"Phenotypic and genotypic characterisation of inflammatory bowel disease presenting before the age of two years"

Jochen Kammermeier [1, 2], Robert Dziubak [2], Matilde Pescarin [2], Suzanne Drury [1, 2], Heather Godwin [2], Kate Reeves [2], Sibongile Chadokufa [2], Bonita Huggett [2], Chela James [1], Nikki Acton [2], Fevronia Kiparissi [2], Mamoun Elawad [2], Phil Beales [1], Neil J Sebire [4], Kimberly Gilmour [3], Holm H Uhlig [5], Chiara Bacchelli [1] and Neil Shah [2]

1. Genetics and Genomic Medicine (GGM) Unit,

Institute of Child Health, UCL, London, UK

2. Department of Gastroenterology,

Great Ormond Street Hospital, London, UK

3. Department of Immunology

Great Ormond Street Hospital, London, UK

4. Department of Histopathology

Great Ormond Street Hospital, London, UK

5. Transitional Gastroenterology Unit, Nuffield Department of Medicine and Oxford Children's Hospital, Oxford, UK

6. NE Thames Regional Genetics Laboratory, Great Ormond Street Hospital, London, UK

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Corresponding author

J. Kammermeier (j.kammermeier@ucl.ac.uk)

University College London (Institute of Child Health) and Great Ormond Street Hospital, 30 Guilford Street, London WC1N 1EH, England, UK

Abstract

Background: Very early onset inflammatory bowel disease (VEOIBD) is an umbrella term for a heterogeneous group of phenotypes typified by chronic intestinal inflammation in children with disease-onset before the sixth year of life. Disease stratification poses considerable challenges given the difficulties in integrating VEOIBD phenotypes into conventional IBD categories: children are more likely to present with more extensive, unclassifiable and treatment-resistant disease when compared to adults with IBD. Furthermore, a diverse group of monogenic conditions can present with IBD-like intestinal inflammation.

Design: We retrospectively reviewed phenotype data of VEOIBD patients and recruited candidates for Next Generation Sequencing of established VEOIBD genes (Whole Exome Sequencing (WES) and Targeted Gene Panel Sequencing (TGPS)).

Results: Over a period of 15 years, 80 VEOIBD patients (55% male) were managed and genetically screened. The median disease onset was six months of age [IQR: 1 to 22] with 78% developing symptoms before the second year of life (infantile-onset IBD). The most common overall diagnosis was IBDU (65%). CD and UC-like IBD were diagnosed in 28% and 8% respectively. Eighty-one percent developed pan-colonic inflammation, 16% extensive perianal inflammation and 9% stricturing disease. Thirty-three percent required parenteral nutrition and 15% underwent abdominal surgery. In 28% of children, TNF-alpha blockade with concomitant immunomodulator therapy was not sufficient to sustain remission. Hematopoietic stem cell transplantation (HSCT) was performed in 23% of patients. Prior to the launch of our Next Generation Sequencing (NGS) program, nine patients were

diagnosed through Sanger sequencing (genes: IL10, IL10RA, IL10RB, XIAP, IPEX). Since then, NGS revealed 10 diagnoses in six genes (EPCAM, IL10RA, TTC37, SKIV2L, LRBA, TTC7A).

Conclusion: Comprehensive immunological, histological and genetic assessment is important to support diagnosis and management. Genetic screening is particularly advisable in consanguineous patients and children with infantile disease-onset, evidence of severe perianal disease, abnormal intestinal epithelial morphology or presence of other associated syndromic features. Particularly for children with disease-onset before the 2nd year of life, the phenotype heterogeneity is not sufficiently addressed by currently available IBD classification tools.

Introduction

Very Early Onset Inflammatory Bowel Disease (VEOIBD) refers to children with disease-onset before the sixth year of life [1]. This age group represents the minority of worldwide reported IBD cases with an estimated incidence of 4.37 per 100000 children and a prevalence of 14 per 100000 children [2]. Consistent with adults with IBD, children present with abdominal pain, intestinal bleeding, diarrhoea and weight loss [3]. Of greater concern in this age group however, are the effects of chronic inflammation on growth and global development [4]. In paediatric IBD and particularly in VEOIBD, it is therefore paramount to establish effective diagnostic and management strategies early in the disease course [5]. This poses considerable challenges given the difficulties in integrating VEOIBD phenotypes into conventional IBD categories: children are more likely to present with more extensive, unclassifiable and treatment-resistant disease compared to adults with IBD [6 7]. IBD classification has recently been modified to address the younger age group but this might not be sufficient to accommodate the heterogeneous phenotype of VEOIBD [8]. Some children with IBD-like disease may not benefit from conventional stratification into Crohn's disease (CD), ulcerative colitis (UC) or IBD unclassified (IBDU).

IBD-like intestinal inflammation can also present as a feature of a phenotypically diverse group of monogenic conditions including primary immunodeficiencies (affecting T/B cells), phagocyte defects, hyper- and autoinflammatory disorders, immune regulation- and epithelial barrier defects [9]. The majority of monogenic IBD-like diseases are classified as IBDU due to the lack of pathognomonic features of CD or UC [10]. Large-scale genetic screening programmes will shed light on the still

unknown overall incidence and prevalence of monogenic IBD. The feasibility of comprehensive genetic screening in VEOIBD beyond patients with obvious syndromic features or consanguineous background has yet to be established [11].

The aim of this retrospective cohort analysis was to map the heterogeneous phenotypes of VEOIBD and to establish new disease stratification tools. In addition, we have interrogated the results of a Next Generation Sequencing (NGS) pipeline to relate phenotypic data with known genotypes in the largest cohort of genetically screened VEOIBD patients reported to date.

Methods

Patient Cohort:

We retrospectively reviewed case notes, electronic case records and archived microfilms from 80 VEOIBD patients who have been managed at Great Ormond Street Hospital for Children (GOSH) over the last 15 years. We extracted anthropometric data, clinical features and laboratory results at presentation when available.

50/80 patients were diagnosed at our centre with the remaining children having undergone primary diagnostic work-up elsewhere.

Definitions:

1. Age of onset: time point from birth at which IBD related and/or gastrointestinal symptoms became apparent.

2. VEOIBD: disease with chronic-relapsing gastrointestinal symptoms with histological features of chronic with or without acute inflammation of the intestinal mucosa (after exclusion of infectious and allergic/eosinophilic intestinal diseases) and with symptom-onset before the sixth year of life.

3. VEOIBD-CD and UC: this study defines children as "CD-like" or “UC-like” based on clinical and/or histopathological features (as established elsewhere: see [10]) regardless of their underlying genetic screening result.

4. VEOIBD-U: IBD which cannot be classified as CD or UC [10]. This heterogeneous group contained children with localized or panenteric chronic-with or without acute inflammation, evidence of increased apoptotic activity and/or abnormal epithelial morphology on histological examination.

For anthropometric data and laboratory markers, we only considered values from treatment naïve symptomatic patients at the time of diagnostic endoscopy (n=50). We prospectively recruited candidates for further molecular evaluation, including NGS, as part of the PETIT Study (Patients with Early-onseT Intestinal inflammaTion Study).

NGS:

In a previous published study we evaluated two different NGS technologies (Targeted Gene Panel Sequencing (TGPS) and Whole Exome Sequencing (WES)) to screen for mutations in known VEOIBD genes [11]. Over a two-year period up to the time of writing, 25 patients were recruited for WES and 55 for TGPS (for in-depth methodology see: [11]).

Ethical approval:

Patients were informed and consented for functional studies and NGS as part of the "PETIT Study". Ethical approval was obtained from the National Research Ethics Service Committee London, Bloomsbury.

Statistical analysis:

IBM SPSS Statistics for Windows, version 22 (Armonk, NY) was used to perform statistical analysis. Continuous data is presented as medians with interquartile ranges and categorical data is presented as rates and proportions.

Mann-Whitney U Test and Kruskal-Wallis were used to compare continuous data between groups. Spearman's rho was used to interrogate correlation between two continuous data sets. To compare proportions between groups we used Pearson Chi-square and Fisher's Exact Test where appropriate. All tests were two-tailed and significance level was set at 5%.

Weights were converted to weight-for-age z-scores using WHO Anthro- and WHO Anthro Plus software.

Multiple logistic regression analysis was used to assess the probability of monogenic VEOIBD when presented with specific phenotype features. Only variables with p-values < 0.05 were included in the model. Goodness-of-fit of logistic regression model was based on Hosmer-Lemeshow test.

Results

Over the last 15 years, 80 VEOIBD patients were treated at our centre, 55% of whom were male. Fifty-four percent of our patients were White Europeans, 14% were Middle Eastern/Arab States -, 11% Pakistani-, 8% Indian-, 6% Bangladeshi-, 4% African- and 4% of mixed ethnic origin. Twenty-six percent (21/80) were offspring from consanguineous unions.

Overall, 19/80 cases (X%) were associated with specific monogenic mutations. Children from consanguineous families had earlier disease onset compared to children from non-consanguineous backgrounds (1 month [IQR: 0 to 12] vs. 9 months [IQR: 3 to 28], p = 0.003) and they had a greater rate of identified monogenic VEOIBD (48% (10/21) vs. 15% (9/59), p = 0.006).

Eighteen per cent of patients had a positive family history for IBD amongst first-degree relatives and 14% had at least one sibling affected with IBD. The overall mortality rate was 4% (1st Patient: IPEX syndrome, idiopathic pneumonitis with multiorgan failure 12 months post HSCT; 2nd Patient: XIAP deficiency, progressive JC virus encephalitis 28 months post HSCT; 3rd Patient: IL10RA deficiency, pulmonary hypertension 6 weeks post HSCT).

The overall observational period for all 80 patients (first endoscopy to last contact) was 28 months [IQR: 14 to 66]. For patients diagnosed at our centre (n=50) the median time of observation (diagnostic endoscopy to last contact) was 34 months [IQR: 15 to 74].

Symptoms at presentation:

The median disease onset for all 80 patients was six months of age [IQR: 1 to 22] with 78% developing symptoms before the second year of life (infantile-onset IBD). Figure 1 describes the most common gastrointestinal symptoms at presentation.

A subgroup of patients exhibited additional atopic features (atopic dermatitis: 20%; drug allergy: 14%; food allergy: 10%; asthma: 4%).

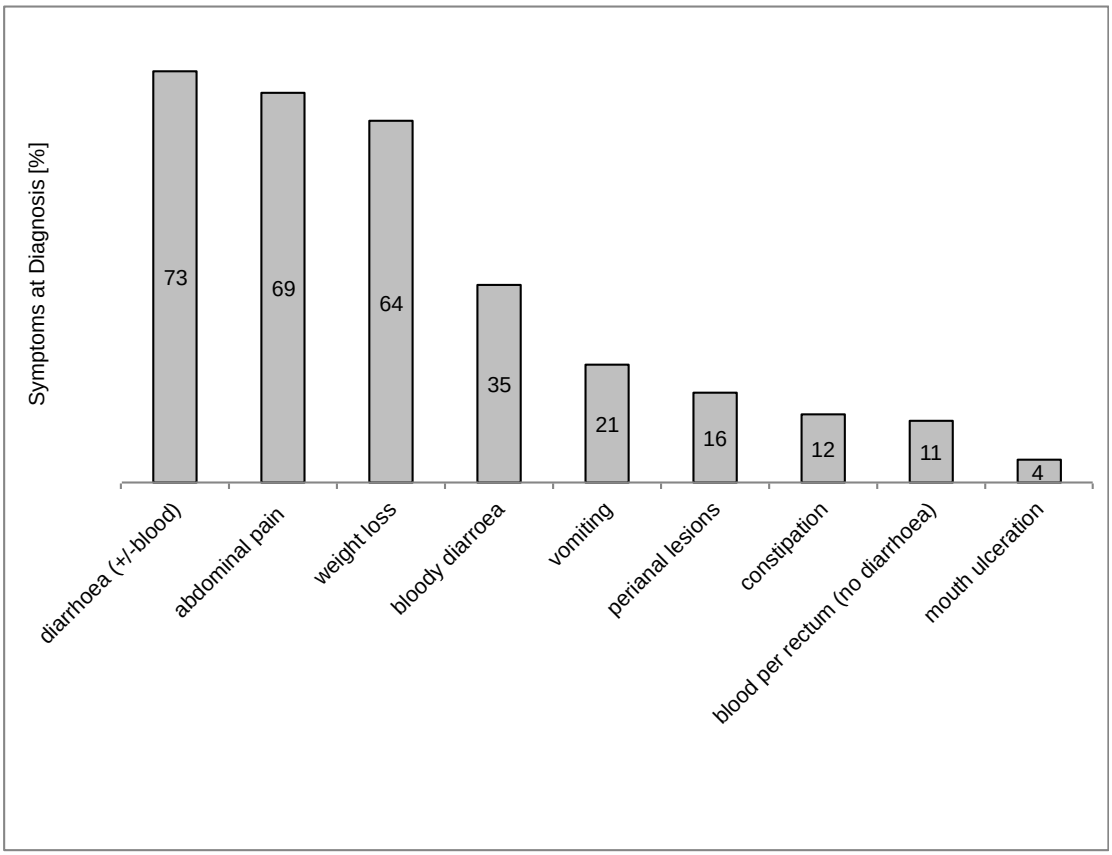

Figure 1: symptoms reported at diagnosis (in % of cases) for 80 patients with VEOIBD.

Weight-for-age at time of diagnosis:

At the time of first endoscopy anthropometric data was analysed for all patients diagnosed at our centre (n=50). The median weight-for-age z-score was -1.15 [IQR: -

2.85 to 0.42]. Thirty-two per cent (16/50) of children had moderate or severe malnutrition (10 cases below -3 z-scores).

Children with earlier disease-onset presented with lower weight-for-age z-scores at diagnosis (rho = 0.486, $p < 0.0001$) but weight-for-age z-scores did not correlate with time between disease onset and weight measurement (rho = -0.153, p = 0.366). Children with monogenic VEOIBD had lower z-scores at diagnosis (-3.9 [IQR: -4.51 to -1.05] vs. -0.97 [IQR: -2.22 to 0.55], p = 0.009).

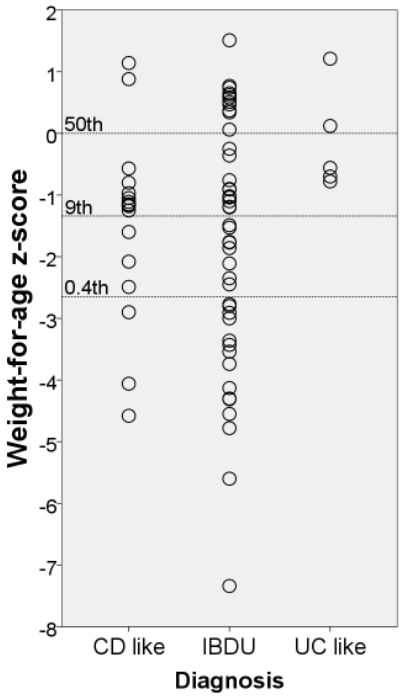

Figure 2: Weight for age z-scores for children diagnosed with CD, UC and IBDU. Dotted horizontal lines represent 50th, 9th and 0.4th weight-for-age centiles (no statistical difference of median weight and number of malnourished children between the three groups).

Endoscopic and histological features:

Patients who had primary diagnostic work up at our centre (n=50) had a median age at onset of symptoms of 13 months [IQR: 2 to 29] and underwent endoscopy at a median age of 30 months [IQR: 10 to 44]. Longitudinal histology data (diagnostic endoscopy to latest endoscopy) for patients diagnosed at our centre was available for an observational period of 20 months [IQR 4 to 63].

Histological data sets were analysed for all patients in this study (n=80) and cumulative endoscopic features were extracted and stratified (Table 1).

The most common histological pattern was IBDU (65%). Features suggestive of CD and UC were diagnosed in 28% and 8% respectively. Age at disease onset differed between diagnoses ($p = 0.027$), with post-hoc analysis revealing a statistically significant difference between symptom onset in IBDU and UC (5 months [IQR: 1 to 16] vs. 23 months [IQR: 14 to 36], $p = 0.023$). Pancolonic disease was seen in 81% with the rates not significantly different amongst the three different histological subgroups (UC, CD and IBDU, data not shown). Eleven cases of CD diagnosed at our centre developed pancolonic disease; in 10 patients this was already evident at diagnostic endoscopy. Furthermore, all four UC patients presented with pancolonic disease on diagnostic endoscopy. Eighteen out of 32 IBDU patients diagnosed at our centre developed panenteric disease over time. Amongst those, 13 children had panenteric inflammation at first (diagnostic) endoscopy.

Chronic +/- acute inflammation	IBDU (n=52)	CD (n=22)	UC (n=6)
Oesophagus	12/52 (23%)	11/22 (50%)	2/6 (33%)
Stomach	40/52 (77%)	16/22 (73%)	3/6 (50%)
Duodenum	41/52 (79%)	11/22 50%)	0/6 (0%)
Terminal Ileum (66/80)	14/39 (36%)	12/22 (55%)	0/5 (0%)
Pancolonic	43/52 (83%)	16/22 (73%)	6/6 (100%)
Isolated small bowel	9/52 (17%)	0/22 (0%)	0/6 (0%)
Small bowel and pancolonic	34/52 (65%)	9/22 (41%)	0/6 (0%)
Other histological features			
Villus atrophy	20/52 (38%)	4/22 (18%)	0/6 (0%)
Epithelial abnormalities	9/52 (17%)	0/22 (0%)	0/6 (0%)
Increased apoptosis	8/52 (15%)	0/22 (0%)	0/6 (0%)
Granulomata	0/52 (0%)	14/22 (64%)	0/6 (0%)
Other endoscopic and clinical features			
Perianal	0/52 (0%)	13/22 (59%)	0/6 (0%)
Stricturing	4/52 (8%)	3/22 (14%)	0/6 (0%)
Fistulising	0/52 (0%)	2/22 (9%)	0/6 (0%)

Table 1. Cumulative clinical and histological features and anatomical distribution of inflammation in patients with IBDU, CD and UC.

Sub-analysis of the IBDU cohort (n=52) revealed that 65% developed panenteric disease (inflammation of the upper and lower gastrointestinal tract), 17% had exclusively pancolonic and 17% exclusively small bowel disease. Histological features only found in the IBDU-panenteric patients were: abnormal epithelial morphology (Irregular surface epithelium, tufting-like changes and epithelial cell layer non-adherent to the basal membrane, n=9) and increased apoptotic activity (n=8).

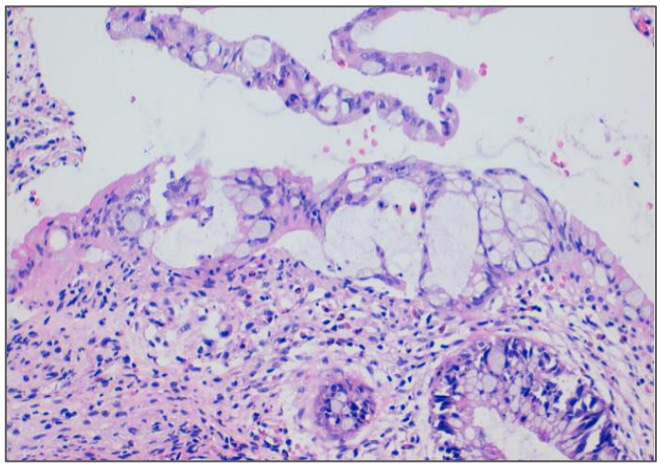

Figure 3. Hematoxylin and eosin stain of intestinal biopsy from patient with TTC7A deficiency with evidence of morphologically abnormal epithelium and separation with abundance of chronic and acute inflammatory cells in the lamina propria.

Laboratory markers:

Full blood count, inflammatory markers and albumin levels were available from treatment naïve children who underwent primary diagnostic endoscopy at our centre (n=50).

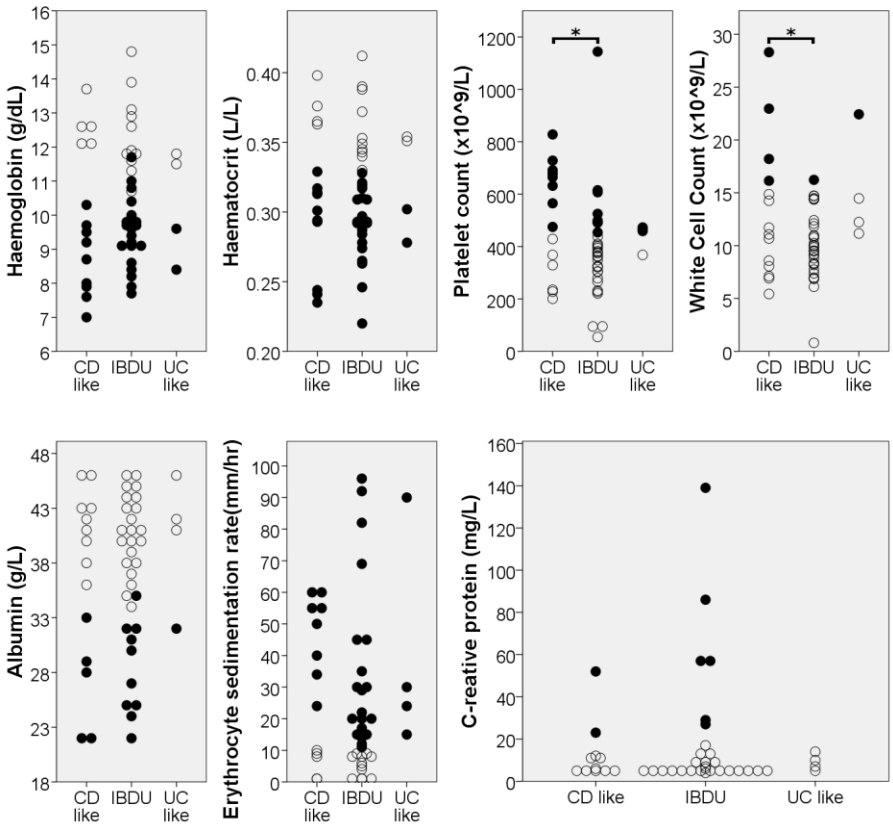

Figure 4. Scatter plot for laboratory markers measured at diagnosis. Black, filled dots represent age-dependant abnormal results. There was no statistically significant difference between the three disease groups (haemoglobin, haematocrit, albumin, ESR and C-reactive protein) with the exception of raised platelet count (CD-like vs. IBDU) and white cell count (CD-like vs. IBDU) ($p<0.05$)*.

Immunology work-up was reviewed for all patients in our cohort (n=80). Basic lymphocyte subsets (CD3+; CD3+CD4+; CD3+CD8+; CD19+ and CD56+CD16+), immunoglobulin profiles (IgG, IgA and IgM) and nitro blue tetrazolium chloride test results (NBT) for diagnosing chronic granulomatous disease (CGD) could be ascertained for 54%, 70% and 34% of patients respectively. All NBT test results were normal.

Total IgE was measured in 73% of children. In 31% of cases (18/58), total IgE was above the upper limit of normal range (adjusted to varying normal range: exceeding

the upper limit of normal range by 257% [IQR: 24% to 545%]). All three children with IPEX disease presented with high IgE levels (282 i/U, 369 i/U and 34404 i/U). Transient hypogammaglobulinaemia (with concomitant low serum albumin levels) in patients with acute intestinal inflammation was observed in 14% of patients (median % below normal range was 29 [IRQ: 19 to 54%]). Five patients were found to have consistently abnormal immunology profiles: One patient with LRBA-deficiency presented with abnormally raised γδ-cells on T-cell panel (after alternative T-cell expansion was suspected on basic lymphocyte subset panel). Two TTC7A deficient patients presented with lower than normal T-cell counts (reduced total CD3+ and CD4+ cells respectively) in addition to low IgA levels in the former patient. One patient with negative genetic screening was found to have persistently reverse CD4+/CD8+ ratio.

Genetics:

Genetic screening in 80 VEOIBD patients revealed 19 positive cases (24%). Overall, children with monogenic VEOIBD developed symptoms earlier when compared with the screening negative cohort (1 month [IQR: 0 to 4] vs. 12 months [IQR: 3 to 29], $p < 0.0001$).

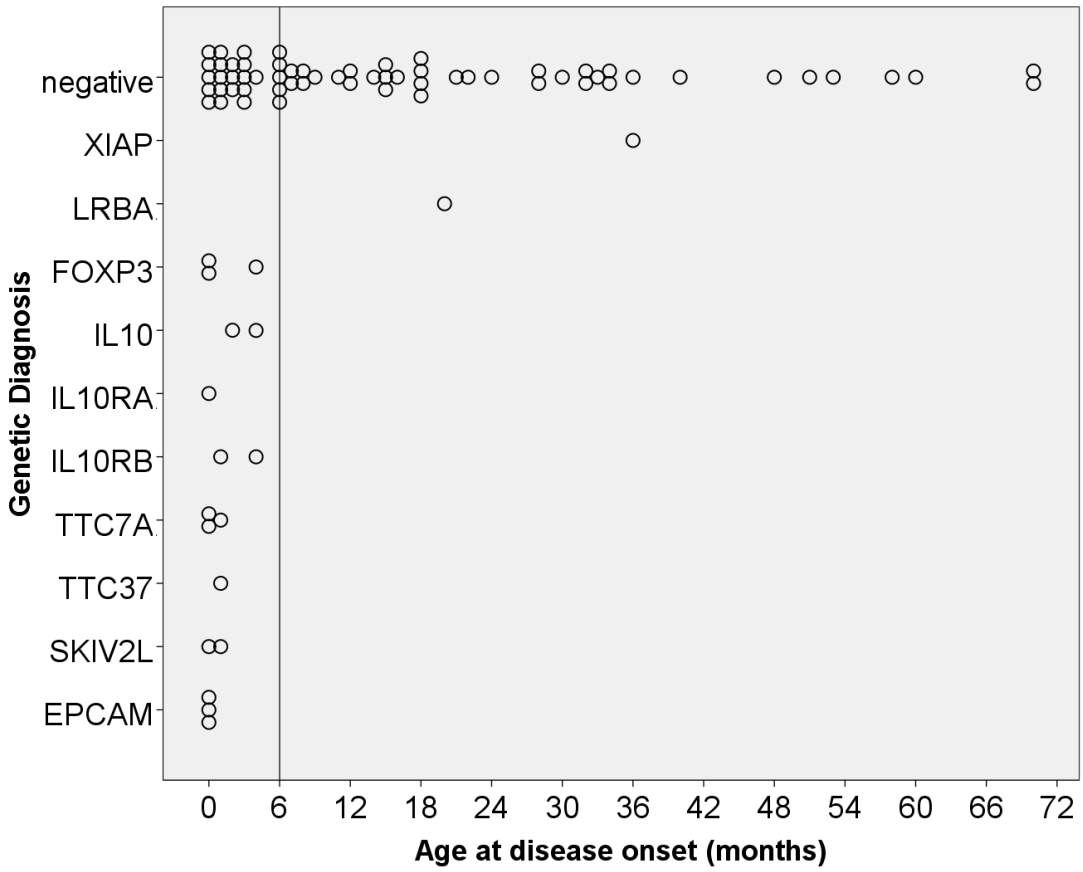

Figure 5. Onset of disease of all 19 monogenic VEOIBD cases versus screening negative patients (for detailed mutation description see supplement 1).

Prior to the launch of the PETIT Study, nine patients were diagnosed through Sanger sequencing (causative mutations established in five genes: *IL10, IL10RA, IL10RB, XIAP, IPEX*). Since then, NGS (TGPS and/or WES) revealed nine new diagnoses in six genes (*EPCAM, IL10RA, TTC37, SKIV2L, LRBA, TTC7A*) [9] [5]. One patient with TTC7A

deficiency was diagnosed externally by Sanger Sequencing (for detailed genetic data see supplement 1).

To establish the likelihood of underlying monogenic disease, we have interrogated the following phenotypic variables: age at onset, gender, consanguinity, positive family history, symptoms at presentation (see figure 1), disease distribution and specific histological features (see table 1).

The logistic regression model (Figure 5) predicts the likelihood of underlying monogenic disease when interrogating the three most significant variables (Hosmer-Lemeshow: p = 0.621): combined model: factor B: -3.264, significance < 0.0001, EXP(B) = 0.038), disease onset ≤ 6 month of age (B = 1.864, significance = 0.028, EXP(B) = 6.45), histological evidence of abnormal epithelial morphology (B = 3.479, significance = 0.003, EXP(B) = 32.439) and the presence of perianal disease (abscesses, fistulae and inflamed skin tags; B = 1.526, significance = 0.045, EXP-B = 4.60).

Group	Condition	Gene and Mutation		
Epithelial Barrier Dysfunction	TTC7A Deficiency	*TTC7A*	*HOM*	p.Gly45fs
		TTC7A	*CH*	p.Glu191fs/p.1854Phe
		TTC7A	*Hom*	
	Congenital Tufting Enteropathy	*EPCAM*	*CH*	p.Gln167fs/c.492-2A>G
		EPCAM	*HOM*	p.Gln167fs
		EPCAM	*CH*	p.Cys135Phe/p.Thr234Lysfs*2
Immune Dysregulation	IL10 Deficiency	*IL10RA*	*HOM*	p.Cys223Arg
		IL10RB	*HOM*	p.Leu59fsX72
		IL10RB	*HOM*	p.Trp18fsX29
		IL10	*HOM*	p.Gly113Arg
		IL10	*HOM*	p.Gly113Arg
	IPEX	*FOXP3*	*HZ*	
		FOXP3	*HZ*	c.-23G>A
		FOXP3	*HZ*	
T/B-Cell Deficiency	CVID	*LRBA*	*HOM*	p.Ser993*
Hyper- and Autoinflammatory Disorders	XIAP Deficiency	*XIAP*	*HZ*	p.Pro225SerfsX227
Others	Tricho-Hepato-Enteric Syndrome	*SKIV2L*	*HOM*	c.355-2A>C
		SKIV2L	*CH*	p.Arg324Trp/p.Arg1041His
		TTC37	*CH*	p.Trp936*/p.Gly673Asp

Supplement 1. 80 VEOIBD patients harboured 19 disease-causing mutations. (Patients 6-11, 17, 19 previously reported [11-14]). HOM: homozygous, CH: compound heterozygous, HZ: hemizygous.

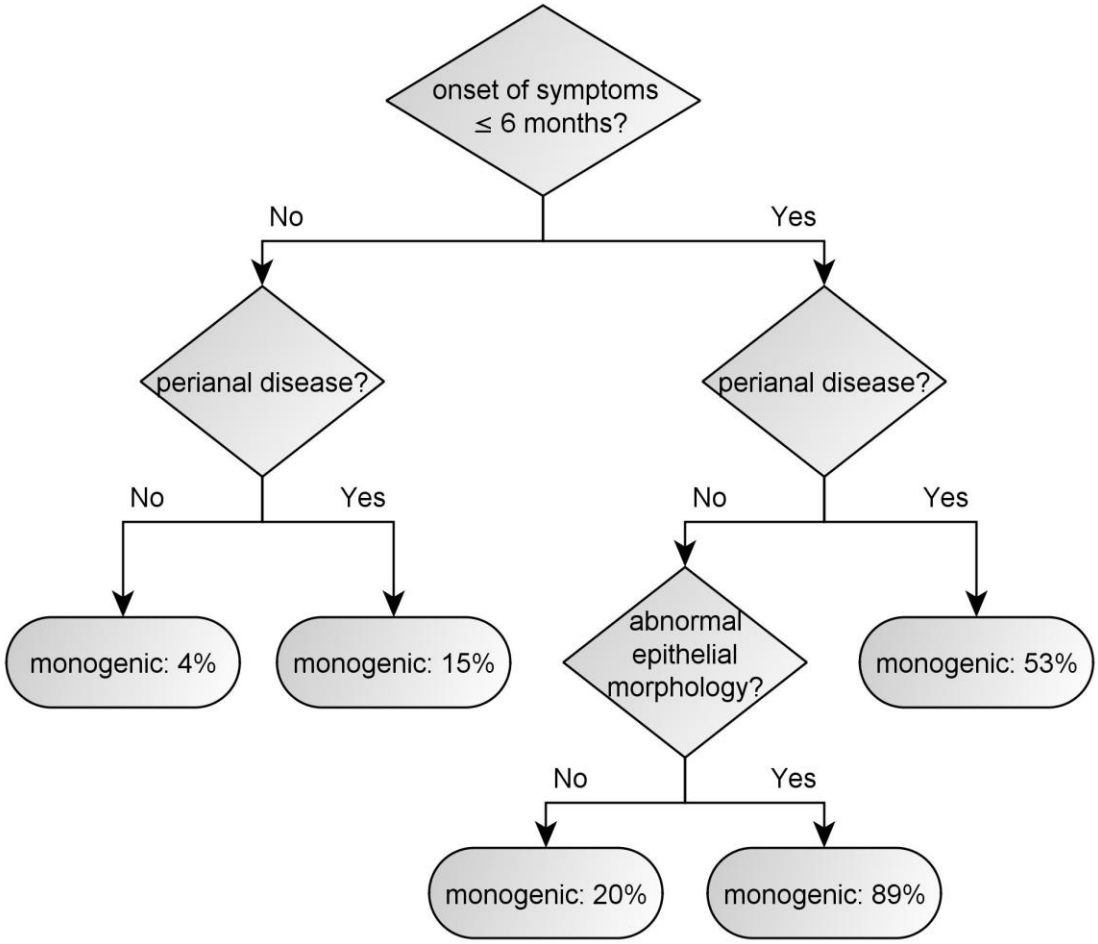

Figure 6. Likelihood of underlying causative mutations in patients with VEOIBD based on phenotype data from patients managed at our gastroenterology centre – multiple logistic regression model.

Medicines in VEOIBD:

Medications used to treat VEOIBD included corticosteroids (86%), immunomodulators (85%; azathioprine, 6-MP, methotrexate, cyclosporine,

tacrolimus, sirolimus or thalidomide), salicylates (48%) and biologics (39%; infliximab, adalimumab or basiliximab).

In addition to TNFα blockade (infliximab, adalimumab) and one first line immunosuppressant drug (azathioprine, 6-mercaptopurine or methotrexate), 28% of patients received further immunomodulation throughout their monitored disease course (≥3 Immunosuppressant Agents (ISA) = ISA ≥3). This cohort of children (ISA ≥3) did not have earlier onset of disease (6 months vs. 7 months p = 0.791), did not receive parenteral nutrition significantly more frequently (41% vs. 29%, p = 0.323) and did not have a significantly increased likelihood of harbouring mutations in known VEOIBD genes (27% vs. 22%, p = 0.648) than the group of children on two or less immunomodulators/biologics (≤2 ISA).

Children requiring ≥3 ISA did not have significantly longer disease duration (age at onset of symptoms to last contact) comparing to children who required ≤2 ISA (72 months [IQR: 57 to 107] vs. 49 months [IQR: 27 to 87], p = 0.075).

Parenteral nutrition in VEOIBD:

A third of children (33%) required parenteral nutrition (PN) throughout their monitored disease course (≥ 50% of calorie requirement for ≥ 28 days; introduced for enteral food intolerance/gut rest and/or inadequate weight gain). Patients who required PN had earlier disease-onset when compared to the non-PN group (1 month [IQR: 0 to 4] vs. 15 months [IQR: 4 to 31], p < 0.0001).

Haematopoietic Stem Cell Transplantation in VEOIBD:

Hematopoietic stem cell transplantation (HSCT) was performed in 18 children of whom 14 were transplanted for failure to conventional treatment, three with a prior-known HSCT-amenable genetic diagnosis (IPEX and IL10/RA deficiency) and one

gene-screening negative patient with a concomitant diagnosis of acute myeloid leukaemia. Two patients in our cohort are currently undergoing HSCT work-up. Patients who underwent HSCT had earlier disease-onset (2 months [IQR: 0 to 5] vs. 12 months [IQR: 2 to 28], p = 0.004) and more intensive immunosuppression (ISA ≥3) when compared to the cohort of non-transplanted children (61% vs. 18%, p = 001).

Surgery:

Fifteen per cent (12/80) of patients underwent abdominal surgery: hemi/subtotal colectomy with stoma formation (n=8), stoma formation alone (n=3), surgery for isolated small bowel atresias (n=1). Eighteen per cent of children had percutaneous gastrostomy tube insertion.

Summary of treatment strategies:

Forty-two children were considered the most treatment resistant requiring parenteral nutrition, abdominal surgery or extensive immunosuppression (ISA ≥3) throughout the observed time period. Figure 6 depicts the application of those three treatments in this most resistant treatment group (n=42) sub-classified into three histological phenotypes: CD-like, UC-like and IBDU.

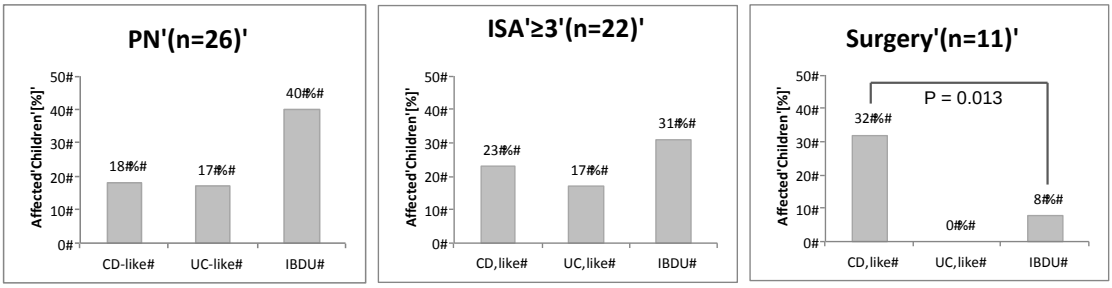

Figure 7. VEOIBD patients (n=42) with disease phenotype requiring intensified management defined as need for **6A**: parenteral nutrition (PN), **6B**: ISA ≥3 or **6C**: abdominal surgery. There was no statistically significant difference between the

three disease groups and treatment strategies with the exception of the need for surgery (CD-like vs. IBDU).

Discussion

In this retrospective cohort study we aimed to fine-map VEOIBD phenotypes in order to evaluate potential disease stratifying tools. VEOIBD is different from conventional (adult onset/idiopathic) IBD: Children have more disseminated intestinal inflammation, higher rates of treatment resistance and rapid disease progression [15] [7]. Within this age group, categorizing IBD by means of clinical severity scores (Paediatric CD Activity Index or Paediatric UC Activity Index) or anatomical distribution (Paris Classification) is difficult and applying such scores presumes histological differentiation into CD or UC, which is often not apparent in VEOIBD. Consistent with previously published paediatric reports, VEOIBD patients present with diarrhoea, abdominal pain, weight loss and rectal bleeding [3]. Extended disease at presentation has profound effects on growth and weight gain as confirmed in our study: 30% of children presented with moderate to severe weight impairment (below -2 z-scores weight-for-age). Chronic inflammation has a significant effect on the patients' nutritional status as evidenced in our study by the high prevalence of hypoalbuminaemia (32%) and anaemia (62% of patients were anaemic at the time of diagnosis) [16].

Inflammatory Markers and Immune Screening:

To support the clinical suspicion of VEOIBD we interrogated three commonly available inflammatory markers: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and thrombocytosis. In our cohort, ESR was raised in 65% of VEOIBD cases being the most frequently abnormal systemic marker for intestinal inflammation when compared to thrombocytosis (38%) and raised CRP (18%). This differs from the trend seen in conventional IBD [17] [18].

Performing basic immune screening in our patients had minimal diagnostic benefit. There is however a strong argument to investigate basic immune components and function given that numerous primary immune deficiencies can present with IBD-like disease [5 9].

Molecular analysis revealed one patient with combined immune deficiency (CID) due to LRBA-deficiency (LPS-responsive beige-like anchor). The patient presented with chronic diarrhoea, evidence of intestinal inflammation, pancytopenia and mildly abnormal basic immune work-up (similar phenotype described in [19]): Albeit normal CD4+ and CD8+ cell counts the sum of both fractions fell short of the total CD3+ count suggesting an expansion of an additional CD3+ cell line. Extended T-cell phenotyping established an increased γδ-T-cell fraction raising the possibility of an immune dysregularory process secondary to defects in recombination or apoptosis pathways. The final diagnosis was established by NGS and confirmed by immunoblot (data not shown). LRBA-deficiency has been previously reported to present with IBD-like disease as well as result in an IPEX phenotype [20 19].

All three IPEX patients (due to *FOXP3* mutations) in our cohort presented with unremarkable lymphocyte subsets and significantly raised total IgE consistent with previous reports [21]. TTC7A deficiency has been recently recognised to cause VEOIBD [22]. It has also been established that patients with TTC7A deficiency can suffer from a wide range of immunological abnormalities including SCID-like disease [23]. Two TTC7A-deficient patients in our cohort presented with low T-cell counts (decreased CD4+ cells and decreased CD3+ cells) as well as persistently low IgA levels in one child. One patient with negative genetic screening, exhibited persistent reversal of the CD4/8 ratio, possibly due to concomitant chronic EBV infection.

It is well established that VEOIBD patients can suffer from immune dysregulation, which is not necessarily reflected by aberrations of basic immune screening tests: Some conditions require specific functional analysis in order to confirm suspected defects in pathways as seen in IL10 pathway- or XIAP- deficiency [5 14 24 25].

Histological examination:

The high prevalence of pancolonic disease and increased rate of IBDU in the paediatric population has been previously described and is confirmed in the present study [6 26]. Pancolonic disease was present in 81% of all cases. Forty-one percent of unclassifiable VEOIBD presented with panenteric inflammation at diagnosis.

In 2013, the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published a widely adopted approach to diagnosing suspected paediatric IBD [10]. When interrogating our cohort only 35% presented with features consistent with either CD (n=22) or UC (n=6). In addition, already sparsely prevalent CD-pathognomonic features such as perianal/fistulising disease (16%, 13/80 of cases) and granulomata on histology (18%, 14/80 of cases) have also been reported in a variety of monogenic conditions such as Chronic Granulomatous Disease (CGD), IPEX, XIAP- or IL10 pathway deficiency and can therefore not reliably be considered specific for idiopathic CD in this age group [27 28 14 25]. As expected, our group of patients originally diagnosed with early onset CD contained all five children with IL10 pathway defects. The heterogeneous group of unclassifiable VEOIBD constitutes the largest group in our study and probably the most challenging both, in terms of establishing a molecular diagnosis and clinical management (A general diagnostic approach to confirm monogenic VEOIBD can be found here [5]).

In this group of children, microscopic mucosal assessment revealed a distinct subgroup of children with abnormal epithelial morphology and concomitant chronic or acute on chronic mucosal inflammatory cell infiltration. This feature carried a high positive predictive value and should prompt the physician to consider screening for genes involved in epithelial integrity [22] [29]. As previously reported, very early onset intestinal inflammation can be due to causative *EPCAM* mutations, which result in tufting enteropathy (TE). The epithelial changes in TE can initially be localised and subtle with inflammatory cell infiltration being a dominant feature. In these cases, inclusive genetic screening might reveal unexpected molecular diagnoses [30] [11]. Mutations in the gene *TTC7A* have recently been reported to cause IBD in children with stricturing intestinal disease and severe epithelial abnormalities on histology [22] [23]. Abnormal epithelial morphology and increased apoptotic activity was evident in all three of our patients. Prominent epithelial surface irregularities and increased apoptotic activity were also present in our patients with *SKIV2L* and *TTC37* mutations. Causative variants in *TTC37* and *SKIV2L* genes, both involved in the RNA decay pathway of the cell (exosome complex) [31] have previously been reported to cause tricho-hepato-enteric syndrome (THES) [32] [33].

Genetic Screening:

Monogenic conditions with IBD phenotype are commonly inherited in autosomal recessive fashion [5] and therefore not surprisingly, are enriched in the cohort of children from consanguineous unions. As an international referral centre, our cohort is subject to referral bias and as a consequence might over-represent ethnic populations with higher prevalence of consanguinity. An increased pre-test

probability will therefore overestimate the prevalence of monogenic IBD as compared to population-based studies.

However, our experience and previously published data suggests that comprehensive genetic screening regardless of consanguinity or ethnicity can reveal unexpected genotypes [11]. Given the large number of potential candidate genes and overlapping phenotypes, single gene sequencing appears less and less appropriate in cases with VEOIBD. The availability and processing time of NGS in the diagnostic setting has improved making it the modality of choice for most paediatric gastroenterology centres. In figure 4 we present a logistic regression model, which estimates the probability of screening positive for monogenic IBD based on three variables: onset of disease younger then 6 months, perianal disease and abnormal epithelial morphology. In the absence of specific syndrome-associated features such as extensive eczema and diabetes mellitus in patients with *FOXP3* mutations it might, according to our model, be reasonable to prioritise children with early disease onset and abnormal epithelial morphology or perianal disease for prompt genetic screening. Exclusively screening the infantile IBD subgroup would however not account for the numerous IBD associated monogenic diseases, which can present beyond infancy and childhood best exemplified by late-onset XIAP deficiency in patients with CD-like phenotype [24].

Establishing a genetic diagnosis has obvious advantages and potentially enables the physician to tailor treatment, predict outcome and counsel patient and parents effectively. For example, genetic screening might reveal genotypes amenable for HSCT in patients with primary immune deficiencies/dysregulation syndromes [13 21 34] offering a potentially curative treatment option.

Surgical and Medical Management:

Surgical intervention was required in 15% of our patients. All three patients with TTC7A deficiency required bowel resections and stoma formation as previously reported in the literature [22] [23]. From five children with IL10 pathway deficiency, three required extensive abdominal surgery; two were diagnosed within months after presentation and underwent HSCT prior to needing intensification of medical or surgical management [13] [12]. Three children with no genetic diagnosis underwent abdominal surgery and eventually HSCT for uncontrollable disease.

Similar to conventional IBD, the majority of VEOIBD patients have been exposed to steroids (86%) in addition to immunomodulators (85%) and TNFα blockade (39%) as long-term therapies. In addition, approximately half of the patients in our cohort required parenteral nutrition, abdominal surgery or further escalation of medical therapy to control disease (figure 6). This is in keeping with previously published data highlighting IBD in this age group as particularly therapy resistant [35] [36] [37]. Children with symptom onset in infancy and unclassified panenteric inflammation had an increased requirement for PN and escalation of medical treatment. In this sub group, we retrospectively established causative mutations in genes involved in epithelial barrier integrity (*EPCAM and TTC7A*) as well as in the RNA-decay pathway (*SKIV2L* and *TTC37*). Confirming the genotype even in these conditions currently lacking well-established treatment options enables the physician to offer less conventional treatments and facilitates the sharing of disease-specific management experience with the national and international expert community for the benefit of the patient.

Stratifying VEOIBD:

Stratifying VEOIBD by establishing a molecular basis was successful in 24% of our patients. Our cohort contained only a small proportion of classifiable patients (CD and UC). Conventional IBD classification is difficult to apply in infants and very young children with IBD: VEOIBD has been established as an umbrella term for diseases with chronic intestinal inflammation in childhood, which may or may not resemble or in time evolve into conventional IBD [5] [1].

As previously shown, our data suggests that infants with features suggestive of perianal-fistulising CD-like disease benefit from genetic screening to investigate, amongst others, mutations in the IL10-pathway.

In the present cohort, we only observed a small number of patients with UC-like IBD (n=6) and none harboured causative mutations in known VEOIBD genes. All patients presented with pancolonic disease and symptom onset was significantly later when compared to the IBDU cohort.

VEOIBD unclassified comprises a multitude of conditions such as apoptotic enterocolitis (e.g. TTC7A deficiency), autoimmune enteropathy (e.g. IPEX and IPEX-like syndromes), tufting enteropathy (EPCAM deficiency), THES (TTC37 and SKIV2L deficiency) as well as others yet undefined chronic intestinal inflammatory conditions. Autoimmune diseases which affect the gut (e.g. causative mutations in the genes *FOXP3*, *STAT1* and *IL2R* or *LRBA*) present with concomitant autoimmune features such as diabetes mellitus, thyroid disease, adrenal insufficiency or cytopenias [38] [39] [40] [20]. In our cohort, genetic screening revealed mutations in the *FOXP3* and *LRBA* gene in four patients who also expressed autoimmune features. A

large cohort of children with unclassified VEOIBD however, lacked evidence of autoimmunity could not be further classified based on clinical, histological, laboratory markers [41] nor harboured likely disease-causing mutations in known VEOIBD genes. The value of anti-enterocyte antibodies (unavailable in our cohort) might provide further evidence of autoimmunity. Doubts remain regarding their sensitivity and whether they merely are a secondary phenomenon after mucosal damage in some individuals [42].

Conclusion

Children with VEOIBD present with clinical and laboratory features reminiscent of conventional IBD but suffer from particularly extensive and treatment resistant disease. Particularly children with symptom-onset before the 2nd year of life should be undergo comprehensive immunological and genetic screening and a diagnosis beyond conventional IBD should be considered. We have previously suggested a mnemonic for features associated with monogenic IBD (YOUNG AGE MATTERS MOST: YOUNG AGE onset, Multiple family members and consanguinity, Autoimmunity, Thriving failure, Treatment with conventional medication fails, Endocrine concerns, Recurrent infections or unexplained fever, Severe perianal disease, Macrophage activation syndrome and hemophagocytic lymphohistiocytosis, Obstruction and atresia of intestine, Skin lesions and dental and hair abnormalities, and Tumors) [5].

Currently available classification tools are insufficiently addressing the heterogeneous phenotype of these patients and it is therefore advisable to have a low threshold for revision of conventional disease terminology. Choosing descriptive

disease terminology might therefore be more appropriate considering the heterogenetic and unpredictable disease evolution of VEOIBD.

Key messages:

- VEOIBD is an umbrella term for a heterogeneous group of children presenting with evidence of chronic-relapsing intestinal inflammation.
- VEOIBD patients frequently suffer from severe and treatment unresponsive disease.
- Comprehensive immunological, histological and genetic assessment is important to support diagnosis and management.
- Genetic screening is particularly advisable in consanguineous patients and children with infantile disease-onset, evidence of severe perianal disease, abnormal intestinal epithelial morphology or presence of other associated syndromic features.
- Particularly for children with disease-onset before the 2nd year of life, the phenotype heterogeneity is not sufficiently addressed by currently available IBD classification tools.

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