Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by thrombocytopenia, eczema and immunodeficiency. WASP, the gene responsible for WAS, has been identified by positional cloning, contains a PH domain, a GBD domain, a proline rich region, and a verprolin/cofilin homology domain. Subsequent studies suggest that WASP is involved in signal transduction and in the regulation of the cytoskeleton.

**Key words:** Wiskott-Aldrich syndrome, signal transduction, cytoskeleton

**Clinical Manifestations of WAS**

**Immune Defect**

The severity of the immune deficiency may vary; in most cases, both the cellular and humoral immune systems are affected. Lymphopenia due to a net loss of T lymphocytes is usually present by age six to eight years. Serum IgG levels are often normal, IgM levels are moderately depressed, and IgA and IgE are elevated. Low isohemagglutinin titers are persistent findings and antibody responses to polysaccharides and to many protein antigens are depressed. Abnormal T cell function is suggested by diminished but not absent lymphocyte responses to mitogens, depressed proliferative responses to allogenic cells and immobilized anti-CD3 monoclonal antibody. The surface of peripheral blood lymphocytes from WAS patients is void of microvillous projections when compared with normal lymphocytes. Although the number of circulating neutrophils and monocytes are normal, in vitro chemotaxis of WAS neutrophils and monocytes has been deficient.

**Platelet Abnormalities**

The platelet defect, thrombocytopenia and small platelet volume, is a consistent finding in patients with mutations of the WASP gene. Several mechanisms responsible for the platelet abnormalities were proposed, including reduced survival, abnormal metabo-
lism\textsuperscript{12} and ineffective thrombocytopenia\textsuperscript{13}. Characteristically, platelet counts and volume increase after splenectomy, but not in response to prednisone or high dose intravenous immunoglobulin\textsuperscript{14}.

Other Manifestations
Eczema is a characteristic finding in WAS patients but is very mild or absent in XLT patients\textsuperscript{2}. Autoimmune disorders are frequent and the incidence of malignancies especially lymphoma is high and increases with age\textsuperscript{3}.

Identification of the WASP Gene
The WASP gene, which is responsible for WAS, was identified by positional cloning (gene bank accession number U12707)\textsuperscript{15}. The gene consists of 12 exons spanning 9 Kb of genomic DNA (Fig. 1). The 1,821 basepair cDNA contains an open reading frame of 502 amino acids and generates a protein with a predicted molecular weight of 54 kDa. A mouse homologue of the WASP gene has been isolated\textsuperscript{16}, and a neural homologue of WASP was cloned by purifying Ash/Grb2-binding proteins from bovine brain\textsuperscript{17}. A human and rat N-WASP has been recently identified\textsuperscript{18}. WASP is constitutively expressed in all hematopoietic stem cell-derived lineages whereas N-WASP is present in various non-hematopoietic tissue extracts\textsuperscript{15,17}. Bee1, a protein that is critical for the assembly of cortical actin skeleton in yeast, was found to be a homologue of WASP\textsuperscript{19}.

Structure of WASP
A number of investigators have identified functional domains within WASP including a WH1 (WASP-Homology 1) domain /PH (pleckstrin homology) domain, a GBD (GTPase binding domain)/CRIB (cdc42 or Rac-interactive binding) motif, a proline-rich region, a verprolin homology domain, and a cofilin (cdc42 or Rac-interactive binding) motif, a proline-rich domain, a GBD (GTPase binding domain)/CRIB motif, located in exons 7 and 8 of the WASP gene, has been identified. WASP, like other proteins containing a CRIB motif, recognizes the GTP but not the GDP-bound form of Cdc42. A functional role of the CRIB motif by its interaction with Cdc42 is suggested by the observation that over-expression of WASP in transfected cells causes a clustering of polymerized actin that can be inhibited by simultaneous over-expression of a dominant negative mutant form of Cdc42; and by the observation that micro-injections of WASP into different cell types consistently causes a profound effect on actin polymerization\textsuperscript{20}. However, a subsequent study demonstrated that Y40C mutant of Cdc42, which does not bind to WASP, still induces filopodium formation, suggesting that interaction of Cdc42 with WASP may not be essential for cytoskeletal organization\textsuperscript{20}. Consistent with this observation, it was demonstrated that the Y40C mutant of Cdc42 binds to N-WASP GBD/CRIB motif but not to WASP GBD/CRIB motif\textsuperscript{29}. N-WASP induces long actin microspikes if co-expressed with Cdc42, while WASP fails to induce filopodium formation, despite the structural similarities. In addition, the binding affinity of WASP with Cdc42 is low\textsuperscript{29}. Thus, the precise role of the interaction between WASP and

GTPase binding domain/CRIB motif
Several investigators have independently reported the interaction of WASP with the small GTPase, Cdc42\textsuperscript{20,25,26}, Cdc42, Rac, and other Rho-like GTPases are key elements in the dynamic organization of the actin cytoskeleton\textsuperscript{27}. A GTPase binding domain (GBD), also referred to as CRIB motif, located in exons 7 and 8 of the WASP gene, has been identified. WASP, like other proteins containing a CRIB motif, recognizes the GTP but not the GDP-bound form of Cdc42. A functional role of the CRIB motif by its interaction with Cdc42 is suggested by the observation that over-expression of WASP in transfected cells causes a clustering of polymerized actin that can be inhibited by simultaneous over-expression of a dominant negative mutant form of Cdc42; and by the observation that micro-injections of WASP into different cell types consistently causes a profound effect on actin polymerization\textsuperscript{20}. However, a subsequent study demonstrated that Y40C mutant of Cdc42, which does not bind to WASP, still induces filopodium formation, suggesting that interaction of Cdc42 with WASP may not be essential for cytoskeletal organization\textsuperscript{20}. Consistent with this observation, it was demonstrated that the Y40C mutant of Cdc42 binds to N-WASP GBD/CRIB motif but not to WASP GBD/CRIB motif\textsuperscript{29}. N-WASP induces long actin microspikes if co-expressed with Cdc42, while WASP fails to induce filopodium formation, despite the structural similarities. In addition, the binding affinity of WASP with Cdc42 is low\textsuperscript{29}. Thus, the precise role of the interaction between WASP and
Cdc42 remains unclear.

Proline-rich region

WASP is unusually rich in proline motifs derived from exon 10, and in motifs corresponding to the PXXP binding consensus for SH3 domains. Several groups have demonstrated that WASP interacts with the SH3 domains of selected signaling molecules, including cytosolic adapter proteins, Grb2 and P47phox; Fyn; cFgr; Lck; c-Src and p47phox; the Tec family cytoplasmic tyrosine kinases, Btk, Tec; PLCγ1; and Itk. Peptides that correspond to proline-rich regions within WASP inhibit binding between WASP and several SH3-containing proteins, e.g., Src, Fyn, PLCγ1, Btk. The most effective peptides included two PPPXXRG-based SH3 binding motifs. These data suggest that WASP, through its interaction with the SH3 domain of selected molecules, plays an important role in intracellular signaling of hematopoietic cells.

In a recent study, Wu, et al. have demonstrated that the cytoskeletal-associated protein PSTPIP binds with its SH3 domain to the proline-rich region of WASP. Phosphorylation of a tyrosine residue in the SH3 domain of PSTPIP results in decreased binding of WASP. Furthermore, co-expression of PSTPIP with WASP results in a loss of WASP-induced actin bundling activity, suggesting that the interaction of PSTPIP with WASP, which is regulated by tyrosine phosphorylation, is involved in cytoskeletal organization.

Profilin, an actin-binding protein that promotes actin polymerization, was shown to bind with N-WASP. Since WASP contains Gly(Pro)5, the putative profilin binding motif, it is likely that WASP through its proline-rich region associates similarly with profilin.

Verprolin/cofilin homology domain

The C-terminal region of WASP and N-WASP contain a verprolin homology domain (a.a.430-446) and a cofilin homology domain (a.a.469-487). Verprolin, a yeast protein, is involved in the regulation and maintenance of actin cytoskeletal organization. Cofilin, an actin binding protein, has been shown to depolymerize actin filaments. Over-expression of wild-type WASP in COS-7 cells results in the formation of large cytoplasmic clusters of WASP and polymerized F-actin. However, if COS-7 cells were transfected with a mutated WASP cDNA lacking the C-terminal 59 amino acids that include a verprolin and cofilin homology domain, clustering of WASP and F-actin did not occur. The clustering of F-
actin observed in WASP over-expressing cells is dependent on actin polymerization, suggesting that the C-terminal portion of WASP is important for actin polymerizing activity. This interpretation is further supported by the observation that the co-immunoprecipitated with WASP from lymphocyte extracts, WASP Interactive Protein (WIP), which co-immuno-reacts with actin and regulates actin polymerization.

**WASP Interactive Protein (WIP)**

Using a yeast two-hybrid system, a proline rich WASP Interactive Protein (WIP), which co-immunoprecipitated with WASP from lymphocyte extracts, was identified. The WIP gene encodes a 503 amino acid proline-rich protein with a calculated molecular mass of approximately 52 kDa. The N-terminal region contains two stretches that are highly homologous to the corresponding amino acid sequences in the N-terminal region of the yeast protein verprolin and contains the actin binding KLLK motif. In addition, WIP contains two APPPPP sequences which have been shown to bind to profilin known to regulate actin polymerization. WIP binds to WASP in vivo and in vitro at a site distinct from the Cdc42 binding site. Unlike WASP, which is only found in hematopoietic cell lineages, WIP is widely expressed in many cells and may interact with N-WASP which is also widely expressed in non-blood cells. Overexpression of WIP in human B cells increased polymerized actin content similar to cells overexpressing WASP, and induced the appearance of actin-containing cerebriform projections on the cell surface. It appears that WIP acts downstream of WASP and plays an important role in linking WASP to the actin cytoskeleton.

**The Function of WASP**

The complex clinical phenotype of classic WAS is difficult to explain by a single gene mutation. The gene product, WASP, has a number of unique domains that suggest a "multifaceted" function. Accumulating observations indicate that WASP is involved both in the cytoskeleton and the cytoplasmic signaling system of hematopoietic cell lineages.

Several signaling molecules including tyrosine kinases bind to WASP, suggesting that WASP plays a role in signal transduction pathways. A consistent finding has been the proliferative response of WAS-T cells if stimulated with anti-CD3 mAb. It was also found that WASP co-immunoprecipitates with the activated epidermal growth factor receptor (EGFR) after EGF stimulation, and that Grb2 enhances the association of WASP with EGFR, suggesting that WASP may be located downstream of the EGF receptor. The finding that carrier females show non-random X inactivation in CD34+ cells, in addition to non-random X-inactivation in T cells, B cells, neutrophil, and monocytes indicates that WASP provides a growth advantage for these cells, suggesting that WASP is involved in the growth and survival of CD34+ hematopoietic stem cells.

The interaction of WASP with the small GTPase Cdc42, a key element in the dynamic organization of the actin/cytoskeleton has been interpreted that WASP plays an important role in the regulation of the actin/cytoskeletal system. It is presently unknown whether WASP interacts directly with actin or through actin-binding proteins, and if mutations of WASP result in a transmembrane signaling defect. Using WAS T cell lines immortalized by Herpesvirus Saimiri, it was demonstrated that WAS T cells failed to polymerize and reorganize actin in response to anti-CD3 stimulation. The observation that actin bundling is necessary for T cell activation by anti-CD3 antibody and that Cdc42 is required for the polarization of T cells toward antigen-presenting cells suggests that the abnormal antibody responses characteristic for classical WAS patients are a direct consequence of defective T/B cell interaction: in the absence of functional WASP, T cells fail to provide adequate help to B cells, resulting in impaired B cell function. Recently, Miki et al. reported that megakaryocyte differentiation and microvesicle formation is dependent upon the interaction of WASP with actin filaments, a process in which WASP associates with tyrosine phosphorylated Shc, suggesting that WASP controls the assembly of actin filaments required for microvesicle and pro-platelet formation.

These observations suggest that WASP may play a role in linking tyrosine kinases to the actin cytoskeleton probably by associating with the active form of Cdc42.

**Mutation Analysis, Genotype/Phenotype Correlation**

The scoring system, described in Table 1, is based on the postulate that patients with WAS/XLT have in common thrombocytopenia and small sized platelets and that most, if not all, develop some form of immunodeficiency, although to a different degree. The extent of eczema may be difficult to assess. Lack of a history of eczema or mild, transient eczema responding well to treatment, indicated by (+) in Table 1, and mild, infrequent infections not resulting in sequelae (+) are consistent with XLT (score 1 or 2). Severe, treatment resis-
tant eczema, recurrent infections in spite of optimal therapy, autoimmune diseases, and malignancies are characteristic for classic WAS and are scored as “mild” (score of 3), “moderate” (score of 4), or “severe” (score of 5).

Using these criteria, patients with missense mutations affecting exons 1, 2, and 3 (WH1/PH domain) (Fig. 1) have mild disease. Other mutations observed in patients with mild disease include splice anomalies resulting in multiple splicing products. All mutations associated with a mild phenotype have in common the expression of a normal sized or truncated protein in various quantities\textsuperscript{34}. Thus, missense mutations that affect the WH1/PH domain (exons 1-3) and leave intact the 3’ regions containing the Cdc42 binding site, the SH3 binding motifs, and the WH2 domain, are associated with a mild phenotype (XLT). Most other mutations result in a classic WAS phenotype\textsuperscript{34}.

**Conclusions**

The clinical phenotype of WAS is complex and varies from mild to severe. Although all hematopoietic cell lineages express WASP and are affected by mutations of the WASP gene, the most prominent symptoms are related to defective lymphocyte, especially T cell, function, and thrombocytopenia. WASP has unique domains that support a number of crucial cell functions including signal transduction and interaction with the cytoskeleton. Mutations affecting certain domains of the WASP gene may result in two characteristic clinical phenotypes, classic WAS and XLT. Future research will focus on the better understanding of the function of WASP and the identification and functional analysis of the multiple “scaffolding” molecules known to interact with WASP. Transgenic and WASP knockout mice may provide experimental tools to better understand the in vivo function of WASP.

**References**


