**Systems Biology in Inflammatory Bowel Diseases: Ready for Prime Time**

Christos Polytarchou[#], Georgios Koukos[#], Dimitrios Iliopoulos*

*Center for Systems Biomedicine, Division of Digestive Diseases, David Geffen School of Medicine, UCLA, Los Angeles, CA;*

[#]these authors contributed equally to this work

***Corresponding author:** Dimitrios Iliopoulos, Ph.D., Center for Systems Biomedicine, Division of Digestive Diseases, David Geffen School of Medicine, University of California at Los Angeles, 650 Charles E. Young Dr. South, CHS 44-133, Los Angeles, CA 90095-7278. Tel: 310-825-8856; E-mail: diliopoulos@mednet.ucla.edu.

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**ABSTRACT**

**Purpose of review:** Ulcerative colitis (UC) and Crohn's Disease (CD) are the two predominant types of inflammatory bowel disease (IBD), affecting over 1.4 million individuals in the US. IBD results from complex interactions between pathogenic components, including genetic and epigenetic factors, the immune response and the microbiome through an unknown sequence of events. The purpose of this review is to describe a system biology approach to IBD as a novel and exciting methodology aiming at developing novel IBD therapeutics based on the integration of molecular and cellular "omics" data.

**Recent Findings:** Recent evidence suggested the presence of genetic, epigenetic, transcriptomic, proteomic and metabolomic alterations in IBD patients. Furthermore, several studies have shown that different cell types, including fibroblasts, epithelial, immune and endothelial cells together with the intestinal microbiota are involved in IBD pathogenesis. Novel computational methodologies have been developed aiming to integrate high-throughput molecular data.

**Summary:** A systems biology approach could potentially identify the central regulators (hubs) in the IBD interactome and improve our understanding of the molecular mechanisms involved in IBD pathogenesis. The future IBD therapeutics should be developed on the basis of targeting the central hubs in the IBD network.

**Keywords:** systems biology, IBD, network, high throughput analysis, data integration

## Introduction

IBD is a multifactorial disease with several components contributing to its pathogenesis[1]. Alterations in the gut microbiota[2-5], activation of different immune cell types[6-8], changes in the vascular endothelium[9, 10] and alterations in the structure of tight junctions in colonocytes[11-13] perturb the gut cellular interactome resulting in IBD development. Each cell population in the gut has its own complex molecular interactions (interactome), consisting of genetic, epigenetic, transcriptional, protein and secreted factors. The isolated modulation of one component in the cellular and molecular gut interactomes might be insufficient to provide an effective therapeutic solution for IBD patients. A major problem that researchers in the IBD field face is how to integrate the continuously generated data derived from novel technologies and collectively characterize and quantitate the roles, relationships and actions of various types of molecules, referred to as "omics", and develop novel therapeutics that would target these interactomes instead of a specific signaling pathway or cellular population. In the last five years, the explosion in the development of novel computational and high-throughput technologies[14, 15] has enabled us to study different human diseases in a genome-wide level. Here, we will discuss how these technologies, through integration of high throughput cellular, molecular and clinical data, could contribute to our understanding of IBD pathogenesis and the development of novel drugs for IBD patients.

## Systems Approach in IBD

A system is an entity that maintains its existence through the interactions of its parts. A systems approach consists of four major steps: a) identify the parts of the system; b) characterize the properties of the parts; c) identify the interaction between the parts; and d) evaluate the interactions between the parts and the environment[16]. A systems approach can be applied in IBD, aiming at identifying and understanding the complex interactions between different cell types (parts) in the gut (system) and determine whether these interactions are deregulated during IBD pathogenesis. The development of high throughput technologies to analyze the entire set of genes (genome), epigenetic modifications (epigenome) and transcriptional regulation (transcriptome), and the identification of novel algorithms and software able to integrate the "omics" data enabled the genesis of the systems biology field. During IBD development there are perturbations of the molecular and cellular gut interactomes. In this review, we will present the state of the art high throughput technologies that are available to study the IBD interactomes and describe novel computational tools able to integrate the "omics" information. The systems biology strategy is very promising aiming to expedite the drug discovery process in IBD.

## Molecular Systems Approach in IBD

Previous studies have revealed the significance of genetic variations in IBD pathogenesis by performing large genome-wide association studies (GWAS)[17]. However, the genetic variants that have been associated with UC and CD can only explain the 20-25% of all IBD cases, suggesting the presence of additional contributing factors[18]. One of the striking aspects of IBD is its substantially increased incidence during the last decades, suggesting the potential involvement of environmental (epigenetic) factors in IBD pathogenesis[19]. Moreover, the completion of the ENCODE project last year[20] identified >100,000 novel transcripts that may be involved in IBD development. To build and characterize the IBD molecular interactome, we should first characterize its components (**Figure 1**) and then identify their complex interactions, by performing computational analysis.

### A. Characterization of the IBD Genome

Genomics is defined as the study of genetic alterations in the genome-wide level. GWAS has been performed in large numbers of IBD patients, resulting in the identification of novel variants[21]. Recently an assay, called ImmunoChip, has been developed including 200,000 single nucleotide

polymorphisms (SNPs) relevant to IBD and other immune-mediated diseases[22]. Studies of SNPs and insertion-deletion polymorphisms identified a total of 163 loci associated with IBD and revealed important pathways involved in IBD pathogenesis such as host-microbe interactions and autophagy[5, 21]. These studies identified genomic regions that influence the risk of disease but cannot on their own determine specific causative roles. In fact, from the 163 loci only a handful has shown functional IBD-associated SNPs[23]. Exome sequencing analysis is informative for the whole spectrum of variation within the protein coding sequence of genes and carries the potential of identifying the missing heritability in complex diseases such as IBD. Recently, whole exome sequencing (the sequencing of the complete coding regions of the genome) applied in CD and pediatric IBD patients revealed novel low-frequency variants in known IBD genes[24, 25]. Although all these approaches have contributed significantly to our knowledge of genetic alterations in IBD patients, up to date there is no study of sequencing the whole genome of IBD patients. This analysis will provide a comprehensive view of genetic alterations in IBD patients and potentially will identify novel SNPs and mutations in the genomes of IBD patients.

**B. Studying the IBD Epigenome**

In addition to the genomic analysis, modifications could occur in the epigenetic level, affecting gene expression. Epigenetics is defined as cellular information that is heritable during cell division but is not encoded in the sequence of the genome. Pathologic epigenetic changes, such as DNA methylation and histone modifications are increasingly considered as alternatives to mutations and chromosomal alterations in altering gene function[26]. ***a) IBD DNA Methylome***: The increasing interest of the role of DNA methylation in IBD pathogenesis coincides with advances in platform-based DNA methylation array technologies. Employment of the Illumina 27K chip to analyze peripheral blood samples revealed fifty genes that were differentially methylated between CD patients with CD and controls[27,28]. Furthermore, another study in 20 monozygotic twins discordant for UC identified 61 differentially methylated loci, including genes involved in inflammation[29]. However, none of these array technologies are able to study the DNA methylome at a genome-wide level. On the other hand, the DNA methylation-sequencing methodology will reveal the global DNA methylation alterations in IBD patients. A recently developed technology, called reduced representation bisulfite sequencing (RRBS)[30], requires low RNA amounts (10ng), even from paraffin-embedded samples, and is highly applicable to a wide range of IBD clinical samples. ***b) IBD Chromatin State:*** In addition to DNA methylation, histone modifications play a critical role in gene regulation by affecting chromatin packaging. A recent study revealed that cytokines could induce chromatin modifications in COL1A2 gene in intestinal mucosal endothelial cells[31]. Global histone modifications have been extensively studied in cancer and neurodegenerative diseases but despite evidence on their role in inflammation, their genome-wide distribution and function in IBD is still unknown. To follow a systems biology approach, it is essential to perform chromatin immunoprecipitation-sequencing (ChIP-seq) analysis for active and suppressive chromatin marks in tissues and blood from IBD patients.

**C. Evaluation of the IBD Transcriptome** The genome-wide expression catalogues the complete set of RNA transcripts, called transcriptome, produced by the genome, including coding and non-coding RNAs. cDNA microarray has been the main tool used to profile the global gene expression in IBD tissues or blood. Previous studies have identified a gene signature in UC patients that correlates with disease activity[32]. In addition to the coding genes, recent evidence suggested the presence of non-coding RNAs in the human genome, including microRNAs and large intergenic non-coding RNAs (lincRNAs)[20]. By employing microRNA expression arrays and use of novel microRNA high-throughput profiling platforms, such as Nanostring[33], microRNAs such as miR-23a, miR-21, miR-29a, miR-124, and miR-192 have been found to be deregulated in colonic tissues and blood from IBD patients, and investigation of their mechanistic implications is currently underway[34-37]. To date there is no study regarding the potential role of lincRNAs in IBD

pathogenesis. Future studies should perform integrated expression analysis of coding and non-coding RNAs in IBD patients by using the RNA-sequencing technology[38]. This technology will reveal all the transcript variants that are potentially involved in IBD pathogenesis.

**D. Revealing the IBD Proteome**

Proteome is the complete set of expressed proteins, including their different isoforms and modifications. The proteome corresponds to the structure and function of proteins and their higher-order complexes as well as their localization and translocation[39-40]. A wide selection of proteomic approaches has been applied to characterize IBD pathogenesis by investigating the dynamic nature of the proteome[41], including surface-enhanced laser desorption ionization (SELDI) or matrix-assisted laser desorption (MALDI)-time of flight (TOF) mass spectrometry (MS) and liquid chromatography (LC/MS) combined with two-dimensional gel electrophoresis. Such proteomic studies have been performed for IBD using epithelial cell lines[42-44], epithelial cells from colonic biopsies [45-47] and serum samples[48-51]. Currently, the greatest interest in proteomic applications lays in identifying biomarkers in biofluids (such as serum and urine) or tissue samples (such as colonic biopsies)[41] that are specific for UC and CD diseases and correlate with disease activity and other clinicopathological parameters.

**E. Constructing the IBD Metabolome**

The metabolome encompasses all the small molecules chemically transformed during cellular metabolism and provide a readout of cellular biochemistry[52, 53]. The analytical techniques used to study the metabolome, include gas chromatography (GC), liquid chromatography (LC) and high/ultra-performance liquid chromatography (H/UPLC) followed by mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR)[54]. Different metabolomic approaches have been applied in human biomaterials, including fecal water extracts, blood serum and urine samples, aiming to discriminate IBD patients from control subjects[55-62]. Furthermore, $^{1}$H NMR spectroscopy and LC-MS have revealed distinct metabolic profiles between different intestinal compartments[63]. These data are very promising, suggesting the deregulation of the metabolome during IBD pathogenesis. Additional studies should determine the significance of these findings in large cohorts of IBD patients, aiming to develop prognostic and diagnostic tests in the near future. It is essential to integrate the proteomic and metabolomics data from IBD patients in order to fully characterize the biochemical map and its alterations during IBD development.

**Integration of IBD "Omics" Data**

Although there is a continuous generation of molecular "omics" data from biomaterials derived from IBD patients, there is a major challenge on how to analyze and integrate these data in order to identify the central regulators of IBD pathogenesis. The network analysis could contribute to the construction of the IBD molecular interactome[64]. The network theory is a branch of applied mathematics that uses the concepts of graph theory[65, 66]. The development of the network theory was led by applications to the real-world examples, such as the social networks and technological (internet) networks. The structure of complex molecular interactions in IBD can be represented by networks and not by linear signaling pathways, a methodology followed by most researchers today. A network could reveal the positive and negative feedback loops and information exchange between the different signaling pathways. Overlooking the loop circuits led to several failures of drugs developed by pharmaceutical companies[67]. In an IBD network, nodes could correspond to genes, proteins or metabolites, while the edges will represent the interactions, causal influences or correlations between them (**Figure 2**). To detect the central molecular regulators in IBD pathogenesis, the IBD network could be compared to random graphs with defined statistical properties[68]. We can build networks based on the specific characteristics described above. For example, we can build an IBD metabolic network of the metabolites deregulated in IBD patients and the chemical reactions that connect these metabolites. An IBD

transcriptional network can be constructed by identifying transcriptional interactions between deregulated coding and non-coding RNAs. A protein network will include the deregulated protein-protein interactions in specific cell types. The next step would be to integrate all these networks aiming to construct the IBD molecular interactome.

**IBD Network Construction and Visualization**.

An important question is how practically could we construct and visualize these IBD molecular networks? The first step would be the generation of the "omics" data from IBD patient biomaterials. Next, the dynamics and regulatory patterns of the potential gene, protein and metabolite interactions should be described by a mathematical graph. The graph could be constructed using Boolean network analysis, Bayesian network analysis, ordinary and partial differential equation systems or stochastic processes[69-73]. A graph consists of a discrete set of nodes (N) and edges (E), which are defined as pairs of nodes (**Figure 2**). The nodes could be genes, proteins or metabolites deregulated in IBD and the edges will show direct or indirect interactions between the nodes. Each network will be characterized by its statistical properties[74]. The node that shares an edge with another node is called neighbor and the number of neighbors is called the degree or node size (k). A distinction between in-degrees ($k^{in}$) and out-degrees ($k^{out}$) refers to incoming and outgoing edges, respectively. The gene, protein or metabolite with the high number of incoming and outgoing edges is called central regulator or hub[75]. The hubs identified by the network analysis could be used as IBD drug targets since they will central regulators of the IBD networks.

In the last few years multiple software programs have been developed for systems biology purposes. A visit to the software guide at www.sbml.org website will reveal more than 100 different softwares developed for network and modeling analyses. JSIM is a Java-based software able to building IBD quantitative networks and can be used from a web browser[76]. In addition, the CellDesigner is another Java-based tool that could show visually appealing graphical representations of the IBD networks[77]. Furthermore, E-Cell is a Python-based software able to model, simulate and analyze large-scale IBD networks and systems[78]. These tools require high knowledge of mathematics and computer programming, revealing the importance of integrating computational biologists in IBD research. In addition to computational tools, there are commercially available tools that do not require computing knowledge. A user-friendly software, called Ingenuity Pathway Analysis (IPA), constructs molecular networks based on experimental and literature-based data[79]. These computational tools can integrate molecular "omics" data into networks for each of the cellular populations involved in IBD pathogenesis.

**Cellular Systems Approach in IBD**

Gut physiology is maintained through balance between the epithelial, immune, endothelial cells, fibroblasts and microbes. Mucosal immunity is an important component of IBD pathogenesis, however, it is only a part of the IBD cellular interactome. Gut microbiota can affect the function of regulatory T cells in the gut, contributing to IBD pathogenesis, while the proportion of the different luminal bacterial species changes significantly in IBD[80, 81]. Investigating the changes in the number and the identity of the microbiome in IBD patients has revealed distinct patterns that could be linked to disease pathogenesis[82, 83]. Lately, studies have focused on the functional consequences of IBD-associated dysbiosis. Using a novel technique to isolate the microbes and the proteins[84] or the metabolites[85] from specific areas of the colon, investigators have shown an interdependence and a bi-directional influence of certain bacteria populations with specific pathways, thus identifying potentially important host–microbe interactions in IBD pathogenesis.

Recent evidence showed the importance of vascular and lymphatic endothelium in IBD pathogenesis. Blocking the intestinal angiogenesis has a beneficial effect in experimental colitis[86], while inhibition of a major lymphangiogenic receptor exacerbates colonic inflammation[87]. Novel studies contribute to our understanding on the role of colonic epithelial cells and tight

junction structure and function in IBD pathogenesis[88-90]. Thus, it is essential to continue characterizing these cellular populations and identify their molecular links, aiming at constructing the IBD cellular interactome. The construction of the IBD cellular interactome should follow the same principles described for the IBD molecular interactome. This will be realized by integrating the molecular networks in each of the gut components (cells, microbes) and construct a larger network which will reveal interactions between these different components. The nodes in this network that link two different gut components would reveal how a perturbation in one component would affect the dynamics and properties of another component.

**IBD Drug Discovery and Systems Approach**

Although different cells and microbes are involved in IBD pathogenesis, modulation of one of these components alone may not lead to an IBD cure. The mucosal immune system has been the focus of multiple therapeutic interventions in IBD, however the degree of success varies[18]. Alterations of the gut microbiota by probiotics have been used in IBD studies, but the benefits are variable and, more importantly, not permanent. Thus, it is becoming more evident that manipulation of a specific cellular or microbial population in the gut may not lead to an IBD cure. Since the different cellular and microbial components are parts of the IBD cellular interactome, identification of drugs perturbing the IBD cellular interactome using systems biology approaches may have a greater therapeutic potential. We propose to develop an IBD drug-molecular interactome map in the near future, based on the connectivity map platform[91]. This software tool will merge drug response hub signatures with the IBD interactome data, linking for the first time chemical compounds to IBD molecular networks. This methodology could potentially identify novel and highly specific IBD drug targets that could expedite the IBD drug development process.

**Conclusion**

Taken together, it is essential in the near future to establish an IBD Interactome Consortium through collaboration between top IBD research centers in various countries. This consortium should generate extensive “omics” knowledge from biomaterials (tissue, blood, urine, stool) derived from a well-characterized IBD cohort of patients, aiming to construct the IBD interactome in the near future, expediting the development of novel drugs for IBD patients through targeting the essential networks in this interactome. We should take advantage of the exciting discoveries in biomedical technology field and transform them in novel therapeutics for IBD patients.

**Key Points**

- Integration of molecular and cellular “omics” is needed for a systems approach in IBD.
- Development of novel therapeutics by targeting the central hubs of the IBD network.
- To build IBD-interactome we should characterize its components and identify their interactions.
- The drug-molecular interactome map will link chemical compounds to IBD molecular networks.

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** This study includes integrated "omics" data (metabolomic and microbiome data).

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**Figures**

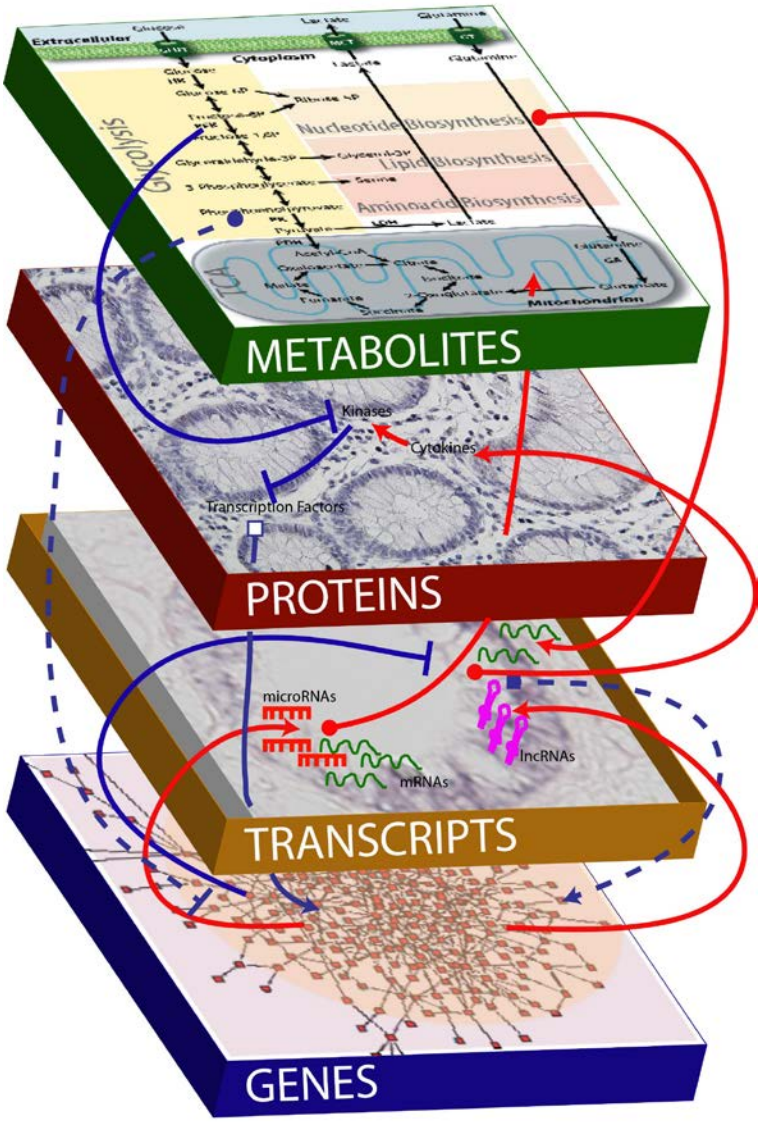


**Figure 1. Components of the molecular interactome**

The molecular interactome consists of alterations at the genomic, transcriptomic, proteomic and secretomic level. In this interactome there are coding genes translated into proteins and non-coding genes, such as microRNAs and lincRNAs, functioning as RNA molecules in the cell. There are multiple complex interactions between transcripts, protein complexes and metabolites.

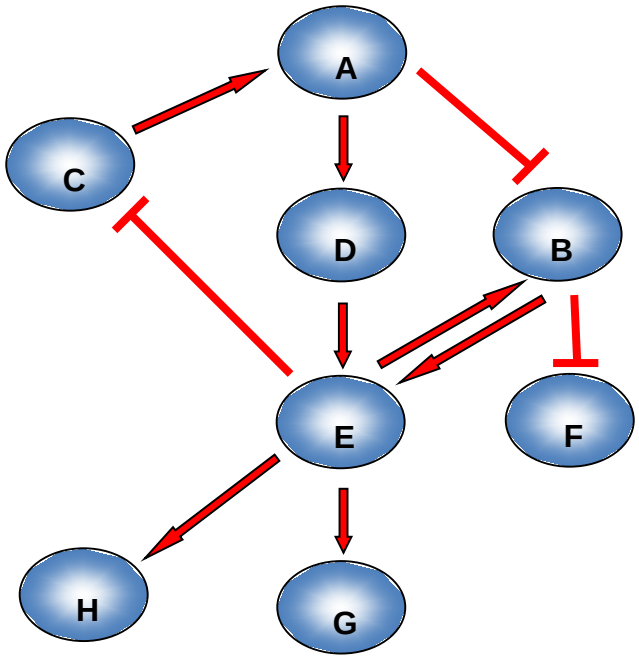


**Figure 2. Structure and graphical representation of a network**

A graph representing a network of nodes (A-H) and edges (arrows and lines). There are 8 nodes and 11 edges. The nodes could be genes, RNAs, proteins and metabolites while the edges would represent their interactions. Network analysis could reveal the central regulator of the network. Manipulation in the expression levels of the central regulator would affect the whole network. In this graph, node E has the highest number of edges, a total of seven, and can be called the central regulator or the hub of this specific network.