Identification of 5 novel WASP Mutations in Chinese Families with Wiskott-Aldrich Syndrome

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The Wiskott-Aldrich Syndrome (WAS) is an X-linked recessive immunodeficiency caused by mutation in the gene encoding WAS protein (WASP). The disease is characterized by eczema, thrombocytopenia and severe immunodecificency and is associated with extensive clinical heterogeneity. Mutation studies indicated that the mutated genotypes are also highly variable. In this study, we performed PCR-direct sequencing analysis of the WASP gene in 6 unrelated Chinese families. Five novel mutations identified included 2 nonsense mutations (506C→T, 1388G→T), a small insertion (685-686insCGCA) and 2 single base deletions (384delT, 984delC). All of the mutations is predicted to lead to premature translational termination of WASP.

KEY WORDS: Wiskott-Aldrich syndrome, WAS, WASP, immunodeficiency, mutation analysis, Chinese

INTRODUCTION

The Wiskott-Aldrich Syndrome (WAS; MIM# 301000) is an X-linked recessive immunodeficiency characterized by thrombocytopenia, eczema, and recurrent infections due to a combined cellular and humoral immunodeficiency (Wiskott, 1937; Aldrich et al., 1954). Unless cured by bone marrow transplantation, the patients usually die before adolescence. The gene responsible for WAS, located on the short arm of chromosome at Xp11.22-Xp11.23, was identified by positional cloning (Derry et al. 1994; Kwan et al. 1995). The Wiskott-Aldrich Syndrome Protein (WASP) gene (GenBank NM_000377) consists of 12 exons, spanning 9kb of genomic DNA, encoding a protein of 502 amino acids. The WASP is expressed mainly in hematopoietic cells and has a role in signal transduction (Rivero-Lezcano et al., 1995; Cory et al., 1996; Snapper and Rosen, 1999) and cytoskeletal organization in response to external stimulus (Kolluri et al., 1996; Symons et al., 1996). Though the precise functions of WASP remain unclear, mutations in gene encoding WASP were identified to be responsible for a wide range of clinical phenotypes from isolated thrombocytopenia to severe WAS (Schwarz et al. 1996). Although the mutations in WASP is highly variable, cluster of missense mutations were identified to associate with milder forms of WAS and X-linked thrombocytopenia (XLT) (Schindelhauer, et al., 1996). In this study, screening for mutations of 5 unrelated Chinese families with 8 individuals suffering from WAS were reported.

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MATERIALS AND METHODS

Patients and their clinical features

Eight male patients referred were diagnosed to have classical WAS. The severity of the phenotypic manifestations was scored from 1 to 5 by the system proposed by Zhu et al 1995 (see Table 1). Score 1 was given to a patient with thrombocytopenia with small platelets without any other symptoms, clinical findings or laboratory abnormalities. Score 2 was given to patient with platelet abnormalities, mild or transient eczema with or without minor infections. Patient with persistent but manageable eczema or recurrent infections or both would be given a score of 3. Patient with persistent and difficult-to-control eczema and frequent life-threatening infection would be scored 4. A score of 5 would be given to those with eczema and/or frequent infection who also developed autoimmune diseases or malignancies. Among the 8 patients, they all presented within the first month of life with the typical clinical traids and suffered from recurrent infections. They are all Chinese patients from 6 unrelated families in Hong Kong, Macau and Mainland China. Patient 3 and 4 are cousins while patient 5 and 6 are twin brothers.

Table 1. Clinical features and mutations identified in patients

Patients	Exon	cDNA Mutation [a]	Codon Change	Age of Onset	Score [b]	Phenotype
1	3	384delT	S117fsX126	At Birth	5	Classical
2	5	506C→T	Q158X	1 month	4	Classical
3 *	7	685-686insCGCA	P218fsX222	1 month	4	Classical
4 *	7	685-686insCGCA	P218fsX222	1 month	4	Classical
5 #	10	984delC	P317fsX444	1 month	4	Classical
6 #	10	984delC	P317fsX444	1 month	4	Classical
7	11	1388G→T	E452X	1 month	4	Classical
8	-	-	-	1 month	5	Classical

* Patients 3 and 4 are cousins.
\# Patient 5 and 6 are twin brothers.
[a] All nucleotide numberings are according to Derry et al. (1994), accession no in Genbank U12707.
[b] The severity of the phenotypic manifestations was scored from 1 to 5 by the system proposed by Zhu et al 1995.
del: deletion, ins: insertion, fs: frameshift, X: condon stop.

PCR and Sequence Analysis

Genomic DNA was isolated from peripheral blood collected in EDTA according to Miler et al. 1988. Four separate PCR reactions, including all the coding sequence and flanking splice sites of the WASP 12 exons, were performed for each patient using 0.1μg of genomic DNA as template (see Table 2 for the PCR primers). The products were purified by ethanol precipitation and directly sequenced using either the original PCR primers or internal intron flanking primers (see Table 2 for the sequencing primers) with Bigdye Terminator Cycle

Sequencing Kit v2.0 (PE Applied Biosystems). Homology analysis of the sequencing data obtained from ABI Prism 377 DNA Sequencer (PE Applied Biosystems) were performed using MacDNASIS v2.0 (Hitachi).

Table 2. Primer pairs used for amplification and sequencing of WASP gene

Exon	PCR Primer Pairs	Sequencing Primers	Product Sizes (bp)
1-2	WAS-EXON1-1588F(S) CCTTGCTGCTCATTGCGGAAG WAS-INTRON2-2330R(AS) CTGAGGTCTTGAAGCTATGGAC	WAS-EXON1-1588F(S) CCTTGCTGCTCATTGCGGAAG WAS-2F(S) GTACCCCTGACCAGACTCCA W1R4(AS) GGAGATCCCCAGCT WAS-INTRON2-2330R(AS) CTGAGGTCTTGAAGCTATGGAC	755
3-7	WASINTRON2-3238F(S) ATCTCCAAACCAGACTATGAGGC WAS-INTRON7-4885R(AS) ACTCAGCCACCCAGCCATGTG	WAS-INTRON2-3238F(S) ATCTCCAAACCAGACTATGAGGC W6L-26(S) GTTGGTAAGTGGGTCAA WAS-INTRON6-4002R(AS) CCTCCATCCACTCTTACCCATC WAS-INTRON7-4885R(AS) ACTCAGCCACCCAGCCATGTG	1648
8-11	WAS-INTRON7-5787F(S) GAGAGGGCAAGAGGGTTTCAC WAS-INTRON11-7330R(AS) TATTGGTGGTGACTGCTGGGAT	WAS-INTRON7-5787F(S) GAGAGGGCAAGAGGGTTTCAC W9L-15(S) GAGAGTTACAGCTATGTGTT W10L-16(S) TAAGCCCTCTGTGCTGAT WAS-8B(AS) CTTGGGCTAGAGAAGGGAGC W9R+23(AS) CAGAGTCCTAGACCCCCAAT WAS-INTRON11-7330R(AS) TATTGGTGGTGACTGCTGGGAT	1544

12	W11L-10(S) GGGCATCTTATCTTTCTCTT W11R11(AS) ACAGGGCAGCAAGTAACT	W11L-10(S) GGGCATCTTATCTTTCTCTT W11R11(AS) ACAGGGCAGCAAGTAACT	110

(S) Sense Primer
(AS) Antisense Primer

RESULTS

Sequencing revealed 5 different types of novel mutations in 5 unrelated families (patient 1 to 7 , see Table 1 for details of cDNA mutations and amino acid codon changes). No mutation was detected in patient 8. Two families (patient 1, 5 and 6) demonstrated single nucleotide deletion. One family (patient 3 and 4) demonstrated small insertion. Two families (patient 2 and 7) were found to have different single nucleotide nonsense mutations. All the mutations found resulted in predicted synthesis of truncated WASP.

DISCUSSION

Using PCR-direct sequencing, we have successfully identified 5 novel mutations in 5 unrelated Chinese families. All the patients have the classical phenotype of WAS with phenotypic severity from score 4 to 5. Three mutations resulted in frameshift and all consequently caused a predicted synthesis of truncated WASP. We have no patient with the mild form, X-linked thrombocytopenia. This finding is also supportive for the observation that milder phenotypes tends to occur with missense mutations in the exon 1-4 (WH1 domain) (Schindelhauer et al., 1996). However, in one patient with phenotypic severity of score 5, no mutations was identified within the coding region and flanking splice sites of the WASP gene. We had also performed PCR-direct sequencing for the primary promotor region of WASP genomic DNA (Hagemann and Kwan, 1999) but no mismatch had been found (data not shown). Hidden mutation may be present in the regulatory regions or introns of WASP, or in other uncharacterized WAS causing genes. The effect of genetic heterogeneity may also be the cause (Rocca et.al., 1996). We had performed heterozygous carrier detection among the family members by PCR-direct sequencing according to the mutation identified. Clinical counselling was provided for the carriers. We also successfully performed pre-natal WAS molecular diagnosis for the pregnant mother of patient 4 by PCR-direct sequencing using genomic DNA extracted from chorionic villus biopsy.

Together with our previous report of a 11-bp deletion in exon 10 of WASP gene (Chan et al. 1999), we had identified a total of 6 novel mutations in Chinese patients with typical features of WAS. The finding supports that WAS mutated genotype is highly variable. However, more patients are required to be studied in order to see whether the uneven distribution of mutations, i.e. missense mutations preferentially located in the amino-terminal while nonsense and frameshift mutation in the carboxyl end of the protein, could be observed in Chinese patients.

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