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Clinical Aspects and Genetic Analysis of Taiwanese Patients with Wiskott–Aldrich Syndrome Protein Mutation: The First Identification of X-Linked Thrombocytopenia in the Chinese with Novel Mutations

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Abstract
Background Wiskott–Aldrich syndrome (WAS) is an X-linked immunodeficiency characterized by microthrombocytopenia, eczema, and recurrent infections. However, the more than 500 patient mutations described are mainly based on Caucasian and Japanese populations. This study investigated Taiwanese patients with WASP mutations since 1985 as part of a long-term comprehensive study in primary immunodeficiency diseases (PIDs) covering 23 million inhabitants.
Methods Clinical manifestations, immunologic functions, and WASP gene sequencing and expressions were analyzed in WAS patients. And, those patients with idiopathic thrombocytopenic purpura and "small" thrombocytopenia were enrolled.
Results Of 16 patients studied in 1993–2009, 12 presented as classic WAS phenotype and four had X-linked thrombocytopenia (XLT). Almost all correlated to the WASP expression level and severity of infections. Causes of mortality in the 12 classic WAS patients were mass bleeding, *Staphylococcus aureus* sepsis, and cytomegalovirus (CMV) pneumonitis in three non-transplant cases, and EBV-associated lymphoproliferative disorder and CMV pneumonitis in two non-engrafted transplant patients. Splicing mutations of Int 8 (+5) G>A in cousins and insertion of 1023 C in unrelated families presented as both

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XLT and classic WAS phenotype in the same mutations. Four XLT patients, including two novel mutations of 1023 Ins C (in 2) and "double" missense mutations of 1378 C>T and 1421 T>C had relatively higher CD4+ memory cells and/or activated lymphocytes (CD3+CD69+) compared with those of classic WAS patients.

Conclusions The lower ratio of XLT to classic WAS patients underestimates the burden of Taiwanese patients with WASP mutations, especially the XLT phenotype. A clustering pattern on exon 1 and five unique mutations (deletion of 45-48 ACCA, IVS 1 (−1) G>C, large deletion of promoter and exon 1 and 2, insertion 1023 C, and 1378 C>T and 1421 T>C) explain the genetic variations in different ethnic groups, despite the possibility of selection and ascertainment bias.

Keywords Wiskott–Aldrich Syndrome (WAS) · X-linked thrombocytopenia (XLT) · primary immunodeficiency diseases (PIDs) · Taiwan · Chinese · genetic analysis

Introduction

Wiskott–Aldrich syndrome (WAS) is an X-linked platelet and immune deficiency disease characterized by thrombocytopenia, bloody diarrhea, susceptibility to infections, and eczema [1, 2]. It is usually lethal in the first two decades of life through hemorrhage, overwhelming infection, or malignant transformation [3]. The gene responsible, the WAS protein (WASP), consists of 12 exons containing 1,823 base pairs. It encodes a 502-amino acid protein expressed selectively in hematopoietic stem cell-derived lineages and involves cell signaling and cytoskeleton reorganization [4], which affects the functions of T, B, NK, polymorphonuclear, and dendritic cells and platelets [5, 6].

Symptoms of immune dysfunction appear shortly after birth in some patients with the severe phenotype of classic WAS, but may be delayed in patients with the relatively mild phenotype of X-linked thrombocytopenia (XLT) [5, 7–11] (http://homepage.mac.com/kohsukeimai/wasp/WASPbase.html). Based on approximately 500 Caucasian and Japanese patients with WASP mutations, the ratio of XLT patients to classic WAS patients ranges from 8/12 [12] and 23/27 [10] to 88/79 [13] and 110/148 [14], respectively. To date, only severe classic WAS phenotypes, not the XLT phenotype, are recognized in ethnic Chinese patients with WASP mutations. Thus, there may be an underestimation [15–22].

This study is a component of a long-term, comprehensive investigation of Taiwanese patients with primary immunodeficiency diseases (PIDs). Genetic analysis of the WASP gene was determined for prompt diagnosis and early intervention through hematopoietic stem cell transplantation, if necessary, to improve survival [23]. Simultaneously, the study examined the possibility that Taiwanese XLT patients with WASP mutations were masked under the diagnosis of chronic idiopathic thrombocytopenic purpura (ITP) because of the absence of recurrent infections and eczema.

Patients and Methods

Patients

Since 1985, 187 patients from 177 families were enrolled from five tertiary medical centers: Chang Gung Memorial Hospital, Taiwan University Hospital, Mackay Memorial Hospital, China Medical University Hospital, and Kaohsiung University Hospital [24]. Based on the eight updated categories, there were 65 patients with "other well-defined immunodeficiency syndromes", 49 with "predominant antibody deficiencies", 32 with "T and B cell immunodeficiency", 22 with "congenital defects of phagocytes", 15 with complement deficiencies, and four with "disease in immune dysregulation". There was no patient with "auto-inflammatory disorders" or "defects in innate immunity".

WAS was classified in the category of "other well-defined immunodeficiency syndromes". Patients with such characteristics were assigned scores based on the clinical severity of the disease using a previously described scoring system [25, 26]. Furthermore, to search for XLT patients under-diagnosed as chronic ITP, WASP genetic analysis and protein expression were conducted in chronic ITP patients with small-sized platelets and thrombocytopenia despite of intravenous immunoglobulin (IVIG) and steroid treatment.

Immunologic Assessment

After obtaining institutional review board approval and informed consent, 20 ml peripheral blood were obtained from candidate patients and age-matched controls. To induce lymphocytic proliferation, peripheral blood mononuclear cells (PBMCs) (10^5/well) were incubated with indicated concentrations of PHA, ConA, and PWM for 3 days, or the *Candida* antigen, BCG vaccine, and tetanus toxoid for 7 days. Lymphocyte subsets (all antibodies purchased from Pharmingen, San Jose, CA), including CD3+, CD4+, CD8+, CD19+, CD45, CD45RO+, CD45RA+, CD27+, CD16+CD56+ (natural killer cells), and CD69, were assessed by flow cytometry.

Sequencing Analysis

Total RNA was isolated from the PBMCs or from established lymphoblastoid cell lines with TRIzol

Table I The Immunoglobulin Levels, Lymphocyte Subsets, and Clinical Features in 16 Taiwanese Patients (All Had Microthrombocytopenia[e]) with WASP Mutations

Patient		Age			Immunoglobulin				Percentage of lymphocytes (%)[a]								Lymphocyte[d] proliferation	Infection-associated events[b]	Autoimmunity or/ and malignancy	Other presentations
(Family history)	Diagnosed year/ referring hospital				mg/dl		IU/L		CD3	CD4	CD8	CD19	CD16/ 56	Memory cell		Activated lymphocyte				
		Onset	Tested	Now	IgM	IgG	IgA	IgE						CD4	CD19					
P1 (+)	1994/TU	5 M	5 M	4Y1M	46	1,154	45	110↑	73	10	2↓	13	11	11.4	1.8	35.4	Normal	RSI		
P2 (+)	2004/CG	NB	2 M	5 Y	76	1,880↑	228	14,282↑	41	23	17	8	18	14.2	5.1	45.4↑	NA	RSI, bronchiectasis, chronic diarrhea, *Pseudomonas aeruginosa* sepsis, severe skin infections, failure to thrive lymphoproliferative disorder	EBV-associated	
P3-1 (+)[c] 1	1993/TU	3 M	1 Y	1Y10M (dead)	42	1,352	112	657↑	49	41	9	16	15	NA	NA	NA	NA	RSI, bronchiectasis, chronic diarrhea, *S.pneumoniae* sepsis, severe skin infections,*Aeromonas enteroclitis*, left orchitis, meningitis	AIHA	Hematuria
P3-2 (+)[c] 1	2006/CG	NB	27 D	2Y2M	44	1,190	71	5	53	49	6↓	21	24	5.0	0.4↓	7.2	Impaired	RSI		
P4 (+)	2009/CG	3 D	1 M	3Y8M	18↓	1,480	50	1,452↑	53	47	6↓	28	4	4.5↓	1.1↓	22.9	Impaired	RSI, failure to thrive		Cerebellar hemorrhage
P5 (−)	2001/TU	NB	3 D	6Y (dead)	38↓	1,171	115	692↑	64	32	24	7↓	12	NA	NA	NA	NA	RSI		
P6 (−)	2007/CG	3 M	5 M	11 M(dead)	128	1,870	167	7,870↑	55	25	30	37	7	11.3	2.8	34.7	Impaired	RSI, failure to thrive, CMV pneumonitis, *S. aureus* sepsis		Epistaxis
P7 (+)	1998/CG	10 D	23 D	2Y5M (dead)	17↓	820	123	1,436↑	58	30	14	11	4	NA	NA	NA	NA	RSI, sepsis, severe skin infections lymphoproliferative disorder, AIHA	EBV-associated	
P8 (+)	2003/CG	18 D	1 Y	2Y(dead)	146	1,540↑	103	2,172↑	48	28	5↓	3↓	8	8.2↓	1.2↓	49.2↑	Impaired	RSI, *Streptococcus* group B and *S. aureus* sepsis, severe skin infections, failure to thrive, osteomyelitis, CMV pneumonitis, meningitis, seizure, HCV hepatitis	AIHA	Duodenal ulcer, GI bleeding
P9 (−)	2005/TU	1 M	2 M	4Y11M	54	825	24↓	462↑	51	31	16	17	8	9.4	2.1	24.5	NA	RSI, failure to thrive		
P10-1 (+)[c] 2	2008/CG	1 Y	12 Y	13Y2M	24↓	622	269	1,520↑	77	35	39	7↓	17	21.1	0.8↓	42.4	NA	*S. pneumoniae* sepsis		
P10-2 (+)[c] 2	1995/TU	2 M	5 M	14Y4M	127	798	234	1,768↑	64	37	28	15	8	14.2	1.2↓	48.4↑	NA	RSI		
P11 (+)	1999/CG	3 D	1 M	3Y7M	572	2,510↑	1,510↑	2,457↑	67	57	23	3↓	38	14.3	2.5	35.2	Normal	RSI		
P12-1 (+)[c] 3	2008/CM	3 M	7Y5M	8Y1M	37↓	1,070	687	1,542↑	62	32	25	10	7	12.4	4.2	54.0↑	Normal	hepatosplenomegaly	IgA nephropathy, AIHA	Hematuria,
P12-2 (+)[c] 3	2004/CM	4 M	23 Y	25Y9M	29↓	1,276	228	532↑	72	33	28	8↓	4	14.2	5.8	49.5↑	Normal			GI bleeding
P13 (−)	2007/CG	4 D	10 Y	12Y6M	67	694	285	7	66	42	22	15	18	9.5	1.0↓	55.1↑	Normal	Failure to thrive		Cerebral palsy, mental retardation

+ positive family history, −negative history, *TU* Taiwan University Hospital, *CG* Chang Gung Memorial Hospital, *CM* China University Hospital, *NA* not available, *GI* gastro-intestinal, *CMV* cytomegalovirus, *HCV* hepatitis C virus, *AIHA* autoimmune hemolytic anemia, *RSI* recurrent sinopulmonary infections

P10, P12-1, P12-2 and P13 had X-linked thrombocytopenia phenotype which were diagnosed as chronic idiopathic thrombocytopenic purpura before WASP gene sequencing

[a] ↑ or ↓ means higher or lower than healthy controls; activated lymphocytes means the CD3+CD69+ subset [41, 42]

[b] RSI means recurrent sinopulmonary infections and includes sinusitis, otitis, and pneumonia

[c] 1, 2, and 3 are cousins from the same families

[d] T cell proliferation including mitogens (PHA, PWM, or/and ConA) and antigens (Candida or/and Tetanus) was performed in nine patients, and impaired in four patients (P3-2, P4, P6, and P8). NK activity was detected in 14 patients and four (P8, P1, P3-2, and P8) decreased.

[e] The volume of platelet was below 8 fl (normal range between 8.5 and 15.2 fl)

(GIBCO-BRL) and polymerase chain reactions (RT-PCR) were performed as previously described [27, 28]. If a specific mutation was identified in a gene, the genomic DNA was amplified and confirmed again.

Western Blot Analysis or Flow Cytometry for WASP Expression

The WASP expression by PBMCs, B-LCL, or IL-2-dependent T cell lines was evaluated by immuno-staining as described previously [25, 29, 30]. Briefly, fresh PBMCs were stained with PE-labeled anti-CD3 mAb (Ancell, Bayport, MN) at 4°C for 20 min, washed, made permeable, and incubated in the presence of FITC-labeled anti-WASP monoclonal antibody (D1, Santa Cruz Bio, Santa Cruz, CA) at 1 μg/mL. In some patients, WASP expression was also measured by Western blot analysis. The blots were incubated with 1:1,000 anti-WASP polyclonal antibody (H-250, Santa Cruz Bio) or rabbit anti-WASP antibody (Ab 503, a gift from Ochs HD MD and Zhu Q MD at the University of Washington, Seattle, WA) [14] at room temperature.

Results

Clinical Features and Immunologic Function

During the 16-year study period (1993–2009), 13 males from ten unrelated families met the inclusion criteria (Table I) after excluding one male mortality case who refused genetic analysis and two females with mild form of WAS but normal WASP gene. Three (P12-1, P12-2, and P13) of six chronic ITP patients with "borderline"-sized platelets had WASP mutations and decreased expression.

Autoimmune disorders included autoimmune hemolytic anemia (four patients), lymphoproliferative disorders (two), and glomerulonephritis (one), while immune dysfunctions included lower IgM (six of 16 patients), higher IgE (14/16), lower CD8 counts (4/16), lower CD19 counts (5/16), decreased lymphocyte proliferation (4/9), decreased NK cytotoxicity (4/14), decreased CD4+T-memory (2/13), and B-memory (6/13) cells (Table I). Six patients had increased lymphocyte activation, suggesting persistent and ongoing autoimmune response.

Scoring, Treatment, and Prognosis

Classic WAS patients with scores of 3 or higher were aged <1 year while four XLT patients (P10-1, P12-1, P12-2, and P13) with scores of 2 or lower were aged 7.4, 10, 12, and 20 years, respectively (Table III). Classic WAS patients with scores of 4 or higher received regular IVIG treatment, platelet transfusion, and prophylactics for bronchiectasis.

Table II Stem Cell Transplantation in Taiwanese WAS Patients

	Age onset/ transplantation	Year performed	Myeloablation	Source (match)	Engraftment (post-transplant day)			GVHD prophylaxis	Cell dose (10^5/kg)		Status	
					Neutrophil	Hemoglobulin	Platelet		Nucleated cell	CD34+	Prognosis (mortality)	Chimerism of donor percentage
P1	8 M/17 M	1994	CYC, Bus	Sibling bone marrow (6/6)	14	14	22	MTX, CsA	5,640.0	NA	Alive	All donor
P3-1	3 M/6 M	1993	ATG, CYC, Bus	Sibling bone marrow (6/6)	Engraftment failure			Cys, MP	4,800.3	NA	Mortality (EBV-associated lymphoproliferative disorder)	ND
P3-2	NB/2 M	2006	ATG, CYC, Bus	Unrelated cord blood (4/6)	17	60	71	Cys, MP	1,114.2	3.0	Alive	47%
P4	3 D/12 M	2009	ATG, CYC, Bus	Unrelated cord blood (6/6)	18	54	67	Cys, MP	1,416.8	3.4	Alive	All donor
P7	1 M/7 M	1998	CYC, Bus	Father bone marrow (3/6)	Engraftment failure			Cys, MP	5,723.4	NA	Alive by IVIG and prophylactics (CMV pneumonitis)	ND
P11	4 M/6 M	1999	CYC, Bus	Sibling bone marrow (6/6)	12	24	45	Cys, MP	5,208.7	NA	Alive	All donor

ATG antithymocyte globulin, *Bus* busulfan, *CYC* cyclophosphamide, *MTX* methothrexate, *Cys* cyclosporine A, *MP* methylprednisolone, *IVIG* intravenous immunoglobulin, *NA* not available, *ND* not done

Table III Molecular Analysis in Taiwanese Patients with WASP Mutations

Patient	Tested age	Score[a]	Exon involved	Mutation type	gDNA mutation[c]	cDNA mutation (if different from gDNA)	Predicted protein change[c]	Domain	Western blot or flow cytometry
P1	5 M	4	Exon 1	Nonsense	37 C>T		Arg 13 stop	PH	Absent
P2	2 M	5	Exon 1	Deletion	Del 45–48 ACCA[b]		Phe 16 Arg, Fs, stop at 44	PH	Absent
P3-1/P3-2	1 Y/NB	5/3	Exon 1	Missense	91 G>A		Glu 31 Lys	PH	Absent/NA
P4	1 M	4	Exon 1	Nonsense	100 C>G		Arg 34 stop	PH	Absent
P5	27 D	4	Exon 1	Nonsense	121 C>T		Arg 41 stop	EVH1	Absent
P6	5 M	4	Exon 1	Nonsense	121 C>T		Arg 41 stop	EVH1	Absent
P7	23 D	5	Exon 1	Splice	IVS 1 (−1) G>C[b]	Del 43 aa and Del exon 2		EVH1	Absent
P8	1 Y	5	Promoter, exon 1 and exon 2	Deletion	Huge deletion, including promoter, exon 1 and 2[b]		Non-detectable mRNA	Whole	Absent
P9	2 M	4	Exon 2	Missense	245 C>T		Ser 82 Pro	EVH1	Absent
P10-1/P10-2	12 Y/5 M	2/4	Int 8	Splice	Int 8 (+5) G>A	Del exon 8	Fs, stop at 246	GBD	Absent/NA
P11	1 M	4	Exon 10	Insertion	1023 Ins C[b]		Ins 342 Leu, Fs, stop 494	PPPP	Decreased
P12-1/P12-2	7Y5M/23Y	2/1	Exon 10	Insertion	1,023 Ins C[b]		Ins 342 Leu, Fs, stop 494	PPPP	Decreased
P13	10 Y	1	Exon 11	Missense (double)	1,378 C>T[b] 1,421 T>C[b]		Pro 460 Ser, Met 474 Thr	VCA	Decreased

Fs frameshift, *Ins* insertion, *NA* non-available, *PH* Pleckstrin homology, *EVH1* Ena/VASP homology 1, *GBD* GTPase binding domain, *PPPP* proline-rich region, *VCA* verpoline/cofilin homology domains/acidic region

[a] Briefly, a score of one was assigned to patients with isolated thrombocytopenia and small platelets; a score of 2 was assigned to patients with microthrombocytopenia who had a history of localized eczema that responded promptly to standard therapy and/or occasionally suffered from uncomplicated upper respiratory infections; a score of 3 was given when both criteria, therapy-responsive eczema and frequent infections requiring intermittent antibiotics, were present. A score of 4 was assigned to patients with microthrombocytopenia, persistent and difficult-to-treat eczema requiring continuous treatment with steroid ointment and occasionally oral antibiotics for superinfection of the eczema, and/or severe life-threatening infections. A score of 5 was assigned to patients with WAS who developed autoimmunity or malignancy

[b] Unique mutations identified in Taiwanese patients only

[c] The beginning number 1 is based on the first nucleotide ("A"TG) of the first amino acid code (Met) according to den Dunnen JD, Antonarakis E: Nomenclature for the description of human sequence variation. *Hum Genet* 2001:109:121–4.

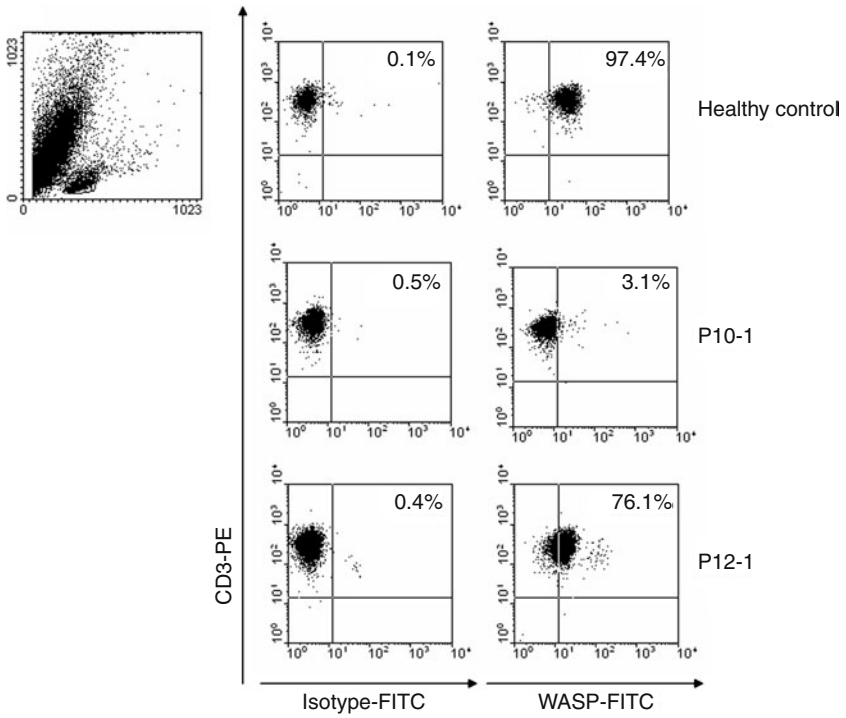

Fig. 1 Flow cytometry showed WASP protein expression in patients and healthy controls. In dot plot exhibition of forward (FSC) and size scatter (SSC), lymphocyte gating showed that intracellular WASP expression of CD3+ cells was almost absent in patient P10-1 (3.1%), reduced in patient P12-1 (76.1%), but all positive (97.4%) in the healthy control

Splenectomy was performed in two patients (P2 and P3-1) for refractory thrombocytopenia before stem cell transplantation became standard in tertiary hospitals in the 1990s. Since then, six patients received stem cell transplantation (Table II). In the four successful transplants, two were engrafted by an unrelated umbilical cord (P3-2 and P4) and two by full-matched bone marrow (P1 and P11). Hematopoietic stem cells from the bone marrow took less time to engraft. However, fully matched and haploid-identical parental bone marrow failed in two patients, who subsequently died of EBV-associated lymphoproliferative disease (P3-1) and CMV pneumonitis (P7), respectively.

To treat CVM pneumonitis (P6), 1 month of intravenous gancyclovir decreased viral load, which became undetectable by RT-PCR after an additional 1 month of oral famcyclovir. This combination of gancyclovir and famcyclovir might be recommended to eradicate CMV for further stem cell transplantation. Unfortunately, the patient died of *Staphylococcus aureus* sepsis at 2 years of age while awaiting a suitable stem cell.

Mutation Analysis and Protein Expression

Five unique Taiwanese mutations were found in 11 identified mutations, but not in 50 Taiwanese healthy females (100× chromosomes; Table III). Three patients had detectable WASP expression (Figs. 1 and 2): P12-1 and P12-2 with insertion 1023C in exon 10 causing frameshift from 342 (Leu342Thr) and stop at 494 (Fig. 3a) and P13 with "double" missense mutations (Pro460Ser and Met474Thr) located in exon 11 (Fig. 3b).

Interestingly, splice mutation Int 8(+5) G>A in P10-1 produced an undetectable WASP truncation (<5% in Fig. 2, middle lane), with subsequent exon 8 skipping, frameshift, and stop at 246. He once had *Streptococcus pneumoniae* sepsis at age 12 years and presented as XLT phenotype, in contrast to his cousin (P10-2) who presented as classic WAS phenotype.

Female pregnancy carriers from five families (P2, P3-1/3-2, P6, P8, and P10-1/10-2) received pre-natal genetic analysis, which revealed one fetus (P3-2) with missense mutation of Glu31Lys. Early unrelated cord blood stem cell transplantation saved him when he was 2 months old [31].

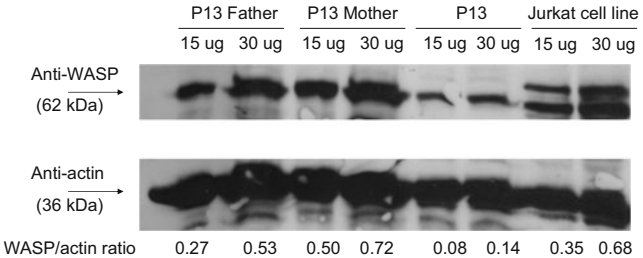

Fig. 2 Western blot compared the WASP expression in patient P13 presenting as XLT phenotype with that from Jurkat T cell lines and his parents' lymphocytes. Whole lymphocyte protein extraction were resolved on the SDS-PAGE gel and probed with antibodies against WASP and actin. The amount of WASP/actin ratio was more detectable by radiography-exposure intensity in Jurkat cell lines and the parents' lymphocytes than that from patient P13 despite 30 ug total loading

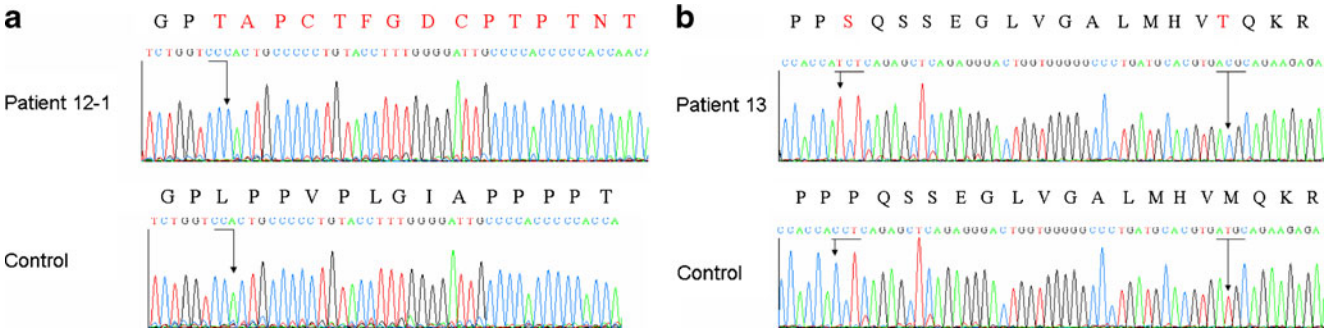

Fig. 3 **a** One nucleotide C insertion into the 1023rd location of exon 10 in patient 12-1 caused a frameshift mutation from 342 (Leu342Thr) and stop at the 494th amino acid. **b** Two nucleotides of 1378 C and 1421 T at the same allele of exon 11 in patient 13 (P13) were substituted by T and C, leading to "double" missense mutations of Pro460Ser and Met474Thr

The other three male healthy fetuses were wild-type, while one female was a carrier.

Discussion

This study first identified four Taiwanese XLT patients with or without detectable WASP expression. It provided an opportunity to compare clinical aspects and immune dysfunctions between Taiwanese XLT and WAS patients. In this series, the classic WAS or XLT phenotype is most influenced by the effect of the mutant protein expression in the patient lymphocytes, which is consistent with other reports [10, 13, 14]. WASP regulates the actin cytoskeleton, transmits, and integrates for cell motility and cell shape change in most immune cell lineages [32, 33]. Thus, profoundly defective or absent WASP expression for cell migration and interaction leads to more severe lymphopenia, impaired lymphocyte proliferation, decreased polysaccharide response [2, 34, 35], inhibited suppressor function of regulatory T [36–39] and invariant NKT cells [40], and disturbed dendritic cell function [6]. Such impaired WASP-related immunity makes patients vulnerable to life-threatening infections and autoimmunity.

However, the concept is not sufficient to predict the clinical course of patients with specific mutations of Int 8 (+5) G>A (in the same family) and 1023 Ins C (in two different families) in this study as well as previously mentioned Val75Met and Phe84Leu [13], all of which can present as XLT and classic WAS phenotype in the same mutations irrespective of WASP expression level. In these kinds of patients, XLT and classic WAS phenotype can be distinguished by the number of activated lymphocytes (CD3+CD69+) and memory cells. The activated lymphocytes in XLT patients (3/4) are higher than in classic WAS patients (1/12). The lower CD4+-memory cells accompanying impaired lymphocyte proliferation function in subgroup of classic WAS patients (P4 and P8, Table I) increases susceptibility to infections.

It may be inferred that normalized CD4+-memory cells and/or increased activated lymphocytes may, to some extent, compensate for WASP-mutation induced immune defects, as seen in family 10 without detectable WASP expression (Table I). In this family, P10-1 with higher CD4+ T-memory cells can present as XLT phenotype (P10-1) compared with P10-2 with relatively lower CD4+ T-memory who presents as classic WAS phenotype (21.1% vs. 14.2%). Whether the phenotypes of XLT or classic WAS in the same mutation are proportionally related to the number of CD4+ T-memory cells in the same family and to the number of activated lymphocytes in different families (P11 vs. P12-1 and P12-2) warrants further investigation.

Genetically, eight hot-spot mutations, defined as occurring in six or more unrelated families with E31K, T45M, V75M, R86N, E133K, R211stop, IVS 6 (+5) G>A, and IVS 8 (+1) G>A mutations, have been concluded from approximately 500 non-Chinese patients (http://homepage.mac.com/kohsukeimai/wasp/WASPbase.html) [12–14]. In Lau's 18-year study covering 1,300 million people in mainland China, only a hot-spot nonsense mutation of R211stop has been identified in three unrelated patients from 26 families of classic WAS phenotype [22]. In Taiwanese WASP mutations, the clustering pattern on exon 1 (56.3%, 9/16 individuals and 66.7%, 8/12 families), five unique mutations, and two hot-spot-like mutations of Arg41stop and 1023 Ins C (two each) may explain the genetic variations between Taiwan and China.

Based on an ongoing study of Taiwanese PIDs patients, the incidence of PIDs is estimated to be 2.17 per 100,000 live births [27] and those with WASP mutation have been recognized in 16 of 187 PIDs patients. Applying the incidence rate of PIDs (2.17 per 100,000 live births) and the distribution (16/187) of those with WASP mutation to 23 million Taiwanese, it can be estimated that at least 42

patients with WASP mutation should be recognized. However, only 12 patients with classic WAS phenotype and four with XLT phenotype have been diagnosed. This still reflects an underestimation of the disease burden in Taiwan. Moreover, regarding the phenotype of patients with WASP mutations, the ratio of XLT to classic WAS is much lower than those in Japanese and Caucasian reports [10, 12–14]. It can be surmised that there is an issue of underdiagnosis of XLT in patients treated as chronic idiopathic thrombocytopenia through ascertainment bias even though genetic variation may exist.

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