P816

Functional rare variants influence the clinical response to anti-TNF therapy in Crohn's disease

M. Chaparro*[1], A. Aterido[2,3], I. Guerra[4], M. Iborra[5], J. Cabriada[6], L. Bujanda[7], C. Taxonera[8], V. García-Sánchez[9], I. Marín-Jiménez[10], M. Barreiro-de Acosta[11], I. Vera[12], M. Martín-Arranz[13], B. Hernández-Breijo[14,15], F. Mesonero[16], L. Sempere[17], F. Gomollón[18], J. Hinojosa[19], A. Algaba[4], B. Beltrán[5], A. Rodríguez Pescador[6], J. Banales[7], D. Olivares[8], P. Aguilar-Melero[9], L. Menchén[10], R. Ferreiro-Iglesias[11], I. Blázquez Gómez[12], B. Benitez García[13], L. Guijarro[14], A. C Marín[1], D. Bernardo[1], S. Marsal[2], A. Julia[2], J. P Gisbert[1]

[1]Hospital Universitario de La Princesa, ISS-IP, Universidad Autónoma de Madrid and CIBEREHD, Gastroenterology Unit, Madrid, Spain, [2]Vall d'Hebron Research Institute, Rheumatology Research Group, Barcelona, Spain, [3]Universitat Pompeu Fabra, Experimental and Health Sciences, Barcelona, Spain, [4]Hospital Universitario de Fuenlabrada, Instituto de Investigación de La Paz (IdiPaz), Gastroenterology Unit, Madrid, Spain, [5]Hospital Universitario y Politécnico de La Fe and CIBEREHD, Gastroenterology Unit, Valencia, Spain, [6]Hospital Universitario de Galdakao, Gastroenterology Unit, Galdakao, Spain, [7]Hospital Universitario de Donostia, Instituto Biodonostia, UPV/EHU, Ikerbasque and CIBEREHD, Gastroenterology Unit, San Sebastián, Spain, [8]Hospital Universitario Clínico San Carlos and IdISSC, Gastroenterology Unit, Madrid, Spain, [9]Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Universitario Reina Sofía/Universidad de Córdoba, Gastroenterology Unit, Córdoba, Spain, [10]Hospital Universitario Gregorio Marañón e IiSGM, Gastroenterology Unit, Madrid, Spain, [11]Hospital Universitario Clínico de Santiago, Gastroenterology Unit, Santiago de Compostela, Spain, [12]Hospital Universitario Puerta de Hierro Majadahonda, Gastroenterology Unit, Madrid, Spain, [13]Hospital Universitario La Paz, Gastroenterology Unit, Madrid, Spain, [14]Universidad de Alcalá, Alcalá de Henares, Spain, [15]IdiPaz. Hospital Universitario La Paz, Immuno-Rheumatology Research Group, Madrid, Spain, [16]Hospital Universitario Ramón y Cajal, Gastroenterology Unit, Madrid, Spain, [17]Hospital Universitario Alicante, Gastroenterology Unit, Alicante, Spain, [18]Hospital Lozano Blesa, IIS Aragón and CIBERehd, Gastroenterology Unit, Zaragoza, Spain, [19]Hospital Universitario Manises, Gastroenterology Unit, Valencia, Spain

Background: Loss-of-function (LoF) variants are one of the most interesting forms of rare functional genetic variations as they impair the function of a gene and are more likely to lead to extreme phenotypes. Our aim was to know the impact of functional rare variants in clinical response to anti-TNF therapy in Crohn's disease (CD).

Methods: CD anti-TNF naïve patients starting anti-TNF treatment due to active disease (CDAI>150) were included. The whole genome was sequenced using the Illumina Hiseq4000 platform. Clinical response was defined as a CDAI score < 150 at Week 14 of anti-TNF treatment. Low-frequency variants were annotated and classified according to their damaging potential. The whole genome of CD patients was screened to identify homozygous LoF variants. The TNF signalling pathway was tested for overabundance of damaging variants using the SKAT-O method. Functional implication of the associated rare variation was evaluated using cell-type epigenetic enrichment analyses.

Results: 41 CD patients were included -61% had remission and 24% were primary non-responders (Table 1); 3,250 functional rare variants (2,682 damaging and 568 LoF variants) associated with response to anti-TNF therapy were identified (Table 2). The strongest damaging impact was detected in 10 LoF SNPs (Table 3). Two homozygous LoF mutations were found in HLA-B and HLA-DRB1 genes associated with lack of response and remission, respectively. Genome-wide LoF variants were enriched in epigenetic marks specific for the gastrointestinal tissue (colon, $p = 4.11e{-}4$; duodenum, $p = 0.011$). The burden of damaging variation in the TNF signalling pathway was associated with response to anti-TNF drugs ($p = 0.018$); damaging variants were enriched in epigenetic marks from CD8+ ($p = 6.01e{-}4$) and CD4+ ($p = 0.032$) T cells.

Conclusions: Functional rare variants are involved in the response to anti-TNF therapy in CD. Cell-type enrichment analysis suggests that the gut mucosa and CD8+ T cells are the main mediators of this response. These findings provide new insights into the underlying heterogeneity of CD, revealing the basis of TNF-dependent biological mechanisms.

Men (%)	22 (53.7)
Location (%)	
Ileal	14 (34)
Colonic	6 (14.6)
Ileocolonic	17 (41.5)
Behavior (%)	
Inflammatory	21 (51.2)
Stricturing	5 (12)
Fistulizing	11 (26.8)
Perianal disease (%)	8 (19.5)
Extraintestinal manifestations (%)	14 (34)
Previous surgery (%)	17 (41.5)
Smoking habit (%)	16 (39)
Steroids	7 (17)
Immunomodulators (%)	
Thiopurines	30 (73)
Methotrexate	3 (7.3)
Anti-TNF type (%)	
Adalimumab	16 (39)
Infliximab	25 (61)

VARIANT ANNOTATION	VARIANTS*
Start lost	8
Stop gained	2
Stop lost	2
Synonymous	30
Missense	2,590
Splice donor	2
Splice region	89
Structural interaction	50
Long intergenic non-coding RNA	56
Sense intronic	17
Small nuclear RNA	12
3' UTR	479
5' UTR	86
Nonsense-mediated decay	1
Nonstop decay	9

*Number of low-frequency damaging variants showing the indicated annotation. Given that a single genetic variant can impact different genes, the annotated variants outnumber those predicted as damaging

SNP	CHR	COORD	A1	A2	GENE	VARIANT TYPE	ANNOTATION	CD (%)	RESP	NRESP
rs150581659	1	16447908	G	A	*NECAP2*	exonic	stop gained	1 (2.4)	1	0
rs761330653	1	39639623	C	T	*HEYL*	exonic	start lost	1 (2.4)	1	0
rs764641613	1	39639624	A	C	*HEYL*	exonic	start lost	1 (2.4)	1	0
chr2:88173152	2	88173152	T	C	*THNSL2*	exonic	start lost	1 (2.4)	0	1
rs41272317	3	132618633	C	A	*NPHP3-ACAD11*	intronic	splice donor	1 (2.4)	0	1
rs138856042	3	137998869	A	G	*CLDN18*	exonic	start lost	1 (2.4)	1	0
rs151314696	6	31719303	C	T	*LY6G6C*	exonic	start lost	1 (2.4)	1	0
rs61732354	6	42890786	C	T	*C6orf226*	exonic	start lost	2 (4.8)	1	1
rs192561318	11	61920191	A	G	*RAB3IL1*	intronic	start lost	1 (2.4)	1	0
rs79556405	17	29941799	G	A	*EFCAB5*	intronic	start lost	4 (9.7)	2	2

Abbreviations: A1, reference allele; A2, alternative allele that causes the LoF of the protein encoded by the indicated gene; CD, number of CD patients carrying the LoF variant; Chr, chromosome; Coord, SNP coordinates in GRCh38/hg38, Nresp, number of patients that carry the LoF variant and did not show a significant clinical response to anti-TNF therapy; Resp, number of patients that carry the LoF variant and responded to anti-TNF therapy; SNP, single nucleotide polymorphism.

P817

Profiles of somatic mutations in tissue of IBD and IBD-associated carcinomas revealed by a targeted next-generation sequencing (NGS) tumour panel confirm notable differences from sporadic colorectal carcinomas

P. Minarikova*[1], L. Benesova[2], B. Belsanova[2], A. Semyakina[2], M. Kasalicky[3], M. Bortlík[4], M. Lukáš[4], M. Zavoral[1], M. Minarik[1,2]
[1]*Military University Hospital and Charles University, Department of Internal Medicine, First Faculty of Medicine, Prague, Czech Republic,* [2]*Genomac Research Institute, Center for Applied Genomics of Solid Tumours (CEGES), Prague, Czech Republic,* [3]*Military University Hospital and Charles University, Surgical Clinic, Second Faculty of Medicine, Prague, Czech Republic,* [4]*ISCARE, a.s., IBD centre, Prague, Czech Republic*

Background: Inflammatory bowel diseases (IBD) present an increased risk of developing colorectal carcinoma. Neutrophil-released chemicals in the immune response to inflammation causes mutagenesis, and its long-term effects may result in the development of tumour-specific DNA mutations that are the initiators of malignant conversion of intestinal tissue cells. The subsequent molecular changes within the affected gastrointestinal mucosa induce focal changes of the tissue morphology. The molecular mechanisms of this malignant conversion show specific differences from similar mechanisms leading to other types of colorectal carcinoma. The aim of the project is to trace tissue-specific somatic DNA mutations by massively parallel next-generation sequencing using an extensive panel of 50 carcinoma-associated genes (oncogenes and tumour suppressors). Furthermore, the purposes was to compare the resulting profiles obtained from IBD (Crohn's disease, Ulcerative colitis) and IBD-associated carcinomas to those obtained from tissue of sporadic colorectal tumours.
Methods: The group consisted of 25 patients with IBD and 5 patients with sporadic colorectal cancer covering samples from primary tumour, metastases with both MSI and MSS status. For each tumour DNA was extracted from either a biopsy or resected tissue (native or FFPE) and subjected to NGS performed on Illumina MiSeq sequencer using SureSeq™ Solid tumour hybridisation-based enrichment panel (Oxford Gene Technology, Oxfordshire, UK). NGS data were processed by NextGENe sequence analysis suite (Softgenetics, State College, PA).
Results: We mapped the incidence and frequency of major control oncogenic mutations in tissue samples of IBD patients. In general, a difference was observed when comparing mutational spectra among IBD, IBD-associated carcinomas and sporadic carcinomas. As expected, we have confirmed an inverse succession of mutations affecting oncogenes and tumour-suppressors from traditional sporadic pathway. Furthermore, we have revealed a high incidence of somatic mutations of the NOTCH1 and EPAS1 genes, which have previously been shown to be related to their activation and inflammatory processes in the tissue.
Conclusions: Investigation of the presence of specific mutations in inflammatory tissue of IBD patients represents a qualitatively new approach to disease characterisation, including the prediction of the risk of malignant conversion. The study was supported by Czech Ministry of Defense research project MO1012.

P818

Polymorphisms in C1orf106, IL1RN, IL10 are associated with postinduction infliximab trough level in Crohn's disease patients

J. Tang*[1], C. Zhang[2], X. Wang[2], X. Gao[1]
[1]*The Sixth Affiliated Hospital of Sun Yat-sen University, Department of Colorectal Surgery, Guangzhou, China,* [2]*Institute of Clinical Pharmacology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China*

Background: The post induction serum infliximab trough concentration was associated with short-term and long-term response to infliximab (IFX), but it has large interindividual difference.
Methods: The present study investigated the effects of genetic (polymorphisms within FCGR3A, ATG16L1, C1orf106, OSM, OSMR, NF-κB1, IL1RN, IL10) and nongenetic (sex, weight, baseline albumin and combination therapy) factors on IFX therapeutic threshold (3 μg/ml) after 14 weeks-induction therapy.