Different phenotypes caused by a *STAT3* variant in a Chinese pedigree

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Signal transducer and activator of transcription 3 (STAT3, OMIM #102582) is involved in the early development, proliferation, and differentiation of cells. Gain-of-function (GOF) variants in *STAT3* cause a higher binding force with DNA compared with the wild-type and lead to abnormal signal transduction of STAT1 and STAT5, developmental disorders of regulatory T cells, and promote the proliferation and activation of Th17 cells, which eventually result in autoimmune responses (1). Regarding clinical manifestations, germline GOF variants in *STAT3* cause infantile-onset multisystem autoimmune disease-1 (also known as ADMIO1, OMIM #615952), while somatic GOF variants have been reported in 27% - 72% of a large granular lymphocytic leukaemia (LGLL) patient cohort (1-4). ADMIO1 is characterised by a spectrum of autoimmune disorders that affect multiple organs (5) along with inflammatory bowel disease, neonatal diabetes, primary hypothyroidism and autoimmune interstitial lung disease and other features, such as fever, skin lesions, arthritis, and recurrent infections caused by hypogammaglobulinaemia. Here, we report a patient and her father who carried the same *STAT3* variant (c.454C>T; p.R152W) but had distinct phenotypes.

A 17-year-old girl was referred to our medical centre and received regular follow-up visit. She has suffered from monthly periodic fever and rash since the 40th day after birth. The frequency of fever was remarkably reduced after 8 years of age but relapsed after 16 years of age, when the maximum body temperature reached 38°C. Non-steroidal anti-inflammatory drugs were used to maintain normal temperature. In addition to fever, she had a recurrent runny nose, itchy throat, and irritating dry cough since childhood. Another major symptom was atopic dermatitis, which occurred at the ages of 5 months, 8 years and 14 years. The specific appearance included red papules on the trunk, limbs, and scalp, which was accompanied by itching and purulent appearance after scratching and desquamation (Fig. 1A). Dark red patches covered with greasy scales or scabs were found on the body by dermatoscope. Pain in multiple joints has occurred since 15 years of age, and it was accompanied by swelling of the bilateral knees and ankles, left elbow, and joints of the hands and toes. B-scan ultrasonography showed joint effusion and synovial hyperplasia. Bone marrow oedema was revealed in hip joint and knee joint by magnetic resonance imaging (Fig. 1B-C). Swollen submandibular lymph nodes were found by ultrasound scan. Mild splenomegaly was detected through positron emission tomography.

Test results of purified protein derivative skin test and T-SPOT.*TB* test were negative. Dermoscopy examination did not find any bacterial or fungal infections. Skin biopsies were not performed because of the proband's refuse. No abnormality was detected in blood culture test, Epstein-Barr virus DNA test, cytomegalovirus DNA test and chest computed tomography scan. These results excluded the possibility of infection. The complete blood count, basic metabolic panel and urinalysis were normal. The erythrocyte sedimentation rate and C-reactive protein were elevated, while autoantibody tests, including antinuclear antibodies, anti-neutrophil cytoplasmic antibodies, and anti-cyclic citrullinated peptide, were negative. The serum IgA level slightly decreased, while the serum levels of IgG, IgM, IgE, and complement were normal. Bone marrow examination found no evidence of haematological diseases. She did not have dental or endocrine system symptoms, and her growth was not affected.

The proband's father had suffered from seborrheic dermatitis, polyarthralgia and low back pain since young. Strikingly, he developed a high fever and severe anemia only one week before the proband was transferred and was then diagnosed with LGLL by bone marrow examination. The proband's mother had no symptoms. Complete medical records, including the pedigree and disease histories of the kindred, were collected and documented. Genomic DNA was collected from both peripheral blood and cheek swabs of the proband and her parents, and whole-exome sequencing (WES) was performed with

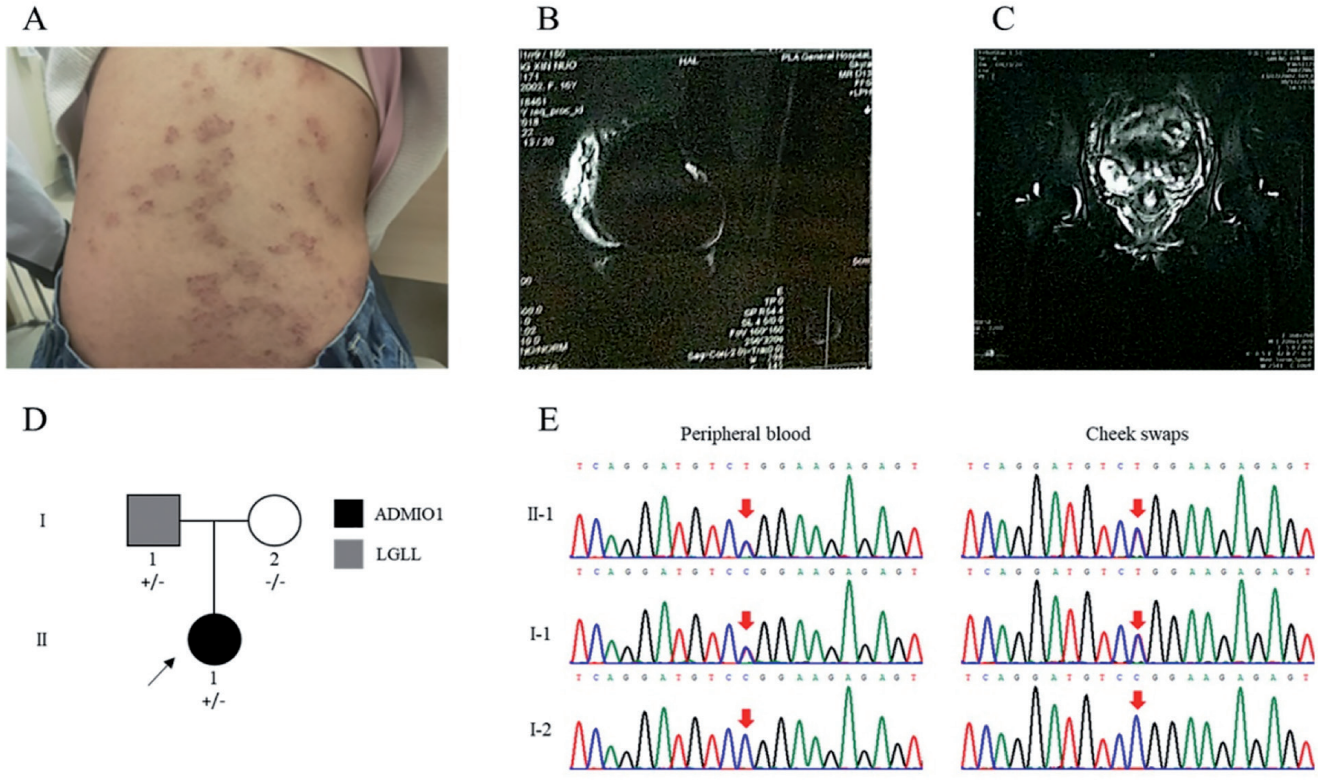

Fig. 1. **A**: Red papules on the trunk of the proband. **B**-**C**: Bone marrow oedema in knee joint (B) and hip joint (C) of the proband. **D**: Pedigree of the proband's family. Black arrow indicates the proband; square mark and circle mark indicate male and female subjects; plus symbol and minus symbol indicate whether subjects carry the c.454C>T variant in *STAT3*. **E**. Sanger sequencing diagram of the *STAT3* variant in the proband and her parents. The red arrow represents the c.454C>T variant in *STAT3*.

DNA extracted from peripheral blood. This research was approved by the Institutional Review Board of Peking Union Medical College Hospital and performed according to the Declaration of Helsinki. Informed consent was obtained from the participants.

WES identified a *STAT3* variant (NM_139276.3: c.454C>T; p.R152W) in exon 5 in both the proband and her father (Fig. 1D). Sanger sequencing with DNA from both peripheral blood and cheek swaps indicated existence of the *STAT3* variant between the proband and her father (Fig. 1E). This variant was reported to be the genetic cause of ADMIO1 (1). Frequency of the *STAT3* variant was not reported in the gnomAD and 1000 Genomes databases. In addition, it is predicted to be "probably damaging" or "damaging" by several *in silico* analysis tools, including SIFT, Polyphen2, CADD, and REVEL, and was categorised as pathogenic in ClinVar. Based on the clinical manifestations, laboratory results and gene testing results, the proband was diagnosed with ADMIO1. The proband was given 0.5 mg/kg/d prednisone along with sulfasalazine (SASP), methotrexate and leflunomide. Her fever, dermatitis, and arthritis initially improved, although the symptoms recurred after prednisone was tapered off. SASP was then replaced by tofacitinib. And after 6 months of treatment, her symptoms and inflammatory markers were relieved, and the maintenance dose of prednisone was set at 10 mg per day.

ADMIO1 is caused by heterozygous variants in *STAT3* and mainly manifests as lymphoproliferation and early-onset multisystem autoimmunity. Our patient presented with lymphadenopathy, hepatosplenomegaly, recurrent fever, polyarthritis, dermatitis, low IgA levels, and ruled out main causes of infection, which were consistent with the features of ADMIO1. Furthermore, Milner *et al*. (1) reported the same *STAT3* c.454C>T variant that caused ADMIO1 in a 25-year-old male patient; however, the clinical manifestations were not identical and the patient of their case mainly presented as autoimmune haemolytic anaemia, autoimmune thrombocytopenia, insulin-dependent diabetes mellitus, alopecia, lung nodules, lymphadenopathy, and hepatosplenomegaly. Our cases expanded the understanding of correlation between ADMIO1 clinical features and the *STAT3* variant.

More importantly, in our study, the proband's father, who carried the same *STAT3* variant, was diagnosed with LGLL, a different phenotype compared with the proband's. According to the report of Jefferson *et al*. (5), a germline *STAT3* variant (c.1975A>C; p.I659L) was found in an ADMIO1 patient while a somatic variant at the same position (c.1975A>C; p.I659L) has been reported to cause LGLL. In several studies, GOF somatic variants in *STAT3* were associated with haematological tumours, such as aplastic anemia, myelodysplastic syndrome and LGLL, and patients sometimes presented repeated infections and mild spleen enlargement (3, 5-7). However, c.454C>T variant was never reported among LGLL patients. In our case, the proband's father was diagnosed with LGLL and exhibited seborrheic dermatitis, joint pain, and low back pain. Thus, we collected DNA samples of the trio using both cheek swab and peripheral blood specimens and verified the variant by Sanger sequencing. According to the sequencing results, the variant was found in at least two germ layers (ectoderm and mesoderm), and the ratio of the wild type (C) to mutant type (T) was almost 1:1; based on this result, there was no evidence supporting the possibility of somatic variants in the proband's father for now. Taken together, although LGLL is usually caused by somatic variants in *STAT3* and AMOD1 is usually caused by germline variants, our cases indicated that other factors are required to decide the phenotype caused by *STAT3* GOF variants.

The prognosis of ADMIO1 is poorly understood. However, early treatment is necessary to slow the progression of the disease, especially among pediatric patients. Precise therapeutic guidelines for ADMIO1 are currently absent, although the use of anti-IL-6 receptors and JAK inhibitors is promising. Th17 cell differentiation is mediated by the interleukin (IL) 6-STAT3 axis (7). In the study of Milner *et al*. (1), the anti-IL6R monoclonal antibody tocilizumab was used to treat an ADMIO1 patient. After the treatment, the patient exhibited remarkable relief of previously fixed flexion contractures of his distal interphalangeal joints. In our study, the symptoms and inflammatory markers of the proband were well improved after treatment with tofacitinib.

In conclusion, although the proband and her father shared the same variant in *STAT3*, they presented different clinical manifestations, which suggests the complexity of *STAT3* variants. This study expands the phenotype spectrum of ADMIO1, explores the effect of tofacitinib in treatment, and emphasises the need for and potential usefulness of genetic testing in differential diagnosis.

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References

1. MILNER JD, VOGEL TP, FORBES L *et al*.: Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood* 2015; 125(4): 591-9. https://doi.org/10.1182/blood-2014-09-602763
2. HAAPANIEMI EM, KAUSTIO M, RAJALA HL *et al*.: Autoimmunity, hypogammaglobulinemia, lymphoproliferation, and mycobacterial disease in patients with activating mutations in STAT3. *Blood* 2015; 125(4): 639-48. https://doi.org/10.1182/blood-2014-04-570101
3. FLANAGAN SE, HAAPANIEMI E, RUSSELL MA *et al*.: Activating germline mutations in STAT3 cause early-onset multi-organ autoimmune disease. *Nat Genet* 2014; 46(8) :812-4. https://doi.org/10.1038/ng.3040
4. MOOSIC KB, PAILA U, OLSON KC *et al*.: Genomics of LGL leukemia and select other rare leukemia/lymphomas. *Best Pract Res Clin Haematol* 2019; 32(3): 196-206. https://doi.org/10.1016/j.beha.2019.06.003
5. TERRY J, LANGLOIS S, RUPPS R, GILL H: Prenatal autoimmune disease, multisystem, infantile onset-like phenotype and proximal renal tubular dysplasia associated with STAT3 mutation. *Pediatr Dev Pathol* 2020; 23(4): 306-11. https://doi.org/10.1177/1093526619890734
6. LOBBES H, DERVOUT C, TOUSSIROT E *et al*.: Rituximab for rheumatoid arthritis-associated large granular lymphocytic leukemia, a retrospective case series. *Semin Arthritis Rheum* 2020; 50(5): 1109-13. https://doi.org/10.1016/j.semarthrit.2020.05.020
7. O'SHEA JJ, PLENGE R: JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. *Immunity* 2012; 36(4): 542-50. https://doi.org/10.1016/j.immuni.2012.03.014