# Autoinflammatory syndromes mimicking Behçet's disease with gastrointestinal involvement: a retrospective analysis

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## Abstract

### Objective

*This retrospective study aimed to investigate the clinical characteristics and genetic findings in paediatric patients with gastrointestinal involvement in Behçet's disease (BD), elucidating the spectrum of autoinflammatory syndromes mimicking BD in this young population.*

### Methods

*Fifty paediatric patients diagnosed with BD between January 2016 and December 2022, including 24 (48%) with gastrointestinal involvement, underwent comprehensive analysis. Clinical presentations, laboratory examinations, gastrointestinal endoscopy, and genetic tests were conducted, with patients stratified based on genetic results for rigorous comparative clinical analysis.*

### Results

*The cohort, with a median age of disease onset at 4.0 years, predominantly manifested with recurrent oral ulcers (100%). Gastrointestinal symptoms were prevalent in 83.3%, with abdominal pain (70%) and haematochezia (16.7%) being notable. Endoscopic evaluations unveiled lesions primarily in the terminal ileum and ileocecal region, with diverse ulcers across various anatomical sites. While 70.8% initially met ICBD criteria, only 41.6% fulfilled new paediatric classification criteria. Genetic analysis in 18 patients unveiled pathogenic variants in 7, with the genetic-positive group exhibiting earlier onset and more atypical symptoms. Noteworthy cases included X-linked deficiency in ELF4, A20 haploinsufficiency, and Majeed syndrome, with two cases revealing chromosomal abnormalities such as trisomy 8 syndrome. Comparative analysis underscored earlier disease onset, heightened inflammatory markers, and distinctive gastrointestinal lesions in the genetic-positive cohort.*

### Conclusion

*Identification of monogenic diseases and chromosomal abnormalities resembling BD underscores the imperative of precise diagnosis for tailored treatment and genetic counselling. Expanding genetic screening initiatives holds promise for enhancing our comprehension of the genetic landscape associated with BD.*

**Key words**

Behçet's disease, gastrointestinal involvement, autoinflammatory syndrome, childhood-onset

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## Introduction

Behçet's disease (BD) is a complex multisystem inflammatory disorder characterised by recurrent oral and genital ulcers, ocular inflammation, and skin lesions. Although BD is more commonly diagnosed in young adults aged 20–40 years, it has also been observed in paediatric populations, with a reported prevalence ranging from 2.7% to 26% (1, 2). Previous research suggests that paediatric BD and adult BD exhibit clinical differences, including a higher incidence of gastrointestinal and central nervous system involvement, more arthralgia, and less vascular involvement (3, 4).

Diagnosing BD in children poses significant challenges due to its rarity, absence of specific laboratory diagnostic tests, and the frequent presence of nonspecific features like headache, arthralgia, and abdominal pain (5). As a result, the diagnosis primarily relies on clinical judgment, which may lead to diagnostic delays and misclassification with other inflammatory conditions. To aid in diagnosis, various clinical diagnostic criteria have been developed, including the International Study Group (ISG) classification criteria (6), the International Criteria for Behçet's Disease (ICBD) classification criteria (7), and the Paediatric Behçet's Disease (PEDBD) classification criteria (8). However, the utility of these criteria in paediatric cases remains a subject of debate, warranting further research and validation (9). Moreover, the aetiology of BD is not fully understood and is believed to involve a complex interplay of genetic and environmental factors. Genome-wide association studies (GWAS) have identified multiple genetic susceptibility loci, with HLA-B51 being the most widely studied genetic factor (10). Additionally, some monogenic autoinflammatory disease like Haploinsufficiency of A20 (HA20) may also present with BD-like symptoms early in life and may be mistaken for BD (11), further emphasising the genetic complexity underlying the disease (12).

In this study, we included 24 BD patients with gastrointestinal involvement from a single Tertiary Referral Centre in China, analysing their clinical characteristics, laboratory features, and gastrointestinal endoscopy findings. Among these patients, 7 children were diagnosed with single gene or chromosomal abnormalities through Whole Exome Sequencing (WES) and/or karyotype analysis. We conducted a comparative analysis of clinical characteristics between individuals with positive and negative genetic testing results to illuminate potential disparities in disease manifestation. Understanding the clinical and genetic features of paediatric BD patients is crucial for early and accurate diagnosis, guiding appropriate management strategies, and advancing our knowledge of this complex autoinflammatory disorder in this population.

## Methods

### *Patients*

A retrospective analysis was undertaken to examine patients diagnosed with BD presenting gastrointestinal involvement, who were admitted to the Department of Rheumatology at Children's Hospital of Fudan University during the period from January 2016 to December 2022.

This study was approved by the Ethics Committee of Children's Hospital of Fudan University (project no.: 2023-279) and was conducted in strict accordance with the Declaration of Helsinki and all applicable laws and regulations in China. All patients provided informed consent prior to the inclusion of their personal information and data. The study employed a cross-sectional design, utilising comprehensive electronic medical records, and adhered to strict procedures to ensure patient anonymity and data de-identification during analysis. Organ involvement was assessed through a comprehensive evaluation of clinical symptoms, past medical history, physical examination findings, laboratory investigations, imaging studies, and endoscopic findings, as previously documented (13). Routine check-ups for patients included endoscopy, ocular examination, and imaging investigations of blood vessels and the central nervous system (CNS).

Within this series, two distinct sets of BD classification criteria were collected to evaluate their performance. The first set was the ICBD criteria, whereby a score of 4 or more, fulfilling the specified criteria, indicated BD diagnosis

**Table I.** Clinical characteristics of BD with gastrointestinal involvement.

| Case | Gender | Age (onset) | Age (diagnosis) | Initial clinical presentation | GI lesions | Oral aphthosis | Genital aphthosis | Skin involvement | Ocular involvement | Vascular signs | Neurological signs | ICBD fulfilled (Y/N) | PEDBD fulfilled (Y/N) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 6y | 9y6m | oral aphthosis | oesophageal ulcer, small intestine ulcer | Y | Y | N | Y | N | N | Y | Y |
| 2 | M | 2y1m | 6y7m | abdominal pain | pharyngeal ulcer, large ileocecal ulcer, rectal ulcer | Y | N | N | N | N | N | N | N |
| 3 | F | 3y | 3y3m | diarrhoea, abdominal pain | small intestine and colonic ulcers, terminal ileal ulcer | Y | Y | N | N | N | N | Y | N |
| 4 | F | 3y | 5y | oral aphthosis | antral gastritis, duodenitis, colitis, terminal ileitis | Y | Y | N | N | N | N | Y | N |
| 5 | M | 0.3m | 12y7m | genital aphthosis | oesophagitis, rectal mucosal erosion | Y | Y | Y | N | Y | N | Y | Y |
| 6 | M | 12y3m | 14y2m | oral aphthosis | eosophagitis | Y | Y | Y | Y | N | N | Y | Y |
| 7 | F | 12y | 14y1m | oral aphthosis | gastric antrum mucosal erosion, deep large ileocecal ulcer | Y | Y | Y | N | N | N | Y | Y |
| 8 | F | 1y10m | 2y4m | fever, haematochezia | small intestine ulcers, colonic ulcer, terminal ileitis | Y | Y | N | N | N | N | Y | N |
| 9 | F | 6m | 3y9m | oral aphthosis | duodenal ulcer, colonic ulcer, terminal ileitis | Y | Y | N | N | N | N | Y | N |
| 10 | M | 9y | 13y10m | oral aphthosis | duodenal ulcer, terminal ileal ulcer | Y | Y | N | N | N | N | Y | N |
| 11 | M | 11y | 14y5m | fever, abdominal pain | small intestine ulcers, terminal ileal ulcer | Y | Y | Y | N | N | N | Y | Y |
| 12 | M | 4y5m | 5y | arthralgia | gastric antrum ulcer, terminal ileitis, colitis, small intestine ulcers | Y | N | N | N | N | N | N | N |
| 13 | M | 3y | 4y | abdominal pain | deep large ileocecal valve ulcer, scattered ulcers in the sigmoid colon | Y | N | N | N | N | N | N | N |
| 14 | M | 7y2m | 11y | oral aphthosis | rectal erosion and ulcer, large ileocecal valve ulcer | Y | N | Y | N | N | Y | Y | Y |
| 15 | M | 4 | 5y10m | oral aphthosis | large ileocecal ulcer | Y | Y | N | N | N | N | Y | N |
| 16 | F | 6y1m | 9y6m | oral aphthosis | duodenitis, terminal ileal ulcer | Y | N | N | N | N | N | N | N |
| 17 | F | 3y | 4y | oral aphthosis | large terminal ileal ulcers | Y | Y | Y | N | Y | N | Y | Y |
| 18 | M | 3y2m | 13y | abdominal pain | multiple erosions in the upper jejunum | Y | Y | N | N | N | N | Y | N |
| 19 | F | 11y | 12y | sore throat | pharyngeal ulcer, large oesophageal ulcer | Y | N | N | N | N | N | N | N |
| 20 | M | 13y | 13y | abdominal pain | large ileocecal valve ulcer | Y | N | Y | N | N | N | N | N |
| 21 | M | 2y5m | 10y | oral aphthosis | oesophageal ulcer, erosive gastritis, large ulcer in the proximal ascending colon | Y | N | Y | N | y | N | Y | Y |
| 22 | M | 10y | 14y | oral aphthosis | small erosions and ulcers in the ileocecal region | Y | Y | Y | N | y | N | Y | Y |
| 23 | F | 1y3m | 6y9m | oral aphthosis | pangastritis, multiple duodenal ulcers, ileocecal ulcer | Y | N | N | N | N | N | N | N |
| 24 | M | 9y | 13y | oral aphthosis | punctate erosions in the rectum, scattered ulcers in the terminal ileum | Y | N | Y | N | Y | N | Y | Y |

F: female; M: male; GI: gastrointestinal; Y: yes; N: no.

(13). The second set consisted of the PEDBD criteria, necessitating the presence of at least 3 out of 6 predefined items to classify a patient as having paediatric BD (14).

*Whole exome sequencing and karyotype analysis*

A combination of WES or Karyotype analysis (peripheral blood or bone marrow) was utilised to ascertain genetic diagnoses. The predicted functional impact of the identified genetic variants was evaluated using the in-silico tools SIFT (http://sift. bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.

**Table II.** Genetic variants characterised in patients presenting BD-like symptoms.

| Case | Gene | Nucleotide change | Amino acid change | Predicted pathogenicity [a] | CADD score | Allele frequency | Zygosity | Final diagnosis | Treatment and outcome |
|---|---|---|---|---|---|---|---|---|---|
| 2 | *ELF4* | c.700C>T | p.R234* | D/-/D | 35 | 1.82e-6 | Hemi | DEX | prednisone, 5-ASA, adalimumab switched to infliximab and SASP with good response |
| 8 | *TNFAIP3* | c.721C>T | p.Q241* | D/-/D | 36 | - | Het | HA20 | prednisone and etanercept with good response |
| 9 | *TNFAIP3* | c.1012G>T | p.E338* | D/-/D | 40 | - | Het | HA20 | prednisone and adalimumab with good response |
| 12 | *LPIN2* | c.200T>G,<br>c.2342_c.2346delAGAAA | p.I67R<br>p.K781Tfs*7 | D/D/D<br>D/-/D | 28.2<br>33 | -<br>4.97e-6 | Het,<br>Het | Majeed syndrome | canakinumab, remission |
| 14 | *ELF4* | c.677C>T | p.P226L | D/D/D | 28.4 | - | Hemi | DEX | prednisone and etanercept switched to adalimumab with good response |

[a]Prediction (polyphen2/SIFT/Mutation Taster).
D: damaging or deleterious; DEX: deficiency in ELF4, X-linked; HA20: haploinsufficiency A20; 5-ASA: 5-aminosalicylic acids; SASP: sulfasalazine; MTX: methotrexate.

**Table III**. Comparison of clinical features between genetically positive group and negative group.

| Characteristics | Positive, n=7 | Negative, n=11 | *p* |
|---|---|---|---|
| Male sex, n (%) | 5 (71.4) | 7 (63.6) | 1.000 |
| Age at onset, y, median (IQR) | 2.0 (1.8-7.0) | 4.0 (3.0-11.0) | 0.157 |
| Age at diagnosis, y, median (IQR) | 6.0 (3.0-8.0) | 9.6 (4.7-13.3) | 0.248 |
| Family history, n (%) | 5 (71.4) | 3 (27.3) | 0.145 |
| Clinical presentation, n (%) | | | |
| Fever | 7 (100.0) | 8 (72.3) | 0.245 |
| Oral aphthosis | 7 (100.0) | 11 (100.0) | 1.000 |
| Genital ulcer | 4 (57.1) | 6 (54.5) | 1.000 |
| Skin involvement | 4 (57.1) | 5 (45.4) | 1.000 |
| Ocular involvement | 0 (0.0) | 1 (9.1) | 1.000 |
| Vascular signs | 1 (14.3) | 3 (27.3) | 1.000 |
| Neurological signs | 1 (14.3) | 0 (0.0) | 0.388 |
| Gastrointestinal symptoms | 6 (77.8) | 10 (90.9) | 1.000 |
| Other[#] | 5 (71.4) | 2 (18.2) | 0.049* |
| Initial clinical presentation, n (%) | | | |
| Oral aphthosis | 4 (57.1) | 7 (63.6) | 1.000 |
| Genital aphthosis | 0 (0.0) | 0 (0.0) | 1.000 |
| Gastrointestinal symptoms | 1 (14.3) | 3 (27.3) | 1.000 |
| Fever | 1 (14.3) | 1 (9.1) | 1.000 |
| Laboratory data, median (range) | | | |
| WBC count (K*10^9/L) | 19.2 (5.7-25.8) | 10.9 (2.5-22.7) | 0.151 |
| Haemoglobin (g/L) | 107.0 (84.0-147.0) | 118.0 (70.0-144.0) | 0.520 |
| Platelet count (K*10^9/L) | 502.0 (50.0-749.0) | 399.0 (110.0-536.0) | 0.120 |
| CRP (mg/L) | 118.0 (43.0-160.0) | 20.0 (0.5-79.0) | 0.003** |
| ESR (mm/h) | 90.0 (33.0-120.0) | 40.0 (3.0-83.0) | 0.004** |
| Endoscopy | | | |
| Ulcer at terminal ileum/ileocecal valve | 3 (42.9) | 8 (72.3) | 0.322 |
| Number of ulcer sites (n≥2) | 7 (100.0) | 4 (36.4) | 0.013* |

Other[#]: haematologic system involvement, arthritis, arthralgia, hearing loss; *indicates $p<0.05$, **indicates $p<0.01$.

harvard.edu/pph2/), Mutation Taster (http://www. mutationtaster.org/) and CADD (https://cadd.gs.washington.edu/) (14). Interpretation of variants was based on recommended standards from the American College of Medical Genetics and Genomics (ACMG), and all variants were categorised as pathogenic, likely pathogenic, variants of unknown significance, likely benign or benign. Variants detected using NGS were also confirmed using Sanger sequencing, to exclude false positives.

*Statistical analysis*

The statistical analysis was conducted utilizing SPSS v. 22.0 (SPSS Inc., Chicago, IL, USA). To evaluate data distribution normality, the Shapiro-Wilk test was performed as a preliminary step. Categorical variables were presented as percentages or ratios, while qualitative variables were depicted as medians along with their corresponding interquartile range (IQR) representing the 25–75% range. For the analysis of quantitative variables, the Mann-Whitney U-test was employed, and for categorical variables, Fisher's exact test was used. A statistical significance level of $p<0.05$ was considered to indicate significant differences.

## Results

*General and clinical characteristics of BD patients with gastrointestinal involvement*

From January 2016 to December 2022, 50 patients were diagnosed with BD at our centre, among which 24 were accompanied with gastrointestinal involvement, accounted for 48% of all BD patients. The median follow-up duration was 11.5 months. Among the 24 patients, 14 were males and 10 females,

for a male to female ratio of 1.4:1. The minimum age of onset is 10 days after birth, the median age of disease onset was 4.0 years (IQR: 2.25-9.75 years), and the median age of diagnosis was 9.87 years (IQR: 5.03 to 13.38 years). Except for two patients, the remaining cohort of 22 individuals (91.6%) exhibited varying degrees of elevated C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR) levels upon disease onset, with ESR elevation demonstrating a more pronounced pattern. Among the entire cohort, the manifestation of recurrent oral ulcers was universally observed, encompassing all 24 cases (100%). Genital ulcers were noted in 13 children, while skin lesions were evident in 11 instances. Additionally, vascular involvement was apparent in 5 cases, whereas 4 cases displayed concomitant joint manifestations. Furthermore, hematologic involvement was evident in 4 children, ocular uveitis was observed in 2 cases, and alterations within the nervous system were detected in a solitary case. 17/24 patients (70.8%) fulfilled ICBD criteria at the first presentation, compared with 10/24 (41.6%) fulfilling the new paediatric classification criteria (Table I).

Gastrointestinal symptoms were evident in 20 cases (83.3%), encompassing abdominal pain in 17 instances, haematochezia in 4 cases, vomiting in 4 cases, and diarrhoea in 2 cases; a subset of 4 patients displayed an absence of overt gastrointestinal symptoms throughout the disease course. Among the cohort, gastrointestinal symptoms materialised as the initial presentation in 8 cases (33.3%). Every patient underwent a gastrointestinal endoscopy assessment. The predominant anatomical sites of lesion localisation encompassed the terminal ileum and ileocecal region, evidenced in 14 instances, including 7 cases of large ulcers, and 4 of these cases had pseudo polyp formation around the ulcers. Additional notable manifestations included 2 cases of formidable pharyngeal ulcers, 3 cases of oesophageal ulcerations, 1 occurrence of gastric antrum ulcers, 3 cases of duodenal ulcers, 5 cases of small intestinal ulcers, 5 occurrences of colonic ulcers, and 2 instances of rectal ulcers.

*Genetics*

Eighteen of the 24 participants, who had symptoms onset before the age of 5 and/or displayed incomplete or atypical BD features, underwent additional molecular evaluation. The median follow-up duration was 15.5 months. Among these, 7 patients were attributed a definitive genic aetiology aligning with their clinical phenotype. Detailed phenotypes and their corresponding genotypes are elaborated upon. (Table I and II).

*Cases with pathogenic variants and likely pathogenic genetic variants*

Case 2 involved a 6-year-old boy presenting with recurrent unexplained fever, oral ulcers, abdominal pain, and diarrhoea for three months. Erythema nodosum was observed during the physical examination. Despite normal ophthalmological and vascular assessments, gastrointestinal endoscopy revealed ulcers in the pharynx, terminal ileum, and rectum with moderate to severe chronic active inflammation (Fig. 1a-b). Diagnosed with intestinal BD, he received corticosteroid, cyclophosphamide, and 5-aminosalicylic acid (5-ASA) treatment for 11 months, leading to initial improvement. However, symptoms recurred later after self-discontinuation of the medication, with substantial ulcers observed in the pharyngeal wall, rectum, and cecum. Adjusted treatment with corticosteroids, infliximab, and 5-ASA proved effective, ultimately healing the ulcers. Trio-WES identified a pathogenic nonsense mutation (c.700C>T: p.234*) in the *ELF4* gene (15), inherited from his mother. This mutation, situated in ELF4's ETS domain crucial for DNA binding, led to X-linked deficiency in *ELF4* (DEX), an inborn error of immunity (IEI) resembling BD (16).

In another case (Case 14), a 10-year-old boy presented with recurrent oral ulcers, skin rash, abdominal pain, fever, and elevated inflammatory markers. Flow cytometry of peripheral blood mononuclear cells showed intermittently low NK cells. Inflammatory markers and IgA were significantly increased. Gastrointestinal endoscopy revealed extensive ulcers (Fig. 1c). Treatment with corticosteroids, Thalidomide, and 5-ASA initially improved his condition. However, the disease recurred during the steroid taper. Trio-WES identified a novel missense mutation (c.677C>T, p.P226L) in *ELF4*, also within the ETS domain, inherited from the mother. This amino acid site is extremely conserved among species (PhyloP score 5.236) (Fig. 1g), thereby supporting a deleterious effect of this amino acid substitution. This missense mutation has not been reported in the ExAC, 1000G, or gnomAD databases The CADD score of 28.4 suggests a predicted pathogenicity, given that a CADD score above 20 is indicative of potential pathogenicity (14). Additionally, the SIFT, PolyPhen-2, and MutationTaster tools all predicted this variant as deleterious. Next, we co-transfected HEK293T cells with flag-tagged WT and the mutant ELF4 construct, confirming the damaging effects of the variant (Fig. 1h). Based on the genetic study, adalimumab treatment was added, which significantly improved the intestinal lesions.

A 4-year and 5-month-old girl (Case 8) presented with recurrent haematochezia, abdominal pain, and intermittent fever since the age of 1 year and 8 months. She also had recurring oral and perianal ulcers, limb joint pain, and liver dysfunction. Due to persistent liver abnormalities, we conducted an extensive investigation to exclude other potential causes of chronic liver damage, such as hemochromatosis, environmental factors, toxins, viruses, autoimmune conditions, metabolic disorders, and iatrogenic factors. The liver biopsy revealed findings indicative of interface hepatitis, including focal hepatocyte necrosis, increased portal tract fibrous tissue, and lymphocyte and plasma cell infiltration. She subsequently received treatment with corticosteroids, etanercept, and glycyrrhizin and responded positively. Additionally, she had bilateral moderate hearing loss via auditory brainstem response, shared by her father and brother, who also had recurrent oral ulcers and liver function issues. Concurrently, another case (Case 9) involved a 3-year-old girl with recurrent oral and perianal ulcers since the age of 6 months, intermittent abdominal pain,

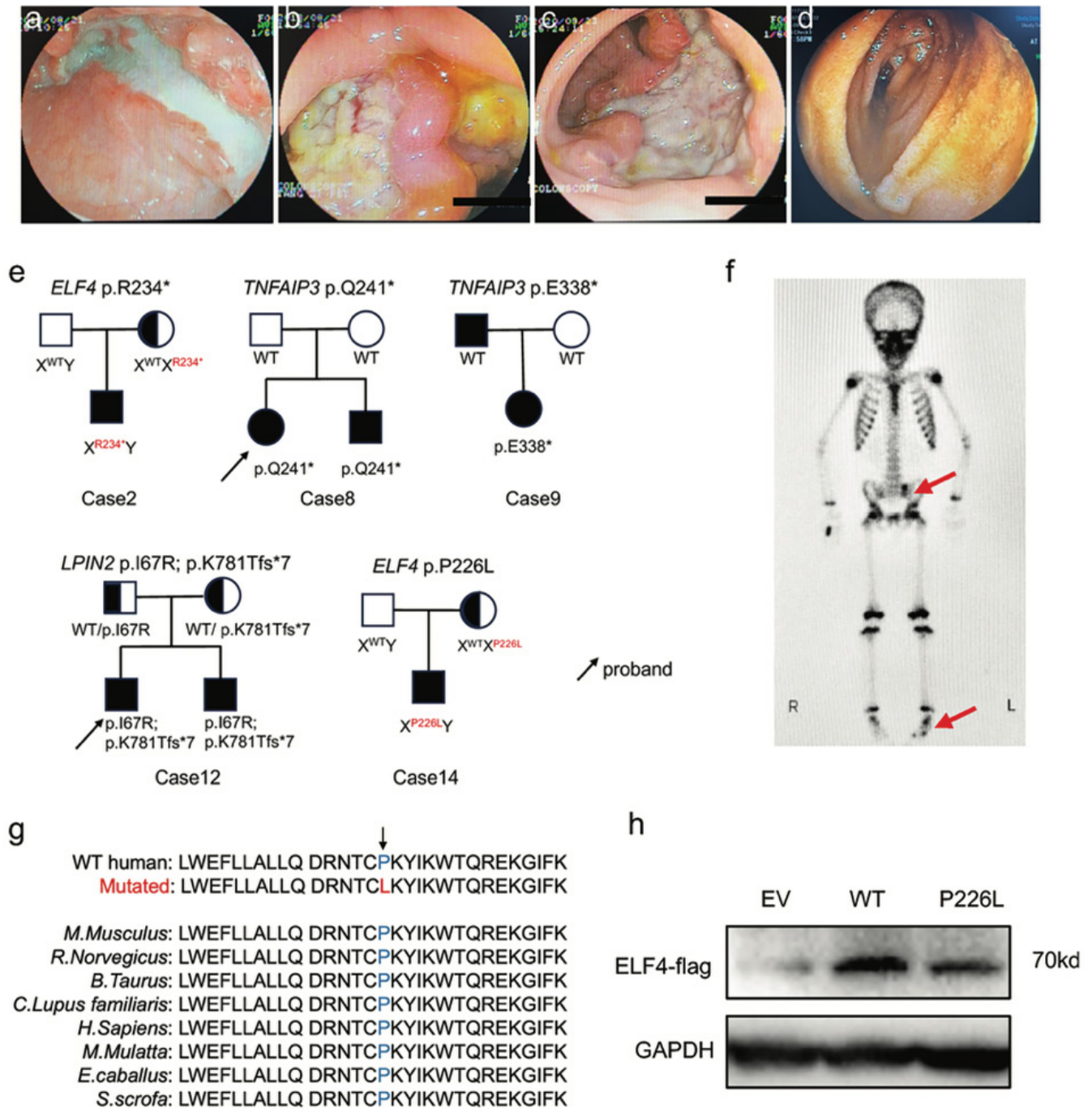


**Fig. 1**. Clinical manifestations in patients with genetic findings. **a**) a massive ulceration on the posterior pharyngeal wall in Case 2. **b**) Severe ileocecal ulceration in Case 2. **c**) a massive ulceration in the terminal ileum (cecum) in Case 14. d. Intestinal stricture in Case 21. **e**) Pedigrees of Case 2, Case 8, Case 9, Case 12 and Case 14. **f**) Whole-body bone scans reveal lesions in the left sacroiliac joint and small joints of the left foot in Case 12. Red arrows indicate abnormal signals. **g**) Amino acid sequence alignment of ETS domains across species. The arrow indicates the position of the Pro226Leu variant. **g**) Plasmids constructs mimicking novel variant of *ELF4* were expressed in HEK293T cells and determined by western blot.
EV: empty vector, WT: wild type.

and endoscopically confirmed gastrointestinal damage. Despite initial corticosteroid and methotrexate treatment, she experienced symptom recurrence, eventually responding to adalimumab.

Both the patients and their parents underwent Trio-WES, which provided significant genetic insights. In Case 8 we identified a *de novo* nonsense variant, c.721C>T: p.Q241*, and in Case 9 another novel nonsense variant was detected, c.1012G>T: p.E338*, in *TNFAIP3*. Both variants cause a premature stop codon in the ovarian tumour (OTU) domain, resulting in a putative haploinsufficiency of the protein. These variants were absent from the ExAC, 1000G, or gnomAD databases. Same variants were identified in the affected brother of Case 8 and in the affected father of Case 9. According to the ACMG guidelines (24), the two variants are classified as pathogenic (PVS1, PM2, PP3, PP4), leading to

their definitive diagnosis of A20 haploinsufficiency (HA20).

In another distinct case, a 5-year-old boy (Case 12) presented with recurrent symptoms, including ankle pain, rash, oral ulcers, abdominal pain, and constipation. Laboratory tests indicated anaemia, significantly elevated CRP and ESR levels, and negative autoantibodies. Initially suspected as BD and juvenile idiopathic arthritis (JIA), he was treated with prednisone, 5-ASA, and MTX, with initial relief. However, he later experienced haematochezia and recurring ankle pain, with whole-body bone scans revealing asymmetric and uneven radiotracer distribution, along with lesions in the left sacroiliac joint and small joints of the left foot (Fig. 1f). Tofacitinib treatment had limited efficacy.

To further elucidate the aetiology, whole exome sequencing (WES) was conducted. After sequencing, we identified a paternal missense variant c.200T>G (p.I67R) and a maternal frameshift variant c.2342_c.2346delAGAAA (p.K781Tfs*7) in the *LPIN2* gene. The c.200T>G (p.I67R) variant has not been reported in the ExAC, 1000G, or gnomAD databases, and this amino acid site is conserved among species (Phylop score 5.004). The in-silico tools SIFT, PolyPhen-2, and MutationTaster predicted the variant to have a deleterious consequence. The high CADD score of 28.2 also suggests a damaging effect of the variant. Based on the ACMG guidelines, this variant is classified as likely pathogenic (PM1, PM2, PM3, PP3). The c.2342_c.2346delAGAAA variant, located in exon 18, leads to a premature translational stop signal (p.Lys781Thrfs*7) in the *LPIN2* gene. It is expected to result in an absent or disrupted protein product. Loss-of-function variants in *LPIN2* are known to be pathogenic. This variant is present in population databases (rs762442011, gnomAD 0.006%). The CADD score is 33 and classified as pathogenic in accordance with ACMG guidelines (PVS1, PM2, PP3). Thus, this patient was re-diagnosed with Majeed syndrome and started on treatment with the monoclonal anti-IL-1β antibody, canakinumab, with significant clinical and laboratory improvement.

*Cases with chromosomal abnormalities*

In July 2020, a 13-year-old boy (Case 10) presented with recurrent oral and scrotal ulcers, anaemia, thrombocytopenia, and elevated CRP and ESR levels. Antibiotics had limited effect, but glucocorticoid therapy, including prednisolone, improved his condition. Intermittent abdominal pain and elevated inflammatory markers led to a gastrointestinal endoscopy, revealing terminal ileum ulcers, raising suspicion of intestinal BD. Genetic testing did not provide a definitive cause. At six, he had been diagnosed with aplastic anaemia, treated with cyclosporine and prednisolone. No improvement or worsening occurred. A bone marrow biopsy at an older age revealed cytogenetic abnormalities (47, XY, +8) and a diagnosis of myelodysplastic syndrome (MDS) was made. He was treated with prednisone and thalidomide with poor response and was recommended for haematopoietic stem cell transplantation (HSCT).

In 2022, a 10-year-old boy (Case 21) came to our hospital with an 8-year history of recurrent oral ulcers, 2 years of abdominal pain, and folliculitis-like skin eruptions. Vascular assessments identified femoral vein thickening and strictures. Gastrointestinal endoscopy revealed extensive oesophageal and colonic ulcers, bringing about the diagnosis of intestinal BD. At 2, he had been diagnosed with "aplastic anaemia" and treated with cyclosporine for nearly 3 years. Platelet count gradually declined, and genetic analysis revealed a trisomy on chromosome 8, confirmed by karyotype examination leading to a trisomy 8 syndrome diagnosis. Treatment with corticosteroids and 5-ASA initially helped, but stopping steroids worsened symptoms. Follow-up endoscopy showed worsening intestinal lesions and intestinal stricture (Fig. 1d). Corticosteroids, sulfasalazine, and adalimumab provided partial relief, but symptoms recurred after 10 months, including skin lesions, ulcers, abdominal pain, and vomiting.

To offer early clinical insights into genetic testing, we undertook a comparative analysis between patients with genetic causes and unknown causes (Table III), despite the inherent limitations of a small sample size. While both groups presented similar initial symptoms, a notable discrepancy emerged regarding the timing of disease onset and diagnosis. Specifically, individuals with positive genetic test results experienced disease onset and diagnosis at earlier median ages compared to those with negative results. Interestingly, although most clinical features exhibited no significant differences between the two groups, the positive genetic test cohort displayed elevated incidences of specific manifestations including arthritis or arthralgia, abnormal liver function, and hearing loss. Furthermore, laboratory assessments revealed higher levels of inflammatory markers, particularly ESR and CRP, in the positive genetic test group, suggesting a more pronounced inflammatory response in these individuals. Endoscopic evaluations of gastrointestinal lesions revealed distinct patterns, with the positive genetic test group showing a predominance of ulcers in multiple sites compared to the negative genetic test group, where lesions were primarily localised to the distal ileum and ileocecal valve.

## Discussion

In our study of 24 BD patients with GI involvement, genetic testing identified monogenic diseases or chromosomal abnormalities mimicking BD in 7 out of 18 tested patients. These individuals had an earlier median onset age of 2 years, with 5/7 (71.4%) having a family history of the condition. Common symptoms included recurrent oral ulcers, and approximately half also exhibited genital ulcers and skin lesions. Ocular, vascular, neurological, and hematologic involvement is less common. Notably, the genetically positive group showed higher inflammatory markers and a greater proportion of atypical presentations (5/7, 71.4% *vs*. 2/11, 18.2%, $p$=0.049) compared to the negative group. Initially, 70.8% of all patients met ICBD criteria for BD, while 41.6% met PEDBD criteria, consistent with prior reports. The genetic testing-positive group displayed similar rates, with 5/7 (71.4%) meeting ICBD criteria and 2/7 (28.6%) meeting PEDBD criteria.

Among the seven patients, two were diagnosed with DEX, a recently identified inborn immune dysregulation disorder (17). Globally, there have been 14 reported cases of DEX (18), including five from China, with eleven distinct variants (c.115C>T, c.443delG, c.465delG, c.553C>T, c.636G>T, c.691T>C, c.700C>T, c.743C>T, c.752G>C, c.1015delG, Deletion exons 2-7). Thirteen of the previously reported cases exhibited BD-like features, while one showed a lupus-like phenotype (15). Common immunological signs included reduced memory B or total B cells, low NK cells, and elevated inflammatory markers (17, 19). In our cohort, these two DEX patients exhibited severe BD-like symptoms, characterised by persistent extensive terminal ileum ulcers, spanning over half of the intestinal circumference, and accompanied with pseudo polyp. The persistent presence of volcano-type ulcers, absence of mucosal healing in two of the patients during follow-up may suggest a potentially poorer prognosis (20). Their disease courses were protracted and relapsing, with suboptimal responses to prednisone and conventional immunosuppressants. Both received TNF-α antagonists, with initial responses to adalimumab observed in two patients. Subsequent relapses led to a switch to infliximab, resulting in disease stabilisation.

HA20, stemming from a loss-of-function *TNFAIP3* mutation identified in late 2015, disrupts the inhibitory function of NF-κB, promoting the overproduction of inflammatory cytokines and leading to systemic inflammatory disease. Currently, HA20 stands as the most prevalent monogenic condition exhibiting BD-like features (21, 22). In our cohort, we presented two girls, each harbouring a distinct truncating *TNFAIP3* mutation, ultimately diagnosed with HA20. Intriguingly, Case 8 displayed not only classical BD symptoms such as fever, oral and perianal ulcers, and gastrointestinal involvement but also persistent liver abnormalities and sensorineural hearing loss. Her brother, sharing the same mutation, also experienced liver abnormalities and hearing loss. In contrast, their father, without the *TNFAIP3* mutation, suffered from hearing loss but had normal liver function. Recent research suggests that HA20 patients may develop chronic liver complications characterised by hepatic fibrosis, hepatocyte injury, and the infiltration of inflammatory T lymphocytes, which aligns with our findings (23). Notably, hepatic Behçet involvement is exceedingly rare (24). When BD affects the liver, it may manifest as conditions like hepatic arteritis, hepatic arterioma, hepatic venous thrombosis, hepatic abscesses, or Budd-Chiari syndrome (25, 26). In cases where other potential causes are excluded, and inflammatory liver lesions are evident, HA20 should be considered. Additionally, it is worth mentioning that sensorineural hearing loss has not been previously reported in HA20 patients. Given the family history, it is plausible that hearing loss in this family may be attributed to factors beyond *TNFAIP3* mutations, including other genetic factors or environmental influences.

In addition to the previously mentioned two BD-like syndromes, our cohort unveiled other monogenic autoinflammatory diseases that mimic BD. Among them, there was a male patient initially diagnosed with BD and JIA but was subsequently confirmed to have Majeed syndrome. Majeed syndrome is a rare multisystem inflammatory disorder characterised by chronic multifocal osteomyelitis (CRMO), congenital dyserythropoietic anaemia (CDA), with or without neutrophilic dermatosis (27). Case 12 in our cohort exhibited classical features of Majeed syndrome, including CRMO, CDA, skin involvement, and growth retardation. And joint symptoms were his initial presentation. However, this patient also manifested recurrent oral ulcers, abdominal pain, haematochezia, and multiple gastrointestinal ulcers, which resemble the intestinal manifestations seen in BD. Notably, despite treatment with corticosteroids and DMARDs, his gastrointestinal symptoms continued to worsen. Gastrointestinal involvement is uncommon in Majeed syndrome, with only two previously reported cases in Indian children experiencing recurrent abdominal pain or diarrhoea (28). However, detailed gastrointestinal endoscopy was lacking in these reports, leaving the extent of intestinal involvement uncertain. These findings imply that gastrointestinal symptoms should be considered within the clinical spectrum of Majeed syndrome. Furthermore, when BD presents alongside significant bone and joint lesions, Majeed syndrome should be contemplated, with IL-1 antagonists potentially offering therapeutic benefits for disease management (29).

The association between MDS and BD has been recognised for over 25 years. Patients with both conditions tend to experience more fever episodes and intestinal lesions compared to those with BD alone. Trisomy 8 is a common finding (81.3%) in patients with both BD and MDS, leading to the term "trisomy 8-associated autoinflammatory disease" (TRIAD) (30). In our cohort, we identified two cases of BD coexisting with MDS. These individuals were initially diagnosed with "aplastic anaemia" during childhood and later developed BD and MDS in their older years. In one case (Case 21), the patient presented with intractable intestinal BD, characterised by recurrent abdominal pain, rectal bleeding, and extensive ileocecal ulcers involving a significant portion of the intestinal circumference (4/5). Despite treatment attempts with glucocorticoids, mesalazine, and TNF-α inhibitors (adalimumab and infliximab), the progression of intestinal lesions remained uncontrolled. This aligns with previous observations that many patients with BD and MDS do not respond well to standard BD treatments, indicating differences in treatment responses compared to BD alone (31, 32). Treatment strategies focusing on MDS appear to offer more favourable outcomes for patients with both MDS and BD. The exact reasons behind the refractoriness of BD with trisomy 8-positive MDS to conventional therapy remain unclear. HSCT is considered the only curative treatment for MDS and has been successful in cases where trisomy 8-positive MDS coexists with BD (33). For individuals like Case 21, HSCT might be a potential therapeutic option. When managing BD patients with concurrent haematological

abnormalities, the presence of trisomy 8 should not be underestimated.

This study is limited by the lack of functional analyses conducted on all newly identified mutations, despite their predicted pathogenicity by multiple prediction tools (SIFT, PolyPhen-2, MutationTaster and CADD). Furthermore, the sample size is insufficient, as genetic testing was not performed on all BD patients in our cohort. Among the 50 BD patients diagnosed concurrently, genetic testing was only conducted on 24 patients, all of whom with positive genetic findings had gastrointestinal involvement. Genetic testing was initiated in BD patients at our hospital over the past decade; however, some patients diagnosed earlier were lost to follow-up, leading to potential inclusion of BD mimics in our cohort.

In summary, this study highlights the range of monogenic diseases and chromosomal abnormalities that can resemble BD, particularly when gastrointestinal symptoms are present. Factors such as early onset, positive family history, atypical clinical features, multi-site intestinal involvement observed during endoscopy, and inadequate response to standard therapies should prompt consideration of early genetic screening. However, we acknowledge the study's limitation due to a small sample size, preventing definitive conclusions. Recognizing the underlying cause, whether treatable or not, is crucial for prognosis assessment, genetic counselling, and the potential avoidance of unnecessary and potentially harmful immunosuppressive treatments. A broader approach to genetic screening may offer a more nuanced understanding of the genetic landscape of BD.

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