compared to ileal biopsies from normal controls (NC). A histopathological score was created based on the degree of active (polymorphonuclear) and chronic (mononuclear) infiltrates (1-normal, 5-major infiltrate). Gene expression analysis was performed using microarrays, and validated by real-time PCR. Gene ontology and clustering were evaluated using bioinformatic tools.

**Results:** Thirty-six subjects (7 NP, 15 pouchitis (10 CP, 5 CLDP) and 14 NC) were recruited. Significant differences of histopathology scores within the pouch were noticed: median activity scores: 1/5 NP, 2/5 CP, 4/5 CLDP (P = 0.001), median chronicity scores: 2/5 NP, 3/5 CP, 3/5 CLDP (P = 0.015). Histopathological differences in the proximal ileum were less pronounced. Nonetheless, significant (fold change $\geq 2$, corrected P < 0.05) molecular alterations were detected in the normal appearing proximal ileum of all pouch groups compared with NC: NP (n = 9), CP (n = 80) and CLDP (n = 230). DUOX2 alteration magnitude in the proximal ileum was highest: an increase of 6.0, 9.8 and 21.7 fold change in NP, CP, CLDP respectively, followed by MMP1 and SLC6A14. Moreover, gene alterations in the proximal ileum overlapped with those observed within the pouch: 76% and 97% overlap in CP and CLDP, respectively. Gene ontology analysis for proximal ileal alterations revealed association with multiple biological processes including active and anion transmembrane transporter activity and metallopeptidase activity. These findings may suggest that the inflammatory processes occurring in pouch patients are not limited to the surgically manipulated region (the pouch) but rather extend to the proximal ileum, potentially exposing it to further inflammation.

**P656**

Fcgamma Receptor Type IIIa polymorphisms and their correlation with clinical outcome in patients with inflammatory bowel disease during a long term follow up

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**Background:** A total of 20-30% of patients with active Crohn’s disease (CD) do not respond to anti-TNFalpha treatments and up to 40% of patients in chronic therapy experience a loss of response. Furthermore about 50% of patients with ulcerative colitis (UC) experience a loss of response to anti-TNFalpha therapy after one year. The cause of this limited efficacy is unclear, but past studies hypothesized that the individual variation of drug metabolism may play an important role. Thus, given the limited data available, the role of FcgammaIIa receptor (i.e. one of the four receptors involved in the catabolic pathway of anti-TNFalpha drugs) polymorphisms should be further explored.

**Aim:** The aim of this prospective, long-term follow up study was to evaluate the correlation between FcgammaIIa receptor polymorphisms and clinical outcome in IBD patients undergoing biologic therapy.

**Methods:** We enrolled consecutive IBD patients who achieved clinical remission by anti-TNFalpha therapy. Blood samples were collected at the beginning of biological therapy. The assessment of IBD activity was based on the Harvey-Bradshaw Index score (HBI, remission $<5$, mild disease 5–7, moderate disease 8–16, severe disease >16) for CD patients and on the Mayo score (Mayo $<2$ remission, mild disease 2–3, moderate/severe disease 6–12) for UC patients. Biochemical evaluation and clinical score were assessed every 8 weeks. For the genotyping analysis we used a Light Snips (Tib-Molbiol, Genova, Italy) and the Real-Time PCR Technique developed by Light Cycler 480 instrument (Roche, Mannheim, Germany).

**Results:** We prospectively included 39 patients (12 UC/27 CD, 16F/23M) with a median follow-up of 66.8 weeks (10–112). A total of 25 (64.1%) (10UC/15CD) patients kept in remission during the whole follow-up period, while 14 (28.6%) (2UC/12CD) experienced disease relapse. As shown in the Table, four out of 14 (28.6%) (1UC/3CD) patients who experienced disease relapse, had FcgammaIIa-158 V/V receptor polymorphism, whereas the remaining 10 (71.4%) (9CD/1UC) had FcgammaIIa-158 F/V or F/F receptor polymorphisms. Of 25 patients who kept in remission, 3 (12%) (1CD/2UC) had FcgammaIIa-158 V/V receptor polymorphism, whereas the remaining 22 (88%) (14CD/8UC) showed FcgammaIIa-158 F/V or F/F receptor polymorphisms.

**Conclusions:** The evaluation of FcgammaIIa-158 V/V receptor polymorphism does not seem useful in identifying patients who are more likely to lose anti-TNFalpha response during a long term period. However, further larger studies are necessary to investigate the role of FcgammaIIa receptor polymorphisms.

**P657**

Effects of isotretinoin treatment on epigenetic programming in T cells

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**Background:** Retinoids are essential nutrients involved in the maturation of the immune system. The majority of in vitro and in vivo studies provided evidence that retinoid treatment exhibits anti-inflammatory properties and is effective acne therapy. However, there is a controversial discussion about a causal relationship between isotretinoin treatment and the onset of inflammatory bowel disease (IBD). Some patients have claimed that they developed acute intestinal inflammation during isotretinoin treatment or had an onset of IBD weeks or even years after cessation of the medication. We have previously shown that isotretinoin treatment has no adverse effects in two mouse models of inflammatory bowel disease [1]. Here we investigated the influence of isotretinoin treatment on genetic imprinting in two T cells subsets as a potential mediator of long-term effects of isotretinoin treatment on the immune system.

**Methods:** Balb/c mice were treated with isotretinoin (30 mg/kg bodyweight) or vehicle orally for 2 weeks and kept for further 4 weeks to study potential direct and long-term effects. Naive T cells and regulatory T cells were isolated directly after the treatment period and at the end of the study by magnetic cell sorting. After isolation of genomic DNA, microRNA and mRNA, samples were sequenced with the Illumina® technique to study changes in methylation patterns, microRNA and mRNA expression. For predicting target genes of determined microRNAs the software Target Scan and Trarget Scan Custom were used. For identification of pathways significantly affected by isotretinoin treatment the software Meta Core® was applied.

**Results:** Analysis of epigenetic modifications in naive and regulatory T cells revealed potential long-term effects in both T cells subsets. In regulatory T cells mainly the methylation pattern was altered in T cells isolated four weeks after cessation of treatment. In naive T cells on the
other hand predominantly microRNA expression was altered in T cells isolated after four weeks without treatment. Pathway analysis by Meta Core revealed that pathways of immune responses, concerning antigen presentation and T helper cell differentiation were affected. Further functional analysis of affected pathways is currently under investigation.

**Conclusions:** Preliminary results identified changes in methylation pattern and microRNA expression in naive and regulatory T cells which might mediate potential long-term effects after isotretinoin treatment, yet differences between the different T cell subsets were far more pronounced than differences induced by isotretinoin treatment.

**Reference(s)**

[1] Frey-Wagner I. (2013), Effects of retinoids in mouse models of colitis: benefit or danger to the gastrointestinal tract?

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**P658**

**Effects of allopurinol on thiopurine metabolism and gene expression levels in HepG2 cells**

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**Background:** Combination therapy with thiopurines (TP) in reduced dose and the xanthine oxidase (XO) inhibitor allopurinol (AP) is safe and effective in patients with previous intolerance to TP. It reverses an unfavorable TP metabolite profile with high mETIMP and low 6TGN concentrations, thereby increasing the therapeutic 6TGN.

In this study we aimed to elucidate the effects of AP on the TP metabolism and gene expression levels in the liver cell line HepG2.

**Methods:** HepG2 cells (+/- transiently transfected to express XO) were incubated with 6-mercaptopurine (6MP) 6 μM, AP 130 μM or 6MP+AP. Genes previously identified in a microarray study of TP treated patients and genes with a potential relationship with TP metabolism [Haglund S et al. PLoS ONE 8(2): e56989 2013] were analyzed using quantitative real-time PCR. Metabolites (MMP base of meTIMP, 6TG base of 6TGN, 6MP base of TIMP and TX; stems from both TXMP and thioxanthine) were analyzed with IP-RP-HPLC. Experiments were run in triplicates. In Student’s t-test a P < 0.05 was considered significant after Benjamini-Hochberg correction for multiple testing. Interactions between gene products identified here and prioritized genes present at susceptibility loci identified for IBD [Jostins L et al. Nature 2012 Nov 1; 491(7422): 119–124] were evaluated using the STRING database v 8.3.

**Results:** No differences in metabolite concentrations were observed between 6MP vs. 6MP+AP in HepG2 cells +/- XO. However, large variation in metabolite determinations over triplicates was observed.

In HepG2 cells not transfected to express XO 6MP resulted in an up-regulation of DPP4, ENTPD1, GMPR1 and SLX1A. No genes were regulated in these cells by AP alone. In a combined treatment (AP+6MP) compared with 6MP alone, these cells expressed reduced levels of DPP4, ENTPD1, FAM156A, GNBD4 and SLC29A2, and increased levels of AOX1, MOCS1 and PPAT. Of these 10 regulated genes 5 interacted with 9 genes present at 9 IBD susceptibility loci. FAM156A, SLX1A1 and SLC29A2 were not present in any network. No genes were regulated by AP, 6MP or the combination treatment in HepG2 cells transfected to express XO.

**Conclusions:** The effect of AP on gene expression levels seems to require presence of 6MP. The interactions between genes identified here and candidate IBD susceptibility genes represented mainly biological processes associated with purine metabolism, T-cell activation, chemokine signaling and inflammation. Further exploration of these genes in vivo may lead to a better understanding of the mechanisms of AP in modulating the thiopurine metabolism. Possibly an effect of AP on metabolite concentrations in HepG2 cells (+/- XO) was obscured by the large variation in metabolite determinations and the small study number.

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**P659**

**Crohn’s disease in an Irish population – a pilot genotype-phenotype analysis**

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**Background:** Loss of function mutations in NOD2 (G908R, R702W, 1007fs) have been associated with Crohn’s disease (CD) and, in particular, with severe phenotypes. Earlier identification of severe disease may allow for tailoring of treatment. NOD mutations are thought to occur less frequently in Celtic populations and their association with phenotype is unclear.

Our aim was to assess NOD2 mutation status in an Irish cohort.

**Methods:** Following informed consent, the frequency of selected NOD2 mutations in blood were compared using genotyping analysis between subjects with established CD and controls. Montreal phenotype was recorded for CD subjects. Individuals with a normal surveillance ileo-colonoscopy, negative histology and lack of symptoms were recruited as controls. The frequency of mutations was compared between groups and among phenotypes using McNemar’s test.

**Results:** To date, 40 subjects have been enrolled: 30 (75%) with CD, 24 (60%) women with a mean age of 37 years. Ileo-colonic, ileal, colonic, strictureing and penetrating phenotypes occurred in 18 (60%), 8 (26%), 4 (13%), 16 (53%) and 5 (16%) respectively, while 19 (63%) had undergone surgery. In total, 7 (23%) CD patients and no controls displayed a NOD2 mutation (p < 0.001). Table 1. No patient had more than one mutation. Strictureing and ileocolonic phenotype and surgery were strongly correlated with a NOD2 mutation (OR 10, p < 0.01, 12, p < 0.01 and 7, p < 0.01 respectively).

**Table 1. Genotype-phenotype comparison**

<table>
<thead>
<tr>
<th>Montreal Classification</th>
<th>G908R</th>
<th>R702W</th>
<th>1007fs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. A2/L3/B2</td>
<td>HH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. A2/L3/B3p</td>
<td>HH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. A1/L1/B1</td>
<td>HZ</td>
<td></td>
<td></td>
</tr>
</tbody>
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HH, homozygous; HZ, heterozygous.

**Conclusions:** In our cohort, NOD 2 mutations appear more prevalent than previously described in the Celtic population. Single NOD2 SNP’s are associated with complicated disease and may be a useful biomarker of disease severity. Genetic testing in a larger cohort is warranted to further assess genetic influence on CD phenotypes.