HK J Paediatr (new series) 2006;**11**:186-190

Haematopoietic Stem Cell Transplant for Wiskott-Aldrich Syndrome

BHY ***Chung***, TL ***Lee***, CF ***Chan***, A ***Chiang***, KW ***Chan***, SY ***Ha***, YL ***Lau***

Abstract We reviewed retrospectively 7 Chinese children diagnosed with Wiskott-Aldrich syndrome (WAS) and managed at the Department of Paediatrics & Adolescent Medicine of Queen Mary Hospital from 1988 to 2005. All patients presented with the classical triad of bleeding tendency, recurrent infections and infantile eczema from neonatal period to 2-3 months of age. The median lag time between diagnosis and presentation was 7 months. Thrombocytopenia and small platelet volume were consistent findings and present in all patients. Findings in immunoglobulin level, lymphocyte subset and lymphocyte proliferative studies were heterogeneous. Four mutations were found in 5 (2 cousins shared the same mutation). Haematopoietic stem cell transplant (HSCT) had been performed for all patients. All 7 had complete immune reconstitution with no major long-term complications in a median follow-up of 9.3 years. Early diagnosis and selection of appropriate donor for HSCT were important strategies for improved survival of patients with WAS.

Key words Chinese; Haematopoietic stem cell transplant; Immunodeficiency; Wiskott-Aldrich syndrome

Introduction

Wiskott-Aldrich syndrome (WAS; MIM# 301000) is an X-linked recessive immunodeficiency characterised by thrombocytopenia and small platelet volume, eczema and recurrent infections due to combined cellular and humoral immunodeficiency. It is a rare disorder, occurring with a frequency of 4 per million male births.[1,2] Phenotypic expression is highly variable. In some patients, they express the full triad of clinical manifestations, while others showed a milder phenotype which is known as X-linked thrombocytopenia. In a multi-institutional survey of 154 unselected patients with WAS, the classic triad was seen only in 27% of the study population, while 5% and 20% had only infectious manifestations and haematological manifestation (thrombocytopenia) before diagnosis.[3] WAS is also associated with autoimmunity and malignancies.[4,5]

Major laboratory features of WAS, besides decreased platelet count and mean platelet volume, include progressive lymphopenia after 6 years of age, normal IgG, normal or low IgM, elevated IgA and elevated IgE, poor antibody response to polysaccharide antigens and diminished T cell response to a range of stimuli.[4] The gene responsible for WAS, located on the short arm of chromosome X at Xp11.22-Xp11.23, was identified by positional cloning in 1994.[6] The Wiskott-Aldrich Syndrome Protein (WASP) gene (GenBank NM_000377) consists of 12 exons, spanning 9 kb of genomic DNA, encoding a protein of 502 amino acids. Different researchers have looked into the function of WASP in the haematopoietic system. Recent evidence suggests that it belongs to the cytoplasmic scaffolding protein family and is functionally implicated in the regulation of the actin cytoskeleton.[7] However, the mechanisms how mutations in WASP result

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong, China

BHY **Chung** （鍾侃言） *MRCPCH*
TL **Lee** （李子良） *MRCP(UK)*
CF **Chan** （陳志峰） *MD, FRCP(Edin)*
A **Chiang** （蔣國誠） *MRCP(UK), PhD*
KW **Chan** （陳冠榮） *BSc, MPhil*
SY **Ha** （夏修賢） *MRCPath(UK), FRCP(Edin)*
YL **Lau** （劉宇隆） *MD, FRCP(Lond)*

Correspondence to: Prof YL **Lau**

Received May 18, 2006

in the specific clinical features in WAS are still not well understood.

Traditionally, adequate supportive treatment with IVIG and antibiotic prophylaxis together with splenectomy enables good survival and quality of life in the short and medium term for patients with WAS.[8] However, risk of malignancies does not change despite these treatments. Nowadays, haematopoietic stem cell transplant (HSCT) is the treatment of choice if an HLA-identical donor is available. The survival rate for patients receiving transplants from related HLA-identical donors is about 90% compared to 34% and 65% for haplo-identical donors and matched-unrelated donors (MUDs) respectively.[8] It has recently been shown 5-year survival of up to 80% can be achieved with matched unrelated donors in boys younger than 5 years old.[9]

In this study, we report on the clinical presentations, laboratory findings, management and outcomes of paediatric patients with WAS in our centre.

Methods

Seven patients with the diagnosis of WAS were registered during the period from July 1988 to December 2005 at the Department of Paediatrics & Adolescent Medicine of Queen Mary Hospital. All patients had clinical manifestations compatible with the classical WAS and the diagnosis was supported by laboratory findings and in 5 cases confirmed by mutational analyses. Among them, patients 1-6 have been included in the report of our Primary Immunodeficiency (PID) Registry.[10]

Laboratory analysis included complete blood picture, blood smear, mean platelet volume, immunoglobulin (Ig) levels, lymphocyte subsets and proliferation response. Genetic analysis was performed for all patients and their selected family members. Genomic DNA was isolated from the peripheral blood of patients and their family members and polymerase chain reaction (PCR)-directed sequencing analysis was performed. Details on methodology has been reported in our previous publications.[11,12]

Haematopoietic stem cell transplantation has been performed in all 7 patients. Type of transplantation and clinical outcome were identified.

Results

There were a total of 7 patients. Patients 4 and 6 are cousins. All of them belonged to the classic type of WAS and there was no patient with X-linked thrombocytopenia in our registry. The median age of presentation was approximately 1 month of age while the median age of diagnosis was 8 months (Table 1). Bleeding tendency due to platelet abnormalities was the major presenting complaint in our patients. The median platelet count and mean platelet volume were 19 x 10^9/L and 4.3 fl (normal range 7-10 fl) respectively.

Family history was present for patients 3 and 5. Patient 3 had a maternal cousin with "skin disease and bleeding problem" who died at 2 years old, while patient 5 had a maternal uncle who died at 5 years old due to uncontrolled bleeding. Patient 6 was a younger cousin of patient 4 and he was promptly referred and diagnosed at 4 months of age.

All patients had infantile eczema of different severity. They had multiple episodes of bleeding, predominantly mucosal bleeding which included bloody diarrhoea, haemetemesis, haematuria and epistaxis. For patients 5 and 6, they had severe intracranial haemorrhage presented with expressive aphasia and recurrent convulsions respectively. Both required neurosurgical interventions. All patients also had recurrent infections, ranging from upper and lower respiratory tract infections to cutaneous/soft tissue abscesses and septicaemia.

Immunological investigations at presentation were summarised in Table 2. IgG, IgA and IgE levels ranged from normal to mild elevation. Interestingly, IgM levels are normal in all of our patients. The lymphocyte subset studies were heterogeneous among our patients and in 4 out of the 7 patients there was diminished lymphocyte proliferative response to mitogens.

DNA sequencing revealed 4 different mutations (Table 3). Patients 4 and 6 are maternal cousins sharing same mutations. No mutation was detected in patient 2. Two patients (patients 1 and 3) had small deletion while other 2 (patients 4 and 6) had small insertions. Patient 5 had single nucleotide substitution. All the mutations found were predicted to result in the production of pre-maturely truncated WASP. Genetic testing had been offered as prenatal diagnosis to the family of patient 6. However, eventually WASP sequencing became unnecessary eventually as the fetus was confirmed to be of female gender.

Patient 2 developed EBV-related high grade B large cell lymphoma at 3 years old. It was incidentally discovered from unexplained cardiomegaly from chest radiograph post-splenectomy. The median age of performing splenectomy

Table 1 Clinical features of our patients with WAS

Patients	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Age of presentation	Neonatal period	Neonatal period	2 months	2 months	Neonatal period	Neonatal period	Neonatal period
Presenting features	Petechiae	Bloody diarrhoea	Haemetemesis	Haematuria	Petechiae	Bloody diarrhoea	Screening for maternal thrombocytopenia
Platelet count	18 x 10^9/L	10 x 10^9/L	26 x 10^9/L	47 x 10^9/L	15-23 x 10^9/L	15 x 10^9/L	30 x 10^9/L
Platelet volume	5 fl	3 fl	4.4 fl	4.2 fl	4 fl	4.3 fl	5.1 fl
Age of diagnosis	10 months	24 months	24 months	8 months	6 months	4 months	7 months
Age when splenectomy performed	19 months	36 months	40 months	15 months	7 months	7 months	Not done
Type of transplant	Haplo-identical (from mother)	Matched unrelated	Haplo-identical (from father)	Matched unrelated	Matched-sibling	Matched unrelated	Matched unrelated (cord blood)
Age when HSCT performed	20 months	53 months	55 months	11 months	8 months	13 months	10 months
Conditioning regimen	Bu/Cy/ Campath-1G	VP-16/ B-CNU/Cy	Bu/Cy/ Campath-1G	Bu/Cy/ATG	Bu/Cy/ATG	Bu/Cy/ATG	Bu/Cy/ATG
GVHD prophylaxis	CSA/MTX	CSA/MTX	CSA/MTX	CSA/MTX	CSA/MTX	CSA/MTX	CSA/MTX
Engraftment (ANC >500/mm^3)	Day 19	Day 16	Day 13	Day 17	Day 18	Day 12	Day 21
Present age	14 y.o.	17.5 y.o.	16.5 y.o.	9.5 y.o.	10 y.o.	7.5 y.o.	1.5 y.o.

Bu = bulsulphan, Cy = cyclophosphamide, Campath-1G = an anti-CD52 monoclonal antibody, CSA = cyclosporine, MTX = methotrexate, VP-16 = etoposide, B-CNU = 1,3-Bis (2-chloroethyl)-3-nitrosourea, ATG = anti-thymocyte globulin, ANC = Absolute neutrophil count.

Table 2 Immunological workup of our patients with WAS

Patients		**1**	**2**	**3**	**4**	**5**	**6**	**7**
Immunoglobulin pattern	IgG	↑	↑	↑	normal	↑	↑	↑
	IgA	↑	↑	↑	normal	↑	↑	↑
	IgM	normal	normal	normal	normal	normal	normal	normal
	IgE	↑	↑	normal	not done	↑	↑	not done
Lymphocyte subset		Low lymphocyte count with low T and B cells	Low lymphocyte count. Low T cell with decreased CD4:CD8	Low lymphocyte count. Low T cell with decreased CD4:CD8 and low B cells	Normal lymphocyte count with normal T and B cell ratio	Low lymphocyte count. Low T cell with decreased CD4:CD8	Low lymphocyte count with low T and B cells	High lymphocyte count with low B cells
Lymphocyte proliferation studies (response to mitogens including PHA, con A, PWM)		decreased	decreased	normal	normal	normal	decreased	decreased

PHA = Phytohaemagglutinin, con A = concanavalin A, PWM = pokeweed mitogen

Table 3 Spectrum of WASP mutations in our patients with WAS

Patients	**1***	**2**	**3****	**4***	**5***	**6***
Exon	Exon 3	Nil	Exon 10	Exon 7	Exon 5	Exon 7
Mutation	T deletion at nucleotide position 384	Nil	GCCGGGGGCCT deletion start at nucleotide position 1298	CGCA insertion at nucleotide position 685	C>T at nucleotide position 506	CGCA insertion at nucleotide position 685
Amino acid change	Frameshift start at phenylalanine 117, stop codon (TGA) created at position 126	Nil	Frameshift start at alanine 422, stop codon (TGA) created at position 490	Frameshift start at proline 218, stop codon (TAG) created at position 222	Glutamine codon (CAG) change to stop codon (TAG)	Frameshift start at proline 218, stop codon (TAG) created at position 222

* Novel mutations reported in reference 12; ** Novel mutation reported in reference 11

was 17 months for patients 1 to 6 (splenectomy was not performed in patient 7). It was performed earlier for patients 5 and 6 with significant bleeding complications. For patient 4, it was performed after HSCT for medically intractable autoimmune thrombocytopenia.

HSCT had been performed for all patients. Only 1 was from matched sibling. Two were from parents while all others from matched unrelated donors. The median age of performing BMT was 17 months of age. Five developed mild acute GVHD of the skin. Patient 3 developed limited chronic GVHD with cutaneous manifestations of vitiligo and alopecia. Three developed immune-mediated phenomenon post BMT. Patient 4 developed autoimmune haemolytic anaemia and thrombocytopenia at day 60 and day 120 post-HSCT respectively. Patient 6 developed thrombocytopenia 2 months post HSCT and anti-platelet antibodies were positive. Patient 7 developed autoimmune haemolytic anaemia and thrombocytopenia at 2 months post HSCT. Interestingly, at 3 months post HSCT, he also developed a nephritic-nephrotic picture with significant proteinuria, microscopic haematuria, deranged renal function and hypertension and required 2 courses of plasmapheresis, mycophenolate mofetil (MMF) and rituximab. Despite all these, full immune recovery was achieved in all patients and there was no mortality in a median follow-up period of 9.3 years.

Discussion

We have reported 7 Chinese children with Wiskott-Aldrich syndrome. All patients have the classical triads of thrombocytopenia and small platelet volume, recurrent infections and eczema. Bleeding tendency is the major presenting feature which begins to occur from birth to 2-3 months of age. The median time interval between presentation and diagnosis was 7 months and in patient 2 it is as long as 23 months. Suggestive family history or presence of relatives known to have WAS are important and can significantly shorten the lag time in diagnosis. In fact, this also allows the opportunity of prenatal diagnosis when coupled with the advances in molecular diagnosis. Sex determination followed by either direct sequencing or DHPLC (Denaturing High Performance Liquid Chromatography) analysis has been suggested as the strategy[13] and this has been applied to the family of patient 6.

Besides clinical features, platelet abnormalities (thrombo-cytopenia and low platelet volume) is a consistent and almost pathognomonic finding in WAS. In our cohort, platelet defect is present in all 7 patients. Immunologic findings are less consistent. Depressed IgM, which is a commonly reported finding, is not observed in any of our patients. Occurrence of lymphopenia, decreases in T-cell and B-cell subsets all correlate with increasing age and is present in some of our patients only. About 57% (4 out of 7) have diminished proliferative response to mitogens.

Presence of WASP gene mutation is the next consistent finding in WAS after platelet abnormalities. No mutation can be detected for patient 2 within the coding region and flanking splice sites of the WASP gene. We had also performed PCR-direct sequencing for the primary promoter region of WASP genomic DNA but no

mismatch has been found. A hidden mutation may be present in the regulatory regions or introns of WASP, or in other uncharacterised WAS-causing genes. Thus summarising our data, classical clinical features (± family history), platelet count with platelet volume together with genetic testing seems to provide the best diagnostic strategies for patients suspected to have WAS.

Early diagnosis of WAS is of utmost importance because of the treatment implication. A five-year survival rate of 87% could be achieved by HSCT from matched sibling donors.[9] Comparable survival can be achieved even using unrelated HLA-matched donor, given that it is performed early in life (younger than 5 years old).[9] This is an invaluable option for families of WAS as the probability of having a HLA-matched and disease-free sibling is quite low. Thus, with early diagnosis and more readily available donor marrows from various marrow banks around the world, the need for HSCT with haplo-identical donors, which has a much lower survival (52% in 5 years), could be minimised.

More recently, unrelated umbilical cord blood has been used as an alternative source of stem cells for children with WAS when a suitable HLA-matched donor is not available (as in our patient 7). Although a direct comparison of the efficacy of unrelated cord blood versus unrelated marrow transplant is still lacking, preliminary reports have shown successful engraftments with development of less severe acute or chronic GVHD and other major adverse complications.[14,15] Other advantages of cord blood stem cells include ready availability, no risk to donor, and lower rate of viral contamination. Its disadvantages include low stem cell dose for larger patients and lack of stem cells for boost infusion following the initial procedure. However, successful immune reconstitution has already been reported using 2 unrelated cord units in 1 patient with WAS.[16] All these demonstrate the great potential of cord blood stem cells in treating patients with WAS.

With the clinical variability of WAS and the number of various treatment options available, there is still no treatment guideline for WAS.[17] There are still a lot of unanswered questions in areas like the basic molecular function of WASP, genotype-phenotype correlation, prognostication of WAS and efficacy of newer treatment options like gene therapy.[18,19] Despite all the unknown answers, the outcome of patients with WAS has become increasingly favourable with early diagnosis and HSCT with selection of appropriate donor.

References

1. Perry GS 3rd, Spector BD, Schuman LM, et al. The Wiskott-Aldrich syndrome in the United States and Canada (1892-1979). J Pediatr 1980;97:72-8.
2. Somerville C, Forsyth KD. Wiskott Aldrich syndrome: an immunodeficiency syndrome not rare in Western Australia. Pediatr Allergy Immunol 1993;4:65-72.
3. Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott-Aldrich syndrome. J Pediatr 1994;125(6 Pt 1):876-85.
4. Orange JS, Stone KD, Turvey SE, Krzewski K. The Wiskott-Aldrich syndrome. Cell Mol Life Sci 2004;61:2361-85.
5. Dupuis-Girod S, Medioni J, Haddad E, et al. Autoimmunity in Wiskott-Aldrich syndrome: risk factors, clinical features, and outcome in a single-center cohort of 55 patients. Pediatrics 2003; 111(5 Pt 1):e622-7.
6. Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. Cell 1994 ;78:635-44.
7. Bompard G, Caron E. Regulation of WASP/WAVE proteins: making a long story short. J Cell Biol 2004;166:957-62.
8. Lee TL, Lau YL. Management of Primary Immunodeficiency. J Pediatr Obs Gyn 1998:5-12.
9. 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.
10. Lam DS, Lee TL, Chan KW, Ho HK, Lau YL. Primary immunodeficiency in Hong Kong and the use of genetic analysis for diagnosis. Hong Kong Med J 2005;11:90-6.
11. Chan SY, Hui YF, Lau YL. An 11-bp deletion in exon 10 (c1295del11) of WASP responsible for Wiskott Aldrich Syndrome. Hum Mutat 1999;13:502. MPR#56 Online.
12. Chan KW, Lee TL, Chung BH, Yang X, Lau YL. Identification of five novel WASP mutations in Chinese families with Wiskott-Aldrich syndrome. Hum Mutat 2002;20:151-2.
13. Siminovitch KA. Prenatal diagnosis and genetic analysis of Wiskott-Aldrich syndrome. Prenat Diagn 2003;23:1014-6.
14. Knutsen AP, Steffen M, Wassmer K, Wall DA. Umbilical cord blood transplantation in Wiskott Aldrich syndrome. J Pediatr 2003;142:519-23.
15. Kernan NA, Schroeder ML, Ciavarella D, Preti RA, Rubinstein P, O'Reilly RJ. Umbilical cord blood infusion in a patient for correction of Wiskott-Aldrich syndrome. Blood Cells 1994;20: 245-8.
16. Slatter MA, Bhattacharya A, Flood TJ, Abinun M, Cant AJ, Gennery AR. Use of two unrelated umbilical cord stem cell units in stem cell transplantation for Wiskott-Aldrich syndrome. Pediatr Blood Cancer 2005; Epub ahead of print.
17. Conley ME, Saragoussi D, Notarangelo L, et al. An international study examining therapeutic options used in treatment of Wiskott-Aldrich syndrome. Clin Immunol 2003;109:272-7.
18. Chinen J, Puck JM. Perspectives of gene therapy for primary immunodeficiencies. Curr Opin Allergy Clin Immunol 2004;4: 523-7.
19. Chinen J, Puck JM. Successes and risks of gene therapy in primary immunodeficiencies. J Allergy Clin Immunol 2004;113: 595-603.