the bioinformatic approach a subset of SNVs and SVs were selected for verification by Sanger capillary sequencing and PCR respectively.
Results A minimum of 50-fold mappable sequence data were generated for each of the 56 genomes. 161/167 (96%) of predicted SNVs were confirmed as somatic, two were miscalled germline variants while four were undetectable in either sample. For 2/75 (3%) SVs PCR amplicons could not be generated, for 18 of 75 SVs (24%) a PCR amplicon was detectable in the normal showing them to be germline polymorphisms. The True positive rate for SV detection was therefore 73%. Comparison of SNV information across all 24 samples revealed many recurrently mutated genes. These include previously reported mutations in TP53, CDKN2A and APC among others. No genes were significantly associated with chemotherapy-treated or chemotherapy-naive samples.
Conclusion Analysis of the Illumina bioinformatic pipeline suggests it is highly specific (96% true positive rate) for somatic SNVs. A true positive rate of 73% for SV detection is comparable to recent literature. Further analysis to determine the sensitivity of this pipeline is ongoing including resequencing of putatively non-mutated genes in samples sent for WGS and the application of alternative bioinformatic approaches for the calling of SNVs, INDELs and SVs. Initial analysis of the SNV data from 32 tumour genomes has revealed several recurrently mutated genes known to be altered in OAC validating the ability of our approach to detect candidate "driver" genes.

Competing interests None declared.

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OC-009 HUMAN ANTI-MICROBIAL Vδ2+ T-CELLS ARE NOVEL INTESTINAL LYMPHOCYTES WITH FUNCTIONAL RELEVANCE IN CROHN'S DISEASE

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Introduction Vγ9Vδ2+ "unconventional" (Vδ2) T-cells are a population of circulating anti-microbial lymphocytes found only in higher primates and whose role in human intestinal immunity is unknown. In macaques, microbe-activated Vδ2T-cells expand and accumulate in mucosal tissues, and human Vδ2T-cells can produce key mediators of intestinal inflammation such as IFNγ, TNFα and IL-17A in response to bacterial species present among the gut microbiota. We therefore hypothesised that Vδ2T-cells might contribute to the pathogenesis of Crohn's disease (CD).
Methods Disaggregated intestinal biopsies and peripheral blood were analysed by flow-cytometry in CD patients (n=22), and healthy controls (n=36). Blood and biopsy-derived cell suspensions were stimulated with microbial phosphoantigen (HDMAPP) and IL-2 in vitro to determine Vδ2T-cell phenotype, cytokine production and proliferative potential in the presence or absence of azathioprine.
Results Blood Vδ2T-cells proliferated, expressed "gut-homing" integrin β7, and produced IFNγ and TNFα upon activation with HDMAPP and IL-2 in vitro. Vδ2T-cells were also identified by confocal microscopy in both healthy and inflamed colonic lamina propria. In contrast to their blood counterparts, mucosal Vδ2T-cells expressed high levels of CD103 integrin, which is implicated in interactions with the intestinal epithelium. Although the frequency of mucosal Vδ2T-cells was low, these cells proliferated rapidly and up-regulated CD70 co-stimulatory molecule upon exposure to HDMAPP and IL-2 in vitro, consistent with responsiveness to the gut microbiota. In CD patients receiving azathioprine therapy, Vδ2T-cells were selectively lost from the blood and were markedly depleted from the lamina propria. Accordingly, physiological concentrations of azathioprine were sufficient to block HDMAPP activation of Vδ2T-cells in vitro.
Conclusion Human Vδ2T-cells primarily reside in the blood but display gut-homing potential upon microbial activation and can be detected in the intestinal mucosa. Intestinal Vδ2T-cells may contribute to local pro-inflammatory responses against the gut microbiota but are depleted by azathioprine therapy in CD.

Competing interests None declared.

OC-010 DETAILED ANALYSIS OF ATG16L1 DEMONSTRATES GENE-WIDE EXTENT OF ASSOCIATION WITH CROHN'S DISEASE SUSCEPTIBILITY

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Introduction ATG16L1 has been implicated in the susceptibility to Crohn's disease (CD), notably the T300A (rs2241880, exon 9). In ATG16L1-deficient and hypomorphic mice, autophagy, Paneth-cell homeostasis and IL-1b secretion were dependent on ATG16L1 (Saitoh *et al* Nature 2008, Cadwell *et al* Cell 2010). In contrast, studies focusing on T300A have shown conflicting results (Kuballa *et al* PLoS ONE 2008, Fujita *et al* JBC 2009). The association at other susceptibility loci (eg, NOD2 and IL23R) consists of common and rare variants (Rivas *et al* Nat Genet 2011). Exon-sequencing studies have not demonstrated rare variants within the 18 exons of ATG16L1. Our aim was to analyse the ATG16L1 association signal, specifically focusing on common variants.
Methods 38 single nucleotide polymorphisms (SNPs) spanning the ATG16L1 gene were imputed for 1735 subjects (800 CD/935 controls) (Franke *et al* Nat Genet 2010). Single SNP and haplotype frequency (>1%, solid spine of Linkage disequilibrium (LD), D'>0.8) association and permutation (n=10 000) analyses were performed (Haploview). Logistic regression (using SNPs $p<0.05$ on permutation analysis) was performed (SPSS). Monte Carlo simulation (n=10 000, R) was used to assess the difference in the number of haplotype blocks, based on D'>0.8, between CD and controls.
Results Single SNP permutation analysis yielded association of 16 markers ($p<0.001$), from intron 1 (rs6752107) to the 3'UTR (rs1045100). Analysis of D' and r^2 characteristics, showed 12 SNPs were in complete LD with rs2241880 (D' and r^2 0.98–1). rs6758317 ($r^2=0.25$, intron 2), rs3792106 ($r^2=0.76$, intron 11), rs4663396 ($r^2=0.25$, intron 12) and rs1045100 ($r^2=0.84$) demonstrated strong association, independent of rs2241880. Regression analysis retained rs3792106, rs6754677 (intron 14) and rs1045100 (3'UTR) ($p<0.05$). A Monte Carlo simulation showed no significant difference in the number of haplotype blocks between CD and controls ($p=1$). A strong association with CD was shown for the haplotype block containing the rs2241880 and for the block containing rs1045100, both $p<10^{-5}$.
Conclusion We demonstrated that the rs2241880 alone is not sufficient to explain the strong ATG16L1 association. Additional variants, independent of rs2241880, could implicate any of the coiled-coil domain, the WD domain and/or the 3' UTR, in CD susceptibility.

Competing interests None declared.