Conclusions: A significant reduction in CT and GMV in cortical areas has been shown in patients with active CD compared with HCs. This observation may be associated with a chronic inflammatory response, attributed to high levels of circulating neurotoxic cytokines. Investigating structural brain changes in active CD will aid our understanding of the cross-linking between chronic inflammation, brain structural changes and an unexplained symptoms such as fatigue in CD. This will inform new medical and psychological therapies.

**P007**

**Activation of pH-sensing receptor OGR1 (GPR68) induces ER stress and autophagy in an intestinal epithelial cell model**

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**Background:** OGR1 (also known as GPR68) is a pH-sensing G-protein coupled receptor previously identified to play an important role in physiological pH homeostasis. A local decrease in pH frequently occurs at sites of intestinal inflammation. A variety of stimuli, including acidosis, can induce endoplasmic reticulum (ER) stress. ER stress can activate autophagy, and both play important roles in gut homeostasis and contribute to the pathogenesis of IBD. To cope with stressful conditions and to ensure correct protein folding, eukaryotic cells have evolved the unfolded protein response (UPR). In a human intestinal epithelial cell (IEC) model, we investigate if the previously observed protective effect of GPR68 deficiency in experimental colitis is in part due to differences in UPR regulation, ER stress and autophagy.

**Methods:** Caco-2 cells stably overexpressing OGR1 were subjected to an acidic pH shift for 24 h (compared with vector control cells and to control pH). A novel small molecule OGR1 inhibitor and c-Jun N-terminal kinase (JNK) inhibitor (SP600125) were used to delineate the different signalling pathways. Expression of ER stress markers (binding immunoglobulin protein (BiP), inositol required 1-α (IRE-1α), phospho IRE-1α), apoptosis markers (caspase 3 and poly (ADP-ribose) polymerase (PARP)), and autophagy markers (microtubule-associated protein 1A/1B-light chain 3 (LC3)), were determined by RT-qPCR, immunoblotting and immunocytochemistry (ICC).

**Results:** Proton-activated OGR1-mediated signalling led to a significant upregulation in the ER stress markers BiP and phospho-IRE-1α, after treatment for 24 h. The induction of ER stress was reversed in the presence of an OGR1 inhibitor and a JNK inhibitor. Furthermore, caspase 3 and PARP were not cleaved, indicating that apoptosis was not induced. In addition, LC3-II protein levels and LC3 fluorescent puncta, observed by immunoblotting and ICC respectively, increased significantly in our proton-activated OGR1 cell model, supporting our finding that autophagy is induced by OGR1-mediated signalling.

**Conclusions:** As inhibition of JNK activity is usually associated with suppression of autophagy, our results imply that OGR1-mediated signalling pathways induce ER stress and autophagy. In an IEC model, a small molecule OGR1 antagonist reversed OGR1-mediated ER stress and autophagy suggesting that OGR1 inhibition might be a novel therapeutic approach for the treatment of IBD.

**P008**

**Differences in macrophage infiltration and Wnt ligands expression between strictureing and penetrating behaviour in Crohn’s disease**


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**Background:** Fibrosis and fistula development constitute the main complications associated to Crohn’s disease. The Wnt signalling pathway induces fibroblast activation and EMT which are involved in these complications. We aim to analyse here the pattern of Wnt ligands expression in surgical resections from stenotic and fistulising CD patients and to determine the potential role of these ligands in favouring fistula development over fibrosis.

**Methods:** CD patients (n = 43) were categorised according to Montreal classification. mRNA was isolated from resections of patients presenting a strictureting (B2) or a penetrating (B3) behaviour or from unaffected mucosa of patients with colorectal cancer. The expression of macrophage markers (CD206, CD86 and CD16), all Wnt ligands and Frizzled receptors, and DKK1, Lgr5, and cmyc was analysed by RT-PCR or flow cytometry. ECM deposition was analysed by staining. Correlations between data were analysed using Pearson’s correlation coefficient (*p < 0.05). The mRNA expression of EMT genes were analysed in isolated crypts treated with Wnt2b peptide for 7 h.

**Results:** A higher percentage of CD16 (B2: 36.12 ± 5.8%; B3:69.7 ± 24.4%) or CD86 (B2: 30.58 ± 10.9%; B3:88.8 ± 18.4%) positive macrophages and a higher mRNA expression of pro-inflammatory cytokines (IL-1, IL-6, Table 1) was detected in intestinal tissue from B3 compared with B2 CD patients. A higher mRNA expression of Vimentin and lower expression of E-cadherin was detected in CD patients compared with controls (Table 1) while no differences in the expression of these markers were detected between the fistulising and stenotic behaviour which exhibited a similar ECM deposition. CD patients presented a generalised overexpression of WNT (Wnt2, Wnt3, and Wnt6) ligands with the exception of Wnt2b which was upregulated in the fistulising

Abstract P008 - Table. Relative (Gene/b-actin) mRNA expression (fold induction vs. control group) of genes with detectable levels. Data are expressed as mean ± SEM with n ≥ 7 in all groups and analysed by ANOVA + Kewman–Keuls test. (*p < 0.05 vs. B2.)

<table>
<thead>
<tr>
<th></th>
<th>IL-1</th>
<th>IL-6</th>
<th>Vimentin</th>
<th>E-Cadherin</th>
<th>Wnt2b</th>
<th>CS6</th>
<th>CD16</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2</td>
<td>3.6 ± 1.0</td>
<td>5.0 ± 0.8</td>
<td>4.1 ± 1.0</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>4.5 ± 0.6</td>
<td>4.8 ± 0.9</td>
</tr>
<tr>
<td>B3</td>
<td>8.9 ± 2.0*</td>
<td>8.7 ± 1.8*</td>
<td>5.1 ± 1.0</td>
<td>2.5 ± 0.4*</td>
<td>7.7 ± 1.3*</td>
<td>7.2 ± 1.1</td>
<td></td>
</tr>
</tbody>
</table>