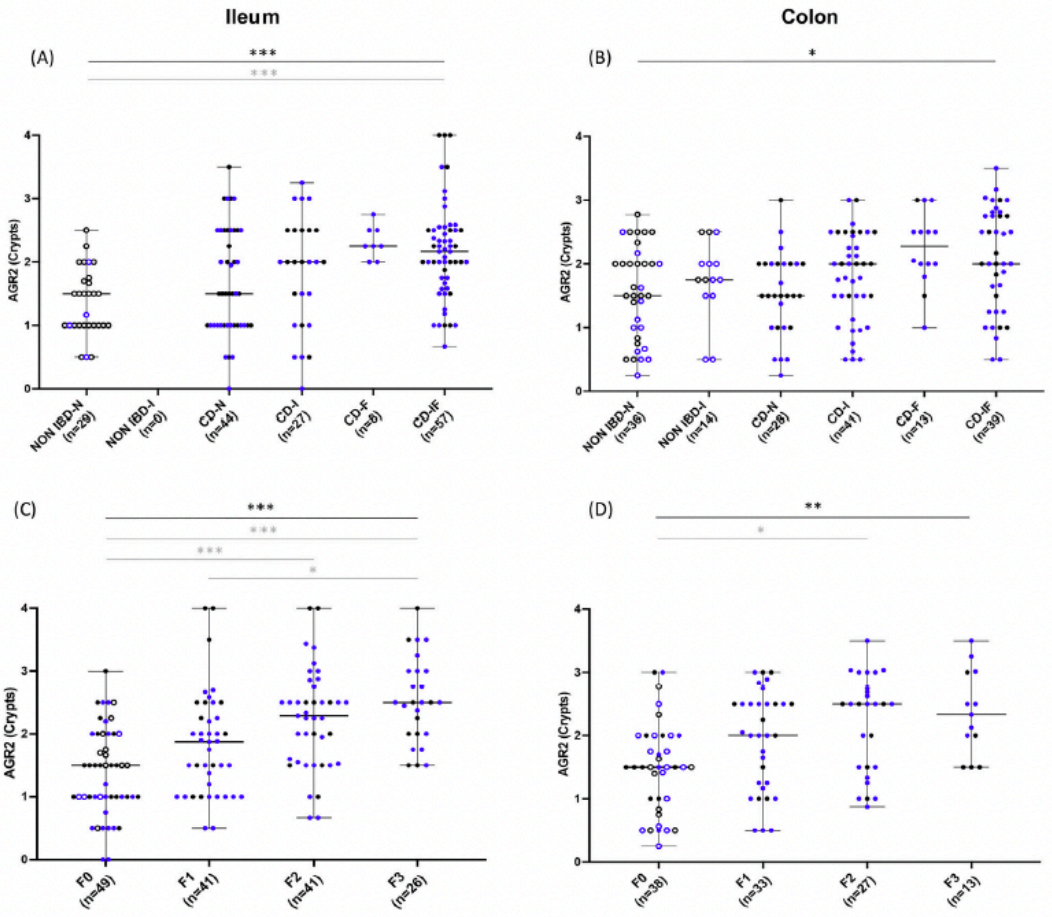

Fig. 2. **(A) (B)** Distribution of AGR2 IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients. **(C) (D)** Distribution of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

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).

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

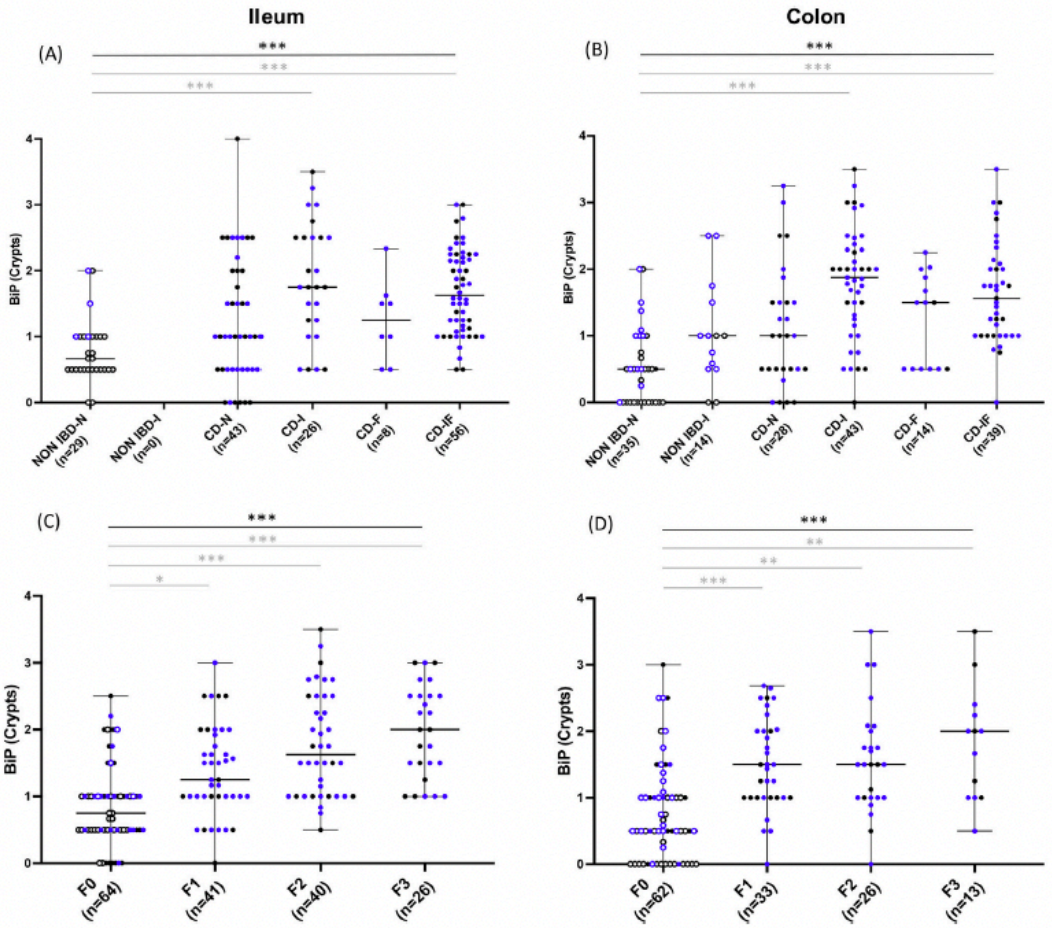

Fig. 3. **(A)(B)** Distribution of BiP IHC scores in ileal and colonic crypts of normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) or tissues with inflammation and fibrosis (IF) in non IBD cases and CD. **(C)(D)** Distribution of BiP IHC scores in 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.
ª Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

pure inflammation (without fibrosis), but not in tissues with pure fibrosis.

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).

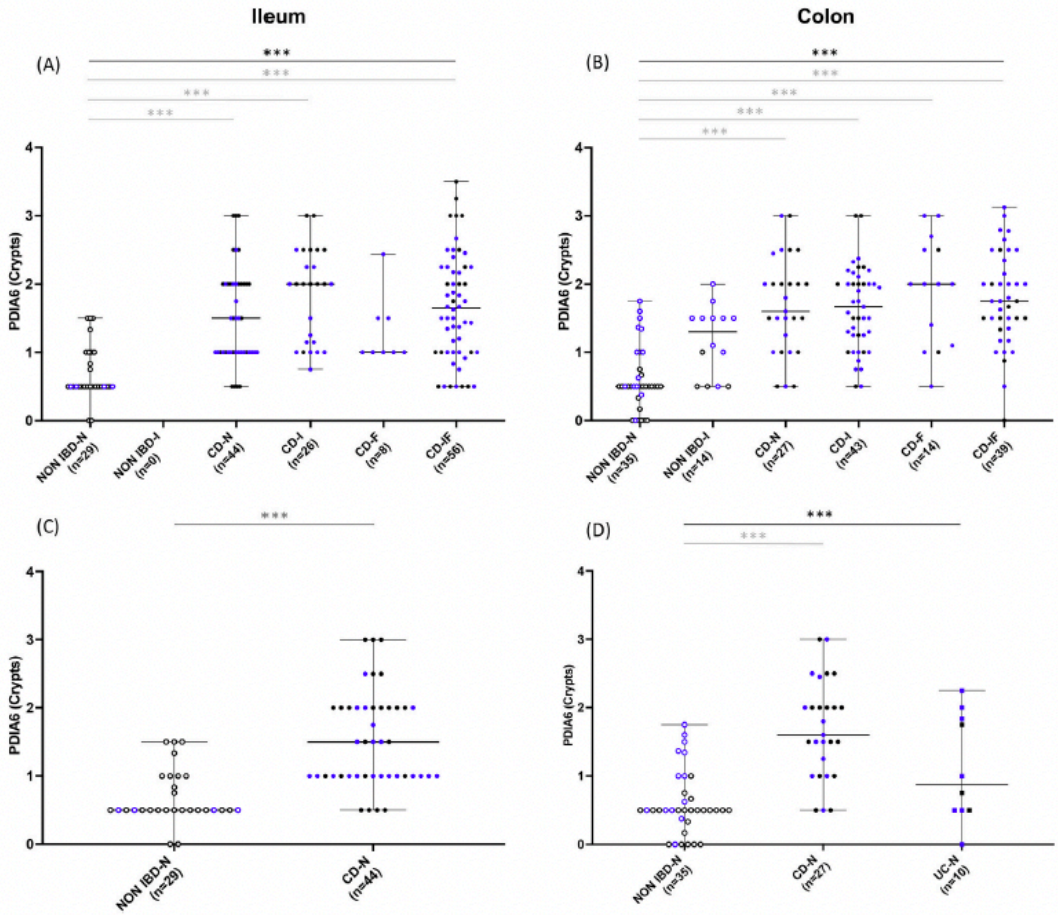

Fig. 4. **(A)(B)** Distribution of PDIA6 IHC scores in ileal and colonic crypts, including normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and tissues with both inflammation and fibrosis (IF) in non IBD cases and CD patients. **(C)(D)** Distribution 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, Supplementary 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).

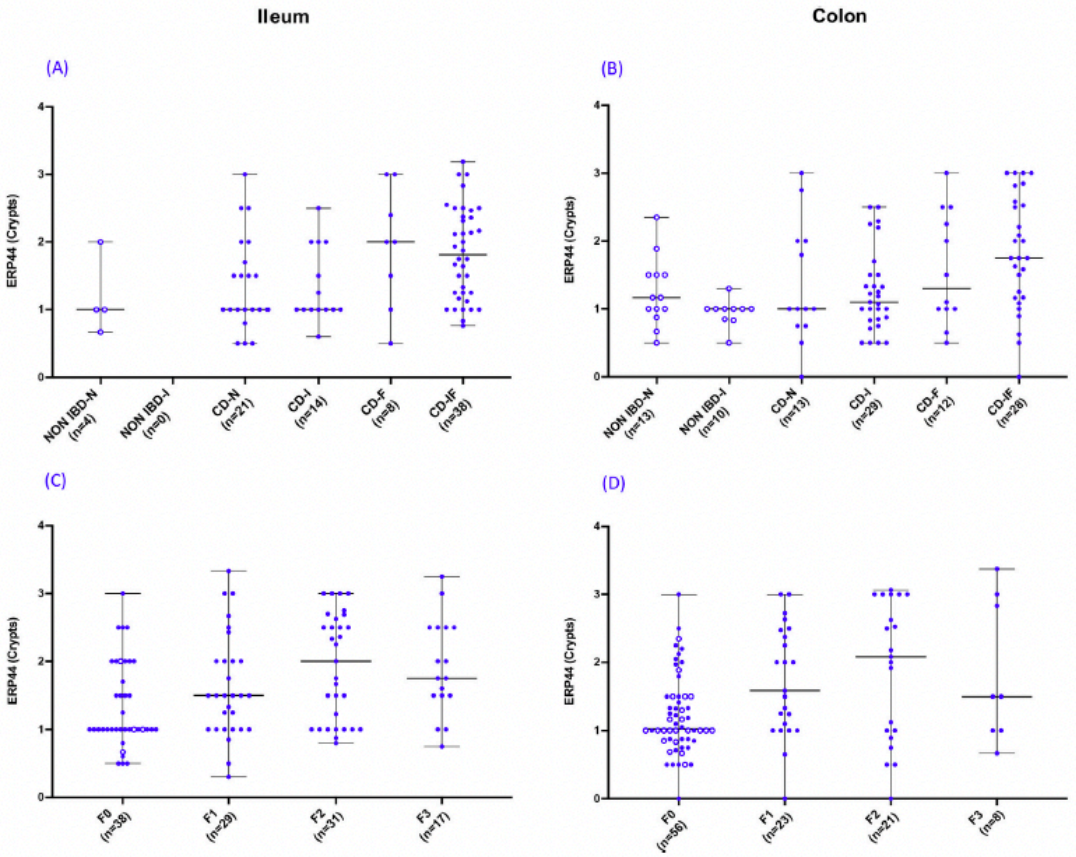

Fig. 5. **(A)(B)** Distribution of ERP44 IHC scores in ileal and colonic crypts, including normal tissues (N- no inflammation nor fibrosis), tissues with pure inflammation (I), pure fibrosis (F) and tissues with both inflammation and fibrosis (IF) in pediatric non IBD cases and pediatric-onset CD patients.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

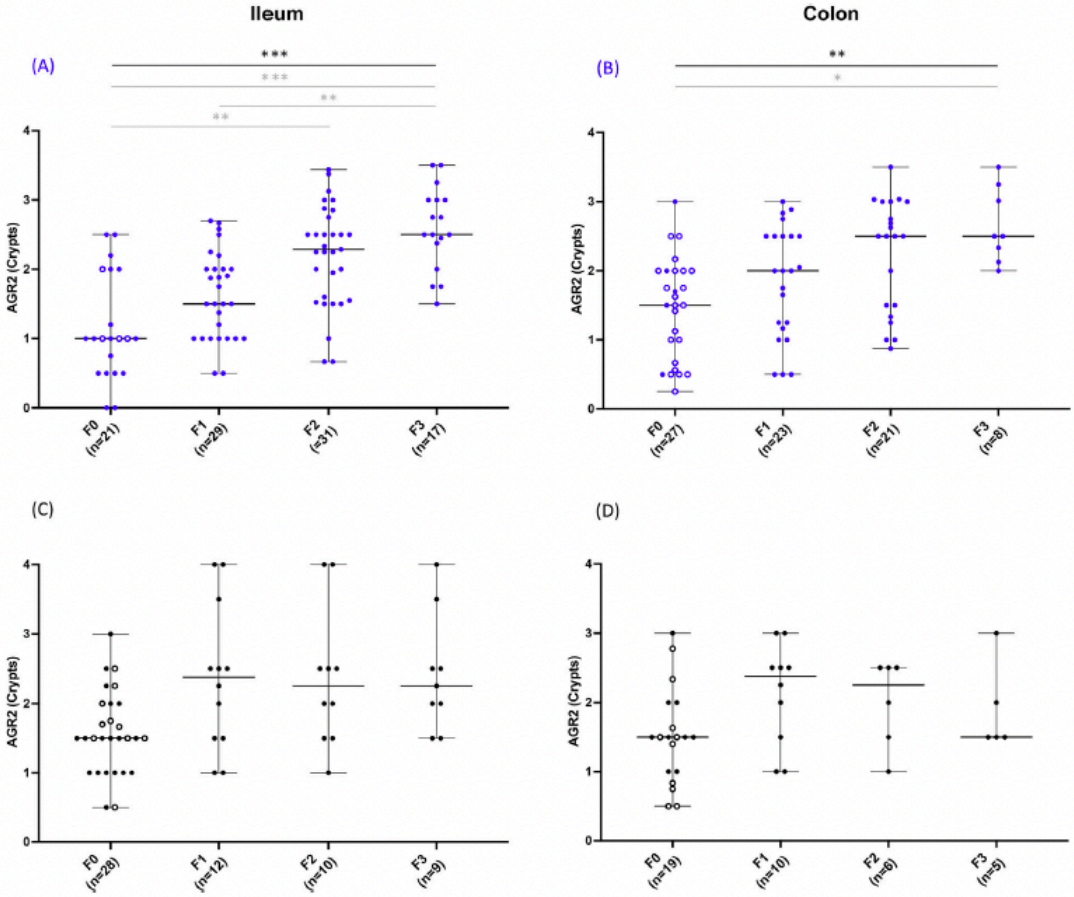

Fig. 6. **(A-B)** Distribution of AGR2 IHC scores according to fibrosis scores in pediatric-onset cases and **(C-D)** in adult-onset cases in ileal and colonic crypts.
The same shape and color coding has been used for all the figures.
● Adult-onset CD.
○ Adult control cases.
● Pediatric-onset CD.
○ Pediatric control cases.
* Statistical significance based on ANOVA test results after Bonferroni correction.
* Statistical significance based on Kruskal-Wallis post hoc test after Bonferroni correction.

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).

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, micro-

bial 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 contributes 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 downstream 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 multicenter 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.

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Supplementary materials

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

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