Diagnostic Approach to Monogenic Inflammatory Bowel Disease in Clinical Practice: A Ten-Year Multicentric Experience

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Background and aims: Multiple monogenic disorders present as very early onset inflammatory bowel disease (VEO-IBD) or as IBD with severe and atypical features. Establishing a genetic diagnosis may change patients' management and prognosis. In this study, we describe the diagnostic approach to suspected monogenic IBD in a real clinical setting, discussing genetic and phenotypic findings and therapeutic implications of molecular diagnosis.

Methods: Information of patients with VEO-IBD and early onset IBD with severe/atypical phenotypes (EO-IBD s/a) managed between 2008–2017 who underwent a genetic workup were collected.

Results: Ninety-three patients were included, and 12 (13%) reached a genetic diagnosis. Candidate sequencing (CS) was performed in 47 patients (50%), and next generation sequencing (NGS) was performed in 84 patients (90%). Candidate sequencing had a good diagnostic performance only when guided by clinical features specific for known monogenic diseases, whereas NGS helped finding new causative genetic variants and would have anticipated one monogenic diagnosis (XIAP) and consequent bone marrow transplant (BMT). Patients with monogenic IBD more frequently were male (92% vs 54%; $P = 0.02$), had extraintestinal findings (100% vs 34%; $P < 0.001$), and had disease onset ≤1 month of life (25% vs 1%; $P = 0.006$). Genetic diagnosis impacted patient management in 11 patients (92%), 7 of whom underwent BMT.

Conclusion: A genetic diagnosis can be established in a significant proportion of suspected monogenic IBD and has an impact on patients' management. Candidate sequencing may be deployed when clinical findings orientate toward a specific diagnosis. Next generation sequencing should be preferred in patients with nonspecific phenotypes.

Key Words: very early onset IBD, monogenic IBD, next generation sequencing

INTRODUCTION

Up to 15% of patients with very early onset inflammatory bowel diseases (VEO-IBD), defined as IBD rising before the age of 6 years, may have a rare monogenic disorder.[1–3] Although more rarely monogenic defects, such as XIAP deficiency or neutrophil defects, have been observed also in later onset IBD.[4, 5]

The spectrum of monogenic disorders presenting with intestinal inflammation is quite varied, and to date, causative genetic variants have been recognized in more than 50 genes involved in innate and adaptive immune functions, inflammatory homeostasis, and intestinal epithelial barrier functions.[6] Irrespective of the age at disease onset, monogenic IBD tends to have a more severe prognosis compared with conventional polygenic IBD due to the extent of gastrointestinal involvement and the presence of extraintestinal manifestations.[7] Early genetic diagnosis of monogenic IBD is essential to determine the correct prognosis and adequate treatment strategy that often differs from conventional polygenic IBD and might include bone marrow transplantation (BMT). However, due to the wide phenotypic and genetic heterogeneity of these conditions and the lack of specific endoscopic or histological findings, it is often difficult to reach a molecular diagnosis. In the last decade, with the advent of next generation sequencing (NGS) techniques, the diagnostic approach to monogenic IBD has shifted from deep phenotyping followed by sequential candidate gene sequencing toward early parallel candidate

Received for publications April 22, 2019; Editorial Decision July 6, 2019.

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Supported by: This work was supported by the Institute for Maternal and Child Health "Burlo Garofolo," Trieste, Italy (grant number RC 17/14 and RC 24/17), Italian Ministero della Salute (grant number NET-2011-02350069), Childrens' Hospital Bambino Gesù, Rome, Italy (RC number 2017-02P003966), and Fondazione Telethon (TIGET Core grant C6).

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sequencing using targeted gene panels sequencing (TGPS) or whole exome sequencing (WES) as the first line molecular diagnostic tool.

Next generation sequencing offers the advantage to look simultaneously at multiple genes and seems to be less time consuming and less expensive compared with sequential sequencing in patients presenting nonspecific clinical phenotype.[1]

In the present study, we aim to describe the diagnostic approach to suspected monogenic IBD in a real clinical setting during a 10-year period, discussing the genetic findings and therapeutic implications. Based on our observation, we analyze advantages and disadvantages of different diagnostic strategies and suggest a practical diagnostic strategy to suspected monogenic IBD for the clinical practice.

MATERIALS AND METHODS

Patients Population and Study Design

This was a multicenter observational cohort study. Patients diagnosed with VEO-IBD and patients with early onset IBD with severe/atypical phenotypes (EO-IBD s/a) managed at 2 main pediatric gastroenterology centers in the last 10 years (2008 to 2017) and patients referred for a genetic workup from 9 external gastroenterology facilities were included.

The definition of severe/atypical phenotype was applied when at least one of the following clinical findings were present: 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. Demographic data and information on gastrointestinal disease, extraintestinal manifestations, and treatments were retrieved from medical records. In the first part of the study, information of interest was retrospectively collected from medical records and included in a dedicated database. Starting from 2015, newly diagnosed patients with VEO-IBD and EO-IBD s/a and patients without a previous definite genetic diagnosis were prospectively recruited for genetic workup. The work was conducted in accordance with the revised Declaration of Helsinki and was approved by Burlo Garofolo and the Bambino Gesù Ethics committee. Written informed parental consent was obtained for genetic analysis.

Diagnostic Workup

In the prospective phase of the study, patients enrolled for genetic workup were screened using NGS technologies, with the exception of patients with well-defined phenotypes, suggestive of a specific monogenic disorder, for whom single gene sequencing was chosen. A targeted gene panel sequencing (TGPS) analysis was performed in the majority of patients as the first line diagnostic tool. Beginning in 2017, WES replaced TGPS due to a significant decrease in WES costs. Whole exome sequencing analysis was initially restricted to a set of 400 genes plus the list of genes associated with primary immunodeficiency and related pathways as described by Kelsen et al.[8] Trio whole exome sequencing (trioWES) was used in selected cases of patients with infantile-onset IBD (IO-IBD) and severe disease when parental DNA was available. Basic immunological workup included complete blood count, immunoglobulin levels, lymphocyte subsets, and neutrophil function studies.

Targeted Gene Panel Design

Two custom-made panels for TGPS were designed: the first panel, designed at Burlo Garofolo, included 30 genes (Panel A); the second panel, designed at Bambino Gesù Hospital, included 43 genes (Panel B). The full list of genes included in the panels and gene coverage is illustrated in online supplementary material (Table 1S). Gene selection for both panels was based on lists of genes suggested by Kammermeier et al,[1] Uhlig et al,[6] and Christodoulou et al.[9] Genes associated with diseases presenting with well-defined phenotypes that had valid structured functional tests, such as Wiskott-Aldrich syndrome (WAS) and Hyper IgM syndrome (HIGM), were not included in the panels. See the online supplementary material for a description of DNA library preparation and raw data analysis.

Variant Selection and Validation

Data were filtered selecting nonsynonymous, nonsense, frameshift, splicing (about 10 nucleotides from the splice site), and variants, which were either absent or had a minor allele frequency (MAF) <0.02 (in case of recessive model) or MAF <0.001 (in case of dominant inheritance model and in case of de novo variants). Minor allele frequency selection was based on 1000 GenomesProject (1000genomes.org) database and ExAC browser (exac.broadinstitute.org). Moreover, all variants were interrogated by Genomic Evolutionary Rate Profiling (GERP) score as a measure of the conservation of the genomic position.[10] Genetic variants were classified according to the American College of Medical Genetics (ACMG) guidelines[11] into "pathogenic," "likely pathogenic," or "variants of uncertain significance" using dedicated tools.[12] Nonsynonymous variants were further selected according to 5 different in silico prediction tools, namely CADD (score > 15),[13] Mutation Taster,[14] Polyphen-2,[15] SIFT,[16] and LRT.[17] Among the selected variants, those with a pathogenic prediction in at least 2 out of the 4 tools were retained. Human Splicing Finder v3.1 (umd.be/HSF3) was used to predict the effect of splicing variants.

The clinical significance of variants, already described in public databases, and the association with specific phenotypes were investigated using OMIM (omim.org), ClinVar (ncbi.nlm.nih.gov/clinvar), and HGMD (Human Gene Mutation Database) professional. For novel mutations, pathogenicity was established with a functional assay, when available, or inferred from similar mutations with known clinical significance or based on the presence of highly specific clinical features. See

the online supplementary material (Figure 1S) for variant selection process flow-chart.

Variants considered to be causative, according to the clinical phenotype and the mode of inheritance, were validated by Sanger Sequencing in patients and their parents, when available, after visualizing the read coverage of each mutation using the Integrative Genomics Viewer (IGV) (software.broadinstitute.org/software/igv).[18, 19] Primers were designed using Primer Blast tool (ncbi.nlm.nih.gov/tools/primer- blast) and synthesized by Eurofins Genomics (eurofinsgenomics.eu). DNA regions were amplified by standard PCR protocols and sequenced in both directions. Sequences were evaluated using CodonCode Aligner 6.0.

Statistical Analysis

Statistical analyses were made using GraphPad Prism version 8. Categorical variables were summarized as frequency and percentage and were compared across independent groups by the Fisher exact test. Numerical variables with asymmetrical distribution were summarized by median and interquartile range (IQR) and were compared by the Kruskal-Wallis test. A P value <0.05 was considered for significance.

RESULTS

Patients Population

A total of 93 patients diagnosed with VEO-IBD and EO-IBD s/a were collected; of these, 55 patients (59%) had disease onset within the first 2 years of life, and 6 patients (6%) had disease onset above 6 years. Fifty-five patients (59%) were males; 7 patients (8%) had a family history of IBD among first degree relatives; 2 patients (2%) had a sibling who had died in infancy or early childhood.

Genetic Workup and Diagnoses

Forty-seven patients (50%) underwent Sanger sequencing of 1 or multiple genes over time. In 8 patients, single gene sequencing was guided by the presence of specific clinical and immunological features. Next generation sequencing was performed in 84 patients (90%) and consisted of TGPS in 69 of 84 patients (82%), WES in 16 (19%), and trio-WES in 5 (6%). Of the patients who underwent NGS, 38 (45%) had been studied previously with a single gene approach and had remained without a genetic diagnosis. The proportion of patients who underwent NGS as the first molecular analysis has increased over time. Among patients diagnosed with IBD before the year 2011, only 25% (7 of 29 patients) underwent NGS as the first genetic analysis; the proportion raised to 45% (16 of 35) between 2011 and 2014 and to 79% (23 of 29) after 2015.

Genetic analysis revealed 12 cases (13%) of monogenic IBD. The clinical and genetic characteristics of patients diagnosed as monogenic IBD are summarized in Table 1. A subdivision of monogenic patients according to functional defect category is illustrated in Figure 1. A single gene approach was diagnostic in 8 out of 47 patients (2WAS, CYBA, CYBB, FOXP3, 2CD40L, XIAP). In 7 out of the 8 patients diagnosed with Sanger sequencing, the analysis was guided by the presence of disease specific features. One patient with XIAP deficiency who had nonspecific presentation underwent sequential sequencing of multiple genes over a period of 15 months. During this time, the patient experienced recurrent bouts of HLH, failed several immunosuppressive therapies, became dependent on parenteral nutrition, and ultimately underwent a total colectomy. After the diagnosis of XIAP deficiency, he received BMT that led to a complete cure.[20]

Next generation sequencing was performed as a first step in 46 patients and revealed causative genetic defects, all of them through TGPS, in 3 patients (6%) (ie, TTC37, DKC1, XIAP). Thirty-eight patients underwent NGS as a second step. Among these, only 1 patient with WAS, in whom Sanger sequencing had not revealed mutations, was diagnosed elsewhere by whole genome sequencing that showed a large genomic inversion.[21] Additionally, with the use of WES, a rare homozygous variant on NOD2 nucleotide-binding domain was found in 1 male patient with IBD onset at the age of 5 months and associated arthritis. Even though he could not be included in the monogenic group, the role of such variant could be better defined through bioinformatics and functional studies, which demonstrated that the consequence of the mutation was an auto-activation of NOD2-mediated NF-kB signaling, similar to that described in patients with Blau Syndrome.[22]

The diagnostic steps and the rates of monogenic diagnosis with the different diagnostic approaches are summerized in Figure 2.

Genetic diagnosis impacted patient management in 11 patients (92%): 7 patients (2XIAP, 2WAS, 2CD40L, FOXP3) underwent BMT; 1 patient with WAS gene inversion introduced anti IL-1 antagonist (anakinra), which led to the resolution of a severe pyoderma gangrenous and arthritis before undergoing gene therapy;[21] 2 patients with chronic granulomatous disease (CGD) introduced anti-infective prophylaxis; and the patient with dyskeratosis congenita (DKC1) introduced danazole as a telomere elongating therapy.

Clinical, Endoscopic, and Laboratoristic Findings

Within the entire cohort, 69 patients (74%) presented with bloody diarrhea; failure to thrive was present in 53 patients (58%). The intestinal disease was isolated to the colon in 50 patients (54%), involved the colon plus the small bowel and/or the perianal area in 38 patients (40%), and was isolated to the small bowel in 2 patients (2%); perianal disease was present in 20 patients (22%). The initial endoscopic diagnosis was consistent with IBD-U in 26 patients (28%), CD in 21 patients (23%), and UC in 18 patients (19%). Eighteen patients were classified as CD-like phenotypes (19%); 9 patients (10%) were

TABLE 1. Clinical and Genetic Characteristics of Patients Diagnosed as Monogenic IBD

Patient (sex)	IBD Onset (months)	Initial Endoscopy	GI Disease	Extraintestinal Findings	Lab Workup	Treatment	Genetic Variant (zygosity)	Impact of Genotype
1 (M)	2	AI	Extensive colitis Apoptosis	Persistent fever, CMV infection, HLH	Normal	EN, steroids, AZA, Anti-TNF, tacrolimus, colectomy,	XIAP; NM_001167, c.1021_1022delAA:p.N341YfsX7 (hem)	BMT
2 (M)	108	CD-like	Colitis, p.	Arthritis, cutaneous vasculitis, PG, uveitis, nephritis	↓ PLT, ↑IgA,↓IgM,IgG	Steroids, anti-TNF, MTX cyclosporine, thalidomide, fistulotomy, colectomy	WAS gene inversion (hem)	Anti IL-1, gene therapy
3 (M)	0	EOS	Extensive colitis	CMV infection	↓ PLT	Steroids	WAS: NM_000377, c.257G>A:p.R86H (hem)	BMT
4 (F)	96	CD-like	Colitis, p	Trichorhexis nodosa, syndromic facies, epatopathy	↑ Ig A, ↓ MBC	Anti-TNF	TTC37: NM_014639, c.4497-1G>A (hom)	Genetic counseling
5 (M)	16	CD-like	Enterocolitis, apoptosis	Leukoplakia, nail dystrophy, skin reticulate	↓ NK, B	Steroids, 5-ASA, anti-TNF, thalidomide, colectomy	DKC1: NM_001363, c.146C>T:p.T49M (hem)	Danazole
6 (M)	20	CD-like	Enterocolitis, p, ileal fistulas	Recurrent respiratory infections	↓Treg & B, ↑IgM,↓IgA,IgG	EN, ileostomy	CD40L: NM_000074, c.585dupA:p.L195fs (hem)	BMT
7 (M)	48	IBD-U	Colitis	Sclerosing colangitis, cryptosporidium	↓ B, ↓ Ig, ↑Eos	EN, steroids,	CD40L: NM_000074, c. 410-2A>T (hem)	BMT, liver transplant
8 (M)	10	IBD-U	Enterocolitis	Liver abscess, eczema	DHR defective	EN, steroids, 5-ASA	CYBA NM_000101 del ex6 (hom)	Antibiotic prophylaxis
9 (M)	30	CD-like	Colitis, p	Skin granulomas, systemic infections	DHR defective	EN, steroids, 5-ASA, AZA	CYBB NM_000397, c.252G> A 3' exon 3^{++}(hem)	Antibiotic prophylaxis
10 (M)	70	CD-like	Enteropathy	Complicated EBV, HLH	↓ Ig	EN, steroids, AZA, anti-TNF	XIAP: NM_001167, c.566T>C:p.L189P (hem)	BMT
11 (M)	1	CD-like	Enterocolitis	Candidiasis, psoriasis, opportunistic infections	↓ PLT	EN, steroids	FOXP3 NM_014009, c.1078C>T:p.L360F (hem)	BMT
12 (M)	1	CD-like	Enterocolitis	Arthritis, severe infections, eczema	↓ PLT, ↓ WBC	EN, steroids, 5-ASA, cyclosporine.	WAS, na° (hem)	BMT

Abbreviations: AI, autoimmune enteritis; AC, allergic colitis; EOS, eosinophilic enteropathy; p, perianal disease; PG, pyoderma gangrenosum, PLT, platelets, WBC, white blood cells; EN, enteral nutrition; AZA, azathioprine; MTX, methotrexate; BMT, bone marrow transplantation; MBC, Memory B cells; $^{++}$splice-site mutation; °not available.

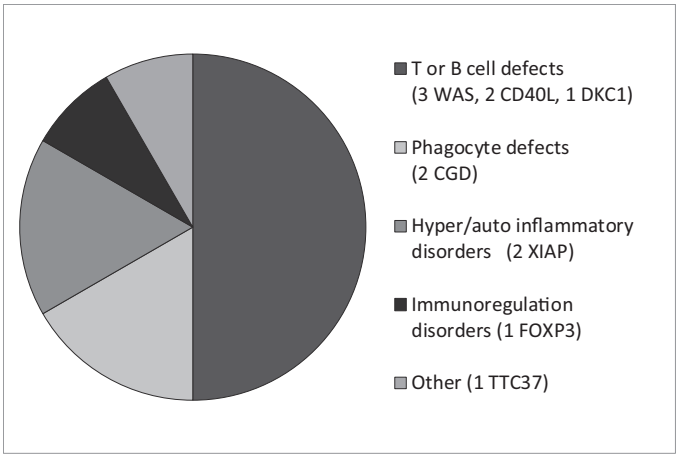

FIGURE 1. Functional defect category of monogenic patients.

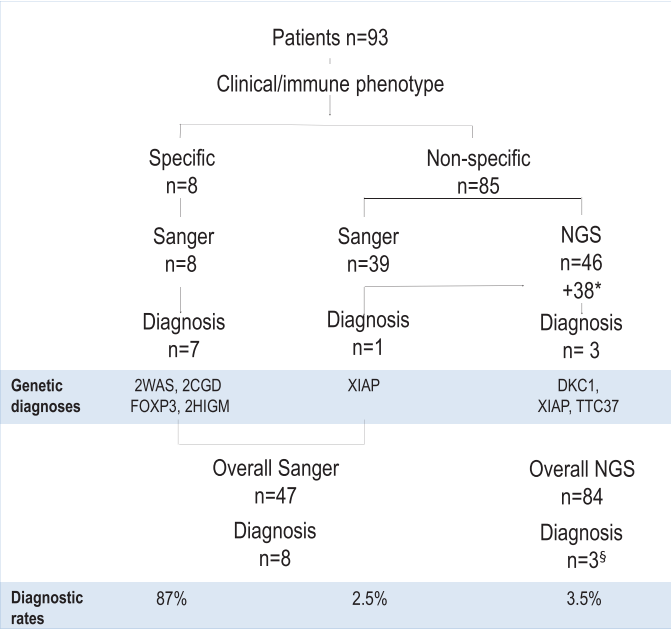

FIGURE 2. Diagnostic steps and rates of monogenic diagnoses with the different diagnostic approach. (*patients undiagnosed with previous Sanger; §plus 1 patient diagnosed elsewhere with WAS gene inversion through whole exome sequencing)

diagnosed as allergic or eosinophilic colitis, and 1 patient (1%) received an initial diagnosis of autoimmune enteropathy. Fifty-nine patients (63%) had severe intestinal disease (as defined by specific clinical indexes for CD or UC), and 17 of 39 patients (44%) with CD or CD-like phenotypes had a complicated disease course (14 structuring disease, 4 internal penetrating disease). Extaintestinal manifestations were reported in 40 patients (43%), and these were severe/atypical/recurrent infections in 20 patients, skin rash or skin/annexes abnormalities in 14 patients, macrophage activation syndrome/HLH in 5 patients, and extraintestinal autoimmune manifestations in 9 patients; 12 patients had "classical" IBD-associated extraintestinal manifestations (ie, erythema nodosum, uveitis, arthritis, sclerosing cholangitis, pyoderma gangrenosum); 4 patients had metastatic CD; and 4 patients had associated dysmorphic features or congenital malformations. A monogenic diagnosis could be established in 12% of patients with VEO-IBD and 15% of patients with infantile onset IBD (IO-IBD). Three out of 4 patients (75%) presenting with intestinal inflammation within the first month of life were diagnosed with a monogenic condition, and disease onset ≤1month had a positive predictive value of 75% to predict monogenic IBD (sensitivity of 25%, specificity of 98%). The clinical features of patients in whom a monogenic defect was diagnosed vs those in whom no causative genetic defects were observed are summarized in Table 2. The distribution of patients with a monogenic diagnosis according to the age at IBD onset is illustrated in Figure 3. Four out of the 88 patients (5%) for whom differential blood count and Ig subclasses were available had low immunoglobulin levels, and all of them were diagnosed with a monogenic IBD (2CD40L, WAS, XIAP). Lymphocytes subsets were available for 64 patients and were altered in 6 (10%); 4 of these received a genetic diagnosis (2CD40L, TTC37, DKC1). Neutrophil function assay was performed in 59 patients and was diagnostic in all of the 2 patients with CGD.

DISCUSSION

In our cohort, the diagnostic approach to suspected monogenic IBD has changed over time. Most of the patients with IBD onset before 2011 underwent a single gene approach. More recently, NGS has been used as the first line diagnostic step in most of the patients. In our study, the molecular diagnostic yield of NGS was 6% when performed as a first diagnostic step and 3.5% overall. These rates are lower than previous observations in VEO-IBD cohorts by Kammermeier et al who reported a diagnostic yield of 16% using a TGPS with 40 genes[1] and Charbit-Henrion et al who, using a TGPS with 66 genes, reported a variable diagnostic yield of 14% to up to 26.5% when TGPS was used either as a second line investigation or as a first screening, respectively.[3] These differences can be explained by a few factors. First, in both cohorts, the majority of patients had a disease onset before the age of 2 years, and the study by Charbit-Henrion et al[3] included only patients with a severe disease course; thus, patients in both cohorts might have had a higher pretest probability for a monogenic disease. Also, it should be noted that the 2 TGPS used in our study did not include at least part of the genes known to be associated with recognizable phenotypes or have valid functional tests for which a single gene approach has been used. Including these genes within the target gene panels would probably result in a higher diagnostic yield of NGS. In our study, a single gene approach had a good diagnostic performance when oriented by clinical or immunological features that were specific for known monogenic defects, such as CGD, WAS, or HIGM, but performed

TABLE 2. Clinical Characteristics of Patients With Monogenic and Nonmonogenic IBD

Clinical Features	MonoIBD (n = 12)	NonmonoIBD (n = 81)	*P*
IBD onset*, median (IQR)	27 (10–48)	24 (8–48)	ns
Age group			
≤ 1 month, n(%)	3 (25)	1(1)	0.006
≤ 6 months, n(%)	4 (33)	17 (20)	ns
≤2 years, n(%)	8 (67)	47 (58)	ns
≤6 years, n(%)	10 (83)	77 (95)	ns
Males, n (%)	11 (92)	44 (54)	0.02
Family history IBD, n(%)	3 (25)	6 (7)	ns
Endoscopy			
CD/CD-like, n(%)	8 (67)	31 (38)	ns
UC, n(%)	0	18 (22)	ns
IBD-U, n(%)	2 (16)	24 (30)	ns
Other, n(%)	2 (17)	8 (10)	ns
Perianal, n(%)	5 (42)	15 (19)	ns
Disease Location			
Colon only	2 (17)	48 (59)	0.01
SB§ only	1 (8)	1 (1)	ns
Colon+ other location	9 (75)	28 (35)	0.01
Colon + p°	3 (25)	10 (12)	ns
Colon+ SB§	4 (33)	14 (17)	ns
Colon+ p° + SB§	2 (17)	4 (33)	ns
Severe GI, n(%)	8 (67)	51 (63)	ns
Growth failure, n(%)	10 (83)	43/78 (55)	ns
Extraintestinal features, n(%)	12 (100)	28 (34)	<0.001
Infections, n(%)	9 (75)	11 (14)	<0.001
HLH/MAS, n(%)	3 (25)	2 (2)	0.02
Skin, n(%)	6 (50)	6 (7)	<0.001
Autoimmune, n(%)	3 (25)	6 (7)	ns
Low PLT, n (%)	4 (33)	2 (2)	0.002
Low Ig, n(%)	4 (33)	0	<0.001
Lymph.subset abn#, n(%)	4/11 (36)	2/52 (4)	0.01
TNF-failure, n(%)	2/7 (29)	18/65 (28)	ns
Steroid resistance, n(%)	0/7	13/68 (19)	ns
Surgery, n(%)	3 (23)	21 (26)	ns

*months; §small bowel; °perianal; #abnormalities

poorly in patients with nonspecific phenotypes. In this subgroup, only 1 out of 39 patients (2.5%) could reach a molecular diagnosis of XIAP deficiency, and the diagnostic process in this case implied multiple single gene sequencing over 15 months. During this period, the patient experienced several complications and treatment failures that could have been avoided with the use of NGS at the beginning of his symptoms. In our cohort, a monogenic diagnosis could be established in 13% of the patients combining different genetic approaches. Monogenic IBD accounted for 12% of patients with VEO-IBD and 15% of patients with IO-IBD, reflecting previous reports.[1, 2, 23] However, the frequency of monogenic diagnoses rose significantly among patients with disease onset before 6 months of life and particularly among patients with a disease onset during the first month of life; in these subgroups, a monogenic diagnosis could be established in 19% and 75% of patients, respectively. A molecular diagnosis was also made in 2 out of 6 patients who had started their disease after 6 years. This age group however, included only selected cases, and those 2 who reached a genetic diagnosis (ie, 1 WAS and 1 TTC37 defect) had developed other signs/symptoms that were specific of their genetic condition earlier than IBD. Interestingly, no patients with IL10

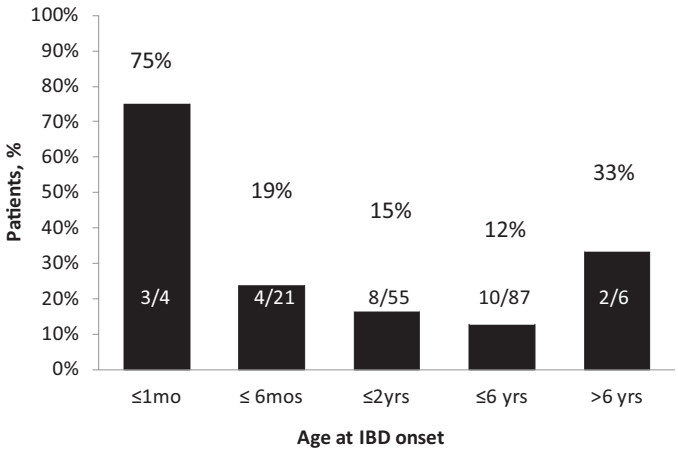

FIGURE 3. Distribution of patients with monogenic IBD within different age groups.

or IL10R defects were found in our cohort. The frequency of IL10 or IL10R mutations in the Italian population is unknown. However, it should be noted that most of the patients with IL10 pathways defect reported so far were of Arab or Asian descent. Moreover, patients of Arab descent had a family history of consanguinity.[24, 25] A possible explanation for our observation is that in our cohort, only 3 patients of non-white ethnicity (2 Arab and 1 Asian) were included, and parental consanguinity was not reported in any of the patients.

Inflammatory bowel disease severity did not seem to differ between patients with monogenic and nonmonogenic IBD nor did the frequency of perianal disease. However, monogenic diagnoses were more frequent among patients with colonic plus small bowel and/or perianal involvement compared with patients with isolated colonic disease. Extraintestinal features were universally present in patients who received a genetic diagnosis in our cohort. The most represented extraintestinal findings were infections that were reported in approximately two thirds of the patients with a genetic diagnosis.

Establishing a genetic diagnosis affected the medical management in the majority of patients. The most frequent consequence was BMT. Introduction of BMT as a potentially curative option for intestinal and extraintestinal manifestations of monogenic IBD has changed the clinical practice, thus identifying patients for whom BMT is indicated and excluding those that are unlikely to benefit from such treatment have become crucial. In patients with IL10 signaling defects and XIAP deficiency, BMT is a treatment of choice, as it resolves the intestinal inflammation and prevents the development of hematologic complications.[26, 27] In patients with epithelial barrier dysfunction such as NEMO deficiency or TTC7A defects, it seems to be a less amenable option because it fails to correct the epithelial defect. In our cohort, we identified 2 patients with genetic defects impacting the epithelial barrier functions (ie, 1 patient had tricohepatoenteric syndrome [TTC37], and 1 patient had dyskeratosis congenita [DKC1]). The patient with dyskeratosis congenita had severe intestinal disease that was refractory to medical therapy and underwent multiple surgeries. For this patient, BMT was considered an option for the treatment of IBD, despite the absence of bone marrow failure, but the idea was abandoned based on previous reports describing a poor outcome after BMT in patients with epithelial barrier dysfunction, including patients with dyskeratosis congenita.[28]

Our study has several limitations: data were collected retrospectively for most of the patients; thus, the quality of data for such patients might be poor; a selection bias may have been introduced given the fact that multiple centers participated in the study and that not all the diagnosed patients meeting the inclusion criteria during the study period may have been sent for genetic analysis.

However, our cohort represents one of the largest studies reporting the genetic profile of patients with suspected monogenic IBD and gives valuable insight on the diagnostic strategies adopted in the clinical setting over the last decade.

CONCLUSIONS

In conclusion, our data provide evidence that genetic diagnosis can be established in a significant proportion of suspected monogenic IBD patients and that establishing a genetic diagnosis impacts a patient's management. Early age at disease onset, the coexistence of extraintestinal manifestation, and male sex are highly suggestive of a genetic defect. When monogenic IBD is suspected, Sanger sequencing may be deployed in patients in whom clinical and immunological findings point toward a specific diagnosis. However, NGS should be preferred in patients with nonspecific phenotypes, especially in infants in whom the probability of a monogenic condition is higher and for whom timely diagnosis, before the full phenotype or complication develops, may have an impact on the patient's management.

SUPPLEMENTARY DATA

Supplementary data is available at *Inflammatory Bowel Diseases* online.

FIGURE 1S.Variant selection work-flow. MAF, minor allele frequency.

ACKNOWLEDGMENTS

The authors are grateful to Erica Valencic and Flavio Faletra for their support with the immunological and genetic analysis and to Maria Chiarico and Bronislava Papadatou who contributed to data analysis and interpretation.

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