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Clinical and Molecular Characteristics of 35 Chinese Children with Wiskott–Aldrich Syndrome

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Abstract
Background Wiskott–Aldrich syndrome (WAS) is a rare primary immunodeficiency disease, with an incidence of 4/1,000,000 live male births. In China, an estimated number of 35 babies with WAS are born each year, but likely many remain undiagnosed.
Objectives The objectives of study were to review the clinical and molecular characteristics of a cohort of Chinese children with WAS and to describe the long-term outcome of those who underwent hematopoietic stem cell transplant (HSCT).
Materials and Method Records of 35 patients diagnosed with WAS during 1991–2008 were reviewed. Genetic diagnosis was established by direct gene sequencing.
Results All patients had classical WAS phenotype. *WASP* mutations were identified in 33 patients from 29 families. Nine patients underwent HSCT at a mean age of 22.1 months (match-unrelated donor, $n=5$; mismatched related donor, $n=2$; matched-sibling donor, $n=2$). Post-transplant immune hemolytic anemia and thrombocytopenia occurred in three patients with complete resolution. All patients survived without significant long-term complications and had full platelet, T and B lymphocyte recovery within 2 years post-transplant.
Conclusion In the past decade, there has been significant improvement in clinical and genetic diagnosis of WAS in Chinese. We demonstrated excellent long-term survival in patients who underwent HSCT. Early workup for transplant should be advocated for children with classical WAS before they suffer from major disease complications and morbidities.

Keywords Wiskott–Aldrich syndrome · WASP · transplant · Chinese · immunodeficiency

PPW Lee and TX Chen were co-first authors and had equal contributions to the study.

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Introduction

Wiskott–Aldrich syndrome (WAS) is a rare X-linked recessive primary immunodeficiency disorder (PID), characterized by thrombocytopenia with reduced platelet volume, eczema, recurrent infections, and autoimmune manifestations. It is caused by an absence or defect in the WAS protein (WASP), a cytoplasmic protein involved in actin polymerization, cytoskeleton reorganization, and intracellular signaling [1]. WASP is expressed in all hematopoietic stem cell-derived lineages and is encoded by the WASP gene located in Xp11.22–11.23 [2]. Different types of WAS gene mutations lead to a variety of clinical phenotypes, including classical WAS, X-linked thrombocytopenia, and X-linked neutropenia. Patients with classical WAS are susceptible to potentially life-threatening complications, including hemorrhage, invasive infections, and malignancies, particularly EBV-related lymphoma. Hematopoietic stem cell transplantation (HSCT) is the definitive cure, and long-term outcome is excellent particularly in those who received HSCT early in their disease course [3, 4].

Clinical severity and overall survival correlated with the degree of WASP expression, and a certain degree of genotype–phenotype correlation has been demonstrated [5–7]. Nonsense or frameshift mutations are often associated with severe disease, while missense mutations or splice site mutations affecting non-invariant sites are associated with the milder phenotype. Molecular diagnosis does not only confirm the clinical diagnosis and aids genetic counseling of the affected families but it also provides important prognostic information.

From the literature, less than 20 cases of genetically confirmed WAS were reported in the Chinese population, and all were classical WAS [8–13]. With an incidence of four per million live male births [14, 15], it is estimated each year that approximately 35 boys with WAS are born in China, and a significant proportion may remain undiagnosed. In the past 10 years, we performed molecular diagnosis for 35 WAS patients. This study summarized the clinical, immunological, and molecular characteristics of these patients and demonstrated excellent long-term outcome in nine patients who received HSCT.

Methodology

Patients

Queen Mary Hospital is a university teaching hospital, and the pediatric immunology unit is a tertiary referral center for patients with PIDs. Genetic studies for PIDs are performed on a research basis for local patients as well as patients referred from centers in China and overseas. Mutation study was performed at no cost to the patients or the referring institutions. Clinical and immunological data at diagnosis was provided by the referring doctors. Consent for genetic study was obtained from parents.

Data Collection

Information on demographics, including the date of birth, age at initial symptom, age at diagnosis, and date of diagnosis, was collected. The clinical features at presentation, type of infections which occurred at or prior to diagnosis, the lowest platelet count ever documented, lymphocyte subsets, and serum levels of IgG, IgA, IgM, and IgE at the time of diagnosis were recorded. Lymphocyte proliferation assay was performed with phytohemagglutinin (PHA), Concanavalin A (ConA), and pokeweed mitogen (PWM) as stimuli.

Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation was performed in nine patients. The clinical features of seven patients managed in Hong Kong were previously reported locally [16]. All patients had clinical scores of 4–5 before transplant. The origin of stem cells included bone marrow (BM, $n=6$), peripheral blood (PB, $n=2$), and cord blood ($n=1$). Five patients received HSCT from HLA-matched unrelated donors, two from matched-sibling donors (MSD), and two from mismatched related donors (MMRD). A conditioning regimen containing busulfan (20 mg/kg total dose), cyclophosphamide (200 mg/kg total dose), and antithymocyte globulin (ATG) was used in five patients, and one patient received busulfan/cyclophosphamide only. One patient received VP-16, cyclophosphamide, and B-CNU for conditioning because of prior chemotherapy for EBV large cell lymphoma. Conditioning with busulphan, cyclophosphamide, and Campath 1G for ex vivo T-cell depletion (Wellcome Biotech, Beckenham, UK) was given to two patients who received bone marrow from MMRD. None of the patients received irradiation. All patients received cyclosporine and methotrexate for GVHD prophylaxis. Neutrophil engraftment was defined as >500 granulocytes per microliter and platelet engraftment as stable or increasing platelet count $>50\times10^9$/L without further necessity of substitution. Post-transplant immunoreconstitution was evaluated by lymphocyte subsets (CD3, CD4, CD8, CD19, CD16/56), lymphocyte proliferation, and immunoglobulins. T-cell recovery was defined as CD3 cell count >1,500/μL and CD4 cell count >1,000/μL and positive lymphocyte proliferations induced by PHA, ConA, and PWM. Several techniques were used for chimerism studies,

including karyotyping, assessment of red blood cell antigens, HLA-typing, XY-FISH, and PCR-based analysis of polymorphic DNA markers.

WAS Gene Analysis

Molecular study for WAS gene mutation was performed for patients, and carrier status of their mother, female siblings, or maternally related female family members was confirmed, according to the method described previously [9]. Briefly, genomic DNA was isolated from peripheral blood and PCR direct sequencing, including all the coding sequences, and flanking splice sites of the 12 exons were performed. Homology analysis with the WAS reference sequence was performed using the NCBI program BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Effect of splice junction mutations was confirmed by RT-PCR or by bioinformatics analysis (Automatic Splice site Analysis, https://splice.uwo.ca/). When a missense mutation was identified, genotyping of 100 alleles would be performed to rule out the possibility of being a polymorphism.

Results

Demographics

All patients were ethnic Chinese. Seven patients were diagnosed and treated in Hong Kong, including one patient who was referred from Macau. Twenty-eight patients were managed in seven hospitals in China and were referred to us for genetic diagnosis. Thirty-one kindreds were identified (Table I); there were two pairs of twins (Patients P14a/P14b and P16a/P16b) and one pair of siblings (P20a/P20b), and two patients were cousins (P12a/P12b).

All patients were symptomatic by the age of 8 months, and 18 patients (51.4%) were worked up because of thrombocytopenia in the neonatal period. The rest presented with bleeding, eczema, and recurrent infections. The median age at diagnosis was 11 months (range, 1–42 months), and the average time at diagnosis from initial presentation was 9.8±11.3 months. Twelve (34.3%) patients had family history of bleeding tendency or recurrent infections in their male siblings (n=7) or maternally related male family members (n=5), but the mean age at diagnosis of WAS between those with or without family history was not statistically different (12.3±8.5 months and 13.3±10.7 months, respectively, p=0.78).

Hematological and Immunological Features

At presentation, vast majority of patients had anemia of variable severity. Major bleeding events included intracranial bleeding (n=2, both complicated by seizures) and massive hemetemesis (n=1), while the others had chronic per-rectal bleeding (n=19, 54.3%), recurrent epistaxis (n=11, 34.1%), and petechiae (n=12, 34.3%). Eczema was present in all patients. Six patients (17.1%) were documented to have autoimmune hemolytic anemia (AIHA). Six (17.1%) patients had neutropenia. The absolute lymphocyte count was $4.1\pm2.4\times10^9$/L at presentation, and five patients (14.3%) had lymphopenia according to age-matched reference.

Data on IgG/A/M and IgE were available in 27 and 23 patients, respectively, as shown in Fig. 1. Raised serum IgG (>1,000 mg/dL) and IgA (>100 mg/dL) was found in 59.3% and 40.7%, respectively. Low serum IgM (<50 mg/dL) was present in 51.8% of patients, while two (7.4%) had raised IgM, and serum IgE level was elevated in 87% of them.

Data on lymphocyte subset were available for 26 patients, but the pattern was heterogeneous. Diminished CD4+ lymphocyte count was found in 16 patients, and reversed CD4/CD8 ratio was found in ten patients. Twelve patients had raised NK cell count. Lymphocyte proliferation test was performed in 11 patients, and four of them had reduced response. A detailed discussion of the immunophenotypes of P18 and P30 was previously published [17].

Infections

Most patients had recurrent upper respiratory tract infection (n=12), otitis media (n=6), pneumonia (n=13), and skin abscess (n=7). Three patients had bacteremia caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and streptococcus species, respectively. P30 had herpetic gingivostomatitis and CMV interstitial pneumonitis as previously described [17].

Malignancy

Lymphoma was diagnosed in P30 at 3 years of age. He was found to have persistent right lower zone opacification on chest X-ray, and bronchosopy revealed right bronchial obstruction and biopsy confirmed to be high-grade large B-cell lymphoma. Lung biopsy specimen revealed clonal population of B lymphocytes and Ebstein–Barr virus, suggesting that the lymphoma was EBV-related. He was treated with chemotherapy (UKCCSG-902) with good response. In view of the high recurrence rate of B-cell lymphoma in WAS, match-unrelated donor (MUD) transplant was performed 6 months after completion of chemotherapy.

Mutations

WAS mutations were found in 33 patients from 29 families and are summarized in Table I. The types of mutations and

Table I Clinical Data of 35 Patients with WAS

Patient	Age of onset	Lowest platelet count ($\times 10^9$/L)	Autoimmunity	Exon/intron	Genomic/cDNA mutation	Predicted codon change	Unique/recurrent mutation	Maternal carrier status
Nonsense								
1	Neonate	6	No	Exon 1	c. 155C>T	p.R41X	Recurrent	Carrier
2	Neonate	15	No	Exon 5	c. 506C>T	p.Q158X	Unique [9]	Not done
3	1 month	44	No	Exon 7	c. 665C>T	p.R211X	Recurrent	Carrier
4	Neonate	17	No	Exon 7	c. 665C>T	p.R211X	Recurrent	Not done
5	Neonate	20	No	Exon 7	c. 665C>T	p.R211X	Recurrent	Not done
6	1 month	11	No	Exon 11	c.1388G>T	p.E452X	Unique [9–11]	No
Frameshift								
7	Neonate	18	AIHA	Exon 3	c. 384del T	p.F117fsX126	Unique [9]	Carrier
8	3 months	42	No	Exon 4	c. 444–453del	p.F137fsX257	Unique[a]	Not done
9	Neonate	8	No	Exon 4	c.470delC	p.Q146fsX260	Unique[a]	Not done
10	Neonate	24	No	Exon 7	c. 600delC	p.P189fsX260	Unique[a]	Carrier
11	1 month	36	No	Exon 7	c.621–622GGdel	p.G196fsX205	Unique[a]	Carrier
12a	2 months	10	AIHA	Exon 7	c.685–686insCGCA	p.P218fsX222	Unique [9]	Carrier
12b	1 month	3	AIHA	Exon 7	c.685–686insCGCA	p.P218fsX222	Unique [9]	Not done
13	8 months	20	No	Exon 7	c.693–694insCAGCACCTGGACC	p.P220fsX225	Unique[a]	Carrier
14a	1 month	15	No	Exon 10	c. 984delC	p.P317fsX444	Unique [9–11]	Carrier
14b	1 month	15	No	Exon 10	c. 984delC	p.P317fsX444	Unique [9–11]	Carrier
15	5 months	10	No	Exon 10	c. 1029–1030insT	p.V332fsX335	Recurrent	Carrier
16a	Neonate	19	No	Exon 10	c. 1040–1041AAdel	p.P336fsX444	Unique [12]	Carrier
16b	Neonate	6	No	Exon 10	c. 1040–1041AAdel	p.P336fsX444	Unique [12]	Carrier
17	Neonate	18	No	Exon 10	c. 1126delA	p.R364fsX444	Unique[a]	Not done
18	2 months	15	No	Exon 10	c. 1295–1305 delCCTGCCGGGGG	p.P421fsX490	Unique [9]	Carrier
Splice site								
19	Neonate	16	AIHA, neutropenia	Intron 1	g.IVS+1_+2insACGAAAATGCTTGG, c.166_167insGACGAAAATGCTTG	p.T45fsX66	Unique[a]	Carrier
20a	5 months	24	No	Intron 1	g.IVS1+1G>T	Predicted splice error	Unique [11]	Carrier
20b	Neonate	46	No	Intron 1	g.IVS1+1G>T	Predicted splice error	Unique [11]	Carrier
21	2 months	23	No	Intron 3	g.IVS3+1G>A	Predicted splice error	Recurrent	Not done
22	Neonate	10	No	Intron 7	g.IVS7–1G>A 2 transcripts detected, c.769–811del (skipped exon 8) and c.769delG	p.K245fsX246 p.K245fsX260	Unique[a]	Carrier
23	3 months	3	No	Intron 7	g.IVS7–1G>A	Predicted splice error	Unique[a]	Carrier
24	Neonate	21	AIHA	Intron 7	g.IVS7–1delG	Predicted splice error	Recurrent	Carrier
25	Neonate	7	No	Intron 8	g.IVS8+3_+6delGAGT	Predicted splice error	Unique[a]	Carrier
26	Neonate	5	No	Intron 9	g.IVS9+2 T>C; c.812-965del (skipped exon 9)	p.V260fsX392	Recurrent	Carrier
27	Neonate	8	No	Intron 11	g.IVS11+1G>C	Predicted splice error	Unique[a]	Carrier
Missense								
28	4 months	28	No	Exon 2	c.251 T>C	p.C73R	Recurrent	Carrier

Table I (continued)

Patient	Age of onset	Lowest platelet count ($\times 10^9$/L)	Autoimmunity	Exon/intron	Genomic/cDNA mutation	Predicted codon change	Unique/recurrent mutation	Maternal carrier status
Complex								
29	5 months	9	No	Exon 1	c.69G>C+c.96delA	p.G12A+ p.N21fsX44	Both were unique[a]	Carrier
No Mutation								
30	Neonate	10	AIHA	–	No mutation found	–		–
31	1 month	75	No	–	No mutation found	–		–

Mutation was identified in 33 patients. Unique mutations were those only identified in the Chinese Population

AIHA autoimmune hemolytic anemia

[a] Denoted novel mutation

the distributions across different domains are illustrated in Fig. 2. There were 34 mutation events of which two different mutations occurred in one single patient (P29, p.G12A+p.N21fsX44). Eight mutations occurring in 12 patients were previously reported by our group [8, 9], Jiang et al. [10, 11], and Zhang et al. [12]; all of them were unique mutations within this patient population. Thirteen were novel mutations. Out of the 34 mutation events, 16 were frameshift (47.1%), and ten were splice site (29.4%) mutations. Genetic study for maternal carrier status was carried out in 22 mothers; 21 were confirmed to be heterozygous for the mutation detected in their child/children, and four of them had more than one affected male offspring.

Single nucleotide insertions or deletions were found in seven families, and two-nucleotide deletions were found in two families. Insertion or deletion of ≥10 nucleotides occurred in four patients. P19 had insertion of 14 nucleotides in the exon 1/intron 1 junction, and revertant mosaicism was identified. The mutant was found in the B lymphocytes and CD3-mononuclear cells, while the wild-type sequence was present in the CD3+ T lymphocytes (manuscript in preparation).

Hematopoietic Stem Cell Transplantation

The clinical features and transplant course of the nine patients who received HSCT were summarized in Table II. All patients underwent HSCT before their fifth birthday, with a mean age of 22.1±18.6 months. Splenectomy was performed in five patients before HSCT (P2, P7, P12a, P19, and P30).

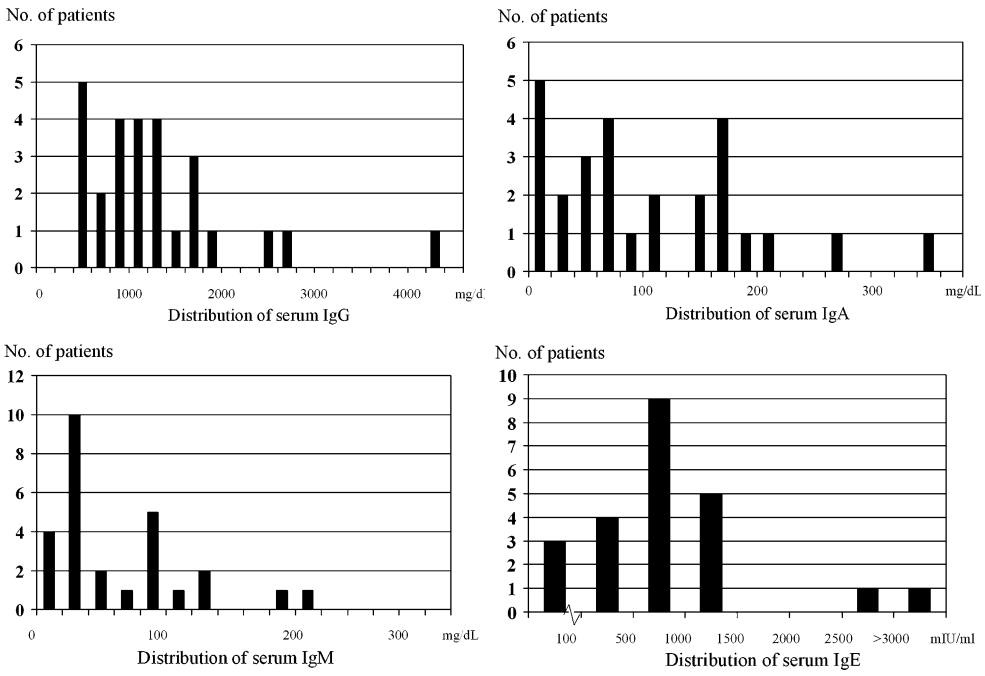

Fig. 1 Serum immunoglobulin levels in patients with WAS

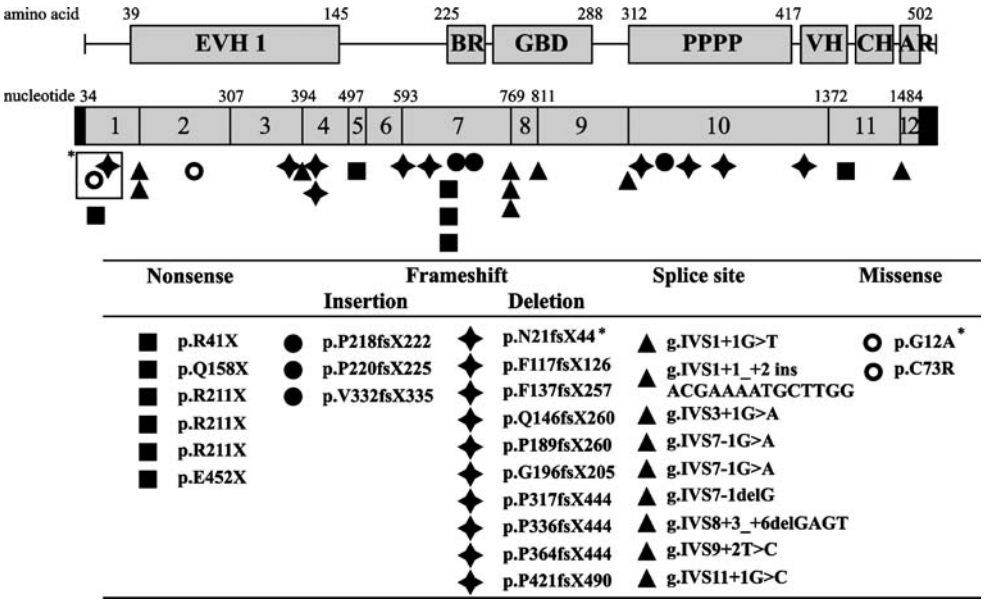

Fig. 2 WASP mutations identified in 29 kindreds, as indicated according to their positions in the protein sequence and exon/intron junctions. *Asterisks* G12A and N21fsX44 occurred in the same patient, otherwise, each *symbol* represents a single family with a WASP mutation. EVH 1, *Ens/VASP* homology 1 domain, *BR* basic region, *GBD* GTPase bending domain, *PPPP* proline-rich region, *VH* verprolin homology, *CH* cofilin homology, *AR* acidic region

Engraftment and Hematopoietic Reconstitution

None of the patients had graft rejection. All patients had neutrophil engraftment by D+30 (mean=16.6±3.2 days). Except three patients, all discontinued platelet transfusion by D+30. Post-transplant immune-mediated hemolysis and thrombocytopenia occurred in three patients, and details were shown in Table III. P12a had AIHA on D+68, which resolved with steroid, but on D+108, he developed immune thrombocytopenia, which was refractory to IVIG and steroid, and he finally underwent splenectomy. P12b developed immune thrombocytopenia and hemolytic anemia on D+30 and D+36, respectively. Anti-platelet antibodies were present, while his hemolytic anemia might be mediated by isohemagglutinins directed against recipient red cell antigens. Donor marrow had been plasma depleted, so the source of anti-A and anti-B antibodies could be other plasma containing products. Both resolved with IVIG. P19 had delayed erythroid (D+35) and platelet (D+43) engraftment with partial donor chimerism (66% on D+29). There was a major ABO mismatch, the post-transplant blood group was persistently of recipient type (O Rh+), and direct anti-globulin test (DAT) was all along positive. However, on D+78, platelet and Hb dropped to 10×10^9/L and 7.3 g/dL, respectively. DAT remained positive, and broad specificity auto-antibodies were identified in indirect anti-globulin test. At the same time, he also developed hypertension, ascites, raised serum creatinine, hypoalbuminemia, and nephrotic-range proteinuria, but without fever or neurological symptoms. The overall impression was an overlap between autoimmune hemolytic anemia and transplant-associated thrombotic microangiopathy (TMA). He received two courses of plasmapheresis followed by pulse methylprednisolone and mycophenolate mofetil but response was suboptimal. Rituximab 375 mg/m^2 weekly for four doses were then given. Improvement in hematological and renal parameters was observed after the third dose and normalized at 5 months post-transplant. Upon last assessment, all patients had normal hemoglobin level, platelet count, and MPV.

Immune Reconstitution

Data on post-transplant immune reconstitution was available for the seven patients who received transplant in Hong Kong (Fig. 3). All patients achieved CD3+ count >1,000/μL by 12 months post-transplant. All patients had normal lymphocyte proliferative response to PHA, ConA, and PWM at a median time of 12 months (6–26 months). P7 had mild lymphopenia before transplant, and his lymphocyte count remained on the low side (1,786–2,011/μL) up to 36 months post-transplant, in particularly CD19 (207–371 μL). Chimerism study revealed that the T cells were predominantly of donor origin, and he remained clinically well. P18 received MMRD and was the only patient with chronic GVHD in this cohort. He had delayed normalization of lymphocyte subsets and function at 26 months, and he had persistently low NK cell count (76–194/μL, 1.6–6%) up to 36 months post-transplant. His CD4/CD8 ratio remained reversed (0.15–0.66). P30 received MUD BMT and had mixed donor chimerism, and he was noted to have gradual drop in ALC especially CD19 cells, and his MPV remained low (5.1 fL) despite normal platelet count. However, his lymphocyte proliferation was normal, and clinically, he remained well after BMT.

Table II Clinical Characteristics of Nine Patients who Underwent Hemopoietic Stem Cell Transplant (HSCT)

Patient	Year of HSCT	Age at HSCT (months)	Type of HSCT (source)	HLA match	Conditioning regimen	GVHD (grade)	Post-HSCT autoimmune manifestations	Chimerism (Donor cells)	Time of cellular immunoreconstitution (months)	IVIG stopped (months post-HSCT)	Current age (years post-HSCT)	Outcome and status at last follow-up
18	1993	55	MMRD (father), BM	4/6	Bu, Cy, Campath-1	Skin (III)	Nil	100%	26	66	19 years 2 months (14 years 6 months)	Chronic GVHD with vitiligo and alopecia. Bronchiolitis obliterans. Otherwise well without functional impairment. Last plt count 226×10^9/L
7	1993	20	MMRD (mother), BM	4/6	Bu, Cy, Campath-1	Nil	Nil	100%	7	84	16 years 6 months (14 years 8 months)	Alive and well. Last plt count 258×10^9/L
30	1993	53	MUD, BM	6/6	VP-16, CY, B-CNU	Skin (I)	Nil	44%	13	77	19 years 5 months (15 years)	Alive and well. Last plt count 177×10^9/L
2	1997	13	Matched-sib donor, BM	6/6	Bu, Cy, ATG	Skin (III)	Nil	100%	10	24	12 years 1 months (11 years 4 months)	Alive and well. Last plt count 676×10^9/L
12a	1997	11	MUD,BM	8/8	Bu, Cy, ATG	Skin (I)	AIHA, thrombocytopenia neutropenia	100%	13	20	11 years 6 months (10 years 7 months)	Alive and well. Last plt count 463×10^9/L
12b	1998	13	MUD,BM	6/6	Bu, Cy, ATG	Nil	AIHA, thrombocytopenia	40%	21	60	10 years 2 months (9 years 9 months)	Alive and well. Last plt count 267×10^9/L
19	2005	10	MUD, cord blood	10/10	Bu, Cy, ATG	Skin (II)	AIHA, thrombocytopenia, nephrotic–nephritic syndrome	70%	12	14	3 years 10 months (3 years)	Alive and well. Last plt count 280×10^9/L
10	2006	6	Matched-sib donor (twin), PBSC	10/10	Bu, Cy	Nil	Nil	100%	6	5	2 years 2 months (21 months)	Alive and well. Last plt count 358×10^9/L
22	2007	18	MUD,PBSC	10/10	Bu, Cy, ATG	Skin (I)	Nil	100%	12	3	2 years 10 months (16 months)	Alive and well. Last plt count 228×10^9/L

Patients 2, 7, 12a, 12b, 18, 19, and 30 received transplant in Hong Kong [16], while patients 10 and 22 received transplant at Xin Hua Hospital, Shanghai, China

AIHA autoimmune hemolytic anemia, *ATG* anti-thymocyte globulin, *B-CNU* 1,3-bis (2-chloroethyl)-3-nitrosourea, *BM* bone marrow, *Bu* busulphan, *Cy* cyclophosphamide, *GVHD* graft-versus-host disease, *MMRD* mismatched related donor, *MUD* match-unrelated donor, *PBSC* peripheral blood stem cells, *plt* platelet

Table III Clinical Details of Three Patients with Post-transplant Immune Hemolytic Anemia and Thrombocytopenia

	P12a	P12b	P19
Type of HSCT/stem cell source	MUD/red cell depleted bone marrow	MUD/plasma depleted bone marrow	MUD/cord blood
Recipient blood group	O+	A+	O+
Donor blood group	B+	O+	A+
History of AIHA before HSCT	No	Yes	Yes
Neutrophil engraftment	D+17	D+12	D+32
Platelet engraftment	D+21	D+19	D+43
Acute GVHD	Acute skin GVHD grade I D+10. Resolved without additional immunosuppressive therapy	No	Acute skin GVHD grade III on D+6. Resolved without additional immunosuppressive therapy
Chronic GVHD	No	No	No
Hemolytic anemia			
Type	Autoimmune	Autoimmune/passive antibody transfer	Overlap of autoimmune and TMA
Onset (days post-transplant)	D+68	D+36	D+78
Drop in Hb (g/dL)	4	2.2	2.4
Blood transfusion required	Yes	Yes	Yes
Direct anti-globulin test	Positive	Positive	Positive
RBC antibody	AHG (polyspecific)+, anti-IgG+, C3b/C3d+	AHG (polyspecific)+, anti-IgG+, anti-complement	AHG (polyspecific)+, anti-IgG+, C3b/C3d+
	Anti-B antibodies absent	Anti-A and Anti-B antibodies present	Anti-A antibodies absent
Highest reticulocyte (%)	25.5	7.9	6.1
Chimerism status	DNA fingerprinting (BM): 100% donor	DNA fingerprinting (BM): 40% donor	DNA fingerprinting (PB): on D+81: 72% donor
Treatment	Steroid	IVIG×3 doses	Poor response to Plasmapheresis and pulse methylprednisolone, gradually resolved after rituximab.
Outcome	Resolved on D+92	Resolved on D+43	Resolved on D+138
Thrombocytopenia			
Type	Autoimmune	Autoimmune	? Immune-mediated or TMA
onset (days post-transplant)	Day+108	D+30	Persistent thrombocytopenia post-transplant; independent of platelet transfusion from D+43 Platelet count dropped to $<20\times10^9$/L from D+80
Lowest platelet count ($\times10^9\times12$/L)	3	25	4
Mean platelet volume (fL)	7.7	Normal	12.5
Anti-platelet antibodies	Negative	Positive	Not done
Bone marrow examination	D+41: normal BM engraftment. D+104: Adequate megakaryocytes and normal in morphology. Stable engraftment	D+30: active erythropoiesis, mildly reduced granulopoiesis with normal maturation, normal megakaryocytes. Normal bone marrow engraftment	D+86: regeneration marrow with active granulopoiesis, but only few megakaryocytes and mildly reduced erythropoiesis
Platelet transfusion required	Yes	Yes	Yes
Chimerism status	100% donor	40% donor	DNA fingerprinting (PB) on D+81: 72% donor
Treatment	Poor response to IVIG and steroid	As above	As above
	Splenectomy done on D+127		Gradually resolved after rituximab
Outcome	Resolved at 6m post-transplant	Resolved on D+43	Resolved at 5m post-transplant

AHG anti-human globulin, *IVIG* intravenous immunoglobulin, *TMA* thrombotic microangiopathy

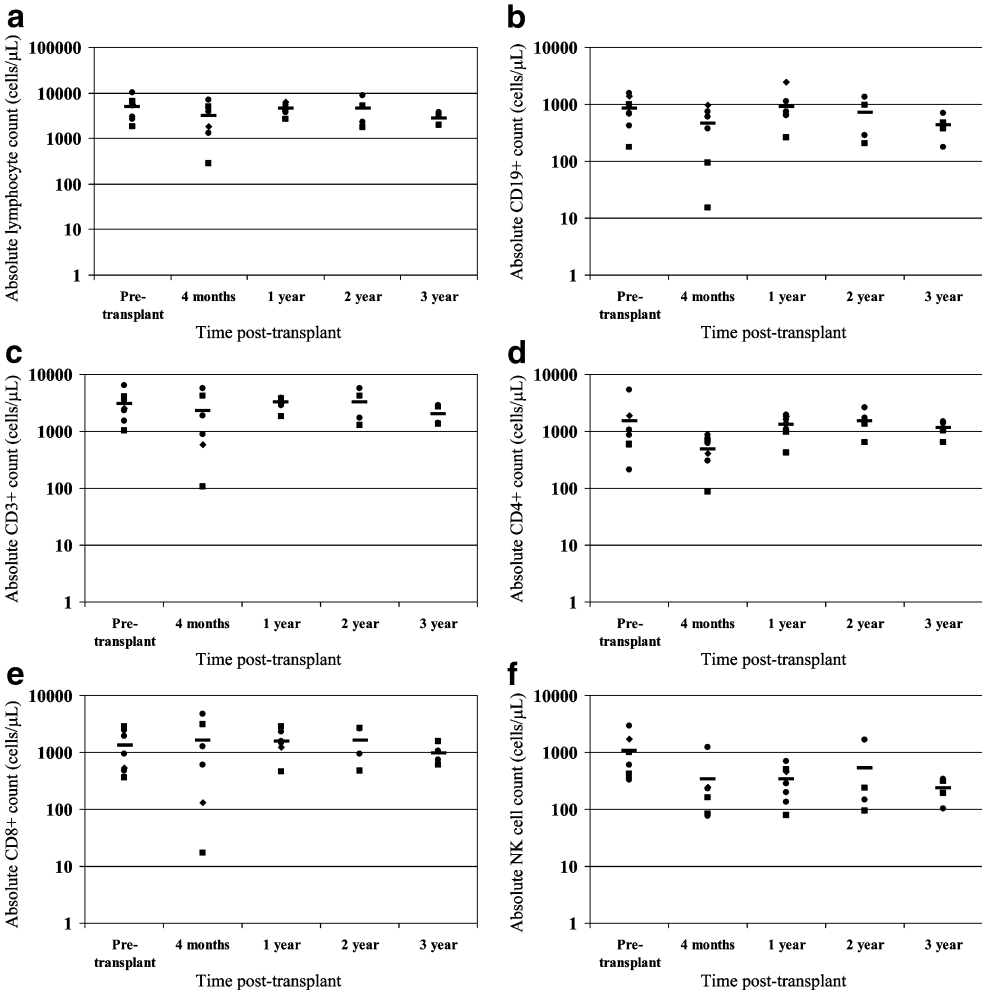

Fig. 3 Immune reconstitution following HSCT for WAS. Reconstitution of circulating T lymphocytes (**a**), B lymphocytes (**b**), CD3+ T lymphocytes (**c**), CD4+ T lymphocytes (**d**), CD8+ T lymphocytes (**e**), and NK cells (**f**) following HSCT are shown. *Diamonds* denote matched-sib transplant (P2), *squares* denote mismatched related transplant (P7and P18), and *circles* denote match-unrelated transplant (P12a, P12b, P19, and P30)

Outcome

The median post-transplant follow-up duration was 24 months (range 5–84 months). Six patients developed acute skin GVHD and three were grade 2 or above. P18 who received T-cell depleted, haploidentical BMT from his father suffered from the most stormy post-transplant course. He developed grade III skin GVHD, CMV viremia, pneumonitis, and enteritis leading to massive gastrointestinal bleeding, as well as reactivation of hepatitis B virus infection leading to liver derangement. The skin GVHD was resistant to high-dose steroid and cyclosporine; he was put on thalidomide but he later developed chronic skin GVHD with alopecia and vitiligo. He also had deranged lung function because of bronchiolitis obliterans. He remained functionally well up to the most recent follow-up. All other patients had complete disease remission, and none of them required second transplant. All the three patients who developed post-transplant autoimmune cytopenia resolved without sequelae. There was no severe infection or malignancy following HSCT, and all patients had normal platelet count and T- and B-cell recovery within 2 years post-HSCT.

Discussion

The present study described the largest cohort of WAS in the Chinese population. There was a predominance of frameshift, nonsense, and splice site mutations, which were predictive of absence or production of non-functional protein. p.R211X, locating at a CpG dinucleotide site, occurred in three unrelated kindreds (three of 29, 10.3%). It was a mutation hotspot and was reported in more than ten families worldwide (http://homepage.mac.com/kohsukeimai/wasp/WASPbase.html). Only two missense mutations were found in this cohort. Amino acid substitution p.G12A (69G>C) occurred in P29 who also had a frameshift mutation p.N21fsX44. G12 is located in the pleckstrin homology domain close to the N-terminal region of WASP and is thought to be important for membrane targeting of the cytoplasmic protein [18]. As both glycine and alanine are tiny and hydrophobic residues, it

could be predicted that the structure and function were largely preserved, so the frameshift p.N21fsX44 was more likely to be the disease-causing mutation in P29. p.C73R was a recurrent mutation previously reported by Lemahieu et al. [19] and was associated with severe WAS phenotype and absence of WASP expression. In contrast to published case series in other ethnic groups [5–7, 20–22], no mild phenotype was found in our cohort. It is uncertain whether this was a genuine characteristic feature in the Chinese population or not, but it was possible that children with X-linked thrombocytopenia were under-recognized and did not receive further investigation.

P30 developed EBV-related large B-cell lymphoma at an early age of 3 years. Patients with WAS are at high risk of developing malignant lymphoma, predominantly non-Hodgkin's lymphoma. The commonest histological subtype was large-cell immunoblastic lymphoma [23], as seen in our patient. In a multi-institutional survey of 154 patients, the average age at onset of lymphoma was 9.5 years, and the youngest developed lymphoma at 2 years of age [24]. Extranodal sites such as brain, small intestine, liver, and lungs were frequently involved. Most were high-grade tumor, and complete remission was difficult to achieve. It was reported that the incidence of lymphoma was highest among patients with splice site mutations (12.1%) compared with those having nonsense/frameshift mutations (9.2%) and missense mutations (3.2%). In particular, lymphoma occurred in three out of seven kindreds with IVS6+5 G>A [25]. However, no mutation could be identified in our patient, despite having the classical WAS phenotype. With advances in HSCT especially with the promising outcome in MUD transplant, an increasing number of patients with WAS receive transplant at an earlier age and may therefore reduce the risk of developing malignancy in the long run. In the two most recently published studies on long-term outcome of 96 (study period 1979–2001) [4] and 57 (study period 1985–2004) [26] WAS patients, respectively, none of them developed malignancy following HSCT.

Post-transplant hemolysis and thrombocytopenia occurred in three patients. They can be caused by multiple mechanisms and may be more complex in WAS patients who had intrinsic tendency to develop autoimmunity. In this cohort, all of these events occurred in patients receiving MUD transplant, but with variability in time and sequence of occurrence. P12a developed AIHA and immune thrombocytopenia 40 days apart, the former resolved with steroid treatment, while the latter did not respond to IVIG and steroid, finally requiring splenectomy. Thrombocytopenia in P12a and P12b occurred after normalization of MPV and stable myeloid engraftment, so was likely to be immune-mediated. The situation in P19 was even more complex; he had prolonged thrombocytopenia and anemia in the early post-transplant period contributed by mixed chimersim and major ABO incompatibility, which accounted for hemolysis and delayed erythroid engraftment. However, hemolysis occurring since D+78 was attributable to multiple mechanisms, including autoimmune and thrombotic microangiopathy. In a recent multi-centered collaborative study in Europe [4], autoimmunity was noted more frequently in recipients of MUD (28%) and MMRD (26%) compared with matched sibling or parent donors (11%). A strong association was noted between autoimmunity and mixed/split chimerism, suggesting that autoimmune manifestations might be mediated by residual host lymphocytes. Our study was limited by the lack of data on immunoreconstitution at the time of autoimmune manifestations, and detailed chimerism study on individual hematopoietic cell lineages was not available. Further studies on the interaction between host and donor B cells and T-regulatory cells may provide insights on post-transplant autoimmunity in WAS patients.

The overall outcome of HSCT in our cohort was excellent. One hundred percent survival within a follow-up duration of 3–15 years was achieved, and six patients were already over 10 years post-transplant. There was no mortality, and all patients had complete disease remission. In keeping with past reports, the patient who received MMRD BMT had the worst outcome and suffered from chronic GVHD and bronchiolitis obliterans as sequelae of BMT. In other published series, the overall 5-year survival rate in various donor types was 70–78.2% [3, 4, 26, 27]. While it has long been recognized that patients receiving MSD transplant had better outcome, the two most recent cohort (Kobayashi et al. 1985–2004 [26] and Pai et al. 1990–2005 [27]) showed that the survival rate in MUD was up to 80% especially in patients who received transplant at younger age. Early diagnosis is therefore pivotal in the overall prognosis of patients with classical WAS. In particular, early initiation of transplant workup and MUD search is important for patients without HLA-matched siblings, as long-term survival equivalent to MSD could be achieved if MUD transplant is performed before 5 years of age [3]. Gene therapy has shown promise in pre-clinical studies and may constitute a novel therapeutic option for WAS in the future [28, 29].

With improved knowledge among pediatricians and better diagnostic facilities, there has been a rapid increase in the number of children diagnosed with PID in China in the past decade. Indeed, over 70% of the patients from Mainland China in this cohort were born within the last 5 years. There has been concerted effort to establish a multi-center network and PID patient registry [30, 31], and HSCT is currently under development in a number of centers. The success of HSCT in PID relies heavily on timely diagnosis, accessibility to spe-

cialist centers, aggressive treatment of infections, supportive care, and financial support. Awareness of the general public and policy makers is equally important so that more resources can be channeled to develop a comprehensive health service and support network for children with PID.

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