

BRIEF REPORT

The Genotype of the Original Wiskott Phenotype

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SUMMARY

The Wiskott–Aldrich syndrome is an X-linked hereditary disorder associated with combined immunodeficiency, thrombocytopenia, small platelets, eczema, and increased susceptibility to autoimmune disorders and cancers. It is caused by mutations in the gene (*WAS*) for the Wiskott–Aldrich syndrome protein (WASP). We investigated family members of the patients originally described by Wiskott in 1937 and identified a new frame shift mutation in exon 1 of *WAS*. This mutation is likely to be the hypothesized genotype that caused the severe form of the Wiskott–Aldrich syndrome in the three brothers described by Wiskott.

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N Engl J Med 2006;355:1790-3.
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IN 1937, ALFRED WISKOTT, A GERMAN PEDIATRICIAN, DESCRIBED THREE brothers who presented shortly after birth with thrombocytopenia, bloody diarrhea, eczema, and recurrent ear infections; all three died early in life from intestinal bleeding and sepsis. Wiskott commented that “the origin of the hemorrhagic diathesis is a dysfunction in the line of the platelets.”¹ The observation that all three brothers were affected, whereas their sisters showed no symptoms, led Wiskott to propose that the syndrome is due to a “hereditary thrombopathia.”¹ In 1954, Aldrich et al. traced six generations of a family and found that 16 of 40 males, but no females, died of the syndrome first described by Wiskott, thus clearly showing an X-linked mode of inheritance.²

The Wiskott–Aldrich syndrome is now known as an X-linked hereditary disorder associated with combined immunodeficiency, thrombocytopenia, small platelets, eczema, and an increased risk of autoimmune disorders and cancers. It has a broad range of phenotypes (Online Mendelian Inheritance in Man no. 301000).³⁻⁶

The severe form of the Wiskott–Aldrich syndrome and its milder manifestations — X-linked thrombocytopenia and X-linked neutropenia — are caused by mutations in the gene for the Wiskott–Aldrich syndrome protein (*WAS*), located at Xp11.22–p11.23 and cloned in 1994.⁷⁻¹⁰ *WAS* and several related proteins are involved in the reorganization of the actin cytoskeleton by activating the actin-related protein 2/3 complex that mediates actin polymerization in all cells of the hematopoietic system.^{3,11-15} Mutations in the *WAS* gene result in truncated or absent WASP in these cells, but there is no strict correlation between the mutant genotype and the expression of WASP or the phenotype of the syndrome.^{11,15-18} The disorder can be cured through hematopoietic stem-cell transplantation.¹⁹

We recruited members of the family described by Wiskott in 1937 in order to identify the hypothesized mutation in *WAS* that caused the severe phenotype of the Wiskott–Aldrich syndrome in the three brothers. Genetic testing for the mutation was carried out in three generations of the kindred.

METHODS

After obtaining written informed consent, we performed mutation analyses in Subjects III-6, III-8, III-9, III-10, IV-2, IV-3, V-1, and V-2 (Fig. 1). Genomic DNA was extracted from peripheral white cells. After amplification, WAS exons 1 through 12 were analyzed with the single-strand conformation polymorphism method and the aberrant fragment of exon 1 was investigated by means of double-strand sequencing. All other WAS exons from Subjects III-10 and V-1 were re-analyzed through direct sequencing (with the use of a kit from Applied Biosystems). The primer sequences used for amplification and the methods used for the screening of mutations have been described previously.¹⁸ A WAS mutation was ruled out in 400 controls (200 men and 200 women) by means of denaturing high-performance liquid chromatography with the Wave system (Transgenomics); the aberrant fragment was observed in DNA from a female carrier, analyzed with the use of buffer B (on a gradient of 53 to 61%) at a running temperature of 64°C.^{20,21} To study the expression of the mutant allele, RNA was isolated from an obligate female carrier and transcribed into complementary DNA (cDNA) with the use of a first-strand cDNA synthesis kit (Amersham Biosciences). The cDNA was sequenced with the use of a pair of primers: one located in the 5' untranslated region (1cF: 5'TCGCCAGAGAAGAC-AAGGGC3') and one in exon 3 (3cR: 5'CATCTCCAGCGAAGGTGTGG3').

RESULTS

Genetic testing for the mutation revealed a deletion of two nucleotides at positions 73 and 74 in WAS exon 1 (coding sequence, 73–74delAC^{22,23}; the first nucleotide is the A of the ATG translation-initiation codon). This mutation is not listed in WASPbase,²⁴ an Internet-based database of WAS mutations. The deletion results in a frame shift that starts with amino acid 25; the shifted reading frame is open for another 11 amino acids before it results in a stop codon (protein sequence, Thr25ProfsX12^{22,23}) (Fig. 2).

To further characterize the 73–74delAC mutation in the coding sequence, we first sought it in 400 normal subjects (200 men and 200 women) serving as controls; none carried the mutation. This result makes it improbable that the mutation is a polymorphic variant in the normal popula-

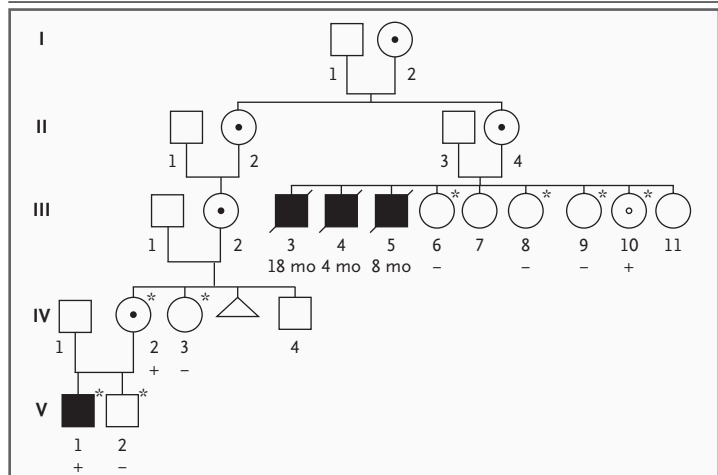


Figure 1. Pedigree of the Three Brothers (Subjects III-3, III-4, and III-5) Described by Wiskott in 1937.

Open symbols represent unaffected family members, solid squares affected men, circles with solid dots obligate female carriers, the circle with an open dot a female carrier identified by gene sequencing, the triangle an aborted fetus, symbols with a slash deceased affected family members, asterisks family members who were genetically tested, a minus sign family members with a negative result on genetic testing for the mutation, and a plus sign family members with a positive result. A positive result indicated that the subject was a carrier of the 73–74delAC mutation in the coding sequence. The age at death is indicated under the symbols representing the three brothers who were originally described by Wiskott.

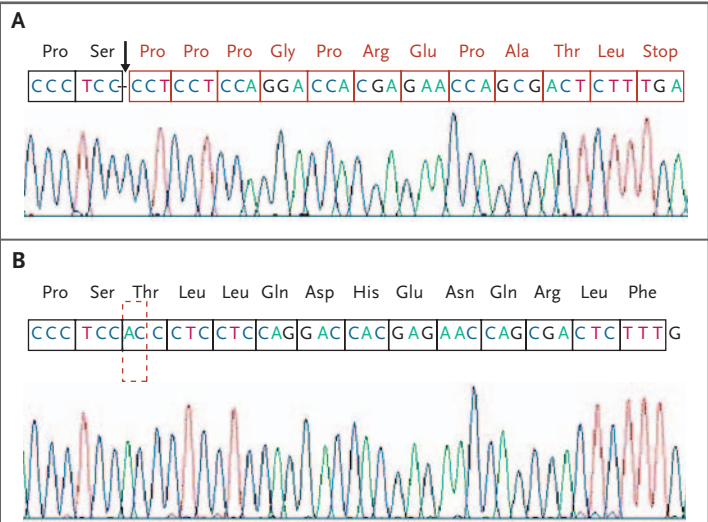


Figure 2. Chromatograms Showing the Mutation in Subject V-1 (Panel A) and the Wild-Type Sequence in the Same Subject after Stem-Cell Transplantation (Panel B).

In Panel A, the black boxes delineate nucleotide triplets upstream of the mutation; the arrow and dashed black line indicate the two nucleotides (AC) in positions 73 and 74 that are deleted in affected persons, causing a frame shift; and the red boxes delineate the nucleotide triplets in the new open reading frame. Panel B shows the position of the nucleotides in the wild type (outlined by dashed box).

tion. Next, we performed RNA analysis in a female carrier (Subject IV-2). She was heterozygous for the aberrant variant in exon 1. However, after the sequencing of exons 1 through 3 of the generated cDNA, we observed monoallelic expression, which indicates the decay of nonsense-mediated messenger RNA (mRNA) (the destruction of mRNA with a premature stop codon) (Fig. 3). This observation is a sign that the 73–74delAC mutation in the coding sequence is likely to result in the complete absence of WASP in affected men.²⁵

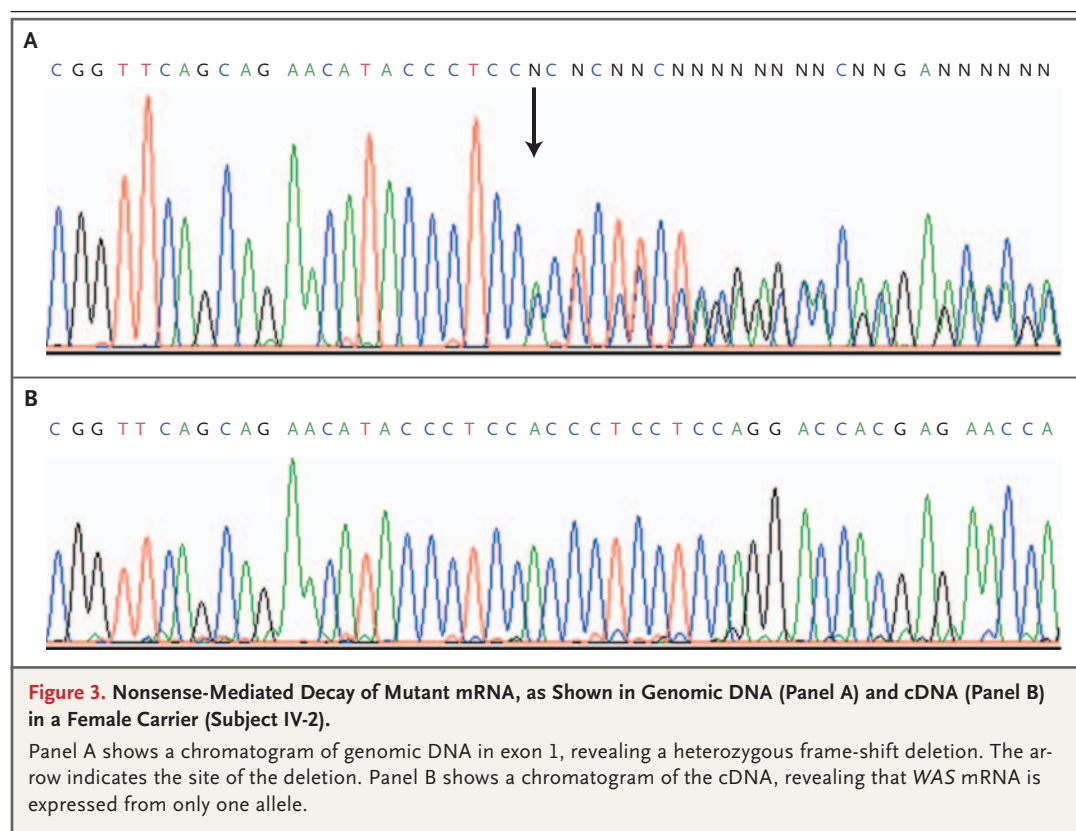
We identified this mutation in three generations of the pedigree: Subjects III-10, IV-2, and V-1. Subject V-1 had presented with symptoms compatible with the severe form of the Wiskott–Aldrich syndrome: bloody diarrhea, severe infections, eczema, and thrombocytopenia with small platelets. He is alive and well after receiving a hematopoietic stem-cell transplant from an HLA-matched unrelated donor (Fig. 2B). Subject III-10, a sister of the three affected brothers described by Wiskott (Subjects III-3, III-4, and III-5), was identified as a carrier of the mutation, but three

of her sisters do not carry the mutation. As expected, Subject IV-2, the mother of Subject V-1, also carries the X-linked mutation (Fig. 1).

DISCUSSION

Our analysis indicates that Subjects II-2 and II-4 were obligate carriers of the 73–74delAC mutation in the coding sequence after they inherited either a germ-line WAS mutation from one of their parents (Subjects I-1 and I-2) or the mutation from their mother (Subject I-2, the grandmother of Wiskott's patients), who could have been a silent carrier of an ancestral mutation. Since Subjects III-10 and IV-2 are carriers of the mutation, Subject III-2 must be an obligate female carrier.

Our findings indicate the improbability of a spontaneous mutation in Subject V-1 and provide strong evidence that the three affected brothers (Subjects III-3, III-4, and III-5) also had the 73–74delAC mutation in the coding sequence of WAS. Almost 70 years after Wiskott's initial clinical description of the Wiskott–Aldrich syndrome in



three brothers, we found that a mutation in the X-linked WAS gene caused the severe phenotype.

Supported by Deutsche Krebshilfe.

No potential conflict of interest relevant to this article was reported.

We are indebted to Drs. W. Friedrich and K. Schwarz, Department of Pediatrics, University Hospital Ulm, Ulm, Germany, for providing information about the molecular analysis and stem-cell transplantation performed in Subject V-1, as well as for providing blood samples for additional analysis.

REFERENCES

1. Wiskott A. Familiärer, angeborener Morbus Werlhofii? *Monatsschr Kinderheilkd* 1937;68:212-6.
2. Aldrich RA, Steinberg AG, Campbell DC. Pedigree demonstrating a sex-linked recessive condition characterized by draining ears, eczematoid dermatitis and bloody diarrhea. *Pediatrics* 1954;13:133-9.
3. Ochs HD, Rosen FS. The Wiskott-Aldrich syndrome. In: Ochs HD, Smith CIE, Puck JM, eds. *Primary immunodeficiency diseases: a molecular and genetic approach*. New York: Oxford University Press, 1999:292-305.
4. Thrasher AJ, Kinnon C. The Wiskott-Aldrich syndrome. *Clin Exp Immunol* 2000;120:2-9.
5. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott-Aldrich syndrome. *J Pediatr* 1994;125:876-85.
6. Belohradsky BH, Griscelli C, Fundenberg HH, Marget W. The Wiskott-Aldrich syndrome. *Ergeb Inn Med Kinderheilkd* 1978;41:85-184. (In German.)
7. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. *Cell* 1994;78:635-44.
8. Derry JM, Kerns JA, Weinberg KI, et al. WASP gene mutations in Wiskott-Aldrich syndrome and X-linked thrombocytopenia. *Hum Mol Genet* 1995;4:1127-35.
9. Devriendt K, Kim AS, Mathijs G, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. *Nat Genet* 2001;27:313-7.
10. Ancliff PJB, Gale RE. Activating mutations in the Wiskott-Aldrich syndrome protein may define a sub-group of severe congenital neutropenia (SCN) with specific and unusual laboratory features. *Blood* 2001;98:439. abstract.
11. Ochs HD, Notarangelo LD. Structure and function of the Wiskott-Aldrich syndrome protein. *Curr Opin Hematol* 2005;12:284-91.
12. Burns S, Cory GO, Vainchenker W, Thrasher AJ. Mechanisms of WASp-mediated hematologic and immunologic disease. *Blood* 2004;104:3454-62.
13. Notarangelo LD, Ochs HD. Wiskott-Aldrich Syndrome: a model for defective actin reorganization, cell trafficking and synapse formation. *Curr Opin Immunol* 2003;15:585-91.
14. Thrasher AJ. WASp in immune-system organization and function. *Nat Rev Immunol* 2002;2:635-46.
15. Imai K, Nonoyama S, Ochs HD. WASP (Wiskott-Aldrich syndrome protein) gene mutations and phenotype. *Curr Opin Allergy Clin Immunol* 2003;3:427-36.
16. Imai K, Morio T, Zhu Y, et al. Clinical course of patients with WASP gene mutations. *Blood* 2004;103:456-64.
17. Jin Y, Mazza C, Christie JR, et al. Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation. *Blood* 2004;104:4010-9.
18. Schindelhauer D, Weiss M, Hellebrand H, et al. Wiskott-Aldrich syndrome: no strict genotype-phenotype correlations but clustering of missense mutations in the amino-terminal part of the WASP gene product. *Hum Genet* 1996;98:68-76.
19. Filipovich AH, Stone JV, Tomany SC, et al. Impact of donor type on outcome of bone marrow transplantation for Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program. *Blood* 2001;97:1598-603.
20. Underhill PA, Jin L, Lin AA, et al. Detection of numerous Y chromosome biallelic polymorphisms by denaturing high-performance liquid chromatography. *Genome Res* 1997;7:996-1005.
21. Ramser J, Abidi FE, Burckle CA, et al. A unique exonic splice enhancer mutation in a family with X-linked mental retardation and epilepsy points to a novel role of the renin receptor. *Hum Mol Genet* 2005;14:1019-27.
22. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations: a discussion. *Hum Mutat* 2000;15:7-12.
23. Human Genome Variation Society. Recommendations for the description of sequence variants. (Accessed September 29, 2006, at <http://www.hgvs.org/mutnomen/recs.html>.)
24. Imai K. WASPbase: database of published WAS gene mutations, updated 29 April 2004. (Accessed September 29, 2006, at <http://homepage.mac.com/kohsukeimai/wasp/WASPbase.html>.)
25. Maquat LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. *Nat Rev Mol Cell Biol* 2004;5:89-99.

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