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Clinical and Mutation Description of the First Iranian Cohort of Infantile Inflammatory Bowel Disease: The Iranian Primary Immunodeficiency Registry (IPIDR)

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ABSTRACT
We describe a cohort of 25 Iranian patients with infantile inflammatory bowel disease (IBD), 14 (56%) of whom had monogenic defects. After proper screening, patients were referred for whole exome sequencing (WES). Four patients had missense mutations in the IL10RA, and one had a large deletion in the IL10 RB. Four patients had mutations in genes implicated in hostmicrobiome homeostasis, including TTC7A deficiency, and two patients with novel mutations in the TTC37 and NOX1. We found a novel homozygous mutation in the SRP54 in a deceased patient and the heterozygous variant in his sibling with a milder phenotype. Three patients had combined immunodeficiency: one with ZAP-70 deficiency (T'B'NK'), and two with atypical SCID due to mutations in RAG1 and LIG4. One patient had a G6PC3 mutation without neutropenia. Eleven of the 14 patients with monogenic defects were results of consanguinity and only 4 of them were alive to this date.

KEYWORDS
Very-early-onset inflammatory bowel disease; whole Exome Sequencing; IL-10 Receptor; TTC37; G6Pc3; SRP54

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**Introduction**

Early-onset inflammatory bowel disease (EO-IBD) is a heterogeneous subgroup of IBD spectrum, characterised by age of onset below 10 years old, predominance of pancolic involvement, high rate of strictures, need for early use of biologic drugs and colectomy surgery, and higher morbidity and mortality (Avitzur et al. 2014; Duricova et al. 2014). EO-IBD patients with an age of onset under 6 years old have a particularly higher rate of carrying monogenic defects and usually present with a unique disease phenotype, leading to the introduction of the “very early-onset” IBD (VEO-IBD) subtype (onset < 6 years old). VEO-IBD in turn subsumes the neonatal (onset < 28 months old) and infantile (onset < 2 years old) categories (Levine et al. 2011). Considering the aggressive course of disease, identification of monogenic defects in patients with VEO-IBD opens new therapeutic avenues, namely hematopoietic stem cell transplantation (HSCT) and gene replacement therapy that could prove curative (Kotlarz et al. 2012).

IBD can also present as part of the clinical spectrum of other primary immunodeficiency disorders (PID) including atypical forms of severe combined immunodeficiency (SCID), X-linked immune dysregulation, polyendocrinopathy, enteropathy (IPEX) and IPEX-like syndromes, X-linked inhibitor of apoptosis protein (XIAP) deficiency, as well as tetratricopeptide repeat domain 7A (TTC7A), and 37 (TTC37) deficiencies, and defects in other genes associated with intestinal epithelial barrier defects (Hartley et al. 2010; Kelsen and Sullivan 2017). Meanwhile, IBD can be the sole presenting feature in IL10 and IL-10 receptor (IL10R) gene defects, and in the trichohepatoenteric syndrome (THES) associated with TTC37 (Kelsen and Sullivan 2017). In the latter category, patients typically present during infantile period, have a more aggressive disease, higher frequency of perianal involvement and high morbidity and can be rightfully categorized as infantile IBD (Kelsen and Sullivan 2017). Given the curative role of HSCT in patients with IL10 and IL10R mutations and other PIDs associated with IBD, as well as the crucial role of early initiation of parenteral nutrition and immunoglobulin replacement therapy in patients with THES (Engelhardt et al. 2013; Alexandre Fabre et al. 2017; Karaca et al. 2016), comprehensive immunologic evaluation is considered a mandatory work up for every patient with infantile or VEO-IBD.

Herein we describe clinical features and mutation analysis of the first cohort of 25 pediatric patients with infantile IBD from the Iranian Primary Immunodeficiency Registry (IPIDR). Patients were screened for infectious causes of colitis and immunodeficiencies, before being referred for whole exome sequencing (WES).

**Methods**

**Patients**

Clinical and genetic data were gathered from patients enrolled in this study as part of the Iranian Primary Immunodeficiency Registry (IPIDR). Pediatric practitioners from pediatric health centers all around country, including pediatric gastroenterologists and immunologists, have established a prospective registry-based in the Children’s Medical Center (CMC) hospital in Tehran, Iran, to collect demographic, clinical and epidemiologic data from patients with PID since 2002 (Aghamohammadi et al. 2014). The registry for pediatric patients with VEO-IBD started on January 1, 2015 and is running through the date of this
article final submission, recruiting pediatric patients with infantile and VEO-IBD referred to the CMC hospital from all over the country. Inclusion criteria for the current cohort were (1) onset of chronic diarrhea before the age of 6 years (VEO-IBD, infantile IBD or neonatal IBD), associated with (2) signs of active colitis on ileocolonoscopy and (3) biopsy findings compatible with either Crohn disease (CD), ulcerative colitis (UC), or inflammatory bowel disease unclassified (IBDU), based on the revised Porto criteria (Levine et al. 2014). All patients underwent through clinical examination and history taking by allergy and immunology subspecialty fellowships at the time of registration in order to identify associated clinical signs corroborating the diagnosis of IBD, and to rule out mimicking differential diagnoses such as allergic responses to dietary antigens or gastrointestinal infections. We adopted the screening approach proposed by Uhlig et al. (Uhlig et al. 2014), except for the fact that due to non-availability of flowcytometry study for FOXP3 and XIAP expression, patients suspected for IPEX or XLP were directly referred for WES study. Patients had all undergone ileocolonoscopy and upper gastrointestinal (GI) endoscopy with at least two biopsy samples taken from each functional segment of the gastrointestinal tract at the time of IBD diagnosis.

Overall 25 patients met the inclusion criteria and were included in the registry, along with both parents and siblings if any. Venous blood sample was obtained from patients and their parents after obtaining informed written consent from both parents, and was used for DNA extraction at the research center for immunodeficiency (RCID) lab in the CMC hospital. The samples were then referred to our partner labs in Dr. von Hauner Children’s Hospital in Munich and Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases in Vienna for WAS study. The protocol of the study, data collection and anonymization all adhered to the declaration of Helsinki and were approved by local ethics review board at the CMC hospital and Tehran University of Medical Sciences, as well as institutional review boards of partnering institutes.

**DNA preparation and whole exome sequencing**

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs), isolated by Ficoll–Paque density gradient centrifugation (Lymphodex, Kronberg/Taunus Innotrain, Germany), using the Phenol-Chloroform method (Di Pietro et al. 2011).

Whole exome sequencing (WES) was performed for each patient, using Agilent V5 + UTR library preparation and an Illumina NextSeq 500 sequencing platform. The bioinformatics analysis pipeline uses Burrows-Wheeler Aligner (BWA 0.7.15) Genome Analysis Toolkit (GATK 3.6), Variant Effect Predictor (VEP 89) and frequency filters with public and in house databases (ExAc, GnomAD, and GME). Subsequently Sanger sequencing was used to confirm the results.

**Results**

**Description of cohort**

From January 1, 2015 through September 1, 2019, we recruited 30 consecutive patients with an initial diagnosis of infantile/VEO-IBD, 5 of whom were excluded as they were proven to have either severe food protein-induced enteropathy or various types of PIDs associated
with chronic diarrhea. Twenty-five patients met the inclusion criteria and enrolled in the study (Table 1). First symptoms of the disease started before 2 years old in all but two patients, meaning that 92% of patients had infantile IBD. Mean age of symptoms onset was 6.6 ± 9.8 months, ranging between few days after birth to up to 37 months. Patients P6 and P7, who were siblings, and patient P10 were the only patients with a positive family history of IBD in their siblings. Nineteen patients had a diagnosis of CD, four had IBDU, one had UC, and two siblings (P6 and P7) had an undetermined severe apoptotic colitis. Eleven patients with Crohn disease had a history of perianal abscess (36%), six patients (24%) had perianal (rectovaginal, rectoperineal or rectovesical fistula), three had anal fissures (12%), and the remaining patients had retractive oral or esophageal thrush and oral aphthous lesions, as IBD associated symptoms. Diagnostic delay – time between the onset of symptoms and diagnosis of IBD – ranged from less than a month to up to 48 months, while most patients (88.4%) were diagnosed within 12 months of the onset of symptoms.

Regarding other GI-related manifestations, 20% of patients had cow's milk allergy, 8% of whom had multiple food allergies to dairy, nuts, seafood, etc., which was diagnosed through standardized allergic panel. Three patients (P2, P3, and P21) with the diagnosis of CD had skin involvement in form of folliculitis and pustular lesions on limbs. None of the patients had any form of arthropathy or axial skeletal involvement. All patients developed growth failure, and four were diagnosed and treated for cytomegalovirus (CMV) colitis during follow-ups. Blood count and immunological workup revealed abnormal count of WBCs, B cells, or T cells in five patients, three of whom were later identified to have known mutations associated with SCID, and one with a mutation previously associated with severe congenital neutropenia (SCN). WES revealed a monogenic defect in 14 out of 25 patients (56%). Eleven (78%) out of 14 patients with monogenic defects were born from consanguineous parents, compared to 2 (18%) out of 11 patients in whom no mutation was identified (P15-P25).

**Mutations in IL10 RA or IL10 RB**

WES revealed mutations in IL10 R genes in five patients, four in the IL-10 receptor subunit alpha (IL10RA) gene, and one in the IL-10 receptor subunit beta (IL10RB) gene. Two patients carried a homozygous variant in IL10RA that was previously reported in association with EO-IBD (c.537 G > A, p.T179 T) (Yanagi et al. 2016), while two novel homozygous mutations were identified in patients P3 and P4 (c.113 T > G, p. 38 F > C & c.809 T > C, p.270 L > P, respectively). Patient P5 proved to have a large deletion in the IL10RB encompassing exon 3 through 7 (Figure 1). Patient P1 is now an 8-year-old girl with full recovery of IBD symptoms 12 months after HSCT from a living unrelated donor. She suffers from chronic graft-versus-host disease with cutaneous manifestations and elevated liver enzymes.

**Other mutations associated with syndromic diarrhea and IBD**

Patients P6-P11 had mutations in genes other than IL10RA and IL10RB. Patients P6 and P7 were siblings with onset of severe diarrhea early after birth and evidence of severe enterocyte apoptosis, crypt abscesses and loss of microscopic architecture in colonic biopsy (Avitzur et al. 2014). Unfortunately, P6 died due to sepsis, but her sister is currently alive with partial disease control on mesalazine and hypoallergenic diet, despite being severely
<table>
<thead>
<tr>
<th>ID</th>
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</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>F</td>
<td>No</td>
<td>16 d</td>
<td>11 mo.</td>
<td>Crohn</td>
<td>Perianal Abscess &amp; Rectovaginal Fistula</td>
<td>EBV Infection</td>
<td>HSCT at 7 y Clinical Remission Cutaneous GVHD</td>
<td>IL10RA, c.537 G &gt; A, p.T179 T</td>
</tr>
<tr>
<td>P2</td>
<td>M</td>
<td>No</td>
<td>4.5 mos.</td>
<td>8 mos.</td>
<td>Crohn</td>
<td>Anal Fissure &amp; Perianal Abscess</td>
<td>Widespread Skin Pustular Lesions</td>
<td>Died at 30 mos. due to Sepsis</td>
<td>IL10RA, c.537 G &gt; A, p.179 T T</td>
</tr>
<tr>
<td>P3</td>
<td>M</td>
<td>Yes</td>
<td>14 d</td>
<td>7 mos.</td>
<td>Crohn</td>
<td>Onset and Perianal Abscess Imperforated Anus Biliary Atresia</td>
<td>Multiple Food Allergies, Pustular lesions on limbs, &amp; PDA</td>
<td>Died at 9 mos. due to Sepsis</td>
<td>IL10RA, c.113 T &gt; G, p.38 F &gt; C</td>
</tr>
<tr>
<td>P4</td>
<td>F</td>
<td>Yes</td>
<td>1.5 mos.</td>
<td>7 mos.</td>
<td>Crohn</td>
<td>Perianal Abscess</td>
<td>-</td>
<td>Unavailable</td>
<td>IL10RA, c.809 T &gt; C, p.270 L &gt; P</td>
</tr>
<tr>
<td>P5</td>
<td>F</td>
<td>Yes</td>
<td>1 mo.</td>
<td>&lt; 1 mo.</td>
<td>Crohn</td>
<td>Recurrent Anal &amp; Perianal Abscesses</td>
<td>Recurrent Pneumonia</td>
<td>Died at 11 mos. due to sepsis</td>
<td>IL10RB full exon 3 to exon 7 deletion</td>
</tr>
</tbody>
</table>

**Mutation in Non-IL10R, EO-IBD Associated Genes**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>P6*</td>
<td>F</td>
<td>Yes</td>
<td>Early after birth</td>
<td>&lt; 1 mo.</td>
<td>Sever apoptotic enterocolitis</td>
<td>Non-Bloody Diarrhea &amp; CMV Colitis</td>
<td>-</td>
<td>Died at 11 mo. due to Candida Sepsis</td>
<td>TTC7a, c.2494 G &gt; A, p.832A&gt;T</td>
</tr>
<tr>
<td>P7*</td>
<td>F</td>
<td>Yes</td>
<td>Early after birth</td>
<td>&lt; 1 mo.</td>
<td>Sever apoptotic enterocolitis</td>
<td>Non-Bloody Diarrhea &amp; Oral Candidiasis</td>
<td>-</td>
<td>Alive, Partial Control on S-ASA and Hypoallergenic Diet, Growth delay</td>
<td>TTC7a, c.2494 G &gt; A, p.832A&gt;T</td>
</tr>
<tr>
<td>P8</td>
<td>M</td>
<td>Yes</td>
<td>6 mos.</td>
<td>&lt; 1 mo.</td>
<td>Non-specific Colitis</td>
<td>Refractory Metabolic Acidosis Cow's Milk Allergy Prenatal and Postnatal Growth Delay</td>
<td>-</td>
<td>Alive, Full Control of Intestinal Manifestations on Hypoallergenic Diet, Growth and Developmental delay</td>
<td>TTC37, c.85-T</td>
</tr>
<tr>
<td>P9</td>
<td>M</td>
<td>Yes</td>
<td>6 mos.</td>
<td>&lt; 1 mo.</td>
<td>Crohn</td>
<td>-</td>
<td>-</td>
<td>Unavailable</td>
<td>NOX1, c.827 C &gt; T, p.276P&gt;L</td>
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### Table 1. (Continued).

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<tr>
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</thead>
<tbody>
<tr>
<td>P10</td>
<td>M</td>
<td>Yes</td>
<td>36 mos.</td>
<td>&lt; 1 mos.</td>
<td>Crohn</td>
<td>Anal Fissure and Rectoperineal fistula</td>
<td>Cutaneous Reaction to Infliximab Change to Adalimumab</td>
<td>Died at 8 y</td>
<td>SRPS4, c. 35 C &gt; T, p. 12 S &gt; L</td>
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<td></td>
<td></td>
<td>ZAP70, c. 552 C &gt; A</td>
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<td>RAG1, c. 1856 C &gt; T, p. 619P &gt; R</td>
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<td>LIG4, c. 1282 G &gt; C, p. 428 G &gt; R</td>
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<td></td>
<td>G6PC3, c. 479 C &gt; T, p. 160 S &gt; L</td>
</tr>
<tr>
<td>P11</td>
<td>F</td>
<td>Yes</td>
<td>4 mos.</td>
<td>9 mos.</td>
<td>Crohn</td>
<td>Perianal Abscess &amp; Refractory Oral Candidiasis</td>
<td>Lymphopenia and Low CD8 + Counts</td>
<td>Died at 14 mos. due to Candida Sepsis</td>
<td>ZAP70, c. 552 C &gt; A</td>
</tr>
<tr>
<td>P12</td>
<td>M</td>
<td>Yes</td>
<td>8 mos.</td>
<td>48 mos.</td>
<td>Crohn</td>
<td>Perianal Abscess &amp; Refractory Oral Candidiasis</td>
<td>Low CD4+ &amp; B-cell Counts and Low IgG Levels</td>
<td>Died at 8 y due to Sepsis</td>
<td>RAG1, c. 1856 C &gt; T, p. 619P &gt; R</td>
</tr>
<tr>
<td>P13</td>
<td>M</td>
<td>No</td>
<td>12 mos.</td>
<td>36 mos.</td>
<td>Crohn</td>
<td>Refractory Oral Candidiasis</td>
<td>Low CD4+ Counts &amp; Low IgG Levels Visceral Leishmaniosis &amp; Cirrhosis</td>
<td>Died at 6 y due to Cirrhosis Complications</td>
<td>LIG4, c. 1282 G &gt; C, p. 428 G &gt; R</td>
</tr>
<tr>
<td>P14</td>
<td>F</td>
<td>Yes</td>
<td>Early after birth</td>
<td>12 mos.</td>
<td>Crohn</td>
<td>Rectovaginal &amp; Rectoperineal Fistula</td>
<td>ASD type 2 &amp; Inguinal Hemia</td>
<td>Alive, Poorly Controlled IBD due to Poor Family Compliance</td>
<td>G6PC3, c. 479 C &gt; T, p. 160 S &gt; L</td>
</tr>
<tr>
<td>P15</td>
<td>F</td>
<td>No</td>
<td>16 mos.</td>
<td>18 mos.</td>
<td>Ulcerative colitis</td>
<td>-</td>
<td>Multiple Food Allergies &amp; Low CD4+ Percentage</td>
<td>Alive, Remission Under PDN &amp; 5-ASA</td>
<td>None</td>
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<tr>
<td>P16</td>
<td>M</td>
<td>No</td>
<td>1 mos.</td>
<td>12 mos.</td>
<td>Non-specific Colitis</td>
<td>CMV Colitis</td>
<td>-</td>
<td>Alive, Full Control with No Medication</td>
<td>None</td>
</tr>
<tr>
<td>P17</td>
<td>M</td>
<td>No</td>
<td>37 mos.</td>
<td>3 mos.</td>
<td>Crohn</td>
<td>CMV Colitis &amp; Candida Esophagitis</td>
<td>Pancreatitis with AZT</td>
<td>Alive, Remission on PDN &amp; Tacrolimus</td>
<td>None</td>
</tr>
<tr>
<td>P18</td>
<td>F</td>
<td>Yes</td>
<td>7 mos.</td>
<td>6 mos.</td>
<td>Crohn</td>
<td>Cow’s Milk Allergy Oral Aphthous Ulcers Perianal Abscess &amp; Bloody Diarrhea Oral Aphthous Lesion</td>
<td>-</td>
<td>Alive, Remission Under PDN &amp; 5-ASA</td>
<td>None</td>
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<tr>
<td>P19</td>
<td>F</td>
<td>No</td>
<td>3.5 mos.</td>
<td>1 mos.</td>
<td>Non-specific colitis</td>
<td>Cow’s Milk Allergy</td>
<td>Cow’s Milk Allergy</td>
<td>Alive, Full Control on PDN, 5-ASA, &amp; Dairy Avoidance</td>
<td>None</td>
</tr>
<tr>
<td>P20</td>
<td>M</td>
<td>No</td>
<td>Early after birth</td>
<td>12 mos.</td>
<td>Crohn</td>
<td>-</td>
<td>-</td>
<td>Alive, Partial Control on PDN, AZT, &amp; Dairy Avoidance</td>
<td>None</td>
</tr>
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<tr>
<td>P21</td>
<td>F</td>
<td>No</td>
<td>5 mos.</td>
<td>1 mo.</td>
<td>Crohn</td>
<td>Perianal Abscess &amp; Rectovaginal Fistula</td>
<td>Recurrent Fever, Recurrent Pustular Lesions on Face &amp; Limbs</td>
<td>Poorly Controlled with 2nd Course Adalimumab</td>
<td>None</td>
</tr>
<tr>
<td>P22</td>
<td>M</td>
<td>Yes</td>
<td>2 mos.</td>
<td>1 mo.</td>
<td>Crohn</td>
<td>Perianal Abscess, Recurrent Watery diarrhea and Vomiting</td>
<td>Hepatosplenomegaly</td>
<td>Died at 6 mos. Due to Sepsis</td>
<td>None</td>
</tr>
<tr>
<td>P23</td>
<td>F</td>
<td>No</td>
<td>20 d.</td>
<td>6 mos.</td>
<td>Crohn</td>
<td>Anal Fissure</td>
<td>Multiple Food Allergies</td>
<td>Alive, Partial Control with 5-ASA, and PDN</td>
<td>None</td>
</tr>
<tr>
<td>P24</td>
<td>M</td>
<td>No</td>
<td>7 mos.</td>
<td>5 mos.</td>
<td>Crohn</td>
<td>Oral Aphthous Lesions</td>
<td>-</td>
<td>Alive, Full Control with PDN and AZT</td>
<td>None</td>
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<tr>
<td>P25</td>
<td>M</td>
<td>No</td>
<td>4 mos.</td>
<td>11 mo.</td>
<td>Crohn</td>
<td>Perianal Fistula</td>
<td>-</td>
<td>Alive, Well Controlled with 5-ASA</td>
<td>None</td>
</tr>
</tbody>
</table>

EO-IBD: early-onset inflammatory bowel disease, IL10RA/B: interleukin 10 receptor subunit alpha and beta; HSCT: hematopoietic stem-cell transplantation; GVHD: graft-versus-host disease; PID: primary immunodeficiencies; CMV: cytomegalovirus; EBV: Epstein-Barr virus; PDA: patent ductus arteriosus; TTC7A/TTC37: tetratricopeptide repeat domain 7A and 37 genes; ZAP70: zeta-chain-associated protein kinase gene; NOX1: NADPH oxidase 1; SRP54: signal recognition particle 54; IgG: immunoglobulin G; ASD: atrial septal defect; mos.: months; d: days; PDN: prednisolone; 5-ASA: 5-aminosalicylic including mesalazine; AZT: azathioprine.
underweight and stunted. P8 had a single nucleotide deletion in the TTC37 gene, resulting in premature termination of transcription. P9 had a novel missense mutation in the NAPDH oxidase 1 (NOX1) gene which is responsible for neutrophilic production of superoxide species, and P10 had a novel homozygous missense mutation in the signal recognition particle 54 (SRP54) gene. P10 died due to sepsis at the age of 8 years. The older brother of patient P10 also has CD with a later age of onset around 5 years old and a milder phenotype. He has been started on 6-mercaptopurine (6-MP) after the onset of widespread cutaneous CD involving axially and genital areas. He has achieved relative remission of intestinal symptoms and is now 10 years old. Interestingly, both parents and patient’s affected older brother carried the heterozygous form of this variant in SRP54.

**Mutations associated with PIDs**

Patients P11 to P14 were shown to have aberrancies in their immunologic work-up in form of reduced CD4, CD3 and/or CD8 + T cells, or CD19 + B cells counts. WES revealed a novel homozygous mutation in the zeta-chain-associated protein kinase (ZAP70) gene in P11, who presented with intractable diarrhea and recurrent oral thrush. We also revealed a novel missense mutation in the recombination-activating 1 (RAG1) in P12 [http://exac.broad institute.org/variant/11-36596710-C-T], and a novel missense mutation in DNA ligase 4 (LIG4) in P13 that had never been reported in association with EO-IBD before. Patient P14 had a possibly destructive missense mutation in the glucose-6-phosphatase catalytic subunit 3 (G6PC3) gene. She presented with intractable diarrhea, and refractory rectovaginal and rectoperineal fistula, along with atrial septal defect and congenital inguinal hernia, both requiring surgery early after birth, but had no apparent abnormalities in blood count.
Discussion

Here we described the clinical features and mutations in the first cohort of pediatric patients with infantile IBD from the IPDR registry. We identified a monogenic cause for IBD in 56% of the patients, much higher than reports from previous cohorts (24–32%) (Charbit-Henrion et al. 2018; Kotlarz et al. 2012). Nine patients had monogenic defects that were potentially amenable to HSCT, including IL10RA, IL10RB, ZAP70, RAG1, LIG4, and G6PC3 mutations. However, only one patient had a successful HSCT with full remission of symptoms. The high prevalence of monogenic defects in this cohort might partly reflect our selection protocol that excluded patients in whom the IBD phenotype was associated with any evidence of other differential diagnoses, including food-protein-induced enterocolitis syndrome, congenital glucose/galactose malabsorption, or PID. This approach enriched our cohort with monogenic forms of infantile and VEO-IBD. Meanwhile, we screened patients for EO-IBD mimickers, including chronic granulomatous disease (CGD), Wiskott Aldrich syndrome, IPEX and IPEX-like syndromes, typical SCIDs, and STAT1 gain-of-function mutation, effectively excluding patients with PID from the EO-IBD registry. On the other hand, mean age of onset in our patients was about 6 months old, which is significantly lower than both of the previous reports (Charbit-Henrion et al. 2018; Kotlarz et al. 2012). This could partly justify the enrichment of monogenic defects in our population as well as the presence of other PID associated genes such as NOX1, ZAP70, RAG1, LIG4, and G6PC3 in our cohort. Low survival chance of VEO-IBD patients with monogenic defects compared to their peers, as seen in the current report, reiterates the importance of early identification of these patients, who could potentially benefit from specific immunotherapy and/or HSCT.

Twenty percent of our patients had monogenic defects in IL-10 related genes; IL10RA and IL10RB, which are the most common monogenic forms of solitary VEO-IBD (Kammermeier et al. 2017; Kelsen and Sullivan 2017). Two patients (P1 and P2) had a known mutation in exon 4 of the IL10RA which confers splicing aberrations and impaired signalling in the IL-10 receptor (P1 and P2) (Fang et al. 2018; Yanagi et al. 2016). The two novel mutations identified in IL10RA in patients P3 and P4 are potentially detrimental to IL-10R subunit alpha tertiary structure, as amino acid replacements are likely non-conservative. Both of these patients are pending completion of functional analyses. Importantly, only one patient with IL10RA mutation (P1) underwent successful HSCT and is the only alive patient with monogenic defect with full remission of IBD. This reiterates the necessity of early identification of monogenic causes of EO-IBD and the curative role for HSCT (Murugan et al. 2014).

P6-P10 had single gene mutations associated with maintenance of gut epithelial barrier and infantile syndromic diarrhea. P6 and P7 were sisters with identical homozygous mutations in the TTC7A gene (Avitzur et al. 2014), who presented with severe diarrhea early after birth along with evidence of severe apoptotic colitis, but no apparent immuno-deficiency or intestinal atresia similar to what has been reported in patients with TTC7A deficiency before (Broome et al. 2019; Lien et al. 2017). The A832 T mutation results in amino acid substitution in a highly conserved site for mutation in TTC7A, the tetratricopeptide repeat domain. This has proved to be deleterious to protein plasma membrane expression and intracellular singling (Avitzur et al. 2014). Despite reports showing that homozygous variants of TTC7A often prove fatal in early childhood (Broome et al. 2019),
P7 is alive at 8 years old and doing well on hypoallergenic, and dairy and nuts restricted diet, and parenteral nutrition. Patient P8 similarly presented with a typical syndromic diarrhea phenotype and had a mutation in the TTC37 gene which has been associated with the THES syndrome type 1 in the literature (Hartley et al. 2010; Rider et al. 2015). THES is characterized by a classic pentad of intractable diarrhea, facial dysmorphism, trichorrhexis nodosa, immune abnormalities, and growth retardation (A. Fabre et al. 1993), although several patients have been described with atypical forms including predominance of IBD-like features, or combined immunodeficiency associated with late-onset diarrhea (Busoni et al. 2017; Hosking et al. 2018; Vely et al. 2018). The classic pentad of THES was absent in our patient, who instead presented with refractory metabolic acidosis, severe vomiting and diarrhea and pre and postnatal growth delay. He was born at 35 weeks of gestation with a birth weight of 1500 g and is currently a 7-year-old boy with developmental delay and weight and height both below 10th percentile. P9 had a novel mutation in NOXI gene, which was associated with VEO-IBD in two other patients (Hayes et al. 2015; Lipinski et al. 2019; Schwert et al. 2018). NADPH oxidase is crucial to maintain gut epithelial barrier towards microbiota through generation of superoxide species and modulation of colonic epithelial proliferation and postmitotic differentiation (Coant et al. 2010; Kawahara et al. 2004). The importance of the reactive oxygen species generated by the NADPH oxidase system in gut barrier function is further exemplified by a higher prevalence of IBD-like symptoms in CGD, which is caused by inactivating mutations in components of the phagocyte NADPH oxidase complex (Uhlig 2013). Between 40% and 74% of patients with CGD develop IBD, often with CD-like phenotype and with symptom onset above 2 years old (Khangura et al. 2016; Marks et al. 2009).

We also reported a novel homozygous mutation in the SRP54 gene in patient P10, which was present in homozygous form in his parents and older sibling. Heterozygous mutations in SRP54 have been described in four patients with autosomal dominant form of SCN associated with Shwachman-Diamond syndrome (SDS) phenotype (Bellanne-Chantelot et al. 2018; Carapito et al. 2017), but never in EO-IBD. The SRP54 gene encodes a component of the signal recognition particle (SRP), a ribonucleoprotein responsible for co-translational recognition of secretory molecules and targeting them for the endoplasmic reticulum (ER) (Pool et al. 2002). Dysfunctional SRP leads to ER stress, autophagy and hence P53-dependent apoptosis of promyelocytes in bone marrow of SDS patients, culminating in severe neutropenia (Bellanne-Chantelot et al. 2018). Interestingly, duodenal inflammatory atrophy is seen in up to 50% of patients with SDS and one patient with SDS has been reported with comorbid CD in the literature (Nissen et al. 2019). We speculate that the numeral defect in neutrophils can hinder formation of normal gut barrier against colonic microbiota and predispose patients with SRP54 mutation to IBD, similar to the effect of functional defects in CGD. Nonetheless, preliminary results from our Polyphen and SIFT phenotype prediction algorithms predict the patients p.12 S > L substitution as being benign or tolerated, adding to confusion on the pathophysiology of IBD in this patient. The significance of the homozygous mutation in SRP54 in our patient, and the heterozygous form in his parents and mildly affected sibling, and its association to IBD pathology is pending to be validated through functional studies by our group.

In our cohort we identified three patients with SCID, all of whom presenting with refractory diarrhea, with perianal abscess and/or oral candidiasis, but no recurrent infection or other stigmata of SCID. These patients developed lymphopenia and characteristic
lymphocyte subset aberrancies during follow-ups, and unfortunately, all passed away from complications of recurrent infections before receiving HSCT.

The product of ZAP70 gene, ZAP-70, is essential for T cell receptor (TCR) signal transduction and is expressed predominantly on natural killer cells and T cell (Chan et al. 2016). ZAP-70 deficiency is characterized by the absence of CD8 + T cells in the periphery, defective TCR signalling in CD4+ cells, and defective T cell tolerance induction, the latter being cited for autoimmune manifestations in these patients (Liu et al. 2017). Importantly, the presence of IBD in patients with ZAP70 mutation is associated with a leaky SCID phenotype with residual CD4 + T cell activity (Liu et al. 2017). Single nucleotide variants of ZAP70 have been found to affect IBD risk in some populations (Bouzid et al. 2013). Our patient had low CD8+ counts, recurrent thrush and a CD phenotype and unfortunately died due to complications of candidemia at 14 months old.

Rag1 -/- mice transferred with naïve allogenic CD4 + T cells develop an IBD phenotype in response to gut microbiota (Stepankova et al. 2007), a model that is frequently used to study the dynamics of altered immunologic response to gut microbiota in the pathogenesis of IBD (Britton et al. 2019; Trobonjaca et al. 2001). Hypomorphic mutations in RAG1/2 genes are among frequent causes of the milder, late-onset form of combined primary immunodeficiency, i.e., atypical SCID, with a T low B low phenotype (Felgentreff et al. 2011; Villa et al. 2001). Patients with atypical SCID classically present with granulomatous colitis, autoimmune cytopenia, or other autoimmune conditions but not recurrent infections (Felgentreff et al. 2011). Mutations in other genes involved in VDJ recombination, including LIG4, have also been associated with T low B low phenotype, atypical SCID and autoimmunity (De Azevedo Silva et al. 2014). We hypothesize that the IBD phenotype in patients P12 (with RAG1 mutation) and P13 (with LIG4 mutation) was the heralding sign for atypical SCID in them, who later developed refractory thrush (P12 and P13) and perianal abscess (in P12) and visceral leishmaniosis leading to cirrhosis (in P13). Unfortunately, neither patients survived without HSCT.

Finally, patient P14 had a novel missense mutation in the G6PC3 gene with undetermined significance (National Center for Biotechnology Information, 2019). G6PC3 deficiency results in an autosomal recessive form of SCN, associated with congenital cardiac and urogenital anomalies (Boztug et al. 2009). The variant in patients P14 has never been reported in patients with the G6PC3-related SCN, although phenotype prediction algorithms suggest that the mutation is destructive to protein structure (National Center for Biotechnology Information 2019). Our patient had a history of atrial septal defect as well as inguinal hernia, compatible with cardiac and urogenital abnormalities reported in G6PC3-deficient patients, but had no neutropenia. IBD has been reported in at least 10 patients with G6PC3 deficiency, all resembling a CD phenotype (Begin et al. 2013; Cullinane et al. 2011; Desplantes et al. 2014; Fernandez et al. 2012; Mistry et al. 2017; Smith et al. 2012), as observed in our patient. As mentioned, neutrophils are crucial in maintenance of gut microbiota: epithelial barrier, through production of reactive oxygen species, enzymes, and chemotactic agents which limit microbial invasion, and by timely termination of inflammatory response and preventing epithelial damage (Cullinane et al. 2011). Similar to CGD, it is speculated that impairments in either function or number of neutrophils are the reason behind IBD-like disease in patients with G6PC3 deficiency (Banka and Newman 2013; El-Mokhtar et al. 2020).
Conclusion

We described a cohort of Iranian patients with EO-IBD in more than half of whom we found responsible monogenic mutations. This relatively high rate of monogenic defects in our cohort reflects our patient’s selection pipeline, as well as reiterating the importance of immunologic evaluation in all patients presenting with EO-IBD. Unfortunately, only 4 out of 14 patients with monogenic defect are alive, compared to 10 out of 11 patients with no mutations, and only 1 patient could benefit from stem cell transplantation has received allogenic HSCT. We believe that long diagnostic delay and compilation of complications, such as perianal abscess and hepatitis, contributed to this casualty in our cohort. Interestingly, we introduced siblings one with a heterozygote and one with a homozygote mutation in the SRP54 gene, which has never been reported in EO-IBD patients, but can be functionally associated with IBD due to its role in neutrophil progeny maturation. We recommend functional IL-10 mediated suppression of lipopolysaccharide-induced PBMC activation to be performed in all pediatric patients presenting with infantile or VEO-IBD as part of routine immunologic workup and at the time of diagnosis.

Disclosure statement

The authors declare that they have no conflict of interest.

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