Clinical-molecular characteristics of very early-onset inflammatory bowel disease in Brazilian children

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1. Introduction

Inflammatory bowel disease (IBD) is a multifactorial disease caused by the combination of genetic predisposition, exposure factors (environmental and dietary), immune status, and dysbiosis, and it could present at any age, ranging from newborns to the elderly. Very early onset inflammatory bowel disease (VEOIBD) represents 4 to 10% of pediatric IBD and is characterized by the presence of symptoms onset before the age of 6 years. Unlike older children and adults, who mostly have polygenic involvement in pathogenesis, VEOIBD has mono- or oligogenic involvement. Recent studies have shown an annual increase in incidence of 7% in children under 5 years old with IBD diagnosis over the past decades. Due to the importance of monogenic causes in patients with VEOIBD and their close relationship with IEI, this study aims to identify variants that may be associated with VEOIBD using a molecular panel developed for IEI diagnosis

2. Methods

This is a cross-sectional study with participants aged 0 to 18 years, with IBD onset before age 6, recruited between April 2022 and January 2024. Saliva samples were collected for genetic testing using oral swabs for analysis by INVITAE. The Fleury Genomics laboratory also analyzed a small portion of the samples. Both use NGS techniques via the Illumina platform, based on the GRCh37 version of the Human Genome for variant detection and analysis. The selected genetic panel contains 426 genes. The data generated by sequencing were analyzed through customized bioinformatics processes (pipeline v3.10). The variants were classified according to the American College of Medical Genetics (ACMG) as pathogenic, likely pathogenic, and of uncertain significance (Figure 1)

3. Results

The study included 32 VEOIBD patients, predominantly female, with an average symptom onset at 2 years and 3 months. 37.5% had symptoms before age 2, and diagnostic confirmation took about 11 months. Clinical data and all IBD characteristics are described in Table 1. Lymphocyte counts and immunoglobulin levels are demonstrated in Table 2.

Table 3 describes clinical, endoscopic, laboratory, and genetic findings in patients with pathogenic/likely pathogenic/ or VUS variants in the genes previously associated with VEOIBD. Table 4 describes the same characteristics for patients with variants associated with an increased risk of developing IBD. Table 5 describes findings in patients with variants we considered with probable association with IBD.

Figure 1: Screening of variants through the evaluation of the phenotype previously described for the gene in question (ClinVar, Medgen, OMIM) and the clinical manifestations presented by the patient, also considering inheritance, zygosity, and alterations in the encoded protein

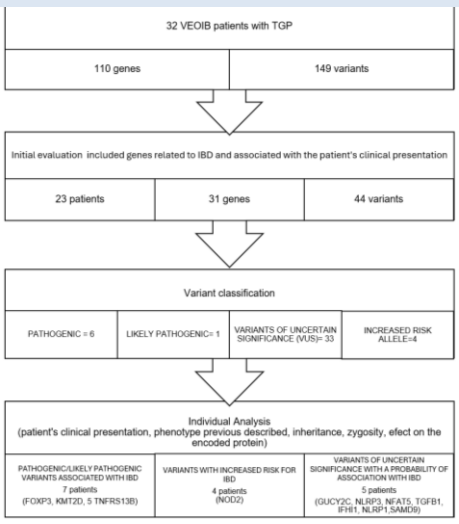

Table 1: Characterization of Brazilian patients with Very Early Onset Inflammatory Bowel Disease (VEOIBD)

Mean age at diagnosis (months)	50.3±34.24
Median time to diagnostic confirmation (months)	10.50 (6 - 17)
Sex N (% female)	21 (65.63%)
Family history of IBD N (%)	7 (21.88%)
Clinical symptons N (%)	
Bloody diarrhea	17 (57.12%)
Abdominal pain	15 (46.87%)
Weight loss	14 (43.75%)
Non-bloody diarrhea	4 (12.50%)
Perianal disease	3 (9.38%)
Extraintestinal manifestations N (%)	13 (40.62%)
Laboratorial findings (mean±SD)	
Hemoglobin level (g/dl)	10.71±2.49
Hematocrit (%)	33.45±6.67
Platelets	477.058±197.438
ESR (mm/h)	41.1±36.60
CRP (mg/dl)	13.51±16.82
Calprotectina (µg/g)	1690 (600-5657)
Disease location N (%)	
Pancolonic	26 (81.25%)
Ileal involvement	7 (21.87%)
Left Colon	5 (15.63%)
Rectum only	1 (3.13%)
Upper gastrointestinal tract involvement	14 (43.75%)
Initial diagnosis N(%)	
Crohn's disease	13 (40.63%)
Ulcerative colitis	16 (50.00%)
Inflammatory bowel disease unclassified	3 (9.38%)
Moderate to severe disease (PUCAI/PCDAI)	27 (84.38%)
Endoscopic findings consistent with VEOIB N (%)	
Focal chronic inflammation	18 (56,25%)
Architectural distortion	16 (50,0%)
Basal lymphoplasmacytosis	14 (43.75%)
Lymphoid accumulation	13 (40.63%)
Increased eosinophils	9 (28.13%)
Increased neutrophils	8 (25.00%)
Nonspecific infiltrate	4 (12,50%)
Granulomas	1 (3.13%)
Perianal disease	3 (9.38%)
Villous atrophy	3 (9.38%)
Moderate to severe histological activity N(%)	21 (77,77%)
Initial therapeutic choice N(%)	
Exclusive Enteral Nutrition (EEN)	3 (9.38%)
Corticosteroids in induction therapy	32 (100%)
Immunosuppressants (Thiopurines)	26 (81.25%)
Mesalazine	3 (9.38%)
Biologic therapy as first choice (top-down)	2 (6,25%
Progression to anti-TNF	16 (50%)
	18 (59.34%)
Other Therapies (Immunoglobulins)	1 (3.13%)
Therapeutic failure N(%)	17 (59.37%)

Table 2: Immunological screening of patients, including immunoglobulin levels and lymphocyte typing, based on references for the Brazilian population.

Immunoglobulins (mg/dl)[a]	**Median (25-75)**	**<P3**	**P3-50**	**P50-97**	**>P97**
IgA (N=26)	158.5 (105.2 -210.0)	1 (3.8%)	5 (19.2%)	14 (53.8%)	6 (23.1%)
IgM (N=22)	127.0 (98.0 - 206.2)	1 (4.5%)	8 (36.4%)	3 (13.6%)	10 (38.5%)
IgG (N=24)	1176.5 (951.7 - 1278.0)	1 (4.2%)	2 (8.3%)	14 (58.3%)	7 (26.9%)
IgE (N=24)	31.8 (11.85 - 64.15)	-	-	-	-
Lymphocyte immunophenotyping (cells/µL)[b]	**Median (25-75)**	**<P10**	**P10-50**	**P50-90**	**>P90**
CD3 (N=23)	2736.0 (1683.6 - 3673.5)	4 (17.4%)	5 (21.7%)	7 (30.4%)	7 (30.4%)
CD4 (N=20)	1379.2 (992.2 - 1975.2)	4 (20.0%)	2 (10.0%)	4 (20.0%)	10 (50.0%)
CD8 (N=20)	867.5 (572.7 - 1627.2)	2 (10.0%)	3 (15.0%)	10 (50.0%)	5 (25.0%)
CD19 (N=17)	616.0 (353 - 1116.0)	9 (52.9%)	0 (0.0%)	2 (11.8%)	6 (35.3%)
CD56 (N=17)	225.0 (90.0 - 725.0)	7 (41.2%)	2 (11.8%)	2 (11.8%)	6 (35.3%)

[a]Source: Fujimura MD, 1991
[b]Source: Moraes-Pinto MI et al, 2005

Table 3: Genotypic and phenotypic characterization of patients with pathogenic/ likely pathogenic/ VUS variants associated with IBD

Patient	Gene(s)	Variant(s)	DNA effect	Effect on the encoded protein	Zygosity	Inheritance	ACMG classification	Associated phenotype	Sex	Age at diagnosis (months)	FH	IBD Diagnostic	Symptoms	Intestinal findings	Additional findings	Treatments required
7	IRF8	c.418C>T	missense	like to be tolerated	heterozygous	AD	VUS	Immunodeficiency-32B: selective susceptibility to mycobacterial infections	M	24	Yes	CD	Bloody diarrhea, Weight deficit, Perianal Disease	Pancolitis with large elevated-edge ulcers, intense inflammatory infiltrate with granulation tissue, Duodenitis with villous atrophy	recurrent infections, hypogammaglobulinemia (IgM), reduced CD19 lymphocytes	Infliximab from the start (Top-down), monthly Gastrostomy for nutritional recovery
	KMT2D	c.10185_10202dup (p.Met3398_Ala3403dup)	insertion	like to be tolerated	heterozygous	AD	VUS	Kabuki syndrome: peculiar facies, skeletal abnormalities, intellectual disability, growth failure, eczema. Association with IBD								
	TTC7A	Deleção (Exons 1-5)	deletion	disrupted protein (LOF)	heterozygous	AR	Pathogenic	Immunodeficiencies with multiple stresias, severe diarrhea, colitis								
13	FOXP3*	c.1256G>A (p.Arg419Gln)	missense	like to be disruptive	hemizygous	X linked	VUS	IPEX: polyendocrinopathy, enteropathy, dysregulation, DM, dermatitis, eczema	M	23	No	UC	Bloody diarrhea, Abdominal pain,	Microerosive pancolitis, intense inflammation, cryptitis, abscesses	Reduced CD19 lymphocytes	Mesalazine
17	SOCS1	c.143C>T. p. (Pro48Leu)	missense	like to be tolerated	heterozygous	AD	VUS	Familial autoinflammatory syndrome: cytopenia, hemolytic anemia, thrombocytopenia, lymphadenopathy. It may be associated with CD.	F	4	Yes	CD	Bloody diarrhea, Weight deficit, Perianal disease	Pancolitis with serpiginous ulcers, mucosal irregularity, intense inflammatory infiltrate with eosinophils and lymphoid accumulations, fibrosedema	anemia, linfocitose, eczema, infecções de repetição, doença perianal, recusa alimentar	EEN for 3 weeks, Infliximab from the start (Top-down), monthly: Gastrostomy for nutritional recovery
	TNFRSF13B	c.310T>C. p. (Cys104Arg)	missense	disrupted protein (LOF)	heterozygous	AD/AR	Pathogenic	TACI deficiency: recurrent infections, autoimmune manifestations Association with IBD and CVID								
20	TNFRSF13B	c.118T>C. p. (Trp40Arg)	missense	deleterious in silico	heterozygous	AD/AR	VUS	TACI deficiency: recurrent infections, autoimmune manifestations. Association with IBD and CVID	M	16	No	UC	Bloody diarrhea, Abdominal pain	Pancolitis, intense of T inflammatory infiltrate with neutrophils and eosinophils	Anemia, reduction of T lymphocytes CD3, CD4, and CD8	Azathioprine
21	TNFRSF13B	c.310T>C. p. (Cys104Arg)	missense	disrupted protein (LOF)	heterozygous	AD/AR	Pathogenic	TACI deficiency: recurrent infections, autoimmune manifestations. Association with IBD and CVID	M	5	No	CD	Bloody diarrhea, Weight deficit,	Ulcerative pancolitis, infiltrate with neutrophils/eosinophils, architectural distortion. Erosive Gastritis/Duodenitis	Elevated IgE, lymphocytosis, reduction in T lymphocytes CD4, autoimmune manifestations	Azathioprine, stepped up to infliximab
22	TNFRSF13B	c.310T>C. p. (Cys104Arg)	missense	disrupted protein (LOF)	heterozygous	AD/AR	Pathogenic	TACI deficiency: recurrent infections, autoimmune manifestations. Association with IBD and CVID	F	< 1	Yes	IBD-U	Bloody diarrhea	Erosive pancolitis with nonspecific inflammatory infiltrate	Anemia, recurrent infections, lymphocytosis, hypogammaglobulinemia IgM/IgG	Intravenous immunoglobulin
25	TNFRSF13B	c.310T>C. p. (Cys104Arg)	missense	disrupted protein (LOF)	heterozygous	AD/AR	Pathogenic	TACI deficiency: recurrent infections, autoimmune manifestations. It may be associated with IBD and CVID	F	24	No	UC	Bloody diarrhea, Weight deficit,	Erosive pancolitis, intense lymphoplasmacytic infiltrate, neutrophils, crypt distortion	Anemia, hyporthyroidism, reduction in T lymphocytes CD3/CD4	Azathioprine, stepped up to infliximab

Abbreviations: ACGM, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; CD, Crohn's disease; CIVD, common variable immunodeficiency; DM, diabetes mellitus; EEN, enteral exclusive nutrition; FH, family history of IBD; IBD, inflammatory bowel disease; IBD-U, inflammatory bowel disease- unclassified; LOF, loss of function; UC, ulcerative colitis; VEOIBD, very early onset IBD; VUS, variant of uncertain significate

Table 4: Genotypic and phenotypic characterization of patients with variants associated with an increased risk of developing IBD

Patient	Gene(s)	Variant(s)	DNA effect	Effect on the encoded protein	Zygosity	Inheritance	ACMG classification	Associated phenotype (ClinVar, Medgen, OMIM)	Sex	Age at diagnosis (months)	HF	IBD Diagnostic	Symptoms	Intestinal findings	Additional findings	Treatments required
4	NOD2	c.2104C>T (p.Arg702Trp)	missense	like to be tolerated	heterozygous	AD	Increased Risk Allele	NOD2: expressed in intestinal cells of the small intestine, mediates antibacterial effects, releases cytokines with T cell activation. Increased risk for CD	F	10	Yes	UC	Bloody diarrhea	Chronic erosive pancolitis with moderate inflammatory infiltrateo	Elevated IgE, lymphocytosis	Azathioprine
	PEPD	c.692_694del (p.Tyr231del)	deletion	like to be disruptive	heterozygous	AR	Likely pathogenic	Prolidase deficiency: autoantibodies, skin ulcers, eczema, infections.								
9	JAK1	c.1584G>C (p.Lys528Asn)	missense	like to be tolerated	heterozygous	AD	VUS	Autoinflammation, immune dysregulation, eosinophilia, may be present with eosinophilic colitis								
	LRBA	c.3508G>A (p.Glu1170Lys)	missense	like to be tolerated	heterozygous	AR	VUS	CVID 8: autoimmunity, autoimmune enteropathy. Can be associated with VEOIBD	F	72	No	UC	Bloody diarrhea, Abdominal pain	Rectosigmoiditis with intense lymphoplasmacytic inflammatory infiltrate, with the presence of cryptitis	Atopic dermatitis, asthma, elevated IgE	Azathioprine, stepped up to infliximab
	NOD2	c.2104C>T (p.Arg702Trp	missense	like to be tolerated	heterozygous	AD	Increased Risk Allele	NOD2: expressed in intestinal cells of the small intestine, mediates antibacterial effects, releases cytokines with T cell activation. Increased risk for CD								
11	NOD2	c.2722G>C (p.Gly908Arg)	missense	like to be tolerated	heterozygous	AD	Increased Risk Allele	NOD2: expressed in intestinal cells of the small intestine, mediates antibacterial effects, releases cytokines with T cell activation. Increased risk for CD								
	NOD2	c.697C>T (p.Gln233*)	stop sign	disrupted protein (LOF)	heterozygous	AD	VUS	Blau syndrome: uveitis, granulomatous synovitis, rash, 30% develop CD	M	48	Yes	CD	Bloody diarrhea, Weight deficit	Pancolitis with patchy erosions, mild lymphoplasmacytic infiltrate, villous atrophy of the small intestine, bullute, and erosive gastritis	Anemia, lymphopenia, hypogammaglobulinemia (IgM)	Azathioprine, stepped up to adalimumabe
	ORAI1	c.776G>A (p.Arg259His)	missense	inconclusive	heterozygous	AR	VUS	Immunodeficiency 9: recurrent infections, myopathy, ectodermal dysplasia								
	ZAP70	c.572C>T (p.Pro191Leu)	missense	like to be tolerated	heterozygous	AR	VUS	Autoimmune, multisystemic disease. It may be associated with VEOIBD and CVID								
15	BACH2	c.979G>A (p.Ala327Thr)	missense	like to be tolerated	heterozygous	AD	VUS	Immunodeficiency 60: inflammatory bowel disease and recurrent sinopulmonary infections								
	NFAT5	c.3044C>T (p.Ser1015Phe)	missense	like to be tolerated	heterozygous	AD	VUS	Autoimmune enteropathy with immunodeficiency, may be associated with CVID								
	NOD2	c.3019dup (p.Leu1007Profs*21)	stop sign	like to be disruptive	heterozygous	AD	Increased Risk Allele	NOD2: expressed in intestinal cells of the small intestine, mediates antibacterial effects, releases cytokines with T cell activation. Increased risk for CD	F	72	No	UC	Abdominal pain, Weight deficit	Rectosigmoiditis with moderate inflammatory infiltrate with plasma cells, eosinophils, and interspersed neutrophils, with ileal involvement	Recurrent oral ulcers, increased IgE, reduced CD19 cells	Mesalazine
	RELA	c.917A>G (p.Tyr306Cys)	missense	like to be tolerated	heterozygous	AD	VUS	Behcet-like autoinflammatory disease: mucocutaneous ulceration, ileitis								
	RTLE1	c.958+3A>G (Intronic)	splice site	like to be tolerated	heterozygous	AD	VUS	Congenital dyskeratosis: nail dystrophy, abnormal skin pigmentation, mucosal leukoplakia, colitis, enteropathy								

Abbreviations: ACGM, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; CD, Crohn's disease; CIVD, common variable immunodeficiency; DM, diabetes mellitus; FH, family history of IBD; IBD, inflammatory bowel disease; LOF, loss of function; UC, ulcerative colitis; VEOIBD, very early onset IBD; VUS, variant of uncertain significate

Table 5: Genotypic and phenotypic characterization of patients with variants of uncertain significance with probable association with IBD

Patient	Gene(s)	Variant(s)	DNA effect	Effect on the encoded protein	Zygosity	Inheritance	ACMG classification	Associated phenotype (ClinVar, Medgen, OMIM)	Sex	Age at diagnosis (months)	HF	IBD Diagnostic	Symptoms	Intestinal findings	Additional findings	Treatments required
1	GUCY2C	c.1490G>A (p.Arg497Gln)	missense	like to be disruptive	heterozygous	AD	VUS	Congenital familial diarrhea. Susceptibility to VEOIBD	M	33	No	UC	Bloody diarrhea	Pancolitis with ileocecal valve involvement		Mesalazine
	NLRP3	Entire coding sequence	copy number gain	like to be tolerated	heterozygous	AD	VUS	Autoinflammation, dyskeratosis, arthritis, autoimmunity. Susceptibility to IBD								
10	NFAT5	c.3383C>T (p.Pro1128Leu)	missense	like to be tolerated	heterozygous	AD	VUS	Autoimmune enteropathy with CVID	F	60	No	DC	Bloody diarrhea, Weight loss	Enanthematic pancolitis, cobblestone appearance with rigidity of the ileocecal valve, intense inflammatory infiltrate	Significant increase in IgE, reduced CD19 B lymphocytes and NK cells, autoimmune hepatitis, atopic dermatitis	Azathioprine, stepped up to infliximab, monthly
	TGFB1	Exon 1, c.85G>A (p.Gly29Arg)	missense	like to be tolerated	heterozygous	AD	VUS	Severe colitis, association with IBD, recurrent infections, encephalopathy								
18	IFIH1	c.1211T>C. p. (Val404Ala)	missense	deleterious in silico	heterozygous	AD/AR	VUS	MDA5 deficiency: characterized by increased susceptibility to infectious and VEOIBD	F	36	No	CD	Bloody diarrhea, Abdominal pain, Weight loss	Erosive pancolitis with moderate inflammatory activity, ileal substenosis	Ileal substenosis, IgA and IgG = P97, reduction of CD3, CD4, and CD8 T lymphocytes.	Azathioprine, stepped up to infliximab
29	NLRP1	c.1531A>G. p. (Lys511Glu)	missense	inconclusive	heterozygous	AD/AR	VUS	Autoinflammation, dyskeratosis, arthritis, autoimmunity. Susceptibility to IBD	M	72	No	UC	Bloody diarrhea	Pancolitis with moderate neutrophilic infiltrate, architectural distortion, and goblet cell depletion. Villi slightly shortened and enlarged in the small intestine		Azathioprine
32	SAMD9	c.4007T>C. p. (L=C46+C1:C44+C=C2:C4)	missense	deleterious in silico	heterozygous	AD	VUS	MIRAGE syndrome: myelodysplasia, infections, growth retardation, adrenal hypoplasia, genital abnormalities, enteropathy	F	36	No	UC	Bloody diarrhea	Extensive colitis with dense inflammatory infiltrate rich in eosinophils and neutrophils, lymphoid aggregates, architectural distortion. Duodenitis and chronic gastritis	Elevated IgE, reduction in CD19 B lymphocytes and NK cells	Azathioprine, stepped up to infliximab

Abbreviations: ACGM, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; CD, Crohn's disease; CIVD, common variable immunodeficiency; FH, family history of IBD; IBD, inflammatory bowel disease; UC, ulcerative colitis; VUS, variant of uncertain significate

4. Conclusions

In this study, it was possible to establish a well-defined genetic diagnosis in 11 patients (34.4%), including 7 patients (21.8%) with monogenic disease, who presented pathogenic or likely pathogenic variants for IBD, and 4 patients (12.8%) with increased risk variants for IBD. Five Patients presented suspicious variants, included in Table 5, that we considered were probably associated with VEOIBD.

In our study, we have found 149 variants that were analyzed in a monogenic disease perspective, excluding them according to previous relation with VEOIBD, zygosity, and potential pathogenicity. On the other hand, recent studies have shown that the interaction between variants, some considered benign when isolated, can be associated with diseases when present together with other variants of the same immune pathway or acting synergistically. Gene variant interaction is a new challenge for understanding the influence of genetics on inflammatory diseases. Pediatric IBD represents a spectrum that can range from extreme monogenic variants to complex polygenic variants, and new AI tools could improve the way to study these interactions.

In conclusion, the genetic evaluation of patients with VEOIBD with target genetic panels to immunologic genes can improve the understanding and the treatment of patients.

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