

# Unique and Recurrent WAS Gene Mutations in Wiskott-Aldrich Syndrome and X-Linked Thrombocytopenia

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**Abstract:** Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are allelic phenotypes caused by defects of the WAS gene. Fourteen distinct mutations including seven novel gene defects in 16 WAS and four XLT patients were identified by single strand conformation polymorphism analysis and DNA sequencing of the WAS gene. Eleven (79%) of these mutations are located within exons 1 to 4 with clustering in exon 2. Carrier detection in 33 at-risk females and prenatal diagnosis at 12 weeks gestation in one family with a novel WAS mutation was performed by direct mutation analysis. A remarkably high frequency (72%) of point mutations involved CpG dinucleotides. C→T or G→A transitions at CpG sites were identified in all isolated WAS cases (n=7). Allele frequencies for the dinucleotide repeat at locus DXS6940 were determined in Northern European, African and Asian populations. Mutation screening alone or in combination with analysis of polymorphic loci DXS6940 and DXS255 delineated the germline origin of a unique insertion mutation and four recurrent CpG mutations, three of which arose spontaneously during maternal gametogenesis.

**Keywords:** Wiskott-Aldrich syndrome, X-linked thrombocytopenia, X-linked disease, mutation, prenatal diagnosis, CpG, polymorphism

## INTRODUCTION

Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) are allelic disorders caused by mutations in the WAS gene (1-10; MIM 301000). Thrombocytopenia accompanied by reduced platelet volume and survival (11) is common to both disorders. Classical WAS is distinguished by the presence of immunodeficiency and eczema. Intermediate phenotypes, for which the term attenuated WAS has been designated, are also recognized.

The WAS gene comprises 12 exons spanning 9kb of genomic DNA located to Xp11.23-p11.22 (12, 13; GenBank accession numbers U19927, U12707). The precise function of the 502 amino acid WAS protein remains unknown despite the identification of several unique binding domains. The WAS protein localizes predominantly in the cytoplasm of peripheral blood mononuclear cells and platelets (14) and contains a consensus binding sequence (amino acids 238-257) for the guanosine triphosphatase binding protein Cdc42Hs implicated in the regulation of

cytoskeletal organization (15). Other domains identified include a proline rich region (amino acids 311-422) thought to behave as a Src homology 3 binding site (16), an acidic region (amino acids 485-502) and sequences conserved in proline rich proteins known to be involved in the organization of the actin cytoskeleton (17). One such domain resides at the amino terminal, WASP-homology domain 1 (WH1, amino acids 47-137) and another at the carboxy terminal, WASP-homology domain 2 (WH2, amino acids 423-449) of the WAS protein.

The causative WAS gene mutations in 20 unrelated families with variable disease phenotypes were characterized. Novel mutations identified include four missense, two frameshift and one large deletion. Mutation analysis in 33 at-risk females detected 18 carriers of either WAS or XLT enabling prenatal diagnosis to be performed at 12 weeks gestation in one family with a novel WAS mutation.

Allele frequencies for the short tandem repeat (STR) at locus DXS6940 (18) within 30kb of the WAS gene were determined in three ethnic

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groups (Northern European, African and Asian). Three novel alleles were found in addition to the three previously described. Haplotype analysis performed in 17 WAS and XLT extended families established the informativity of the DXS6940 STR which was compared with that of the variable number tandem repeat (VNTR) at locus DXS255 (Xp11.3-Xcen; 19).

## MATERIALS AND METHODS

### *Patients and Isolation of Genomic DNA*

Twenty unrelated Northern European (British Isles) male patients, 16 with WAS and four with XLT were studied. Ten cases were familial. Eight patients had classical WAS characterized by thrombocytopenia, reduced mean platelet volume (MPV), eczema and evidence of immune deficiency. A further eight patients exhibiting a milder hemorrhagic diathesis associated with persistent thrombocytopenia, reduced MPV, mild eczema and/or increased frequency of infection were defined as attenuated WAS. Genomic DNA was extracted from blood samples obtained after informed consent from patients, family members and 130 unrelated normal subjects. Chorionic villus sampling was undertaken at 12 weeks gestation and DNA extracted from pooled villi.

### *DNA Amplification and Single Strand Conformation Polymorphism (SSCP) Analysis*

All 12 exons and flanking splice sites of the WAS gene were amplified by the polymerase

chain reaction (PCR) method using oligonucleotide primers shown in Table 1. Each 30  $\mu$ l PCR reaction contained 60 pmol of each primer, 200  $\mu$ M of each dNTP, 1U *Taq* DNA polymerase (Promega, Southampton, UK), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1  $\mu$ Ci  $\alpha$ -<sup>33</sup>P-dCTP (Dupont, Stevenage, UK) and 0.5-1.0  $\mu$ g of genomic DNA. Dimethyl sulfoxide (10%) was included in the reaction mixture for amplification of exons 7, 8, 9 and 11. PCR conditions were initial denaturation for 5 min at 94 C followed by 35 cycles of denaturation for 1 min at 94 C, annealing for 1 min at 54 to 62 C depending on the primers used (Table 1), extension for 1 min at 72 C with a final 10 min extension at 72 C. PCR products for exons 3/4 and 10 were digested with a restriction enzyme (Table 1), prior to SSCP gel electrophoresis. PCR amplified DNA was mixed in a 1:5 ratio with loading buffer (80% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol, 1 mM EDTA and 10 mM NaOH) denatured at 95 C for 5 min, rapidly cooled on ice and 3  $\mu$ l aliquots applied to a non-denaturing 4.5% polyacrylamide gel. Electrophoresis was at constant power (20W) and temperature (4 C). SSCP gels were transferred to Whatman 3MM paper, dried at 80 C and exposed to X-ray film (Kodak Biomax, Anachem Limited, Luton, UK) at room temperature.

### *DNA Sequencing*

PCR product was either cloned into a TA vector (LigATor; R&D Systems Europe Ltd,

**Table 1.** Primers for DNA amplification and sequencing

Name	5' Primer	3' Primer	Exon(s) amplified	Annealing temperature <sup>a</sup>	Size (bp) <sup>b</sup>	Restriction endonuclease <sup>c</sup>
WASPE1	CCAGAGAAGACAAGGGCA	AGAAACGGTGGGGACG	1	62	204	-
WASPE2	CCTGACCAGACTCCACTACC	GGTTGAGAAGCTGGCTTGC	2	58	224	-
WASPE3/4	AGTGCCACTGTGCCTCC	CCAACTTCCTTCTCTCCC	3/4	60	358	<i>Ava</i> II
WASPE5/6	GGCTCCAATCCATCTATC	ACTCTTACCCATCCATCCAG	5/6	62	262	-
WASPE7	GTTGGTAAGTGGGTCAATAGC	CCAGGAATCTGTGGGTCC	7	54	254	-
WASPE8	TGAGTCTCTTACCTCTCCC	GAGTCCAAGAGACTCTGAAGTTAC	8	54	92	-
WASPE9	GGCCTTTTCTCTCTGG	GCGTATGGAAGCAGGGTC	9	54	197	-
WASPE10	GAAATCAATGAGAGTTACAGCTATG	CAGAGTCTAGACCCCAATC	10	60	495	<i>Rsa</i> I
WASPE11	CCTAAGCCCTCTGTGCTG	TGGAGCCAGGTTTGAG	11	60	187	-
WASPE12	GCATCTTATCTTCTCTTCCC	GTTGGTGGGGAAGAAATG	12	54	208	-

<sup>a</sup>PCR annealing temperature (C) and <sup>b</sup>product size are shown. <sup>c</sup>Restriction enzymes used to digest PCR amplified DNA fragments are also indicated.

Abingdon, UK) or purified (Wizard minicolumns; Promega) prior to sequencing. Double stranded DNA sequencing incorporating sense or antisense PCR amplification primer (Table 1), 5'-end labelled with  $\gamma$ - $^{32}$ P-ATP (10  $\mu$ Ci; Amersham, Little Chalfont, UK), was by dideoxy cycle sequencing (fmol, Promega). PCR products were resolved by electrophoresis on an 8% denaturing polyacrylamide gel, dried and exposed to X-ray film (Kodak Biomax) at  $-80$  C overnight. All mutations were confirmed by restriction or amplification refractory mutation system analysis of PCR amplified genomic DNA.

#### *Characterization and Analysis of Locus DXS6940 STR*

The STR at locus DXS6940 was PCR amplified from 0.5-1.0  $\mu$ g of genomic DNA using the oligonucleotide primers described (18). Samples sequenced to determine the length of the dinucleotide repeat (CA)<sub>n</sub> were used as DNA size markers. Each 50  $\mu$ l PCR contained 30 pmol of each primer, 200  $\mu$ M of each dNTP, 2U *Taq* DNA polymerase (Promega), 1.5 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, and the PCR primer 5'-end  $^{32}$ P-labelled (1.7  $\mu$ Ci  $\gamma$ - $^{32}$ P-ATP, Amersham). Initial denaturation was at 94C for 5 min followed by 25 cycles denaturation at 94 C for 1 min, annealing at 54 C for 1 min and extension at 72 C for 1 min with a final extension at 72 C for 6 min. PCR product (30  $\mu$ l) was concentrated and loading buffer added to a total volume of 15  $\mu$ l prior to electrophoresis on an 8% denaturing polyacrylamide gel. The gel was dried and exposed to X-ray film (Kodak Biomax) at  $-80$  C overnight.

#### *Analysis of Locus DXS255 VNTR*

Genomic DNA (6  $\mu$ g) was restricted with *Pst* I at 37 C overnight. Digestion products were resolved by agarose gel (0.8%) electrophoresis in tris borate ethylenediaminetetraacetic acid buffer, pH 7.2, at 30-50V for 24-30 hours. DNA was

transferred to hybond N (Amersham) by Southern blot using an alkali transfer method. The nylon membrane was prehybridized for 4 hours at 65 C in 2 x SSC (1 x SSC = 150mM sodium chloride, 15mM sodium citrate, pH 7.0), 10 x Denhardtts, 0.1% sodium dodecyl sulfate (SDS) and 100  $\mu$ g/ml single stranded salmon sperm DNA (Life Technologies Ltd, Paisley, UK). The probe, a 1.9kb *EcoR* I/*Hind* III fragment of M27 $\beta$  cloned into pUC 9, was labelled with  $\alpha$ - $^{32}$ P-dCTP using an oligo labelling kit (Pharmacia biotech, St. Albans, Hertfordshire, UK). The blot was hybridized at 65 C overnight in fresh prehybridization buffer containing denatured probe. The filter was then washed with 2 x SSC, 0.1% SDS at 65 C for 30 min and exposed to X-ray film (Kodak Biomax) at  $-80$  C overnight.

## RESULTS

### *Mutation Screening*

All WAS and XLT patients showed abnormal banding patterns after SSCP analysis of WAS gene PCR product. In each case a single PCR or digestion product migrated abnormally on SSCP analysis. Direct sequencing of PCR product identified 14 different WAS gene mutations (Table 2). Eleven mutations lie within exons 1 to 4, six of these in exon 2. Seven novel mutations include four missense mutations (Fig. 1; patients 9, 14, 15 and 17), two single base insertions (patients 6 and 19) and a large DNA deletion (Table 2; patient 20). Novel missense mutations were excluded as neutral polymorphisms by PCR-SSCP analysis of 50 X-chromosomes from normal subjects. A single guanine insertion after nucleotide 218 at codon 62 (Fig. 1, patient 6) results in a frameshift leading to premature termination 16 codons downstream. Insertion of cytosine within consecutive cytosines at codons 326-328 CTC CCC CGG (Fig. 1, patient 19) creates a stop signal at codon 335. The deletion identified in one case (patient 20) spans the

**Table 2.** Mutations in WAS and XLT identified in subjects from 20 unrelated families

Patient	Phenotype	Family history	Exon	Nucleotide position <sup>a</sup>	Amino acid substitution	CpG site	Restriction endonuclease <sup>e</sup>
1	attenuated	no	1	134C→T	Arg34TERM	yes	<i>Tsp45</i> I (+)
2	attenuated	no	2	168C→T	Thr45Met	yes	<i>Hga</i> I (-)
3	attenuated	yes	2	168C→T	Thr45Met	yes	<i>Hga</i> I (-)
4	XLT	yes	2	168C→T	Thr45Met	yes	<i>Hga</i> I (-)
5	XLT	yes	2	168C→T	Thr45 Met	yes	<i>Hga</i> I (-)
6	classical	no	2	<b>218-219insG<sup>b</sup></b>	<b>Glu62Gly78TERM<sup>d</sup></b>	no	<i>Dde</i> I (-)
7	classical	yes	2	257G→A	Val75Met	yes	<i>Nla</i> III (+)
8	XLT	yes	2	257G→A	Val75Met	yes	<i>Nla</i> III (+)
9	attenuated	yes	2	<b>284T→C<sup>b</sup></b>	<b>Phe84Leu</b>	no	<i>Mnl</i> I (+)
10	XLT	yes	2	290C→T	Arg86Cys	yes	<i>Fok</i> I (-)
11	attenuated	no	2	291G→A	Arg86His	yes	<i>Bgl</i> I (-)
12	attenuated	yes	2	291G→A	Arg86His	yes	<i>Bgl</i> I (-)
13	classical	no	2	291G→A	Arg86His	yes	<i>Bgl</i> I (-)
14	classical	yes	3	<b>365A→C<sup>b</sup></b>	<b>Thr111Pro</b>	no	- <sup>f</sup>
15	attenuated	yes	3	<b>377C→T<sup>b</sup></b>	<b>His115Tyr</b>	no	<i>Xcm</i> I (-)
16	attenuated	no	4	431G→A	Glu133Lys	yes	<i>Mnl</i> I (-)
17	classical	no	4	<b>447G→C<sup>b</sup></b>	<b>Arg138Pro</b>	yes	<i>Apa</i> I (-)
18	classical	no	7	665C→T	Arg211TERM	yes	<i>Nla</i> III (+)
19	classical	no	10	<b>1016insC<sup>b</sup></b>	<b>Arg328Pro335TERM<sup>d</sup></b>	no	<i>Mwo</i> I (-)
20	classical	no	11-12	- <sup>b</sup>	<b>Exons 11 &amp; 12 deleted</b>	-	-

<sup>a</sup> Nomenclature as recommended by Antonarakis et al (20). <sup>b</sup> Novel WAS gene mutations. <sup>c</sup> Position of last C in a stretch of identical nucleotides. <sup>d</sup> Frameshift mutation in the first codon results in premature termination (TERM) at the second amino acid position. The creation (+) or abolition (-) of a recognition site for the restriction endonuclease is shown in parentheses. <sup>e</sup> Presence of mutation 365A→C was confirmed by amplification refractory mutation system.

last two exons of the WAS gene with the proximal breakpoint located within intron 10.

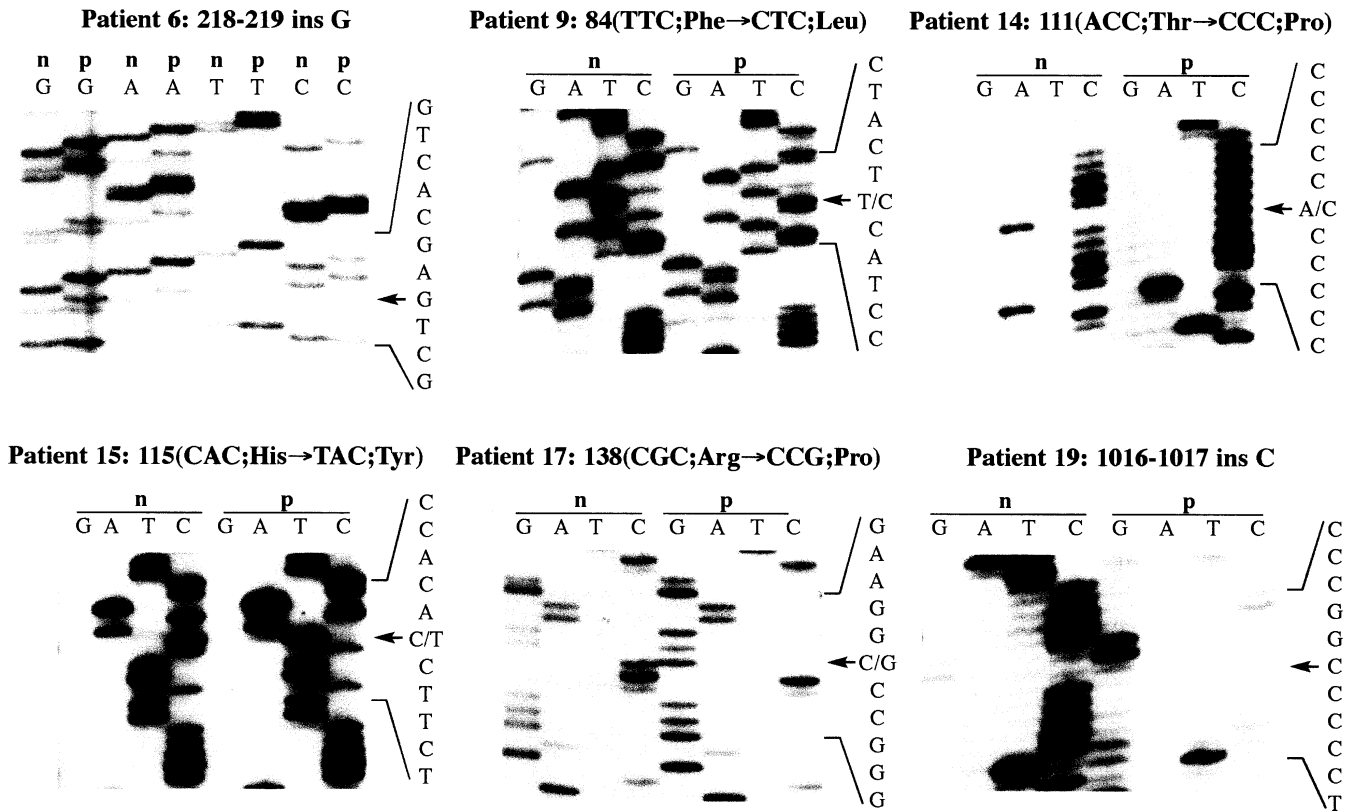
#### Germline Origin of WAS Gene Mutations

All point mutations of the WAS gene in cases without a family history of WAS occurred at CpG dinucleotide sites (Table 2; patients 1, 2, 11, 13, 16, 17 and 18). Independent C→T or G→A base changes at five sites (nucleotides 134, 168, 291, 431 and 665) predict the mutation mechanism to be deamination of 5-methylcytosine. The germline origin of WAS point mutations was determined in five cases where requisite family members were available. Three mutations at CpG sites (Table 2; patients 1, 2 and 13) were shown by direct mutation analysis to have occurred spontaneously during maternal gametogenesis. Combined mutation and linkage analysis using markers at loci DXS6940 and DXS255 identified two mutations of grandpaternal origin, one at a CpG

site in exon 2 (Table 2; patient 11) the other a novel insertion within the same exon (patient 6). Different gender origin of germline mutation is highlighted in cases 13 and 11 where mutation 291G→A has arisen independently during maternal and grandpaternal gametogenesis respectively (Fig. 2).

#### Carrier Detection and Prenatal Diagnosis

Direct mutation analysis established carriership (18 carriers, 15 non-carriers) in female relatives, on the basis of which prenatal diagnosis was performed at 12 weeks gestation in one family. The mother was confirmed by *Dde* I restriction analysis to be a carrier of the novel guanine insertion at position 218 identified in her affected son (Table 2; patient 6). Analysis of DNA extracted from chorionic villi identified the male fetus to be hemizygous for the mutation responsible for severe WAS in this kindred.

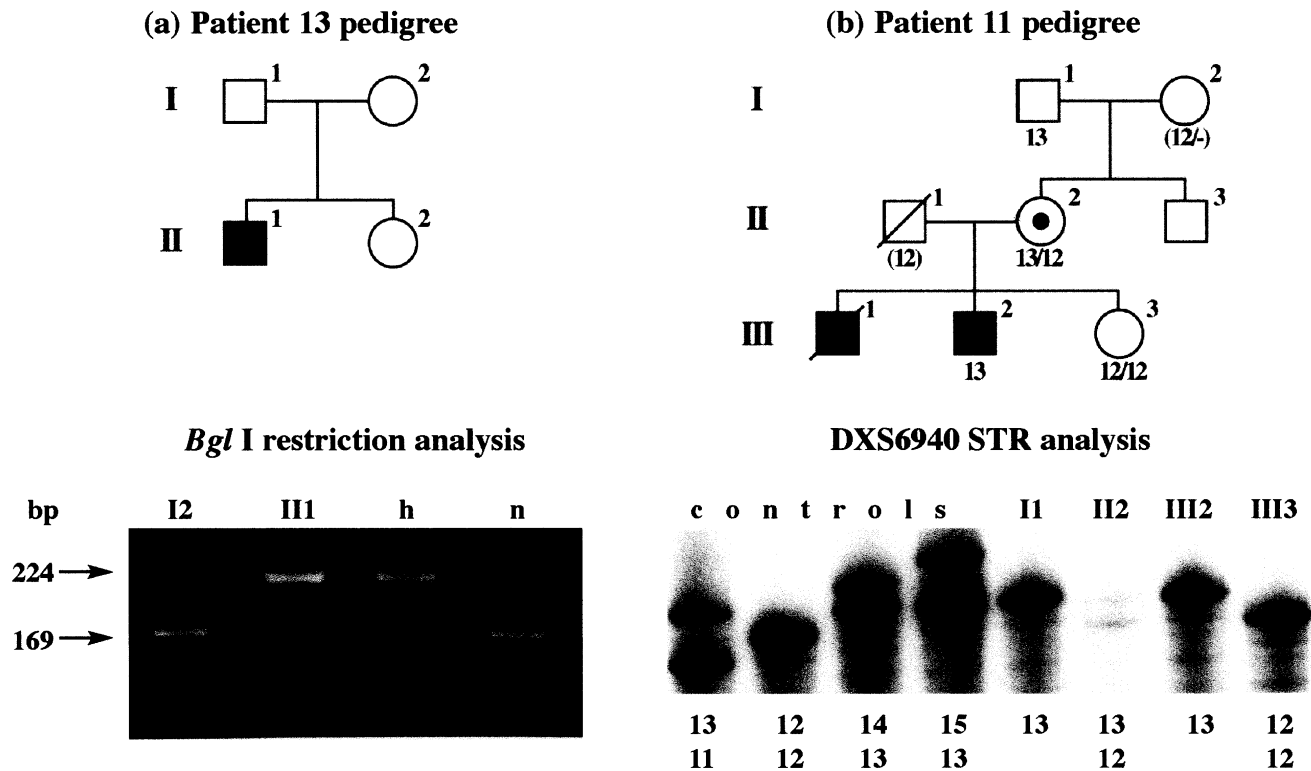


**Figure 1.** WAS gene DNA sequence showing six novel mutations. DNA sequence was compared between individual WAS patients (p) and a normal subject (n). Sense sequence is shown in all cases except for patient 17. Single base pair insertions, 218-219insG (patient 6), 1016-1017insC (patient 19), and single base changes are indicated by an arrow. Sequencing reactions for individual nucleotides common to the control and patient 6 were run in parallel lanes to highlight the point of insertion and consequent frameshift.

### DXS6940 STR Analysis

Sequence analysis identified the number of CA dinucleotides present within the variable length DNA products previously defined by PCR amplification of the DXS6940 STR (18). A novel (CA)<sub>15</sub> allele in addition to the three known alleles (CA)<sub>12</sub> (168bp), (CA)<sub>13</sub> (170bp) and (CA)<sub>14</sub> (172bp) was identified in the Northern European population studied (Figure 2b). In this population the most frequent allele (70%) is (CA)<sub>13</sub> (Table 3). In contrast, (CA)<sub>12</sub> is prevalent in the African population (56%). (CA)<sub>13</sub> predominates in the Asian population (92%) in which there is a high degree of homozygosity

(83%). Two additional novel alleles were identified (CA)<sub>9</sub> in the African population and (CA)<sub>11</sub> in four additional subjects of Afro-Caribbean and Asian Indian origin (not included in the study; Fig. 2b). Informativity of the DXS6940 STR was determined in 37 females from 17 WAS and XLT kindreds. Nine (24%) were informative for the DXS6940 STR. The number of subjects observed to be homozygous for the DXS6940 STR is similar to that expected for the Northern European and Asian groups, but lower than calculated for the African group on the basis of allele frequencies in the respective populations.



**Figure 2.** Germline origin of WAS gene mutation 291G→A by pedigree analysis using *Bgl* I restriction and DXS6940 STR analysis. (a) Upper, pedigree for patient 13. Lower, products of *Bgl* I restriction analysis after agarose gel electrophoresis. PCR amplified DNA from the mother (I2) and a normal control (n) show complete digestion (169bp DNA fragment) confirming the absence of the G291A mutation clearly seen to be present in her affected hemizygous son (II1) and a heterozygous control (h; a female carrier relative of patient 12). The 291G→A mutation has thus occurred spontaneously during maternal gametogenesis. (b) Upper, pedigree of subject 11. Lower, autoradiograph of DXS6940 STR analysis. The affected child (III2) with the 291G→A mutation has inherited the DXS6940 STR allele (CA)<sub>13</sub> linked to the mutation, from his unaffected grandfather (II1) for whom *Bgl* I restriction analysis of PCR amplified DNA confirmed the absence of the mutation (inferred alleles are shown in parentheses). Thus in this case the WAS gene defect arose spontaneously during grandpaternal gametogenesis.

DISCUSSION

Fourteen different WAS gene mutations in 16 WAS and four XLT patients were identified including nine missense, two nonsense, two frameshift and one large deletion (Table 2) enabling carrier screening in 33 at-risk females and prenatal diagnosis in one family. Seven of the observed WAS gene defects are novel. Missense mutation 284T→C resulting in substitution of phenylalanine by leucine at residue 84 of the WAS protein is associated with attenuated WAS in patient 9. Substitution of threonine by proline at position 111 of the WAS protein caused by the 365A→C transversion in exon 3 is associated

**Table 3.** DXS6940 STR allele frequency in three ethnic groups

(CA) <sub>n</sub>	Northern European	African	Asian
9	0.0	0.03	0.0
11	0.0	0.0	0.0
12	0.15	0.56	0.03
13	0.70	0.38	0.92
14	0.14	0.04	0.05
15	0.01	0.0	0.0
X-chromosomes	81	77	60
Observed homozygosity	0.57	0.28	0.83
Expected homozygosity <sup>a</sup>	0.53	0.46	0.85

<sup>a</sup>calculated using Hardy Weinberg equilibrium.

with a classical WAS phenotype in patient 14 compared with the attenuated phenotype of patient 15, in which the 377C→T transition replaces the basic histidine with a neutral tyrosine at residue 115 of the protein. The 447G→C transversion in exon 4 replaces arginine with proline at residue 138 probably affecting the overall hydrophobicity of the WAS protein and thereby contributing to the classical WAS phenotype seen in patient 17. Comparison of the primary structure of human and mouse WAS proteins reveals that the four newly described missense mutations affect residues conserved in the murine WAS protein (19), which shares 86% amino acid sequence homology with the human protein. The novel insertion and deletion mutations, the latter one of only four large deletions described (9, 22, 23) in the WAS gene, were identified in three cases with no family history of WAS (Table 2) and as expected give rise to a severe clinical phenotype. Interestingly, a reported guanine insertion between nucleotides 1030 to 1035 resulting in a frameshift and premature termination codon at position 335 identical to patient 19 was associated with a family history of attenuated WAS in conjunction with Fanconi anemia (24). The remaining seven point mutations identified occurred at CpG sites known to be mutation hotspots in the WAS gene. These recurrent mutations were found in 13 unrelated patients, six of whom had no previous family history of either WAS or XLT. This suggests a high incidence of spontaneous mutation.

Extensive molecular heterogeneity accounts in part for the phenotypic variability associated with WAS and XLT. However, interfamilial variation in disease manifestation was observed in patients with the same causative missense mutation. Codon 86 is the most frequent site of missense mutation in the WAS gene. The 291G→A transition (Arg86His) is associated with both attenuated (patients 11 and 12) and classical WAS phenotypes (patient 13; Table 2) and has independently been identified in X-linked

thrombocytopenia (9), attenuated (5, 6) and classical WAS (1, 2, 5, 6, 11). Transition 290C→T (Arg86Cys), while associated in patient 10 with isolated thrombocytopenia consistent with previous findings (2-5, 7, 9, 10) has also been observed in classical WAS (6). These two mutations have to date been reported in 20 unrelated WAS and XLT patients. By contrast, substitution of Arg86 by leucine (12) or proline (6) has been reported associated only with classical WAS. Other mutation hotspots, 168C→T (Thr45Met) and 257G→A (Val75Met), are also associated with variable phenotypic expression. Mutation 168C→T identified in patients 2 and 3 with attenuated WAS and patients 4 and 5 with XLT confirm previous reports (3, 5, 7, 9, 22, 25). Similarly the 257G→A transition detected in patient 7 with classical WAS and in patient 8 with congenital thrombocytopenia is consistent with reports of this mutation being associated with classical WAS (1, 3) or XLT (1, 2, 9, 10, 25). In this study mutations at codons 45, 75 and 86 account for 50% of families including 30% of sporadic cases. The distribution of mutation hotspots within the WAS gene (8) provides a rationale for targeted screening of molecular defects at codons 45, 75 and 86 prior to extensive mutation analysis.

The 431G→A WAS gene missense mutation in patient 16 with an attenuated phenotype has been described in severe WAS (2, 5, 7, 10, 18). By contrast, phenotypic concordance was observed between patient 18 in which the 665C→T transition produces a premature stop signal at codon 211 (Table 2) and other cases of classical WAS in which this mutation has been found (3, 6, 7, 9, 26). Extensive clinical heterogeneity among WAS patients cannot be ascribed to WAS genotype alone. Other genetic or environmental factors are likely to modify phenotypic expression of mutation of the WAS gene. Candidates include genetic variation in other host defence mechanisms (27) which may influence the severity of the underlying immunodeficiency.

Of the 14 individual mutations identified, 11 (79%) occurred within the first four exons of the WAS gene, with six clustering in exon 2 encompassing the putative WH1 domain. Recently a plekstrin homology (PH) domain (6-105 amino acids) involved in the regulation of actin binding proteins has been found at the amino terminal end of the protein encoded for by exons 1 to 3 of the WAS gene (28). Missense mutations in the PH domain associated with a mild clinical phenotype may cause an abnormal cytoskeletal rearrangement in the megakaryocyte and hence defective platelet formation consistent with the mild variants of WAS.

In this study 72% of point mutations (8/11) in WAS and XLT occur at CpG dinucleotide sites. All nucleotide substitutions in isolated WAS cases occurred at CpG dinucleotide sites and with the exception of one case (patient 17) are consistent with deamination of 5-methylcytosine. The frequency of reported WAS mutations at CpG sites is striking. Three mutations at CpG sites were shown to have arisen during maternal gametogenesis (Table 2; patients 1, 2 and 13). The possibility in these cases of gonadal mosaicism and the consequent risk of transmitting the defect more than once has implications for genetic counselling. The pattern of methylation at the WAS locus in females may contribute to increased frequency of germline mutation. Linkage analysis in one kindred (patient 11) using the DXS6940 STR provides evidence of CpG mutation in the male germline. Spontaneous mutation at CpG sites may also occur as a post-zygotic event giving rise to germline mosaicism as previously described in this syndrome (29). Such an event would require consideration of prenatal diagnosis in subsequent pregnancies.

The apparent departure from Hardy-Weinberg equilibrium resulting from the greater number of observed heterozygous (72%) African subjects for the DXS6940 STR compared to that calculated (54%), based on allele frequencies (Table 3), suggests a selective advantage for the heterozygous state in this population. DXS6940

STR analysis by PCR amplification will facilitate rapid tracking of WAS in nearly half the families with the condition complementing the DXS255 VNTR by Southern blot analysis (30). The proximity of the DXS6940 locus to the WAS gene makes haplotyping errors due to meiotic recombination less likely than with the DXS255 VNTR in which recombinational events have been reported (30).

In the Northern European families studied mutations in the WAS gene are heterogeneous and cluster within the first four exons of the gene. Prediction of clinical phenotype based on WAS genotype alone is problematic. Identification of specific WAS gene mutations facilitates carrier detection and prenatal diagnosis and contributes to an understanding of WAS protein domains in lymphocyte and platelet function.

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