

Review

Clinical Genomics in
Inflammatory Bowel DiseaseHolm H. Uhlig^{1,2,*} and Aleixo M. Muise^{3,4,5}

Genomic technologies inform the complex genetic basis of polygenic inflammatory bowel disease (IBD) as well as Mendelian disease-associated IBD. Aiming to diagnose patients that present with extreme phenotypes due to monogenic forms of IBD, genomics has progressed from 'orphan disease' research towards an integrated standard of clinical care. Advances in diagnostic clinical genomics are increasingly complemented by pathway-specific therapies that aim to correct the consequences of genetic defects. This highlights the exceptional potential for personalized precision medicine. IBD is nevertheless a challenging example for genomic medicine because the overall fraction of patients with Mendelian defects is low, the number of potential candidate genes is high, and interventional evidence is still emerging. We discuss requirements and prospects of explanatory and predictive clinical genomics in IBD.

IBD as an Immune Disorder with Complex Genetic Architecture

In Mendelian disorders, genomic technologies change current clinical practice by identifying disease-causing variants, discovering causative functional mechanisms, and highlighting potential drug targets. **Exome sequencing** (see [Glossary](#)) [1] has identified the genetic basis of a large group of Mendelian disorders including inborn errors of immunology [2,3] and gastroenterology [4,5]. The focus of this review is the transition from genomic research to clinical genomics in IBD.

IBD is a diverse group of disorders with complex pathogenesis. The three main subgroups of IBD are Crohn's disease (CD), ulcerative colitis (UC), and IBD unclassified (IBDU). These are chronic inflammatory disorders associated with defects in the epithelial barrier and the innate immune responses as well as adaptive immune dysregulation [6,7]. The genetic basis is similarly complex [8,9]. **Genome-wide association studies** (GWAS) as well as meta-analysis of loci have been instrumental in defining over 230 disease loci linked to polygenic IBD [10–12]. GWAS have informed on disease mechanisms by the identification of genetic variants and candidate gene networks that affect host–microbe interactions, in particular microbe sensing within the NOD2 pathway; they have also identified autophagy as pathogenic mechanism in IBD, and have highlighted the key role of inflammatory signaling pathways such as IL-23-driven T helper cell responses [8–12]. GWAS have highlighted similarities and differences among ethnicities [11–13], as well as genetic variation among the IBD subtypes CD, UC, and IBDU (in particular disease location and the moderate effects of age) [14]. Comparison of IBD with other inflammatory disorders such as ankylosing spondylitis, psoriasis, and primary sclerosing cholangitis suggests overlapping functional pathways and identified pleiotropy (genetic loci affect multiple phenotypes) [15–18].

Common variants in IBD risk loci explain only a fraction of the expected heritability. Among all risk loci, only a restricted set of causal variants and causative genes are known (including

Trends

Mendelian forms of IBD are a diagnostic challenge due to a spectrum of 50+ conditions that can present with IBD.

Genomics is transitioning from research into the clinic with extraordinary opportunities and practical challenges for bioinformatics variant identification, pathogenicity classification, and translation into therapies.

A genetic diagnosis offers opportunities for personalized care including bone marrow transplantation, surgery, or pathway-specific biologics.

Functional validation of genetic variants plays a crucial role in clinical decision-making.

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NOD2, *ATG16L1*, *IRGM*, *IL23R*, *CARD9*, *RNF186*, and *PRDM1*), with most risk loci containing several viable candidate genes [19]. Recently, genetic association studies have identified variants that are associated with IBD disease severity such as progression to surgery [20]. GWAS also offer opportunities to understand the genetic and mechanistic basis of adverse responses to medications that IBD patients receive [21]. Gene-variant enrichment scores that assess the IBD variant burden help statistical discrimination between CD and UC phenotypes and provide a genetic basis to place IBDU between colonic CD and UC [14].

Genomic Research in IBD

Whereas GWAS have investigated the contribution of common genetic variation, next-generation sequencing technologies offer the possibility to study rare and ultra-rare genetic variants [22]. The contribution of rare protein-coding variants in GWAS loci was investigated by resequencing technologies. Rare variants in candidate genes associated with common susceptibility loci add to disease risk but cannot explain a major percentage of heritability [23–26].

Exome- or **genome-sequencing** studies have further investigated the genetic basis of IBD as recently reviewed by Petersen *et al.* [22]. The role of *NOD2* variants, initially discovered in 2001 as CD-associated susceptibility variants [27,28], has been revisited by sequencing studies [26,29]. Collectively, these studies suggest that compound recessive complementation by rare *NOD2* variants plays a larger role than anticipated. Exome sequencing identified multiple genetic variants including missense variants in the *PRDM1* and *NDP52* [30], *IL17REL* [31], and *CSF2RB* genes [32]. In contrast to variants that confer susceptibility and provide insight into the pathoetiology, the identification of protective variants offers the opportunity to identify drug targets. For instance, a loss-of-function variant in the *RNF186* gene confers protection against UC, suggesting that drugs targeting RNF186 could theoretically be used for UC [33]. Beyond individual genes, recent studies suggest an increased gene-variant burden among immune genes in patients with very early onset IBD (VEOIBD) [34].

Whereas most studies have focused on exome sequencing as the most cost-effective genomic strategy to investigate large patient cohorts, low-coverage genome sequencing might provide an interim stage for genome sequencing in large IBD cohorts. This has been demonstrated by the identification of an IBD-associated protein-coding variant in the *ADCY7* gene [35].

Genomics for the Identification of Mendelian Disorders Associated with IBD

In addition to the large group of patients with polygenic IBD, some rare Mendelian disorders can present with IBD-like intestinal inflammation. The intestinal pathology in those patients is often indistinguishable from the more common polygenic IBD. The terms monogenic IBD and IBD-like inflammation are currently largely used interchangeably throughout the literature, reflecting the overlap in histological features but highlighting different genetic architectures compared to polygenic IBD.

The identification of genetic defects in the IL-10 receptor (encoded by *IL10RA* and *IL10RB*) and the *IL10* gene itself as the cause of severe infantile IBD by family association studies and candidate sequencing [36,37] was a starting point for the identification of multiple high-impact monogenic disorders with high penetrance. Largely by genomic technologies, >50 Mendelian disorders were identified that present with intestinal inflammation as one phenotype [5,38].

Between 2015 and 2017 alone the genetic basis of several diverse genetic disorders that present with intestinal inflammation has been identified by exome sequencing. It is evident that several of the novel defects affect pathways implicated in previously identified Mendelian disorders. This illustrates the concept of shared networks that drive intestinal inflammation.

Glossary

Gene panel sequencing: parallel sequencing of a defined number of genes that are preselected based on known disease relevance.

Genome and exome sequencing: the use of DNA sequencing technologies aiming to sequence the entire human genome or exome (i.e., the fraction of the genome that is transcribed into mature RNA).

Genome-wide association studies (GWAS): analysis of genome-wide sets of genetic variants to probe association with a disease trait or disease outcome/response to therapy.

Hematopoietic stem cell transplantation (HSCT): transfer of hematopoietic stem cells derived from bone marrow, peripheral blood, or in some cases umbilical cord blood into a patient. To correct a genetically driven immune disorder causing intestinal inflammation in a patient, stem cells from a healthy donor are used (allogeneic HSCT).

RNA sequencing (RNA-seq): whole-transcriptome sequencing that allows relative gene expression, alternative splice transcripts, and gene variants to be investigated.

Among the recently identified defects, congenital diarrhea and intestinal inflammation are caused by defects in the *SLC9A3* gene, encoding the sodium hydrogen exchanger 3 that is expressed at the luminal intestinal epithelium [39,40]. The functional consequence of loss-of-function *SLC9A3* gene variants (i.e., reduced sodium uptake and proton exchange at the luminal surface) is similar to that of gain-of-function variants in the *GUCY2C* gene [39–41]. These *GUCY2C* variants caused an increase in the levels of cGMP, a known inhibitor of the *SLC9A3* encoded protein. Animal models suggest that defective *SLC9A3* gene activity drives increased susceptibility to intestinal inflammation via intestinal dysbiosis [42].

An immunodeficiency with a Wiskott–Aldrich syndrome-like phenotype and intestinal inflammation is caused by genetic defects in the *ARPC1B* gene [43]. Similarly to Wiskott–Aldrich syndrome caused by defects in the *WAS* gene, defects in *ARPC1B* affect actin filament formation, causing microthrombocytopenia, defects in platelet function, invasive infections, IBD, vasculitis, and eosinophilia.

In addition, the central role of the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) signaling pathway for IBD is illustrated by the recent identification of additional downstream gene defects including *TRIM22*, encoding a ubiquitin ligase that ubiquitinylates NOD2 [44]. Interestingly, mutations in the Niemann–Pick type C type 1 protein encoded by *NPC1* cause not only neurological and hepatic defects associated with classical Niemann–Pick disease type C but also CD-like intestinal inflammation affecting NOD2-dependent bacterial killing by macrophages [45].

A immune dysregulation syndrome with intestinal inflammation has been described in patients with *MALT1* gene defects [46]. Other recent discoveries suggest distinct gene functions and phenotypes. Mutations in the *SLCO2A1* gene encoding a prostaglandin transporter cause an enteropathy with distinct intestinal ulcerations [47,48]. Intronic mutations in the *POLA1* gene locus affect DNA polymerase- α expression and cause an autoinflammatory X-linked reticulate pigmentary disorder, including infantile IBD, due to defective intranuclear RNA:DNA primer synthesis and type I interferon regulation via cytosolic RNA:DNA [49,50].

To illustrate the opportunities that the application of genomics offers and the complex considerations involved, we discuss X-linked inhibitor of apoptosis (XIAP) deficiency as one of 50+ Mendelian disorders with IBD-like features. Genetic defects in XIAP are associated with innate and adaptive immune defects including granulomatous colitis and perianal disease [51]. The penetrance of IBD-like intestinal inflammation in patients with XIAP deficiency is about 30–40%, ranging from neonatal to adult-onset IBD. Multiple loss-of-function *XIAP* mutations are linked to pathology [51–55]. Many patients with *XIAP* deficiency carry stop-codon, frameshift, or non-synonymous amino acid exchange mutations that are clustered in the BIR2 domain. This domain is required for binding to receptor-interacting serine/threonine-protein kinase 2 (RIPK2) that becomes ubiquitinylated by XIAP and mediates nuclear factor κ B (NF- κ B) activation, and also drives antimicrobial activity [53,54]. XIAP is an indispensable molecule within the NOD2 signaling pathway, but XIAP-deficient patients show only a partially overlapping phenotype with loss-of-function *NOD2* variants. Defective NOD1 signaling potentially accounts for additional cellular phenotypes, such as defective lymphocyte response to Epstein–Barr virus or hemophagocytic lymphohistiocytosis, and might contribute to the complications that *XIAP* patients experience after **hematopoietic stem cell transplantation** (HSCT). As a first example of genomic medicine with immediate therapeutic consequences, exome sequencing identified a genetic defect in *XIAP* in a patient with therapy-resistant IBD, permitting curative HSCT [54]. Heterozygous females may present with XIAP deficiency due to non-random X-inactivation [56]. While the percentage of pediatric male IBD patients with *XIAP* variants may approach 4% [56–58], appropriate functional testing is crucial to distinguish variants of unknown significance

from pathogenic variants. The importance of functional validation and correct genetic diagnosis cannot be understated because there are significant risks of morbidity and mortality with HSCT in XIAP deficiency. The incorrect reporting of novel variants (in disease databases and literature) without the appropriate genetic and functional validation can therefore result in a real risk to patient safety.

Actionable Genetic Information – The Emerging Clinical Care Pathways

Identification of pathogenic genetic variants in IBD patients offers individualized treatment pathways including the appropriate use of HSCT, pathway-specific biologic therapies, and informed use of elective surgery. Furthermore, it allows family counseling and screening for tumors and infections.

The most dramatic use of clinical genetics is observed in IBD patients with predominant immune defects that can be cured through allogeneic bone marrow transplantation. After the original description of causative mutations in *IL10RA* and *IL10RB* genes, many young children with severe intestinal inflammation and perianal disease have successfully received curative HSCT [37,59–61]. *IL10R* mutations have also been observed in early-onset IBD patients after the development of B cell lymphoma [62], revising the clinical pathway from planned autologous to allogeneic HSCT [63].

Genomic diagnostics allows the identification of patients with epithelial defects associated with mutations such as in the *EPCAM* [64,65] or *TTC7A* genes [66,67] where HSCT is not helpful. In contrast to Mendelian disorder-associated IBD, in polygenic IBD autologous HSCT or mesenchymal stroma stem cell transplantation is currently being investigated as a potential way to reset the immune system and modify the stromal microenvironment [68,69].

Molecular diagnosis further informs on potential therapeutic targets. Effective targeting of IL-1 has been described in individual IBD patients with hyperinflammation and inflammasome activation due to mevalonate kinase deficiency [70], *NLR4* gene defects [71], or, most recently, IL-10 receptor deficiency [72]. Moreover, enterocolitis due to *NLR4*-associated hyperinflammation can be treated with IL-18 (and IL1R) inhibition [73]. A fusion protein containing the extracellular domain of CTLA4 suppressed the adverse effects of CTLA4 deficiency *in vitro* [74,75], and might be beneficial for patients with immune dysregulation [76]. In patients with lipopolysaccharide-responsive and beige-like anchor protein (LRBA) deficiency, a defect that regulates CTLA4 endosomal vesicular trafficking in FOXP3⁺ regulatory T cells and activated T cells, the therapeutic CTLA4 fusion protein can restore immune homeostasis [77].

These exemplars illustrate that understanding the cellular mechanisms enables personalized medicine.

Implementation of Genomics for Monogenic Forms of IBD in Clinical Practice

Genomic medicine goes far beyond the technical aspects of diagnostic sequencing (sample preparation, sequencing, sequence alignment to a reference genome, and post-sequencing analysis of sequence variants) in certified laboratories performed within routine clinical care [22,78]. It also requires accurate patient assessment, including family history and patient phenotype assessment, as well as counseling in the pre-sequencing phase. After variant identification, genetic and functional validation of variants, and phenotype–genotype assessment, reporting of variants to patient and clinicians and post-test counseling is required [78–80]. Many of the challenges in implementing genomics in clinical practice are universal and not specific to IBD. With the increased complexity of genetics, immunology, and gastroenterology,

Table 1. Application of Genomics to the Investigation of Mendelian Disorder-Associated IBD

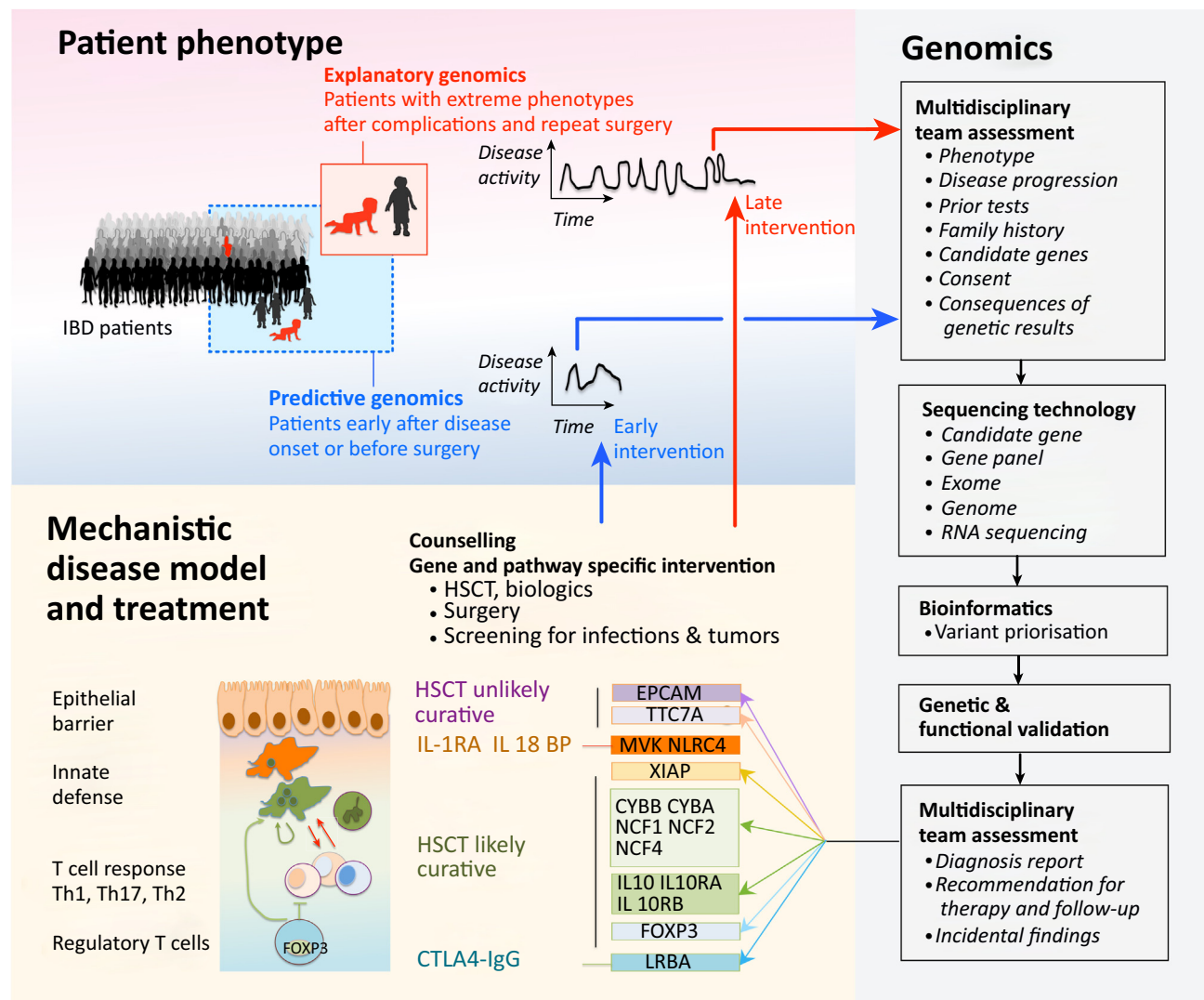
	Advantage	Disadvantage
Pre-analysis and post-genomic multi-disciplinary team discussion	Full assessment of the clinical phenotype, genetic methods, and clinical care plan	Time-consuming/labor intensive
Screening technology		
Panel sequencing	High coverage/excellent diagnostic accuracy	Restricted to pre-defined genes
Exome sequencing	High coverage, most genes available	Some genes are difficult to sequence (<i>IKBK</i> , <i>NCF1</i>)
	'Virtual panel' analysis and research	Restricted to exonic parts
Genome sequencing	Scalable, even coverage, copy-number analysis 'Virtual panel' analysis and research	Comparatively high costs
RNA-seq	Covers isoforms, rare variants, splice variants, and cell- and tissue-specific gene expression	High costs if multiple cell types are analyzed
Selection of the candidate gene panel		
High-confidence genes only ^{a,b}	High confidence, best predictive value	Might miss variants in other genes
Extensive IBD panel (all known genes ^c)	Higher sensitivity/helps to accumulate evidence	More variants of unknown significance and uncertain predictive value
Patient selection		
Infantile IBD/VEOIBD	Enrichment for a Mendelian disorder	IBD in several disorders can present at a later age
Focus on severe cases (Young age MATTERS MOST ^d)	Enriched in Mendelian disorders	There is often a preexisting history of complications
Timing of genomics		
At time of IBD diagnosis	Potential for early diagnosis and targeted care avoiding tissue damage (secondary prevention)	Change in phenotype possible, a large number of patients must be screened, higher number of variants of unknown significance
In severe IBD cases before resections or HSCT	Allows informed treatment decisions	Limited timeframe available
In severe cases with multiple complications	Reasonable patient preselection, allows the phenotype to be explained and guides subsequent therapies	Already significant irreversible tissue damage
Prenatal or at birth	Potential for prenatal diagnosis in affected families or diagnosis after birth	Only relevant for genes/variants with complete penetrance (such as IL-10 signaling defects) No primary prevention strategies yet
Genomics setting		
Research	No cost to the patients/expertise	Clinical-grade validation needed
	Highest potential to identify novel variants	Needs a clear pathway for actionable results
Clinical genomics	Clinical-grade sequencing in CLIA-certified lab	High costs/reimbursement required Functional validation often in research setting
Direct-to-consumer genomics	Patient-initiated genomics; moderate costs	Clinical interpretation and validation required

^aAll gene names in this article are according to Human Genome Organization Gene Nomenclature.

^bHigh-confidence genes according to ClinGen criteria include gene defects where multiple families have been described, independent reports are available, no opposing population-based data are present, and functional validation data are available. Examples: *IL10RB*, *IL10RA*, *XIAP*, *CYBB*, and *FOXP3*.

^cDiscussed previously and adapted from [38]. Epithelial barrier: *COL7A1*, *FERMT1*, *IKBK*, *TTC7A*, *ADAM17*, *GUCY2C*, *SLC9A3*, and *SLCO2A1*; phagocyte defects, hyper- and autoinflammatory: *CYBB*, *CYBA*, *NCF1*, *NCF2*, *NCF4*, *SLC37A4*, *G6PC3*, *ITGB2*, *XIAP*, *TRIM22*, *NPC*, *MVK*, *PLCG2*, *NLR4*, *MEFV*, *STXBP2*, *SH2D1A*, *HPS1*, *HPS4*, and *HPS6*; T and B cell selection and differentiation defects, IPEX (-like) syndrome: *ICOS*, *LRBA*, *IL21*, *CTLA4*, *BTX*, *PIK3R1*, *CD40LG*, *AICDA*, *DCLRE1C*, *ZAP70*, *RAG1*, *RAG2*, *IL2RG*, *LIG4*, *ADA*, *CD3G*, *DKC1*, *RTEL1*, *TGFB1*, *TGFB2*, *PIK3R1*, *PIK3CD*, *PTEN*, *WAS*, *ARPC1B*, *MALT1*, *FOXP3*, *IL2RA*, *STAT1*, and *STAT3*; IL-10 signaling defects: *IL10RA*, *IL10RB*, *IL10*; and others: *MASP2*, *SKIV2L*, *TTC37*, and *POLA1*.

^dFeatures indicating that a monogenic cause/Mendelian disorder should be considered include (i) very young age of IBD onset, (ii) multiple affected family members and consanguinity, (iii) autoimmunity, (iv) failure to thrive, (v) treatment with conventional medication fails, (vi) endocrine concerns, (vii) recurrent infections or unexplained fever, (viii) severe perianal disease, (ix) macrophage activation syndrome and hemophagocytic lymphohistiocytosis, (x) obstruction and atresia of the intestine, (xi) skin lesions with dental and hair abnormalities, and (xii) tumors (Young age MATTERS MOST).



Trends in Genetics

Figure 1. Clinical Genomics Integrates Diagnostic and Therapeutic Pathways in Mendelian Disorder-Associated Inflammatory Bowel Disease (IBD). Conventional explanatory genetics is focused on patients with extreme phenotypes (such as infantile onset), long-term therapy-resistant course, and multiple complications. Genomic medicine aims to find an explanation for the therapy-resistant inflammatory activity in these patients. Subsequent genetic investigations can establish a genetic diagnosis but can lead to targeted interventions late in the disease course when irreversible tissue damage has already been caused. By contrast, predictive genomics aims to identify causal genetic variants early in the disease course, thereby allowing targeted treatments that prevent severe complications and tissue damage in a proportion of children, avoiding a long diagnostic and therapeutic odyssey. This strategy requires screening of a larger group of patients. Both strategies integrate interdisciplinary pre- and post-genomic patient assessments. A molecular diagnosis allows counseling of families, screening for infections and tumors in subgroups of patients, and gene- and pathway-dependent interventions. Abbreviations: HSCT, hematopoietic stem cell therapy; Th1/2/17, type 1/2/17 T helper cells

an interdisciplinary approach is crucial to provide the best diagnostic and therapeutic opportunities for patients (summarized in Table 1 and Figure 1).

Gene Panel Selection and Patient Selection for Clinical Genomics

The probabilistic nature of genetic information of Mendelian disorders with variable penetrance and phenotype is one of the biggest challenges in the field of immune-mediated disorders including IBD. The disease is not present at birth and the full phenotype develops over time. The ratio of patients with monogenic conditions is moderate to very low, depending on the age

group and patient selection, but certainly lower compared to other diseases such as developmental disorders [81]. Mendelian disorders were identified in 31% of infantile IBD in a tertiary referral center [65,82]. In other pediatric IBD cohorts only a small percentage or no Mendelian disorders were recorded [34,57,83,84]. Mendelian disorder-associated IBD is enriched in infantile and VEOIBD but does not exclusively present in this age group.

A major clinical challenge for genomic programs is to decide on the prioritisation of genes for gene panel analysis and the timing of genomic analysis. The use of genomics shifts the classical diagnostic model from a pre-genetic test differential diagnosis mode to a post-test diagnostic assessment [80,85], and from explanatory towards predictive genomics. The inclusion of more than 70 monogenic disorders that have ever been described with IBD into the screening is one option, but selecting only genes with high clinical validity and therapeutic consequences is a rational alternative. The anticipated penetrance of the disorder, the severity of the defect, and the availability of a cure favor different screening strategies in different settings.

(i) Most research studies have so far focused on the analysis of patients with an extreme severe phenotype. This explanatory strategy focuses on patients with a severe course, and where failure of conventional treatment to control the disease results in complications and a need for surgery. This strategy increases the discovery rate, but restricts the opportunities of targeted therapeutic interventions because of existing irreversible organ damage.

(ii) Clinical genomics at time of diagnosis (when the full phenotype is not present) is an alternative approach that permits early diagnosis of Mendelian disorders, allowing early adaptation of therapeutic pathways with the potential of avoiding complications. Large numbers of patients need to be screened and validation of variants of unknown significance is a greater challenge in this predictive genomics approach.

(iii) A compromise is to screen those patients with IBD onset under the age of 1 or 2 years (i.e., an age group enriched in Mendelian disorders) as well as patients who require major therapy escalation such as planned colostomies or severe perianal disease (i.e., a patient group that might benefit most).

(iv) A congenital (or even prenatal) screen is currently only relevant in a setting of positive family history and for selected candidate genes (which exhibit complete penetrance, present with extreme early onset, and where efficacy of the therapeutic intervention is well documented – such as *IL10RA/IL10RB*, *IL10*, and *FOXP3*). In families with a history of a known Mendelian disorder of high mortality and morbidity (such as IL-10 signaling defects) or no available current cure (such as *TTC7A* defects), early prenatal diagnosis might be justified.

Targeted Panel, Exome, and Genome Sequencing

High-coverage targeted **gene panel sequencing** [65,82,86,87], exome sequencing [34,57,65,82–84], and genome sequencing [81] have been used aiming to identify patients with Mendelian disorder-associated IBD.

Owing to its high diagnostic accuracy and moderate costs, targeted panel sequencing is a very practical screening method. Exome and genome sequencing are more cost-intensive at comparable coverage, but can prioritize ‘virtual panels’ for initial screening and only later extend the analysis to novel genes. Inherent technical difficulties of exome sequencing include the challenge of detecting structural variants such as indels, non-coding variants, copy-number variations, inversions, and translocations. Some IBD-relevant genes, in particular *IKBKG* and *NCF1*, are difficult to capture by exome-sequencing techniques and are under-represented [65]. Adapted exon kits can improve capture of splice regions and compensate for uneven

representation [88]. High-coverage genome sequencing offers more even sequencing coverage across the genome, coverage of promoter and enhancer binding regions, as well as analysis of copy-number variants including deletions [22,81,88,89].

Application of additional sequencing technologies such as long-range single-strand sequencing across exons can distinguish between mono- and biallelic variants using patient samples when parental samples are not available or a *de novo* mutation is suspected. In a recent analysis of Nanopore sequencing that allows single DNA strands to be sequenced, 4.5% of the reads had a length of at least 10 000 nt, and some reads covered 50 000 nt [90]. Once these long-read single-cell technologies improve towards the required clinical grade accuracy they might even become point-of-care applications [91].

To assess the impact of variants that affect RNA splicing or gene expression level, **RNA-sequencing** (RNA-seq) technologies complement the spectrum of genomic technologies. RNA-seq can significantly improve the diagnostic yield, as shown in muscular disorders where RNA-seq identified coding and non-coding pathogenic variants that caused splicing defects [92].

Beyond Sequencing: Assessing the Plausibility of Prioritized Genetic Variants

Once genetic variants have been identified, prioritization is key to select likely pathogenic or pathogenic variants. Bioinformatics prioritization is largely based on low-frequency variants (Mendelian disorders are *per se* rare), evolutionary conservation, and the predicted impact on protein function. After variant confirmation by Sanger sequencing, cDNA sequencing, or PCR-based methods, variants with sufficient genetic evidence and previous functional characterization might have direct clinical consequences. However, owing to the large number of private and novel mutations identified in clinical practice, in many cases variants must be functionally evaluated. Reliable functional tests have been established for subgroups of disorders. IL-10 receptor signaling defects can be confirmed by an absence of IL-10-dependent STAT3 phosphorylation or by lack of IL-6, IL-8, or TNF cytokine responses in patient-derived blood mononuclear cells in the presence of exogenous IL-10 [37,61]. Disease-causing variants in the NOD2–RIPK2–XIAP pathway can be assessed via muramyl dipeptide stimulation assays [45,93]. Patients with suspected chronic granulomatous disease are assessed by intracellular reactive oxygen production in neutrophils [94]. Additional immune defects can be reliably identified by their immune phenotype based on numeric differences in lymphocyte or neutrophil counts or immunoglobulin levels using standard laboratory methods. However, functional validation is still a diagnostic bottleneck because it is time-consuming, and for a substantial number of genetic defects no standard validation benchmark tests exist to predict the functional outcome. For many genes only a small number of specialized laboratories worldwide perform functional assays using non-validated research technologies. In patients where a distinct phenotype can be explained by a plausible rare predicted damaging variant this is less relevant. However, lack of functional validation gives rise to a diagnostic dilemma in patients with combinations of phenotypes not described before (phenotype expansion?) or when difficult therapeutic decisions depend on a genetic result suggestive of a potentially damaging mutation.

Patient Benefit and Cost-Effectiveness of Genomic Medicine in IBD

The prospect of genomics is that early diagnosis in a small proportion of patients might prevent unnecessary operations, severe infections, or tumors, and allows progression to targeted therapies. Increased diagnostic accuracy and the rapidly decreasing material costs of exome and genome sequencing provide a basis for increased use of genomic technologies. In this

setting, genomics is likely to be both time- and cost-effective. Formal cost-effectiveness studies would require not only comparison between costs of genomics versus conventional sequential genetics but also associated and follow-up costs for hospitalizations, functional assays, and empiric versus genomically informed interventions. In children with syndromic and immunodeficiency disorders, exome sequencing ended the diagnostic odyssey of multiple previous genetic tests [95], attesting to the cost-effectiveness of the technology.

The Challenges Ahead

To improve genotype–phenotype associations and understand long-term disease progression, integration of genomic results with electronic medical records and clinical decision support is required (Figure 1 and Table 1). The potential for linking large-scale exome sequencing and electronic healthcare records has been demonstrated by the DiscovEHR study that included patients with IBD [96].

Mendelian IBD gene variants require careful classification of pathogenic, potentially pathogenic, unlikely pathogenic, or non-pathogenic variants. Genomic research studies in several pediatric IBD patient cohorts [34,57,65,82–84,97,98] have employed a very variable degree of variant classification and functional validation, and can only partially serve as examples for clinical care pathways. Databases such as ClinVar (www.ncbi.nlm.nih.gov/clinvar) or ClinGen (www.clinicalgenome.org) aim to implement standards for assigning the level of evidence supporting a gene–disease relationship. Although genomic resources in IBD are emerging (IBD Exomes Portal; <http://ibd.broadinstitute.org>), currently there is no single database that allows access to comprehensive IBD-related Mendelian variants.

When genomic investigations in IBD are performed in a research setting, clear rules for the exchange of data between research and clinical care settings, reporting of incidental findings, and clinical follow-up are necessary, and require patient consent. Direct-to-consumer genomics is another emerging pathway, where genomic data might become available outside the clinical setting; however, highly specialized clinical evaluation is necessary to understand the results and initiate follow-up.

To date, protein-coding changes account for the large majority of pathogenic variants identified by genomic technologies. The potential role of synonymous variants is only emerging because nearly all variant-identification algorithms focus on protein-coding mutations. However, a synonymous variant in *IL10RA* (p.T179T) that causes a splice variant has been identified as pathogenic in patients with infantile IBD in Japan, Korea, and China [99–101].

There is an increasing number of disorders with haploinsufficiency gene defects such as in *CTLA4* and *BACH2* [74,75,102,103]. Haploinsufficiency disorders with incomplete penetrance and very variable phenotype pose major diagnostic challenges for predictive genomic screening approaches.

In most settings, genomic screening still requires several weeks to months, and subsequent functional validation leads to further delay. In contrast to life-threatening metabolic disorders that can be diagnosed by genomics as quickly as by biochemical screening tests [104], ‘emergency’-type genomic analysis is rarely needed in patients with IBD. Urgent results are desired in IBD patients where genetic findings would influence proceeding towards elective colectomy or autologous or allogeneic HSCT.

Although the definition of Mendelian disorder-associated IBD indicates a monogenic contribution, in both IBD and many other Mendelian disorders the mutations act in combination with the ‘genetic background’ of the patient that modulates joint protein networks [80,105]. The concept of

Mendelian mutations combined with phenotype-modifying IBD variant ‘burden’ is supported by emerging data that patients with established chronic granulomatous disease and IBD carry an increased burden of common IBD-associated variants compared to chronic granulomatous disease patients without IBD, but a lower variant burden than polygenic IBD [106].

Final integration of genomics into clinical IBD practice will be achieved when clinical societies recommend support for reimbursed genomic diagnostics and evidence-based pathways of care.

Concluding Remarks and Future Perspectives

Whereas clinical decision-making in many disorders including IBD was historically rarely influenced by genetic results, this has changed with progress in targeted treatments. The concept of precision medicine is not only a future perspective but is now a reality for a small fraction of patients. The large spectrum of rare Mendelian disorders that can present with IBD makes the clinical use of genomic methods necessary, practical, and even cost-efficient. Careful genetic and functional evaluation of variants via established pathways will be necessary to implement genomics as a routine technology in the clinic (see Outstanding Questions). In the future we envisage:

- (i) Large-scale population-based high-coverage exome and genome-sequencing studies will reveal the extent of rare variants that contribute to IBD and identify Mendelian disorders with IBD-like intestinal inflammation.
- (ii) Gene and variant databases for intestinal and extra-intestinal immunological and epithelial phenotypes will allow further transition of research-based genomics to clinical genomics.
- (iii) Understanding gene–environment (microbiome, virome) interactions in Mendelian disorder-associated IBD, assessment of di- or oligogenic contributions, epigenetic imprinting, and mosaicism, as well as the genetic burden of common IBD risk loci, will further improve diagnostics and predictive models beyond classical Mendelian genetics.
- (iv) Genomics will shift classical explanatory genetics with Mendelian models towards probabilistic and predictive models for subgroups of patients.
- (v) Research combining genetics and immunology with systems biology by analysis of genomic, proteomic, metabolomic, transcriptomic, and microbiomic profiles will allow patient stratification based on functional pathways.
- (vi) Single-cell DNA and RNA-seq technologies may provide an powerful tool to assess the impact of gene variants, splice variants, and mosaicism within different immune, stromal, and intestinal epithelial cell populations based on a biopsy sample.

Acknowledgments

A.M.M. and H.H.U. are supported by the Leona M. and Harry B. Helmsley Charitable Trust. A.M.M. is supported the Canadian Institute of Health Research (CIHR). HHU is supported by the Crohn’s and Colitis Foundation of America (CCFA), the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN), and the National Institute for Health Research (NIHR) Biomedical Research Centre, Oxford. We would like to thank Athena Cavounidis for critical reading the manuscript.

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Outstanding Questions

Current phenotypic IBD classification systems for CD, UC, and IBDU have limitations, and a molecular classification is needed.

For several monogenic IBD genes, causality is assumed based on a very limited number of patients, and hence definitive evidence of causality is lacking.

Clinical standards for application of genomic technologies, standardized functional testing and evidence-based therapeutic pathways are only beginning to emerge.

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