31-5p as a candidate master regulator of gene expression pathways associated with ileum-like CD, a CD phenotype that is associated with the use of anti-TNF in the post-operative setting.

Conclusions: Our results show for the first time that miRNA levels in colon tissue segregate patients into 2 clinically distinct forms of CD. Specifically, our findings suggest that miR-31-5p contributes most to the discrimination between ileum-like and colon-like phenotypes. miR-31-5p is secreted into the circulatory system, therefore it is possible that plasma miR-31-5p levels could serve as an effective biomarker of CD subtypes potentially providing an important clinical diagnostic and prognostic indicator of disease phenotypes.

P-309
IBD Causal Variant rs1887428 in the Promoter of JAK2 Demonstrates Differential Allelic Expression
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Background: Work by the International IBD Genetics Consortium (preprint by Huang et al) has used dense genotyping on the Immunochip and Bayesian statistical analysis to elucidate 18 loci that could be fine-mapped to single SNPs with greater than 95% posterior probability of causality. Not surprisingly, 8 of these SNPs were coding variants due to their strong phenotypic impact. However, 10 other loci were identified in which the causal SNP has no known motif or feature that suggests its mechanism of action, such as being contained within a consensus transcription factor binding sequence, a chromatin modification region, or a cis expression quantitative trait locus (c-eQTL). We used functional experiments to determine if any of these causal SNPs could show allelic differences in expression.

Methods: We took 8 loci—including LRRC2, HNF4A, IL2RA, IKZF1, GPR35, NKX2-3, JAK2, and PRDM1—of approximately 700 bp in size centered on the credible SNP, and cloned both the reference and alternate allele into a luciferase reporter vector with a minimal promoter. Expression was assayed by the Dual Luciferase kit (Promega) in HEK 293T cells. We also assessed the full-length JAK2 promoter containing variant rs1887428 in the dual luciferase assay. Electrophoresis mobility shift assay (EMSA) was used to determine if sequence-specific binding proteins could recognize these SNPs.

Results: Six of the loci did not show any expression of luciferase above the background level of the empty vector. However, both JAK2 and PRDM1 region SNPs showed significantly above-baseline expression. The JAK2 SNP lies within the promoter region 556 bp from the transcriptional start site and showed a 20% allelic difference in expression using a promoter fragment but no significant difference using the full-length promoter. Both JAK2 and PRDM1 probes containing the causal SNPs could be bound by sequence-specific DNA-binding proteins.

Conclusions: These results suggest that credible set SNPs can mediate differences in gene expression even when known functional annotations are lacking or the mechanism of action is unknown. Disease-associated SNPs that confer allelic differences in gene expression are more likely to contribute to disease pathogenesis.

P-310
Y1
Characterizing Gut Metagenome Variation with Host Contamination Using Nanopore Sequencing
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Background: Metagenome sequencing has been used to great effect to characterize the diversity of microbial communities, including the variation in gut microbiota associated with the development and severity of inflammatory bowel diseases (Kostic et al, 2014). However, modern sequencing-by-synthesis such as Illumina produces short reads that are often difficult to accurately assign to a taxonomic unit and most of which are not informative to differentiate closely related taxa. Recent long-read single-molecule sequencing technologies such as Pacific Biosciences and Oxford Nanopore Technologies (ONT) permit the identification of taxa with greater specificity and sensitivity than short reads, but are limited by high cost and low throughput.

Methods: To assess the accuracy of nanopore sequencing-based metagenome classification, we constructed a mock microbiota colony by mixing ten cultured bacteria species at approximately equal cellular volume. We sequenced the mock community using ONT’s MinION sequencer in 2 replicates. We also constructed a DNA sample simulating a host contamination by mixing the mock community with Arabidopsis thaliana at a ratio of 10% bacterial DNA and 90% host DNA. We used Kraken (Wood and Salzberg, 2014) to assign OTUs to the sequenced reads.

Results: Mock community replicates A1 and A2 produced 55,027 and 84,178 reads, respectively. The mock host/A1 mixture sequencing produced 7531 reads. Across replicates A1 and A2, 25% and 47% of reads were identified as bacterial and classified by phylum. Of those classified, over 90% were assigned to a single species or strain. All 10 bacterial species in the mock community were accurately identified and no false positives were found with a threshold of 3 reads. Identified species varied from 0.01% to 9.45% relative abundances, perhaps due to differences in cell size and cellular density in medium of the mock community. The lowest abundance member was accurately identified to the species level in only 3 reads. The mock host contamination data produced only 311 reads identified as bacterial due to the 90% “host” DNA and low sequencing throughput. All but the lowest abundance member was accurately identified. We saw high correlation between estimated taxa abundances between both community replicates (r² = 0.861) and A1 versus host+A1 (r² = 0.942).

Conclusions: We have shown that whole-metagenome shotgun sequencing using ONT’s MinION nanopore sequencing platform allows identification of known species with greater than 95% posterior probability of causality. Recent genetic variants can also determine changes in vitamin D mechanisms of action and affect the clinical course of IBD. In Brazil, there are a lack of data concerning VDR polymorphisms in IBD patients and its use as a predictor of poor prognosis. The aim of this study was to evaluate the association of VDR gene polymorphisms, vitamin D status and the poor prognostic factors in a multiracial IBD population.

Methods: A case control study recruited 107 patients with Crohn’s disease (CD), 43 patients with ulcerative colitis (UC) and 81 control healthy subjects from a tertiary center in Rio de Janeiro. The VDR polymorphisms Apal, (‘Aa’ ‘aa’), TaqI (‘TT’ ‘Tt’ ‘tt’) were evaluated in IBD and non IBD groups. The genotyping was performed by polymerase chain reaction (PCR) in real time technique. Serum 25(OH)D was measured using chemiluminescence immunoassay. Demographic, clinical features and poor prognostic factors of IBD patients were obtained from the chart review.

Results: The genotype ‘aa’ (Apal) (49.5%) and ‘TT’ (TaqI) (50.5%) were more frequent in CD group. The distribution of polymorphisms was similar between UC and the control group. In CD group, the “a” allele carrier status of Apal appeared to be a protective factor against the use of immunosuppressors (OR = 0.225; 95% CI, 0.071–0.712). The “t” allele carrier status of TaqI was protective factor against the use of steroids: at the time of CD diagnosis (OR = 0.242; 95% CI, 0.065–0.901) but it was associated with the need of more than 2 steroids courses in UC group (OR = 4.19; 95% CI, 1.104–15.901) The 25(OH)D level was measured in 44 IBD patients, and low levels were detected only in 36.6% of cases. No association was observed between clinical features, VDR polymorphisms and vitamin D deficiency.

Conclusions: We have shown that host contamination can skew the results of conventional functional genomics. We also observed that VDR polymorphisms were associated with CD and a poor prognostic factors. Although the polymorphisms have not been associated with UC, the presence of TaqI polymorphism was associated with poor prognosis predictor in this group. No associations were detected between VDR polymorphisms and vitamin D deficiency in IBD groups.

P-312
Mucosal Gene Expression in Pediatric and Adult Patients with Ulcerative Colitis
Permits Modeling of Ideal Biopsy Collection for Transcriptomic Analysis
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