

literature that body sites once believed to be sterile do indeed have an endogenous microbiome at least in disease such as CD. Further studies are required but the goal in CD subjects may be to develop an effective personalised treatment based on knowledge of the individual's microbiota profile (both gut and adipose tissue microbiota).

## DOP079

### Heat-shock protein GP96 is essential for self-renewal of the intestinal epithelial barrier

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**Background:** The intestinal epithelium constitutes an important barrier that separates the body from the external environment to prevent access of external pathogens or potential hostile agents. Defects in epithelial barrier function are a characteristic clinical feature of intestinal disorders, such as inflammatory bowel disease (IBD). Genome-wide association studies identified the gene locus encoding heat-shock protein GP96 (also known as GRP94) as a risk factor to develop IBD, and recent in vivo data suggest a role for GP96 in maintaining intestinal barrier function and gut homeostasis.

**Methods:** To induce an intestinal epithelial cell-specific deletion of GP96, mice with a loxP flanked GP96 gene were bred with VillinCre mice (constitutive deletion) or VillinCre-ERT2 mice (inducible deletion). To induce GP96 deletion in GP96-VillinCre-ERT2 mice ( $N = 11$ ), tamoxifen was injected at five consecutive days (1 mg/day).

**Results:** Constitutive deletion of GP96 in the intestinal epithelium turned out to be embryonically lethal. Conditional GP96 deletion upon tamoxifen application resulted in the development of severe diarrhoea and rapid weight loss after 5–6 days after treatment. Colonoscopy revealed macroscopic signs of inflammation, including increased granularity of the colonic mucosa, fibrin deposits, and strong vascularisation of the colon. While the wall of the small intestine appeared transparent and fragile, the colon was thickened and significantly shortened. Histological analysis revealed severe lesions throughout the small intestine, characterised by a loss of goblet cells, decay of crypts and villi, as well as immune cell infiltration, culminating in complete eradication of the intestinal epithelium and subversion of the mucosal architecture. Morphological changes in the proximal and the distal colon were also observed, but less prevalent. Staining for cleaved caspase 3 disclosed a comparable low number of apoptotic cells throughout the whole intestinal epithelium. However, staining for the proliferation marker Ki67 revealed the absence of proliferating cells in the bottom of the remaining crypts.

**Conclusions:** GP96 plays a crucial role in the maintenance of the intestinal epithelial architecture, self-renewal and regeneration of the mucosa, possibly via mediating proliferation and differentiation of intestinal stem cells. Our hypothesis is in line with clinical studies, reporting elevated GP96 expression levels in the intestinal epithelium of IBD patients, where recurring damage and inflammation of the intestinal surface require intense regeneration and constant epithelial restitution. Therefore, GP96 may serve as a new target for therapy in IBD, offering the potential to induce mucosal healing.

## DOP080

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

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**Background:** Intestinal fibrosis in Crohn's disease (CD) is complex and its initiation and progression are linked to chronic inflammation and lead to bowel damages. Bordering epithelium involvement in this process remains unravelled. Our purpose was to address proteomic changes occurring in the bordering epithelium of regions with increasing degrees of sub-mucosal inflammation and fibrosis. Proteins differentially abundant could be potential actors eliciting fibrogenic pathways and potential therapeutic targets.

**Methods:** Formalin fixed paraffin embedded tissue sections from CD patients with ileal stenosis ( $n = 5$ ) were treated by laser capture microdissection to isolate bordering epithelial cells. Paired regions were selected in Normal, inflammatory and mildly fibrotic, as well as inflammatory and moderately to severely fibrotic areas. Protein digests were analysed by label free proteomics. Immunohistochemical (IHC) evaluation on independent CD cases was done ( $n = 32$ ). Moreover, the epithelial colonic cell line HT29 was used for in vitro culture experiments in order to characterise the selected protein in the epithelium and its potential impact on a simple model of intestinal fibrosis.

**Results:** Proteomics identified 1249 proteins from which 257 showed a distribution varying significantly with the degree of fibrosis. Anteriority gradient protein homolog 2 (AGR2), related to endoplasmic reticulum (ER) stress and mucus secretion, was the most significant one. AGR2 IHC confirmed a significant increase in ileal CD ( $n = 19$ ) with inflammation and a stronger one with fibrosis. In the colon ( $n = 13$ ) there was no significant difference between simple inflammation and inflammation with fibrosis. ER stress induction in HT29 cells enabled AGR2 increase as measured by RT-Q-PCR and WB, while TGF $\beta$ , the main fibrogenic elicitor, induced a decrease of this protein.

**Conclusions:** AGR2 increase in the epithelium of fibrotic CD ileum and in epithelial cell line upon ER stress induction suggest a potential role for epithelium and ER stress (including AGR2) in the development of surrounding submucosal fibrosis in ileal CD.

## DOP081

### Genital granulomatosis in Crohn's disease: results of the ECCO CONFER study

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