Pathogenic mechanisms and clinical implications of congenital neutropenia syndromes

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Purpose of review
The purpose of this review is to summarize pathogenic mechanisms and clinical implications of the most illustrative genetic entities of congenital neutropenia syndromes.

Recent findings
Congenital neutropenia comprise monogenetic entities with or without additional immunologic and extrahaematopoietic syndromatic features. Continuous careful explorations of known entities such as ELANE, GFI1, HAX1, G6PC3 deficiency and XLN help to define principles controlling differentiation and function of neutrophil granulocytes. Furthermore, the identification of novel genetic defects associated with congenital neutropenia, such as VPS45 deficiency, broadens our understanding of neutrophil biology. Pathogenic mechanisms imply protein and vesicle mistrafficking, endoplasmic reticulum stress, the unfolded protein response, destabilization of the mitochondrial membrane potential, disturbed energy metabolism, dysglycosylation and deregulated actin polymerization.

Summary
Advanced genetic and biochemical techniques have helped to expand our knowledge of congenital neutropenia syndromes. Known and novel genetic entities shed light on fundamental biological processes important for the homeostatis and functioning not only of the neutrophil granulocyte but as well of the entire haematopoietic system. Furthermore, treatment decisions become more tailored and might pave the road towards personalized molecular medicine.

Keywords
elastase neutrophil expressed, glucose 6 phosphatase catalytic subunit 3, growth factor independent 1 transcription repressor, haematopoietic cell specific LYN substrate 1 associated protein X-1, severe congenital neutropenia, vacuolar protein sorting 45 homolog, X-linked neutropenia

INTRODUCTION

Neutrophil granulocytes are the most abundant type of leukocytes and their inherited quantitative disorders are referred to as congenital neutropenia with or without additional immunologic and extrahaematopoietic manifestations (Table 1 [1–14,15▪,16–41,42▪▪,43–53,54▪,55–61,62▪▪,63▪,64▪▪,65–76,77▪,78–95,96▪▪,97–102]). The absolute neutrophil count (ANC) in the peripheral blood is used for dividing congenital neutropenia into mild ($1.0–1.5 \times 10^9$/l), moderate ($0.5–1.0 \times 10^9$/l) and severe ($<0.5 \times 10^9$/l) subtypes. Neutropenia may be constant, intermittant or periodic such as in cyclic neutropenia (CyN). Frequently, in congenital neutropenia, compensatory monocytosis is seen [103▪,104–106].

Patient registries founded in the early 1990s have allowed calculating an estimated incidence of $10–15/1 \times 10^6$ birth and a prevalence of more than $10/1 \times 10^6$ inhabitants. Concerted international collaborations have facilitated the discovery of genetic defects associated with severe congenital neutropenia (SCN). However, even in the era of next-generation sequencing, the genetic cause of congenital neutropenia remains unknown in 30–50% of patients [107▪].

Clinically, congenital neutropenia are characterized by acute and life-threatening invasive bacterial and fungal infections such as cellulitis, pneumonia and sepsis and chronic stomatologic infections such as recurrent aphthosis, paradontopathy and tooth

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KEY POINTS

- Congenital neutropenia comprises monogenetic entities with or without additional immunologic and extrahaematopoietic manifestations.
- Pathogenic mechanisms imply RNA processing, protein and vesicle mistrafficking, endoplasmic reticulum stress, the unfolded protein response, destabilization of the mitochondrial membrane potential, disturbed energy metabolism and dysglycosylation, as well as deregulated actin polymerization.
- Molecular diagnosis is important for risk stratification, treatment choice and prognosis.

loss [107▪]. Moreover, congenital neutropenia is considered a premalignant state of haematopoietic stem or progenitor cells and may degenerate into myelodysplastic syndrome (MDS) and acute myeloblastic leukaemia (AML) [108,109].

Granulocyte colony stimulating factor (G-CSF), which has been commercially available since 1993, has emerged as an effective first-line treatment and importantly reduced the mortality of congenital neutropenia [110,111]. However, a cumulative G-CSF dose of more than 10 000 μg/kg and a mean G-CSF dose per injection of more than 10 μg/kg are significantly related to malignant transformation [108,109]. The individual phenotype and the risk of malignancy vary for each genetic subtype of congenital neutropenia [112].

MAJOR PROGRESS

Qualitative neutrophil disorders such as myeloperoxidase deficiency (MPD), chronic granulomatous disease (CGD) and leukocyte adhesion deficiencies (LADs) are reviewed in great depth elsewhere. This review focuses on recent major progress in science on diseases associated with reduced numbers of neutrophil granulocytes [113▪–115▪].

BENIGN NEUTROPENIA

Benign neutropenia is a mild to moderate ($0.5–1.5 \times 10^9$/l) congenital neutropenia without numerical or morphological bone marrow abnormalities and without increased risk of bacterial or fungal infections [14,15▪,116,117]. Benign neutropenia is prevalent in approximately 4.5% of black and 0.8% of white Americans and can be found in the Arabian Peninsula and in various European regions as well [118–120].

In individuals from geographic regions where *Plasmodium spp.* are endemic, benign neutropenia is associated with an autosomal recessive promotor polymorphism in the Duffy blood group chemokine receptor gene (*DARC* c.T-46>C) that abrogates the binding of the transcription factor GATA binding protein 1 (GATA1) and leads to a loss of DARC expression on erythrocytes [1–3]. Even though the resulting Duffy null blood group has been found protective against *Plasmodium vivax* infection and is associated with an increased susceptibility towards human immunodeficiency virus (HIV) infection, the precise molecular mechanism of benign neutropenia remains elusive [4–6,121▪]. Current attempts to explain the phenotype of benign neutropenia often invoke nonhaematopoietic cells such as the endothelium and consecutive aberrant response to chemokines and transendothelial migration of neutrophil granulocytes [122,123].

Genome-wide association studies (GWAS) [124,125▪] for benign neutropenia in the remaining ethnicities have identified a locus on chromosome 17q and further genetic regions.

ELANE DEFICIENCY

Heterozygous mutations in the elastase neutrophil expressed gene (*ELANE*) coding for the neutrophil elastase cause CyN and SCN type 1 (SCN1) in an autosomal dominant way and are the most common cause of congenital neutropenia in the Caucasian population (41% in Europeans and 55.6% in North Americans) [7,8]. More than 100 defined genetic variants in *ELANE* have been associated with SCN1 [126▪▪].

CyN is characterized by an ANC that oscillates with a periodicity of approximately 21 days between almost normal values and complete disappearance of neutrophil granulocyte and that exhibits a reverse monocyte cycling, that is monocytes increase when neutrophil granulocytes decrease [9]. SCN1, however, displays a permanent bone marrow maturation arrest at the promyelocyte stage [7].

Even though specific *ELANE* mutations may lead to CyN and SCN1, respectively, there is no established firm genotype–phenotype correlation and we face a continuum rather than a clear-cut separation [7,8,10]. This might be due to inappropriate ANC monitoring, G-CSF treatment related cycle modification, incomplete mutation spectrum data, phenotype diversification by modifier genes and additional somatic mutations, especially in the colony stimulating factor 3 receptor gene (*CSF3R*) [11–14,15▪,127,128].

Nevertheless, on the basis of recent findings, several general conclusions may be justified. First, all known mutations lead to protein expression, so mere haploinsufficiency is not causing *ELANE*

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Table 1. Genetic defects causing congenital neutropenia syndromes.

Disorder	Gene mutated	Inheritance	Haematopoietic manifestations	Extrahaematopoietic manifestations	Pathophysiology	Ref.
Benign neutropenia						
DARC polymorphism	*DARC*	AR	Mild to moderate CN, no severe infections, protection against *P. vivax*, susceptibility for HIV	–	In erythrocytes, abrogated GATA1 binding to *DARC* leads to LOE (Duffy null blood group), cause of CN unknown	[1–6]
Severe congenital neutropenia						
ELANE deficiency (CyN and SCN1)	*ELANE*	AD	CyN with 21 day periodicity, CN with permanent bone marrow maturation arrest, predominantly SCN1: MDS, AML	–	ELANE mistrafficking and/or UPR leads to autophagy and enhanced apoptosis of myeloid cells	[7–14,15▪,16–20]
GFI1 deficiency (SCN2)	*GFI1*	AD	SCN and lymphopenia	–	Disturbed myeloid cell differentiation	[21–24]
HAX1 deficiency (SCN3)	*HAX1*	AR	CN, MDS, AML	Epilepsy, neurological impairment in some patients	Destabilization of mitochondrial membrane potential, enhanced apoptosis of myeloid and neuronal cells	[25–34]
G6PC3 deficiency (SCN4)	*G6PC3*	AR	CN, thrombocytopenia	Congenital heart and urogenital defects, failure to thrive, endocrinopathy, facial dysmorphy, inner ear hearing loss, hyperelastic skin, decreased subcutaneous fat tissue livedo reticularis	Impaired intracellular glucose homeostasis, dysglycosylation and UPR lead to enhanced apoptosis of myeloid cells	[35,36]
X-linked neutropenia (XLN, SCN-XL)	*WAS*	XL	CN, lymphopenia, MDS, AML	–	Disturbed actin polymerization, defective mitosis and cytokinesis	[37–41]
VPS45 deficiency (SCN5)	*VPS45*	AR	CN and neutrophil dysfunction, myelofibrosis, hypergammaglobulinaemia	Hepatosplenomegaly, nephromegaly (with extramedullary haematopoiesis)	Defective endosomal trafficking leads to impaired differentiation and motility and increased apoptosis of myeloid and mesenchymal cells	[42▪▪]
Hypopigmentation disorders comprising congenital neutropenia						
Chédiak-Higashi syndrome	*LYST*	AR	CN, defective cytotoxicity, HLH, mild bleeding tendency, enlarged granules	Oculocutaneous albinism, neurodegeneration	Defective biogenesis of lysosomes, cytotoxic granules and melanosomes	[43–49]
Hermansky-Pudlak syndrome type 2	*AP3B1*	AR	CN, defective cytotoxicity, HLH, mild bleeding tendency, enlarged granules	Oculocutaneous albinism	Defective lysosomal protein sorting, for example of elastase and tyrosinase	[50–53,54▪]
Griscelli syndrome type 2	*RAB27A*	AR	CN, defective cytotoxicitiy, HLH	Oculocutaneous albinism	Defective priming of cytotoxic granules and melanosomes	[55–58]
LAMTOR2 deficiency	*LAMTOR2*	AR	CN, defective cytotoxicity, lymphoid immunodeficiency	Oculocutaneous albinism, growth failure	Aberrant distribution of late endosomes	[59]

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Complex disorders comprising congenital neutropenia						
AK2 deficiency	*AK2*	AR	CN and SCID, that is reticular dysgenesis	Inner ear hearing loss	Defective mitochondrial adenine nucleotide homeostasis, enhanced apoptosis	[60,61]
STK4 deficiency	*STK4*	AR	CN, lymphopenia, CMC, warts	Congenital heart defects	Disturbed mitochondrial membrane potential, enhanced apoptosis	[62▪▪,63▪,64▪▪]
Shwachman-Bodian-Diamond syndrome	*SBDS*	AR	CN, bone marrow failure, MDS, AML	Exocrine pancreatic insufficiency, skeletal dysplasia, hepatic and cardiac disorders	Mitotic spindle destabilization, genomic instability, enhanced apoptosis	[65–68]
WHIM syndrome	*CXCR4*	AD	CN, warts, hypogammaglobulinaemia, infections, myelokathexis	–	Constitutively activated CXCR4 impairs chemokinesis of NG, DC and B cells	[69–73]
Poikiloderma with neutropenia (Clericuzio-type)	*USB1*	AR	CN, bone marrow abnormalities	Poikiloderma, photosensitivity	Disturbed biogenesis of U6 snRNA, impaired cell viability	[74–76,77▪]
Cartilage-hair hypoplasia	*RMRP*	AR	CN, variable cellular and humoral immunodeficiency, bone marrow failure	Short-limbed dwarfism, sparse hair, dysspermatogenesis, neuronal intestinal dysplasia, cancer susceptibility	Defective ribosome assembly, aberrant cell cycle control and telomere function	[78–84]
CD40LG deficiency	*CD40LG*	XL	Intermittent CN, class-switch recombination deficiency, combined immunodeficiency	–	Abrogated CD40LG:CD40-signalling	[85,86]
Barth syndrome	*TAZ*	XL	CN (in most patients)	Cardioskeletal myopathy, growth impairment, carnitine deficiency	Mitochondrial dysfunction, increased apoptosis in myeloid cells	[87,88]
Cohen syndrome	*VPS13B*	AR	Intermittent CN	Chorioretinal dystrophy, postnatal microcephaly, myopathy	Altered vesicle sorting and transport	[89–91]
GATA2 deficiency	*GATA2*	AD	CN, MDS, AML, MonoMAC syndrome	Sensory deafness, lymphoedema, pulmonary alveolar proteinosis	Complex ontogenic dysregulation of haematopoiesis and vascularization	[92–95,96▪▪]
Glycogen storage disease Ib	*SLC37A4*	AR	CN, MDS, AML	Glycogen overload of the liver, hypoglycaemia, fasting hyperlactacidaemia	Impaired intracellular glucose homeostasis, probably as in G6PC3 deficiency	[97–99]
Pearson syndrome	Deletion of mtDNA	MI	CN, bone marrow failure, vacuoles in erythroid precursors	Exocrine pancreas insufficiency, hepatopathy, nephropathy, endocrinopathy, neuromuscular degeneration	Variably sized mtDNA deletion, variable heteroplasmy, and mosaicism lead to metabolic disorder/energy failure and apoptosis in affected tissues	[100–102]

AD, autosomal dominant; AML, acute myeloblastic leukaemia; AR, autosomal recessive; CMC, chronic mucocutaneous candidiasis; CN, congenital neutropenia; CyN, cyclic neutropenia; DC, dendritic cell; HLH, haemophagocytic lymphohistiocytosis; LOE, loss of expression; MDS, myelodysplastic syndrome; MI, maternal inheritance; mtDNA, mitochondrial deoxyribonucleic acid; NG, neutrophil granulocyte; SCID, severe combined immunodeficiency; SCN, severe congenital neutropenia; snRNA, small nuclear ribonucleic acid; UPR, unfolded protein response; XL, X-linked recessive; XLN, X-linked neutropenia. Adapted with permission from [103▪].

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deficiency. Second, truncating mutations in the terminal exon 5 lead to expression of neutrophil elastase that lacks a binding site for the adaptor protein 3 (AP3) and are predominantly associated with SCN1. Third, missense mutations of the consensus splice acceptor in intron 4 lead to the use of a cryptic splice site in exon 5 and to the deletion of the catalytic site (Δ161–170) and are predominantly associated with CyN. Fourth, the common p.G815R mutation leads to G-CSF-refractory SCN1 with frequent progression to MDS and AML [15▪,16,17].

The monomeric serine chymotryptic-type protease neutrophil elastase is a major component of neutrophil granulocyte azurophilic granules and is named after its first known substrate elastin [129]. Neutrophil elastase is synthesized as an inactive zymogen of 267 amino acid (aa) residues and is activated by limited proteolysis at both its amino and carboxy-terminus. The cleavage of the N-terminal 27 aa pre and two aa pro-signalling sequences and a C-terminal 20 aa propeptide is fundamental to neutrophil elastase trafficking and enzymatic function [130,131]. Neutrophil elastase targets a variety of bacterial virulence factors and processes cytokines, chemokines, G-CSF and G-CSFR. Thus, it is involved in bacterial killing and in various signalling and homeostatic circuits [132,133]. Activated neutrophil elastase is kept in check by serine proteinase inhibitors, that is serpins, such as α1-protease inhibitor [134].

How exactly *ELANE* mutations lead to CyN and SCN1 remains a matter of debate and the three principal hypotheses that have emerged are not mutually exclusive. The inhibitor hypothesis assumes that *ELANE* mutations produce a negative feedback loop that leads to periodic impairment of neutrophil production and differentiation and uses mathematical models to demonstrate the 21-day periodicity of CyN [135].

The mislocalization hypothesis posits that neutrophil elastase is an AP3-cargo and trafficking through the *trans*-Golgi network towards the azurophilic granules is hampered as a consequence of C-terminal (truncating) mutations. Consequently, neutrophil elastase devoid of correct posttranslational processing accumulates in erroneous subcellular compartments [16,18,19]. How this interferes with (cyclic) neutrophil maturation arrest is not resolved and the answer might, at least partially, come from the unfolded protein response (UPR) hypothesis.

The UPR hypothesis proposes that *ELANE* mutations lead to neutrophil elastase misfolding during protein biosynthesis and cause an endoplasmic reticulum stress response as deduced by the upregulation of the stress marker heat shock 70-kDa protein 5 (HSPA5) and the splicing of X-box-binding protein 1 (XBP1) mRNA. Endoplasmic reticulum stress then initiates autophagy or apoptosis of neutrophil granulocyte and causes SCN1 as illustrated in cell culture systems and transgenic murine models [19,20,136].

Even though it has become clear the *ELANE* mutation induced cell death is pivotal for the phenotype of congenital neutropenia, it is still unclear which factors determine CyN versus SCN1, why G-CSF treatment can overcome the observed maturation arrest and why MDS and AML preferentially develop in SCN1, but are scarce in CyN. It is noteworthy that leukaemogenesis in SCN1 can proceed as a consequence of somatic *CSF3R* mutations. The very mechanisms that deviate *ELANE* mutations towards CyN versus SCN1 may also control the threshold for selective pressure and additional somatic hits required for clonal outgrowth of leukaemia cells [137▪].

GFI1 DEFICIENCY (SCN2)

Very rarely, congenital neutropenia has been found to be associated with mutations in the transcription factor growth factor independent 1 transcription repressor (*GFI1*). A murine knockout model for the growth factor independent 1 gene (*Gfi1*) has been found to cause myeloid differentiation arrest and to lead to SCN as well [138,139]. In addition, the differentiation and function of further haematopoietic lineages such as dendritic cells, B cells and T helper cells are disturbed in Gfi1-deficient mice [140–143]. Gfi1 is a predominantly repressive zinc-finger transcription factor that governs haematopoietic stem cell homeostasis and especially myeloid differentiation by regulating a plethora of biological processes such as trancriptional networks, microRNA processing and degradative ubiquitination-mediated apoptosis [144–147].

Almost paralleling these findings, heterozygous human mutations in *GFI1* leading to the dominant negative p.N382S and p.K403R substitutions and reducing the expression level of neutrophil elastase have been described to cause SCN2 with an additional lymphocyte proliferation defect [21,22]. Mechanistically, NEDD4-binding protein 2-like 2 (N4BP2L2) has been shown to interact with neutrophil elastase and GFI1 and might link *GFI1* mutations to reduced *ELANE* expression in SCN2, even though there has not yet been any *N4BP2L2* mutation found in SCN [23].

Interestingly, the very same mutations have been found as monoallelic de-novo mutations in one patient presenting with CyN. The authors found cycling cytotoxic T cell responses against HLA-A2 presented neutrophil elastase epitopes as causing

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this autoimmune CyN and their study [24] expands the pathogenetic mechanisms probably leading to CyN.

HAX1 DEFICIENCY (SCN 3)

Autosomal recessive mutations in haematopoietic cell specific LYN substrate 1 (HCLS1)-associated protein X-1 (*HAX1*) cause SCN3, classically known as Kostmann disease, and contribute to approximately 15% of SCN with a preponderance in the Turkish and Middle Eastern population [25,26,148].

HAX1 deficiency is characterized by SCN (often $<0.2 \times 10^9$/l) resulting from a maturation arrest at the promyelocyte stage and presenting during the first weeks of life with severe and life-threatening bacterial infections [27]. Some patients additionally display neurological impairment such as mental retardation and epilepsy and this is thought to result from *HAX1* mutations that do not only affect isoform A but also isoform B [28,29]. However, there might be a more complex genotype–phenotype relation, because compound heterozygous *HAX1* mutations targeting both transcripts have been described in SCN3 patients not affected by neuropathology [149,150▪▪].

HAX1 was initially identified as a ubiquitously expressed binding partner of HCLS1, which is involved in SRC-kinase signalling downstream of the B cell antigen receptor (BCR) [30]. HAX1 is an intracellular 35-kDa protein that predominantly localizes to the mitochondria but that can be detected in the endoplasmic reticulum and nuclear envelopes as well [30]. Biochemical analyses established a role of HAX1 in stabilizing the mitochondrial membrane potential ($\Delta\psi m$) and were congruent with increased apoptosis observed in HAX1-deficient myeloid and extrahaematopoietic cells [26,31]. HAX1 interacts with presenilin-associated rhomboid-like (PARL) and HtrA serine peptidase 2 (HTRA2) that activate the pro-apoptotic BCL2-associated X protein (BAX) pathway [31,32]. In addition, HAX has been shown to protect the antiapoptotic X-linked inhibitor of apoptosis (XIAP) from ubiquitination-mediated proteasomal degradation [33]. Further evidence for an antiapoptotic role of HAX1 stems from the observation that it interacts with and downregulates the protein level of ATPase Ca^{2+} transporting cardiac muscle slow twitch 2 (ATP2A2) that regulates the endoplasmic reticulum Ca^{2+}-concentration [34].

In line with an important function for HAX1 in cellular homeostatis is the observation that a number of viruses such as Epstein-Barr virus (EBV), human herpesvirus 8 (HHV-8), HIV and hepatitis C virus (HCV), which partially establish latency, code for HAX-1 interacting proteins, even though the mechanistic consequences of these interactions remain elusive [151–156].

Additional HAX1–protein interactions that are coupled to particular biological processes have been elucidated and it seems that HAX1 is involved in Ras homolog family member A (RHOA) mediated neutrophil cell adhesion and migration as well as in posttranscriptional mRNA processing, stability and localization [157,158].

Taken as a whole, HAX1 seems to balance proapoptotic and antiapoptotic signalling networks and to modulate further biological processes, but the precise mechanisms of increased apoptosis in developing myeloid cells have still to be determined.

G6PC3 DEFICIENCY (SCN4)

Autosomal recessive mutations in glucose 6 phosphatase catalytic subunit 3 (*G6PC3*) cause SCN4, a predominantly quantitative disorder of neutrophil granulocytes with variable thrombocytopenia and associated syndromatic features [159,160▪▪]. The most common extrahaematopoietic manifestations of G6PC3 deficiency comprise congenital heart and urogenital defects, failure to thrive, endocrine disorders, facial dysmorphy, inner ear hearing loss, hyperelastic skin, decreased subcutaneous fat tissue and livedo reticularis [160▪▪]. Mutations in *G6PC3* cluster in exons 1 and 6, which are coding for the C and N-terminus of G6PC3, respectively. However, the current mutational spectrum in a limited number of patients has not yet allowed a clear genotype–phenotype correlation [160▪▪].

G6PC3 is a ubiquitously expressed metabolic enzyme of the glucose 6 phosphatase (G6P) family, that is G6PC1–3, which localizes to the endoplasmic reticulum membrane where it is supposed to form a functional complex with glucose-6-phosphate transporter (G6PT), also known as solute carrier family 37 member 4 (SLC37A4) [161,162]. Neutrophil granulocyte G6PC3 deficiency deranges glucose recycling between the endoplasmic reticulum and the cytoplasm and leads to reduced levels of glucose uptake and its energy-carrying catabolites obtained by glycolysis [163]. In an experimental setting, this metabolic starving induces pronounced apoptosis of peripheral neutrophil granulocyte, whereas other haematopoietic lineages are less affected [159]. Mechanistically, a nutrient-sensitive network governed by the proapoptotic and antiapoptotic molecules glycogen synthase kinase 3 beta (GSK3B) and myeloid cell leukaemia sequence 1 (MCL1), respectively, in case of starvation targets the outer mitochondrial membrane and induces the intrinsic apoptotic pathway [164,165].

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Importantly, defective endoplasmic reticulum glucose metabolism additionally interferes with proper glycosylation patterns of various proteins, such as components of the NADPH oxidase, which are important for neutrophil granulocyte functioning [35]. Furthermore, apoptosis in G6PC3-deficient myeloid cells might be linked to the UPR, but whether this is linked to dysglycosylation has not yet been examined [160▪▪].

In G6pc3-deficient murine neutrophil granulocytes, metabolic alterations and increased apoptosis can be counteracted by application of G-CSF [36]. It is tempting to speculate that the favourable clinical response towards G-CSF reported for G6PC3-deficient patients might follow similar correction mechanisms [160▪▪]. Currently, for G6PC3 deficiency, no malignant transformation has been reported, and in light of the particular pathogenesis and comorbidity, this might be an important feature of SCN4 and influence our therapeutic approach [160▪▪].

X-LINKED NEUTROPENIA

Mutations inactivating the Wiskott–Aldrich syndrome (*WAS*) gene product Wiskott–Aldrich syndrome protein (WASP) cause classical Wiskott-Aldrich syndrome (WAS) or X-linked thrombocytopenia (XLT). WAS and XLT determine a spectrum rather than two distinct nosological entities [166,167]. The former is characterized by lymphoid and myeloid immunodeficiency, autoimmunity, predisposition to malignant lymphoma, eczema and microthrombocytoepnia, while the latter presents predominantly with microthrombocytoepenia and bleeding diathesis [166,167].

WASP is central to actin dynamics and in the haematopoietic system couples the membrane-proximal signalling layer to the cytoskeletal layer [168]. Consequently, a variety of cellular processes such as immunological synapse and kinapse formation, TCR and BCR signalling, integrin inside-out signalling, vesicle trafficking and cellular morphogenesis are critically hampered [169]. An important means of WASP regulation is an autoinhibitory conformation that is based on an intramolecular interaction between the verprolin homology domain-cofilin homology domain-acidic region (VCA) and the GTPase-binding domain (GBD). This closed conformation interferes with binding of the actin-related protein 2/3 (ARP2/3) complex and with monomeric actin to the carboxyl terminus of WASP [169].

Contrasting to WAS and XLT wherein inactivating *WASP* mutations lead to absent or residual protein expression, activating *WASP* mutations that target the GBD and interfere with autoinhibition cause X-linked neutropenia (XLN, SCN-XL) [37–39]. Constitutively active WASP results in intensified and misplaced actin polymerization and results in defective mitosis and cytokinesis as well as apoptosis-prone proliferation, especially in myeloid lineages [40]. Apart from neutropenia and monocytopenia, XLN shows reduced natural killer (NK) and T cell counts, impaired T cell proliferation and a genomic instability with a predisposition to MDS [38–41].

In summary, distinct *WAS* mutations lead to various disease entities and impressively highlight the multifaceted biological roles of WASP at the interface of the membrane-proximal signalling layer with the cytoskeletal layer in the haematopoietic system.

VPS45 DEFICIENCY (SCN-5)

Lately, autosomal recessive mutations in vacuolar protein sorting 45 homolog (*VPS45*) have been ascribed to a congenital neutrophil defect syndrome (SCN5) in seven patients from five consanguineous families [42▪▪].

VPS45 controls membrane trafficking through the endosomal system and defective endosomal trafficking and protein sorting/recycling appear to be the pathogenic mechanisms [42▪▪,170,171].

Elegant phenotypic and functional analyses performed with VPS45-deficient primary neutrophil granulocyte and fibroblasts found quantitative and qualitative neutrophil impairments due to hampered development, decreased motility and increased apoptosis of myeloid and mesenchymal cells [42▪▪]. In addition, complementation assays reversed the phenotype in fibroblasts and a morpholino-injected zebrafish model recapitulated the SCN found in man [109].

Clinically, the patients presented SCN, thrombocytopenia, and anaemia, the latter probably due to an important myelofibrosis. In addition, extramedullary haematopoiesis leads to hepatosplenomegaly and nephromegaly [42▪▪]. All patients were refractory to G-CSF treatment, and in one of the affected kindred, additional neurodevelopmental sequelae were noted [42▪▪].

VPS45 deficiency highlights the importance of vesicle trafficking in the haematopoietic system, but especially the pathogenic mechanism of myelofibrosis awaits further clarification.

POIKILODERMA WITH NEUTROPENIA (CLERICUZIO-TYPE)

Mutations in the U6 snRNA biogenesis 1 (*USB1*) gene cause an autosomal recessive genodermatosis that

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associates poikiloderma, that is erythematous rash, irrregular skin pigmentation, teleangiectasia and hyperkeratosis, with neutropenia (PN), bone marrow and peripheral blood abnormalities [74,75]. The syndrome was first described by Clericuzio in Navajo Indians and consecutively found in Apache Indians and other kindred [74,76,172]. Infrequently, short stature, nail dystrophy and MDS have been reported as well [173].

USB1 is a phosphodiesterase and its yeast ortholog is involved as a RNA exonuclease in posttranslational processing of spliceosomal small nuclear RNA (snRNA) that is important for cell viability [77▪]. The clinical phenotype of PN overlaps with those of dyskeratosis congenita and Rothmund-Thomson syndrome (RTS), and even though in PN telomere shortage has not yet been described, this might be a common pathomechanism [173,174].

CONCLUSION

With the advent of joint databases, commonly available G-CSF treatment and advanced genetic and biochemical techniques, our knowledge of congenital neutropenia syndromes is constantly growing. Novel genetic entities pinpoint towards fundamental biological processes important for the homeostatis and functioning not only of the neutrophil granulocyte but also of the haematopoietic system and its niche. Even though not all aspects of molecular pathogenesis have been elucidated up to now, treatment decisions, that is G-CSF application versus haematopoietic stem cell transplantation, become more sophisticated and might open up into personalized molecular medicine.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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