A Novel Mutation W252X in the WAS Gene in a Korean Patient with Wiskott-Aldrich Syndrome

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Abstract

Wiskott-Aldrich syndrome (WAS) is a primary immunodeficiency disorder characterized by recurrent infection, eczema, and microthrombocytopenia. WAS is inherited in an X-linked recessive pattern, and various mutations in the WAS gene on the X chromosome are the genetic basis of WAS. A 7-month-old Korean boy presented with recurrent bloody diarrhea, eczema, and persistent thrombocytopenia with small platelets. Direct sequence analysis of the entire coding region of the WAS gene showed a novel nonsense mutation with a G-to-A substitution at the nucleotide position 756 on exon 8, leading to a premature termination at codon 252 (c.756G>A; p.W252X). Family study revealed that neither of the parents had the mutation, indicating the de novo occurrence of the mutation.


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Key words: Wiskott-Aldrich syndrome; WAS gene; Mutation; Korea

1. Introduction

Wiskott-Aldrich syndrome (WAS) is an X-linked platelet and immunodeficiency disorder characterized by recurrent infection, eczema, and microthrombocytopenia [1]. The causative gene, WAS, is located on the chromosome band Xq11.23 and encodes a 502-amino acid intracellular protein, which consists of 5 distinct domains [2,3]. Until now, more than 400 patients with WAS or related disorders such as X-linked thrombocytopenia, an attenuated form of WAS, have been reported to carry different mutations in the WAS gene [4], and genotype-phenotype correlations have been recognized [5].

In this report, we describe a Korean boy with a classic phenotype of WAS with a novel de novo mutation in the WAS gene. This is the fifth case of WAS with a mutation in the WAS gene in Korea, and the mutation identified occurred de novo in a mutational cold spot.

2. Case Report

2.1. Clinical and Laboratory Findings

A 7-month-old Korean boy was admitted because of fever and tachypnea. He had a history of recurrent mucous bloody diarrhea, recurrent infection, and persistent thrombocytopenia since birth. Physical examination revealed whole body petechiae and eczema. On laboratory examination, the Hb level was 9.9 g/dL, and the white blood cell count was 4.5 x 10^9/L with neutrophils at 35% and lymphocytes at 49%. The platelet count was decreased at 10 x 10^9/L, and the mean platelet volume was 7.6 fL, below the lower limit of the reference range (9.0-13.0 fL). A peripheral blood smear revealed normocytic normochromic anemia and thrombocytopenia with small platelets. The serum immunoglobulin (Ig) levels were all within reference ranges for IgG, IgA, IgM, and IgE. The lymphocyte subset analyses revealed whole body petechiae and eczema. On laboratory examination, the Hb level was 9.9 g/dL, and the white blood cell count was 4.5 x 10^9/L with neutrophils at 35% and lymphocytes at 49%. The platelet count was decreased at 10 x 10^9/L, and the mean platelet volume was 7.6 fL, below the lower limit of the reference range (9.0-13.0 fL). A peripheral blood smear revealed normocytic normochromic anemia and thrombocytopenia with small platelets. The serum immunoglobulin (Ig) levels were all within reference ranges for IgG, IgA, IgM, and IgE. The lymphocyte subset analyses revealed CD4+ T-cells at 21% and CD8+ T-cells at 67% with a reversed CD4+/CD8+ ratio at 0.31 due to the decrease of CD4+ cells. Both B-lymphocytes and NK cells were decreased at 3% and 1%, respectively. Bone marrow studies revealed granulocytic hyperplasia and slightly increased megakaryocytes without apparent dysplastic features. A chest x-ray showed haziness in the right lung field. The patient was treated with platelet transfusions and intra-
venous administration of immunoglobulins, but the thrombocytopenia was refractory to the treatment. The patient died at the age of 8 months, 1 month after admission, from severe pneumonia.

2.2. Genetic Analysis of the WAS Gene

After we had obtained informed consent from the parents, the blood sample was collected from the patient, and genomic DNA was isolated from peripheral blood leukocytes using the Wizard genomic DNA purification kit according to the manufacturer’s instructions (Promega, Madison, WI, USA). All 12 coding exons and their flanking intronic sequences of the WAS gene were amplified by polymerase chain reaction using the primers designed by the authors (available upon request). Cycle sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA, USA) on the ABI 3100 Genetic Analyzer (Applied Biosystems). The sequence variation was described according to the recommendations of the Human Genome Variation Society [6].

The proband was revealed to have a nonsense mutation in exon 8 of the WAS gene, a G-to-A substitution replacing tryptophan with a termination codon at codon 252 (c.756G>A; p.W252X) (Figure 1). The sequencing analyses were performed on the parents and demonstrated that neither of the parents had the mutation, indicating de novo occurrence of the mutation in the patient.

3. Discussion

The causative gene for Wiskott-Aldrich syndrome, the WAS gene, encodes a 502-amino acid protein, which includes 5 distinct domains: the N-terminal WASP-homology domain 1 (WH1), the GTPase-binding domain (GBD), the proline-rich region (PRR), the verprolin homology domain (V), and the cofilin-homology sequence (C), from 5' to 3' (Figure 2) [3]. Although different mutations have been reported throughout the entire coding region, the distribution of mutations is uneven, with the majority of mutations occurring in the first 5'-domain, WH1, especially in exon 2 [4]. Genotype-phenotype correlations have shown that missense mutations of the first 3 exons result in detectable WAS protein and mild disease, while deleterious mutations such as nonsense or frameshift mutations are associated with classic WAS and severe disease [5,7]. The mutation identified in our patient was a nonsense mutation on codon 252 in exon 8, which is a part of the GBD, a relatively cold spot for muta-

![Figure 1](image.png)