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Wiskott-Aldrich syndrome: A new synonym mutation in the WAS gene

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SUMMARY Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive primary immunodeficiency disorder. Mutations in the WAS gene are considered to be the primary cause of WAS. In this work, we report a boy who presented with intracranial hemorrhage (ICH) as an initial symptom and detects a novel pathogenic synonymous mutation in his *WAS* gene. His mother was a carrier of the mutant gene. The mutation, located at position c.273 (c.273 G>A) in exon 2, is a synonym mutation and predicted to affect protein expression by disrupting gene splicing. This study summarizes the diagnosis and treatment process of the patient and expands the genetic spectrum of WAS.

Keywords Wiskott-Aldrich syndrome, synonymous mutation, newborn, hematopoietic stem cell transplantation, intracranial hemorrhage

Wiskott-Aldrich syndrome (WAS) is a rare X-linked recessive disorder which is characterized by thrombocytopenia, microplatelets, eczema, recurrent infections, and an increased risk of autoimmunity and malignance (*1,2*). This disease results from mutations in the *WAS* gene which is located on the short arm of the X chromosome (Xp11.22-p11.23) and contains 12 exons. A wide range of mutations in the *WAS* gene cause WAS protein (WASp) deficiency, leading to immune function defects (*3*). Depending on the type of WAS gene mutation, WAS can ultimately manifest as multiple clinical phenotypes, including classical WAS, X-linked thrombocytopenia (XLT), intermittent X-linked thrombocytopenia (IXLT), and X-linked neutropenia (XLN) (*4,5*).

Intracranial hemorrhage (ICH) is an important cause of neonatal morbidity and mortality (*6*). IHC can be particularly harmful in the neonatal period as this is a critical stage for brain development (*7,8*). Reported cases of WAS that start with ICH are rare. Here, a neonatal patient diagnosed as WAS with a novel gene mutation developed ICH and eventually died of ICH. The aim of this study was to enhance clinical practitioners' awareness of the rare disease onset in WAS patients and to further our understanding of the pathogenesis of WAS. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University School of Medicine. The informed consent has been obtained from the patient's guardian.

A 14-day-old neonatal boy was the first and only child of a Han nationality parents with full-term normal delivery. His mother had a history of thrombocytopenia during the pregnancy. Initially, he was admitted to other hospital because of intracranial hemorrhage, thrombocytopenia, anemia and prolonged activated partial thromboplastin time (APTT). After the surgical removal of intracranial hematoma was used to clear intracranial hematoma, he was transferred to our hospital with eczema. Physical examination showed that the newborn did not have hepatosplenomegaly and lymphadenopathy but had significant eczema on the face and chest. Some rash had ruptured and been infected just looked like the impetigo. The result of hematological examination revealed that the platelet count (PLT, 45.00 $\times 10^9$/L) and plateletcrit (0.04%) were significantly decreased and mean platelet volume (MPV, 7.20 fL) level was at the lower limit of normal complicated with mild anemia (Hb91g/L). Coagulation functions were normal.

Serum IgA, IgM levels were normal except for obvious increased IgE (1,110 IU/mL) and moderately increased IgG (17.50g/L). The percentages of peripheral blood lymphocyte subsets were as follows: CD3+ 66.97%, CD3+CD4+ 60.49%, CD3+CD8+ 5.90%, CD4+/CD8+ 10.25, CD3−CD16+CD56+ 25.79%, and CD3−CD19+ 2.62%. Antinuclear antibodies (ANA) were positive with cytoplasmic (granular) pattern at a 1/100 titer. Antibodies to extractable nuclear antigens (ENA) and complement C3 were negative. Bone marrow biopsy was found to be normal. Evidence of EB virus or CMV virus infection were not found. The case did not find obvious infection except for the impetigo herpetiformis that were cured by systemic antibiotic. The patient was put on intravenous immunoglobulin (IVIG) at the total dose of 2.32 g/kg and the platelet count increased to 61.00 × 10^9/L. Having atypical thrombocytopenia and eczema in a male infant suggested the initial diagnosis of WAS. Then the WAS gene testing confirmed our diagnosis. One month later, the child was re-admitted to the hospital because of thrombocytopenia (PLT25 × 10^9/L). Following IVIG infusion at the dose of 200mg/kg, the child's platelet count raised to 85.00×10^9/L and he was discharged home. Later on, he did not have regular follow-up and eventually died of ICH at the age of 1 year.

As for WAS gene test, DNA extracted from blood samples of the patient were analyzed using next-generation sequencing technologies. The results showed that the patient carries a new hemizygous mutation in the *WAS* gene (Figure 1, b). A G-to-A substitution at position c273 in exon 2 of the *WAS* gene resulted in the mutation (c.273 G>A) (Figure 1, a). This is a silent mutation induced by a base substitution that has no effect on the amino acid it encodes. Meanwhile, Sanger sequencing of the *WAS* genes of the child's parents showed that his mother had a heterozygous mutation in her X chromosome, a G-to-A substitution at position c273 in exon 2 of the *WAS* gene. His father and maternal grandmother revealed wild-type gene sequencing. Therefore, we deduced that the patient's *WAS* C.273 G>A was passed down from her mother, who was an asymptomatic carrier of the mutant gene (Figure 1, c).

The newly discovered mutation did not result in amino acid substitution due to the codon's degeneracy. To explore whether this gene mutation was deleterious, we used online prediction software for preliminary investigation. In Combined Annotation Dependent Depletion (CADD) testing, the mutation site had a RawScore of 3.430166 and a Phred score of 24.8 (Figure 2, b). Both the Spidex and Mutation Taster software prediction results showed that the mutation would result in a splicing change, which would affect the transcript (Figure 2, a).

To further confirm this prediction, we carried out transcriptome sequencing on the patient's and his parents' genes. The results indicated the alteration of the *WAS* gene transcript (Figure 2, c). A novel transcript called ONT.4932.1 was highly expressed in the patient's and his mother's genomes compared to the normal population. WAS-201 was the most highly expressed transcript in the normal population, but it was expressed at a relatively low level in patients. According to the sequence variant interpretation guidelines established by the American College of Medical Genetics and Genomics (ACMG), combined with the clinical manifestations of the child, this variant can be assessed as "pathogenic".

WAS is a rare X-linked recessive disease which threatens the survival and growth of children. Until now, more than 400 different types and sites of mutations in the *WAS* gene have been identified (*9*). The most common type of mutation is missense mutations clustered mainly in exons 1-4, followed by splicing mutations which mainly occur in exons 6-10. The deletion and insertion mutations are relatively rare which

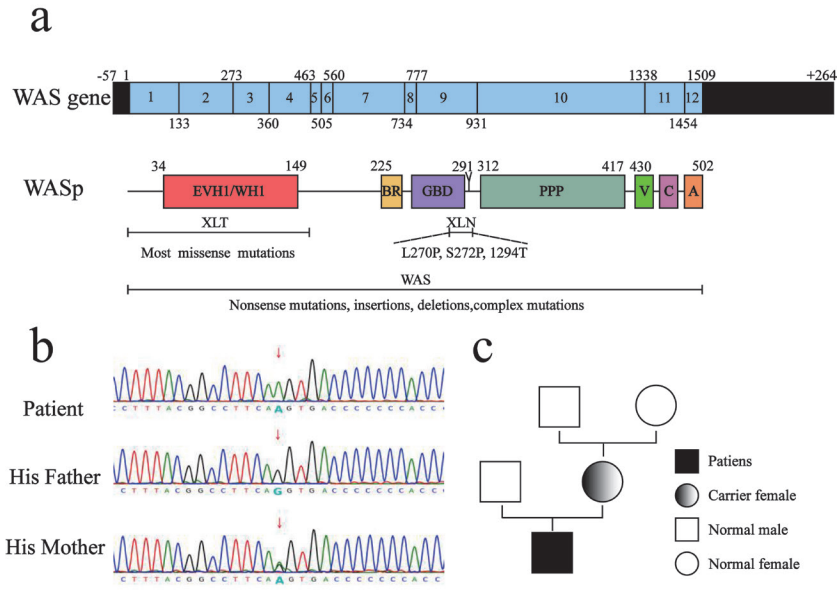
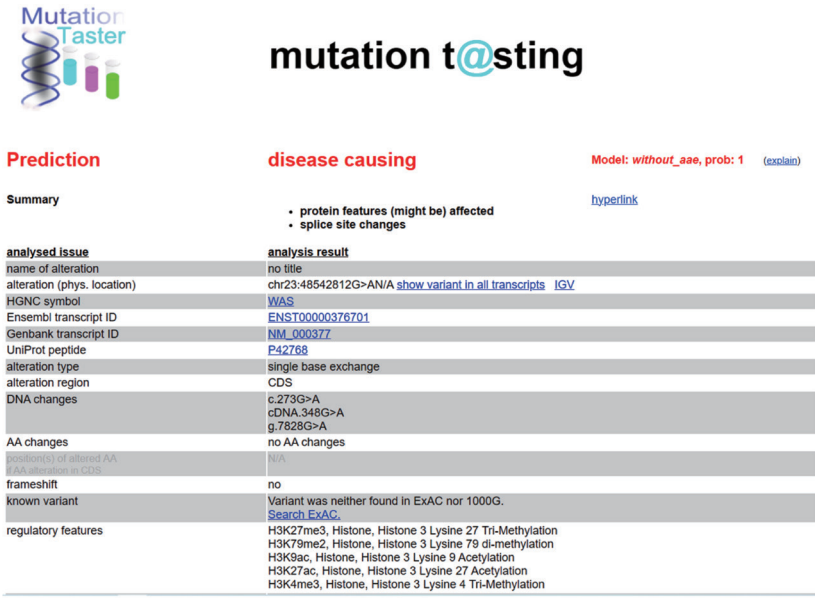


Figure 1. (a) Schematic diagram of the WAS gene and WASp structure. *WAS* gene contains 12 exons and *WAS* c.273 G>A (in patient) occurred on exon 2. WASp consists of five functional domains, namely Ena-VASP Homologous 1 domain (EVH1), base domain (B), guanosine triphosphatase-binding domain (GBD), proline-rich domain (PRD), and verprolin homologous/central hydrophobic C/ acidic domain A domain (VCA). The domain encoded by exon 2 is EVH1. **(b) Sanger sequencing of *WAS* gene in c.273 G>A. (c) Genetic family map of this WAS patient.**

a



b

Chrom	X
Pos	48542812
Ref	G
Alt	A
RawScore	3.430116
PHRED	24.8

c

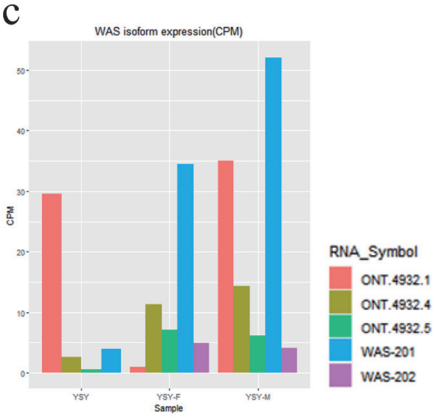


Figure 2. (a) The results of Mutation Taster. (b) The results of CADD testing. (c) The results of transcriptome sequencing.

are distributed in the entire WAS gene (*10,11*). The present study identified a novel synonymous mutation (c.273 G>A), which has not been reported in the HGMD and Clinvar database as a known variant in the general population.

In the process of exploring the pathogenicity of this mutation, next-generation sequencing, SPIDEX and Mutation Taster software as well as transcriptome sequencing were used. At the same time, we traced the origin of the mutation and found that his mother was a carrier of the mutant gene. Therefore, the mutation was considered to be "pathogenic", combined with the child's symptoms and family history. Nonsense mutations had previously been reported at this locus (c273 G>C), but the substituted bases and pathogenicity are different from those in this study (*12*). This finding has important implications for understanding the pathogenicity of mutations in the *WAS* gene and expands the genetic spectrum of WAS.

The treatment depends on the main symptoms, severity and the expected prognosis of WAS (*13*). Hematopoietic stem cell transplantation (HSCT) is currently recognized as the only potentially curative strategy that provides lifelong benefits for typical WAS patients (*14*). As a typical WAS patient, after receiving supportive treatments including antibiotics, immunoglobulins, and platelet transfusion, the clinical symptoms of the patient were temporarily controlled, and humoral immune status was improved. However, the patient eventually died due to recurrent episodes and the lack of curative hematopoietic stem cell transplantation.

Gene therapy for WAS has been rapidly developed in recent years and its therapeutic effect has been widely recognize (*15,16*). Gene therapy involves gene editing of autologous hematopoietic stem cells and reinfusion, which avoids the risks of allogeneic transplantation and effectively overcomes the realistic obstacles of bone marrow matching (*17,18*). With the advancement of treatment methods, the overall survival rate and prognosis of WAS patients has increased. The child might benefit from gene therapy, but don't have the time to receive this therapy and eventually died. Therefore, the effect of gene therapy on WAS caused by this mutation remains to be verified.

In conclusion, the synonymous mutation (c.273 G>C) of exon 2 in the *WAS* gene is pathogenic. Genetic testing

and family history tracing should be done as soon as possible on patients with suspected WAS. In this study, the patient presented with ICH as the first symptom, which is uncommon in patients with WAS. Therefore, infants with ICH as the initial presentation should alert clinicians to the possibility of WAS. Once the diagnosis is established, symptomatic treatment should be combined with early HSCT to improve the prognosis of the patient.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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