

DATA REPORT

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# Novel *TNFAIP3* microdeletion in a girl with infantile-onset inflammatory bowel disease complicated by a severe perianal lesion

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## Abstract

A20 haploinsufficiency (HA20), a disease caused by loss-of-function *TNFAIP3* mutations, manifests various autoinflammatory and/or autoimmune symptoms. Some cases of HA20 were initially diagnosed as very early onset inflammatory bowel disease (VEO-IBD). We performed whole-exome sequencing (WES) for a Japanese girl with infantile-onset IBD and a severe perianal lesion and detected a novel de novo 119 kb microdeletion containing only *TNFAIP3* (arr[GRCh37] 6q23.3(138125829\_138244816) × 1).

Very early onset inflammatory bowel disease (VEO-IBD), defined as IBD with an onset before 6 years of age, is challenging, with difficulties in diagnosis and management. Over 60 genes associated with monogenic IBD have been described worldwide with the widespread use of whole-exome sequencing (WES)<sup>1</sup>. Among them, *TNFAIP3* suppresses nuclear factor-kappa B, and its germline mutations lead to HA20<sup>2</sup>, presenting with IBD and symptoms similar to those of Behçet disease<sup>3</sup>. However, the diagnostic approach with genetic testing has not been well established, and the genotype–phenotype correlation in HA20 remains unclear. Here, we report a case of infantile-onset HA20 with a severe perianal lesion and a novel de novo microdeletion spanning the *TNFAIP3* gene as determined by WES and copy number variation (CNV) analysis.

The patient was an 8-month-old Japanese girl with no consanguinity or other significant family medical history. Her perinatal history was unremarkable. From the age of

3 weeks, she developed an intermittent high fever every 1–2 months that resolved without intervention. The patient's serum C-reactive protein levels remained elevated, and abdominal computed tomography revealed hepatosplenomegaly. Screening tests previously performed at 1 month of age were not consistent with primary immunodeficiency, autoinflammatory disease, or hemophagocytic syndrome.

The patient started experiencing diarrhea more than ten times a day at 6 months of age, and active perianal fistulae appeared at the age of 7 months. She had impaired growth at 8 months of age, with a height of 63 cm (−2.5 standard deviations (SDs)) and weight of 5.3 kg (−3.0 SDs), but was not developmentally delayed. Although she was afebrile, laboratory tests indicated an abnormal condition of the autoimmune system (Supplementary Table S1). The patient had a few aphthous ulcers in the oral cavity, perianal fistulae in the right labia majora and right anterior of the anus, and anal ulcers at the anterior and dorsal sides of the anus (Fig. 1A). Esophagogastroduodenoscopy findings were unremarkable. However, ileocolonoscopy revealed aphthous ulcers throughout the colon with punched-out ulcers over the descending colon to the rectum (Fig. 1B). The terminal ileum was normal. External fistula openings ran directly to the rectal ulcers just

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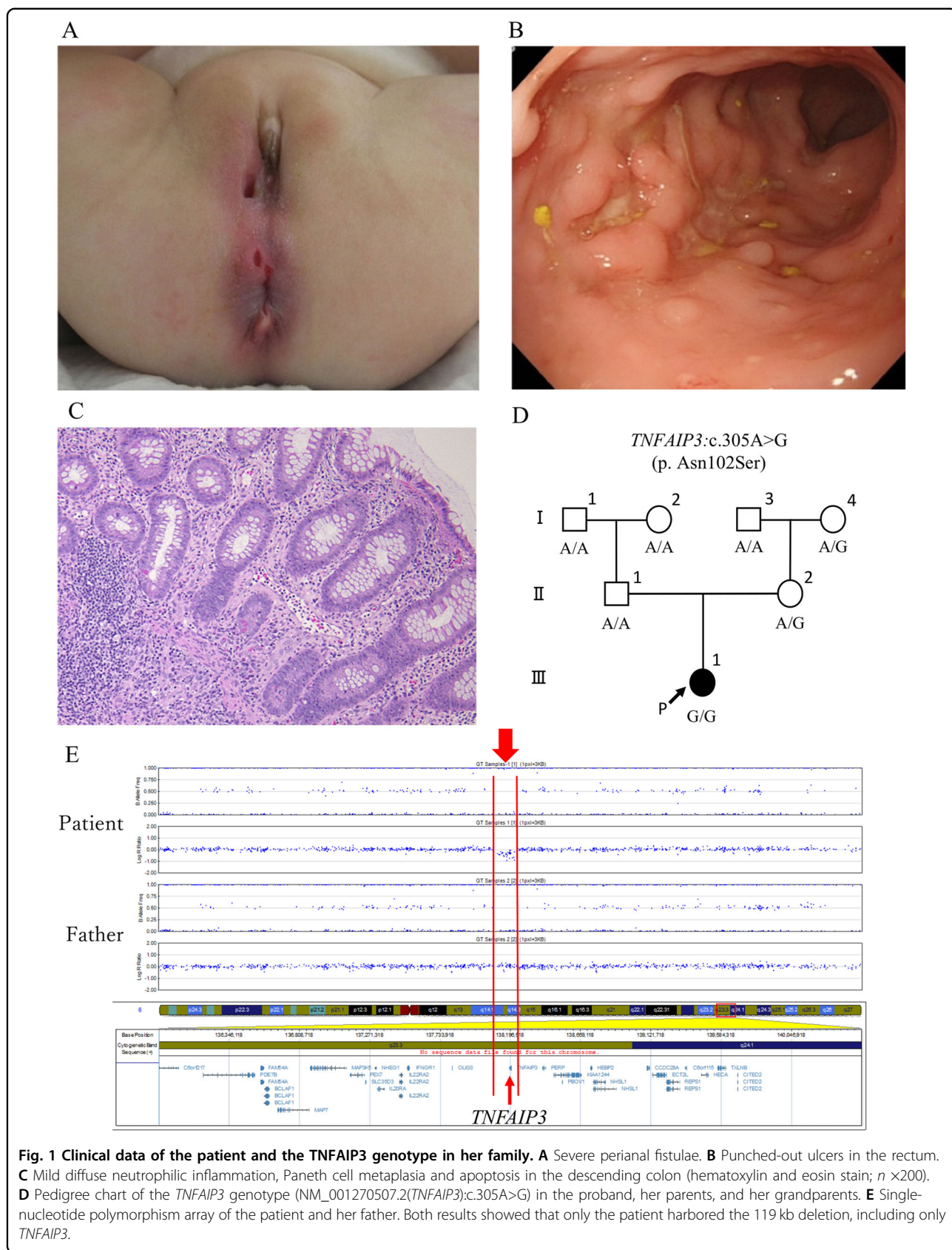
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**Table 1 Comparison of reported patients with HA20 and their deletion type in the literature.**

|                                 |   |  |   |   |   |
|---------------------------------|---|--|---|---|---|
| Location                        | 6q23.3  | 6q23.3   | 6q23.3  | 6q23.3  | 6q23.2–6q24.3   |
| Range of deletion (GRCh37/hg19) | chr6:138125829_138244816  | —  | chr6:138192201–138428412  | chr6:138192201–138428412                              | chr6:134387945–147518246  |
| Size of deletion                | 119 kb  | 5 kb   | 236 kb  | 236 kb  | 13.13 Mb  |
| Involved genes                  | TNFAIP3   | TNFAIP3 (exons 2–3)  | TNFAIP3, PERP   | TNFAIP3, PERP   | SGK1, ALDH8A1, HBS1L, MYB, AHI1, PDE7B, MIFR2, BCLAF1, MAP7, MAP3K5, PEX7, SLC35D3, IL20RA, IL22RA2, IFNGR1, OLIG3, TNFAIP3, PERP, ARFGF3, PBOV1, HEBP2, NHSL1, GVQW2, CCDC28A, ECT2L, REPS1, ABRACL, HECA, TXLNB, CITED2, NIMBR, VTA1, ADGRG6, HIVEP2, AIG1, ADAT2, PEX3, FUCA2, PHACTR2, LTV1, ZC2HC1B, PLAGL1, SF3B5, STX11, UTRN, EPM2A, FBXO30, SHPRH, GRM1, RAB32, ADGB |
| Gender                          | Female  | Male   | Female  | Female  | Male  |
| Initial symptoms                | Periodic fever  | Recurrent oral and perianal ulcer  | Periodic fever  | Oral ulcer  | Upper respiratory infections, gastroenteritis, febrile episodes   |
| Symptom onset                   | 3 weeks   | 6 years  | 2 months  | 1 year  | 1 year  |
| Age at diagnosis                | 8 months  | 9 years  | 11 years  | 16 years  | 12 years  |
| Phenotype                       | Periodic fever, abdominal pain, diarrhea, bloody stools, failure to thrive, digestive ulcers, perianal fistulae | Recurrent episodes of abdominal colic with fever, headache, vomiting, and oral/perianal ulcers | Periodic fever, abdominal pain, diarrhea, bloody stools, weight loss, digestive ulcers, acute anterior uveitis, pharyngalgia, enlarged tonsil | Oral ulcer, folliculitis, abdominal pain, thyroiditis | Neutrophilic dermatosis, growth and psychomotor delay, oral aphthae, diarrhea, perianal ulcers  |
| Treatment                       | Colchicine, 5-aminosalicylic acid, surgical placement of setons   | Adalimumab   | Colchicine, cimetidine, mesalazine, PSL, NSAIDs, MTX, corticosteroid eye drops  | Levothyroxine   | Etanercept  |
| References                      | This report   | (Shimizu et al. <sup>10</sup> )  | (Tsuchida et al. <sup>8</sup> )   | (Tsuchida et al. <sup>9</sup> )                       | (Franco-Jarava et al.)  |

NSAIDs nonsteroidal anti-inflammatory drugs, MTX methotrexate, PSL prednisolone.

above the dentate line. Setons were placed to drain each fistula. Although the recurrent oral aphthous ulcers, arthritis, and intestinal punched-out ulcers observed in this patient met the International or Japanese criteria for diagnosing Behçet disease, these symptoms did not fulfill the criteria for the complete or incomplete type of Behçet disease. Colonic histopathology showed mild diffuse inflammation that was primarily neutrophilic, Paneth cell metaplasia, and crypt distortion throughout the colon; further, apoptosis was observed in the sigmoid colon and descending colon. A granuloma was also noted in the hepatic flexure. Despite macroscopic inflammation, no crypt atrophy or basal plasmacytosis was present (Fig. 1C).

The study was approved by the Institutional Review Board (IRB) of the National Center for Child Health and Development (IRB number: 926). Infantile-onset IBD with perianal lesions is a well-known manifestation of monogenic IBD, particularly in patients with IL10 signaling defects<sup>4</sup>; therefore, we performed WES to identify the genetic etiology of the disease as described previously<sup>5</sup> after obtaining written informed consent from the patient's family. DNA was extracted from the peripheral blood leukocytes of the patient, her parents, and her grandparents. Among the candidate variant genes, *TNFAIP3* was notable, as it had been previously reported to be associated with monogenic IBD. We could not detect any homozygous *IL10* and *IL10R* variants suspected as pathogenic in the present patient. The patient was homozygous (A/A) for *TNFAIP3* (c.305A > G; NM\_001270507.2). This variant was also observed in the heterozygous state (A/G) in her mother (II-2) and is relatively common (MAF = 0.0117) in the Japanese population<sup>6</sup>. However, her father (II-1) was homozygous for the reference allele (G/G) (Fig. 1D), suggesting that the patient had a deletion in this region that includes *TNFAIP3*. Subsequent CNV analysis conducted with WES data using EXCAVATOR2<sup>7</sup> revealed that the patient had only a single de novo 187 kb microdeletion within the *TNFAIP3* gene (Supplementary Fig. S1). Genome-wide single-nucleotide polymorphism array analysis of the patient and her father using the Infinium Asian Screening Array-24 v1.0 BeadChip Kit (Illumina, San Diego, CA, USA) validated that the patient had a de novo 119 kb microdeletion involving only *TNFAIP3* (arr[GRCh37]6q23.3(138125829\_138244816) × 1, accession number SCV001424911 in ClinVar) (Fig. 1E). Both WES and CNV analyses indicated that the patient had lost the paternal allele of the *TNFAIP3* gene, leading to HA20.

We detected a novel de novo microdeletion in the *TNFAIP3* gene region of a patient with infantile-onset IBD complicated by a severe perianal lesion. The present case suggests that HA20 caused by *TNFAIP3* heterozygous microdeletion should be considered when determining the cause of infantile-onset IBD with perianal lesions.

The majority of prior studies on VEO-IBD associated with HA20 have reported point mutations<sup>3</sup>. However, including this case, recent studies have revealed several *TNFAIP3* deletions in patients with HA20, and these are summarized in Table 1<sup>8–10</sup>. Based on a comprehensive case review<sup>3</sup>, nine other reports<sup>8–16</sup>, and the present study, five of 69 (7.2%) patients diagnosed with HA20 carried a deletion predicted to result in HA20. As approximately 7% of HA20 cases appear to be caused by deletions in *TNFAIP3*, we propose that CNV analysis should be included in the genetic testing for monogenic IBD, especially when suspecting HA20. However, Shimizu et al. reported a case possessing a very small deletion of only 5 kb in *TNFAIP3*; therefore, it is necessary to develop a smaller CNV detection algorithm using WES data to detect *TNFAIP3* microdeletion.

For her infantile-onset perianal lesion, seton drainages were required for 1 year after their placement. Although infantile-onset IBD with perianal lesions is a well-known manifestation of monogenic IBD, particularly in patients with IL10 signaling defects<sup>4</sup>, this patient did not have any homozygous *IL10* and *IL10R* mutations despite low serum IL10 levels. Some HA20 cases show perianal lesions, but their severity is variable, and cases with severe perianal fistula, as in this case, are very rare. As VEO-IBD cases undergoing WES are increasing<sup>8,12–15</sup>, more cases including the deletion type will be accumulated in the future. It is also expected that the phenotype-genotype correlation will be elucidated. Overall, HA20 should be included as a differential diagnosis for infantile-onset IBD with perianal lesions, and CNV analysis in addition to WES should be considered to evaluate the *TNFAIP3* deletion.

In conclusion, patients with HA20 caused by *TNFAIP3* microdeletions can present infantile-onset IBD with severe perianal lesions. New genomic technologies to analyze microdeletions should be used to further investigate previously undiagnosed cases of monogenic IBD.

#### HGV database

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <https://doi.org/10.6084/m9.figshare.hgv.2903>.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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