

Online supplementary material

MATERIALS AND METHODS

Patients

Patients were recruited to the Oxford IBD Cohort, a prospective IBD cohort established for the evaluation of outcomes, treatments and predictors in IBD. The cohort is built from a range of referral sources covering 620,000 people within Oxfordshire. IBD was confirmed in all patients by standard methods, including endoscopy, radiologic, laboratory, and clinical evaluation. Phenotypic classification was based on Montreal classification. Patients were selected for DNA sequencing based on their age at diagnosis (> 7 years of age) and severity of disease, indicated by need for surgery and/or therapy progression to biologics.

Informed consent was obtained from all patients and the study was approved by the local ethics committees (Inflammatory Bowel Disease in Oxford: prospective cohort for outcomes, treatment and predictors. Research Ethics Committee Reference: 09/H1204/30 (Granted June 2009).

Exome sequencing

Exome sequencing was performed at the Wellcome Sanger Institute (WSI). In short, genomic DNA (1-3µg) was extracted, exon enriched and sequenced using the HiSeq 2000 platform (Illumina) according to the manufacturer's protocol. The Burrows-Wheeler Aligner was used for alignment to the human reference genome build UCSC hg19/GRCh37 (1000Genomes_hs37d5). Variants were called using GATK Haplotype Caller (version 3.4) and alleles with low genotype quality (<20) or coverage (<8x) were excluded. Variants were annotated with allele frequency (AF) information taken from several reference datasets (1000 Genomes Phase 1 integrated callset 2012-07-19 (N=2,504), NHLBI Exome Sequencing Project 6,500I (N=6,500), UK10K whole-genome sequenced cohorts (N=3,781), UK10K exomes (N=4,818) and Exome Aggregation Consortium r0.3 (ExAC) (N=60,706).

Sanger sequencing for genotype validation was performed using standard techniques.

Variant screening

To prioritize rare IBD associated variants of clinical significance, 59 genes associated with Mendelian forms of IBD were screened ⁹. We focused on rare (initially MAF<1%)

deleterious or potentially deleterious variants that were consistent with the reported inheritance pattern of the given gene.

We performed two independent analyses. First, we screened VCF files manually using custom scripts. We investigated functional variants with transcript ablation, stop gained/lost, stop retained, splice donor/acceptor/region, frameshift, inframe insertion/deletion, initiator codon and missense variants with a PolyPhen-2 and SIFT pathogenicity predictions of 'possibly damaging/deleterious' (or greater). Second, we performed the same analysis as described above using the Ingenuity Variant Analysis (Qiagen) platform.

XIAP expression analysis

Fluorescence-activated cell sorting (FACS) was used to quantify XIAP protein expression as previously described ¹⁰.

L18 MDP stimulation assay

Intracellular TNF staining was carried out as described ¹¹. Briefly, monocytes from patients and healthy donors were rested overnight in RPMI media plus 10% fetal calf serum. Following day, monocytes were stimulated either with L-18 MDP (200ng/ml) or LPS (200ng/ml) or left unstimulated for 2.5 hours in the presence of golgi-plugin. Following stimulation, monocytes were stained with L/D stain, CD14 and HLADR (surface) and TNF (intracellular). Sample acquisition was carried out on BD Fortessa and data analysis was performed using FlowJo.

Additional suppl. references

9. Uhlig HH, Schwerd T. Inflamm Bowel Dis 2016; 22: 202-12.
10. Gifford CE, et al. Cytometry. Part B, Clinical cytometry 2014; 86: 263-71.
11. Schwerd T, et al. Gut 2017; 66: 1060-1073.