MONOGENIC MIMICS OF BEHÇET'S DISEASE IN THE YOUNG

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Abstract

Objectives: Monogenic autoinflammatory disorders (AID) and primary immunodeficiencies can present early in life with features that may be mistaken for Behçet’s disease (BD). We aimed to retrospectively describe the clinical and laboratory features of 11 paediatric cases referred for suspected BD who turned out to have an alternative, monogenic disease mimicking BD.

Methods: Retrospective, paediatric BD specialist multicentre case series. Next generation sequencing (NGS) or conventional candidate gene screening approaches were utilised, facilitated in some cases by functional assays.

Results: Eleven children referred with suspected BD underwent genetic screening because of atypical BD features, and/or presentation before age 5-years. Eight patients (73%) were Caucasian; 2 were Pakistani; and 1 was Turkish; 55% were female. A positive family history of BD was reported in 54% cases. The median age of disease onset was 0.6 (range 0.2-2.3) years. All had systemic inflammation and oral ulceration; 5/11 had genital ulceration; 3/11 had ocular involvement; and 9/11 had cutaneous manifestations. Nine/11 had known disease-causing genetic mutations in: TNFAIP3 (n=2), WDR1 (n=2), NCF1, AP1S3, LYN, MEFV, and GLA. The remaining 2 cases each had novel variants in STAT1 and TNFRSF1A.

Conclusion: Rare monogenic diseases can mimic BD, particularly when presenting early in life. These observations are now informing a strategy to explore screening for genetic mimics of BD in a UK cohort of children and adults to better understand the proportion of UK BD patients who may in fact have an underlying monogenetic diagnosis.

Keywords: Behçet’s Disease, child, autoinflammatory disease, next-generation sequencing, A20 haploinsufficiency, Fabry disease, Immunodeficiency, chronic mucocutaneous candidiasis disease

Key messages:

1. A wide range of monogenetic diseases may present with a BD-like phenotype.
2. Early disease onset, positive family history or atypical presentation constitute ‘red flags’ and should prompt genetic screening.
3. Next generation genetic sequencing should be considered as part of the diagnostic work up for young patients with suspected BD.
Behçet’s disease (BD) is a multi-systemic inflammatory variable vessel vasculitis affecting virtually any type of blood vessel but particularly veins, typically associated with recurrent oral and genital ulceration, ocular, and skin inflammation as cardinal features. Almost any organ can be affected, including the gastrointestinal, cardiovascular, renal, pulmonary, musculoskeletal and central nervous system (1). BD typically affects young adults 20 to 40 years of age but also affects children (2, 3) with onset under the age of 16 years in 4-26% (2, 4), and with median disease onset 4.9-12.3 years in different paediatric cohorts (3). The diagnosis is challenging since clinical presentation is heterogeneous and may vary by gender, ethnicity or age of disease onset (5). Clinical diagnostic criteria have therefore been devised but perform sub optimally, particularly in children at their first presentation (3).

The exact aetiology of BD is unknown. The prevailing hypothesis is that BD is the result of genetic susceptibility and environmental triggers, which subsequently cause dysregulation of the immune system. Several studies have demonstrated a multifactorial polygenic contribution (6). Genome-wide association (GWAS) and (to a lesser extent) next generation sequencing (NGS) studies have provided evidence for the role of genes involved in both innate and adaptive immunity (7). The human leukocyte antigen (HLA) loci, most importantly HLA-B*51 in the major histocompatibility complex (MHC) on chromosome 6, is considered the strongest known genetic risk factor for BD, an association that has now been replicated in multiple populations (8, 9). HLA-B*51 interacts with another susceptibility gene polymorphism, p.Arg725Gln in ERAP1, resulting in reduced peptide trimming activity, thus altering the peptides available for MHC class I binding (10), an example of epistatic genetic contribution to BD susceptibility. Associations between BD and other genetic variants around the MHC class I polypeptide-related sequence A (MICA) region have also been reported (9). Non-HLA susceptibility loci include: the intergenic region between IL23R and IL12RB2; IL10 (11); IL1A-IL1B, IRF8, and CEBPB-PTPN1 (9). An inherent and important limitation of GWAS, however, is that it focuses on common variants with allele frequencies greater than 5%; rare genetic variants with allele frequencies less than 1% are excluded from association tests because of inadequate power to evaluate the effects in studies typically involving a few thousand individuals (10). This means that patients with monogenic diseases masquerading as BD will not be detected by GWAS.
Next generation sequencing (NGS) is increasingly impacting on the clinical care of patients in virtually every aspect of medicine, including those with autoinflammatory or autoimmune diseases (AID). Recently, an AID with a familial BD-like phenotype has been reported in association with monoallelic mutation in \textit{TNFAIP3} resulting in haploinsufficiency of A20 protein, a regulator of NFKB activation (12). Other monogenic AID and some primary immunodeficiencies can also present early in life with Behçet-like symptoms and may be mistaken for BD. The aim of this study was therefore to describe the clinical and laboratory features of 11 cases referred to Great Ormond Street Hospital NHS Foundation Trust (GOSH) for suspected BD, who ultimately turned out to have an alternative monogenetic diagnosis.

\textbf{Methods}

This was a retrospective case notes review collating anonymised clinical information obtained as part of routine clinical care. As such, patients were not required to provide written consent as per national and institutional ethical guidelines. Patients who underwent additional next-generation sequencing (i.e. if no genetic diagnosis was revealed by standard genetic testing) as part of their diagnostic work up did so with full written consent obtained and with full ethical approval from the National Research Ethics Service, Bloomsbury Committee, London (ethics number 08H071382).

A total of 11 patients were included, all referred to GOSH for advice and further management of suspected BD. Case notes were reviewed retrospectively, and pre-specified clinical and laboratory data were collected using a Microsoft Excel spreadsheet. Two BD definition scores were collated to explore their performance in this series: the ICBD criteria (\textit{supplementary table 1}) with a score of 4 or more satisfying criteria for BD diagnosis (13); and the proposed new (2015) paediatric classification criteria (\textit{supplementary table 2}), where 3/6 items are required to classify a patient as having paediatric BD (14).

Data analyses were descriptive, with numeric data summarised using median and interquartile range (IQR); as such no formal comparative statistical testing was required. Specific metrics scrutinised included the time to molecular diagnosis, calculated from the date of the first clinical review at GOSH to the date of confirmed genetic diagnosis (with functional study
A combination of NGS or conventional candidate gene Sanger sequencing approaches were utilised to ascertain genetic diagnoses, facilitated in some cases by functional immunological or biochemical assays. NGS approaches included targeted exome sequencing (the “Vasculitis and Inflammation Panel”, VIP) (15); or whole exome sequencing (WES). Targeted gene panel sequencing, bioinformatic analyses, and pathogenicity assessment of identified genetic variants were performed using standard methodologies, as previously described (15). The identified variants were individually assessed and classified into pathogenicity groups (Class 1: clearly not pathogenic; Class 2: unlikely to be pathogenic; Class 3: unknown significance; Class 4: likely to be pathogenic; Class 5: clearly pathogenic), according to the Association of Clinical Genetics Science Practice Guidelines (ACGS) 2013 guidelines (16). The level of evidence was assigned using the 2015 American College of Medical Genetics guidance (17). Variants detected using NGS were also confirmed using Sanger sequencing, to exclude false positives.

Results

Demographics and clinical manifestations at presentation

A total of 11 patients (6 females and 5 males) with a median age of 5 years (IQR 3-9 years) were identified. Eight patients (73%) were Caucasian; 2 were Pakistani; and 1 was Turkish. The demographics and clinical features of all 11 cases are summarised in Table 1. Six out of the eleven had a family history of BD. Cases 4 and 5 were siblings from a consanguineous Pakistani kindred with suspected familial BD. Family trees for all 6 index cases with a positive family history are provided in Supplementary Figure 1.

The median age of disease onset was 0.6 (range 0.2-2.3) years, and the median age at first review at GOSH was 4.1 years (IQR 2.2-4.8 years). A genetic diagnosis was made a median of 4.5 (IQR 0.9-9.7) years after the first clinical visit at GOSH. All had suspected BD as the reason for referral to GOSH, and underwent further molecular testing because of symptom onset <5 years of age and/or incomplete or atypical BD features. All had systemic inflammation and oral ulceration. Episodes of fevers (affecting 9/11 cases) or systemic inflammation were
recurrent rather than continuous, but typically non-periodic for this cohort. Detailed descriptions of these 11 patients are provided in Tables 1 and 2. Eight/11 patients (73%) fulfilled ICBD criteria at the first presentation, compared with 6/11 (55%) fulfilling the new Paediatric classification criteria (Table 2).

Genetic testing

Nine out of 11 cases were considered to have a definite monogenic cause that accounted for their phenotype. In the remaining 2 cases (cases 3 and 9), variants of uncertain clinical significance were identified as the suspected, but as yet unproven cause for the phenotype (Table 1). NGS was performed in 10 cases (gene panel in 7; WES in 3), with Sanger sequencing confirmation of identified class 5 variants and for familial segregation analyses. In the remaining patient (case 7) Sanger sequencing alone revealed the molecular diagnosis. More detailed descriptions of the phenotypes and associated genotypes classified according to pathogenicity (17) are provided, as follows.

Clearly pathogenic variants (Class 5)

Six/11 cases (55%) had at least one class 5 (clearly pathogenic) variant (Table 1). The genetic variants harboured by these 6 cases fulfilled the pathogenicity criteria from literature evidence and pertinent functional laboratory immunological data supporting disease-genotype concordance (17).

A diagnosis of haploinsufficiency of A20 (HA20) was made in case 1 (Figure 1; Table 1). She was heterozygous for the highly penetrant loss-of-function nonsense mutation in TNFAIP3, (p.R271X), recently reported by Zhou et al as the cause of HA20 (18). The mother also had this mutation, and was being investigated for a milder, uncharacterised inflammatory phenotype (Supplementary Figure 1A).

We previously reported 2 Pakistani girls (Cases 4 and 5) born of consanguineous parents (pedigree B in Supplementary Figure 1) and diagnosed with familial BD beginning in the first weeks of life (19). Both had severe recurrent oral inflammation, which caused scarring
and acquired microstomia in Case 4 (Figure 2). Both siblings were found to have a novel homozygous missense p.L293F mutation in the actin regulatory gene *WDRI* detected using WES, causing periodic fevers with immunodeficiency and thrombocytopenia (PFIT) (19).

Case 7 was referred with suspected BD with a history of pustules and erythematous rashes, recurrent oro-genital ulceration, gastrointestinal bleeding and high inflammatory markers from the age of 2.5 years (Figure 3A and 3B). Nitroblue tetrazolium (NBT) test revealed absent neutrophil respiratory burst, compatible with CGD (20); Sanger sequencing confirmed compound heterozygote mutation in *NCF-1*: c.75_76 del GT mutation in exon 2 (21, 22), and a splice site mutation c.682+1G>C mutation at the end of exon 7, a site previously associated with CGD (23). Two further siblings were subsequently also diagnosed with CGD.

Case 8 was found to have the p.F4C mutation in *AP1S3* which is associated with pustular psoriasis and impaired Toll-like Receptor 3 trafficking (24). She was commenced on infliximab 6mg/kg 4 weekly with a good response, later switching to adalimumab.

Case 11 was referred aged 15 years with fever of unknown origin, recurrent mouth ulcers suggestive of BD, myalgia, fatigue, lymphadenopathy, colitis and skin rash reminiscent of panniculitis (but without histological confirmation), and weight loss. Investigations revealed raised inflammatory markers and microcytic anaemia (Hb=9.3 g/dL, CRP=46 mg/L, ESR=100 mm/hr). Upper and lower gastrointestinal tract endoscopies excluded inflammatory bowel disease. Selective visceral catheter arteriography angiography revealed subtle changes affecting the small intrarenal arteries suggestive of a vasculitic process, for which he was treated with corticosteroids and cyclophosphamide, followed by azathioprine maintenance immunosuppression. Despite that he went on to develop sagittal and bilateral cortical vein thromboses and bilateral parietal venous infarctions complicated by parenchymal haemorrhage on the left (Figure 4). At that point, cerebral venous thromboses complicating atypical BD was diagnosed, and treatment with infliximab was introduced. At the age of 25 years, genetic screening using our VIP gene panel revealed the heterozygous class 5 *GLA* p.G271S mutation causing Fabry disease (FD) (25), a lysosomal storage disease associated with cerebral vein thromboses (26). Assessment of leukocyte alpha-galactosidase A enzyme activity using fluorogenic method (27) revealed complete absence of enzyme activity (<1% of
control), confirming the diagnosis and the mutation was confirmed by Sanger sequencing in the regional genetics laboratory. There were no other Class 4 or class 5 genetic mutations in any of the AID genes contained on VIP (supplementary Table 3). Alpha-galactosidase A enzyme replacement therapy has now been commenced.

Likely pathogenic genetic variants (Class 4)

Three/11 subjects (27%) with likely pathogenic variants (class 4) are summarised in Table 1. Case 2 presented with a history of recurrent panniculitis, abdominal pain, mouth ulcers and testicular pain associated with fever and elevated acute phase reactants. IL-1 blockade followed by tocilizumab provided only partial responses. Genetic testing revealed the p.Y508F variant in the tyrosine-protein kinase \( \text{LYN} \) gene, recently reported to cause a novel autoinflammatory syndrome with similar phenotype with our case, due to loss of the phosphorylation site (28). An animal model also confirms that this tyrosine residue at position 508 has important regulatory function, as mice with the p.Y508F mutation have enhanced enzymatic activity and present with haemolytic anaemia (29), lethal autoimmune glomerulonephritis and positive autoreactive antibodies (30).

We identified a novel \( \text{STAT1} \) gain-of-function variant (p.L280W) in case 6, compatible with the diagnosis of chronic mucocutaneous candidiasis disease (CMCD), mimicking mucocutaneous Behçet’s disease. Her symptoms began at the age of 7 months, with (initially) non-infective oral and genital ulcerative lesions (Figure 3C), associated with recurrent fevers. Treatment with colchicine, corticosteroids, azathioprine and adalimumab provided little symptomatic relief. Severe oral and oesophageal candidiasis emerged despite stopping all immunosuppression; she then developed recurrent chest infections, and episodes of paronychia requiring repeated courses of antibiotics. At the age of 6 years she developed acute adrenocortical insufficiency, confirmed on synacthen test, with absence of adrenal cortex autoantibodies. To explore the pathogenicity of this variant, a functional \( \text{STAT1} \) phosphorylation assay was performed, adapted from an established \( \text{STAT5} \) phosphorylation assay (31). We demonstrated increased \( \text{STAT1} \) phosphorylation in response to IFN\( \alpha \) stimulation in the lymphocytes of this patient compared to control (Supplementary Figure 2), providing strong supporting evidence for the pathogenicity of the identified p.L280W \( \text{STAT1} \).
variant, and the diagnosis of CMCD. At the time of writing, treatment with a Janus Kinase inhibitor is being considered (32).

Case 10, with familial BD, was given a final diagnosis of HA20, also confirmed in the father. The paternal uncle and grandmother had also been previously diagnosed with severe BD (Supplementary Figure 1E). He and his father had a novel heterozygous mutation in exon 2 of TNFAIP3, c.292delA, predicted to cause a frameshift leading to a premature stop codon p.N98Tfs*25. Anakinra is being considered for this case at the time of writing.

Variants of uncertain clinical significance (class 3)

Two/11 patients had no class 5 or 4 variants but had notable class 3 variants in known AID genes. Case 3 first presented aged 3 years with severe recurrent oral ulceration from the age of 1 year but becoming progressively more severe over time. One episode required hospital admission due to a large ulcer involving his entire hard palate; and with punched-out skin ulcerations in his right axilla, with tender cervical lymphadenopathy and pyrexia. He subsequently developed recurrent scarring ulcers to his skin, and features of pathergy. His symptoms responded partially to oral corticosteroids and colchicine. VIP targeted gene panel screening revealed the p.K265R variant in exon 9 on the TNFSFR1A gene. Treatment with Anakinra has led to considerable improvement in his symptoms and improved quality of life.

Case 9, a Turkish male, presented with recurrent oral ulceration, and acneiform lesions from early in life. These symptoms responded well to colchicine. His mother had identical symptoms from early in life and had been diagnosed with BD (Supplementary Figure 1D). Genetic testing using our VIP targeted gene panel revealed a heterozygous p.I591T variant in MEFV, also present in the mother (confirmed with Sanger sequencing).

Discussion

In this retrospective study, we report eleven paediatric cases referred with suspected BD who ultimately turned out to have a monogenic disease mimicking BD. These cases had early onset
of disease at median 0.6 years of age; and 54% had a positive family history, similar to previous reports of BD in the paediatric population (6). Although the clinical presentation was broad, all of the cases had recurrent aphthous stomatitis and evidence of systemic inflammation. At the time of first presentation to us, 8/11 (73%) fulfilled the ICBD criteria for BD, while 6/11 (55%) fulfilled the Paediatric Criteria for BD. Ten out of 11 were considered to have monogenic diseases of the immune system; the remaining case had FD, a lysosomal storage disease. Two were diagnosed with A20 haploinsufficiency. Both of them had recurrent oral ulceration and one also presented with genital ulceration, which are considered hallmarks of HA20 (33). One case was diagnosed with LYN associated autoinflammation. Regulation of immune cell function by the tyrosine kinase lyn has been previously demonstrated to be important for immune diseases (34); indeed dysregulation of lyn can lead to autoimmune diseases in mice, reminiscent of SLE, asthma and psoriasis; and recently a case with an early-onset autoinflammatory phenotype has been reported in a patient with a de novo nonsense mutation in LYN (35). Other diagnoses included CGD, PFIT, CMCD and pustular psoriasis.

For the remaining 2 cases, one had a novel variant in TNFRSF1A, and the other a monoallelic I591T variant in the MEFV gene which segregated with disease in the mother.

Case 11 with FD was the only one out of our series that presented to us at an older age (15 years of age). Disease course was characterised by evidence of chronic inflammation, oral ulceration, rashes, lymphadenopathy and gastrointestinal involvement, compatible with autoinflammation. Undegraded substrates in lysosomes stimulate various pathogenic cascades that can ultimately cause autoinflammation, regardless of the specific lysosomal deposits (36). In FD, the autoinflammation is suggested to be primarily driven by glycolipid accumulation (Gb3 and lyso-Gb3), which is recognised as a danger signal. These lysosomal deposits may behave as damage associated molecular patterns (DAMPs), or cause DAMP production by injured cells, driving autoinflammation. Indeed, the addition of Gb3 to normal control cells induces apoptosis and cytokine secretion (37, 38). Thus, FD joins other emerging lysosomal storage diseases that recently have been associated with an autoinflammatory phenotype (39, 40). Cerebral vein thrombosis is well described in FD (41). Recently, Lenders et al (42) reported a high thromboembolic event rate for patients with FD that may be reduced by enzyme replacement therapy (42). Lysosomal accumulation of glycosphingolipids, primarily globotriaosylceramide (GL-3), in vascular endothelium may contribute mechanistically to the pathogenesis of thromboembolism in FD (43); other factors may include the presence of a concurrent prothrombotic state, abnormalities in cerebral blood flow velocity, and autonomic
dysfunction (44). Thus, we highlight FD as an important mimic of BD which we suggest should be screened for, particularly for work up of suspected neuro-BD, and for BD patients with thrombo-embolic complications.

A disproportionately high prevalence of \textit{MEFV} mutations has been demonstrated in BD patients compared with the healthy population (45, 46). Moreover, the coexistence of both FMF and BD has been reported (47). A recent meta-analysis of 8 studies with a total of 2538 BD patients and 2792 healthy controls demonstrated that M694V and M680I, two highly penetrant \textit{MEFV} mutations, were associated with BD; whereas E148Q, a low-penetrance pyrin mutation, was not linked to BD (46). A higher prevalence of \textit{MEFV} mutations has been observed in BD patients with vascular complications (45). The I591T \textit{MEFV} mutation identified in case 9 was also found in his mother who had mucocutaneous features of BD. This variably penetrant \textit{MEFV} mutation has been reported to cause FMF in both monoallelic and compound heterozygous states (48, 49). It is thus possible that case 9, and his mother, do have a familial form of BD with this \textit{MEFV} variant contributing to the pathogenesis, as suggested for the M694V and M680I \textit{MEFV} variants.

Most of the genetic studies done thus far in BD have been GWAS (9, 11). GWAS has markedly advanced our knowledge about the genetic determinants underlying the development and progression of BD, with the discovery of several polymorphisms. Although GWAS studies can serve as a powerful tool for elucidating associations between genes and traits, there are important limitations, particularly the aforementioned issue of excluding rare genetic variants. Additionally, population stratification, linkage disequilibrium, and DNA pooling can bias the results of GWAS. Therefore, GWAS studies of BD populations would not detect rare monogenic diseases masquerading as BD. That said, GWAS did suggest that polymorphisms (i.e. common variants) in \textit{TNFAIP3} could be important in BD (50) emphasising that GWAS and NGS studies can be complementary for understanding the genetic architecture of BD.

The highest level of analytic validity occurs when a variant in a gene has been previously associated with a particular disease, combined with pertinent functional assays supportive of pathogenicity. In our study, where available, we performed functional assays (i.e. NBT, STAT1 phosphorylation, and α-galactosidase activity) to support our genetic findings. On the whole, however, rapid progress in genomic technologies has been faster than development of functional laboratory assays used to confirm or refute pathogenicity of genetic variants in
routine clinical care, an area that requires ongoing effort as we move forwards in the post genomics era.

The main limitation of our study is the possibility of referral bias resulting in us receiving more atypical BD cases in our specialised paediatric BD clinical service (3). Secondly, we did not apply NGS genetic screening to all BD paediatric referrals we received at GOSH, mainly due to the practical fact that this approach has only relatively recently been introduced. Furthermore, we do not yet know how our findings will extrapolate to adult-onset BD. Therefore, our results do not yet allow us to provide firm guidance on which patients with BD should undergo genetic screening with NGS. Thus, whilst we await the results of a planned systematic NGS screening programme for UK BD patients, we suggest that patients with disease onset aged <5 years; with family history of BD; consanguinity; or atypical clinical course should be considered for NGS screening for monogenic disease. If a targeted gene panel approach rather than WES is used, clinicians should be aware of which genes are included to understand any potential limitations if the genetic screen is negative. Lastly, more extensive genetic screening with whole genome sequencing might in the future provide an even more accurate picture of the true frequency of monogenic diseases mimicking BD.

In conclusion, we highlight a range of monogenic mimics of paediatric BD and preliminarily suggest criteria for selecting patients for genetic screening. These data are now informing a strategy to begin to explore screening for genetic mimics of BD in a UK cohort of children and adults to better understand the proportion of UK BD patients who may have an underlying genetic diagnosis. This is important since treatment of monogenic BD mimics may differ significantly from standard BD treatment, as highlighted by our case series. Additionally, the determination of the underlying cause, whether treatable or not, has significance from the point of view of prognostication, genetic counselling, and in some instances avoidance of unnecessary and potentially harmful immunosuppression.
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References

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<th>Amino acid change*</th>
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<td>Cauc</td>
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<td>TNFAIP3</td>
<td>811C&gt;T</td>
<td>R271X</td>
<td>T/-/D</td>
<td>Het</td>
<td>5</td>
<td>Uveitis, mouth ulcers, vasculitic rash, lupus nephritis class III and IV [Intermittent high intermittent] ESR, normal CRP/SAA Hydroxychloroquine, azathioprine, mycophenolate mofetil unresponsive</td>
<td>HA20; genetic counselling; alive, and being considered for IL-1 blockade</td>
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<td>15</td>
<td>LYN</td>
<td>1523A&gt;T</td>
<td>Y508F</td>
<td>D/D/D</td>
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<td>Recurrent but non-periodic fevers, panniculitis, abdominal pain,</td>
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<td>TNFRSF1A</td>
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<td>K265R</td>
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<td>Het</td>
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<td>Intermittent (but typically non-periodic) systemic inflammation, recurrent oral ulceration, pathergy, poor wound healing, chronic vocal cord inflammation; undefined autoinflammatory disorder (carrying a variant of uncertain clinical significance in TNFRSF1A); alive</td>
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headaches, conjunctivitis, arthralgia, mouth ulcers, intermittently elevated CRP/SAA. Anakinra and tocilizumab unresponsive, Colchicine partial response.
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<td>3</td>
<td>WDR1</td>
<td>877C&gt;T L293F Hom P/D/D</td>
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<td>intermittent but non-periodic systemic inflammation, recurrent orogenital ulceration, arthritis and fevers, recurrent upper respiratory tract infections, negative workup for immunodeficiency, intermittent high ESR/CRP; underwent PFIT; alive and off all immunosuppression post allo-HSCT 6 years</td>
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### Patient 5
- **Race/Cauc:** Pakist
- **Gender:** Y F
- **Age:** 12
- **Gene:** WDR1
- **Mutation:** 877C>T L293F
- **Phenotype:** Recurrent (non-periodic) fever, orogenital ulceration, uveitis, thrombocytopenia, pyoderma gangrenosum-like rash, arthritis, high ESR, CRP, SAA, initially good response to anakinra; death from sterile inflammation and multi-organ failure age 14 years
- **Outcome:** PFIT; died age 14 years

### Patient 6
- **Race/Cauc:** Cauc
- **Gender:** N F
- **Age:** 3
- **Gene:** STAT1
- **Mutation:** 839T>G L280W
- **Phenotype:** Recurrent (non-periodic) fever with CMCD
- **Outcome:** CMCD; alive
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with corticosteroids, cyclophosphamide and azathioprine with poor response; infliximab added with partial initial response, but later stopped due to loss of efficacy.

*Since each gene may have multiple splicing isoforms, the variants were annotated according to the RefSeq transcript in supplementary table S3

**Age at the time of this study.

†Prediction (polyphen2/SIFT/MutationTaster); B = Benign, D = damaging or deleterious, P = probably damaging, T = tolerated, n = neutral, A = disease causing automatic for MutationTaster.

Abbreviations: Cauc = caucasian, Pakist = Pakistani, CRP = C-reactive protein, SAA = Serum amyloid A, ESR = erythrocyte sedimentation rate, MTX = Methotrexate, DMARDS = Disease-modifying anti-rheumatic drugs, AID = autoinflammatory disease, CMCD = Chronic Mucocutaneous Candidiasis Disease, CGD = Chronic granulomatous disease, HA20 = A20 haploinsufficiency, PFIT = Periodic fevers, immunodeficiency, and thrombocytopenia, TRAPS = TNF receptor-associated autoinflammatory syndrome, Consan = Consanguinity (Y = yes, N = no, U = unknown), Sex (F = female, M = male). HSCT = Haematopoietic stem cell transplantation, CS = corticosteroid (including pulses of intravenous methylprednisolone or oral prednisolone); GI = gastrointestinal, PR = per-rectal, FMF = Familial Mediterranean Fever, IL1 = Interleukin 1
Table 2. Comparison of the scores for the International Criteria for Behçet's Disease (ICBD) and Paediatric Criteria for Behçet's Disease (2015)

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<tr>
<th>Case</th>
<th>Oral aphthosis</th>
<th>Genital aphthosis</th>
<th>Ocular involvement</th>
<th>Vascular signs</th>
<th>Neurological signs</th>
<th>Skin involvement</th>
<th>Pathergy Test (+)</th>
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<th>Paediatric BD Criteria fulfilled (Y/N)</th>
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Figure 1. Case 1: vasculitic rashes of feet and hand (A, B).
**Figure 2.** Acquired microstomia (A) in case 6 aged 12 years and 10 months, the consequence of recurrent sterile oral inflammatory episodes. At 13 years of age, she developed a severe necrotizing cellulitis of the neck caused by *Streptococcus pneumoniae* (B).
Figure 3. Case 7 presented with mouth ulcers (A) and pustular erythematous rash (B). Significant oral ulceration requiring hospital admission in case 6 (C).
**Figure 4.** Unenhanced CT brain, coronal (A) and axial (B) reformats, shows hyperdensity and expansion of the superior sagittal sinus (dashed arrows) and emissary cortical veins (arrows), in keeping with venous thrombosis. MRI brain, coronal FLAIR (C) and axial T2 weighted imaging show bilateral brain infarctions in the parietal lobes.