Multiomics Analyses to Deliver the Most Effective Treatment to Every Patient With Inflammatory Bowel Disease

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For many chronic, complex human diseases like inflammatory bowel disease (IBD), there are limited treatment options. Although the 21st century has seen remarkable advances in our understanding of the biology of these disorders, translation to new medicines has been slow. More than 90% of new drug candidates ultimately fail the required registration trials. Nevertheless, IBD presents a unique opportunity to build personalized therapies for a complex, chronic disease: >250 genetic associations have been discovered, diseased tissues and cells are readily accessible through routine clinical care (blood draws, stool samples, and endoscopy with biopsies), and it is a classic example of the role of the gut microbiome in disease.[1,2] Using these opportunities to better understand the molecular biology of IBD may enable more targeted use of the increasing range of partially effective therapies that already exist.

Multiple research groups and consortia throughout the world are currently generating large datasets comprising extensive clinical data combined with genome, transcriptome (often tissue and cell specific), methylome, proteome, metabolome, or gut microbiome data. Although all these datasets are able to answer important questions individually, an integrative worldwide collaborative effort is needed to leverage all the data and knowledge and address 2 key questions:

1. How can the rapid pace of large-scale data generated in IBD biology be harnessed to prioritize the best new targets for early stage drug development or microbiome directed interventional strategies?
2. How can we discover clinical biomarkers that capture the biological heterogeneity of IBD and hence deliver an optimized, personalized treatment approach?

In April 2018, the Royal Netherlands Academy of Arts and Sciences held an Academy Colloquium on "Delivering the most effective treatment to every patient with inflammatory bowel disease" in Amsterdam, the Netherlands. This colloquium brought together 33 clinical and basic scientists with expertise in human genetics and genomics, clinical gastroenterology, microbiome analysis, and drug discovery as well as representatives of IBD patient organizations. The program was centered around 5 topics: genetics, microbiome, immunology, biomarkers, and drug development. Forward-looking views on possible new data, experiments, and collaborations that could push forward IBD research were discussed.

Genetics

After a decade of successful genome-wide association studies, the major challenge has turned from the identification of associations to dissecting their causal variants, genes, and mechanisms. Two main approaches are proposed to tackle this challenge. First, regulatory target analysis is used to identify the cell- or tissue-specific mechanisms of action for common regulatory variants. This method requires bringing together public resources, such as the ENCODE project, as well as generating data on which genes are expressed and enhancers active in relevant IBD tissues. Making connections between variants and target genes also requires considering a broader set of transcriptional consequences of genetic variation. For example, 1 IBD risk variant is not associated with changes of overall expression of any gene, but is associated with transcript isoform usage of the gene *ADAM15*, which would have been missed with earlier approaches. Building both the types of data and analysis techniques for regulatory target analysis will improve our ability to understand IBD biology.

Second, the rapid increase in exome and genome sequencing in IBD offers an opportunity: If very rare, damaging variants (usually affecting protein sequence) can be identified, they might offer insight into the functional implication of associated genes. The previously published example of *CARD9* in which functional follow-up of a protective variant aids in the identification of a small molecule

Abbreviations used in this paper: **FMT, fecal microbiota transplantation; GWAS, Genome Wide Association Study; HMP, Human Microbiome Project; IBD, inflammatory bowel disease.**

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mimicking its effect offers a nice illustration of how this approach might be applied.[3] Interpreting the wealth of new sequence data can also benefit from regulatory target analysis because in combination these 2 types of data can reveal allelic series, where multiple variants affecting the same gene with different degrees of severity (weak regulation vs loss of function, for example) can provide strong support for an overall hypothesis. These sequence datasets might also allow the identification of additional monogenic forms of IBD, although the discussion noted that such cases had so far been relatively rare, and even severe forms (such as very early onset) were often largely polygenic in nature.

Both of these concepts will rely on the same foundation that served the genome-wide association studies era so well: large, international collaborations and data sharing. Because even larger sample numbers will be required for rare variant analyses, developing a mechanism for joint analyses of sequence datasets will be crucial. Early progress was made in agreeing how such sharing would happen, and it was stressed that other interested parties with valuable data who would be interested in participating in joint analyses are encouraged to contact the consortium.

The Gut Microbiome

Key Challenges in gut microbiome studies in IBD were identified.

Reproducibility and Standardization

Within the microbiome community, efforts are being made for standardization of sampling, storage, and DNA extraction methods. Although it is becoming clear how different protocols behave, this does not necessarily always mean that one can compare or combine them.

High Interpersonal Heterogeneity

Most microbiome changes seem to occur early in the disease course and may be both causal and responsive. There is a huge amount of interindividual heterogeneity and (in the HMP2 data) little influence on disease progression, behavior, and disease activity measurements.[4] This also makes model organism or systems biology studies more complicated.

The Role of Environmental Factors (Exposome) and Confounders

It is increasingly becoming clear that the influence of environmental factors such as diet and commonly used IBD drugs have an influence on the gut microbiome and that these confounding factors should be incorporated in all analyses.

Temporal Variation

Longitudinal data are still scarce in IBD. It is unclear what the frequency of sampling should be, but sampling every 3 months seems to be a minimal requirement, depending on the question that need to be addressed.

Cohort Sizes and Statistical Power

In contrast with genetic studies, where large well-powered cohorts are needed to find associated genes, in the IBD microbiome community this need for, as an example, meta-analyses to identify more IBD associated bacteria is less imminent. There is, however, a need for well-powered longitudinal studies in specific contexts (eg, fecal microbiota transplantation [FMT]) or specific IBD phenotypes.

In addition key areas where to focus research efforts in IBD microbiome studies were identified.

Understanding the Results of Fecal Microbiota Transplants in IBD

FMT has been shown to be successful in $\geq$3 randomized, controlled trials in patients with ulcerative colitis, whereas results in those with Crohn's disease are inconsistent. Engraftment seems to be host and donor specific, and there is a need for consensus on isolation and manufacturing methods, donor and recipient status, mode of delivery, dosage and frequency of administration, and pretreatment of patients. Understanding successful mechanisms in FMT will also allow us to understand potential causative roles of specific species or strains.

How Can We Induce Beneficial Shifts by Other Means Than FMT?

Should there be a focus on removing potential pathobionts and/or do we need to add potential beneficial species? A combined strategy including targeted immunomodulation is likely to be beneficial.

Can the Gut Microbiome Be Used for Treatment Response Prediction in IBD?

Because it is becoming clear that the gut microbiome plays a role in drug efficacy with the potential benefit of modulating it before treatment, this factor needs to be further explored in the context of IBD treatment.

What Dark Matter Are We Missing? (Also in the Context of FMT Efficacy)

Further research is needed into the virome, uncharacterized sequences, unlabeled metabolites, fungi, and so on. Although fungi are low in number, they are 100-fold larger than typical microbial cells and make up a significant biomass within the gut with unique metabolic functions. Better reference databases for viruses, phages, and fungi are needed.

Mechanistic Studies

Mechanistic studies—both in vitro screens and mouse studies—are needed to study the functional properties of

specific IBD-associated species (or specific strains) and microbiome-derived metabolic compounds.

Immunology

An overview of how genetic findings have been translated mechanistically into biological explanations and how these variants could contribute to aberrant immunologic responses and disease development was given. It is acknowledged that translating genetic findings into pathogenic mechanisms is a difficult process and each (group of) gene(s) would require a deep functional assessment by using different sets of laboratory techniques to study each gene properly. Given the nature and scale of this work, it is expected that in-depth analyses of IBD risk genes can be done on only a limited number of the >250 IBD risk genes.

The Human Functional Genomics Project (http://www.humanfunctionalgenomics.org) is a large-scale project making use of a systems biology approach to study the consequences of genetic variation and gut microbial features on the immune responses in healthy individuals and in specific disease cohorts. The Human Functional Genomics already identified both genetic variants as well as microbial features that influence cytokine production after different stimuli.[5] The integration of genetic, transcriptomic, and metabolomic information in combination with immune data has the potential to uncover novel disease-associated pathways and potential therapeutic targets. Although there is a lot to be learned from healthy controls, the Human Functional Genomics approach has now been adopted by groups studying specific diseases like human immunodeficiency virus or rheumatoid arthritis and it was acknowledged that IBD should be included.

Biomarkers

An overview of the current and potential future role of biomarkers in IBD was given. Although clinical parameters in combination with, for example, C-reactive protein levels, serology, and fecal calprotectin, are used to follow-up on disease activity, there is a great need for biomarker development in IBD especially toward prediction of disease and therapy outcome.[6] Recent advances in genetic prediction of, for example, anti-tumor necrosis factor response and thiopurine-induced myelosuppression based on large-scale genome-wide association studies or whole exome sequencing studies show great promise for clinical implementation.[7] An integrated multiomics data-driven framework focused on clearly defined clinical questions provides a great opportunity to identify biomarkers with clinical applicability. These biomarkers have to be reliable, reproducible, easy to assess, and easy to interpret by clinicians in daily practice.

Three key areas in biomarker development were identified: (1) disease susceptibility and disease behavior, (2) prediction of drug response and drug toxicity, and (3) molecular reclassification of IBD subphenotypes.

The following challenges to advance potential biomarkers identified by omics research toward clinical practice were discussed.

Focus on Specific Phenotypes or Unmet Needs With Clearly Defined Endpoints in Prospective Well-Phenotyped Standardized Cohorts Across Centers

This point asks for collaborative efforts across multiple centers, adopting standardization of all steps in the process of biomarker discovery.

More Intensified Academia–Industry Partnerships

Pharmaceutical companies have been reluctant to support biomarker discovery partly because of the fear of fragmenting the market, but also owing to a lack of samples or institutional review board approval. With more therapeutic agents becoming available for IBD patients, pharma is increasingly recognizing the need to identify patients that would benefit most from their products. Second, data from controlled and standardized, randomized, controlled studies are ideal to perform "omics" analyses to identify biomarkers for drug response or toxicity as examples. The hope was expressed that regulatory agencies would also support academia–industry collaborative efforts to address these questions. Last, validation, valorization, and implementation of biomarkers toward the market is costly and a complicated process in which support of pharmaceutical companies is needed.

Uniform Definitions of Disease Behavior

The Montreal classification does not capture the clinical heterogeneity that is needed for reliable biomarker development or molecular reclassification. Furthermore, different studies use different definitions of severe disease. The Lemann index is a good alternative capturing cumulative damage in the course of IBD, but is not widely adopted. It was proposed to revisit the Montreal classification.[8]

Drug Discovery

Many of the previous topics have been pursued with the primary aim of understanding disease biology, and they also can form the foundations for a new generation of IBD medicines. There is an enormous opportunity presented by the wealth of data from genetics, microbiome, and immunology. There was also a strong argument to consider the full range of clinical questions related to the use of drugs to treat patients. For example, identifying maximally safe and effective dosing regimens of existing therapies could benefit patients enormously, with far less lead time than development of new drugs.

The challenge facing all of us, for a new medicine to truly change the IBD patient experience, requires either transformational efficacy or an extremely precise biomarker to identify patients who will benefit most. This built on the previous biomarker session, where it had become clear that the range of possible biomarker measurements and current lack of any strongly correlated with drug response is an area that needs strong focus. A challenge and strength in IBD is the need for, and opportunity to collect, evidence of

molecular mechanism in the tissues of action: the gut and immune system.

Conclusions

This meeting covered a broad spectrum of topics but centered around the question how to build on the large-scale data generated in IBD biology to identify clinical meaningful biomarkers and to prioritize new targets for early stage drug development or microbiome-directed interventional strategies. Large-scale cohorts are needed for unbiased discovery efforts, but at the same time the scientific community should perform in-depth "deep-dive" analyses on well-defined clinical subsets or unmet needs in IBD. An effort should be made to harmonize clinical data and sampling protocols across centers. In addition, the currently used Montreal classification should be revisited. Furthermore it was acknowledged that there is a need for better academia–industry partnerships and data sharing. Finally, the entire group felt that the tremendous success the International IBD Genetics Consortium has had in the last decade was due to its open and transparent collaboration across the world. The consortium should now expand in the same spirit and include other disciplines and partners to speed up the translation of multiomics-derived findings to clinical practice.

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Conflicts of interest

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