also has an intermediate response to second line therapy. Both scores are useful tools to aid clinical decision making but do not replace timely multidisciplinary care for these patients.

REFERENCES

Disclosure of Interest None Declared.

OC-003

GENOME-WIDE EPIGENETIC ANALYSIS IN CHILDHOOD-ONSET CROHN’S DISEASE IMPLICATES MIR21


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Introduction DNA methylation influences transcriptional activity and marks sites of active transcription. Technological developments allow the rapid assessment of methylation state at 450,000 sites across the genome. The aim of this study was to identify genes with a possible role in Crohn’s disease pathogenesis, and candidate genes for methylation based diagnostic biomarkers.

Methods Using the Illumina 450k platform we analysed genome-wide DNA methylation in symptomatic children who underwent diagnostic colonoscopy, half of whom were diagnosed with CD and half had no pathology. Replication was performed in children with established CD vs. symptomatic non-disease controls. Further targeted replication by pyrosequencing was performed in adults with CD vs. healthy controls, with qPCR and microarray data to analyse expression.

Results Meta-analysis of the combined paediatric datasets (n = 66) identified 165 individual CpGs with epigenome-wide significance (Bonferroni correction) and 138 differentially methylated regions (DMR). Methylation changes were significantly enriched (p < 0.0001) in proximity to loci implicated by genome-wide association studies (GWAS).

The strongest result by each approach was MicroRNA 21, within the autophagy gene VMP1 (p = 1.2 × 10^-14), 48 kb from GWAS SNP rs1292053. In adults with CD we replicated MIR21 hypomethylation (p = 6.6 × 10^-5, n = 172), and showed increased expression in blood (p < 0.005, n = 66). Intestinal expression increased with inflammation in CD (p = 1.4 × 10^-6, n = 99) but not controls (n = 73).

Linear discriminant analysis of methylation in the paediatric discovery cohort accurately predicted disease state in the paediatric replication cohort (94% sensitivity, 100% specificity) based on methylation at two CpG sites.

Conclusion MIR21 emerges as a target for further investigation based on methylation and expression, further strengthened by other positive findings – notably dysregulation in dysplasia and colorectal cancer in IBD, an established role in T-cell differentiation, and protection from DSS-induced fatal colitis by MIR21 knockdown. These data demonstrate a novel approach for identifying biological variations associated with germ-line variants identified by GWAS, and demonstrate translational potential for biomarker development and therapeutic target discovery.

Disclosure of Interest None Declared.

OC-004

THIOPURINE INDUCED PANCREATITIS IN INFLAMMATORY BOWEL DISEASE: CLINICAL FEATURES AND GENETIC DETERMINANTS


Introduction Pancreatitis is a rare, but important complication of thiopurine treatment. The aims of this project were to a) characterise the clinical features of thiopurine-induced pancreatitis and b) identify clinically useful genetic markers that might predict development of this serious adverse drug reaction.

Methods Patients were identified and recruited from 172 sites (128 UK). Inclusion criteria included a) onset of acute severe abdominal pain within three months of starting thiopurine treatment b) ≥ two-fold rise in amylase or lipase c) medical opinion implicating thiopurine therapy and drug withdrawal.

Results We recruited 303 patients. Following adjudication 48 cases classified as definite (recurrent pancreatitis on rechallenge) and 195 cases classified as probable (temporal relationship and no other cause for pancreatitis) were taken forward for analyses. 46% of patients were smokers at the time of development of pancreatitis. Patients were treated with a thiopurine for a median of 19 days (95% CI: 17 – 21) before development of pancreatitis. Most cases were mild, with only 5 cases developing single organ dysfunction. 70% of patients were hospitalised with a median length of stay of 4 days (95% CI: 3.2 – 4.8). Neither age (p = 0.08), drug dose (p = 0.11), BMI (p = 0.73) nor smoking (p = 0.59) predicted length of hospital stay or severity of pancreatitis in multivariate analysis. Using a control cohort of 4,109 Crohn’s disease and ulcerative colitis cases we conducted a genome wide association study with these 239 patients. A significant variant was identified in the Class II MHC region (Odds ratio 3.03, p = 1.2 x 10^-14), 48 kb from GWAS SNP rs1292053. In adults with CD we replicated MIR21 hypomethylation (p = 6.6 x 10^-5, n = 172), and showed increased expression in blood (p < 0.005, n = 66). Intestinal expression increased with inflammation in CD (p = 1.4 x 10^-6, n = 99) but not controls (n = 73).

Linear discriminant analysis of methylation in the paediatric discovery cohort accurately predicted disease state in the paediatric replication cohort (94% sensitivity, 100% specificity) based on methylation at two CpG sites.

Conclusion MIR21 emerges as a target for further investigation based on methylation and expression, further strengthened by other positive findings – notably dysregulation in dysplasia and colorectal cancer in IBD, an established role in T-cell differentiation, and protection from DSS-induced fatal colitis by MIR21 knockdown. These data demonstrate a novel approach for identifying biological variations associated with germ-line variants identified by GWAS, and demonstrate translational potential for biomarker development and therapeutic target discovery.

Disclosure of Interest None Declared.
identified a significant association within the Class II MHC region.

Disclosure of Interest None Declared.

OC-005 NOVEL PATHWAY-CENTRIC ANALYSIS REVEALS VARIANTS ASSOCIATED WITH TOXICITY AND RESPONSE TO THIOPURINES IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction Thiopurines remain the first line immunosuppressants recommended in the management of inflammatory bowel disease (IBD). Unfortunately, 30–40% of patients prescribed these agents develop adverse drug reactions or fail to derive therapeutic benefit. Candidate gene studies have identified loci that explain some of these treatment failures; however a substantial fraction of the genetic contribution remains undefined. Using whole thiopurine pathway analysis the aim of this study was to identify novel loci associated with toxicity and response to azathioprine (AZA)/mercaptopurine (MP) in patients with IBD.

Methods Genomic DNA was extracted from EDTA blood samples of 472 well-characterised IBD patients treated with AZA/MP. We examined exome array data using the Illumina HumanExome Beadchip and restricted the analysis to variants associated with the thiopurine pathway as defined by the KEGG database (100 genes, 639 single nucleotide polymorphisms). Using a case-control design we firstly tested for genetic associations between patients with (n = 154) and without (n = 258) adverse drug reactions, and secondly for polymorphisms differentiating patients with (n = 188) and without (n = 141) response to thiopurines after 12 months of treatment. One year intervention-free clinical response was defined by 3 investigators (PB, PI, JS).

Results Following adjustment for principal components, the minor alleles at ADK rs946185 (p = 0.0078; OR 1.675), SLC28A1 rs2242046 (p = 0.0168; OR 1.600) and ABCA1 rs4149268 (p = 0.033; OR 1.487) were associated with the development of drug toxicity, whereas the minor alleles at ABCB5 rs2301641 (p = 0.0170; OR 0.608), ABCC4 rs4148549 (p = 0.027; OR 0.652) and AOX1 rs55754655 (p = 0.038; OR 0.549) protected against it. The minor allele at RRM2 rs1130609 (p = 3.80 × 10⁻⁶; OR 0.461), which codes a subunit of ribonucleotide reductase involved in the conversion of thio- guanine nucleotide to deoxy-thioguanine nucleotide, and a higher normalised dose of AZA/MP were associated with protection from non-response. Conversely, the minor allele at ABCA1 rs2230808 (p = 0.008; OR 2.585) and Crohn’s disease (p = <0.001; OR 5.007) were associated with non-response to treatment after 12 months.

Conclusion High-throughput sequencing using exome array technology has revealed new loci, other than thiopurine-S-methyltransferase, explaining toxicity and response to thiopurines. Validation of these markers in separate cohorts will allow the development of biomarker panels to predict outcomes prior to the start of treatment.

Disclosure of Interest None Declared.

OC-006 SAFETY AND EFFICACY OF LOW DOSE AZATHIOPRINE AND ALLOPURINOL CO-THERAPY: A LARGE SINGLE CENTRE EXPERIENCE

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Introduction The effectiveness of full dose azathioprine (FDA) for inflammatory bowel disease (IBD) has been questioned in recent scientific literature. A popular strategy to improve its outcomes recommends the use of low dose azathioprine with allopurinol co-therapy (LDA) for patients profiled as “hyper-methylators” (30% of non-responders). The aim of this study was to determine the safety and efficacy of LDA without using thiopurine metabolite (TM) profiling.

Methods Records of IBD patients treated with LDA were retrospectively analysed.

Patients who had poor response and/or side-effects to FDA were offered LDA by all Consultants whilst a single IBD physician also offered LDA to thiopurine-naïve patients. Azathioprine dose was reduced to 25% of the thiopurine methyl transferase (TPMT) adjusted dose (0.5 mg/kg for wild type and 0.25 mg/kg for heterozygotes) followed by conventional haematological monitoring. Non-adherence was assessed by TM measurements. Full response (FR) was defined as steroid free remission (Harvey Bradshaw index ≤3, Truelove-Witts normal) for greater than 3 months after a 3 month induction period for LDA.

Results Of 300 LDA patients, adequate data was available for 295 cases. Group 1 (G1) were treated 1st line (n=105) and Group 2 (G2) were switched from FDA to LDA (n=190). Overall, for both groups, there were 207 (70%) full responders (FR), 20 partial responders (PR) and 68 non-responders (NR). Full response rate was 78% in G1 and 66% in G2. The commonest indication for switching to LDA was non-response to FDA (n,118).

Analysis of haematological indices revealed significant changes (p < 0.05) in erythrocyte sedimentation rate, white cell count and platelet count after therapy induction. Myelotoxicity occurred in 5 patients (all NR, WCC >2 and <3.5) and 12 patients had asymptomatic hepatotoxicity (ALT range: 100–700) which resolved by increasing allopurinol to 200 mg in 9 patients (all FR).

Time on treatment: 208 patients took LDA for more than twelve months with a median length of therapy of 24 months.

Conclusion Appropriately dosed LDA therapy delivers a therapeutically effective dose of azathioprine without the

Abstract OC-006 Table 1

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<th>FR</th>
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<th>NR</th>
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