Project acronym	**IBD-BIOM**
Grant agreement number	305479
Work programme topic	HEALTH.2012.2.4.5-2: Biomarkers and diagnostics for chronic inflammatory diseases of the joints and/or digestive system FP7-HEALTH-2012-INNOVATION-1
Period report	Final
Dissemination level	Public
Co-ordinating group	University of Edinburgh
Lead Investigator	Prof Jack Satsangi
Scientific representative of the co-ordinating group	Mr Nicholas Ventham
Tel	+44 (0) 131 651 1807
Fax	+ 44 (0) 131 651 1085
Project website	www.ibdbiom.eu

Final publishable summary report

1. Executive summary

The IBD-BIOM collaboration has brought together renowned clinical and scientific partners in an attempt to develop biomarkers in inflammatory bowel disease (IBD). The inflammatory bowel diseases, Crohn's disease (CD) and Ulcerative colitis (UC) are a cause of significant debility amongst young people and are becoming increasingly common. The cause of IBD is thought to have a significant genetic (there are 200 genes associated with developing the disease) and environmental contribution.

The main aim of the collaboration has been to develop biological markers using the latest '-omic' technology in genomics, epigenomics, glycomics, glycoproteomics and activomics (see Box 1 for a definition of these terms. A large biobank of retrospective samples (n>1200) was distributed from clinical centres at the outset of the project to develop early putative biomarkers in glycoproteomics, activomics and epigenomics. In parallel, a large cohort of newly diagnosed IBD patients (n=1,394) has been recruited to provide prospective validation of these markers. The recruitment target was exceeded and the consortium has collated a valuable cohort of biological samples with rich phenotypic data. The consortium has now published several high impact articles detailing these discoveries.

An overarching aim has been to develop bioinformatic methodologies to integrate these large multi-omic datasets in an effort to provide models that can accurately diagnose IBD and to stratify patients according to disease progression and prognosis. A further integral strand of the project has been on quality control with successful study on the impact of sample handling together with facilitating scientific centres to obtain ISO9001 certification.

Key findings from the project have been successfully disseminated throughout the project including over 40 presentations at national and international fora to a collective audience of >5000 people. The project website has provided a platform for dissemination to the public and scientific communities (www. Ibdbiom.eu). Strong

Genomics: The branch of molecular biology concerned with the structure, function, evolution, and mapping of genetic variation
Epigenomics: Epigenomics relates to changes occurring around the DNA (without a change in the underlying code) that can affect the way genes are expressed.
Glycomics: Glycomics is about the comprehensive study of the entire complement of sugars, whether free or present in more complex molecules of an organism, including genetic, physiologic, pathologic, and other aspects.
Glycoproteomics: Glycoproteomics is a branch of proteomics that identifies, catalogs, and characterizes proteins containing carbohydrates as a posttranslational modification
Activomics: Activomics is the proteome-wide analysis of enzyme activities that modify the structure and function of other proteins post-translationally by the addition or removal of chemical groups such as phosphate, acetyl, methyl moieties or by proteolysis.

links have been forged between complementary EC funded projects including collaboration and joint symposia with IBD-CHARACTER, HighGlycan and MIMOmics.

2. Summary description of the project context and objectives

Inflammatory bowel disease (IBD) is an important cause of suffering and distress and affects approximately 2.5-3 million people across Europe (Burisch et al *J Crohns Colitis* 2013;**7**:322–37). Improved patient outcomes are achieved by early diagnosis and treatment. IBD is often difficult to diagnose, and current tests can be invasive and cause radiation exposure. The development of novel, sensitive and specific biomarkers to both diagnose and stratify patients according to risk of severity of disease and treatment response will be a major development. Additionally, the development of biomarkers may provide critical insights into disease pathogenesis.

IBD-BIOM is a multidisciplinary consortium of leading academic and industrial SME researchers in inflammatory bowel disease, genomics, epigenomics, glycomics, glycoproteomics and activomics.

Objectives of the study

The development of a robust biomarker for clinical application in the diagnosis and stratification of IBD patients by;

- Complex phenotyping of IBD patients
- Stratification of patients with IBD, in terms of disease course and response to treatment
- Integration of genomic, epigenomic, immunological, glycomic and activomic data
- To elucidate molecular targets for new therapies for IBD
- To gain insight into disease pathogenesis

3. Main Science &Technology results/foregrounds

Retrospective samples have allowed for early generation of data: biomarker discovery.
A significant advantage of the IBD-BIOM study design is retrospective study arm in parallel with prospective recruitment of newly diagnosed IBD patients. The retrospective arm of the study was a major strength and allowed early generation of data from a large number of high quality, well-phenotyped biobanked patient samples (n > 6500). This retrospective samples now complete allowing the development of putative biomarkers from the outset.

Glycoproteomics an important focus of the IBD-BIOM project, and provides a good illustration of the study design and progress of the project. An early authoritative review was published on glycosylation in IBD, thereby setting the context for future work. (Theodoratou et al. 2014 Nat Rev Gastro Hep 11 (10);588-600). The first significant output of the consortium was the largest project of its kind using retrospective serum samples to analyse the circulating IgG glycome in IBD patients and controls. Statistically significant differences in the IgG glycan structures in IBD patients compared with controls, specifically in galactosylation and sialylation and glycan traits were used to discrimintate IBD patients from controls (Figure 1, Trbojević Akmačić et al, IBD 2015: 21(6); 1237-47).

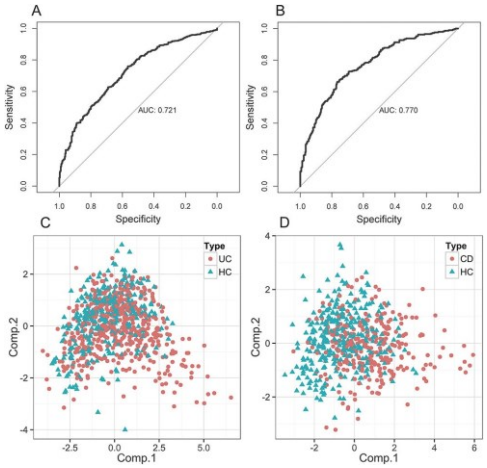


Figure 1– Receive operator characteristic curves and principal component plots demonstrating performance of glycan trait-based models in discriminating disease status in Crohn's disease (CD, A) and Ulcerative colitis (UC, B).

These data have now been replicated using orthogonal techniques (Mass Spectrometry and Ultra-Performance Liquid Chromatography) in two scientific centres (Genos and LUMC) using samples from two clinical centres (USA & Italy).

Associations of IBD with the total plasma *N*-glycome (TPNG) of 3631 patients and healthy controls from two cohorts were established by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) measurements. New associations were found with age and sex due to the use of a newly developed method differentiating the sialic acids linkages. Strong associations were found for all glycans classes (high mannose, hybrid and complexe-type) when comparing IBD patients to healthy controls and some significant associations were found to differentiate CD and UC. Common inflammation and IBD markers found for IgG glycans were replicated in the plasma glycome analysis. Association with disease location and medications were found resulting in several leads for IBD biomarker development.

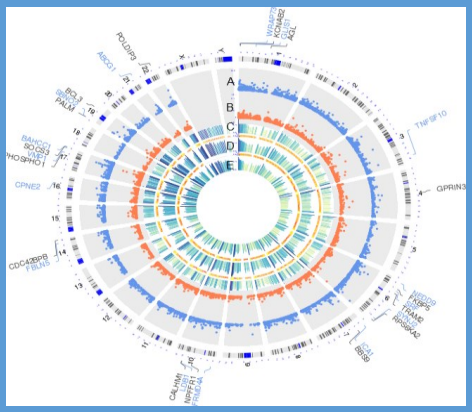
To reflect bowel inflammation of IBD patients, the route of IgA glycosylation was explored as potential plasma proxy for IBD. A new Liquid chromatography–Mass spectrometry (LC-MS) method was developed allowing the identification of four *N*-glycosylation sites as well as an *O*-glycosylated region. Previously reported associations of IBD and IgA glycans (**Inoue *et.al.* 2012**) were not replicated in our study. The newly found associations between IBD and IgA glycans will be replicated in a larger sample set, beyond the time-course of this project.

EPIGENETICS IN IBD-BIOM

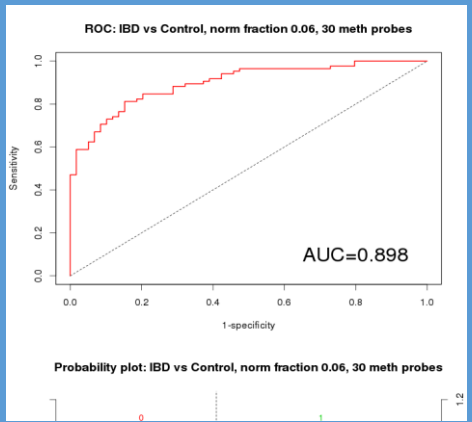
One epigenetic modification, DNA methylation, has been studies extensively within the IBD-BIOM project.

Methylation WAS studies across the genome using microarray technology in a large number of newly diagnosed patients and controls (Ventham et al Nature Comms 2017 [in press]). These data were related to the underlying genotype and gene expression. Moreover, these epigenetic modifications are cell-specific and DNA methylation was studied in isolated blood cell populations (CD4+ & CD*+ T-lymphocytes and CD14+ monocytes).

Significant differences were found in DNA methylation patterns in IBD patients compared with controls. The IBD-BIOM project successfully collaborated with another EU FP7 grant IBD-CHARACTER (www.ibd-character.eu) to use an advanced technique (whole genome bisulphite sequencing) to further characterise these areas of differential methylation.



Differences in DNA methylation were used to translate these data into clinically useful biomarkers. A 30-probe DNA methylation marker panel was accurately able to discriminate IBD cases from controls (figure below), and further work was performed to use methylation data to prognosticate on IBD disease severity and progression.



Methodological and quality control aspects are a strong focus of the study. A methodological analysis of serum N-glycome variability caused by different serum processing methods has also been published (Ventham et al Plos One 2015: 10 (4); e0123028) and will helped to inform subsequent analyses within the project

Glycoproteomic analysis has also been performed in paraffin-embedded gut tissue (Hinneburg H, Methods Mol Bol 2017;1503:131-145) by MPI (Germany). Subsequent work on clinical material provided by clinical partners provides an atlas of O- and N-glycan expression according to anatomical location in the gut, as well has ongoing work to identify differences in IBD cases and controls.

This approach has delivered results with publications from the IBD-BIOM consortium in high impact journals (Box). These novel putative biomarkers have also been tested in the large number of prospectively recruited patient samples.

The project underwent an Ethics review that was facilitated by Professor Sarah Cunningham-Burley, who is the Dean of Molecular, Genetic and Population Health Sciences, University of Edinburgh.

Prospective patient recruitment

In parallel with the ongoing scientific biomarker discovery, prospective recruitment has been successfully completed. The target of 1,200 patients specified in the grant agreement has been met and exceeded (n=1395). Recruitment methodology has been refined and developed throughout the first and second reporting periods, with patients being recruited within 3 months of diagnosis. An additional clinical partner, Maastricht University Medical Centre (UM) has joined the existing three clinical centres (Edinburgh (UK), Cedars-Sinai (US) & UHC Florence (It)) to assist in the successful attainment of the prospective recruitment target. Serial sampling in a subset of patients will allow detailed intra-individual characterisation of potential biomarkers over time, and may assist in relating changes in biomarker activity to changes in disease activity or in response to therapy.

A focus on quality for biomarker discovery systems

Another major strength of the consortium has been a focus on rigorous quality control on biomarker development. Several of the analytical techniques have been tested using different sample processing methods and these results have been published. In development of biomarker discover systems (BDAS), Ludger have performed laboratory visits to scientific SMEs in an attempt assist attainment of ISO9001 certification.

Disseminating the output of IBD-BIOM

Critical to the success of the IBD-BIOM is the communication of the progress and results to clinicians, pharma, the wider scientific community and most importantly the patients and their families. The main interface for achieving this goal has been the IBD-BIOM website (www.ibdbiom.eu, Figure 1) launched during the first period and continually updated during the second reporting period. IBD-BIOM has also been publicised amongst the scientific community with over 40 presentations at national and international forums (to a collective audience of >5000 people). The IBD-BIOM project has promoted synergy with allied EU FP7 projects with a joint meeting and open symposia held with IBD character (www.ibdcharacter.eu) and high glycan projects.

Figure 1 - Screenshot from the IBD-BIOM website (www.ibdbiom.eu)

4. Potential impact main dissemination activities and exploitation of results

This study has generated a large amount of phenotypic, genomic, epigenomic, glycomic and activomic data. Integrative analyses, to help use information gained from scientific analyses to predict patient disease course and treatment response has been performed with promising results. Preliminary models integrating genomic, microbiota, serological and glycomic have been developed by bioinformaticians at Cedars-Sinai Medical Centre, USA.

IBD-BIOM members took part in an EU consortia session at the largest IBD-focused international conference in 2016 (ECCO Amsterdam 16/3/2016). This was an excellent opportunity to discuss existing data, promote discussion, forge new collaborations and develop follow-on studies. (https://www.ecco-ibd.eu/ecco16).

5. Address of the project public website and contact details

Address of the project public website: www.ibdbiom.eu

Contact details	
Co-ordinating group	University of Edinburgh
Lead Investigator	Prof Jack Satsangi
Scientific representative	Mr Nicholas Ventham
Project administrator	Mrs Stephanie Scott
Tel	+44 (0) 131 651 1807
Fax	+ 44 (0) 131 651 1085