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When Actin is Not Actin' Like It Should: A New Category of Distinct Primary Immunodeficiency Disorders

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Abstract
An increasing number of primary immunodeficiencies (PIDs) have been identified over the last decade, which are caused by deleterious mutations in genes encoding for proteins involved in actin cytoskeleton regulation. These mutations primarily affect hematopoietic cells and lead to defective function of immune cells, such as impaired motility, signaling, proliferative capacity, and defective antimicrobial host defense. Here, we review several of these immunological "actinopathies" and cover both clinical aspects, as well as cellular mechanisms of these PIDs. We focus in particular on the effect of these mutations on human neutrophil function.

Introduction

Actin is one of the most highly conserved proteins during the course of evolution and plays a key role in many cellular functions, including cell motility, cell division, and endocytosis. Furthermore, by forming filaments, that is, the polymerization of globular actin (G-actin) into filamentous actin (F-actin), it provides structural support and helps cells to maintain their shape and internal organization [1]. To date, several primary immunodeficiencies (PIDs) have been identified which are caused by mutations in genes which encode for proteins involved in actin regulation, also referred to as "immuno-actinopathies" [2–4]. PIDs can impact the immune system on different levels, from the innate immune system (phagocyte and complement defects) to the adaptive immune system (T-cell and B-cell defects) [2, 3]. In this review, we focus on PIDs in humans caused by defects in the actin cytoskeleton and the impact on phagocytes, specifically neutrophils. Once released from the bone marrow, granulocytes circulate for some hours in the circulation before extravasation for routine tissue surveillance, for instance of the oropharynx or gut, or they will be more massively recruited into tissues during localized or disseminated infection or inflammation. Neutrophils are capable of eliminating pathogens by several mechanisms, that is, by the intracellular production of reactive oxygen species (ROS) and degranulation of antimicrobial proteases into the so-called phagosomes following uptake or phagocytosis of

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microbes or release into the extracellular environment of ROS and proteolytic activity from their (azurophilic) granules, as well as localized accumulation of this activity onto net-like DNA-containing structures, released upon cell death in which microbes might be "trapped," known as neutrophil extracellular traps [5]. As will be illustrated by the severely impaired hematopoietic immune system in patients with dysregulation of their actin cytoskeleton, an efficient host defense is dependent on the rapid reorganization of the actin cytoskeleton.

The Actin Cytoskeleton

The actin cytoskeleton is a highly dynamic network of filamentous proteins, which consists of 4 main components, that is, microtubules, intermediate filaments, septins, and actin filaments [6, 7]. There are 6 mammalian actin isoforms which share nearly identical amino acid sequences. Four of these isoforms are mainly expressed in different types of muscles, namely, α-cardiac, α-skeletal, α-smooth muscle, and γ-smooth muscle actin. The other 2 isoforms are non-muscle actin, also termed β-cytosplasmic and γ-cytosplasmic actin, which are ubiquitously expressed. Although there are similarities between these isoforms (i.e., cytoplasmic actins only differ by 4 amino acids), studies have shown that they have distinct biological roles [8, 9]. Part of the actin is localized in the cytoplasm as monomeric globular or G-actin and part is contributing to filamentous or F-actin which localizes just beneath the plasma membrane, in the cytoplasm to give the cell its basal form (including stress fibers), and in part also in the nucleus in some cells.

As mentioned, these actin filaments are linear polymers of G-actin. F-actin adopts 2 main forms, namely, branched actin networks or linear actin networks (and mixtures of the 2). The formation of either of the 2 major subforms is mediated by distinct actin nucleators. The actin-related proteins-2/3 (ARP2/3) complex, consisting of 7 subunits, mediates the formation of branched actin networks by nucleating a daughter filament to the side of the preexisting actin filaments in an ATP-dependent process [10]. Branched actin networks are needed for the generation of protrusive force that aids in cell adhesion and motility. The ARP2/3 complex is essential in the formation of lamellipodia [11]. Formins form a family of different actin nucleators involved in the formation of linear actin networks but also promote elongation of preexisting filaments [12, 13]. Aside from actin nucleators, actin dynamics are regulated by numerous actin-binding proteins which have diverse functions, for example, stabilizing, capping, bundling, and cross-linking of actin filaments (shown in Fig. 1) [1, 14–16]. Mutations in genes encoding several of these actin-binding proteins have been described to cause PIDs of the innate immune system, and these will be further discussed in this review (Table 1).

PIDs Caused by Defective Cytoskeletal Regulation

The assembly of G-actin into F-actin is mediated by actin nucleators, including the ARP2/3 complex. This complex is inherently inactive and needs activation by Wiskott-Aldrich syndrome protein (WASP) or the WASP-family verprolin homologous protein (WAVE) regulatory complex (WRC) (shown in Fig. 2). Ena/VASP proteins enhance actin filament elongation and can also bind to the WRC to enhance ARP2/3 complex activity [17]. Furthermore, depolymerization of actin filaments is essential for dynamic actin remodeling and mediated by several regulatory proteins (shown in Fig. 1). We will discuss several PIDs, which impair the assembly and disassembly of actin filaments. To our knowledge, there are no disease-causing mutations described in the genes which encode Ena/VASP proteins (i.e., *VASP*, *ENAH*, or *EVL*) in humans, and therefore, these proteins will not be discussed.

ACTB Mutations

Cytoplasmic beta-actin (encoded by *ACTB*, chromosome 7p22.1), is 1 of the 6 isoforms of actin. Most heterozygous autosomal dominant mutations in *ACTB* are de novo alterations associated with Baraitser-Winter syndrome (BRWS type 1; OMIM 243310), a rare disorder characterized by distinct facial features, for example, hypertelorism and a broad nasal bridge, with cerebral malformations and intellectual disability [18, 19]. Apart from *ACTB*, mutations in gamma-actin (*ACTG*, chromosome 17q25.3), the other non-muscle actin, can also cause BRWS (type 2, OMIM 614583) [20]. While most mutations causing BRWS are postulated to be gain-of-function mutations [20], loss-of-function mutations in *ACTB* can result in a pleiotropic malformation syndrome with intellectual disability distinct from BRWS [21]. Although susceptibility to infection is not reported as a general phenotype in these patients, infections, including chronic respiratory infections, recurrent/chronic sinusitis, and frequent otitis media, are being observed, suggesting that mutations in *ACTB* might have an effect on the immune system [21].

To date, the consequence of mutated beta-actin on neutrophil function has been reported in a single patient by Nunoi et al. [22]. This patient suffered from recurrent infections, photosensitivity and mental retardation. A missense mutation in exon 6 of *ACTB* was identified, leading to the expression of both normal and mutated beta-actin in the patient's leukocytes, platelets, and fibroblasts. Compared to control neutrophils, patient's neutrophils demonstrated an impaired chemotactic response toward N-formyl-met-leu-phe (fMLF) and zymosan-activated serum. This impaired mobilization was confirmed by a skin window test, where a poor influx of leukocytes was observed. These neutrophils also showed a reduced ROS production and impaired membrane potential response when stimulated with fMLF [22]. This mutant actin was shown to have a significantly slower polymerization rate than control actin, explaining the impaired chemotaxis [23].

The patient also suffered from nonimmune thrombocytopenia, a symptom also observed in a separate cohort of 6 BRWS patients (of 4 unrelated families) with mutations in exon 5 and 6 of *ACTB* without a history of recurrent infections [24]. Two patients had leukocytosis with an increased eosinophil count, while the patient of Nunoi et al. [22] showed leukopenia with a large percentage of band neutrophils. The concomitant decrease in neutrophil count might explain the difference in phenotype between patients.

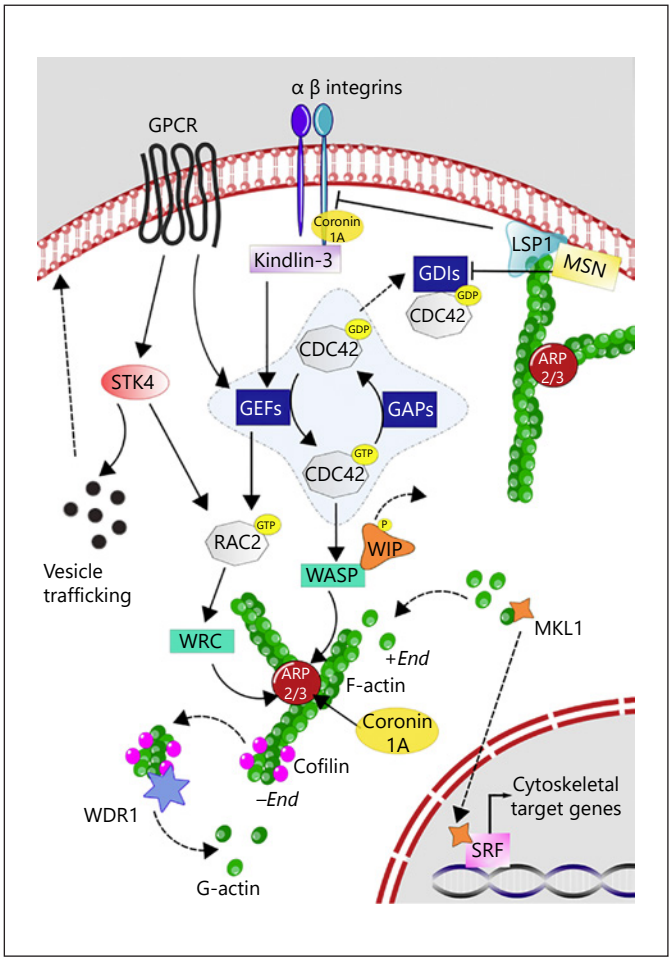

Fig. 1. Summary of actin cytoskeleton dynamics. The proteins involved in PIDs described in this review are depicted. When a neutrophil gets activated (e.g., by ligand binding to GPCRs) several signaling cascades are initiated. STK4 controls the translocation of vesicles containing laminin-binding integrins and neutrophil elastase to the surface (indicated by mice studies). STK4 can also activate RAC via the kinase PKC-α and the GDP-dissociation inhibitor RHOGDI2. RHO GDIs sequester Rho GTPases family members (e.g., RAC2 and CDC42) in the cytosol to render them inactive. Activated GEFs (e.g., DOCK2) mediate the exchange of GDP to GTP, thereby promoting the binding of Rho GTPase family members to their specific effectors (WRC and WASP, respectively). These effectors in turn activate the actin nucleator complex ARP2/3 (which ARPC1B is part of). Also coronin-1A can directly interact with ARP2/3, as well as with integrin beta-2. Upon activation of WASP, WIP dissociates from WASP. Actin depolymerizes at the "pointed (–) end," and the severing and disassembly of actin filaments by cofilin is enhanced by WDR1. Monomers of actin (G-actin) can be incorporated in the growing filamentous actin (F-actin) strand (barbed [+] end), which allows for the dissociation of MKL1 from G-actin, thereby allowing translocation of MKL1 to the nucleus, where it can activate SRF-dependent transcription of cytoskeletal target genes. MSN links actin filaments to the plasma membrane and membrane receptors, and it can initiate activation of Rho family members by reducing the activity of RHO GDI. LSP1 is an actin-binding protein which bundles actin filaments. Also, LSP-1 is thought to act as a negative regulator of integrin beta-2-mediated adhesion (mice studies). The most prominently expressed integrin complex on neutrophils is the integrin alpha-M/integrin beta-2 complex. Upon activation, it will first move from a low to a medium avidity state. The high avidity state is induced by kindlin-3, and this allows for an effective binding of integrins to substrates on the endothelium and extracellular matrix. ARP2/3, actin-related proteins-2/3; ARPC1B, actin-related protein complex-1; CDC42, cell division control protein 42 homolog; DOCK2, dedicator of cytokinesis protein 2; F-actin, filamentous actin; GDP, guanosine diphosphate; GEF, guanine nucleotide-exchange factor; GPCR, G protein-coupled receptors; GTP, guanosine triphosphate; LSP-1, leukocyte specific protein I; MKL1, megakaryoblastic leukemia 1; MSN, moesin; PIDs, primary immunodeficiencies; RAC2, Ras-related C3 botulinum toxin substrate 2; RHO GDI, RHO protein guanine nucleotide-dissociation inhibitor; SRF, serum response factor; STK4, Serine/threonine-protein kinase 4; WASP, Wiskott-Aldrich syndrome protein; WRC, WASP-family verprolin homologue protein regulatory complex; WDR1, WD repeat-containing protein 1; WIP, WASP-interacting protein.

Table 1. List of actinopathies with corresponding protein function, clinical symptoms, and neutrophil defects

Disease	Protein function	Clinical symptoms	Defects reported in primary neutrophils from patients
ACTB mutations	Non-muscle actin isoform	Recurrent infections and mental disability	Impaired chemotaxis and ROS production, lower membrane potential response
MKL1 deficiency	Co-activator of SRF	Severe, recurrent bacterial infections	Reduced actin content and impaired actin polymerization, impaired migration, impaired spreading, enhanced degranulation, and defective endothelial transmigration
ARPC1B deficiency	Component of the ARP2/3 complex	Viral and bacterial infections, bleeding tendency, vasculitis, eczema, allergy, thrombocytopenia, and eosinophilia	Impaired actin polymerization, impaired motility, enhanced degranulation, and impaired podosome formation
Wiskott-Aldrich syndrome (*WAS*)	Nucleation factor for the ARP2/3 complex	Recurrent infections, severe bleeding, eczema, autoimmune diseases, lymphoma, thrombocytopenia, and lymphocytopenia	None reported
X-linked thrombocytopenia (*WAS*)	Nucleation factor for the ARP2/3 complex	Mild immunodeficiency, bleeding, and thrombocytopenia	None reported
X-linked neutropenia (*WAS*)	Nucleation factor for the ARP2/3 complex	Severe neutropenia, monocytopenia, thrombocytopenia, late-onset malignancies, and recurrent infections	Enhanced basal F-actin levels, proliferation, and maturation defect
WIP deficiency (*WIPF1*)	Interactor of WASP and keeps WASP stable and inactive	Recurrent infections, eczema, papulovesicular/ulcerative lesions, bloody diarrhea, and thrombocytopenia	None reported
HEM1 deficiency	Component of the WRC	Recurrent bacterial and viral infections and atopic and allergic disease	Impaired migration
Coronin-1A deficiency	Actin-binding protein, interacts with the ARP2/3 complex, signaling mediator	Recurrent bacterial and viral respiratory infections, skin lesions, chronic warts, and chronic T-lymphopenia	Very short telomere length
LLS/*WDR1* deficiency	Enhances severing and disassembly of actin filaments by cofilin	Severe stomatitis, recurrent infections and moderate neutropenia (periodic), fever, thrombocytopenia, and intellectual impairment	Migration defect, abnormal spreading, increased basal F-actin levels, enhanced ROS production, and abnormal location of the nucleus
RAC2 mutations (activating)	Rho GTPase involved in the respiratory burst and activator of the WRC	Neutropenia, recurrent respiratory infections, and lymphopenia	Impaired chemotaxis, increased basal F-actin levels, increased actin polymerization, and increased ROS production
RAC2 mutations (loss-of-function)	Rho GTPase involved in the respiratory burst and activator of the WRC	Neutrophilia, severe bacterial infections, poor wound-healing, lymphopenia, and hypogammaglobulinemia	Impaired chemotactic response, decreased basal F-actin levels, impaired actin polymerization, decreased ROS production, and absent azurophilic granule release
DOCK2 deficiency	GEF activating RAC	Severe bacterial and viral infections and T-cell lymphopenia	Impaired actin polymerization and impaired ROS production
DOCK8 deficiency	GEF activating CDC42	Severe recurrent viral, bacterial, and fungal infections; eczema; and allergies	Mild motility defect
STK4 deficiency	Kinase for several nuclear proteins and activator of RAC through PKC-α and LyGDI	Recurrent bacterial and viral infections, $CD4^+$ T-lymphopenia, mucocutaneous candidiasis, eczema, molluscum contagiosum, high IgA/E levels, cardiac aberrations, and episodes of neutropenia	Loss of mitochondrial membrane potential and susceptibility to apoptosis

Table 1 (continued)

Disease	Protein function	Clinical symptoms	Defects reported in primary neutrophils from patients
MSN deficiency	Links actin filaments to the plasma membrane and signaling mediator for selectins	Recurrent bacterial and viral infections, recurrent molluscum, eczema, and lymphopenia	None reported
LAD-I (*ITGB2*)	Facilitates cell-cell and cell-ECM interactions	Early onset recurrent bacterial or fungal infections, delayed umbilical cord detachment, omphalitis, poor wound healing, non-pyogenic wounds, and severe neutrophilia	Impaired ROS production, reduced killing of *Candida albicans*, impaired adhesion (static and flow), defective chemotaxis, and defective endothelial transmigration
LAD-III (*FERMT3*)	Key-activator of integrins	Milder immune deficiency compared to LAD-I, but accompanied by a Glanzmann-like bleeding disorder	Impaired ROS production, reduced killing of *C. albicans*, impaired adhesion (static and flow), defective chemotaxis, and defective endothelial transmigration
CalDAG-GEFI deficiency	GEF activating RAP1	Impaired platelet function and bleeding disorder	Defective integrin activation
NAD	Unknown gene	Recurrent fevers, recurrent pulmonary infections, leukocytosis, non-pyogenic infections, hepatosplenomegaly, and thrombocytopenia	Decreased basal F-actin levels, impaired actin polymerization, impaired podosome formation, and increased ROS production

ACTB, β-actin; ARPC1B, actin-related protein complex 2/3 subunit 1B; ARP2/3 complex, actin-related protein complex 2/3; CalDAG-GEFI, calcium and DAG-regulated guanine nucleotide exchange factor I; CD, marker of cell differentiation; CDC42, cell division control protein 42; DOCK, dedicator of cytokinesis; ECM, extracellular matrix; FERMT3, fermitin family homolog 3; F-actin, filamentous actin; GEF, guanidine exchange factor; Ig, immunoglobin; ITGB2, β-2-integrin; LAD, leukocyte adhesion deficiency; LLS, lazy leukocyte syndrome; LyGDI, lysine GDP-dissociation inhibitor; MKL1, megakaryoblastic leukemia 1; MSN, moesin; NAD, neutrophil actin dysfunction; PKC, protein kinase C; RAC, Ras-related C3 botulinum toxin substrate; RAP1, Ras-related protein 1; Rho GTPase, rho guanidine triphosphosphatase; ROS, reactive oxygen species; SRF, serum response factor; STK4, serine/threonine kinase 4; WAS, Wiskott-Aldrich syndrome; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP family verprolin-homologous protein; WRC, WAVE regulatory complex; WDR1, WD repeat-containing domain 1; WIP, WASP-interacting protein.

Megakaryoblastic Leukemia 1 Deficiency

Megakaryoblastic leukemia 1 (MKL1), member of the myocardin-related transcription factor family, is a transcriptional co-regulator of numerous genes involved in actin cytoskeletal dynamics, cell survival, and cell proliferation through activation of serum response factor [25]. In the cell, MKL1 is found in the cytosol (in complex with G-actin) and in the nucleoplasm. Initiation of actin polymerization promotes the dissociation of MKL1 from G-actin, thereby allowing translocation of MKL1 to the nucleus, where it can activate serum response factor-dependent transcription [26].

Loss-of-function mutations in *MKL1* result in a PID, first described by Record et al. [27]. More recently, a second family with 2 siblings with a homozygous frameshift mutation in *MKL1* was identified [28]. The first identified MKL1-deficient patient suffered from severe bacterial infections, and the second MKL1-deficient patient deceased as an infant from progressive and severe pneumonia by *Pseudomonas aeruginosa*. The MKL1-deficient sibling of the latter patient underwent a successful hematopoietic stem cell transplantation (HSCT) shortly after birth.

MKL1-deficient neutrophils showed reduced overall actin content and displayed an actin polymerization defect, subsequently leading to a migratory defect. Furthermore, proteomic and transcriptomic analyses of MKL1-deficient primary neutrophils and an HL-60 myeloid leukemia MKL1 knockdown cell line revealed several actin-related proteins and genes to be downregulated, confirming a role of MKL1 as transcriptional co-regulator. No difference in the capacity of production of ROS was observed in MKL1-deficient neutrophils, while degranulation was shown to be enhanced [27, 28]. In contrast to the first report [27], no phagocytosis defect and an intact killing of various microbial pathogens was found in neutrophils of the MKL1-deficient patient described more recently [28]. The susceptibility to (bacterial) infections observed in MKL1 deficiency could thus be explained by the inability of neutrophils to migrate towards the site of infection as opposed to a killing defect.

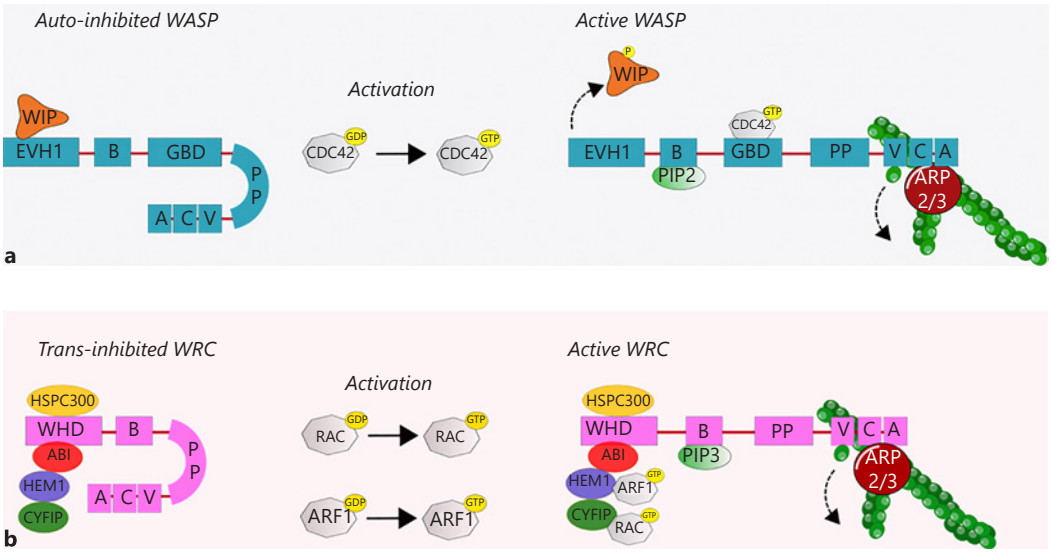

Fig. 2. Activation of WASP and the WRC. WASP consists of 5 domains (**a**): EVH1 domain, Basic domain, GBD domain, PP domain, and the VCA region. The protein is kept in an inactive state through auto-inhibition by interaction of the GBD region with the VCA region. The EVH1 domain of WASP can interact with WIP, which stabilizes the inactive state of WASP. Upon activation of WASP with GTP-CDC42 (which binds on the GBD domain), PIP_2 (which binds to the B domain), or tyrosine phosphorylation of WIP (which causes dissociation of WIP), the VCA region can interact and activate the ARP2/3 complex, thereby initiating actin polymerization. Furthermore, the PP domain of WASP can bind SH3-domain-containing proteins (e.g., cortactin and WISH) and profilin, which enhances ARP2/3 activation (not depicted). **b** The WRC is a protein complex consisting of HSPC300, ABI, HEM1, CYFIP, and WAVE. WAVE has a similar protein structure as WASP, with a B domain, PP domain, and VCA region. The WRC is activated by GTP-RAC or GTP-ARF1, which bind to CYFIP or HEM1, respectively. Subsequently, the VCA region of WAVE can bind G-actin and the ARP2/3 complex, thereby initiating actin polymerization. PIP_3 binds to the B domain, which is important for the localization of WAVE. Also, the protein IRSp53 binds to the PP domain of WAVE. It can also bind to RAC and enhance the activity of the WRC (not depicted). More WASP and WAVE interacting proteins are reviewed by Takenawa and Suetsugu [223]. ABI, Abelson interactor 1; ARF1, ADP-ribosylation factor 1; ARP2/3, actin-related proteins-2/3; CDC42, cell division cycle 42; CYFIP, cytoplasmic FMR1-interacting protein 1; EVH1, Ena-VASP-homology-1; G-actin, globular actin; GBD, GTPase binding domain; GTP, guanosine triphosphate; HEM1, hematopoietic protein 1; HSPC300, hematopoietic stem/progenitor cell protein 300; PIP2, phosphatidylinositol-(4,5)-biphosphate; PIP3, phosphatidylinositol-(3,4,5)-triphosphate; PP, polyproline; SHR, Src-homology-3; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP-family verprolin homologue protein; WIP, WASP-interacting protein; WISH, WASP-interacting SH3-domain protein; WRC, WAVE regulatory complex.

Both MKL1-deficient pedigrees described a mild non-immune thrombocytopenia, which can be explained by the role of MKL1 in megakaryocyte maturation [29]. Proliferative capacity of T-cells, B-cells and NK-cells were normal. Their motility has not been formally tested. Non-hematopoietic MKL1-deficient fibroblasts were not affected in their morphology, F-actin content, and migratory capacity, which is most likely due to compensatory mechanisms of MKL2, also member of the myocardin-related transcription factor family [28]. It is reported that MKL1 and MKL2 could have redundant roles. However, in contrast to fibroblasts, MKL2 is not expressed in neutrophils [28].

Characterization of the complete clinical and immunological phenotype of MKL1 deficiency will involve identification and description of new patients. Furthermore, it would be of interest to identify which pathways are solely dependent on MKL1, considering possible redundancy between MKL1 and MKL2.

Actin-Related Protein Complex-1B Deficiency

The ARP2/3 complex is the key regulator in branching of actin filaments and therefore of importance for many cellular processes, including cell migration [10]. One of the 7 subunits of ARP2/3 is actin-related protein complex-1 (ARPC1), which is present in 2 isoforms in hu-

mans, namely, ARPC1A and ARPC1B [30]. The latter is predominantly expressed in hematopoietic cells, whereas ARPC1A is mostly expressed in non-hematopoietic tissues [31, 32].

Since 2017, several reports on ARPC1B deficiency have been published [31–36], herein describing patients suffering from a combined immunodeficiency (CID), including a neutrophil defect. The disorder is caused by autosomal recessive, mostly homozygous mutations in *ARPC1B* (chromosome 7q22.1; OMIM 617718). A wide range of clinical manifestations has been described; most patients suffer from infections (both bacterial and viral), bleeding tendency, vasculitis, eczema, and allergy. The immunological phenotype is characterized by thrombocytopenia, eosinophilia, high immunoglobulin (Ig)A and IgE levels, an increased number of $CD19^+$ B-cells, and reduced numbers of $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cells [31–36].

ARPC1B deficiency has a major impact on neutrophil motility due to an actin polymerization defect. No defect in ROS production or the phagocytosis and killing of *Escherichia coli* and *Staphylococcus aureus* was found, whereas the azurophilic granule release was also increased by ARPC1B-deficient neutrophils under suboptimal stimulation, similar to our findings in MKL1 deficiency. Investigation of the patient's primary fibroblasts revealed normal migratory behavior, most likely due to the expression of ARPC1A in these cells [31]. Interestingly, ARPC1A is expressed at a very low level in ARPC1B-deficient hematopoietic cells (i.e., platelets and neutrophils) and more so in activated peripheral blood mononuclear cells (PBMCs), compared to control cells as assessed by Western blot [33]. Apart from neutrophil defects, ARCP1B deficiency causes defects in thrombocytes [35], T-cells [33–36], B-cells [35], NK-cells [33], and platelets [32], illustrating the crucial function of this ARPC1 isoform in hematopoietic cells. Apparently, the increase of ARPC1A expression is not sufficient to rescue these cells from the actin polymerization defects.

Wiskott-Aldrich Syndrome

Wiskott-Aldrich syndrome (WAS) was first described in 1937, and it was found 57 years later that a defect in the X-chromosomal *WAS* gene, which was named accordingly, was causative of this disease [37]. The *WAS* gene (chromosome Xp11.23) encodes for WASP, which is expressed solely in the non-erythroid hematopoietic lineage and is a key regulator of actin polymerization [38]. It acts as a nucleator for the ARP2/3 complex to form branched F-actin networks [39] and is important for adhesion, chemotaxis, and phagocytosis of immune cells [40–42]. On the protein level, WASP consists of 5 domains: EVH1 domain, Basic domain, GBD domain, Polyproline domain, and the VCA region. The VCA region is important for the interaction and activation of the ARP2/3 complex, and the protein is kept in an inactive state through auto-inhibition by interaction of the GBD region with the VCA region (shown in Fig. 2a) [43].

The total number of mutations found in the *WAS* gene numbers over 400 and depending on the location of the mutation can result in classic WAS (OMIM 30100), X-linked thrombocytopenia (XLT; OMIM 313900) or neutropenia (XLN; OMIM 300299). Patients suffering from classic WAS have a severe clinical phenotype of immunodeficiency, bleeding, and eczema, and patients who survive infancy have the tendency to develop autoimmune diseases and lymphoid malignancies [44, 45]. In the case of XLT, patients have a similar but milder "bleeding-only" phenotype compared to WAS patients [44]. In contrast to WAS and XLT, patients suffering from XLN have mutations in the GBD domain, which is important for the auto-inhibition of WASP, thus resulting in expression of constitutively active WASP (CA-WASP) [46–52]. First classified as severe congenital neutropenia [53], individuals are usually admitted to the hospital early in life due to recurring bacterial infections due to severe neutropenia, coinciding with monocytopenia, thrombocytopenia, low NK-cell numbers, and abnormal CD4/CD8 ratio [46–52]. Late-onset malignancies have been reported in 1 pedigree [46], but not the other 2 [48, 54]. Infections for all these patients are generally less severe and more manageable than for WAS patients. Individuals are generally put on prophylactic antibiotics after discharge and do not require treatment with G-CSF despite the episodes of neutropenia [44, 45, 54]. A common strategy for definitive treatment in case of classical WAS, but not XLT or XLN, consists of HSCT [44]. Recently, gene therapy for diseases caused by mutated *WAS* has emerged as an alternative treatment option next to HSCT [55, 56]. XLT patients may require platelet transfusions in case of severe bleeding or preoperatively.

Notably, all 3 clinical variants are caused by mutations in the *WAS* gene, but the clinical phenotype differs between mutations. WAS and XLT are generally caused by mutations which affect WASP expression, where WAS is usually characterized by the absence of protein, while patients with XLT generally have a partial decrease in expression and behaves as a hypomorphic variant [44]. This difference also explains the milder clinical phenotype of XLT [44, 57]. It is unclear whether and – if at all – the

downregulation of WASP affects neutrophil function. Most studies do not find any impairment of neutrophil phagocytosis, killing, and migration [58, 59], but 1 study in a single WAS patient showed reduced adhesion under flow and impaired integrin clustering and respiratory burst upon coating on intercellular adhesion molecule-1 (ICAM-1) [40]. Notably, integrin activation was not affected [40]. Additionally, migration seems to be affected toward complement component 5a (C5a) [60], but not to fMLF or interleukin (IL)-8 [58, 61]. Differences between these studies might arise from different mutations, experimental settings, or use of medication. Some of these factors might cause neutrophil dysfunction, while others may not.

In contrast to WAS and XLT, patients suffering from XLN have normal to near-normal protein expression [46, 48]. These patients have mutations in the GBD domain of *WAS* which is important for the auto-inhibition of WASP, thus resulting in the expression of CA-WASP [43, 46–52]. The presence of CA-WASP hampers neutrophil proliferation and maturation of patient-derived $CD34^+$ cells into fully differentiated neutrophils [46, 51, 62], which can be attributed to the increased F-actin polymer content [46, 51]. Also, patient bone marrow progenitor cells ($CD34^+$, $CD33^+$, and $CD15^+$ cells) showed increased levels of spontaneous apoptosis [46]. The maturation defect is the most likely cause of the observed neutropenia in these patients due to the gain-of-function mutations in the WASP GBD domain (L270P, S272P, or I294T). Furthermore, FACS data from XLN patient neutrophils indicate that the ratio $CD11b^+/CD16^{high}$ (mature neutrophils) to $CD11b^+/CD16^{low}$ (metamyelocytes) is lower than healthy controls. The presence of neutrophils in saliva suggests that XLN patient neutrophils have the capacity to migrate to the gingival tissues, thus protecting the oral site from periodontal diseases which could be explained by the faster migratory properties of these cells. Another striking observation is the increased membrane expression of the NADPH oxidase subunit $Gp91^{phox}$ and a strong ROS production upon activation [62], although this may vary among patients [46]. Together with a normal polymorphonuclear morphology, this suggests a reduced bone marrow output of neutrophils with a mature cellular phenotype. Proteomic and transcriptomic analysis would be useful to dissect what causes this distinct phenotype.

WASP-Interacting Protein Deficiency

As the name suggests, the WASP-interacting protein (WIP) interacts with WASP. The EVH1 domain of WASP can interact with WIP, which stabilizes the inactive state of WASP, thus preventing the degradation of WASP [63, 64]. Moreover, WIP is important for correct localization of WASP [65]. Activation of WASP with the small Rho GTPase cell division cycle 42 (CDC42) or tyrosine phosphorylation of WIP causes the dissociation of WIP with WASP (shown in Fig. 2a) [43, 66].

To the best of our knowledge, 3 pedigrees with totally 6 affected individuals have been described with WIP deficiency, caused by autosomal recessive mutations in a gene for WIP family member 1 (*WIPF1*, chromosome 2q31.3, OMIM 614493) [67–69]. Most patients were admitted early in life with recurrent infections, eczema, papulovesicular or ulcerative lesions, bloody diarrhea, and thrombocytopenia [67–69]. The infections are treated depending on the pathogen, and individuals are generally put on prophylactic antibiotics [67, 68]. Malignancies have not been reported to date. HSCT with umbilical cord blood or bone marrow has proven successful as definitive treatment of WIP deficiency [67, 68].

In patients with WIP deficiency, WIP protein expression is found to be absent which also causes WASP expression to be abrogated, thus mimicking WAS [68]. No reports on neutrophil function of WIP-deficient neutrophils have been published, but defects have been described in patient lymphocytes. Reconstitution of WIP in 1 patient's T-cells was found to reconstitute WASP expression [68]. Furthermore, WIP deficiency seems to cause severe abrogation of migration, cell polarity, and morphology of the cell and cytoskeleton in T-cells and B-cells derived from patient material [69].

Hematopoietic Protein 1 Deficiency

Hematopoietic protein 1 (HEM1), encoded by *NCKAP1L* (chromosome 12q13.1), is a hematopoietic cell-specific member of the WRC [70]. This regulatory complex is intrinsically inactive and can be activated by diverse signals (including Ras-related C3 botulinum toxin substrate (RAC), kinases, and phosphatidylinositols) and thereby controls ARP2/3 complex-mediated actin polymerization (shown in Fig. 2b) [71]. Knockout of *NCKAP1L* in HL-60 cells has been shown to result in the concomitant degradation of other subunits of the WRC, thereby impairing cell polarity and motility. Specifically, these cells failed to form lamellipod-like protrusions at the leading edge [72]. Furthermore, loss of HEM1 results in aberrant levels and polarization of RAC activity in chemoattractant-stimulated HL-60 cells [72, 73].

Recently, 5 patients have been identified with loss-of-function mutations in *NCKAP1L*[74]. These bi-allelic missense mutations lead to either a loss of HEM1 protein

or abrogated binding of HEM1 to the GTPase ADP-ribosylation factor 1, thereby leading to destabilization of the WRC or disruption of ADP-ribosylation factor 1-WRC binding and activation, respectively. These patients suffered from a severe immunodeficiency with recurrent bacterial and viral skin infections, otitis media, upper respiratory tract infections, as well as immune system hyperreactivity which included atopic and allergic disease [74].

Neutrophils of these patients showed impaired migration in response to fMLF, C5a, and IL-8. More specifically, HEM1-deficient neutrophils could not form a broad leading edge or maintain directional persistence, but instead became dramatically elongated with competing leading edges moving parallel to or against the chemotactic gradient [74]. Impaired migration is also observed in HEM1-deficient mouse neutrophils, as well as impaired actin polymerization [75].

HEM1 deficiency also resulted in T-cell defects, including defective proliferation and activation, despite normal FAS (tumor necrosis factor receptor superfamily member 6) and T-cell receptor-mediated apoptosis. These cells showed reduced CD11a/CD18 inside-out activation, while adhesion to immobilized ICAM-1 was intact. However, T-cells migrated poorly on ICAM-1-coated surfaces and showed a loss of lamellipodia formation and F-actin at the leading edge. Also, HEM1-deficient T-cells displayed decreased levels of F-actin at immune synapses, and there was diminished immunological synapse stability. This defective F-actin accumulation was also observed in the NK-cell-target synapse. Reduction of the cortical actin barrier in HEM1-deficient T-cells resulted in exaggerated degranulation and IL-10, perforin, and granzyme A and B release, which could explain the patients' autoinflammatory phenotype [74]. Increased degranulation has also been observed in ARPC1B-deficient [31] and MKL1-deficient [28] neutrophils. Although degranulation of neutrophils was not investigated in HEM1 deficiency, it is likely that the cortical actin barrier of neutrophils is also affected, resulting in an increased release of granular proteases which could also contribute to autoimmunity.

Coronin-1A Deficiency

Coronin members constitute a family of evolutionarily conserved regulators of the actin cytoskeleton turnover, represented by 7 members in mammals. They have been grouped into 3 classes based on phylogenetic and functional criteria. Class I includes coronins 1, 2, 3, and 6 (also called 1A, 1B, 1C, and 1D) that associate with the actin cytoskeleton, localize at the leading edge of migrating cells, and participate in various signaling processes [76–79]. The actin-binding protein coronin-1A (encoded by *CORO1A*, chromosome 16p11.2, OMIM 615401), also known as p57, is predominantly expressed in the hematopoietic lineage. It is classified as a 7 beta-propeller protein, which contains 5 WD40 repeats and has a C-terminal coiled-coil leucine-zipper domain connected via an extension (CE) or linker region [80]. Two actin-binding regions are described within the beta-propeller [80, 81], and 1 within the linker region [82, 83]. Mutation of the C-terminal leucine-zipper results in failure to homo-oligomerize [82, 84, 85]. It has been described that coronin-1A can directly interact with the ARP2/3 complex [86], is present at the leading edge during chemotaxis [78, 87], and plays a role in phagocytosis [87] and signal transduction [88]. Class II includes coronin-4 and coronin-5 (also called 2A and 2B), involved in focal adhesion turnover, reorganization of the cytoskeleton, and cell migration [89, 90]. The class III coronin-7 has an unusual structure and plays a role in Golgi morphology maintenance [91, 92].

Several reports have been published listing 10 different mutations in *CORO1A*, which describe patients suffering from either autosomal recessive SCID [85, 93–95] or hemophagocytic lymphohistiocytosis [96, 97], whereas copy number duplication has been linked to autism [98]. Patients diagnosed with SCID suffered from recurrent bacterial and viral infections of the respiratory tracts and lungs, as well as skin lesions and chronic warts [85, 93–95]. Another characteristic is chronic T-lymphopenia of varying T-cells and to a lesser extent (memory) B-cells and NK-cells, but a normal-sized thymus and normal IgG and specific antibodies with a marginally elevated IgE levels [85, 93–95]. Some patients with SCID also presented with attention hyperactivity disorder or other cognitive impairments, which relates to expression of coronin-1A in the hematopoietic system and the brain [94, 95]. Three patients carried *CORO1A* mutations which have developed hemophagocytic lymphohistiocytosis, of which only one had a compound heterozygous mutation in *CORO1A* (CORO1A p.L235V and p.P277R). Telomere length in patient lymphocytes and granulocytes was very low, indicating they suffer from impaired hematopoiesis in the lymphoid and myeloid compartment, although neutropenia has not been described to date [93].

Regarding the function of coronin-1A deficiency in patient neutrophils, there is 1 report evaluating ROS production in coronin-1A-deficient patients, which was found to be normal [93]. One of the 2 siblings described

suffered from tuberculoid leprosy, which is surprising since coronin-1A-deficient macrophages have been described to have increased killing capacity towards mycobacterial pathogens [99–101].

Several studies on the role of coronin-1A in neutrophils have been conducted. An initial study showed that transducing primary neutrophils with the beta-propeller region of coronin-1A changed the F-actin distribution of the cells and reduced neutrophil adhesion, spreading, chemotaxis and phagocytosis [87]. Co-immunoprecipitation of CD18 and coronin-1A has been found in adherent differentiated HL-60 cells, and intravital microscopy in inflamed cremaster muscle venules revealed that leukocyte adhesion and extravasation was severely impaired in coronin-1a-knockout mice, while the number of rolling leukocytes was unaffected [78]. Overexpression of coronin-1 in PLB-985 cells (which were differentiated towards neutrophils) resulted in less apoptosis-induced mitochondrial depolarization. Also, neutrophils from patients with cystic fibrosis showed increased coronin-1 expression and lower apoptosis rates, indicating a pro-survival role for coronin-1 [102].

Notably, several case reports describing SCID patients with coronin-1A mutations have evaluated patient T-lymphocytes or T-cell lines derived from patient cells [85, 93–95], but it is not clear if granulocytes are also affected in this type of SCID to date. Additional class I coronins may, however, compensate for the absence of coronin-1A in granulocytes, similar to platelets. Coronin-1A-deficient platelets retain the ability of the ARP2/3 complex to accumulate at the cell cortex or endosomes and phagosomes, as well as enable the formation of lamellipodia and spreading under some conditions in vitro [103]. Further investigation of the relevance of coronins for granulocyte function could elucidate the importance of this cell type in this SCID and would expand fundamental understanding of coronins in neutrophil function.

WD Repeat-Containing Protein 1 Deficiency (Lazy Leukocyte Syndrome)

The lazy leukocyte syndrome (LLS, OMIM 150550), first described in 1971 [104], derives its name from the observation that neutrophils of these patients show a severe migration defect. Almost half a century later, Kuhns et al. [105] identified autosomal recessive loss-of-function mutations in the gene WD repeat-containing protein 1 (*WDR1*, chromosome 4p16.1). Although the authors mentioned the defect as a possible cause of LLS, this is debatable because consanguinity or the clinical manifestation of severe oral stenosis had not been mentioned in the past. LSS is more descriptive in nature and can have miscellaneous backgrounds representing 1 distinct disorder among many different "actinopathies" in general terms.

WDR1 encodes for actin-interacting protein 1 (AIP1), which plays a major role in enhancing the severing and disassembly of actin filaments by cofilin [106, 107]. Cofilin has been shown to bind to the sides of actin filaments and to destabilize monomer-monomer interactions within the filament, causing actin filaments to break apart. Cofilin activity is regulated by LIM kinase-mediated phosphorylation at Ser3, which disrupts the F-actin-binding domain, resulting in decreased actin depolymerization [108]. The ability to sever actin filaments varies depending on the stoichiometry of cofilin binding (cofilin:actin ratio). When actin filaments are saturated with cofilin, severing is minimal and filaments are stabilized, but in living cells, despite the high concentrations of cofilin, actin filaments undergo rapid severing when bound by coronins, Srv2/cyclase-associated protein, or AIP1 [109–111]. Several patients with WDR1 deficiency have been described to date, and these patients mainly suffer from severe stomatitis, recurrent infections, and moderate neutropenia (homozygous [p.D26 N], compound heterozygous [p.delK7 and p.V424M], and [p.G121R and p.L286V], respectively) [105].

Mutations in *WDR1* are also described to cause an autoinflammatory phenotype with periodic fever, immunodeficiency with increased IL-18 serum levels and thrombocytopenia (PFIT, due to a homozygous missense mutation [c.877C>T] affecting both transcripts of *WDR1*, that is, transcript 1 exon 8 c.877C>T, L293F and transcript 2 exon 5 c.457C>T, L153F) [112]. Also most of the patients described by Pfajfer et al. [113] (which have homozygous missense mutations [p.H145Q] and [p.D572V] or compound heterozygous mutations [p.D572V and p.G501S]) suffered from fever and/or thrombocytopenia, similar to hypomorphic *Wdr1* mutations in mice [114], as well as intellectual impairment. Clearly, not all patients with *WDR1* mutations are reported to be neutropenic, in contrast to LLS patients [112, 113, 115], and may even show elevated neutrophil counts instead.

The pathophysiology of LSS phenotype or PFIT may be different. The mildly neutropenic patients studied by Kuhns et al. [105] did not exhibit persistently elevated levels of IL-18 nor did LPS stimulation of patient PBMCs show exaggerated IL-18 production. Also, there were comparable levels of mutated AIP1 protein in patients compared to normal AIP1 protein in control neutrophils [105]. The mutations causing PFIT were associated with

near-absent levels of WDR1 expression in PBMCs, and only some variable residual WDR1 expression in expanded T-cells, which may suggest that activation of lymphocytes stimulates or stabilizes expression of the WDR1 variants [113]. The other kindred described with PFIT showed mutated WDR1 expression which formed aggregates that co-localized with pyrin [112].

Functionally, neutrophils of patients with WDR1 deficiency show a severe migration defect and abnormal spreading in response to fMLF. Furthermore, these cells showed an enhanced F-actin content, which is illustrating the defective actin depolymerization [105, 113]. No defect was seen in the killing of *S. aureus* [105] and phagocytosis of opsonized *E. coli* [112], as also has been described for LLS [104, 115]. Granule content and release were also reported to be normal, whereas ROS production was, depending on the stimulus used, found to be enhanced [105, 112]. Mitochondrial membrane potential and apoptosis in patient neutrophils was not reported to be affected [113]. Furthermore, around 40–60% of the neutrophils showed abnormal "herniation" of their nuclear lobes being pushed to the perimeter of the cell [105, 113]. Interestingly, depletion of coronin-1a fully restored the adverse effects of WDR1 deficiency (i.e., neutropenia and impaired actin dynamics, motility, and nuclear morphology) in neutrophils of WDR1-deficient zebra fish embryos, which display a similar phenotype as human WDR1-deficient neutrophils [116].

Apart from neutrophils, also defects in monocytes, dendritic cells, and lymphocytes have been reported [112, 113]. These cells also showed increased F-actin content. Monocytes showed an increased spreading capacity, both basally as upon stimulation with LPS, and this was associated with F-actin-rich podosome-like structures [113]. Furthermore, IL-18 production was increased by dendritic cells both basally as upon stimulation, while no difference was observed in IL-1β levels. Increased caspase-1 cleavage was observed in $CD14^+$ PBMCs, indicative of inflammasome activity, which corresponds with the autoinflammatory phenotype observed in these patients and the co-localization of WDR1 mutant protein with pyrin, a protein involved in the classical fever syndrome familial Mediterranean fever [112].

The adaptive immune system is also shown to be affected in WDR1 deficiency. Pfajfer et al. [113] observed abnormalities in the B-cell and T-cell compartment, including profound peripheral B-cell lymphopenia, lack of switched memory B-cells, and reduced clonal diversity. Migration of B-cells was shown to be normal, even though an increased adhesion was observed. Similarly, T-cells showed increased spreading, but unaffected T-cell receptor internalization, migration toward to C-X-C motif chemokine ligand 12 (CXCL12), and killing of target cells by $CD8^+$ T-cells were not affected. Follicular T-helper cells were reduced in all patients described by Pfajfer et al. [113], which can explain the variety of microbial pathogens causing infections in WDR1 deficiency including *Streptococcus pneumoniae* and *Haemophilus influenza*, being pathogens more compatible with typical humoral immunodeficiency instead of a neutrophil defect.

When Signaling Goes Wrong: Actin Polymerization Defects due to Defective Downstream Signaling

There are several PIDs known which are caused by mutations in genes which are essential in the induction of actin polymerization. These proteins are found downstream of receptor activation (e.g., G protein-coupled receptors or integrin receptors) and induce actin polymerization by activation of the WRC or WASP. Key components in these signaling pathways are Rho GTPases, which cycle between an (inactive) guanosine diphosphate (GDP)- and (active) guanosine triphosphate (GTP)-bound state [117]. This is coordinated by 3 groups of regulatory molecules, namely, guanine nucleotide-exchange factors (GEFs), GTPase-activating proteins, and guanine nucleotide-dissociation inhibitors (GDIs) [118]. Also, ezrin/radixin/moesin proteins can directly interact with RHO GDI, thereby reducing its activity (shown in Fig. 1) [119]. Defective downstream signaling will result in impaired actin polymerization and thus reduced cell motility, which subsequently results in the increased susceptibility of these patients to infections.

RAC2 Mutations

RAC2 is a member of the Rho GTPases family, which also includes CDC42, which binds to WASP upon cell activation, and Ras homologous family member A which acts upon Rho-associated protein kinase and mammalian diaphanous-related formin 1 [120]. All 3 proteins can function as molecular switches, thereby regulating signal transduction. In their inactive state, they are GDP-bound, while they become active when bound to GTP [117]. RAC2 (encoded by *RAC2*, chromosome 22q13.1) in particular is shown to be of importance for activating the respiratory burst in neutrophils by interacting with $p67^{phox}$. RAC2 also plays a major role in actin polymerization by activating cofilin and the ARP2/3 complex through the WRC [121, 122].

RAC2 expression is considered to be restricted to the hematopoietic system. Following the first description of a dominant-negative autosomal dominant mutation in *RAC2* as heterozygous de novo $RAC2^{D57N}$ variant [123], subsequent reports have further diversified the clinical spectrum of *RAC2* mutations [124–129]. In neutrophils, RAC2 is the major isoform (95%) with RAC1 being only a minute fraction of total RAC [123].

Mutations in *RAC2* leading to immunodeficiency include both loss-of-function (4 patients described) and activating (9 patients described) mutations. Loss-of-function mutations are described to cause neutrophilia, severe bacterial infections, and poor wound healing, as well as T- and B-cell lymphopenia and hypogammaglobulinemia [123, 125, 126]. Recurrent respiratory infections, severe lymphopenia, and (mild) neutropenia have been described in patients with activating gain-of-function mutations in *RAC2* [127–129]. The activating variant $RAC2^{E62K}$ retains intrinsic GTP hydrolysis, but the GTPase-activating protein failed to accelerate hydrolysis to switch RAC2 activity off [127]. The $RAC2^{G12R}$ mutation was in the GDP/GTP-binding domain and shows strong impact causing another clinical phenotype of early SCID with neutropenia, known as reticular dysgenesis [129].

Both loss-of-function and activating mutations in *RAC2* resulted in a severely impaired chemotactic response by neutrophils [123–127]. Neutrophils did not polarize and showed less or disorganized F-actin in response to fMLF stimulation in case of loss-of-function mutations [123, 125], while neutrophils of patients with gain-of-function mutations showed increased F-actin levels at basal state, as well as upon fMLF stimulation [127, 128]. Also, the respiratory burst of patients' neutrophils was either decreased (loss-of-function mutations) or increased (gain-of-function mutations) in response to fMLF when compared to controls. Patient neutrophils responded normally to phorbol 12-myristate 13-acetate (PMA), irrespective of the presence of a gain-of-function or loss-of-function mutation in the *RAC2* gene, which is indicative of a functional oxidase complex [123, 125, 127, 128]. However, $RAC2^{E62K}$-transfected COS-7 cells (co-transfected with NADPH oxidase components) did show elevated ROS production both at the basal state as when stimulated with PMA [127]. Neutrophil granular content was shown to be normal in 1 patient with a (dominant negative) loss-of-function mutation and no defect in specific granule release was observed. However, release of azurophilic granules was absent as assessed by myeloperoxidase release [123]. In 1 case with a homozygous loss-of-function mutation, neutrophils showed reduced granule content, as well as altered secondary granule morphology [126].

The T- and B-cell lymphopenia seen in these patients also illustrates a role for RAC2 in lymphocyte development. Functionally, Lougaris et al. [128] showed that both B- and T-cells of patients with activating *RAC2* mutations have increased apoptosis rates [128]. It would be of interest to further functionally characterize the different lymphocyte subsets in RAC2 patients.

DOCK2 (and DOCK8) Deficiency

As mentioned previously, Rho GTPases cycle between (inactive) GDP- and (active) GTP-bound states. GEFs mediate the exchange of GDP to GTP, thereby promoting the binding of Rho family proteins to their specific effectors [118, 130]. The GEF dedicator of cytokinesis protein 2 (DOCK2) is an important regulator of the activation of RAC and acts downstream of various mitogenic chemokine receptors [131, 132].

DOCK2 is predominantly expressed in hematopoietic cells [133]. Upon stimulation of neutrophils with fMLF, DOCK2 translocates to the plasma membrane. This process is mediated by the phospholipid phosphatidylinositol 3,4,5-trisphosphate. Another phospholipid, phosphatidic acid, subsequently interacts with DOCK2 and stabilizes the leading edge of the cell, thereby ensuring localized RAC activation during neutrophil chemotaxis [134]. To date, 10 patients from 6 families with autosomal recessive loss-of-function mutations in *DOCK2* (chromosome 5q35.1, OMIM 616433) have been identified. These patients suffer from early-onset invasive bacterial infections, severe viral infections (including vaccine-related varicella zoster infections), and T-cell lymphopenia. Some patients show high IgE levels [135, 136].

Neutrophil function of 1 patient with DOCK2 deficiency has been described by Moens et al. [136]. Patient neutrophils showed impaired actin polymerization upon fMLF stimulation, as well as reduced cell protrusions upon fMLF or IL-8 stimulation. The respiratory burst upon PMA stimulation was impaired [136]. It would be of interest to test the effect of other stimuli (e.g., fMLF) on ROS production, as the PMA-induced respiratory burst was also normal in neutrophils of patients with *RAC2* mutations [123, 125, 127, 128].

Next to neutrophils, Epstein-Barr virus-transformed B-cells, T-cells, NK-cells, and Simian virus 40-immortalized primary fibroblasts from DOCK2 patients were assessed [135, 136]. These B-cells and T-cells showed impaired actin polymerization and migration in response to CXCL12 or chemokine coiled-coil-ligand 21 (CCL21),

respectively. Also, basal F-actin levels were lower in DOCK2-deficient cells compared to control cells [135]. DOCK2-deficient NK-cells showed aberrant degranulation upon stimulation with K562 target cells [135, 136]. Furthermore, these cells displayed lower basal levels of F-actin, as well as impaired actin polymerization, signaling, and interferon-gamma production, which may explain the susceptibility of these patients to viral infections [135]. While DOCK2 is predominantly expressed in hematopoietic cells [133], some DOCK2 expression was found in healthy control fibroblasts, but not in fibroblasts of DOCK2 patients. DOCK2-deficient fibroblasts were found to be more susceptible for virus-induced cell death, although this effect could be rescued by interferon-alfa-2b treatment. Since HSCT resolved the immunodeficiency completely, it seems that this defect in fibroblasts is not contributing to the susceptibility to viral infections when there is a healthy hematopoietic system [135].

Whereas DOCK2 is an important regulator of the activation of RAC, its family member, DOCK8, acts as GEF for CDC42 activation. Mutations in *DOCK8* cause the major form of autosomal recessive hyper IgE syndrome (chromosome 9p24.3, OMIM 243700) [137]. DOCK8 is expressed in the hematopoietic system and coordinates the actin cytoskeleton response to mitogenic and chemokine signals through the reversible activation of CDC42. DOCK8 interacts with signal transducer and activator of transcription 3 and regulates signal transducer and activator of transcription 3-dependent T-helper 17 cell differentiation [138], which may largely explain the recurrent and severe bacterial, viral, and fungal infections, as well as the eczema and environmental allergies in DOCK8 deficiency [139]. Our data with DOCK8-deficient patient neutrophils show by and large normal functions, including neutrophil actin polymerization (Sprenkeler, Tool, Kuijpers, unpublished). Also, neutrophil chemotaxis, phagocytosis, and ROS production was assessed in DOCK8-deficient patients, and these functions were mostly found to be normal (2 out of 6 patients showed a mild to moderate chemotaxis defect) [140].

Mutations in the gene encoding CDC42 have been identified to cause severe disease related to early-onset macrophage activation and defective hematopoiesis with an inherited hemophagocytic syndrome [141]. Directed migration toward CXCL12 by bone marrow $CD34^+$-cells and PBMCs was defective; neutrophils were not analyzed.

The absence of major effect of DOCK8 mutations may also explain the observations that the absence of WASP may not affect actin polymerization and motility in human neutrophils. Although neutrophils by and large show normal actin polymerization to chemotactic stimuli, a much more subtle defect in DOCK8-deficient neutrophils has been observed under specific conditions in which neutrophil motility seems affected (Sprenkeler, unpublished). Nonetheless, the signaling unit DOCK8-CDC42-WASP seems not to be the dominant circuitry involved in actin polymerization and motility in human neutrophils.

Serine/Threonine-Protein Kinase 4 Deficiency

Serine/threonine-protein kinase 4 (STK4; also known as mammalian sterile 20-like kinase 1) is a member of the large family of kinases named after the yeast sterile20 kinase. It is ubiquitously expressed throughout the body, with a high expression in bone marrow and lymphoid tissue.

This cytoplasmic kinase is activated upon stress signals, and upon caspase-cleavage, will translocate to the nucleus where it can phosphorylate multiple proteins, including histone H2B, transcription factors forkhead box O1 (FOXO1) and FOXO3, and microtubule-associated proteins 1A/1B light chain 3B (LC3), an important regulator of autophagy [142–144]. STK4 is a key component of the evolutionary conserved HIPPO pathway, which plays a pivotal role in controlling the balance between cell survival and cell death [145, 146]. Furthermore, upon phagocytosis and TLR signaling, STK4 can activate RAC via the kinase PKC-α and RHOGDI2, the RHO GDI expressed in high levels by hematopoietic cells. Subsequently, activated RAC promotes cytoskeletal rearrangements that recruit mitochondria to phagosomes. Also, mitochondrial- and phagosomal ROS production is promoted by RAC, thereby enhancing the killing of the pathogens [147].

Since 2012, several loss-of-function mutations have been identified in STK4 (chromosome 20q13.12) [148–155]. The prominent future of STK4 deficiency (OMIM 614868) is $CD4^+$ T-cell lymphopenia. Patients suffer from recurrent bacterial and viral infections, mucocutaneous candidiasis, eczema, molluscum contagiosum, high levels of IgE and IgA, and may have some cardiac aberrations [148–155].

A number of patients suffer from intermittent neutropenia [148, 149, 154, 155]. Bone marrow evaluation of STK4-deficient patients showed mature neutrophils, indicating that there is no maturation arrest. However, primary neutrophils of patients showed an enhanced loss of mitochondrial membrane potential, and these cells were more susceptible to apoptosis, which could explain the observed neutropenia [148]. This susceptibility to apoptosis

is also seen for T-cells, and defective IL-7R signaling, reduced expression of the anti-apoptotic protein Beclin-2, and increased FAS expression/signaling are likely mediating this impaired survival. Also, STK4-deficient T-cells showed an impaired proliferative response [148, 152, 155]. Furthermore, STK4 has been shown to play a role in CD11b/CD18 surface mobilization and localization in lymphocytes [156], and T-cells indeed show impaired migratory capacity upon stimulation with CCL19, CCL21, or CXCL11 [150, 155]. Although neutrophil adhesion in flow chambers coated with E-selectin/ICAM-1/CXCL8 was shown to be normal in 2 patients [157], motility was not tested. Mouse studies have shown that mammalian sterile 20-like kinase 1 is critical for neutrophil extravasation by controlling the translocation of vesicles containing laminin-binding integrins and neutrophil elastase to the surface, suggesting normal adhesion but inappropriate motility [157]. It would be of interest to assess whether chemotaxis and extravasation is also defective in humans.

Moesin Deficiency

Moesin (MSN) is part of the ezrin/radixin/moesin protein family of which members contain so called 4-point-one protein, ezrin, radaxin, moesin (FERM) domains capable of binding to lipids [158]. MSN links actin filaments to the plasma membrane and membrane receptors, which increases cell rigidity and polarity [159, 160]. It has also been reported that MSN is important for signal transduction of selectins [161], and that its N-terminal fragment interacts with RHO GDI (i.e., bovine RHO GDI, which is 97% identical to human RHO GDI on the peptide level [162] and 78% identical to RHOGDI2, the RHO GDI expressed in high levels by hematopoietic cells [163]). This initiates activation of Rho family members, including Ras homologous family member A, RAC1, and CDC42, by reducing the activity of RHO GDI to inhibit GDP/GTP exchange (shown in Fig. 1) [119]. During cytoskeletal reorganization, MSN becomes phosphorylated, causing the N-terminus and C-terminus to interact, which obscures them from interacting with the plasma membrane and cytoskeleton, respectively [164, 165].

To our knowledge, 3 reports which describe mutations in the X-chromosomal *MSN* gene (Xq12) have been published, 1 missense mutation in the FERM domain and 1 frameshift mutation close to the carboxyl-terminus [166–168]. The first report introduces the name X-linked MSN-associated immunodeficiency (X-MAID; OMIM 300988) [166]. All patients described having suffered from CID with recurrent bacterial infections of the respiratory, urinary, and/or gastrointestinal tracts and viral infections, including varicella zoster virus infection, recurrent molluscum, and persistent eczema. One case had autoimmune-related thrombotic thrombocytopenic purpura due to anti-ADAMTS13 autoantobodies [166–168]. Notably, bacterial infections could be treated with either immunoglobulin replacement therapy, antibiotic treatment, or both [166–168]. Examination of blood cell counts revealed a severely reduced lymphoid compartment, and especially younger patients suffered from episodes with reduced neutrophil counts [166–168], which was successfully compensated by treatment with granulocyte-colony stimulating factor (G-CSF) in some patients [166, 167]. In addition to lymphopenia, all patients, except 1 [167], were reported to have a normal-sized thymus, which indicates impaired migration of lymphocytes from the thymus [166]. All patients who were studied as children showed a normal cell distribution in the bone marrow which indicates that hematopoietic differentiation was not affected by this genetic disorder [166–168]. Therefore, the most likely cause for the patient's phenotype would be impaired migration of T-lymphocytes or granulocytes [166–168].

For unknown reasons, MSN protein expression in patient granulocytes was not affected by the R171W missense mutation or R553X truncation of the MSN protein, whereas expression was reduced in lymphocytes, which seems to relate to the lifetime of lymphocytes in vivo [166, 167]. No functional assays have been performed using granulocytes from these patients. However, deletion of MSN in mice affected the velocity of neutrophil rolling, impaired neutrophil chemotaxis toward invading bacteria, and diminished neutrophil-mediated microbial killing and inflammation [169, 170]. Patient lymphocytes showed impaired proliferation, migration, and adhesion [166, 167]. These results support the hypothesis that defective lymphocyte egress from the thymus causes the observed lymphopenia. Since patients also suffer from episodes of neutropenia, it would be interesting to perform migration and adhesion assays with patient granulocytes. This could elucidate a role of MSN in neutrophil migration from the bone marrow, which might be separate from G-CSF-induced bone marrow egress based on the positive response of patients upon G-CSF treatment.

Adhesion Deficiencies Resulting in Impaired Chemotaxis

Although leukocyte adhesion deficiencies (LADs) do not result in impaired actin polymerization, the defect in adhesion results in defective chemotaxis which is also

commonly found in actinopathies. Therefore, these patients also have an increased susceptibility to infections. Whereas LAD-II is caused by the absence of fucosylated ligands for selectins resulting in the inability of neutrophils to bind selectins on the endothelium, both LAD-I and LAD-III are caused by mutations in genes involved in leukocyte integrin signaling. For that reason, the latter two will be discussed. Furthermore, we will discuss shortly calcium and DAG-regulated guanine nucleotide exchange factor I (CalDAG-GEFI) deficiency, which is also described to lead to defective leukocyte integrin activation (but not a chemotaxis defect in neutrophils). Mutations in the ankyrin-spectrin network (these proteins link membrane proteins to the cytoskeleton) are reported to cause human disease, including spherocytosis type 1, cardiac arrhythmia, and neurodevelopmental disorder [171, 172]. In mice, disruption of the ankyrin-binding site of CD44 impaired neutrophil rolling on E-selectin [173]. However, to our knowledge, there are thus far no reports on neutrophil dysfunction caused by mutations in ankyrin or spectrin proteins in humans.

LAD-I and LAD-III

For LAD, 3 different variants are described. Here, we will focus on LAD-I and LAD-III, which are caused by mutations in *ITGB2* and *FERMT3*, respectively. Although LAD-I and LAD-III do not encompass an actin defect, these genes are part of the CD11/CD18 complex which links extracellular components to the cytoskeleton and play a major role in adhesion and chemotaxis, both commonly found defective in actinopathies. The membrane protein CD18 (integrin β2) is found as a heterodimer with 1 of 4 different α-integrins, of which complement receptor 3 (CR3; CD11b/CD18) is the most prominently expressed dimer on neutrophils. These β2-integrins are in a dormant state and are activated in a process termed "inside-out" signaling. Inflammatory stimuli and chemoattractants induce β2-integrins from low avidity to medium avidity by talin-1 [174–176] and then to their high avidity state by kindlin-3 [177]. After activation by talin-1, kindlin-3 interacts with the CD18 cytoplasmic tail at a membrane-distal NxxY/F motif [177]. Binding of kindlin-3 induces the transition of CD11b/CD18 from the medium avidity state induced by talin-1 toward the high avidity state [178, 179], which allows these β2-integrins to effectively bind to substrates on the endothelium and extracellular matrix. Upon binding of substrates, CD11b/CD18 forms clusters and relays signals into the cell, also known as outside-in signaling [180]. Furthermore, CD11b/CD18 binds actin through Talin-1 and recruits vinculin [181], which binds actin and recruits the actin-binding protein actinin [182]; thus in these 2 ways, CD11b/CD18 anchors the extracellular matrix and actin cytoskeleton. Additionally, CD11b/CD18 is also involved in the recognition of fungal components in neutrophils, and upon recognition causes release of specific granules [183–185].

There are several commonalities between LAD-I and LAD-III. Patients with either LAD-I or LAD-III are admitted to the hospital early in life or from birth onward with recurrent bacterial and fungal infections, and delayed detachment of the umbilical cord and omphalitis are often early symptoms [186–188]. Early morbidity is high due to poor wound healing, and these lesions do not show puss formation. Patients also exhibit a severe neutrophilia and often fatal early episodes of sepsis [186–188]. Patients who survive early infancy often present with chronic periodontitis and gingivitis [186–188]. Compared to LAD-I patients, LAD-III patients typically show milder immunological defects but suffer from a severe Glanzmann thrombasthenia-like bleeding disorder [189–192]. Additionally, some LAD-III patients exhibit osteopetrosis [178, 193]. Definitive treatment is possible through HSCT [178, 194, 195]. Otherwise, infections in LAD-I and LAD-III patients are treated with antibiotics and anti-fungal medication, and patients commonly receive prophylactic antibiotics.

Although LAD-I and LAD-III patients suffer from similar symptoms, the underlying mechanisms for these diseases differ. LAD-I is caused by mutations in *ITGB2*, which encodes CD18 and generally results in the loss of protein expression [187, 188, 196]. Generation of ROS is impaired in patient neutrophils upon stimulation with unopsonized pathogens, and CD18 has been implicated in NADPH oxidase activity [197]. Furthermore, blocking CD11b/CD18 in neutrophils impairs their in vitro killing capacity of *Candida albicans* [183, 184]. Primary neutrophils from these patients show impaired adhesion in static conditions [188] and under flow, as well as defective chemotaxis [188] and reduced transendothelial migration [198]. The lack of transendothelial migration is the most likely cause of the observed neutrophilia in these patients. This, along with an impaired killing capacity, explains the difficulty of clearing bacterial and fungal infection. Patients suffering from LAD-III have mutations in the *FERMT3* gene, which encodes kindlin-3 [178, 179, 199]. Similar results as in LAD-I regarding adhesion, chemotaxis, ROS production, and killing capacity have been found using primary neutrophils

from LAD-III patients [177, 178, 183, 184, 200, 201]. Similar to the β2 integrins, kindlin-3 expression is restricted to the hematopoietic lineage and is also important for activation of β1 and β3-integrins, of which the latter explains the observed severe Glanzmann-like bleeding phenotype. To effectively discriminate between LAD-I and LAD-III, adhesion of neutrophils can be tested in the presence of dithiothreitol, which forces CD18 into an open conformation. Patient neutrophils with LAD-III will show normal adhesion, while patients with LAD-I will show severely abrogated adhesion due to the absence of CD11b/CD18 dimers [201]. Collectively, these PIDs illustrate the importance of functional β2-integrins in the myeloid compartment for normal immune response and pathogen clearance through facilitating the interaction between the extracellular matrix and actin cytoskeleton.

CalDAG-GEFI Deficiency

RAS guanyl-releasing protein 2 (RASGRP2), also known as CalDAG-GEFI, is part of the RasGRP family. Initially, mutations in *RASGRP2* (chromosome 11q13.1) were proposed to be the cause for LAD-III [202]. The main target of CalDAG-GEFI is Ras-related protein 1 (RAP1), a small GTPase which regulates the activation of integrins in platelets ($\alpha_{IIb}\beta_3$ integrin) and neutrophils (β2 integrin) [203–205].

Several reports on CalDAG-GEFI deficiency (OMIM 615888) have been published, describing patients who suffer from impaired platelet function and bleeding disorder. The main platelet phenotype in these patients was impaired $\alpha_{IIb}\beta_3$ integrin activation upon stimulation (except upon PMA stimulation), impaired RAP1 activation, and defective platelet aggregation [206–210].

Neutrophil function was also investigated in these patients. Some patients showed normal β2 integrin expression, but defective integrin activation as assessed by the ability to bind soluble fibrinogen or the conformation-specific antibody m24 [207, 209], as also described in *Rasgrp2* knockout mice [211]. These patients had homozygous mutations in *RASGRP2*, leading to no or severely reduced expression of CalDAG-GEFI. However, no defects in integrin activation by neutrophils were observed in 2 other pedigrees. The patient described by Kato et al. [208] had compound heterozygous mutations leading to absence of CalDAG-GEFI in platelets, while the pedigree from Canault et al. [206] suffered from homozygous point mutations leading to a mutated version of CalDAG-GEFI with impaired catalytic activity, without any defect in neutrophil ROS production, adhesion, chemotaxis, and transendothelial migration [206]. More importantly, none of the patients suffered from immune defects or susceptibility to bacterial infections associated with neutrophil dysfunction, suggesting that CalDAG-GEFI has an insignificant or redundant role in RAP1 activation/integrin activation in neutrophils.

Undefined "Actinopathies": Neutrophil Actin Dysfunction

There may still be a group of "actinopathies" which remain unsolved, even though some of the functional and biochemical changes have been reported, such as the early description of a defect called neutrophil actin dysfunction (NAD) at the time. Because the genetic nature of NAD is not known, we will discuss here the function of lymphocyte-specific protein 1 (encoded by *LSP1*, chromosome 11p15.5) as it was found to be overexpressed in this PID. LSP1 is found in different types of leukocytes [212]. Known as 47-kDa actin-binding protein in NAD, LSP1 has been implicated in actin-mediated cell function of the hematopoietic lineage such as migration and phagocytosis [213]. It has several actin-binding domains in the latter half of the protein, which includes 2 caldesmon-like and villin-headpiece-like domains [214, 215] and on a molecular level it most likely functions by bundling actin filaments [216]. Postmortem samples indicate that LSP1 is upregulated in the epithelium, endothelium, and leukocytes of septic lungs; thus, it seems to play a role during inflammation in these cell types [17].

Two cases are known of NAD, both characterized by the overexpression of a 47-kDa protein [217, 218]. Additionally, a 89-kDa protein was absent from whole cell lysates of the second patient [217]. Both patients were admitted to hospitals early in life due to recurrent fevers and pulmonary infections [218, 219]. The index patient was first suspected of LAD-I because of the marked leukocytosis and non-pyogenic infections. Although CD11b/CD18 expression on neutrophils was found to be downregulated in the index patient, his mother, and sister, an increased CD11b/CD18 expression was present in the second patient [217–219]. The second patient was part of a large family and had 2 siblings who had died early in life with similar symptoms, suggesting an autosomal recessive genetic defect [219]. In addition to recurrent infections, this patient suffered from hepatosplenomegaly and thrombocytopenia but had normal neutrophil counts.

Moreover, morphology of resting neutrophils was normal, but fMLF stimulation induced abnormal hair-like protrusions. Both patients have been successfully treated by HSCT [218, 219].

In the second patient, the 47-kDa protein was identified as LSP1 [217]. It was then inferred that the first patient most likely also exhibited overexpression of LSP1. Unfortunately, the underlying genetic defect for this disease has not been elucidated. Based on the different phenotype and the absence of an 89-kDa protein in the second cases [219], it is more feasible that these defects are not identical and may neither be restricted nor even causally related to LSP1, although this remains unclear until a genetic cause has been elucidated.

Despite the unknown molecular cause for NAD, neutrophils from both patients have been functionally assessed prior to HSCT [217–219]. Evaluation of actin polymerization in the neutrophils of the index patient revealed that F-actin contents were lower both in the resting state and upon activation. Having a normal morphology in the resting state, these neutrophils produce hair-like protrusions upon stimulation with fMLF and fail to form podosomes or migrate, which seems very similar to neutrophils of ARPC1B-deficient patients [31]. Moreover, the index patient showed an abnormal degranulation [218], while ROS production was increased in neutrophils from the NAD 47/89 patient [219]. Because LSP1 functions as an actin-capping protein, the overexpression of LSP1 probably prevents F-actin formation. The effect of LSP1 overexpression may not be limited to neutrophils but can also affect endothelial and epithelial cells in mouse models [17, 214, 220].

The LLS as well as the 2 NAD cases discussed here indicate that we may well expect additional gene defects related to actin polymerization and motility to be uncovered. Such defects may or not present as a severe neutrophil disorder with an early onset for which HSCT is a potential life-saving cure.

Conclusion

The recent advances in whole-exome sequencing have resulted in the identification of numerous deleterious mutations in genes which are involved in actin cytoskeleton regulation to be causative of PIDs. Not only new PIDs have been described, like MKL1 deficiency and ARPC1B deficiency, but also deficiencies which have been known for decades, now have a known cause. PIDs involving the actin cytoskeleton, or so-called "actinopaties," have greatly increased our knowledge on the role of the actin cytoskeleton in immune function. However, treatment is often challenging, as patients can display combinations of immunodeficiency, auto-immunity, and auto-inflammation. Currently, the only definitive treatment option remains HSCT, which has its obvious challenges. Recent phase I/II clinical trials using lentiviral gene therapy in PIDs, for example, X-linked severe CID [221], X-linked chronic granulomatous disease [222], and WAS [55] showed promising results and demonstrate that gene therapy can be a valuable treatment option for these diseases.

Conflict of Interest Statement

The authors declare that they have no relevant conflicts of interest.

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Author Contributions

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