

A case of Wiskott-Aldrich syndrome with de novo mutation at exon 4

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Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by thrombocytopenia, eczema and immunodeficiency. Clinical features of the disease are highly varied; therefore, the diagnosis is sometimes difficult, especially in solitary cases or cases with milder forms of the disease. However, the identification of the WASP gene has made possible a definite WAS diagnosis for these cases. In this report, we present a 26-month-old boy who had received several ineffective treatments for chronic immune thrombocytopenic purpura. He was then suspected to have WAS because of the early onset of thrombocytopenia and small platelets. The diagnosis became definite with the detection of a de novo mutation at exon 4 of the WASP gene, Arg138Pro, through mutation analysis.

Key words: thrombocytopenia, Wiskott-Aldrich syndrome, WASP gene.

The Wiskott-Aldrich syndrome (WAS, MIM 301 000) is an X-linked primary immunodeficiency disease originally described as a clinical triad of immunodeficiency, eczema and thrombocytopenia with small platelets¹. Although many patients with WAS express all three of these clinical manifestations, others have a partial or variant phenotypic expression. Because of the wide spectrum of clinical findings, the diagnosis of definite WAS can only be done through genetic analysis or the determination of protein expression with a specific antibody^{2,3}.

In this study, we report a new case of WAS who was previously diagnosed as chronic immune thrombocytopenic purpura. A 26-month-old boy, the first child of nonconsanguineous parents, was referred to our clinic with recurrent bruising and petechial lesions since 3.5 months of age. He had received prednisolone and intravenous immunoglobulin treatments until the diagnosis of WAS was considered. The presence of mild transient eczema together with early onset of thrombocytopenia and small platelets supported the diagnosis in this case.

However, he had neither severe infections nor a family history. His height (92 cm, 75-90%) and weight (16 kg, 97%) were normal on physical examination, but he had petechial lesions all over the body. His previous thrombocyte counts were between $38 \times 10^9/L$ - $67 \times 10^9/L$ and MPV: 4.6-7.0 fl. Immunological tests showed a low IgM level with normal level of isohemagglutinin and polio antibody titers. Peripheral blood lymphocyte subsets were found to be normal. Lymphoproliferative response to anti-CD3 was diminished.

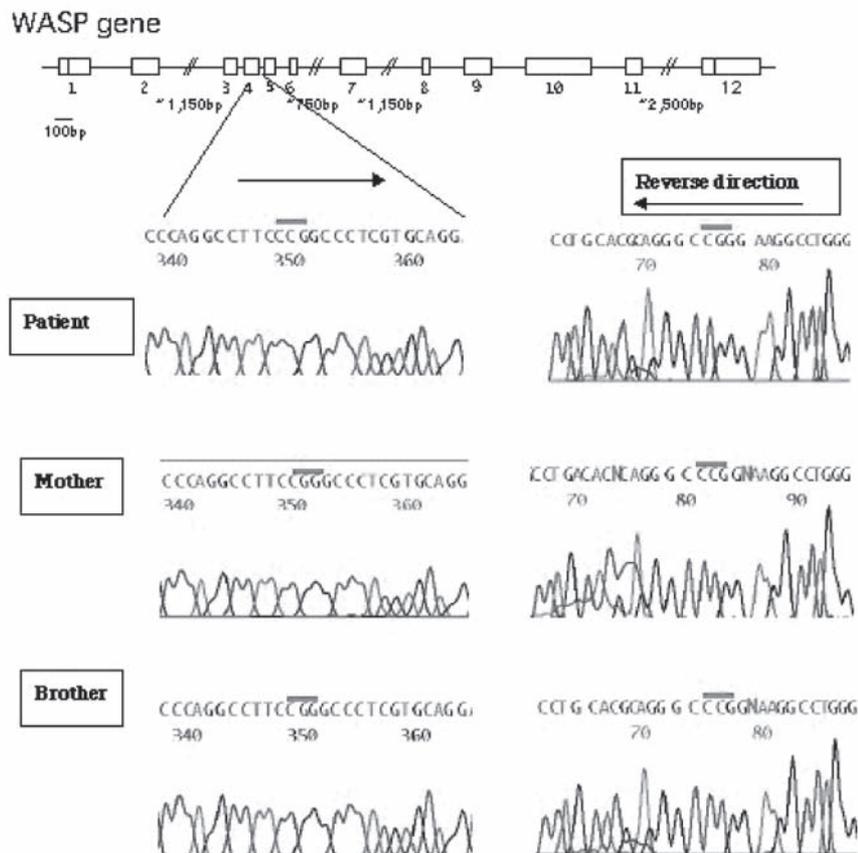
DNA samples were obtained from the patient, his mother and nine-month-old brother using a standard phenol-chloroform extraction technique for molecular analysis. Written, informed consent was obtained from the parents. Mutation analysis of the WASP gene was performed as previously reported². Briefly, 10 fragments amplified by polymerase chain reaction (PCR), in which 12 exons and flanking introns of the WASP gene were included. Each amplified fragment was purified and directly sequenced using ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction kit [Perkin-

Elmer: Foster City, CA] and automated ABI 373A DNA sequencer. Sequencing the WASP gene revealed a rare single base de novo mutation, resulting in a substitution of proline for arginine 138 at exon 4, which confirmed the diagnosis. Unexpectedly, his mother does not have this mutation and he is the only proband in this family (Fig. 1).

Clinical manifestations of WAS are highly variable and the most consistent feature is thrombocytopenia with small platelets. Infections and immunological abnormalities may be absent, mild or severe, and autoimmune diseases and malignancies may develop in some cases¹. The clinical severity of the disease also varies from patient to patient even in the same family or over time in a given patient^{4,5}.

The discovery of the gene has led to a better awareness of the disease and resulted in an increased usage of mutation analyses, especially for newly diagnosed and atypical patients. To date, 150 mutations in more than 300 families with WAS/XLT have been characterized worldwide⁶. Attempts to correlate different WAS phenotypes with specific genotypes have resulted in controversial interpretations. Zhu et al.⁷ designed a scoring system based on characteristic clinical symptoms and examined the effects of individual WASP mutations on the clinical phenotype in 48 unrelated families. Using these criteria, patients with missense mutations affecting the exons 1-3 have mild disease, while the amino acid substitutions in exon 4 result in classic or

WASP gene mutation analysis for AÖ and his family members



Mutation detected; CGG→CCG in exon 4 of WASP gene

Arg138Pro

His mother does not have the mutant allele; she is not a carrier for WAS.

His brother does not have the mutant allele, either. He does not have WAS.

severe WAS⁷. Our patient's symptoms are consistent with a milder disease [score 2]. His detected mutation [Arg138Pro] at exon 4 was previously described in two kindreds with different clinical presentations. The first case was a classical WAS patient⁸ with no family history, while the others were twin brothers with X-linked thrombocytopenia⁹. Therefore, extensive heterogeneity among the WAS patients cannot be ascribed to WAS genotype alone. Other genetic and environmental factors are likely to modify phenotypic expression of mutation of the WASP gene.

Another outstanding feature of this case is that the mother is not a carrier. It seems that a de novo mutation took place in her oocyte, or after fertilization. Although it seems very unlikely, another explanation for this situation could be a germinal mosaicism in the mother.

As known, the only curative treatment for WAS is still bone marrow transplantation, especially in the presence of a matched sibling donor or matched unrelated donor. Transplantation before the age of five has a success rate of 90%¹. In our case, bone marrow transplantation from a fully matched baby brother was planned with the confirmation of diagnosis.

In conclusion, the diagnosis of WAS should be considered in boys with thrombocytopenia and small platelets, whether or not there is a family history. The early confirmation of diagnosis would increase the success rate in the treatment.

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