Analysis of the Clinical and Genetic Characteristics of 3 Cases of A20 Haploinsufficiency

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Research article

Keywords: A20 haploinsufficiency, Behçet-like syndrome, Tumor necrosis factor antagonists

Posted Date: September 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-81339/v1>

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Abstract

Background To evaluate the clinical and genetic characteristics of 3 children with HA20. **Methods** The clinical and genetic testing data of 3 children with HA20 treated at CIP between August 2016 and October 2019 were retrospectively analysed. **Results** Patient 1 presented with arthritis and inflammatory bowel disease, patient 2 presented with axial spinal arthritis and lupus-like syndrome, and patient 3 presented with recurrent oral ulcers, gastrointestinal ulcers, and perianal abscesses. For laboratory examination, In patient 1, the WBC,CRP and AESR showed high. In patient 2, the WBC was normal, but CRP and AESR showed high. In patient 3, the WBC, CRP, and AESR showed high. Patient 2 was positive for antinuclear antibodies, anti-Sjögren's syndrome antigen A, dsDNA, rheumatoid factor and Coombs test. Genetic testing showed that the 3 patients were heterozygous for mutations in the TNFAIP3 gene. For treatment, patient 1 used TNF α antagonist and thalidomide, patient 2 used TNF α antagonist and sulfasalazine, and patient 3 used hormones and thalidomide. Patients 1 and 2 were followed up for 4 and 3 months, respectively. There was improvement in joint and gastrointestinal symptoms, inflammatory indices and RF were normal, dsDNA and Coombs test became negative. Patient 3 was treated at an outside hospital and showed gradual improvement in oral ulcers and perianal abscesses. **Conclusion** HA20 is a single-gene auto-inflammatory disease caused by mutation in the TNFAIP3 gene. It presents clinically as a Behçet-like syndrome and can present as various other autoimmune diseases as well. Hormones and immunosuppressive agents are effective treatments, and cytokine antagonists can be used in refractory cases. Whole-exome genetic testing should be proactively performed for children with an early age of onset or Behçet-like presentation to achieve early diagnosis and accurate treatment.

Background

A20 haploinsufficiency is an autosomal dominant hereditary disease caused by a pathological mutation in the tumour necrosis factor (TNF)- α -induced protein 3 gene. Production of the nuclear factor (NF)- κ B regulatory protein A20 encoded by the TNFAIP3 gene is insufficient, and the hallmark characteristics are similar to those of Behçet's disease (3, 4). However, these characteristics appear earlier in the former, and the age of disease onset is before the age of 10 years in most patients, including recurrent appearance of painful oral, genital, and/or gastrointestinal ulcers. With the extensive use of whole-exome genetic testing in recent years, clinical reports of this disease have gradually increased. In most reports, the clinical presentation of this disease is still primarily a Behçet-like presentation, and there are relatively few reports of complication with other autoimmune diseases such as lupus-like syndrome and autoimmune cytopenia. Hormones and immunosuppressive agents are the primary treatments, and treatment with TNF- α antagonists or other biologics has not been widely reported. The three patients of HA20 recently diagnosed at our hospital had early ages of onset. In addition to presentation with Behcet's disease, one patient presented with lupus-like syndrome and one patient presented with Crohn's disease. Two of the three patients were treated with biologics, which showed significant clinical efficacy and few side effects. The clinical and genetic testing results of the three patients were systematically analysed to improve the clinical understanding of the diagnosis and treatment of this disease.

Methods

Clinical data. The clinical characteristics of 3 children (1 male, 2 female) with HA20 hospitalized and diagnosed at the Affiliated Children's Hospital of Capital Institute of Pediatrics between August 2016 and October 2019 were retrospectively analysed. Medical histories were obtained, including age of onset, time to diagnosis, medical history, physical examination, laboratory tests, electronic endoscopy, and whole-exome genetic testing. Primary observational indices were as follows: (1) Clinical presentation: fever, oral ulcers, genital ulcers, abdominal pain/diarrhoea, skin lesions, eye lesions, arthritis, kidney involvement; (2) Laboratory tests: conventional blood tests, C-reactive protein (CRP), dynamic erythrocyte sedimentation, conventional urine tests, antinuclear antibody (ANA) panel; (3) Arthrography and electronic endoscopy; and (4) Whole-exome genetic testing.

Gene trapping high-throughput sequencing

1. Methods

After obtaining consent from the child's guardian, approximately 2 mL (EDTA anticoagulant) of peripheral blood was collected from each child and the parents, and genomic DNA was extracted using the QIAamp whole blood DNA extraction kit (Qiagen, Hilden, Germany). An exon capture kit (probe synthesised by Twist Bioscience, San Francisco, CA, USA) was used. The Illumina NovaSeq 6000 second-generation sequencer (San Diego, CA, USA) was used to capture and sequence the coding exon regions of the sample gene. BWA software was used to compare the sequences with a reference genome (version hg19), and statistical data analysis of sequencing depth, uniformity, probe specificity, and other data was performed. GATK software was used to detect polymorphic sites from the comparison data of each sample, and statistical analysis of single-nucleotide polymorphism (SNP) and indel mutation data was performed. The SNPs and indels obtained from screening had a frequency of less than 0.05 in the 1000 Genomes Project, ESP6500si, ExAC_ALL, ExAC_EAS, and the myGenostics 1000 normal individual databases. In addition, the results predicted from the SIFT, PolyPhen2, MutationTaster, GERP++, and other databases were pathogenic loci that served as disease-related candidate loci.

2. Results

The following mutations were detected in the TNFAIP3 gene of the 3 patients:

The sites of gene mutation in TNFAIP3 in the 3 patients were c.1906 + 1G>C heterozygous mutation, c.925_927dup heterozygous mutation, and c.547C>T heterozygous mutation. These mutations do not appear in the 1000 Genomes Project, ESP6500si, ExAC_ALL, ExAC_EAS, or myGenostics 1000 normal individual databases. Among these mutations, c.1906 + 1G>C and c.547C>T were predicted by GERP++ to be loci in a conserved region. c.1906 + 1G>C and c.925_927dup were confirmed by Sanger sequencing as spontaneous mutations. The c.547C>T mutation in one of the patients was confirmed by Sanger sequencing as having been inherited from the mother.

As shown in Figure 1, the c.547C>T locus falls within the ovarian tumour (OTU) domain of OTU-like cysteine proteases. This family consists of a group of predicted cysteine proteases homologous to the OTU gene in *Drosophila*, and its members include proteins from eukaryotes, viruses, and pathogenic bacteria. Conserved cysteine, histidine, and possibly aspartic acid residues represent catalytic residues in this putative protease family. These mutations may affect the function of the protein domain. Among them, 1906 + 1G>C may affect the alternative splicing of exons, thereby affecting protein function.

The TNFAIP3 gene has an autosomal dominant mode of inheritance. Based on these results, the 3 patients showed the possibility of pathogenicity at the genetic level.

Results

Table 1 summarizes the clinical presentations of the 3 patients. Table 2 summarizes the laboratory test data.

Patient 1: A girl aged 3 years and 2 months was hospitalized with "swelling and pain in multiple joints for 6 months and intermittent fever with diarrhoea for over 4 months" as the chief complaint. At 6 months before admission, the patient developed swelling and pain in the right ankle with limited mobility without any obvious cause. There was no fever, vomiting, abdominal pain, rash, or eye discomfort, and no treatment was administered. At over 4 months before admission, the patient developed intermittent fever accompanied by diarrhoea. There was no abdominal pain or bloody stool, and joint swelling and pain was as observed previously. Conventional blood tests at the local hospital showed that the WBC was $15.8 \times 10^9/L$, classified primarily as neutrophils, haemoglobin was 106 g/L, CRP was 42.7 mg/L, and dynamic erythrocyte sedimentation rate (AESR) was 50 mm/60 min. Magnetic resonance imaging (MRI) of the joints indicated synovial thickening, and "infectious arthritis" was considered. There was no improvement with anti-infective treatment, and "synovectomy and joint irrigation" was then performed. After operation, the patient still had intermittent fever with diarrhoea, the same stool characteristics as previously, and bilateral knee swelling, pain, and limited mobility. Physical examination after hospitalization and treatment at our department revealed a height of 91 cm, weight of 11.5 kg, fever with flushed appearance, swelling of the fourth proximal interphalangeal joint of the right hand of 4 cm in circumference with mild tenderness and no limited mobility, swelling of bilateral knees of 21 cm in circumference, positive floating patella test, limited flexion, swelling of the right ankle without

tenderness, plantar flexion of 5°, and dorsal extension of 5°. After admission, conventional blood test showed that the WBC was $34.06 \times 10^9/L$, CRP was 150 mg/L, and AESR of 47 mm/60 min. Tests for tuberculosis, fungal infection, parasite infection, Epstein-Barr virus, and other pathogens were negative. The antinuclear antibody (ANA) panel was negative. For humoral immunity, IgA was less than 0.0647 g/L, and IgG and IgM were normal. Cellular immunity was generally normal. Enhanced computed tomography of the thorax and abdomen were negative. Because of the presentation of fever and diarrhoea during the course of the disease, the height and weight of the patient were in the 3rd percentile for the same sex and age, suggesting growth delay. Endoscopic examination indicated multiple colorectal ulcers, and subsequent whole-exome genetic testing indicated the new mutation c.925-927dup (p. Val309dup) in the TNFAIP3 gene (see figure 2 for a map of gene mutations), suggesting a diagnosis of A20 haploinsufficiency. The patient was administered oral thalidomide and two treatments of 6 mg/kg infliximab, and then switched to 20 mg adalimumab subcutaneously every two weeks because of allergy. Currently, the patient has been treated with adalimumab for 3 months, the fever and joint symptoms improved significantly, joint swelling and pain have subsided, conventional blood tests and inflammatory indicators were generally normal, and endoscopic re-examination indicated complete healing of ulcers.

Patient 2: A girl aged 11 years and 7 months was hospitalized with "recurrent low back pain for over two years" as the chief complaint. Over 2 years before admission, the parents of the patient discovered that the child had thoracic kyphosis and recurrent oral ulcers; no special treatment was administered. Later, the patient developed low back pain and an inability to bend downward; there was no fever, vomiting, abdominal pain, rash, joint swelling, eye discomfort, or other presentations. Rheumatoid factor (RF) examination at the local hospital showed a value of 37.5 IU/mL, positivity for HLA-B27, normal WBC, CRP of 42.7 mg/L, and AESR 50 mm/60 min, and the patient was treated at our hospital. Physical examination after hospitalization and treatment at our department revealed a height of 105 cm, weight of 18.4 kg, developmental delay, thin frame, scoliosis, thoracic kyphosis, positive spinous process tenderness, positive Schober's test, and bilateral positive number 4 sign. After admission, conventional blood tests showed a normal WBC, Hb of 100 g/L, CRP of 73 mg/L, and AESR of 87 mm/60 min. The ANA panel showed a ratio of 1:100, anti-Sjögren's syndrome antigen A (SSA)++, and RO 52+++. The Coombs test was positive, and cellular and humoral immunity were generally normal. Conventional urine tests and 24-h urinary protein were normal. Complement was normal. Enhanced sacroiliac MRI indicated inflammatory changes. The patient had a history of recurrent oral ulcers and alopecia recently became more obvious, and systemic lupus erythematosus was strongly suspected. Renal biopsy was not performed. Juvenile idiopathic arthritis was diagnosed, and the patient was administered oral sulfasalazine treatment, after which the low back pain gradually subsided. At 8 months before admission, low back pain developed again, and examination showed an ANA of 1:1000, SSA+++, and positive double-stranded DNA (dsDNA; enzyme-linked immunosorbent assay). Enhanced sacroiliac MRI re-examination suggested exacerbation of the symptoms. Taken together with the presentation of autoimmune haemolytic anaemia and arthritis, a diagnosis of systemic lupus erythematosus was considered in accordance with the 1997 ACR standard. Oral methylprednisolone, hydroxychloroquine, and sulfasalazine were administered for 6 months, and the patient discontinued all medication. Over 2 months before admission, the low back pain of the patient was again aggravated. Enhanced MRI re-examination of the thoracic and lumbar spine at our hospital revealed extensive small pyramidal arthritic changes. Enhanced MRI of the sacroiliac joint revealed re-aggravation of lesions, and the patient was treated with sulfasalazine and infliximab; the joint symptoms subsided significantly. Taken together with the delayed growth in height, with a height of only 130 cm at age 11 years, which is below the 3rd percentile for the same sex and age, a diagnosis of dwarfism was considered. Whole-exome genetic testing indicated a new mutation in TNFAIP3 at c.1906 + 1G>C (see figure 3 for a map of gene mutations), and A20 haploinsufficiency was diagnosed. Infliximab (6 mg/kg) was administered 3 times. The patient did not report back pain, and CRP and AESR were normal at re-examination.

Patient 3: A boy aged 4 years and 5 months was hospitalized with "intermittent fever with perianal abscesses for over 3 years and intermittent fever for 2 years" as the chief complaint. At over 3 years before admission, the patient developed perianal abscesses with fever with no obvious cause. There was no cough, vomiting, abdominal pain, rash, joint swelling and pain, eye discomfort, or other symptoms, and the patient was symptomatically treated with topical and intravenous antibiotics and intermittent hormone therapy at a local hospital, but the symptoms were recurrent after treatment. At 2 years before admission, the patient developed fever with no other accompanying symptoms. The patient was diagnosed with a bacterial infection at the local hospital and showed improvement after antibiotic and hormone therapy. The fever recurred at intervals ranging from 3

days to 2 months. At 6 months before admission, recurrent oral ulcers developed, with 4–5 episodes within six months. There was intermittent abdominal pain and no diarrhoea, blood in the stool, or other discomfort. Dynamic monitoring and conventional blood tests showed a WBC of $17 \times 10^9/L$, CRP of 105 mg/L, and AESR of 31 mm/60 min. The patient was diagnosed at our department with pre-existing pigment deposition in the perianal skin. The patient was negative for tuberculosis and fungal, parasitic, Epstein-Barr virus, and other infections. Antinuclear antibody and antineutrophil cytoplasmic antibody panels were negative. Neutrophil phagocytosis was normal. Whole-exome genetic testing found a c.547C>T (p.Arg183Ter) mutation in TNFAIP3 (see figure 4 for the gene mutation map). The mutation originated from the mother, who also had a history of recurrent oral and genital ulcers for over 20 years. The father did not carry the mutation, and thus a diagnosis of A20 haploinsufficiency was considered. Later, complete endoscopic examination was performed at the local hospital, which indicated ileocecal ulcers; hormone and thalidomide immunosuppressive therapy were added. There was occasional recurrence during hormone dose reduction, which presented primarily as periodic fever. Conventional blood tests during the episodes showed elevated WBC, CRP, and AESR. Currently, hormones have been discontinued, and the disease course is relatively stable.

Discussion

A20 haploinsufficiency (HA20) is an autosomal dominant genetic disease caused by mutation of the TNF- α -induced protein 3 (TNFAIP3) gene (5-7). The cause of this disease is insufficient production of A20 resulting in negative feedback inhibition of the NF- κ B signalling pathway, and it presents with the characteristics of autoinflammatory disease. Laboratory examination of this disease may reveal elevated CRP and AESR during onset as well as the presence of autoantibodies to varying degrees, thus presenting with characteristics of autoimmune disease. Colchicine treatment is effective in some patients, whereas immunosuppressive agents (including systemic glucocorticoids, slow-acting antirheumatic drugs, and cytokine inhibitors) alone or in combination are used in other patients.

NF- κ B is a family of dimeric proteins composed of Rel domains. When stimulated by various inflammatory factors (such as viruses, TNF, B-cell activating factor, lymphotoxin, and others), NF- κ B can induce the expression of various genes, resulting in the production of cytokines that participate in the inflammatory response. These cytokines are associated with inflammation, including chemokines, adhesion factors, and enzymes that produce secondary inflammatory mediators (such as cyclooxygenase and inducible nitric oxide synthase) and promote the inflammatory response process. In addition, some proteins induced by NF- κ B such as TNF- α can act on NF- κ B via negative feedback, thereby resulting in continuous production of inflammatory factors and aggravating the inflammatory response. Continuous activation of the NF- κ B signalling pathway and persistent inflammatory response can cause pathological changes in the body, such as systemic lupus erythematosus, rheumatoid arthritis, cancer, and other diseases associated with inflammation.

The A20 protein, encoded by the TNFAIP3 gene and known as TNF- α -inducing protein 3, was first discovered in 1990 by Dixit et al. in human umbilical vein endothelial cells treated with TNF- α (8-9). Later, A20 was found to be expressed in epithelial cells, dendritic cells, and T and B lymphocytes. A20 protein is a negative regulator of the NF- κ B signalling pathway and plays a fundamental role in a variety of physiological and pathological processes such as immunity, apoptosis, inflammation, and cancer (10). It regulates the immune response by preventing NF- κ B from overstimulation by various exogenous stimuli. The A20 protein consists of an N-terminal OTU structural domain and a C-terminal OTU domain containing 7 zinc finger domains. The deubiquitinating enzyme activity of the OTU domain can cause the receptor-interacting protein in the NF- κ B signalling pathway to undergo deubiquitination (11), inhibiting its degradation by proteases. The ubiquitinase enzyme activity of the repetitive zinc finger structure can cause ubiquitination and degradation of Lys8 in receptor-interacting protein. The deubiquitinating enzyme structural domain of A20 can antagonize ubiquitination upstream of a necessary regulator of NF- κ B (NEMO) by activating various mechanisms; this regulator is a regulatory subunit of the IKK kinase complex. The IKK complex has two catalytic subunits (IKK α and IKK β) and a subunit that regulates the NF- κ B pathway (NEMO/IKK γ). IKK regulates non-canonical pathways through IKK α . A20 ubiquitination of NEMO can down-regulate IKK activation by blocking IKK phosphorylation. IKK activation inhibits the phosphorylation of IKK α protein, resulting in its degradation and dissociates from

NF- κ B, and then translocation to the target gene for transcription. Therefore, loss of A20 leads to NF- κ B pathway activation. Mutations in either the N- or C-terminus of the TNFAIP3 gene result in loss of A20 function.

HA20 is a novel single-gene auto-inflammatory disease first reported in 2016 by Zhou et al. (12), who found that its primary phenotype is Behçet's disease-like symptoms (13), including recurrent oral ulcers, genital ulcers, and gastrointestinal symptoms (14). However, numerous studies have shown that some patients exhibit not only the characteristics of auto-inflammatory diseases, but also those of various autoimmune diseases. In genome-wide association studies, TNFAIP3 polymorphisms were found to be associated with a variety of autoimmune (15) and autoinflammatory diseases (16), such as systemic lupus erythematosus (17), Sjögren's syndrome, Crohn's disease, rheumatoid arthritis, Still's disease in adults, juvenile idiopathic arthritis, psoriatic arthritis, Hashimoto's thyroiditis (18), autoimmune lymphoproliferative syndrome (19), type 1 diabetes, and psoriasis. To date, 24 pathogenic variants of A20 have been reported. Significant clinical heterogeneity exists even in patients with the same variant of TNFAIP3 (20). Therefore, HA20 may have unexpected clinical phenotypes. No cases of HA20 have been reported in China. A search of the PubMed database with the search terms "A20 haploinsufficiency" and "TNFAIP3" (as of October 2019) yielded a total of 24 relevant articles involving 35 patients in 14 families, of which 12 patients were male and 23 patients were female; the male/female ratio was 1:2 and the average age of onset was 6.3 years. Of the 35 patients, 18 had fever, 17 had oral ulcers, 18 had genital ulcers, 5 had lymphadenitis, 12 had gastrointestinal symptoms such as abdominal pain and diarrhoea, 5 had gastrointestinal ulcers, 2 had perianal abscesses, 15 had arthritis, 8 had skin lesions (of which there was 1 case of vitiligo), 7 had Hashimoto's thyroiditis, and 3 had renal involvement (haematuria, proteinuria). There was only 1 case of ocular lesions, 1 case of malignant cancer, 1 case of deep vein thrombosis with arterial inflammation, and 1 case of Raynaud's phenomenon. One case presented with systemic juvenile idiopathic arthritis with macrophage activation syndrome and interstitial pneumonia (presenting as fever, abnormal liver function, hypertriglyceridemia, hypofibrinogenemia, and significant ferritin elevation). One case presented with juvenile systemic lupus erythematosus and lupus nephritis. In conventional blood tests, all 24 patients had elevated WBCs, CRP, and AESR, of which 2 were positive for ANAs, 1 was positive for SSA, 1 was positive for dsDNA, 1 had complement deficiency, 2 were positive for HLA-B27, and 1 had lupus nephritis revealed by renal biopsy, all of which were consistent with the characteristics of the HA20 clinical phenotype. Mouse studies have shown that the disease phenotype may depend on the major cell types affected. Mice homozygous for A20 loss can develop early-onset auto-inflammatory diseases. Mice with bone marrow cells lacking A20 develop polyarthritis similar to rheumatoid arthritis in humans, whereas intestinal cell-specific A20 deficiency can result in intestinal inflammation in mice (21). Cell-specific loss of A20 in B cells and dendritic cells also results in a corresponding clinical phenotype with the production of autoantibodies. In addition, somatic deletion and biallelic mutations of TNFAIP3 have been found in B-cell lymphoma, suggesting that A20 plays a role in suppressing cancer. Therefore, factors such as genetics and the environment may play a determinative role in the disease phenotype. In addition, the heterogeneity of HA20 also appeared in the same family, with clinical manifestations ranging from periodic fever-like disease and Behçet's disease to rheumatoid arthritis.

In this group of 3 patients, patient 1 presented primarily with arthritis and inflammatory bowel disease, patient 2 presented primarily with severe spinal arthropathy and lupus-like syndrome, and patient 3 presented primarily with Behçet-like syndrome. Patients 1 and 3 had recurrent fever that tended to resolve on its own without any fixed cycle, and conventional blood tests during febrile periods showed elevated WBC and inflammation indicators, exhibiting the characteristics of auto-inflammatory diseases. Patient 2 had no fever or gastrointestinal symptoms but was positive ANA, SSA, dsDNA, and Coombs test, and exhibited severe spinal arthritis and lupus-like syndrome, which are characteristic of autoimmune diseases. The 3 HA20 patients in this group also showed diverse clinical phenotypes, in agreement with literature reports. The heterozygous mutation in patient 3 in this group originated from the patient's mother, who had a history of recurrent oral and genital ulcers since childhood, consistent with the characteristics of onset of familial HA20. Both patients 1 and 2 had varying degrees of delayed growth, which was considered as associated with long-term inflammation and diarrhoea in patient 1 and long-term inflammation and limited growth due to severe spinal arthritic joint fusion in patient 2. Because this group of patients with HA20 had such heterogeneous clinical presentations, genetic screening of patients based on clinical characteristics alone is unlikely to be sensitive enough to detect all relevant cases. Whole-exome/genomic sequencing is becoming more widely used for testing. Therefore, potentially pathogenic heterozygous mutations of TNFAIP3 may also be found in patients with no apparent HA20 phenotype.

There is no standard treatment for patients with HA20. Generally, treatment methods are based on the patient's dominant clinical phenotype. The literature has reported that most patients respond to glucocorticoids, but the treatment cycle is often long-term. Interestingly, colchicine, which is traditionally used to treat familial Mediterranean fever, appears to have some effect on HA20, but has little effect in the treatment of other hereditary fever syndromes (22). Slow-acting antirheumatic drugs such as methotrexate, cyclosporine, hydroxychloroquine, and mycophenolate mofetil have been reported in the literature, but most patients require a combination of glucocorticoids to control their symptoms. Because HA20 patients exhibit excessive production of pro-inflammatory cytokines, biologics targeting cytokines are commonly used as second-line treatments, and primarily include anti-TNF- α (infliximab, adalimumab), anti-IL-1 (anakinra, canakinumab), and anti-IL-6 (tocilizumab) (23), which can effectively inhibit systemic inflammatory responses in patients with HA20. Other immunotherapies, such as anti-CD20 monoclonal antibodies (rituximab) and JAK-1 and -3 inhibitors (tofacitinib) have also been reported. Hematopoietic stem cell transplantation can be considered for patients with severe refractory disease. A literature review of 24 cases showed 3 cases of effective treatment with colchicine, 1 case of ineffective treatment with colchicine and switching to tocilizumab, after which symptoms subsided, and 2 cases initially treated with hormonal immunosuppressive agents followed by administration of 25 mg/w etanercept by subcutaneous injection, which achieved disease control, and 4 cases treated with hormones and immunosuppressive agents for disease control. Among the 3 patients in this group, patient 1 experienced an allergic reaction after 2 treatments with infliximab and was switched to adalimumab. At the same time, thalidomide was administered to control gastrointestinal symptoms. Patient 2 was treated with infliximab and sulfasalazine. Two patients showed significant clinical effects after the first application of TNF- α inhibitor. The temperature of patient 1 decreased to normal, diarrhoea disappeared, and joint swelling and pain improved significantly. Low back pain and joint mobility in patient 2 improved significantly. Considering the early age of onset of the patient and serious side effects caused by hormones, hormones were not used in 2 of the patients. As of follow-up at 4 months and 3 months respectively, the joint swelling and pain have disappeared. Patient 1 occasionally developed fever and bloody stool, but the frequency of onset was significantly decreased. Most patients still required low-dose hormone therapy, in contrast to literature reports. Disease control using biologics and immunosuppressive agents in this group was satisfactory, but because of the small number of cases and severity of the disease, treatment responses may differ. Therefore, more cases of TNFAIP3 mutation should be accumulated to elucidate the pathophysiology and treatment strategies for HA20.

Conclusion

In summary, A20 haploinsufficiency (HA20) is caused by a germline mutation with a highly penetrant loss of TNFAIP3 function. The discovery of multiple nonsense mutations and frameshift mutations associated with impaired regulatory functions of TNFAIP3/A20 provides strong evidence that this is the pathogenic mutation. For patients with an early age of onset, whole-exome genetic testing is necessary regardless of whether they present with characteristics of auto-inflammatory or auto-immune disease. For known rheumatic immune diseases of traditional significance, continuous improvement and widespread application of genetic testing technology have led to new diagnostic and therapeutic options for rheumatic immune diseases caused by single-gene mutations. Only early diagnosis and accurate treatment can result in better outcomes for patients.

Abbreviations

HA20: A20 Haploinsufficiency

WBC: white blood cells

CRP : C-reactive protein

AESR: dynamic erythrocyte sedimentation

TNF: tumour necrosis factor

ANA: antinuclear antibody

OUT: ovarian tumour

RF: Rheumatoid factor

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication : Not applicable

Availability of data and materials : Not applicable

Competing interests : The authors declare that they have no competing interests

Funding : Not applicable

Authors' contributions :

GXS analyzed and interpreted the patient data. DZ collected data and was a major contributor in writing the manuscript. ZXZ and JML conceived of the study, and participated in its design and coordination.

All authors read and approved the final manuscript."

Acknowledgements Not applicable

Disclose: none

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Tables

Table 1

	patient1	patient2	patient3
Fever	+	-	+
Oral ulcer	-	+	+
Genital ulcer	-	-	-
Gastrointestinal elcer	+	-	+
rash	-	-	-
oculopathy	-	-	-
arthritis	+	+	-
Renal damage	-	+	-

table2

	WBC ×10 ⁹		CRP mg/L		AESR mm/60min		PCT	Routine urine test/24h proteinuria	ANA
	Prior treatment	Post treatment	Prior treatment	Post treatment	Prior treatment	Post treatment			
patient1	34.06	7.3	150	2.7	47	6	<0.5	-	-
patient2	5.61	3.68	73	0.75	87	8	<0.5	-	ANA 1:320 dsDNA positive
patient3	17	N	17.5	N	31	N	<0.5	-	-

Figures

Figure 1

Structural domains of the TNFAIP3 protein

A : map of gene mutations of Patient 1

B : map of gene mutations of Patient 1's father

C : map of gene mutations of Patient 1's mother

Figure 2

map of gene mutations of Patient map of gene mutations of Patient 1's father map of gene mutations of Patient 1's mother

A : map of gene mutations of Patient 2

B : map of gene mutations of Patient 2's father

C : map of gene mutations of Patient 2's mother

Figure 3

map of gene mutations of Patient 2 map of gene mutations of Patient 2's father map of gene mutations of Patient 2's mother

A : map of gene mutations of Patient 3

B : map of gene mutations of Patient 3's father

C : map of gene mutations of Patient 3's mother

Figure 4

map of gene mutations of Patient 3 map of gene mutations of Patient 3's father map of gene mutations of Patient 3's mother

Figure 5

CT: A, B: reconstruction of thoracic CT sagittal bone window, C: three-dimensional image showing extensive osseointegration of thoracic spine and disappearance of vertebral facet joint space. MRI: D, T1WI sequence of the sacroiliac joint showing that the bilateral sacroiliac joint space is widened, and the zygomatoc side articular surface is jagged. E, FSPD sequence showing multiple patchy high signals below the sacroiliac joint, suggesting bone marrow oedema with the right sacroiliac joint as the focus; F,G, T1WI sequence of enhanced fat suppression scans showing significant enhancement of the bilateral articular surfaces, indicating the presence of active inflammation.