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Spectrum of *WAS* gene mutations in Vietnamese patients with Wiskott–Aldrich syndrome

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Abstract

Background: *WAS* gene mutational analysis is crucial to establish a definite diagnosis of Wiskott–Aldrich syndrome (WAS). Data on the genetic background of WAS in Vietnamese patients have not been reported.

Methods: We recruited 97 male, unrelated patients with WAS and analyzed *WAS* gene mutation using Sanger sequencing technology.

Results: We identified 36 distinct hemizygous pathogenic mutations, with 17 novel variants, from 38 patients in the entire cohort (39.2%). The mutational spectrum included 14 missense, 12 indel, five nonsense, four splicing, and one non-stop mutations. Most mutations appear only once, with the exception of c.37C>T (p.R13X) and c.374G>A (p.G125E) each of which occurs twice in unrelated patients.

Conclusion: Our data enrich the mutational spectrum of the *WAS* gene and are crucial for understanding the genetic background of WAS and for supporting genetic counseling.

KEYWORDS
hemizygous mutation, novel variant, Vietnamese, *WAS* gene, Wiskott–Aldrich syndrome

INTRODUCTION

Wiskott–Aldrich syndrome (WAS; OMIM#301000) is a rare X-linked recessive immunodeficiency disorder characterized by thrombocytopenia and small-sized platelets, eczema, recurrent bacterial, and viral infections, and an increased risk of autoimmune manifestations and malignancies.[1] The gene responsible for WAS was sequenced in 1994. The *WAS* gene is composed of 12 exons with 1823 base pairs, which is mostly expressed in hematopoietic stem cells and plays critical roles in cytoskeleton-dependent cellular processes, immunological synapse formation, and cell migration. The WAS protein consists of a pleckstrin homology (PH) domain, an enabled vasodilator-stimulated phosphoprotein homology (EVH1, also known as WH1) domain at the amino terminal, a short basic domain (B), a Cdc42- and Rac-interactive binding (CRIB) domain, a large proline-rich region (PRR), and a verprolin/central/acidic (VCA) domain at the carboxyl terminal.[2–4]

Patients with WAS may have heterogenous clinical manifestations due to different mutations in the *WAS*

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gene. *WAS* mutations disrupt cytoskeleton-dependent cellular processes, immunological synapse formation, cell migration, and cell signaling, involved in pathogenesis of WAS.[1,3] It is the heterogenous clinical spectrum that makes the initial diagnosis of WAS difficult, and thus investigation of *WAS* mutations is required for a definitive diagnosis.[1,5] In this study, we screened for mutations in the *WAS* gene from 97 unrelated Vietnamese patients clinically diagnosed with WAS. We reported the mutation spectrum with some novel variants in the *WAS* gene. These molecular genetic findings provide an overview of the genetic background involved in Vietnamese WAS and will be useful for advanced genetic counseling.

METHODS

Subjects

Patients with clinical diagnosis of WAS were referred to Center for Molecular Biomedicine, University of Medicine and Pharmacy at Ho Chi Minh City for genetic testing from April 2015 until December 2022. All patients met the WAS diagnostic criteria, which included thrombocytopenia, eczema, and recurrent infections, as described.[6] The protocol for this study was approved by the ethics committee of the University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam (approval number 567/HDDD-DHYD). Written informed consent was obtained from parents or legal guardians of all minors.

Sample collection and DNA extraction

We collected 2 mL of peripheral blood with EDTA anticoagulant from each patient, then DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) and stored at −30°C.

Mutational analyses

Polymerase chain reaction (PCR) and sequencing primers were designed for analyzing the sequence of exons and exon-intron boundaries of *WAS* gene. *WAS* gene reference genomic sequence was obtained from the National Center for Biotechnology Information Consensus Coding Sequence (CDS) database, with accession number NG_007877.1 (https://www.ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi).

The 12 *WAS* exons and exon-intron boundaries were amplified by primers synthesized by IDT (Integrated DNA Technologies), and listed in Table 1. The PCRs (15 μL) contained 25–50 ng of genomic DNA, 0.5 U of Taq Hot Start Polymerase (Takara Bio), 0.1 μM each of forward and reverse primers, 200 μM each of dNTP, 1X PCR Buffer. The reactions were run in SimpliAmp Thermal Cycler (Thermo Scientific) and the annealing temperature was set to 60°C. The PCR products were analyzed on 1.5% agarose gel electrophoresis and then were purified with the ExoSAP-IT reagent (Thermo Scientific). The amplicons were direct sequenced by the Sanger method using Bigdye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) in both forward and reverse directions. The sequencing reactions were analyzed on an ABI 3500 genetic analyzer (Applied Biosystems).

Sequencing results were analyzed with CLC Mainworkbench v5.5 software based on transcript version NM_000377.3 and nucleotides were counted from the first ATG translation initiation codon, calling mutants according to J.T. den Dunnen nomenclature.[7] To determine the pathogenicity of novel identified variants, functional prediction software packages and databases were used, including PolyPhen-2, Clinvar, and Varsome.

RESULTS

A total of 97 male unrelated patients with WAS were recruited in this study. The average age of onset of disease

TABLE 1 Primer sequences for polymerase chain reaction amplification and sequencing.

Primer name	Primer sequence (5′–3′)	Length (bp)	Region
WAS-g1F	CTTCTTACCCTGCACCCAGA	720	Exons 1–2
WAS-g2R	GGTCTGAGGTCTTGAAGCTA		
WAS-g3F	CAGCTAACAAAAGCCTGCCA	1611	Exons 3–7
WAS-g7R	ACCACCCATTTACCCACTCA		
WAS-g8F	GGGTTTCACTATGAAGGGAG	546	Exons 8–9
WAS-g9R	GGACTGAGTGACTTAGTGCG		
WAS-g10F	TGAGCAAAACTGAGGCTCAG	939	Exons 10–11
WAS-g11R	TGGGGCTGATGTCACTATTG		
WAS-g12F	CTCTAGCATGAGACCTCAGA	307	Exon 12
WAS-g12R	AGGGATAACAGCATTGGAGG		

was 5.1 years (range, 1–12 years). All patients fulfilled the clinical diagnostic criteria for WAS. Through comprehensive genetic analysis, we identified 36 distinct hemizygous pathogenic mutations from 38 patients in the entire cohort (Table 2 and Figure 1). Most mutations appeared only once, with the exception of c.37C>T (p.R13X) and c.374G>A (p.G125E), each of which occurred twice in unrelated patients. In detail, the 36 distinct mutations included 14 missense, 12 indel, five nonsense, four splicing, and one non-stop mutations.

Although most missense mutations were located in the first four exons, other kinds of pathogenic variants were distributed widely in nearly every exon and their adjacent splice sites.

Notably, 17 out of 36 pathogenic mutations are novel. The indel group has the highest proportion of novel

TABLE 2 Pathogenic mutations found in the *WAS* gene among 38 patients.

				Pathogenic variants	
No	**Code**	**Age (years)**	**Region**	**Nucleotide change**	**Amino acid change**
1	WAS-32	1	Exon 1	c.35_36insG	p.R13fsX25
2	WAS-14	1		c.37C>T	p.R13X
3	WAS-22	2		c.37C>T	p.R13X
4	WAS-6	1		c.91G>A	p.E31K
5	WAS-29	1		c.92A>G	p.E31G
6	WAS-61	1		c.97C>T	p.Q33X
7	WAS-38	1		c.121C>T	p.R41X
8	WAS-39	1	Exon 2	c.134C>T	p.T45M
9	WAS-99	3		c.151G>C	p.V51L
10	WAS-12	1		c.217T>C	p.C73R
11	WAS-105	1		c.245C>A	p.S82Y
12	WAS-36	2		c.256C>T	p.R86C
13	WAS-65	4		c.257G>A	p.R86H
14	WAS-90	2	Exon 3	c.298_312del	p.E100_Q104del
15	WAS-51	1		c.320A>G	p.Y107C
16	WAS-21	1	Exon 4	c.374G>A	p.G125E
17	WAS-40	1		c.374G>A	p.G125E
18	WAS-30	1		c.374G>T	p.G125V
19	WAS-AP5	1		c.375_376insGG	p.L126fsX2
20	WAS-AP1	1		c.397G>A	p.E133K
21	WAS-88	3		c.402_403insGAGGCC	p.A134_Q135insEA
22	WAS-108	1		c.404A>C	p.Q135P
23	WAS-AP4	1		c.436C>T	p.Q146X
24	WAS-5	1		c.461delG	p.G154fsX107
25	WAS-60	1	Intron 6	IVS6+1G>T	
26	WAS-64	2		IVS6+5G>T	
27	WAS-AP2	1	Exon 7	c.665delC	p.P222fsX39
28	WAS-17	4	Intron 8	IVS8+1_+4delGTGA	
29	WAS-83	6		IVS8+1G>A	
30	WAS-43	10	Exon 9	c.847G>A	p.D283N
31	WAS-24	12		c.913C>T	p.Q305X
32	WAS-100	1	Exon 10	c.1058delC	p.P353fsX92
33	WAS-26	1		c.1148_1149insC	p.P384fsX111
34	WAS-97	2		c.1157delC	p.P386fsX59
35	WAS-AP3	1		c.1271_1272insG	p.L425fsX70
36	WAS-87	1		c.1320_1321>TT	p.Q440fsX2
37	WAS-35	4	Exon 11	Exon 11 deletion	
38	WAS-9	1	Exon 12	c.1507T>C	p.X503R

mutations: only three of the 12 detected mutations have been previously reported, namely p.P222fsX39,[8] p.P353fsX92,[1,2] and p.L425fsX70 mutations.[1,9] Among the nine novel indel mutations, there are two inframe and seven frameshift mutations. Both inframe mutations (p.E100_Q104del and p.A134_Q135insEA) were predicted by Varsome to be likely pathogenic, while the seven novel frameshift indels lead to premature termination protein synthesis and were thus considered as pathogenic. The exon 11 deletion is considered as novel mutation because it is a 306 bp deletion of genomic DNA region that contains entire exon 11 (Figure 2), unlike an intron 11 splicing mutation leading to exon 11 skipping in mRNA transcript previously reported.[10]

Regarding missense mutations, we identified six novel variants including p.V51L, p.S82Y, p.G125V, p.G125E, p.Q135P, and p.D283N. In WAS, other amino acid substitutions have been reported at codons V51, S82, and G125, such as V51F,[11] S82P,[2,12] S82F,[13] and G125R,[2,13] strongly supporting the pathogenicity of novel variants at these sites. In contrast, there have not been any reports of amino acid substitutions at two codons Q135 and D283 in previous studies. Nevertheless, all six of these novel missense mutations have a PolyPhen-2 score of 1.0, predicted to be pathogenic on *WAS*.

Among four splicing mutations, only the IVS6+5G>T is novel. This mutation was predicted by Varsome to be likely pathogenic. Furthermore, IVS6+5G>A has been documented in some well-known previous studies.[2,10] This evidence strongly suggests that IVS6+5G>T is a pathogenic mutation. Unfortunately, we lack mRNA from the patient sample to investigate the splicing effect of the IVS6+5G>T.

Finally, four out of five nonsense mutations (p.R13X, p.Q33X, p.R41X, and p.Q305X) were reported elsewhere.[2,8,11,14] The remaining mutation, p.Q146X, has not been recorded in the literature before. In fact, change at codon Q146 has not been documented in WAS.

To know whether mutations found in the patients were inherited or de novo, we tested eight mothers for the known mutation in their children. These cases include four previously reported mutations (p.E31K, p.E31G, p.R41X, and p.P353fsX92) and four novel mutations (p.R13fsX25, p.G125V, p.G154fsX107, and p.P384fsX111). All mothers tested were found to be carriers (data not shown).

DISCUSSION

The *WAS* gene mutation analysis is crucial for establishing a final diagnosis of WAS. At present, the only

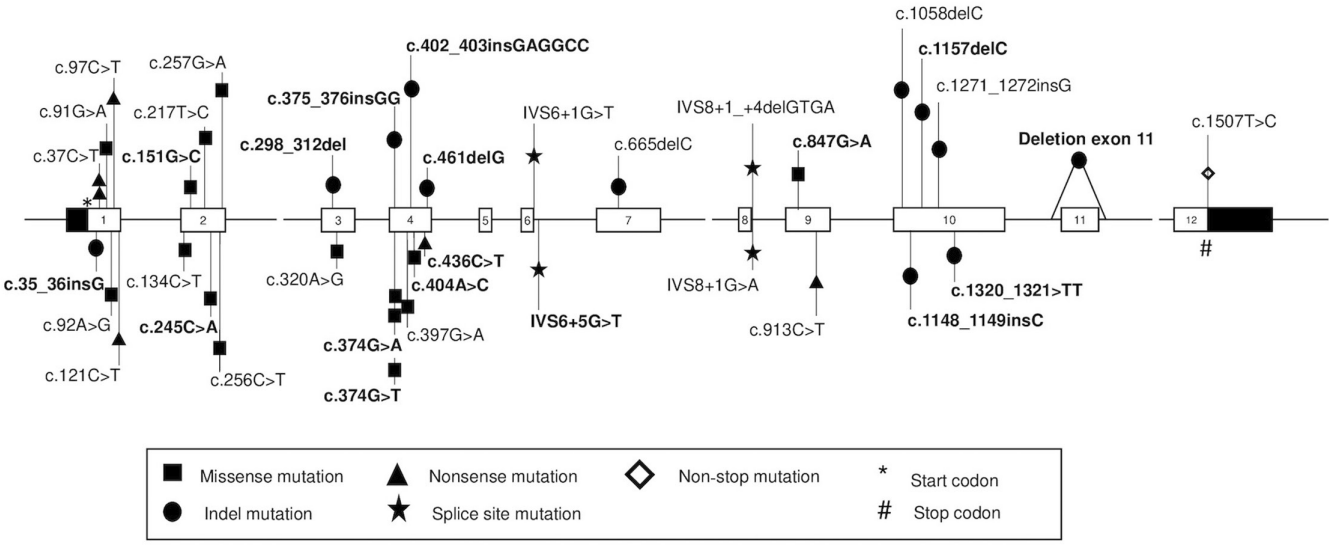

FIGURE 1 Mutation spectrum of the *WAS* gene in this study. The novel identified variants are shown in bold characters. Numbers in boxes indicate exon order.

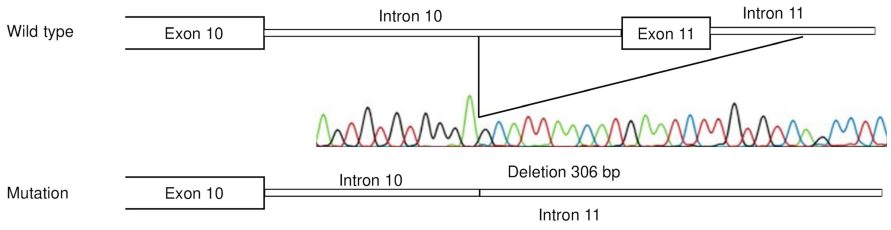

FIGURE 2 The large deletion contains exon 11 of the *WAS* gene.

curative therapy is hematopoietic stem cell transplantation,[15] with good results for patients with a human leukocyte antigen (HLA)-identical donor. Gene therapy is promoted as a valuable alternative treatment and is used to establish WAS prevention strategies.[16,17] We investigated mutations of the *WAS* gene in 97 Vietnamese patients with clinical diagnosis of WAS and revealed that 39.2% (38/97) of patients had the disease-causing mutation. This limited mutation detection rate may be due to poor clinical diagnosis, or due to defects in other genes causing overlap clinical pictures. In fact, Wiskott–Aldrich syndrome protein Interacting Protein (WIP) deficiency lead to similar clinical manifestation with that of patients with WAS protein deficiency.[18]

Although limited by a small number of mothers analyzed for mutations, this study pointed out that *WAS* mutations are inherited, not de novo, as observed in the *RB1* gene mutations causing retinoblastoma in Vietnamese patients.[19] A wide spectrum of mutations, including missense, nonsense, indel, splicing, and even nonstop mutations, were found in *WAS* and distributed over the entire coding region. More interestingly, a significant proportion—roughly half—of the mutations were novel, leading to the complex task of interpreting variants in each unique patient as well as suggesting that DNA sequencing is the most suitable method for searching *WAS* mutations. Among the novel missense mutations, codons Q135 and D283 were first found to be altered in WAS, implying that they are two among the major functional residues of the WAS protein. In addition, some mutated codons have been identified twice or more, including R13, E31, R86, and G125, suggesting that they are mutational hotspots in Vietnamese patients.

Non-stop mutations are relatively rare in genetic disorders, recorded in less than 100 genes.[20] We identified the case of a 1 year-old patient with a p.X503R mutation, which has been reported in Slovak and Thai populations.[21,22] Further research is required to determine the exact mechanism by which a non-stop mutation of the *WAS* gene leads to WAS.

Missense and splicing mutations are frequently associated with milder WAS symptoms, whereas nonsense and frameshift mutations are frequently associated with severe WAS symptoms.[14,23] However, in this study, due to a lack of clinical data we were unable to assess the association between mutation types and disease severity.

In conclusion, we have reported the unique *WAS* gene variant spectrum from 97 Vietnamese patients with WAS. With 36 distinct hemizygous pathogenic mutations including 17 novel ones, our data enriches the mutational spectrum of *WAS* gene and are crucial for an understanding of the genetic background of WAS and for supporting genetic counseling.

AUTHOR CONTRIBUTIONS

Ho Quoc Chuong, Phan Thi Xinh, and Hoang Anh Vu designed the study. Ho Quoc Chuong, Duong Bich Tram, Nguyen Thi Thanh Ha, and Phan Nguyen Lien Anh performed experiments. Tuan Minh Nguyen, Nguyen Dinh Van, Nguyen Hoang Mai Anh, Phu Chi Dung, Huynh Nghia, and Hoang Anh Vu collected and analyzed data. Ho Quoc Chuong, Phan Thi Xinh, and Hoang Anh Vu wrote the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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