WASP (Wiskott–Aldrich syndrome protein) gene mutations and phenotype

Kohsuke Imai[a], Shigeaki Nonoyama[b] and Hans D. Ochs[c]

Purpose of review

Wiskott–Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT), characterized by chronic microthrombocytopenia with and without immunodeficiency, are caused by mutations of the *WAS protein* (*WASP*) gene. WASP has been reported to interact with many cytoplasmic molecules linking cellular signaling to the actin cytoskeleton. In this review we will focus on recent molecular findings that provide a better understanding of the pathogenesis of this complex disease and explore the correlation of genotype and clinical phenotype.

Recent findings

Recent investigations have provided evidence that WASP and several related proteins are involved in the reorganization of the actin cytoskeleton by activating Arp2/3-mediated actin polymerization. This function is controlled mainly by a small GTPase Cdc42. Activated GTP-bound Cdc42 dissociates the intramolecular autoinhibitory loop formation of WASP. In addition, WASP is involved in cytoplasmic signaling by its interaction with a variety of adaptor molecules or kinases and serves as a link to actin reorganization, which is important for immunological synapse formation, cell trafficking and motility. Tyrosine or serine phosphorylation of WASP increases the actin polymerization activity of WASP via Arp2/3. Mutation analysis of WAS/XLT patients has provided evidence for a strong correlation between phenotype and genotype. Gene therapy for WASP-deficient human lymphocytes and Wasp-deficient mice was performed successfully.

Summary

The study of WASP and its mutations has led to a better understanding of the pathogenesis of the syndrome (thrombocytopenia, immunodeficiency, atopic dermatitis, autoimmune and malignant diseases) and the mechanisms required for cell mobility, cell–cell interaction and cytoplasmic signaling, as well as thrombopoiesis and maintenance of the number of platelets.

Keywords

Wiskott–Aldrich syndrome, primary immunodeficiency, congenital thrombocytopenia, autoimmune disease, congenital neutropenia, actin cytoskeleton, gene therapy, small G-protein, genotype–phenotype correlation

Curr Opin Allergy Clin Immunol 3:427–436.

[a]INSERM (The French Institute of Health and Medical Research) U429, Hôpital Necker-Enfants Malades, Paris, France, [b]Department of Pediatrics, National Defense Medical College, Saitama, Japan and [c]University of Washington, Seattle, Washington, USA

Correspondence to Kohsuke Imai, INSERM U429, Hôpital Necker-Enfants Malades, 149, rue de Sèvres, 75015 Paris, France
Tel: +33 1 4449 2507; fax: +33 1 4273 0640; e-mail: imai@necker.fr

Current Opinion in Allergy and Clinical Immunology 2003, 3:427–436

Abbreviations

GBD	small G protein binding domain
NK cell	natural killer cell
N-WASP	neural WASP
PH/WH1/EVH1	pleckstrin homology/WASP homology 1/enabled-VASP homology 1 domain
PI(4,5)P_2	phosphatidylinositol-4,5-bisphosphate
PRR	proline rich region
TCR	T-cell receptor
VCA	verprolin homology/cofilin homology/acidic region domain
WAS	Wiskott–Aldrich syndrome
WASP	WAS protein
WIP	WAS interacting protein
XLT	X-linked thrombocytopenia

1528-4050

Introduction

Wiskott–Aldrich syndrome (WAS) was recognized as a unique clinical entity by Wiskott, who in 1936 delineated this syndrome from idiopathic thrombocytopenia or 'Morbus Werlhofii' by recognizing the association of thrombocytopenia, bloody diarrhea, eczema, and recurrent otitis media in three male infants (but not their sisters). Aldrich rediscovered this syndrome in 1954 and clearly established an X-linked recessive inheritance. Immunodeficiency, increased risk of autoimmunity and malignancies, and abnormalities of platelets were subsequently recognized [1]. Bone marrow (hematopoietic stem cell) transplantation, first reported in 1968 [2], has been shown to be the only curative therapy [3]. In 1994, a multi-institutional survey reported detailed clinical and laboratory findings in 154 patients with classic WAS, confirming the high incidence of autoimmune diseases and often fatal outcome in WAS [4].

In the same year the gene responsible for WAS/X-linked thrombocytopenia (XLT) was identified by positional cloning and designated as the *WAS protein* (*WASP*) gene [5]. The *WASP* gene was found to be mutated not only in classic WAS patients, but also in patients with XLT [6–8], which had been considered for many years to be an attenuated form of WAS because of its congenital thrombocytopenia, but without immunodeficiency or eczema [9]. Recent reports have expanded the *WASP* mutation phenotype to include intermittent XLT [10•], X-linked neutropenia with or without myelodysplasia [11,12] or WAS/XLT in females with heterozygous mutations [13,14•–16•].

WASP was considered a novel protein with unknown function until the discovery of neural WASP (N-WASP)

DOI: 10.1097/01.all.0000104452.09202.9c

[17] and other members of the 'WASP family' of proteins including the WAVE/SCARs (WASP family, Verprolin homology domain containing protein/Suppressor of cAMP receptor) which have been found conserved in a wide variety of species (human, mouse, rat, bovine, *Drosophila*, yeasts). These proteins play an important role in the organization of actin in the cells [18••,19].

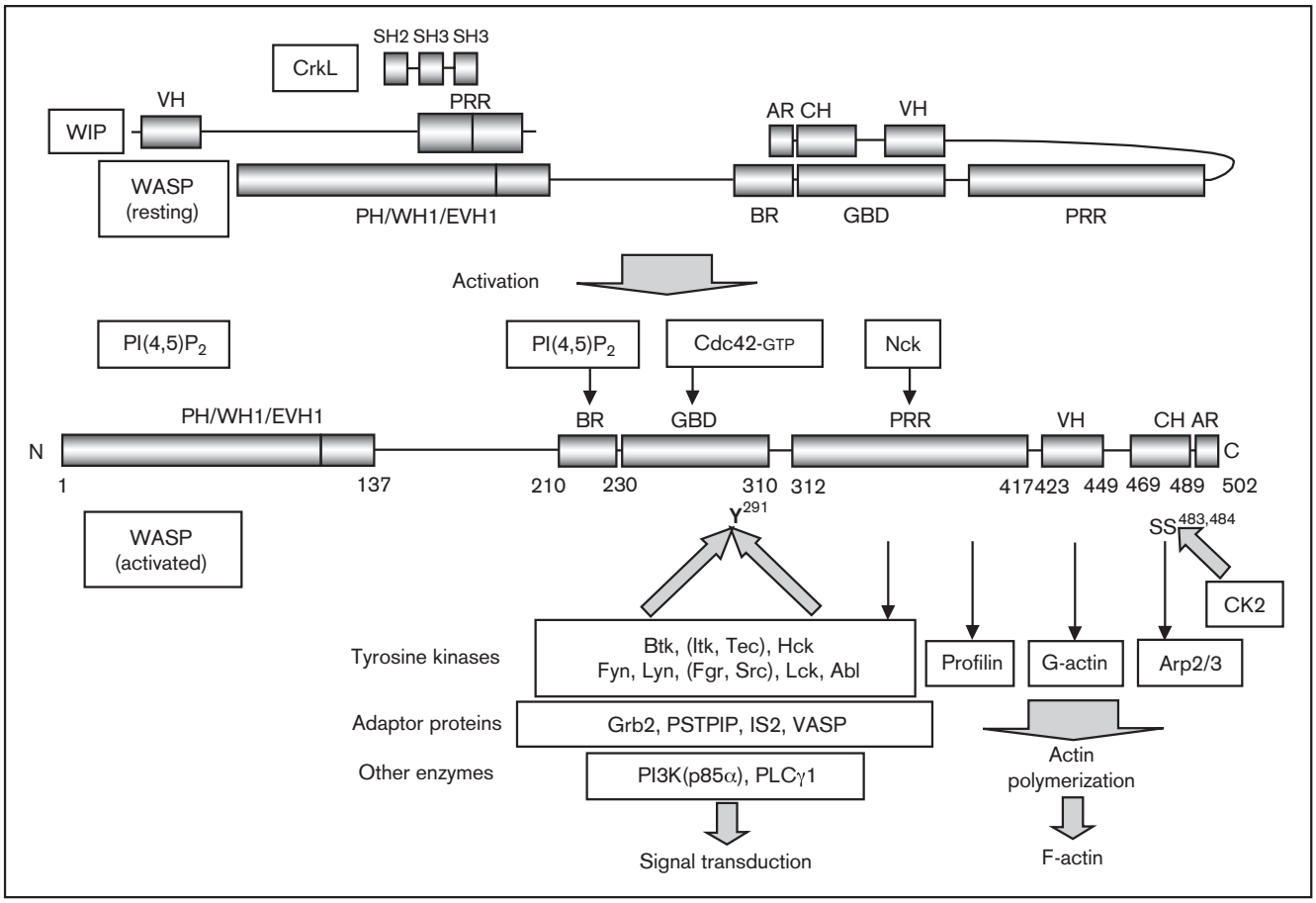
In this review we will focus on recent clinical and molecular findings that provide new insight into the pathogenesis of this complex disease and the correlation of genotype and phenotype.

WASP and WASP-binding molecules

WASP is a hematopoietic cell specific protein with several functional domains (Fig. 1), by which WASP interacts with a number of cytoplasmic molecules (Table 1) involved in signal transduction and reorganization of the cytoskeleton by activating Arp2/3-mediated actin polymerization (reviewed in [18••,20•]).

In its resting state, WASP forms an autoinhibitory loop through binding of the C-terminal region, which contains a globular actin monomer binding verprolin homology (VH) domain, a cofilin homology (CH) domain, and an acidic region (AR), to the hydrophobic core of the Cdc42/Rac GTPase-binding domain (GBD) [21]. The GTP bound activated form of Cdc42 disrupts this autoinhibitory loop and releases the proline rich region (PRR) and the C-terminal region to bind to profilin, globular actin monomer, and the Arp2/3 complex. The Arp2/3 complex, which is composed of seven polypeptides, nucleates actin polymerization which is accelerated by profilin and thus contributes to filamentous actin

Figure 1. WASP functional domains and its binding partners



In the resting state (upper panel), Wiskott–Aldrich syndrome protein (WASP) forms an intramolecular autoinhibitory structure with binding of the verprolin homology/cofilin homology/acidic region (VCA) domain to the small G protein binding domain (GBD). WASP-interacting protein (WIP) binds constitutively with its proline-rich region (PRR) domain to the PH/WH1/EVH1 (pleckstrin homology/WASP homology 1/enabled-VASP homology 1) domain of WASP. WIP also interacts constitutively with CrkL. After activation, GTP bound Cdc42 dissociates itself from the autoinhibitory structure of WASP leading to actin polymerization via Arp2/3 complex. Tyrosine kinases phosphorylate the tyrosine 291 (Y^{291}) residue, and CK2 phosphorylates the serine 483 and 484 residues ($SS^{483,\ 484}$) that enhance the actin polymerization activity of WASP. BR, basic region.

Table 1. WASP binding molecules

	Binding molecules	Binding domains in binding protein	Binding domains in WASP	Methods	Cell line used for analysis	References
Tyrosine kinases	Lyn	SH3	ND	rec. prot.	RBL-2H3	[26]
	Fyn	SH3	ND	IP and rec. prot.	CHRF, U937, Jurkat	[27,28]
	Fgr	SH3	PRR (aa375–388)	rec. prot.	CHRF, U937, Namalwa	[27,29]
	Src	SH3	PRR (aa307–322, 375–388)	rec. prot.	CHRF, U937, Jurkat, Namalwa	[27–29]
	Lck	SH3	ND	rec. prot.	Jurkat	[28]
	Btk	SH3	PRR (aa308–323)/aa. 170–185	rec.prot.	Daudi	[30]
	Itk	SH3	PRR	rec. prot.	Jurkat, Daudi	[28,30]
	Tec	SH3	ND	rec. prot.	Daudi	[30]
	Abl	SH3	PRR	rec. prot.	Jurkat	[28]
	Hck	SH3	PRR	IP and rec. prot.	U937, THP-1	[31•]
Other enzymes	PI3K-p85α	SH3	ND	rec. prot.	CHRF, U937, Jurkat, Namalwa	[27–29]
	PLC-γ1	SH3	PRR (aa307–322, 375–388)	rec. prot.	CHRF, U937, Namalwa, Daudi	[27,29,30]
Adaptor proteins	WIP	C-terminal PRR	PH/WH1/EVH1	two hybrid and IP	lymphocyte	[32]
	Nck	SH3	PRR	IP and rec. prot.	HL-60	[33]
	Grb2/Ash	SH3-N, C	PRR	IP and rec. prot.	CHRF, U937, Namalwa, Daudi, Meg01	[17,27,28,30]
	PSTPIP1	SH3	PRR	two hybrid, IP and rec. prot.	COS	[34]
	intersectin 2	SH3	PRR	IP and rec. prot.	Jurkat	[35]
Small G-proteins	Cdc42Hs	ND	GBD	IP and rec. prot.	neutrophil, HEL, EBV-B blasts	[36–38]
	Rac	ND	GBD	rec. prot.	–	[37]
Actin related molecules	G-actin	–	VH	rec. prot.	COS7	[39]
	Arp2/3	–	CH/AR	rec. prot.	–	[40]
	profilin	–	PRR	IP and rec. prot.	PC12, 3T3	[41]
	VASP	–	PRR	rec. prot.	RBL-2H3, BHK-21	[42]
Phospholipids	PI(4,5)P_2	–	PH/WH1/EVH1, BR	rec. prot.	–	[43,44•]

ND, not determined; rec. prot., recombinant protein; IP, immunoprecipitation; PRR: proline rich region; PH/WH1/EVH1, pleckstrin homology/WASP homology 1/enabled-VASP homology 1 domain; two hybrid, yeast two hybrid method; GBD, small G protein binding domain; G-actin, globular actin monomer; CH, cofilin homology domain; AR, acidic region; BR, basic region; PI(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; PLC: phospholipase C; PSTPIP1: proline-serine-threonine phosphatase-interacting protein 1.

formation (reviewed in [22••]). WASP binding molecules, phosphatidylinositol-4,5-biphosphate (PI(4,5)P_2) and Nck (Table 1) synergize the activation of WASP [23–25].

Phosphorylation of WASP was shown to enhance the actin polymerization activity of WASP. Hck, a hematopoietic cell specific Src-type tyrosine kinase, has been found to be the latest partner of WASP. Hck binds and phosphorylates WASP on tyrosine 291 as does Btk [45], a process that enhances the actin polymerization activity of WASP via the Arp2/3 complex [31•,46•]. Similarly, phosphorylation of serine 483 and 484 in the cofilin homology domain of WASP by casein kinase 2 increases the affinity of the C-terminal region for the Arp2/3 complex and is required for efficient actin polymerization *in vitro* [47•].

N-terminal domains of WASP and N-WASP were originally reported by sequence alignment to be a pleckstrin homology (PH) domain and to bind to PI(4,5)P_2 using an ELISA method and PI(4,5)P_2 specific antibody [17,43]. Subsequent structural analysis revealed that this domain is a distinct member of the PH domain superfamily called WH1/EVH1 (WASP-homology 1/enabled-VASP homology 1) domain which binds to proline rich peptides [48–50] but not to PI(4,5)P_2 when using lipid vesicle binding assays and ultracentrifugation [44•]. Volkman and co-workers [44•] showed that PI(4,5)P_2 binds to basic region and GBD-derived peptides.

WASP-interacting protein (WIP) was found to be a binding partner of WASP by the yeast two hybrid method and by immunoprecipitation from lymphocytes [32]. It is ubiquitously expressed and is reported to participate in fillopodium formation [51], ruffle formation [52] and vesicle movement [53]. WIP has a proline rich region in its C-terminal region which constitutively binds to the PH/WH1/EVH1 domain [44•]. Most missense

mutations (40/51 published mutations) observed in WAS/XLT patients are located in the PH/WH1/EVH1 domain of WASP (Table 2), suggesting that WIP plays an important role as a ligand of the PH/WH1/EVH1 domain. WIP also binds to CrkL constitutively and forms the complex of CrkL–WIP–WASP [54••]. Stimulation of the T-cell receptor and subsequent phosphorylation of ZAP-70 allows the binding of the SH2 domain of CrkL to ZAP-70 and thus initiates the transport of the CrKL–WIP–WASP complex to the lipid rafts to form the immunological synapse [54••]. After the serine phosphorylation of WIP by protein kinase C (PKC) θ, WASP dissociates from WIP and can be activated by membrane bound Cdc42 to initiate Arp2/3 complex-dependent actin polymerization [54••]. Recently the phenotype of WIP-deficient mice was reported [55••], demonstrating that WIP-deficient mice show no gross abnormalities. This is in spite of the fact that WIP is widely expressed and binds to both WASP and N-WASP [32,44•]. Whereas WIP seems dispensable for T- and B-lymphocyte development, WIP is essential for T-cell activation via the T-cell receptor (TCR)/CD3 complex: WIP-deficient T cells show impaired proliferation and IL-2 secretion in response to anti-CD3 stimulation. Addition of IL-2 to the culture system rescued the proliferation in response to anti-CD3 stimulation. WIP-deficient T cells fail to increase the filamentous actin content or to form protrusions and pseudopodia following TCR/CD3 ligation. Alternatively, responses to PMA + ionomycin were normal [55••]. The abnormalities observed in WIP-deficient T cells are similar, but more profound than those seen in WASP-deficient mice [56,57]. These observations suggest a critical role for WIP in T-cell activation via TCR/CD3, and the WASP-deficient T-cell defect reported may be partially due to defective signaling via WIP. Interestingly, B cells of WIP-deficient mice showed increased proliferation and IL-2 receptor expression in response to various stimulations, suggesting a negative regulatory effect of WIP. Antibody responses to a T-independent antigen were normal, but were impaired to a T-dependent antigen [55••].

Table 2. Missense mutations of the *WASP* gene and associated phenotypes

Domain	AA		XLT	WAS	XLN
PH/WH1	6	Met > Ile	2		
PH/WH1	27	Leu > Phe	1		
PH/WH1	31	Glu > Lys/Gly/Asp	1	7	
PH/WH1	35	Leu > His		1	
PH/WH1	39	Leu > Pro	3	1	
PH/WH1	40	Gly > Val		1	
PH/WH1	41	Arg > Gly	1		
PH/WH1	43	Cys > Trp		1	
PH/WH1	45	Thr > Met	15		
PH/WH1	46	Leu > Pro	1		
PH/WH1	47	Ala > Asp	1		
PH/WH1	48	Thr > Ile	1		
PH/WH1	52	Gln > His	1		
PH/WH1	56	Ala > Val	6		
PH/WH1	58	Pro > Arg/Leu	1[a]	1	
PH/WH1	64	Trp > Arg		1	
PH/WH1	70	Gly > Try		1	
PH/WH1	73	Cys > Arg/Tyr		3	
PH/WH1	75	Val > Met	11	3	
PH/WH1	77	Gln > Gly	1		
PH/WH1	82	Ser > Pro/Phe		2	
PH/WH1	83	Tyr > Cys	1		
PH/WH1	84	Phe > Leu	2	2	
PH/WH1	85	Ile > Thr		1	
PH/WH1	86	Arg > Cys/His/Pro/Leu	19	17	
PH/WH1	89	Gly > Asp	1		
PH/WH1	97	Trp > Cys	2	1	
PH/WH1	99	Gln > Arg	1		
PH/WH1	105	Leu > Pro		1	
PH/WH1	107	Tyr > Cys	1	1	
PH/WH1	111	Thr > Pro		1	
PH/WH1	115	His > Tyr		1	
PH/WH1	119	Gly > Glu	1		
PH/WH1	124	Ala > Glu		1	
PH/WH1	125	Gly > Arg		2	
PH/WH1	128	Phe > Leu/Ser		3	
PH/WH1	131	Glu > Lys	1	3	
PH/WH1	133	Glu > Lys	1	13	
PH/WH1	134	Ala > Thr/Val		2	
PH/WH1	138	Arg > Pro	2	1	
BR	187	Gly > Cys	2		
GBD	236	Ala > Glu/Gly	3		
GBD	270	Leu > Pro			1
GBD	294	Ile > Thr			1
GBD	307	Met > Val	1		
PRR	339	Ser > Tyr		1	
PRR	359	Pro > Thr		1	
PRR	373	Pro > Ser		1	
VH/WH2	459	Pro > Ser		1	
CH	476	Lys > Glu		1	
CH	477	Arg > Lys	2		
CH	481	Ile > Asn	1[a]		
CH	485	Asp > Asn	1	2	
		Total	88	79	2

AA, amino acid. [a]Intermittent X-linked thrombocytopenia. Data from WASPbase [82•].

T-lymphocyte and NK cell abnormalities in WAS patients

Reorganization of the actin cytoskeleton is required for the establishment of immunological synapses between T lymphocytes and antigen-presenting cells, as well as between cytotoxic T lymphocytes or NK cells and their targets. On the T-cell side, the immunological synapse comprises a central area containing the TCR complex, and CD2 and CD28 molecules, and a peripheral rim in which adhesion molecules are accumulated. These local modifications are associated with a focal process of actin polymerization. Although the immunological synapse is not involved in the initiation of TCR-mediated signaling, it ensures optimal T-cell activation and localized effector activity. Following TCR ligation, WASP, WIP, CrkL and ZAP-70 are rapidly recruited to lipid rafts that are membrane platforms which allow lateral movements of the TCR complex and associated kinases. WASP is also required for the immunological synapse formation

by binding to the SH3 domain containing cytoplasmic adaptor protein, PSTPIP1/CD2BP1, after stimulation with CD2 [58•]. Recruitment of WASP to the immunological synapse in T cells is independent of its GBD domain but dependent on the PRR [59]. Tyrosine phosphorylation of SLP-76 by ZAP-70 results in the binding of the SH2 domain of Nck and Vav-1. Nck recruits WASP to the immunological synapse and Vav-1, a guanine nucleotide exchange factor for Rho family GTPases, may activate Cdc42 which activates WASP [60••]. Mutated WASP negatively affects the establishment of immunological synapses and T-cell activation via CD3 [61••] and CD2 [58•]. WASP deficiency results in a lower basal level of lipid rafts and in impaired upregulation of lipid rafts following TCR crosslinking [61••]. As a consequence, T lymphocytes from WAS patients are not only impaired in undergoing activation, but also in sustaining activation. Thus WAS patients and WASP-deficient mice show defective TCR-induced T-cell proliferation, defective T-cell capping, and reduced flux of intracellular calcium in anti-CD3 activated T cells [57,58•,61••,62]. These defects may play a major role in the inability of WAS patients to elicit efficient antigen-specific T-cell dependent memory immune responses.

WASP is required for NK cell cytotoxicity. Actin accumulates at the immunological synapse on NK cells, which is defective in NK cells of WAS patients [63•]. This defect may explain the susceptibility of WAS patients to herpes group virus infections and the increased rate of malignancy.

Monocyte/macrophage/dendritic cell and neutrophil abnormalities in WAS patients

WAS patients show various defects in cell motility and homing, including reduced motility of monocytes in response to formyl-methionyl-leucyl-phenylalanine (fMLP), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and impaired podosome expression by macrophages and dendritic cells [18••].

WASP may also be involved in T-lymphocyte trafficking, as indicated by the fact that stromal-derived factor-1α (SDF-1α)/CXCL12, a well-studied T-cell chemoattractant, induces tyrosine phosphorylation of WASP and of some adaptors (Nck, Cas) that are involved in cell migration [64].

The minimal region of WASP required to direct cellular movement is the C-terminal VCA (VH/CH/AR) region, which is also the minimal region required for stimulating actin nucleation [65•]. Phagocytic defects are also seen in WASP-deficient macrophages and neutrophils [66]. Lentiviral-mediated transfection of WASP into WAS macrophages restores these abnormalities and normalizes chemotaxis in response to colony stimulating factor-1 (CSF-1) [67•].

Gene therapy for human WASP-deficient cells and WASP-deficient mice

Although bone marrow transplantation from an HLA-matched donor (sibling or unrelated) has an excellent rate of success, especially if performed during the first 5 years of life (5-year probability of survival 87%) [3], many patients do not have a suitable donor. One source of hematopoietic stem cells is unrelated, partially HLA-matched cord blood and has been performed successfully [68•,69].

Another treatment approach, gene therapy, is being actively investigated. Retrovirus-mediated WASP gene transfer into B-cell lines corrected the cell surface abnormality of glycoproteins and the defective actin polymerization [70,71]. Human WASP-deficient T cells (primary cells [72•] and HTLV-1 (human T lymphotrophic virus–1) transformed T-cell lines [73•]) transduced with retroviral vectors encoding *WASP* expressed WASP that was able to bind to SH3 containing proteins (Grb2, phospholipase C (PLC)-γ1, and Fyn) and to proliferate and polymerize actin in response to anti-CD3 stimulation. No toxic effect as a result of overexpression of WASP was observed with retrovirus-mediated gene transfer.

To test the feasibility and safety of gene therapy, Wasp-deficient mice who in concordance with WAS patients have impaired T-cell receptor-induced proliferation and aberrant cytoskeleton rearrangement [56,57], underwent gene therapy [74•]. Hematopoietic stem cells obtained from WASP-deficient mice were transduced with WASP-expressing retroviruses and transferred into RAG2-deficient mice after total body irradiation (WASP-deficient mice developed severe colitis after total body irradiation and did not survive the procedure). Mature B and T lymphocytes developed in normal numbers and TCR induced proliferation was significantly improved in the RAG2-deficient mice after transplantation of *Wasp* gene transduced hematopoietic stem cells. Competitive repopulation experiments by transplanting a mixture of wild type and WASP-deficient bone marrow cells revealed a selective advantage of wild type cells in spleen but not in bone marrow or thymus [74•]. Although transient ectopic overexpression of WASP has been reported to be associated with dramatic changes in the actin cytoskeleton [36], retrovirus-mediated WASP expression does not seem to have such a toxic effect [70,71,72•,74•].

The observation of spontaneous in-vivo reversion of *WASP* mutations also suggests a growth advantage of T cells with expression of a normal *WASP* gene [75,76].

WAS gene mutations and phenotype

A correlation between clinical phenotype and genotype was reported independently by several investigators [77–79] but not observed by others [80,81].

Over 400 patients with *WASP* gene mutation have been reported to date. The mutations, WASP expressions and the phenotypes reported are accessible on the Internet as WASPbase [82•]. Using a simple scoring system [78,83•], the severity of the clinical phenotype can be assessed (Table 3). All but four patients with *WASP* mutations (two intermittent XLT, two X-linked neutropenia, see below) have chronic thrombocytopenia which is a common finding of WAS (with immunodeficiency, score 3, 4) and XLT (without immunodeficiency, score 1, 2). Forty-six percent of all *WASP* gene mutated patients have null mutations (nonsense mutations, deletions, insertions with frameshift), 42% have missense mutations and 12% have splice anomalies (Table 4). The majority of the missense mutations are located in the PH/WH1/EVH1 domain (Table 2) as reported previously [6]. Majority of the patients with null mutations and splice anomalies involving invariant nucleotides have the classic WAS phenotype (86.4% and 89.8%). Although the majority of XLT patients (74.6%, 88/118 cases) have a missense mutation, only half of the patients with a missense mutation have the XLT phenotype (52.7%, 88/167 cases). This apparent lack of a genotype/phenotype correlation in patients with missense mutations has several explanations. Many patients collected from the literature were scored retrospectively based on incomplete records. Some missense mutations result in lack of WASP expression, expected to result in a classic WAS phenotype. This explanation is underscored by the observation that WASP expression correlates best with the clinical phenotype: 74.2% of the patients with positive WASP expression present with the XLT phenotype, and 86.5% of the patients with negative WASP expression develop the classic WAS phenotype (Table 5). The most frequently reported missense mutation affects Arg86 (36 patients) (Table 2). Data provided in published reports fail to demonstrate a clear correlation of genotype/protein expression and phenotype, since 42% (14/33) of patients with Arg86Cys or Arg86His mutation are WASP positive and have a WAS phenotype. Such a discrepancy was also reported by Schindelhauer *et al.* [81]. Interestingly, Arg86 is an important site for the interaction of WASP and WIP [44•,84]. By contrast, two large referral centers, using the scoring system described above, observed that of 19 patients from 17 unrelated families with missense mutations affecting Arg86, one patient had a score of 3 (WAS phenotype), two had scores of 2–3 and the remaining 16 patients had XLT with scores of 1 (3) and 2 (13). All patients but two had demonstrable WASP by Western blot, although the amount was consistently reduced (L. Notarangelo and H.D. Ochs, unpublished observation). The exclusion of patients with mutations of Arg86 listed in Table 2 improves the correlation of genotype-WASP expression and phenotype: 83.3% of patients with missense mutations other than Arg86 who are also WASP positive have the XLT phenotype.

Table 3. Clinical scores for WAS/XLT patients [78]

	XLT		WAS		
Scores	1	2	3	4	5
Thrombocytopenia	+	+	+	+	+
Immunodeficiency	–	– or +	+	++	–~++
Eczema	–	– or +	+	++	–~++
Autoimmune or malignancy	–	–	–	–	+

XLT, X-linked thrombocytopenia; WAS, Wiskot–Aldrich syndrome.

Table 5. Correlation of WASP expression and phenotype in the patients with a missense mutation of *WASP* gene

	Cases with a missense mutation		%	
WASP expression	XLT	WAS	XLT	WAS
Positive	69	24	74.2	25.8
Negative	5	32	13.5	86.5

WASP, Wiskott–Aldrich syndrome protein; WAS, Wiskott–Aldrich syndrome; XLT, X-linked thrombocytopenia. Data from WASPbase [82•].

Table 4. Correlation of genotype and phenotype from WASPbase [82•]

	Type of mutations (cases)				Type of mutations (%)		
Phenotype	Null	Missense	Splice	Total	Null	Missense	Splice
WAS	159	77	44	280	86.4	46.1	89.8
XLT	25	88	5	118	13.6	52.7	10.2
XLN	0	2	0	2	0.0	1.2	0.0
Total	184	167	49	400			
%	46	42	12	100			

WAS, Wiskott–Aldrich syndrome; XLT, X-linked thrombocytopenia; XLN, X-linked neutropenia.

A recent analysis evaluated the clinical phenotype, the genotype and WASP expression in a large cohort of Japanese *WASP* gene mutated patients. Susceptibility to infections, severe eczema, intestinal hemorrhage, death due to intracranial bleeding and malignancy were highly associated with lack of WASP expression by patient lymphoctyes. Overall survival and survival without intracranial hemorrhage were significantly lower in WASP negative patients. This observation strongly suggests that the long-term clinical outcomes correlated with WASP expression [69].

Autoimmune disease in classical WAS patients is a frequent complication ([4,85•], reviewed in [86•]). The incidence of autoimmune or inflammatory manifestations is reported to be 40% (61/154 patients) in one study [4] and 72% (40/55 patients) in a more recent survey [85•]. In the study from Japan [69], 22% of patients with classic WAS (6/27) presented with autoimmune disease and two of those six patients also developed malignancies. The relatively low incidence of autoimmune disease in the Japanese patients can be explained by the younger age of the patients and the higher percentage (52%) of patients receiving stem cell transplantation at an early age (mean age 2.3 years). Although several cases of autoimmune diseases in XLT have been reported, the incidence of this complication in XLT is unknown. Of the 23 Japanese patients with the XLT phenotype, six (26%) reported autoimmune or inflammatory disease, including five boys who had developed IgA nephropathy [69].

Two unexpected clinical phenotypes due to missense mutations of the *WASP* gene have recently been recognized.

Two Italian families in which affected males presented with intermittent thrombocytopenia were found to have missense mutations in exon 2 (P58R) and exon 11 (I481N), respectively [10•]. Affected patients experienced normal platelet numbers at times, followed by periods of thrombocytopenia. They did not have splenectomy and were originally considered to have recurrent idiopathic thrombocytopenia. The reduced platelet volume, which is not consistent with idiopathic thrombocytopenia, led to *WASP* gene analysis and the discovery of two novel missense mutations affecting the PH/WH1/EVH1 and the CH domains. A normal amount of WASP protein was observed in B-cell lines and platelets from affected males.

Two families with X-linked neutropenia and a maturation arrest at the promyelocyte/myelocyte stage without microthrombocytopenia or eczema were found to have missense mutations within the GBD domain (L270P and I294T, respectively) [11,12]. This mutation resembles the effect of phosphorylation of tyrosine 291 residue resulting in the constitutive active form of WASP [12]. It is not known why the constitutive active form of WASP leads to neutropenia with maturation arrest [11,12] or myelodysplasia [11].

These observations extend the spectrum of the clinical phenotype associated with *WASP* gene mutations beyond the classic features of thrombocytopenia and small platelets commonly observed in the WAS/XLT phenotype.

Conclusion

WASP is a complex protein with multiple functional domains. It is involved in platelet production/removal, cytoplasmic signaling, actin polymerization, cell–cell interaction, immunological synapse formation, cell motility, phagocytosis and neutrophil production/release. The effect of *WASP* gene mutations on protein expression strongly correlates with the clinical phenotype.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan (S.N.), Kawano Masanori Memorial Foundation for Promotion of Pediatrics (S.N.), the National Institutes of Health (HD 17427) (H.D.O.), the DeJoria Wiskott–Aldrich Research Grant (H.D.O.), the Jeffrey Modell Foundation (H.D.O.) and the Immunodeficiency Foundation (H.D.O.).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 Ochs HD, Rosen FS. The Wiskott–Aldrich syndrome. In: Ochs HD, Smith CIE, Puck JM, editors. Primary immunodeficiency diseases: a molecular and genetic approach. Oxford University Press: Oxford; 1999. pp. 292–305.

2 Bach FH, Albertini RJ, Joo P, *et al.* Bone-marrow transplantation in a patient with the Wiskott–Aldrich syndrome. Lancet 1968; 2:1364–1366.

3 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; 97:1598–1603.

4 Sullivan KE, Mullen CA, Blaese RM, Winkelstein JA. A multiinstitutional survey of the Wiskott–Aldrich syndrome. J Pediatr 1994; 125:876–885.

5 Derry JM, Ochs HD, Francke U. Isolation of a novel gene mutated in Wiskott–Aldrich syndrome. Cell 1994; 78:635–644 [published erratum in Cell 1994; 79:922].

6 Schwarz K. WASPbase: a database of WAS- and XLT-causing mutations. Immunol Today 1996; 17:496–502.

7 Zhu Q, Zhang M, Blaese RM, *et al.* The Wiskott–Aldrich syndrome and X-linked congenital thrombocytopenia are caused by mutations of the same gene. Blood 1995; 86:3797–3804.

8 Villa A, Notarangelo L, Macchi P, *et al.* X-linked thrombocytopenia and Wiskott–Aldrich syndrome are allelic diseases with mutations in the WASP gene. Nat Genet 1995; 9:414–417.

9 Canales ML, Mauer AM. Sex-linked hereditary thrombocytopenia as a variant of Wiskott–Aldrich syndrome. N Engl J Med 1967; 277:899–901.

10 Notarangelo LD, Mazza C, Giliani S, *et al*. Missense mutations of the WASP • gene cause intermittent X-linked thrombocytopenia. Blood 2002; 99:2268–2269.
This paper describes two families with intermittent thrombocytopenia due to missense mutations of the WASP gene. This is the mildest phenotype of WASP gene mutations, and expands the clinical spectrum of the disease.

11 Ancliff PJ, Blundell MP, Gale RE, *et al*. Activating mutations in the Wiskott–Aldrich syndrome protein may define a sub-group of severe congenital neutropenia (SCN) with specific and unusual laboratory features. American Society of Hematology 43rd Annual Meeting; Orlando, Florida; 2001.

12 Devriendt K, Kim AS, Mathijs G, *et al*. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 2001; 27:313–317.

13 Parolini O, Ressmann G, Haas OA, *et al*. X-linked Wiskott–Aldrich syndrome in a girl. N Engl J Med 1998; 338:291–295.

14 Inoue H, Kurosawa H, Nonoyama S, *et al*. X-linked thrombocytopenia in a girl. • Br J Haematol 2002; 118:1163–1165.
This is a report of a rare case of XLT in a female with heterozygous mutation of the *WASP* gene.

15 Lutskiy MI, Sasahara Y, Kenney DM, *et al*. Wiskott–Aldrich syndrome in a • female. Blood 2002; 100:2763–2768.
This is a report of a rare case of WAS in a female with heterozygous mutation of the WASP gene. Analysis of WASP expression in her blood cells indicates that activation and maturation of T lymphocytes is associated with preferential expansion of WASP-positive cells.

16 Zhu Q, Christie JR, Tyler EO, *et al*. X-chromosome inactivation in • symptomatic carrier females of X-linked thrombocytopenia. Clin Immunol 2002; 103:S129–S130.
This describes symptomatic carriers for XLT.

17 Miki H, Miura K, Takenawa T. N-WASP a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J 1996; 15:5326–5335.

18 Thrasher AJ. WASp in immune-system organization and function. Nat Rev •• Immunol 2002; 2:635–646.
This is a very detailed review on the biological properties of WASP and related proteins, and offers a nice overview of the cellular defects associated with WASP gene mutations.

19 Takenawa T, Miki H. WASP and WAVE family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. J Cell Sci 2001; 114:1801–1809.

20 Caron E. Regulation of Wiskott–Aldrich syndrome protein and related • molecules. Curr Opin Cell Biol 2002; 14:82–87.
This is a review of WASP family proteins focused on molecular aspects.

21 Kim AS, Kakalis LT, Abdul-Manan N, *et al*. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. Nature 2000; 404:151–158.

22 Weaver AM, Young ME, Lee WL, Cooper JA. Integration of signals to the •• Arp2/3 complex. Curr Opin Cell Biol 2003; 15:23–30.
This review focused on the Arp2/3 complex and its regulation of actin cytoskeleton.

23 Higgs HN, Pollard TD. Activation by Cdc42 and PIP(2) of Wiskott–Aldrich syndrome protein (WASp) stimulates actin nucleation by Arp2/3 complex. J Cell Biol 2000; 150:1311–1320.

24 Rohatgi R, Nollau P, Ho HY, *et al*. Nck and phosphatidylinositol 4,5-bisphosphate synergistically activate actin polymerization through the N-WASP-Arp2/3 pathway. J Biol Chem 2001; 276:26448–26452.

25 Rozelle AL, Machesky LM, Yamamoto M, *et al*. Phosphatidylinositol 4,5-bisphosphate induces actin-based movement of raft-enriched vesicles through WASP-Arp2/3. Curr Biol 2000; 10:311–320.

26 Guinamard R, Aspenstrom P, Fougereau M, *et al*. Tyrosine phosphorylation of the Wiskott–Aldrich syndrome protein by Lyn and Btk is regulated by CDC42. FEBS Lett 1998; 434:431–436.

27 Banin S, Truong O, Katz DR, *et al*. Wiskott–Aldrich syndrome protein (wasp) is a binding partner for c-src family protein-tyrosine kinases. Curr Biol 1996; 6:981–988.

28 Bunnell SC, Henry PA, Kolluri R, *et al*. Identification of itk/tsk src homology 3 domain ligands. J Biol Chem 1996; 271:25646–25656.

29 Finan PM, Soames CJ, Wilson L, *et al*. Identification of regions of the Wiskott–Aldrich syndrome protein responsible for association with selected src homology 3 domains. J Biol Chem 1996; 271:26291–26295.

30 Cory G, Maccarthymorrogh L, Banin S, *et al*. Evidence that the Wiskott–Aldrich syndrome protein may be involved in lymphoid cell signaling pathways. J Immunol 1996; 157:3791–3795.

31 Scott MP, Zappacosta F, Kim EY, *et al*. Identification of novel SH3 domain • ligands for the Src family kinase Hck: Wiskott–Aldrich syndrome protein (WASP), WASP-interacting protein (WIP), and ELMO1. J Biol Chem 2002; 277:28238–28246.
This paper demonstrates that WASP and WIP are major binding partners of Hck, a Src-family tyrosine kinase that plays a major role in regulating cell migration and integrin-mediated signal transduction.

32 Ramesh N, Anton IM, Hartwig JH, Geha RS. WIP, a protein associated with Wiskott–Aldrich syndrome protein, induces actin polymerization and redistribution in lymphoid cells. Proc Natl Acad Sci U S A 1997; 94:14671–14676.

33 Rivero-Lezcano OM, Marcilla A, Sameshima JH, Robbins KC. Wiskott–Aldrich syndrome protein physically associates with Nck through Src homology 3 domains. Mol Cell Biol 1995; 15:5725–5731.

34 Wu Y, Spencer SD, Lasky LA. Tyrosine phosphorylation regulates the SH3-mediated binding of the Wiskott–Aldrich syndrome protein to PSTPIP, a cytoskeletal-associated protein. J Biol Chem 1998; 273:5765–5770.

35 McGavin MK, Badour K, Hardy LA, *et al*. The intersectin 2 adaptor links Wiskott Aldrich Syndrome protein (WASp)-mediated actin polymerization to T cell antigen receptor endocytosis. J Exp Med 2001; 194:1777–1787.

36 Symons M, Derry JM, Karlak B, *et al*. Wiskott–Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell 1996; 84:723–734.

37 Aspenstrom P, Lindberg U, Hall A. Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome. Curr Biol 1996; 6:70–75.

38 Kolluri R, Tolias KF, Carpenter CL, *et al*. Direct interaction of the Wiskott–Aldrich syndrome protein with the GTPase Cdc42. Proc Natl Acad Sci U S A 1996; 93:5615–5618.

39 Miki H, Takenawa T. Direct binding of the verprolin-homology domain In N-Wasp to actin is essential for cytoskeletal reorganization. Biochem Biophys Res Commun 1998; 243:73–78.

40 Machesky LM, Insall RH. Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. Curr Biol 1998; 8:1347–1356.

41 Suetsugu S, Miki H, Takenawa T. The essential role of profilin in the assembly of actin for microspike formation. EMBO J 1998; 17:6516–6526.

42 Castellano F, Le Clainche C, Patin D, *et al*. A WASp–VASP complex regulates actin polymerization at the plasma membrane. EMBO J 2001; 20:5603–5614.

43 Imai K, Nonoyama S, Miki H, *et al*. The pleckstrin homology domain of the Wiskott–Aldrich syndrome protein is involved in the organization of actin cytoskeleton. Clin Immunol 1999; 92:128–137.

44 Volkman BF, Prehoda KE, Scott JA, *et al*. Structure of the N-WASP EVH1 • domain–WIP complex: insight into the molecular basis of Wiskott–Aldrich syndrome. Cell 2002; 111:565–576.
This study analyzes in detail the biochemistry and structure of the WASP–WIP interaction, and evaluates the putative effect that various missense mutations in the WH1 domain of WASP have on this interaction.

45 Baba Y, Nonoyama S, Matsushita M, *et al*. Involvement of Wiskott–Aldrich syndrome protein in B-cell cytoplasmic tyrosine kinase pathway. Blood 1999; 93:2003–2012.

46 Cory GO, Garg R, Cramer R, Ridley AJ. Phosphorylation of tyrosine 291 • enhances the ability of WASp to stimulate actin polymerization and filopodium formation: Wiskott–Aldrich syndrome protein. J Biol Chem 2002; 277:45115–45121.
This study illustrates the important role of phosphorylation of Tyr291 of WASP in regulating formation of cellular podosomes and filopodia.

47 Cory GO, Cramer R, Blanchoin L, Ridley AJ. Phosphorylation of the WASP- • VCA domain increases its affinity for the Arp2/3 complex and enhances actin polymerization by WASP. Mol Cell 2003; 11:1229–1239.
This study demonstrates the phosphorylation of serine within the cofilin homology domain of WASP and its effect on the actin polymerization activity of WASP via Arp2/3 complex.

48 Ponting CP, Phillips C. Identification of homer as a homologue of the Wiskott–Aldrich syndrome protein suggests a receptor-binding function for WH1 domains. J Mol Med 1997; 75:769–771.

49 Prehoda KE, Lee DJ, Lim WA. Structure of the enabled/VASP homology 1 domain-peptide complex: a key component in the spatial control of actin assembly. Cell 1999; 97:471–480.

50 Rong SB, Vihinen M. Structural basis of Wiskott–Aldrich syndrome causing mutations in the WH1 domain. J Mol Med 2000; 78:530–537.

51 Martinez-Quiles N, Rohatgi R, Anton IM, *et al.* WIP regulates N-WASP-mediated actin polymerization and filopodium formation. Nat Cell Biol 2001; 3:484–491.

52 Anton IM, Saville SP, Byrne MJ, *et al.* WIP participates in actin reorganization and ruffle formation induced by PDGF. J Cell Sci 2003; 116:2443–2451.

53 Benesch S, Lommel S, Steffen A, *et al.* Phosphatidylinositol 4,5-biphosphate (PIP2)-induced vesicle movement depends on N-WASP and involves Nck, WIP, and Grb2. J Biol Chem 2002; 277:37771–37776.

54 •• Sasahara Y, Rachid R, Byrne MJ, *et al.* Mechanism of recruitment of WASP to the immunological synapse and of its activation following TCR ligation. Mol Cell 2002; 10:1269–1281.
This paper demonstrates that the constitutive interaction between WASP and WIP is released (permitting WASP-dependent activation of the Arp2/3 complex) upon formation of a macromolecular complex, which also includes Crkl. This complex is recruited to the immunological synapse by ZAP-70. This results in phosphorylation of WIP, and subsequent activation of WASP by GTP-bound Cdc42.

55 •• Anton IM, de la Fuente MA, Sims TN, *et al.* WIP deficiency reveals a differential role for WIP and the actin cytoskeleton in T and B cell activation. Immunity 2002; 16:193–204.
This is a report of WIP knockout mice. The authors focus on the description of T- and B-lymphocyte development and function in WIP-deficient mice. WIP-deficient T cells have impaired activation following TCR stimulation. WIP-deficient B cells show hyperreactivity to B-cell receptor stimulation. T and B cells have a profound defect in the subcortical actin filament network. The authors propose that WIP is important for immunological synapse formation and T-cell activation.

56 Snapper SB, Rosen FS, Mizoguchi E, *et al.* Wiskott–Aldrich syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. Immunity 1998; 9:81–91.

57 Zhang J, Shehabeldin A, da Cruz LA, *et al.* Antigen receptor-induced activation and cytoskeletal rearrangement are impaired in Wiskott–Aldrich syndrome protein-deficient lymphocytes. J Exp Med 1999; 190:1329–1342.

58 • Badour K, Zhang J, Shi F, *et al.* The Wiskott–Aldrich syndrome protein acts downstream of CD2 and the CD2AP and PSTPIP1 adaptors to promote formation of the immunological synapse. Immunity 2003; 18:141–154.
The authors show that CD2 crosslinking induces formation of a macromolecular complex that includes CD2, CD2AP, PSTPIP1, and WASP. WASP directly binds to PSTPIP1 which binds to CD2, CD2AP. This complex is recruited to the immunological synapse and induces actin nucleation. CD2-induced actin polymerization is abrogated in WAS.

59 Cannon JL, Labno CM, Bosco G, *et al.* Wasp recruitment to the T cell:APC contact site occurs independently of Cdc42 activation. Immunity 2001; 15:249–259.

60 •• Zeng R, Cannon JL, Abraham RT, *et al.* SLP-76 coordinates Nck-dependent Wiskott–Aldrich syndrome protein recruitment with Vav-1/Cdc42-dependent Wiskott–Aldrich syndrome protein activation at the T cell-APC contact site. J Immunol 2003; 171:1360–1368.
This study shows the different molecules that participate in the membrane localization and activation of WASP at the immunological synapse. Vav1 is needed to activate Cdc42 as a small G protein enhancing factor that leads to the activation of WASP. Nck is needed to recruit WASP to the immunological synapse. Nck binds to SLP-76, which functions as a scaffold.

61 •• Dupre L, Aiuti A, Trifari S, *et al.* Wiskott–Aldrich syndrome protein regulates lipid raft dynamics during immunological synapse formation. Immunity 2002; 17:157–166.
This study shows that WASP is rapidly recruited to the immunological synapse during T-cell activation, and demonstrates that WAS is associated with reduced basal levels, and failure to upregulate lipid rafts following TCR engagement.

62 Molina IJ, Sancho J, Terhorst C, *et al.* T cells of patients with the Wiskott–Aldrich syndrome have a restricted defect in proliferative responses. J Immunol 1993; 151:4383–4390.

63 • Orange JS, Ramesh N, Remold-O'Donnell E, *et al.* Wiskott–Aldrich syndrome protein is required for NK cell cytotoxicity and colocalizes with actin to NK cell-activating immunologic synapses. Proc Natl Acad Sci U S A 2002; 99:11351–11356.
In this paper, the authors demonstrate that WASP is expressed in human natural killer cells, and is recruited to natural killer cell-activating immunological synapses. They also show the compensatory increase in natural killer cell population in WAS patients.

64 Haddad E, Zugaza JL, Louache F, *et al.* The interaction between Cdc42 and WASP is required for SDF-1-induced T-lymphocyte chemotaxis. Blood 2001; 97:33–38.

65 • Yarar D, D'Alessio JA, Jeng RL, Welch MD. Motility determinants in WASP family proteins. Mol Biol Cell 2002; 13:4045–4059.
In this study, the authors show that the VCA region of WASP is the minimal part of the molecule required both for stimulating acting nucleation and for cell motility.

66 Leverrier Y, Lorenzi R, Blundell MP, *et al.* Cutting edge: the Wiskott–Aldrich syndrome protein is required for efficient phagocytosis of apoptotic cells. J Immunol 2001; 166:4831–4834.

67 • Jones GE, Zicha D, Dunn GA, *et al.* Restoration of podosomes and chemotaxis in Wiskott–Aldrich syndrome macrophages following induced expression of WASp. Int J Biochem Cell Biol 2002; 34:806–815.
This is a study of chemotaxis of WASP-deficient macrophages. The chemotaxis, cell morphology and ability to form podosome was restored by exogenous WASP expression.

68 • Knutsen AP, Steffen M, Wassmer K, Wall DA. Umbilical cord blood transplantation in Wiskott Aldrich syndrome. J Pediatr 2003; 142:519–523.
This is a report of successful unrelated umbilical cord blood transplantation in three WAS patients.

69 Imai K, Morio T, Zhu Y, *et al.* Clinical course of patients with WASP gene mutations. Blood (in press).

70 Huang MM, Tsuboi S, Wong A, *et al.* Expression of human Wiskott–Aldrich syndrome protein in patients' cells leads to partial correction of a phenotypic abnormality of cell surface glycoproteins. Gene Ther 2000; 7:314–320.

71 Candotti F, Facchetti F, Blanzuoli L, *et al.* Retrovirus-mediated WASP gene transfer corrects defective actin polymerization in B cell lines from Wiskott–Aldrich syndrome patients carrying 'null' mutations. Gene Therapy 1999; 6:1170–1174.

72 • Strom TS, Gabbard W, Kelly PF, *et al.* Functional correction of T cells derived from patients with the Wiskott–Aldrich syndrome (WAS) by transduction with an oncoretroviral vector encoding the WAS protein. Gene Ther 2003; 10:803–809.
In this study gene therapy was performed on primary T cells of WAS patients. The authors show the feasibility of gene transfer without toxic effect on T cells.

73 • Wada T, Jagadeesh GJ, Nelson DL, Candotti F. Retrovirus-mediated WASP gene transfer corrects Wiskott–Aldrich syndrome T-cell dysfunction. Hum Gene Ther 2002; 13:1039–1046.
Gene transfer has been performed in HTLV-1 transformed T-cell lines derived from WAS patients.

74 • Klein C, Nguyen D, Liu CH, *et al.* Gene therapy for Wiskott–Aldrich syndrome: rescue of T-cell signaling and amelioration of colitis upon transplantation of retrovirally transduced hematopoietic stem cells in mice. Blood 2003; 101:2159–2166.
This is the first report of correction of T-cell abnormalities of WASP-deficient mice by gene transfer. Furthermore, by using competitive repopulation experiments, the authors have shown that gene-corrected cells have a selected advantage over WASP-null cells in repopulating secondary lymphoid organs.

75 Wada T, Schurman SH, Otsu M, *et al.* Somatic mosaicism in Wiskott–Aldrich syndrome suggests in vivo reversion by a DNA slippage mechanism. Proc Natl Acad Sci U S A 2001; 98:8697–8702.

76 Ariga T, Kondoh T, Yamaguchi K, *et al.* Spontaneous in vivo reversion of an inherited mutation in the Wiskott–Aldrich syndrome. J Immunol 2001; 166:5245–5249.

77 Wengler GS, Notarangelo LD, Berardelli S, *et al.* High prevalence of nonsense, frame shift, and splice-site mutations in 16 patients with full-blown Wiskott–Aldrich syndrome. Blood 1995; 86:3648–3654.

78 Zhu QL, Watanabe C, Liu T, *et al.* Wiskot–Aldrich-syndrome/X-linked thrombocytopenia: WASP gene mutations, protein expression, and phenotype. Blood 1997; 90:2680–2689.

79 Lemahieu V, Gastier JM, Francke U. Novel mutations in the Wiskott–Aldrich syndrome protein gene and their effects on transcriptional, translational, and clinical phenotypes. Hum Mutat 1999; 14:54–66.

80 Greer WL, Shehabeldin A, Schulman J, *et al.* Identification of wasp mutations, mutation hotspots and genotype–phenotype disparities in 24 patients with the Wiskott–Aldrich syndrome. Hum Genet 1996; 98:685–690.

81 Schindelhauer D, Weiss M, Hellebrand H, *et al.* Wiskott–Aldrich syndrome: no strict genotype–phenotype correlations but clustering of missense mutations in the amino-terminal part of the wasp gene product. Hum Genet 1996; 98:68–76.

82 • Imai K. WASPbase. Updated 1 January 2002. http://www.tmd.ac.jp/med/ped/wasp/WASPbase.html. [Accessed 1 August 2003]
This is a database of WASP gene mutations and WAS phenotype from the literature and includes over 300 patients. This database is different from a previously published WASPbase (Schwarz [6])

83 • Ochs HD. The Wiskott–Aldrich syndrome. Isr Med Assoc J 2002; 4:379–384.
This paper has the updated scoring system with detailed explanation.

84 Stewart DM, Tian L, Nelson DL. Mutations that cause the Wiskott–Aldrich syndrome impair the interaction of Wiskott–Aldrich syndrome protein (WASP) with WASP interacting protein. J Immunol 1999; 162:5019–5024.

85 • 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:e622–e627.
This is the largest survey of classical WAS patients from a single center which focuses on the incidence of autoimmune diseases. XLT patients were excluded from this study.

86 • Schurman SH, Candotti F. Autoimmunity in Wiskott–Aldrich syndrome. Curr Opin Rheumatol 2003; 15:446–453.
This is a comprehensive review of the relationship between WAS and autoimmune diseases.