

Predominantly genital-limited phenotype of paediatric haploinsufficiency of A20

Sirs,

We report a girl with haploinsufficiency of A20 (HA20) presenting with predominantly genital-limited recurrent ulcers associated with a novel *TNFAIP3* nonsense variant and impaired NF- κ B regulation. HA20 (OMIM #616744) is a monogenic autoinflammatory disease caused by heterozygous *TNFAIP3* variants and characterised by dysregulated NF- κ B signalling (1-4). Although recurrent oral and genital ulcers, fever and gastrointestinal inflammation are common manifestations, organ-limited paediatric presentations remain poorly recognised (5, 6).

A Japanese girl presented at 12 years of age with recurrent genital ulcers accompanied by only one febrile episode without other systemic manifestations. Herpes simplex virus infection was excluded by cytology and negative cultures. Despite repeated antiviral therapy, the lesions persisted, prompting referral for further evaluation. At presentation, physical examination revealed vulvar vesicular lesions (Fig. 1A) without oral ulcers, ocular involvement, or neurological abnormalities. The leukocyte count was $7.41 \times 10^9/L$, and the erythrocyte sedimentation rate was 14 mm/h (reference range [rr], 0–15 mm/h). C-reactive protein was mildly elevated at 0.80 mg/dL (rr, <0.30 mg/dL). Ophthalmologic examination, chest radiography, and brain magnetic resonance imaging revealed no abnormalities. Because of the family history of periodic fever and autoimmune disease (Fig. 1B), a monogenic autoinflammatory disorder was suspected.

Gene panel testing for hereditary autoinflammatory diseases (K010-14_v13, Kazusa DNA Research Institute), targeting *ADA2*, *NLR4*, *TNFAIP3*, *MEFV*, *TNFRSF1A*, *NLRP3*, *NLRP12*, *MVK*, *PLCG2*, and *NOD2*, identified a heterozygous nonsense variant in *TNFAIP3* (NM_006290.4:c.491G>A, p.Trp164Ter), which was absent from population databases including gnomAD. NF- κ B reporter assays using A20-deficient HEK293 cells (7) demonstrated impaired suppression of TNF-induced NF- κ B activation by the p.Trp164Ter variant compared with wild-type A20 (Fig. 1C). Results were derived from three independent experiments with technical triplicates. Formal statistical comparisons were not performed, given the descriptive nature of this functional validation. According to ACMG/AMP criteria, the variant was classified as pathogenic (PVS1, PS3, PM2). Based on these findings and the clinical phenotype, the patient was diagnosed with HA20.

Given the recurrent genital ulcers, treatment with oral prednisolone in combination with colchicine was initiated. Clinical

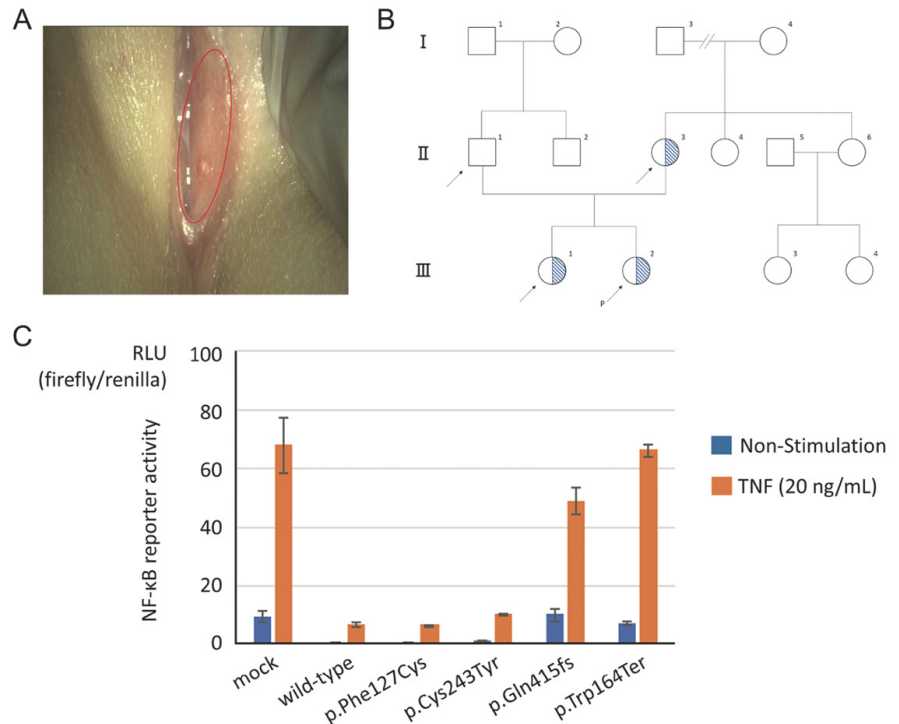


Fig. 1. Clinical features, family pedigree and NF- κ B reporter gene activity assay.

(A) Genital ulcers in the patient. Several small vesicles were observed on the vulva, particularly on the labia minora (red circle).

(B) Pedigree of the patient's family. The black arrow indicates the patient (p) and her family. Individuals with blue diagonal shading represent those with symptoms suggestive of autoimmune disease. II-4, the patient's mother, has had recurrent episodes of periodic fever and erythema since early childhood and experienced recurrent genital ulcers and oral aphthae. III-1, the patient's sister, has a history of periodic fever, IgA vasculitis, and cold urticaria.

(C) NF- κ B reporter activity was assessed using A20-deficient HEK293 cells transiently transfected with *TNFAIP3* expression constructs together with the NF- κ B reporter plasmid. Blue bars represent unstimulated conditions, and orange bars represent TNF-stimulated conditions (20 ng/mL). Compared with the *TNFAIP3* wild-type, the *TNFAIP3* p.Trp164Ter variant showed impaired suppression of TNF-induced NF- κ B reporter activity. Mock, pcDNA3.1 mock vector; wild-type, *TNFAIP3* wild-type; p.Phe127Cys, negative control (*TNFAIP3* single-nucleotide polymorphism); p.Cys243Tyr, positive control (HA20-associated missense variant); p.Gln415fs, positive control (HA20-associated frameshift variant).

cal improvement was observed following treatment with prednisolone and colchicine. Prednisolone was subsequently tapered, and no recurrence has been observed during colchicine monotherapy.

The differential diagnosis of recurrent vulvar ulceration in children includes Behçet disease, non-sexually acquired genital aphthous ulcers, infectious aetiologies such as herpes simplex virus infection, and other monogenic autoinflammatory disorders including *MEFV*-, *NLRP3*-, and *TNFRSF1A*-associated diseases (1, 2, 5, 6, 9). Genital-only presentations appear uncommon in paediatric HA20, with one cohort reporting such a phenotype in only 26.6% of cases (8). Moreover, non-sexually acquired genital ulcers are generally rare in paediatric practice (9). These findings suggest that monogenic autoinflammatory diseases including HA20 should be considered in children with such presentations, particularly when accompanied by a family history of immune dysregulation. However, clinical differentiation may be difficult, and genetic testing should be considered when a monogenic autoinflammatory disease is suspected.

The identified variant localises to the ovarian tumour domain of A20, which is critical for NF- κ B regulation (7). Functional analysis supported loss of A20-mediated NF- κ B suppression, consistent with previous reports showing that variants affecting A20 disrupt NF- κ B regulation and contribute to the broad clinical spectrum of HA20 (7). Therapeutic strategies for HA20 remain empirical. In the present case, clinical improvement was achieved with prednisolone and colchicine (1, 2), although interpretation is limited by the single-patient design and short follow-up period. Biologic agents and Janus kinase inhibitors are generally reserved for severe or refractory cases (2, 10). Accordingly, larger studies are needed to better define the clinical spectrum, treatment, and prognosis of paediatric HA20. Predominantly genital-limited recurrent ulcers may represent an initial manifestation of paediatric HA20. Early recognition and *TNFAIP3* testing may facilitate accurate diagnosis and management.

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