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Clinical Genomics for the diagnosis of monogenic forms of inflammatory bowel disease: A Position Paper from The Paediatric IBD Porto Group of ESPGHAN

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ABSTRACT
Background: It is important to identify patients with monogenic IBD since management may differ from classical IBD. In this position statement we formulate recommendations for the use of genomics in evaluating potential monogenic causes of IBD across age groups.
Methods: The consensus included paediatric IBD specialists from the Paediatric IBD Porto group of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and specialists from several monogenic IBD research consortia. We defined key topics and performed a systematic literature review to cover indications, technologies (targeted panel, exome and genome sequencing), gene panel setup, cost-effectiveness of genetic screening, and requirements for the clinical care setting. We developed recommendations that were voted upon by all authors and Porto group members (32 voting specialists).
Results: We recommend next-generation DNA sequencing technologies to diagnose monogenic causes of IBD in routine clinical practice embedded in a setting of multidisciplinary patient care. Routine genetic screening is not recommended for all IBD patients. Genetic testing should be considered depending on age of IBD onset (infantile IBD, very early onset IBD, paediatric or young adult IBD) and further criteria such as family history, relevant comorbidities and extraintestinal manifestations. Genetic testing is also recommended in advance of hematopoietic stem cell transplantation. We develop a diagnostic algorithm that includes a gene panel of seventy-five monogenic IBD genes. Considerations are provided also for low resource countries.
Summary: Genomic technologies should be considered an integral part of patient care to investigate patients at risk for monogenic forms of IBD.

Key words: Crohn's disease, Ulcerative colitis, primary immunodeficiency, very early onset inflammatory bowel disease, exome sequencing, genetics

INTRODUCTION and BACKGROUND

Genetic technologies have revolutionized the understanding of the genetic basis and subsequent functional understanding of immune mediated disorders such as inflammatory bowel diseases (IBD) which encompasses Crohn's disease, ulcerative colitis and IBD unclassified IBDU) (1, 2). The genetics of IBD is complex with three major areas arising: complex genetics based on hundreds of common polygenic risk variants, rare monogenic IBD genetics and pharmacogenetics. In most patients with IBD, a large number of common genetic variants (>1% allelic frequency in the general population) contribute to disease susceptibility in a polygenic setting (3-8).

Use of genomic sequencing technologies allows identification of previously undiagnosed disorders in patients with multiple conditions, including gastrointestinal, immunological and rheumatologic diseases (9). A growing number of rare monogenic disorders presenting with inflammatory bowel disease (IBD)-like intestinal inflammation have been identified (10-14). In these patients, IBD is caused by high penetrance genetic variants in a single gene (monogenic IBD). The group of monogenic IBD defects includes a large number of primary immunodeficiencies as well as intestinal epithelial cell defects.

It can be challenging to distinguish monogenic IBD from classical IBD based on clinical phenotype alone. Whereas some of these monogenic disorders present with almost pathognomonic features (such as Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome or Trichohepatoenteric syndrome), others do not. Monogenic IBDs have a higher morbidity compared to classical IBD (15, 16). Incomplete penetrance of most causative genes suggests that additional factors such as environmental factors, the microbiome and additional genetic risk factors contribute to the phenotype of monogenic IBD (17, 18).

Next generation sequencing technologies including targeted panel sequencing, exome and genome sequencing are increasingly used in the research and in the clinical setting to screen for monogenic disorders associated with IBD (10, 11, 19). The technologies and analytic approaches are standardised to meet clinical diagnostic needs (20). Panel sequencing is a cost-effective technology to screen for variants in a limited number of genes (typically tens to few hundred genes). This technology results comparably in high coverage of the sequencing reads and the analysis is relatively simple compared to an exome- or genome-wide analysis. Exome sequencing aims to investigate the whole range of protein coding variants in approximately 20000 genes (20). The technology does have limitations in the diagnostic setting due to read coverage deficiencies in some regions, but newer exon capture assays compensate for uneven coverage. Genome sequencing investigates the entire genome of approximately 3 billion base pairs of which most are biallelic (20). In addition to the protein coding regions, it allows analysis of regulatory elements and intronic regions. Due to a more even sequencing coverage of the sequence reads compared to exome sequencing, it allows to investigate copy number variation (CNV, deletions or duplications). Nevertheless, genome sequencing has not yet fulfilled its full potential for clinical genomics since current information of the non-coding elements in IBD is still restricted and its analysis is complex

(21). The diagnostic yield of exome and genome sequencing is similar or slightly higher in genome sequencing (22, 23).

We present a position statement on the application of next generation sequencing for diagnosis of monogenic IBD in clinical practice. We outline indications for genetic testing and discuss the diagnostic yield in different settings. Focusing on next generation sequencing for monogenic IBD diagnosis, we will not discuss emerging genetic applications such as polygenic IBD risk scores (3) or IBD-related pharmacogenetics (24-28).

METHODS

Definition of topics and summary of evidence

Following an open call to the members of the Paediatric IBD Porto and interest group of ESPGHAN, international specialists were selected based on content expertise. Besides Porto group members, external paediatric and adult IBD as well as immunology specialists were invited to participate. These included specialists from several research consortia with a focus on monogenic IBD including the Genius working group of ESPGHAN, the ESPGHAN supported COLORS in IBD research network and the VEOIBD consortium (www.veoibd.org).

Initially, the steering committee identified 7 key topics as part of an online iterative discussion process (**Box 1**). Subsequently, these topics were discussed within working groups, literature was assessed using a PubMed search, and position statements were developed.

Literature search strategy and eligibility criteria

To assess the use for next generation sequencing technologies for the diagnosis of monogenic IBD in patient cohorts, a systematic literature review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement (PRISMA; http://www.prisma-statement.org). The electronic database PubMed was searched for studies (updated June 2020). To achieve the maximum sensitivity of the search strategy in title and abstract, we combined terms that describe the IBD population (monogenic inflammatory bowel disease n=8 articles and Very Early Onset Inflammatory Bowel Disease n=110 articles) with search for IBD related sequencing technologies (Inflammatory Bowel Disease AND Exome Sequencing n=107 articles or Inflammatory Bowel Disease and targeted panel sequencing n=21 articles). Patient cohort studies published between 2010 to 2020 were included when 1) the study was published as original article in a peer-reviewed journal, 2) it was written in English, 3) the study population consisted of at least 10 patients diagnosed with IBD, 4) the aim was to screen for a spectrum of monogenic IBD disorders using targeted panel sequencing, and/or exome and/or genome sequencing. Case reports, conference presentations, reviews, editorials, and expert opinions were excluded. The reference lists of relevant articles and reviews on the topic were screened for additional studies that meet the eligibility criteria. From each selected study the following information was extracted: author name and year of publication, number of patients, cohort description, age at IBD diagnosis, region where participants were recruited and ethnic descent of IBD patients investigated, sequencing technology used, the number of monogenic IBD

genes screened and the number of patients with monogenic IBD identified, whether functional validation was performed and whether the results had therapeutic consequences. The selected studies were assessed for diagnostic costs and health economic data.

To assess clinical features that are significantly associated with the group of monogenic IBD, we selected studies that compared clinical features between cohorts of patients with monogenic IBD (including diverse genetic defects) and control IBD patients in whom no monogenic defect was identified. Significantly associated features were reported according to the statistical analysis described in the respective paper.

To select an extensive list of genes associated with monogenic IBD, we summarised the gene list discussed in four extensive specialist review articles that cover the biology and clinical implications of monogenic IBD (10-13). To reduce selection bias, we included reviews from specialists of different institutions. Based on this summary, a consensus list was generated by representatives of the research consortia based on published evidence.

We assessed different diagnostic algorithms to identify patients with monogenic IBD (11, 12, 29-31).

Definitions

In accordance with previous definitions, we define neonatal IBD as onset within first 28 days of age, infantile (and toddler) onset of IBD (less than 2 years of onset), very early onset IBD (VEOIBD; less than 6 years of age IBD onset), early onset IBD (EOIBD; A1a Paris Classification; less than 10 years of age), paediatric IBD less than 17 years of age onset (A1a and A1b Paris Classification) as well as adult onset IBD 17 year and older (29, 32).

To define the pathogenicity of genetic variants we follow the American College of Medical Genetics (ACMG) five-tier classification system (33). According to this classification (1) Pathogenic and (2) likely pathogenic variants directly contribute to the development of disease or are very likely causative, (3) variants of uncertain significance lack evidence to support a more definitive classification, whereas (4) likely benign or (5) benign variants do unlikely or not cause disease (33).

Statement voting

Position statements were developed based on iterative e-mail and conference call group discussions. The writing group voted on all statements while adding specific comments using a web-based voting platform. A second round of electronic voting and revisions was done, including all members of the Paediatric IBD Porto group of ESPGHAN. In total 32 paediatric IBD specialists voted anonymously on all statements: 23 Porto group members (of whom 9 were authors; Appendix 1, *http://links.lww.com/MPG/C127* names of all Porto group IBD specialists) and 9 non-Porto group authors. All statements were supported by at least 80% of the group.

RESULTS

Next generation sequencing for diagnosis of monogenic IBD

The initial literature search resulted in 185 studies. The use of next generation sequencing for diagnosis of diverse monogenic IBD disorders has been described in 18 cohort studies (**Table 1**). Five studies identified significant clinical and laboratory features of monogenic IBD patients compared to IBD patients in whom no monogenic cause was identified (**Table 2**). The results of the studies are influenced by several factors including the geographical representation of the cohorts, the ethnic background of the study groups, or the preselection of the study population in respect to clinical phenotype (unselected cohorts, cohorts with undiagnosed immune defects or congenital diarrhea, cohorts with or without clinical suspicion of monogenic IBD prior to genetic tests). Further factors that influence the diagnostic yield are the age of IBD onset of the study population (ranging from neonatal onset to adult onset IBD), the use of sequencing technologies (targeted panel sequencing, exome and genome sequencing, with and without prior Sanger sequencing), the study setup (single or multi-centre studies, analysis focused on singleton sequencing in most studies versus family trio analysis in one), and the degree of pathogenic classification and functional validation of the genetic variants (**Table 1 and 2**). The heterogeneity of these studies has so far precluded a formal meta-analysis to establish a hierarchy of factors. However, even in the absence of a multifactorial analysis, current data allow to draw conclusions about the patient populations that will benefit most from genetic screening, and how to optimise the diagnostic pathway in the clinical care setting.

Indications for genetic testing of monogenic IBD informed by clinical phenotypes and therapeutic implications

Establishing a genetic diagnosis is of benefit to patients and their families because it can help to predict the disease course, to anticipate complications, to facilitate genetic counselling and to consider specific treatments, most notably allogeneic hematopoietic stem cell transplantation for some underlying primary immunodeficiencies (10-12) (**Table 1**). Since monogenic IBD is associated with a number of demographic features including age of IBD onset, the family history, clinical phenotypes (in particular comorbidities and extraintestinal manifestations), as well as abnormal laboratory findings (**Table 2**), those features can inform the need for genetic investigations as well as the clinical need to progress with informed treatment options.

Age of IBD onset as a predictor of monogenic IBD

The age of onset of IBD is a strong predictor for the risk of monogenic IBD (**Table 1 and 2**). The majority of patients with monogenic IBD present in the first 6 years of life (i.e. very early onset IBD, VEOIBD) (16, 31, 34, 35). However, the age of onset forms a spectrum and across a large number of gene defects, individual patients present in paediatric care beyond 6 year of age (29).

Studies in patients with infantile-onset IBD (i.e. <2 years of age at disease onset) identified a monogenic cause in 13-41%, in patients with VEOIBD in 0-33%, and in patients with IBD onset between 6 years and 18 years in 0-38% depending on the preselection of the

patients investigated (**Table 1**). Among all paediatric IBD patients aged <18 years in a single centre, monogenic IBD was identified in 3% (36).

Diagnosing a monogenic cause of IBD during adulthood is exceptional. Sequencing studies in adult onset IBD populations have not identified monogenic IBD (37, 38). Among the exceptional rare gene defects that are associated regularly with adult onset IBD is congenital diarrhea due to GUCY2C defects (39). Adult age onset IBD is a rare but consistent finding in patients with XIAP defects, who were either previously symptomatic due to immunodeficiency or even non-symptomatic (40). Hypomorphic variants in XIAP without clinical consequence for the patients are more common in patients with IBD onset >6 years of IBD onset, suggesting a role as modifier variant and a spectrum of pathogenicity (38).

Family history as predictor of monogenic IBD

Consanguinity, a family history of autoimmune disease and family history of suspected or confirmed monogenic disorders are associated with monogenic IBD (16, 36) (**Table 2**). Male predominance is a sign of X-linked disorders such as XIAP deficiency or IPEX syndrome (41). Suspicion is highest if similar disease phenotypes are observed in several family members. However, a positive family history is not specific since multiple affected family members can also be found in classical IBD, males predominate in paediatric-onset Crohn's disease overall, and a quasi Mendelian inheritance pattern has been described in families with NOD2 variants (42, 43). On the other hand, monogenic IBD disorders such as *LRBA* deficiency (44, 45), or CTLA4 haploinsufficiency (46) can present with quite diverse phenotypes reflecting variable expression and incomplete penetrance. In those defects a Mendelian inheritance pattern can be clouded even if several family members are affected.

Clinical features of monogenic IBD including comorbidities and extraintestinal manifestations

Current data suggest a limited diagnostic value of endoscopic IBD classification for the diagnosis of monogenic IBD (**Table 1 and 2**) whereas some histologic features may raise the suspicion for monogenic IBD **(Table 3**).

Comorbidities and extraintestinal features are significantly associated with monogenic IBD (**Table 2**). We use the terms "comorbidities and extraintestinal manifestations" aligned to acknowledge the fact that potentially unrelated comorbidities in patients with suspected monogenic IBD (pre-test definition) are in fact often extraintestinal manifestations in accordance with the broader phenotypic disease spectrum of individual gene defects (post-diagnosis definition). Several reviews have provided an overview of extraintestinal features of the diverse immunodeficiency and epithelial cell disorders that can present with intestinal inflammation (10, 12, 29). Those features include recurrent infections, hemophagocytic lymphohistiocytosis (HLH), autoimmune and dermatological features as well as development of malignancy (**Table 3**).

It is important to note, that not all rare extraintestinal manifestations and comorbidities are a consequence of monogenic IBD and that intestinal inflammation in a rare Mendelian disease is not always caused by the gene defect. For instance, hemophagocytic lymphohistiocytosis can be a medication-induced side effect of thiopurines used to treat IBD

(**Table 3**). D*e novo* intestinal inflammation in patients with Mendelian disorders can be a consequence of treatment of malignancy by immune checkpoint inhibitor (anti-CTLA4 or anti-PD1) or mofetil mycophenolate treatment after solid organ transplantation (47).

Laboratory features associated with immune dysfunction in monogenic IBD

Abnormal immune cell numbers and function and abnormal immunoglobulin levels in a patient with intestinal inflammation can suggest a primary immunodeficiency (**Table 4**). A pragmatic basic workup for patients with IBD and suspected monogenic IBD includes a limited number of essential laboratory tests (**Table 4**). There are no data in support of more extensive laboratory studies in routine clinical practice. However, the infection history, auto-immune phenotype and abnormal immunological laboratory features may trigger more specialized investigations in an interdisciplinary effort.

Genetic screening in advance of interventions associated with high morbidity and mortality

Genetic investigations to establish monogenic IBD can guide appropriate therapies and inform on risk benefit of therapies associated with high morbidity and mortality (**Table 1**).

Results of genetic investigations in patients with suspected monogenic IBD guide the application of allogeneic hemopoietic stem cell transplantation in several aspects. Allogeneic hemopoietic stem cell transplantation has developed into a *de facto* standard of care for several monogenic IBD disorders associated with primary immunodeficiencies, in particular IL10 signalling defects or regulatory T cell defects (48-50). For other monogenic IBD disorders with epithelial defects such as TTC7A or IKBKG defects, allogeneic haematopoietic stem cell transplantation is less or not at all effective (51, 52). This means that for some patients with monogenic IBD defects the likely therapeutic benefit clearly outweighs the transplant associated mortality and morbidity (such as Graft versus host disease, medication toxicity, infections) whereas patients with epithelial conditions will unlikely benefit while still experiencing potential complications. Genetic screening can identify patients with pure epithelial defects and prevent progression to haematopoietic stem cell transplantation (53). In patients with monogenic IBD defects that affect both hematopoietic and non-hematopoietic (epithelial) cell lineages (e.g. *CASP8, IKBKG or RIPK1*), weighing risks and benefits of allogeneic HSCT remains challenging (54-56).

Patients with IL10 signalling defect have an increased susceptibility for lymphoma (57). Whereas in patients with many forms of lymphoma, chemotherapy and autologous hematopoietic stem cell transplantation is a standard of care, autologous transplantation does not correct the underlying genetic driver in IL10 signalling defect. Genetic analysis can therefore prevent autologous haematopoietic stem cell transplantation in patients with monogenic IBD defects and inform progression to *allogeneic* haematopoietic stem cell transplantation (57).

In some patients with monogenic severe therapy refractory Crohn's disease, multiple resections can cause short gut syndrome. Establishing the genetic diagnosis of XIAP deficiency in a patient with short gut syndrome by exome sequencing resulted in change of clinical management from a proposed small bowel transplantation to allogeneic HSCT (37).

Establishing a genetic diagnosis early in the course of the disease may prevent surgery by progressing with curative allogeneic HSCT.

A genetic diagnosis of monogenic IBD can inform on pharmacological treatment options

Preliminary data suggest that patients with distinct monogenic disorders might benefit from specific pharmacologic interventions that are currently not standard of care in patients with classical IBD. Case reports or non-controlled small case series suggest that patients with IL10 signalling defects and mevalonate kinase deficiency might benefit from IL-1 targeting therapies (58, 59), patients with NLRC4 defects from IL-18 or IL-1 targeting treatments (60, 61), whereas patients with CTLA4 and LRBA defects can benefit from CTLA4 fusion protein abatacept (62, 63).

Prenatal testing in families with history of infantile IBD

Families with children affected by severe genetic disorders may ask whether predictive prenatal testing can inform recurrence of the disorder in subsequent pregnancies. A recent survey among clinicians in tertiary centers of 10 countries reported referrals for prenatal genetic testing for IL10 signalling defects, i.e. a form of monogenic IBD with severe phenotype and complete penetrance in four countries (64). Prenatal testing in families with known *IL10RA* defects was performed as targeted preimplantation test after *in vitro* fertilization or as targeted genetics after intrauterine amniocentesis/chorion villus sampling. Prenatal diagnostics requires specific clinical genetic counselling and poses great ethical challenges due to the potential implications of embryo selection or termination of a pregnancy (64).

What monogenic disorders should be included in genetic sequencing panels?

Gene sets for monogenic IBD disorders have been discussed in recent literature reviews (10-13). Among those, there is considerable heterogeneity; 32 genes are discussed in all four reviews (**Table 5**). Similarly, there are differences in the gene panel setup of commercial, clinical and research focused targeted gene panel assays aiming to screen patients with monogenic IBD, infantile IBD, and/or congenital diarrhoea to include between 21 and 160 genes (**Suppl. Table 1,** *http://links.lww.com/MPG/C125*). Those differences can be explained by several factors: i) an increasing number of candidate genes is identified over time, ii) focus on slightly different patient cohorts (i.e. focus on immunodeficiency genes and/or congenital diarrhea depending on the referral population), iii) different definitions on what defines a causative monogenic IBD gene, and iv) inclusion of genes that are (currently) of research interest but not established as monogenic cause of disease, v) some panels include genes informed by polygenic risk loci. Acknowledging those factors, we reviewed the gene list and agreed on a consensus list of 75 monogenic IBD genes (**Table 5)**. Depending on the phenotype and the family history, a variable set of candidate genes can be prioritized for the individual patient. The emerging number of newly described genetic causes and the process of variant validation over time means that this list of monogenic IBD genes will evolve and needs updating. The vast majority of these genes has been recognized as disease-causing by an independent international expert committee of the international union of immunological societies (65).

Are there preferred sequencing technologies?

Due to the increasing number of candidate genes, the use of classical Sanger sequencing of candidate genes has been replaced by parallel sequencing technologies. Sanger sequencing is still an effective, fast and economic way to confirm a suspected genetic diagnosis in a family with a known genetic variant or in a patient with a pathognomonic phenotype. There is now ample evidence that panel sequencing (53, 66), exome sequencing (8, 36, 37, 53, 66, 67), as well as genome sequencing (21, 68, 69) are excellent tools to investigate genetic variants in patients with suspected monogenic IBD. Comparative studies in patients with IBD have confirmed that sequencing read coverage and diagnostic accuracy of panel sequencing is higher than exome sequencing (53, 66). Several clinical genetic laboratories and additional commercial companies offer panel sequencing assays aimed for early onset IBD (**Suppl. Table 1,** *http://links.lww.com/MPG/C125*). It is in the nature of the assay that updating the panel of genes is required over time and retesting of patient samples with a high suspicion of a monogenic IBD is required either with an updated panel or subsequent progression with exome or genome sequencing. Since the first description of **exome sequencing** in a patient with IBD and XIAP deficiency (70), exome sequencing has demonstrated its clinical utility and potential for discovery (11, 19). Using exome sequencing, a specified "virtual" monogenic IBD panel can be used for the initial screening and exome-wide analysis can be performed as part of a subsequent analysis. Genome sequencing has not yet fulfilled its full potential for clinical genomics since current information of the non-coding elements in IBD is still restricted and its analysis is complex (21). Genome sequencing care pathways for infantile onset IBD are currently being evaluated in a formal trial of the National Genomic program in England (https://www.genomicsengland.co.uk). The genetic DNA sequencing technologies might be complemented by copy number variation analysis via multiplex ligation-dependent probe amplification. Other variants might require validation via RNA sequencing to confirm relevant splice variants.

Which sequencing technology to use in clinical practice depends on the clinical setting and the resources available. For example, regional or national health care services may opt for well-designed panel sequencing approaches as a first-line strategy, whereas academic centers (single centers or national research hubs) may prefer standardized exome or genome sequencing platforms.

In the context of exome wide sequencing, analysis of patient and parents (family trios) has a higher discovery rate than singleton patients sequencing (20, 22). Indeed, the use of trio analysis might explain in part the higher diagnostic rate in a paediatric IBD population compared to previous studies (36).

Analysis and interpretation of genomic data should be performed by specialists trained in clinical genomic medicine. All variants within each gene should be classified as either "pathogenic", "likely pathogenic", or "variants of unknown significance". Databases such as Clinvar, ClinGen, or The Human Gene Mutation Database can help to assess variant phenotype relations (71, 72) although there is currently no single repository of classified monogenic IBD gene variants. Deposition of variants of unknown significance via Clinvar

(https://www.ncbi.nlm.nih.gov/clinvar/) or sharing variants via Matchmaker (https://www.matchmakerexchange.org) can initiate communication with interested research groups and facilitate characterization. In some patients, in particular in consanguineous families, occasionally more than one plausible monogenic defect is identified. Since annotation and assembly of the human genome is an ongoing project and computational algorithms are constantly being improved, the identification of genetic variants is also subject to evolution.

Is a functional validation of genetic variants required before genetic variants can be regarded as pathogenic?

The causal relation of a genetic finding in a patient with potential monogenic IBD needs to be established on a gene as well as variant level. Relying solely on genetic screening can be misleading, because computational variant prediction can either fail to detect functional damaging variants (false negative) or predict damaging effects in neutral variants (false positive). It is therefore important that novel variants in known genes are functionally validated and assessment of variants in novel candidate genes typically requires further research. In principle, functional studies are required to show that genetic variants cause disease through loss-of-function, or gain-of-function via altered protein function or expression, or localization of the gene product.

It is a limitation that in routine diagnostic laboratories, functional tests are currently available only for a fraction of genes associated with monogenic IBD. Among those tests used in the routine clinical immunology labs, the dihydrorhodamine test is standardised to detect defective neutrophil NADPH oxidase activity in patients with chronic granulomatous disease. Other functional tests assess IL10 signalling (function of the IL10 receptors - *IL10RA* and *IL10RB* deficiency), or IL10 secretion in response to LPS, and muramyl dipeptide induced signalling (XIAP deficiency) (29). Flow cytometry studies for XIAP, SLAM-associated protein (SAP), CTLA4, LRBA and FOXP3 protein expression will identify a large proportion of patients that harbour gene defects with deletion, stop codon or frameshift variants in those genes (73, 74). In patients with suspected IPEX or IPEX-like syndrome measurement of a range of autoantibodies including anti-thyroid antibodies, type 1 diabetes specific antibodies, and anti-enterocytes antibodies can be adequate (41).

Some genetic defects show cell-type specific effects, some variants affect isoforms, some variants only affect a fraction of cells, i.e. mosaicism (8). With increasing use of sequencing technologies, it becomes clear that there are many hypomorphic defects and it can be challenging to assess whether a gene variant is a pathogenic variant or a risk factor (NADPH oxidase complex, (75); XIAP (38, 76)).

Functional validation usually requires in-depth studies either based on primary patient-derived cells or cell lines that are modified to express patient derived mutations. To assess compound heterozygosity, the effects of both genetic variants should be tested. For a large number of genes, functional validation assays are available in the research setting only. Complex *in vitro* models based on organoids or induced Pluripotent Stem Cells (iPS cells) may be employed to study cell types such as epithelial cells or macrophages (77, 78). I*n vivo* models include animals such as zebrafish or mice (e.g. humanised mouse models) (79). Since

the clinical analysis depends on research findings, translational research studies must adhere to clear standards when reporting variants and associated pathogenicity. Without performing functional validation studies, research results are potentially misleading (80, 81).

Are genomic screening technologies for diagnosis of monogenic IBD cost-effective?

Formal studies on the overall diagnostic costs of genomic diagnostics in IBD are lacking. Among the next generation sequencing technologies, the technical sequencing costs are lowest with panel sequencing and highest with genome sequencing (82). Whereas the technical sequencing costs drop over time aiming for costs less than $1000 per genome and less than $500 per exome, the overall costs of the genetic analysis are still substantial. A systematic literature review that included studies until the year 2016 estimated the cost for a single test of exome sequencing from $555 to $5,169 and for genome sequencing from $1,906 to $24,810 (82). Another study calculated the per-sample costs €1669 for genome sequencing, €792 for exome and €333 for targeted panel sequencing (83). A micro-costing analysis of genome sequencing in a UK National Health Service laboratory processing 399 samples per year estimated the true costs of analysis several times higher than the technical sequencing costs (84). Targeted gene panel sequencing has emerged as a diagnostically accurate and cost-effective technology with approximately 25% the sequencing cost of exome sequencing and less analytic complexity. Petersen et al. performed targeted panel sequencing in a cohort of 71 patients with early onset IBD or early onset chronic diarrhoea and compared the findings to exome sequencing performed in 25 of these patients (66). Costs can differ in different health care settings. Many centers see the trade-off between costs and the expected clinical utility currently in favour of performing targeted panel or exome sequencing as first line analysis.

Health economic studies are currently not available to assess how genomic diagnostic costs in different care pathways compare long term in patients with suspected monogenic IBD. Although not related to monogenic IBD, a recent prospective study demonstrating cost-effectiveness of early exome sequencing in relation to all diagnosis-related investigations including Sanger sequencing in a group of 40 children with a range of suspected Mendelian disorders (85). An early genetic diagnosis may prevent a diagnostic odyssey with associated diagnostic costs, treatments, operations and hospitalization in those patients. Case reports, case series and cohort studies suggest a long diagnostic delay in some patients and confirm that a genetic diagnosis after performance of genomic screening technologies does lead to fundamental change in clinical management, years to even decades after the onset of intestinal inflammation (8, 36, 37, 68).

Multidisciplinary care pathways and clinical genomics in monogenic IBD

In light of the individual benefit for patients and their families, targeted panel sequencing and exome sequencing strategies for patients with suspected monogenic IBD have been implemented in routine clinical care by health care providers in several countries (e.g. Switzerland, UK, Israel, USA).

Several diagnostic algorithms for monogenic IBD have been proposed **(Figure 1, Suppl. Figure 1,** *http://links.lww.com/MPG/C126***)**. The use of genetic technologies

complement the diagnostic algorithms for the diagnosis of paediatric Crohn's disease, UC and IBD unclassified (IBDU) to establish the extent and activity of the intestinal inflammation (32, 86). Due to the continuously increasing number of Mendelian disorders that can cause IBD, the prediction of a single candidate gene based on clinically presentation is unreliable even to the most experienced clinicians. Next-generation sequencing technologies are therefore key to help establish the diagnosis of monogenic diseases associated with IBD.

Qualitative reports and reviews describe how clinical genomics pathways for patients with suspected monogenic IBD are best organised (10, 11, 87). Multidisciplinary care of patients with expected or confirmed monogenic IBD is key to deliver state-of-the-art patient-centered care. Setup of dedicated clinics with focus on VEO-IBD, monogenic IBD and genomic medicine can help to implement such interdisciplinary care (87). This supports provision of specialized paediatric gastroenterologist service care jointly with immunology and genetic services, as well as additional disciplines such as radiology, surgery, rheumatology, dermatology, dietetics, pharmacists (potential use of off licence treatments), psychology (severe chronic disorders, family support), primary care clinicians, as well as ethicists (implications for other family members, novel treatment options, families who consider prenatal screening) and research scientists. Individual specialities such as paediatric radiology (88) have highlighted the specific needs of patients with VEO-IBD and monogenic IBD within their diagnostic algorithms. Virtual multidisciplinary case discussions can be helpful to connect not only local but national and international specialists.

Prior to genetic investigations, it is important to discuss indication, previous diagnostic findings, the genetic screening process, the likelihood to identify incidental findings, and the potential therapeutic consequences **(Figure 1)**. Up-to date standards and guidelines and practice resource for clinical genomics are available via specialist societies such as the European Society of Human Genetics (89) or the American College of Medical Genetics (https://www.nature.com/gim/). There are country-specific rules on handling patient consent, genetic data storage and data protection, as well as incidental findings. Children should always be encouraged to actively engage in the consent/assent process, respecting their rights in light of their age-related cognitive development (90). After the genetic investigations, a multidisciplinary team meeting helps to assess the genetic and functional validation results, to assess the need of additional diagnostic tests and therapeutic implications, to discuss incidental findings, and to prepare communicating genetic findings to patients **(Figure 1)**. Given the complexity of the findings, it is important to communicate the genetic test results to patients and their families as well as non-specialists in a understandable way (91). How to communicate information about less well-understood genetic variants is a matter of debate. In light of the large number of variants of unknown significance there is good reason not to report variants of unknown significance (92). Pre- and post-test counselling on variants of unknown significance and their spectrum of potential consequences can help to reduce misinterpretations, anxiety, as well as decisional regret (93).

Although centralised sequencing and analysis has several advantages, hospital-based analysis was associated with higher diagnostic yield compared to centralised exome analysis

suggesting that local expert knowledge (on patient and phenotype) contributed significantly to the increased diagnostic yield (22).

The urgency of genetic testing is influenced by the clinical condition of the patient and the therapeutic implications of the genetic results. However, the former can change and the latter are difficult to predict before sequencing results are received. This is illustrated by a patient with VEO-IBD who was in stable condition at the time of study participation but died after an unexpected EBV infection response and liver failure due to X-linked lymphoproliferative type 1 disorder before the exome sequencing results became available (8). An "emergency" sequencing is rarely required but technically feasible in critically ill infants (94). In a patient with infantile onset Crohn's disease, such an emergency 24-hour trio-exome sequencing was performed revealing compound heterozygous IL10RA defects allowing to proceed rapidly with curative haematopoietic stem cell transplantation (94).

For young adult patients with suspected or confirmed monogenic IBD defined arrangements for transitional care from paediatric to adult care is important. In some patients, the monogenic IBD diagnosis has been made only when transitioned to adult gastroenterology care emphasizing decades of diagnostic delay some patients face (37, 68).

Recommendations and position statement

There is sufficient evidence to recommend the use of next-generation DNA sequencing technologies to diagnose known monogenic causes of IBD in routine clinical practice. We propose a diagnostic pathway for monogenic IBD that complements the standard IBD guideline workup, facilitates multidisciplinary team assessment of patients with suspected monogenic IBD, supports genetic counselling and consent to research if appropriate, and implements next generation sequencing technologies as well as multidisciplinary team assessment of genetic results (**Figure 1**). This diagnostic pathway involves the full assessment of the patients clinical phenotype, the family history, the test results from previous immunological investigations (**Table 4**), as well as interpretation of results and incidental findings and its implications for prognosis and therapies. We formulated nine statements on the use of genomic technologies in routine clinical care (**Table 6**). Special considerations for low resource countries are formulated to address the fact that specialised clinics with focus on monogenic IBD and clinical genetics next generation sequencing technologies are difficult to access and that socioeconomic conditions such as lack of health insurance coverage prevent access to those services (**Box 3**).

Opportunities, challenges and the need for education and research

A prerequisite for the implementation of genomic diagnostics for monogenic IBD in routine clinical practice is education. Rapid developments in genomic technologies and its ethical implications requires an updated training syllabus for specialty training as well as continued professional education of paediatric and adult gastroenterologists. In a 2017 web-based nationwide survey of UK gastroenterology specialty trainees, 91% of trainees regard their local training program not adequate in regard to genomic medicine (95). In paediatric gastroenterology, the identification and understanding of genetic conditions is part of the

ESPGHAN and NASPGHAN training syllabus but genomic medicine is not currently specified.

Studies on the perspective of patients and patient reported outcome are lacking in the field of monogenic IBD.

Technologies such as RNA sequencing, single cell RNA sequencing, proteomic and metabolomic technologies do complement current clinical genomic technologies. Defined applications will soon reach clinical practice to aid splice defect analysis and understand isoform usage, cellular mosaicism and posttranslational modification.

The field of precision medicine and genomics in rare diseases is heavily research driven. One challenge is to combine clinical genetic practice and research. The clinical genetics perspective is focused to identify known disorders with a strong previously published or accessible evidence (identification of pathogenic variants or likely pathogenic variants based on literature or established databases) whereas translational science aims to identify novel causative genetic variants that are relevant for disease pathogenesis and treatments. Expectations in regard to timely reporting of known and novel genetic results can be challenging since it might take months or years to validate novel findings. Di- and potentially oligogenic forms IBD are not yet robustly defined.

Open access to research genetic data is challenging in times of increasing scrutiny towards personal data protection and genetic confidentiality on one side and opportunities to identify individuals based on ancestry analysis on the other (96). The intimate interaction between clinical genetics and translational science from using genetic technologies and functional validation in the research setting to applying genetically informed treatments off label is only partially sanctioned by law and regulators.

Another emerging challenge is the use of direct-to-consumer genetics (97). Genetic tests initiated and paid for by patients via commercial sequencing facilities can provide IBD relevant genetic results (for instance by providing direct-to consumer genome sequencing Nebula Genomics https://nebula.org/whole-genome-sequencing/). However, in the absence of a multidisciplinary team assessment and disease specific specialist input, interpretation of direct-to consumer genetics test results will be challenging for patients and clinicians alike.

A formal health economic assessment for monogenic IBD is required. This requires assessment of the diagnostic costs in relation with treatment and procedure costs in a group of very diverse monogenic disorders with only gradually emerging standards of care in a setting of centralized and specialized multidisciplinary care. For many disorders, medications are not formally licenced, novel medications that are provided as part of research (no costs can be affiliated yet), and patients with extremely expensive forms of health care utilisation such as multiple operations, parental nutrition and stem cell transplantation will strongly influence the analysis.

SUMMARY

The use of genomic medicine offers essential diagnostic opportunities and has complex medical and scientific as well as ethical, legal, financial and social implications. Implementation of appropriate genomics into clinical practice requires therefore not just the use of evolving clinical genetics technologies but a patient centred multidisciplinary approach.

DISCLAIMER AND QUALIFYING STATEMENT

ESPGHAN is not responsible for the practices of physicians and provides guidelines and position papers as indicators of best practice only. Diagnosis and treatment is at the discretion of physicians. This guidance may be revised as necessary to account for changes in technology, new data, or other aspects of clinical practice. This guidance is intended to be an educational device to provide information that may assist clinicians in providing care to patients. They are not a rule and should not be construed as establishing a legal standard of care or as encouraging, advocating, requiring, or discouraging any particular treatment. Clinical decisions in any particular case involve a complex analysis of the patient's condition and available courses of action. Therefore, clinical considerations may require taking a course of action that varies from the suggestions made as part of this guidance.

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Figure 1: Diagnostic algorithm monogenic IBD.

Patients with endoscopically and histologically confirmed diagnosis of inflammatory bowel disease are assessed for red flag signs that might raise suspicion for a monogenic IBD (age of onset, family history, clinical presentation and laboratory results; Table 3 &4). In patients with suspected monogenic IBD (either < 2 year of onset of IBD or > 2 year of IBD onset plus additional red flag features) a multidisciplinary team assessment will help to establish a diagnostic and therapeutic care plan. After appropriate counselling of the family, clinical sequencing will be performed based on availability of the technologies, the expected coverage of likely monogenic IBD candidate genes and urgency to have results available. The role of suspected genetic variants will be functionally validated und results will be discussed by a multidisciplinary team to assess the therapeutic consequences and to amend the diagnostic and therapeutic care plan of the patient.

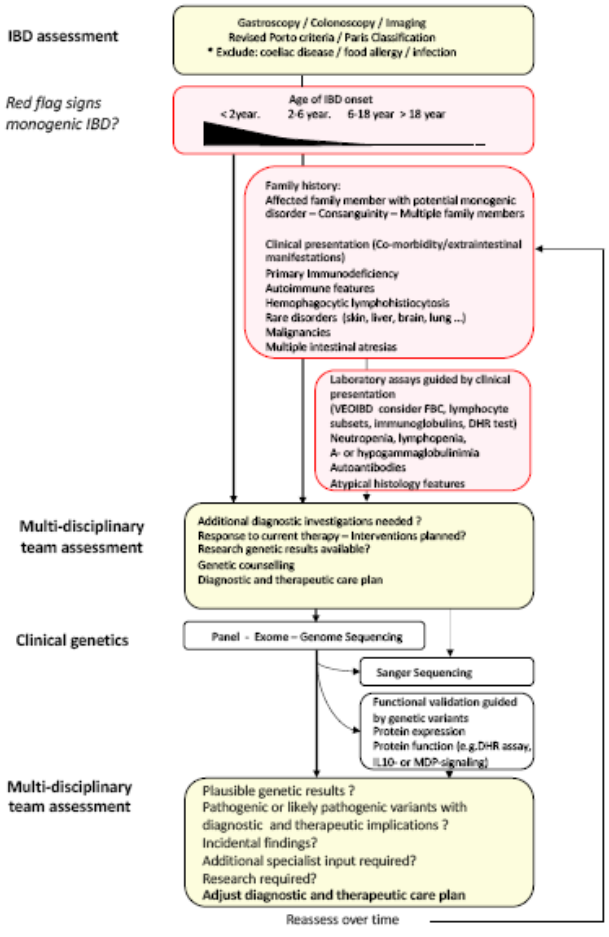

Table 1: IBD cohort studies to investigate a spectrum of monogenic IBD using next generation sequencing

Publication	Year	Cohort description Study setup Patient selection Ethnicity	Age-at-IBD diagnosis - median or mean (range; in years)	Sequencing technology / Number of candidate genes	Number of patients Total (n) / Monogenic IBD (n) / Functional validation Yes/No	Therapeutic consequences of genetic findings and Comment
Taylor et al. (21)	2014	Single center, UK IBD onset < 7 years		WGS 40 genes	Total 15 Monogenic IBD 0	N/A
Kammermeier et al. (53)	2014	Single tertiary referral center; UK Extensive disease (pancolitis or panenteritis) Diagnosis within the first 36 months of life Caucasian n=11, Asian n=14	Median 0.58 years (0.1-1.6)	Sanger sequencing TGPS n=25 WES n=20 40 genes	Total 25 Monogenic IBD 7/25 IO-IBD 19% (4/21)	HSCT assessment initiated and performed
Kelsen et al. (67)	2015	Single tertiary referral center; USA IBD onset under 5 years of age	range 0.06-5	WES 400 genes	Total 125 Monogenic 0 Hypomorphic variants Yes	N/A
Ashton et al. (80)	2016	Single tertiary center; UK Age < 18 years	Median age at diagnosis 12.2 years Median age at onset 11.04 years	WES n=147 51 genes	Total 147 Unclear** No	
Ostrowski et al. (98)	2016	Multi-center; Poland Paediatric IBD No family history of IBD	88 patients with IBD, 43 under 6 years of age, and 45 more than 40 years of age VEO-IBD : age range 1–5; median 3	WES n=43 VEO-IBD and n=45 after 40 y.o. 40 genes	Total 88 Unclear** 2 homozygote variants (*NCF4* and *WAS*) were found in two affected adults, no functional validation	
Xiao et al. (99)	2016	Single tertiary center, China VEO-IBD Chinese n=13	mean age 0.5 range: 0 to 3 years	TGPS 10 genes, including susceptibility genes	Total 13 Monogenic IBD 3 Others not clear IO-IBD 23% (3/13) No	
Petersen et al. (66)	2017	Multi-center; international Early onset IBD or chronic diarrhea Age at diagnosis < 10 years of life Caucasian n=47, Arab n=9, Turkish n=5, Other n=10	Average 3 years	Total 71 TGPS n=46 TGPS+WES n=25 28 genes, including 5	Total 71 Monogenic IBD 5 IO-IBD 13% (4/31) VEOIBD 9.26% (5/54)	HSCT initiated and performed

				susceptibility genes	Yes	
Suzuki et al. (100)	2017	Multi-center; Japan 35 patients age < 16 years, among whom 27 patients under the age of 6 Japanese n=33, Japanese- Chinese n=1, Japanese-Brazilian n=1	Mean 4.50 years	TGPS n=35 55 genes	Total 35 Monogenic IBD 5, IO-IBD 22% (2/9) Paris A1a 13.3% (4/30) Paris A1b 20% (1/5) Yes	HSCT initiated and performed
Kammermeier et al. (16)	2017	Single center, tertiary referral, UK IBD onset < 2 years 52% were White Europeans, 16% were Middle Eastern/Arab States, 8% Pakistani, 8% Indian, 6% Bangladeshi, 5% African, and 5% of mixed ethnic origin; 29% were offspring from consanguineous unions; 18% had a positive family history of IBD	Median 0.25 years (0.1-0.9)	TGPS only n=17 WES only n=37 Sanger only n=8 40 genes	Total 62 Monogenic IBD 19, IO-IBD 31% (19/62) Partial	HSCT initiated and performed
Quaranta et al. (37)	2018	Single center, tertiary referral, UK Age at IBD diagnosis 7- 40 years Severe disease (need for intestinal surgery and/or therapy progression to biologics)		WES 59 genes	Total 503 Monogenic IBD 1, Paris A1a/b 0.19% (1/503) Yes	HSCT initiated and performed
Charbit-Henrion et al. (31)	2018	Multi-center, international Clinical presentation of severe VEO-IBD (n=185) and congenital diarrhea; History suggestive of monogenic disorder (n=22) European n=200, Asian n=2 African n=3, Australia n=2	< 2 years n=144; > 6 years n=22	TNGS n=167 WES n=51 66 genes	Total 207 Monogenic IBD 66, IO-IBD 41% (59/144) VEO-IBD 33.5% (62/185) > 6 years 18% (4/22) Yes	
Amininejad et al. (38)	2018	660 early onset/familial cases among the 2390 cases with Crohn's disease		NGS 23 PID-genes	Hypomorphic variant in XIAP	no
Fang et al. (34)	2018	Single center, China IBD onset before 6 months of age or VEO-IBD accompanied with severe perianal disease, severe malnutrition or growth failure, or resistance to conventional treatment median age of disease onset was 14 mo (IQR: 0 to 72 mo) among 54 patients with VEO-IBD Chinese n=54	Median 2.9 years (0.25-14.4)	TGPS n=12 WES n=6 TGPS and WES n=2 4503 genes	Total Monogenic IBD 9 IO-IBD 19.3% (6/31) VEOIBD 16.6% (9/54) Yes	HSCT assessment initiated
Lega et al. (35)	2019	Multi-center, Italy VEO-IBD and patients with early onset IBD with severe/atypical phenotypes*	Median Monogenic IBD 2.25 years (0.83-4); Non-monogenic IBD 2 years (0.66-4))	Candidate gene n= 47 TGPS n=69 WES n=16 TrioWES n=5 Candidate genes: WES n=400 TGPS A n=30	Total 93 Monogenic IBD 12; IO-IBD 14.5% (8/55) VEO-IBD 11.5% (10/87) > 6 years 17% (2/6) Yes	HSCT n=7 Liver transplant n=1

				TGPS A n=43		
Crowley et al. (36)	2020	Single tertiary center IBD onset Canada under 18 years IBD onset European/Caucasian 566, East Asia n=19, South Asia n=104, Africa n=29, Mixed n=63, American n=65, Asian n=35, West Asian n=21, Unclassified n=103	Median 12.0 years (0-18)	WES (trio analysis) 67 genes	Total 1005 Monogenic IBD 31 Yes IO-IBD 13.8% (4/29) VEO-IBD 6.2% (7/112) 6-9.9 year 1.7% (3/179) 10-17.9 year 2.5% (17/684)	HSCT initiated and performed
Ashton et al. (81)	2020	Single tertiary center; UK Age < 18 years	Median 11.9 years (1.3-17.4)	WES n=68 68 genes	Total 401 Unclear** No	
Serra et al. (8)	2020	Multi-center, international Severe IBD disease course (previous surgery or need for biological therapy) no suspicion of monogenic IBD Caucasian n=99 African n=2, Asian n=21 Jewish n=1 Others/unknown n=22	Median 3.5 years (4-6.8)	WES 67 genes	Total 145 Monogenic IBD 4 VEO-IBD 2.75% (4/145) Mosaicism n=1 (CYBB) Yes	HSCT assessment initiated in several patients
Uchida et al. **JPGN**	2020	Multi-center, Japan Age < 17 years, early-onset diarrhea, refractory to conventional therapies Japanese n=107, Japanese-Laotian n=1	Median age at onset 3.82 (IQR 2.50) years	TGPS 193 genes	Total 108 Monogenic IBD 15 VEO-IBD 9.9% (8/81) IO-IBD 17.1% (7/41) 6-10 years 38.4% (5/13) 10-17 years 14.3% (2/14) Yes	

Abbreviation: WES Exome sequencing, WGS genome sequencing, TPS Targeted panel sequencing, HSCT

*(severe perianal disease, recurrent/atypical infections, skin/annexes abnormalities, abnormal immune status, associated multiple/severe autoimmunity, history of macrophage activation syndrome or hemophagocytic lymphohistiocytosis, intestinal atresia, or early development of tumors); ** in these articles, no functional validation of novel variants was performed. Many variants are found at unexpectedly high allele frequencies (ExAC and gnomAD databases), thus pathogenicity and inheritance pattern is unclear.

Table 2: Clinical features associated with monogenic IBD in cohort studies.

Author	Year	Patient numbers (n)	Genetic defects	Age of onset	Features significantly associated with monogenic IBD
Kammermeier et al. (16)	2017	Monogenic IBD: 19 Control IBD: 43	*EPCAM (n=3)* *IL10 (n=2)* *IL10RA (n=1)* *IL10RB (n=2)* *FOXP3 (n=3)* *LRBA (n=1)* *SKIV2L (n=2)* *TTC37 (n=2)* *TTC7A (n=3)*	Mean age of onset 2 months monogenic 8.3 months control	Consanguinity Disease onset < 6 months Height-for-age z-score < -3 Extensive disease Epithelial abnormality Parenteral nutrition required
Fang et al. (34)	2018	Monogenic IBD: 9 Control IBD: 45	*IL10R (n=5)* *CYBB (n=2)* *XIAP (n=1)* *CVID with TNFRSF13B (n=1)*	Median age of onset 1 months monogenic, 19.5 months control	Incidence of perianal disease Use of Mesalazine Death
Kim et al. (15)	2018	Monogenic IBD: 18 (not all genes specified) Control IBD: 212	*CGD (n =3)* *IPEX (n=2)* *GSD (n=1)* *Congenital neutropenia (n=2)* *Hyper immunoglobulin (Ig)M syndrome (n=1)* *Hypogammaglobulinemia (n=1)* *IL-10 (n=8)*	Mean age of diagnosis 1.6 year monogenic, 11.7 year control	Incidence of surgery per year Hospitalization per year Height < 3rd percentile Weight < 3rd percentile IBD-U
Lega et al. (35)	2019	Monogenic IBD: 12 Control IBD: 81	*XIAP (n=2)* *WAS (n=3)* *TTC37 (n=1)* *DKC1 (n=1)* *CD40L (n=2)* *CYBA (n=1)* *CYBB (n=1)* *FOXP3 (n=1)*	Mean age of onset 27 month monogenic, 24 month control	Males (n) Extraintestinal findings Disease onset </= 1 month Disease location (colon only) Disease location (colon + other location) Extraintestinal: infections Extraintestinal: HLH/MAS Extraintestinal: skin disease Low platelets Low Immunoglobulin

					Lymphocyte subset abnormalities
Crowley et al. (36)	2020	Monogenic IBD: 31 Control IBD: 974	*ALPI (n=1)* *COL7A1 (n=1)* *GUCY2C (n=2)* *SLCO2A1 (n=1)* *TTC7A (n=1)* *ARPC1B (n=2)* *BTK (n=1)* *DKC1 (n=1)* *DOCK8 (n=3)* *LRBA (n=2)* *STAT 1 (n=1)* *HPS1 (n=1)* *PIK3CD (n=1)* *SH2D1A (n=1)* *XIAP (n=5)* *CYBB (n=1)* *CTLA4 (n=1)* *FOXP3 (n=2)* *IL10RB (n=1)* *HSPA1L (n=1)* *MASP2 (n=1)*	Mean age of onset 9.69 years monogenic	Age at diagnosis < 2 years Family history of autoimmune disease Any extraintestinal manifestation > 1 extraintestinal manifestation Progression to surgical therapy

Table 3: ***Phenotypic features of monogenic IBD (exemplars)***

Phenotypic features		*Exemplar disorder and gene defect*
Infection	Recurrent typical (e.g. *Staphylococcus aureus* or single/recurrent atypical infections (e.g. mycobacterial, fungal or cytomegalovirus in patients without immunosuppressive therapy	Primary immunodeficiency e.g. chronic granulomatous disease
	Hemophagocytic lymphohistiocytosis (HLH)	
Immune activation with and without infection		*XIAP(101)* and *STXBP2(102).* *HLH* is not specific since a known complication of cytomegalovirus and Epstein-Barr virus infection in patients receiving immunomodulatory medications including thiopurines (103)
	Immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX or IPEX-like) syndrome	
Autoimmune features	Oral leukoplakia	*FOXP (41), LRBA (45), CTLA4 (63), STAT3 (104),* and *STAT1*(105).
	Ectodermal dysplasia with dysplastic nails in conical teeth	
Dermatological features	Woolly hair with trichorrhexis nodosa	Telomeropathies (106)
	B cell lymphomas	NF kappa B essential modulator (NEMO) *IKBKG* defects (107)
	Gastric adenocarcinomas	
Tumors	Multiple intestinal atresia	Trichoenterohepatic syndrome due to *TTC37A* (108) or *SKIVL2* (109)
		IL-10 signalling defects (110)
		CTLA4 (46) and *LRBA (111)*
Intestine		*TTC7A (112)*
Endoscopic and histology features	Complex perianal fistulizing disease accompanying luminal inflammation, especially if manifest in the first year of life	*IL10, IL10RA and IL10RB* (49), *TGFB1 (113),* or XIAP (40, 70).
	Intestinal epithelial cell apoptosis	

	Tissue eosinophilia	*TTC7A, LRBA, XIAP,* SH2D1A, ARPC1B or *COL7A1 (29, 112, 114)*
	Enteropathy with villous flattening, similar to celiac disease	IPEX, IPEX-like syndrome or *WAS* (115)
	Germinal cell hypoplasia	IPEX, or IPEX-like syndrome and CVID (41, 44, 63)
	Granulomas and pigmented macrophages	Defect in humoral immunity such as in ICOS or BTK deficiency (116, 117) Chronic granulomatous disease

Table 4 Inflammatory markers, immunology, and infectious diseases workup.

	Test	Indication and examples of monogenic IBD disease groups were abnormal results expected
Blood work essential	CBC	
	Neutrophils	Autoimmune neutropenia Neutrophilia (Leucocyte adhesion deficiency)
	Thrombocytes	Autoimmune thrombocytopenia
	Lymphocytes	Congenital neutropenia
	Inflammatory markers (CRP, ESR)	Inflammation activity - non specific
Basic immune blood work	Immunoglobulin classes (IgA, IgG, IgM, IgE; *age-specific normal range)*	CVID Agammaglobulinemia
	Lymphocyte subsets	CVID, Agammaglobulinemia
	DHR testing	Chronic granulomatous disease
Blood tests to consider depending on presentation	TREC/TCR repertoire	Hypomorphic SCID
	Vaccine antibodies (v*accination history)*	Infection susceptibility CVID, Agammaglobulinemia
	Autoantibodies	Exclude coeliac disease
	coeliac screen	Autoimmune enteropathy
	Anti-enterocyte antibodies	IPEX and IPEX-like
	Thyroid function tests	Autoimmune hepatitis, thyroiditis, diabetes etc.

	Liver function test Metabolic workup FOXP3 or XIAP-expression* MDP-monocyte stimulation assay IL10-induced phospho-STAT3 or LPS/IL-10 suppression-assay	 Glycogen storage disease IB Niemann Pick Type C IPEX, XIAP deficiency XIAP deficiency IL-10 signalling defects
Basic infection screen	TB Elispot assay HIV serology	Exclude infections
Stool tests	*Microbiology to exclude bacterial and parasitic enteric infections* Calprotectin stool	Exclude infections Inflammation activity - non specific

Abbreviations: TREC/TCR T-cell Receptor Excision Circles/ T cell receptor oligoclonal expansion, DHR dihydrorhodamin test, CVID combined variable immunodeficiency, IPEX, TB tuberculosis, HIV human immunodeficiency virus, MDP muramyl dipeptide

*A normal expression does not exclude FOXP3 or XIAP deficiency but a substantial proportion of patients can be detected.

Table 5: Monogenic IBD gene panel suggested by specialist reviews and consensus (75 genes).

Table 3 Monogenic IBD gene panel **75**

Gene	Disorder	Inheritance AR/AD/X	Uhlig & Muise 2017	Duabeg et al. 2020	Kelsen et al. 2019	Pazmandi et al. 2019	consensus
ADA	Atypical SCID	AR	+	+		+	+
ADAM17	Inflammatory skin and bowel disease, neonatal	AR	+	+	+	+	+
AICDA	Immunodeficiency with hyper-IgM	AR	+	+	+	+	+
ALPI	Intestinal Alkaline Phosphatase deficiency	AR				+	+
ARPC1B	Wiskott–Aldrich syndrome-like	AR	+	+			+
BTK	Agammaglobulinemia, X-linked 1	XL	+	+	+	+	+
CASP8	Caspase-8 deficiency	AR		+		+	+
CD3G	Atypical SCID	AR	+	+			+
CD40LG	Immunodeficiency, X-linked, with hyper-IgM	XL	+	+	+	+	+
CD55	CHAPLE syndrome	AR		+		+	+
COL7A1	Dystrophic epidermolysis bullosa	AR	+	+			+
CTLA4	Autoimmune lymphoproliferative syndrome, type V	AD	+	+	+	+	+
CYBA	Chronic granulomatous disease	AR	+	+	+	+	+
CYBB	Chronic granulomatous disease	XL	+	+	+	+	+
DCLRE1C	Omenn syndrome	AR	+	+		+	+
DKC1	Dyskeratosis congenita - Hoyeraal Hreidarsson Syndrome	XL	+	+	+	+	+
DOCK8	Dedicator of Cytokinesis 8 (DOCK8) deficiency - atypical?	AR			+		+
DUOX2	Dual Oxidase 2 (DUOX2) deficiency	AR				+	
FCN3	Ficolin 3 defect					+	
FERMT1	Kindler syndrome	AR	+	+		+	+
FOXP3	Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome	XL	+	+	+	+	+
G6PC3	Congenital neutropenia	AR	+	+		+	+
GUCY2C	Familial diarrhea	AD	+	+		+	+
HPS1	Hermansky-Pudlak syndrome (type 1)	AR	+	+			+
HPS4	Hermansky-Pudlak syndrome (type 4)	AR	+	+	+		+
HPS6	Hermansky-Pudlak syndrome (type 6)	AR	+	+			+
ICOS	COS deficiency	AR	+	+	+	+	+
IKBKG	X-linked ectodermal dysplasia, anhidrotic and immunodeficiency	XL	+	+	+		+
IL10	IL10 deficiency	AR	+	+	+	+	+
IL10RA	IL10 receptor deficiency	AR	+	+	+	+	+
IL10RB	IL10 receptor deficiency	AR	+	+	+	+	+
IL21	IL21 deficiency (Combined variable immunodeficiency-like 11)	AR	+	+	+		+
IL2RA	IPEX-like (Immunodeficiency with lymphoproliferation and autoimmunity)	AR	+	+	+	+	+
IL2RB	IL2RB Immune Dysregulation	AR			+		+
IL2RG	Atypical SCID	XL	+	+		+	+
ITCH	ITCH deficiency	AR			+	+	+
ITGB2	Leukocyte adhesion deficiency 1	AR	+	+	+	+	+
JAK1	JAK1	AD		+			
LIG4	Atypical SCID	AR	+	+	+	+	+
LRBA	Combined variable immunodeficiency (CVID 8)	AR	+	+	+	+	+
MALT1	MALT1 deficiency (IPEX-like)	AR	+	+			+
MASP2	Mannan Binding Lectin Serine Peptidase 2 defect	AR	+			+	+
MEFV	Familial Mediterranean fever	AR	+	+	+	+	
MVK	Mevalonate kinase deficiency	AR	+	+	+	+	+
NCF1	Chronic granulomatous disease	AR	+	+		+	+
NCF2	Chronic granulomatous disease	AR	+	+	+	+	+
NCF4	Chronic granulomatous disease	AR	+	+	+	+	+
NFAT5	NFAT5 variant	AD			+		
NLRC4	Autoinflammation with infantile enterocolitis	AD	+		+	+	+
NOX1	NADPH Oxidase 1 deficiency	XL				+	
NPC1	Niemann-Pick type C disease	AR	+	+		+	+
ORAI1	ORAI1 deficiency					+	
PIK3CD	PIK3CD deficiency & PI3K activation syndrome	AR & AD	+	+	+	+	+
PIK3R1	Agammaglobulinemia type 7 & Activated PI3K syndrome	AR & AD	+	+	+	+	+
PLA2G4	Phospholipase A2 defect					+	
PLCG2	Autoinflammation, antibody deficiency, and immune dysregulation syndrome	AD	+	+	+	+	+
POLA1	PDR syndrome (pigmentary disorder, reticulate, with systemic manifestation)	XL	+			+	+
PTEN	PTEN hamartoma tumor syndrome	AD	+			+	
RAG1	Atypical SCID	AR	+			+	+
RAG2	Atypical SCID	AR	+	+		+	+
RIPK1	RIPK1 deficiency	AR		+			+
RTEL1	Dyskeratosis congenita - Hoyeraal Hreidarsson Syndrome	AR / AD	+	+	+	+	+
SH2D1A	X-linked lymphoproliferative syndrome 1 (XLP1)	XL	+			+	+
SKIV2L	Trichohepatoenteric syndrome 2	AR	+	+	+	+	+
SLC37A4	Glycogen storage disease type 1b	AR	+	+			+
SLC7A4	???				+		
SLC9A3	Congenital diarrhea	AR	+	+		+	+
SLCO2A1	Prostaglandin transporter deficiency	AR	+	+		+	+
STAT1	IPEX-like	AD	+	+	+		+
STAT3	Autoimmune disease, multisystem, infantile-onset, 1	AD	+	+	+	+	+
STIM1	STIM1 deficiency				+	+	+
STXBP2	Familial hemophagocytic lymphohistiocytosis type 5	AR	+	+	+	+	+
STXBP3	Syntaxin binding protein 3 defect	AD/AR			+		+
TGFB1	TGFB1 deficiency	AR				+	+
TGFB2	TGFB2 defect					+	
TGFBR1	Loeys-Dietz-Syndrom 1	AD	+	+			+
TGFBR2	Loeys-Dietz syndrome 2	AD	+	+			+
TNFAIP3	Autoinflammatory syndrome, familial, Behcet-like Syndrome	AD				+	+
TRIM22	TRIM22 defect	AR	+	+		+	+
TRNT1	SIFD (sideroblastic anemia, immunodeficiency, periodic fevers and developmental d	AR				+	+
TTC37	Trichohepatoenteric syndrome 1	AR	+	+	+	+	+
TTC7A	TTC7A deficiency	AR	+	+	+	+	+
WAS	Wiskott–Aldrich syndrome	XL	+	+	+	+	+
XIAP	X-linked lymphoproliferative syndrome 2 (XLP2)	XL	+	+	+	+	+
ZAP70	Atypical SCID	AR	+	+		+	+
ZBTB24	Immunodeficiency, centromeric instability and facial anomalies (ICF) syndrome	AR		+		+	+

Gene lists are derived from four review articles (10-13). Inheritance autosomal recessive AR, autosomal dominant AD, X-linked XL.

Table 6: Statements

Statements	Consensus rate
1. The diagnostic process and care of a patient with suspected or confirmed monogenic IBD is best coordinated by a multidisciplinary team of specialists, including gastroenterologists, geneticists, immunologists, and other subspecialists contingent on the individual gene defect, comorbidities and extraintestinal manifestations	32/32 (100%)
2. Next-generation DNA sequencing technologies are recommended to diagnose known monogenic causes of IBD in routine clinical practice	32/32 (100%)
3. Genetic screening for monogenic IBD is recommended in all patients with infantile onset IBD (<2 years) and should be considered in patients with very early onset IBD (<6 years), in particular in those patients with relevant comorbidity, extraintestinal manifestations and/or family history	31/32 (97%)
4. Although a rare or very rare diagnosis, a monogenic form of IBD should be considered in patients with any paediatric or adult age IBD onset if they present with relevant comorbidity, extraintestinal manifestations and/or family history	27/32 (84%)
5. Routine genetic screening for all IBD patients is not recommended since a monogenic cause of IBD in patients with IBD onset over 6 year of age, especially those with adolescent or adult age onset of IBD is exceptional in the absence of relevant comorbidity	32/32 (100%)
6. Genetic investigations to establish monogenic IBD are recommended in advance of hematopoietic stem cell transplantation unless the bowel inflammation can be clearly explained (e.g. drug induced colitis)	32/32 (100%)
7. Panel sequencing, exome, and genome sequencing technologies have complementary diagnostic strength; the first-line technology should be guided by availability and degree of diagnostic suspicion	30/32 (94%)
8. Functional assessment of novel gene defects and variants of unknown significance is necessary to establish causality	32/32 (100%)
9. Patients and their families with suspected or confirmed monogenic IBD should be offered the opportunity to participate in research studies. A therapeutically relevant genetic result established in a research setting should be confirmed in a clinical genetics setting	30/32 (94%)

Comments

*Relevant comorbidities and extraintestinal manifestations and family history are summarised in Box2.

Box 1: Key themes identified to assess utilisation of Clinical Genomics for diagnosis of monogenic IBD.

1. Is there evidence to support the clinical use of genomic sequencing technologies for diagnosis of monogenic forms of IBD?
2. Which patients should be investigated by genomic technologies?
3 What Mendelian disorders (genes) should be included in a screening panel?
4. Are there preferred genomic sequencing technologies (targeted panel sequencing, whole exome and whole genome sequencing)?
5. What is the role of functional validation to establish causality?
6. What is the role of a multidisciplinary team in the care of patients with suspected monogenic IBD (Paediatric/adult gastroenterology, immunology, clinical genetics, other disciplines)?
7. Is there evidence of cost-effectiveness of genomic screening technologies for diagnosis of IBD?

Box 2: Summary of clinical features that should prompt considering a monogenic IBD workup (Red flag signs)

Age of IBD presentation

<2 years IBD symptom onset

<6 years IBD symptom onset in particular when other red flag signs are present

Family history

- Affected family member with a suspected monogenic disorder
- Consanguinity
- Multiple family members with early onset IBD

Comorbidity and extraintestinal manifestations are particularly relevant for monogenic IBD diagnostic considerations when rare or atypical for patient age irrespective of organ manifestation

- Recurrent severe infections or atypical infections consistent with diagnostic criteria of a Primary Immunodeficiency
- Hemophagocytic lymphohistiocytosis
- Autoimmune features in particular features of Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome
- Malignancies
- Multiple intestinal atresias

Box 3: Specific considerations for low resource countries

- To support patients and families with suspected monogenic IBD within low resource settings, knowledge on local health care pathways, health care insurance provision, ethnic and religious considerations are important to choose effective and pragmatic diagnostic pathways and to plan subsequent provision of relevant therapies
- Socioeconomic conditions and regional environmental factors such as infections that can mimic IBD and primary immunodeficiencies within different parts of the world (environmental enteropathy, intestinal tuberculosis etc.) and are therefore important considerations to assess the pre-test risk of monogenic IBD
- Access to multidisciplinary teams might be facilitated by centralised care of children with suspected monogenic IBD within each country and via international collaboration
- Access to multidisciplinary teams might be facilitated via virtual clinics
- Sanger sequencing can be a cost-effective test strategy in particular in regions with enrichment of known genetic variants due to founder effects
- Participation in international research projects can provide genetic testing and functional validation in settings were routine genetic and immunological clinical resources and health care utilisation are not available