

## CASE REPORT

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# Detection of Six Novel Mutations in WASP Gene in Fifteen Iranian Wiskott-Aldrich Patients

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## ABSTRACT

Wiskott-Aldrich syndrome (WAS) is a life-threatening X-linked recessive immunodeficiency disease described as a clinical triad of thrombocytopenia, eczema, and recurrent infections, caused by mutations of the WAS protein (WASP) gene. The milder form of this disease is X-Linked thrombocytopenia (XLT) that presents only as platelet abnormalities. Mutation analysis for 15 boys with Wiskott-Aldrich syndrome was performed. Five previously reported mutations and six novel mutations including G8X, R41X, D283E, P412fsX446, E464X, and AfsX358 were detected.

**Keywords:** Mutations; Thrombocytopenia; Wiskott-Aldrich syndrome

## INTRODUCTION

Wiskott-Aldrich syndrome (WAS) was first described by Wiskott in 1937 and was further characterized by Aldrich in 1954. It is an X-linked recessive immunodeficiency syndrome characterized by recurrent bacterial, eczema, and bleeding diathesis caused by

thrombocytopenia and decreased platelet volume. However, only a third of patients with the syndrome have the classic triad symptoms.<sup>1</sup> The gene for the Wiskott-Aldrich syndrome protein (Wasp) is localized to Xp11.22-23 and consists of 12 exons that has 502 amino acid (53 kD) protein. Wasp is a cytosolic protein that is expressed on all hematopoietic cell lineages and is essential factor for normal antibody function, T-cell responses and platelet production.<sup>2</sup>

It also regulates transcription, actin polymerization and a selective post-transcriptional role in Th2 effector

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function.<sup>3</sup> The most of missense mutations in patients with Wiskott-Aldrich syndrome are distributed in the WIP-binding domain located in the first four exons and might cause dissociation of the WASP-WIP complex and WASP degradation.<sup>4</sup> Usually, splice site mutations are reported through introns 6-10, other mutations consist of insertions and deletions which are distributed throughout the whole gene.<sup>5,6</sup>

## MATERIALS AND METHODS

### Clinical Samples

Fifteen Iranian patients with Wiskott-Aldrich syndrome, who were referred to the Immunology, Asthma and Allergy Research Institute in Tehran, and 20 Iranian controls, were included in this study. All patients and controls were unrelated Iranian Caucasians.

**Table 1. WASP mutations in 11 patients from 11 families**

Case	Exon	Nucleotide position	Amino acid substitution
1	1	C37T	R13X
2	1	C121T*	R41X
3	1	G22T*	G8X
4	2	G208A	G70R
5	2	C167T	A56V
6	2	C167T	A56V
7	4	C381G	N127K
8	9	C849G*	D283E
9	10	1281delC*	P412fsX446
10	10	c.1074-1075ins *	AfsX358
11	11	G1390T*	E464X

\*new mutation

The probable diagnosis of Wiskott-Aldrich syndrome was made by physicians based on clinical findings and laboratory tests.

### DNA Extraction, Polymerase Chain Reaction and DNA Sequencing

Genomic DNA was isolated from peripheral blood, using DNA extraction kit (Roche high pure PCR template preparation KIT). All the 12 exons of the WASP gene were individually amplified with primers described previously.<sup>7</sup> PCR was carried out in a volume of 25 µl with 200 ng of genomic DNA, 10pmol of each primer and 1 unit Taq polymerase. The PCR reactions

were performed in a thermal cycler (MWG-Biotech Primus) for 10 min at 95°C followed by 30 cycles of denaturation for 1min at 95°C, primer annealing for 50 sec at 58°C to 63°C depending on the primers, primer extension for 35 sec at 72°C with a final 3 min extension at 72°C. The amplified fragments were separated by gel electrophoresis in 2% agarose gel electrophoresis and directly sequenced using the same primers used in the PCR reaction. The amplified DNA fragments were separated by agarose gel electrophoresis and direct sequencing was also performed.

## RESULTS

Direct sequencing of WASP gene was performed in 11 patients suffering from Wiskott-Aldrich syndrome. The gene in all patients was genetically screened according to physician requests based on clinical and laboratory features of patients. Ten different mutations were recognized in the 11 patients. The mutations are listed in table 1.

## DISCUSSION

Wiskott-Aldrich syndrome is caused by mutation through the WASP gene. Since 1994 which the causative gene was first isolated and cloned,<sup>8</sup> different unique mutations including missense, nonsense, and splice site mutations and insertions, deletions, and rearrangements have been detected. The WASP gene consists of 502 amino acids, which has an N-terminal WASP homology domain 1(WH1), a Cdc42/Rac GTPase-binding domain (amino acids 238-257),<sup>8</sup> a proline-rich region (amino acids 311-422),<sup>9</sup> a verprolin homology domain (V), and a cofilin-homology sequence(C). WASP regulates the actin cytoskeleton and the C-terminal portion of WASP contains a WH2 domain (amino acids 423-449)<sup>10</sup> and an acidic region (amino acids 485-502)<sup>11</sup> that mediates interactions with Gactin and the Arp2/3 complex.<sup>12</sup> Mediating TCR signals, which plays a pathogenetic role in WAS, is dependent on the expression of WASP and WASP-interacting protein (WIP).<sup>13,14</sup> Most missense mutations are localized in the WH1 domain (amino acids 47-137).<sup>10</sup> A mutated WASP often cannot bind to WIP, leading to defective WASP expression.<sup>15</sup>

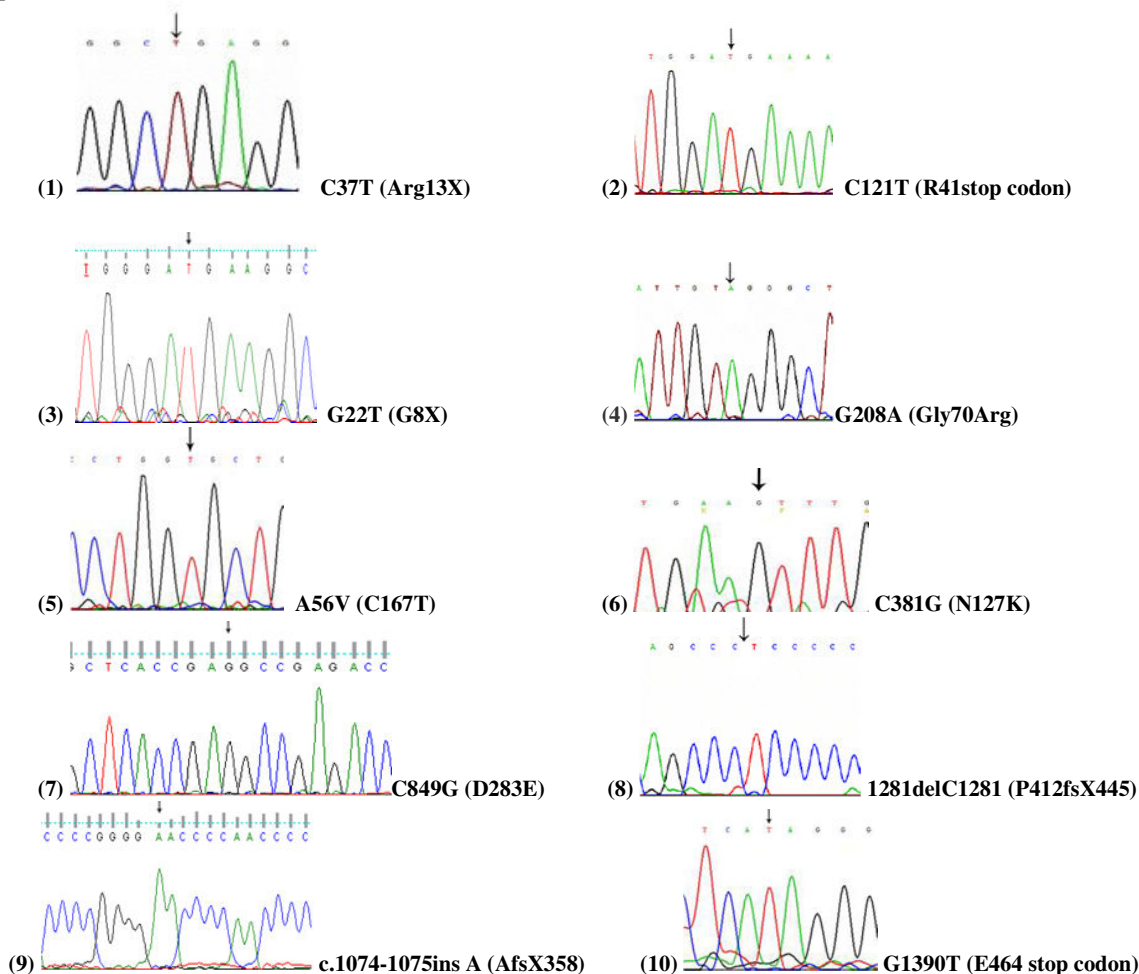
The diagnosis of WAS is based on detecting the mutation by the direct sequence analysis of WASP

## Detection of Six Novel Mutations in WASP Gene

cDNA and genomic DNA. Based on searching the literature and the human gene mutation database, we hereby report six novel WASP gene mutations identified in Iranian Wiskott Aldrich cases, i.e., G8X, R41X, D283E, P412fsX446, E464X, and AfsX358 (Figure 1).

A mutation was found predominantly in exon 1, which leads to changing of Arginine at position 13 to stop codon. The 2 other new mutations in exon 1; R41X, and G8X point mutation lead to a premature stop codon in the WH1 domain. G70R mutation in

exon 2 located in WASP homology I (WHI) domain, interacts with PIP2 and WASP interacting protein (WIP) resulting in missense substitution which was described previously.<sup>16</sup> Point mutation detected in exon 2 (A56V) which leads to an amino acid change in the WH1 domain, had been previously reported.<sup>17</sup> Considering the existence of this unique mutation in two unrelated patients in a few number of cases involved in this study, it may be possible to claim that this mutation is one of the hotspot sites, in WAS gene.



**Figure 1.** Diagrams of WASP mutations identified by sequencing method in patients. WASP gene mutations in WAS patients are identified by arrow: 1) Exon 1 have a substitution of cytosine for thymine 37 (R13X). 2) The mutation in exon 1, resulting in substitution of cytosine 121 to thymine (R41X). 3) The mutation in exon 1, resulting in substitution of guanine 22 to thymine (G8X). 4) The mutation in exon 2, resulting in substitution of guanine 208 to adenine (G70R). 5) The mutation in exon 2, resulting in substitution of cytosine 167 to thymine (A56V). 6) The mutation in exon 4, resulting in substitution of cytosine 381 to guanine (N127K). 7) The mutation in exon 9, resulting in substitution of cytosine 849 to guanine (D283E). 8) The mutation in exon 10, resulting in deletion of cytosine 1281 (P412fsX445). 9) The mutation in exon 10, resulting in insertion of adenine (AfsX358). 10) The mutation in exon 11, resulting in substitution of guanine 1390 to thymine (E464X).

A new mutation was observed in exon 9, that leads to changing of Aspartic acid at position 283 to Glutamic acid. Two new mutations in exon 10 were also identified, which generate frame shift, bringing stop codon including a deletion and an insertion mutation. A new E464X mutation was found in exon 11. The control group was negative for these mutations. A more direct method for diagnosis of new mutations should always be coupled with direct mutation analysis, such as WASP expression to suggest a correlation between the level of WASP expression and variation in expressed phenotypes of clinically affected patients. The data presented here suggests the diagnostic approaches in patients suspected of to WAS disease and also provide further information concerning the spectrum of WASP mutations responsible for Wiskott-Aldrich. Our data are similar to those of previous reports and support the importance of the N-terminal of WASP in its function due to the clustering of WAS mutations within the 2 exons of the gene (WH1 domain).

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