# A novel termination codon mutation of the *WAS* gene in a Thai family with Wiskott-Aldrich syndrome

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Received June 10, 2003; Accepted August 29, 2003

**Abstract.** Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disorder characterized by microthrombocytopenia, eczema, immunodeficiency, and susceptibility to lymphoid malignancy. Loss-of-function mutations in *WAS* gene have been identified to cause disorders with platelet defects including WAS and X-linked thrombocytopenia. Mutations anticipated to yield truncated or no protein have been associated with the more severe presentations of WAS. Activating mutations in *WAS* gene result in an entirely different phenotype, an X-linked severe congenital neutropenia. We describe a Thai family with classic WAS. The proband, a one-year-old boy presented with recurrent mucous bloody diarrhea, recurrent otitis media, chronic eczema, thrombocytopenia, and small platelet sizes. The patient's older brother who also had persistent thrombocytopenia died at the age of seven months from severe pneumonia. Immunoblot analysis demonstrated that the proband's cells lacked WAS protein expression. Mutation analysis of the proband and his mother for the entire coding region of *WAS* identified a novel type of mutation, a termination codon mutation, X503R. The change is expected to result in an elongated mRNA that would code for a WASP of 581 amino acid residues instead of the normal 502 residues. Because of the absence of WASP expression, we speculate that the termination codon mutation causes reduced mRNA stability. Our findings supported that *WAS* mutations resulted in no protein are associated with a severe phenotype of WAS.

## Introduction

Wiskott-Aldrich syndrome (WAS; MIM 301000) is an X-linked recessive disorder with variable clinical phenotypes (1). Patients with a classic WAS phenotype present with microthrombocytopenia, eczema, immunodeficiency, and susceptibility to lymphoid malignancy. Mutations in the *WAS* gene give rise to this disorder (2). Since there is a considerable variation in the severity of clinical disease, the clinical diagnosis of WAS can be difficult and is usually supported by the detection of *WAS* gene mutations or absence of the Wiskott-Aldrich syndrome protein (WASP) in peripheral blood mononuclear cells (3). The *WAS* gene is composed of 12 exons and encodes a 502-amino acid protein (2). The gene product, WASP, is a proline rich intracellular protein with a number of unique domains that suggest a 'multi-faceted' function. Accumulating evidence indicates that WASP is involved both in the cytoskeleton and the cytoplasmic signaling system of hematopoietic cell lineages (4).

Various mutations in the *WAS* gene has been reported (5). Here we report a novel mutation in *WAS* in a Thai family with WAS.

## Materials and methods

*Case report.* A 1-year-old Thai boy presented with recurrent mucous bloody diarrhea since the first week of life. He developed recurrent otitis media at the age of one month, and chronic eczema at two months. Physical examination revealed a small for his age male infant with subacute eczema on the scalp and face, and petechiae on both upper and lower extremities. There was no hepatosplenomegaly or lymph-adenopathy. Laboratory investigation revealed severe persistent thrombocytopenia with small platelet sizes (platelet volume 5 fl). He was diagnosed as having WAS with WAS score of four. The patient's older brother who also had persistent thrombocytopenia died at the age of seven months from severe pneumonia.

WASP protein assay using immunoblot analysis demonstrated that the patient's cells lacked WASP expression (courtesy of Dr Hubert B. Gaspar and Kimberly C. Gilmour, UK) (6).

*WAS gene analysis.* After informed consent was obtained, three ml of peripheral blood of the boy and his mother were obtained. RNA was isolated from white blood cells using QIAamp® RNA blood mini kit (Qiagen, Valencia, CA, USA). Reverse transcription was performed using ImProm-II™ reverse transcriptase (Promega, Madison, WI, USA), according to the company recommendations. PCR amplification of the *WAS* cDNA exons 1-9 was performed using primers and

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*Key words:* Wiskott-Aldrich syndrome, WAS, mutation analysis, a termination codon mutation

Table I. Primer pairs, the optimal annealing temperatures, and the PCR product sizes for mutation analysis of cDNA and gDNA of the *WAS* gene.

| Template: exon | Primers | Annealing temperatures (˚C) | Product sizes |
|---|---|---|---|
| cDNA: exons 1-9 | 5'-GCC TCG CCA GAG AAG ACA AG-3'<br>5'-GCA ATC CCC AAA GGT ACA GG-3' | 60 | 1082 |
| gDNA: exon 10 | 5'-GCA CCT ATA CTG CTT CAG TC-3'<br>5'-TAT CCT GAC TTA GAC GGG AC-3' | 58 | 593 |
| gDNA: exon 11 | 5'-GAG AAA TGC TCC TTT CCC AG-3'<br>5'-TGT TTT GAG GCC AGT GCT AG-3' | 25 | 233 |
| gDNA: exon 12 | 5'-CTA GCA TGA GAC CTC AGA AC-3'<br>5'-AAA GGG ACT TTG GGG GAA AG-3' | 60 | 484 |

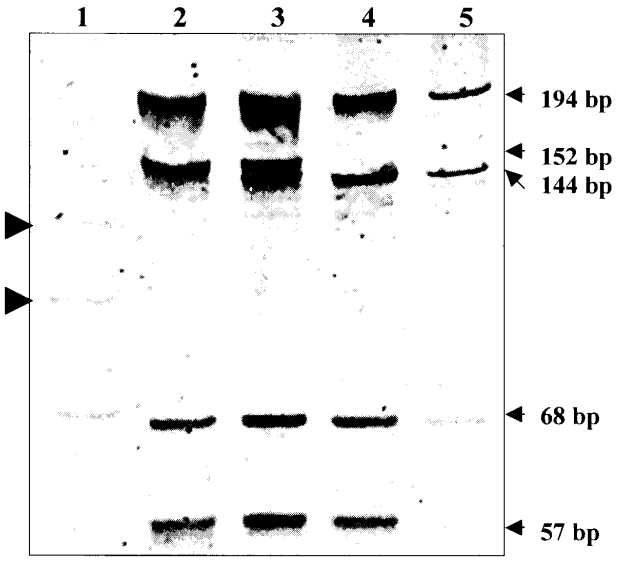


Figure 1. Restriction enzyme detection of the *WAS* 1507T→A (X503R). Lane 1 represents a 25 bp DNA marker with the bands at 100 and 125 bp indicated with arrow heads. Lanes 2-5 are PCR products mixed with *Dde*I electrophoresed on a 12% polyacrylamide gel. Lane 2 is of the proband; lane 3 his mother, lane 4 an unrelated patient with WAS, and lane 5 a normal control. Lanes 4 and 5 show digested 194, 144, 68 and 57 bp bands, while the 13 and 8 bp bands are not seen. The 1507T→A mutation eliminates a restriction enzyme site resulting in a new band of 152 bp in lane 2 demonstrating that the proband is hemizygous for the mutation. Both 152 and 144 bp bands are present in lane 3 indicating that the proband's mother is heterozygous for the mutation.

conditions shown in Table I. We used 1 µl of first-strand cDNA, 1X PCR buffer (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, and 0.5 U Taq DNA polymerase (Promega) in a total volume of 20 µl. Genomic DNA was obtained from whole blood using a standard extraction method. Exons 10-12 of *WAS* gene were amplified using primers and conditions as shown in Table I. PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH, USA), according to the company recommendations, and sent for direct sequencing at the National Science and Technology Development Agency, Bangkok, Thailand. The 1507T→A (X503R) was confirmed by digestion of the PCR products with the restriction enzyme *Dde*I (New England Biolabs, Beverly, MA, USA), according to the manufacturer's instructions. Digested DNA was analyzed by electrophoresis on 12% polyacrylamide gels using Tris-borate buffer and visualized by staining with GelStar Nucleic Acid Stain (Cambrex, East Rutherford, NJ, USA).

## Results

Direct sequencing and the pattern of restriction enzyme analyses of the *WAS* gene revealed that the boy was hemizygous for a T→A transversion at nucleotide 1507 in *WAS* exon 12, while his mother was heterozygous for the same mutation (Fig. 1). The mutation changed the termination codon to the codon for arginine (X503R), giving rise to an elongated mRNA that would code for a WASP of 581 amino acid residues instead of the normal 502 residues.

## Discussion

In our family, the proband had microthrombocytopenia, eczema, and recurrent infections, clinically typical of WAS. His brother, with a history of thrombocytopenia and death in infancy due to a severe infection, was likely to have been also affected with WAS. Unfortunately, he died undiagnosed. Knowing the definite diagnosis and the specific mutation in this family will assist identifying other family members at risk for this deadly disease and provide a method of prenatal diagnosis.

To decrease the expense of mutation detection, we first chose to PCR amplify and directly sequence cDNA. However, we could not amplify the entire coding region of WAS cDNA in one piece. We, therefore, amplified exons 1-9 using cDNA as template, while amplifying exons 10, 11 and 12 using gDNA. We found a unique type of mutation, a termination

codon (X503R) which was confirmed by a restriction enzyme digestion analysis. The mutation was found to be inherited from his mother. To our knowledge, the mutation has not been described (5). Interestingly, there are many unique mutations in genes of several disorders among Thai population, such as Van der Woude syndrome (7) and pseudoachondroplasia (8).

WASP is an important regulator of the actin cytoskeleton, mediating communication between Rho-family GTPases and the actin nucleation/crosslinking factor, the Arp2/3 complex (9). Many *WAS* mutations impair cytoskeletal control in hematopoietic tissues, resulting in functional and developmental defects. Loss-of-function mutations, through decreased transcription or translation of the gene, result in reduced WASP signaling and cause WAS or the related X-linked thrombocytopenia (10). In contrast, a constitutively activating mutation in WASP resulted in an entirely different disorder, X-linked severe congenital neutropenia (XLN). XLN is characterized by severe congenital neutropenia and monocytopenia with normal size and number of platelets and no history of eczema (11).

Our patient exhibit moderately severe phenotype with WAS score of four. Previous reports have suggested that the more severe presentations of WAS are predominantly associated with mutations anticipated to yield truncated or no protein; while most patients with XLT and intermittent X-linked thrombocytopenia have missense mutations within exons 1 and 2, leading to decreased but detectable protein expression (12). Immunoblot analysis of our patient showed absence of WASP expression on peripheral blood mononuclear cells. We speculate that the termination codon mutation causes reduced mRNA stability, as has been shown for other termination codon mutations (13). Our findings supported that *WAS* mutations resulted in no protein are associated with a severe phenotype of WAS. However, previous reports also observed variability of disease phenotype within the same families (14) indicated that other factors such as genetic determinants not related to WASP and environmental factors are influential. Therefore, phenotypic outcome in WAS may not be deduced from WAS genotype alone.

In conclusion, we report a Thai family with classic WAS, in which mutation analysis revealed a novel termination codon mutation resulted in absence of WASP. Our findings supported that *WAS* mutations resulted in no protein are associated with a severe phenotype of WAS.

**Acknowledgments**

We are grateful to Dr Jarungchit Ngamphaiboon for her valuable advice and to Drs Hubert B. Gaspar and Kimberly C. Gilmour for the WASP protein assay. This study was supported by the Molecular Biology Project, Faculty of Medicine; the Development Grants for New Faculty/ Researchers, Chulalongkorn University, and the Thailand Research Fund; Contract grant number: RSA/05/2544.

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