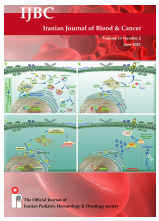
IJBC 2023; 15 (2): 146-171

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir



Review article

# Genetics and pathophysiology of severe congenital neutropenias (SCNs)

Samareh Younesian [1], Amir-Mohammad Yousefi [1], Peyman Eshghi [2], Mohammad Faranoush [3], Pooya Faranoush [4], Bijan Keikhaei [5], Aziz Eghbali [6], Bibi Shahin Shamsian [2], Babak Abdolkarimi [7], Sabahat Haghi [8], Hassan Abolghasemi [2,*], Davood Bashash [1,*]

1 Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2 Pediatric Congenital Hematologic Disorders Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Pediatric Growth and Development Research Center, Iran University of Medical Sciences, Tehran, Iran
4 Pediatric Growth and Development Research center, Institute of Endocrinology, Iran University of Medical Sciences, Tehran, Iran
5 Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur, University of Medical Sciences, Ahvaz, Iran
6 Clinical Research Development Center of Aliasghar Hospital, Iran University of Medical Sciences, Tehran, Iran
7 Department of Pediatrics, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
8 Department of Pediatrics, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran

ARTICLE INFO

**Article History:**
Received: 24/04/2023
Accepted: 18/06/2023

**Keywords:**
Neutropenia
Severe congenital neutropenia
Kostmann disease
ELANE
HAX1

***Corresponding authors:**
Hassan Abolghasemi
MD Pediatric Congenital Hematologic Disorders Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran
**Email:** h.abolghasemi.ha@gmail.com

Davood Bashash
PhD Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
**Email:** david_5980@yahoo.com

## Abstract

Severe congenital neutropenias (SCNs) are the rare heterogenous group of preleukemia bone marrow failure syndromes characterized by impaired differentiation of neutrophilic granulocytes and, as a result, severe chronic neutropenia. Patients with SCN are predisposed to recurrent, often life-threatening bacterial and/or fungal infections beginning in the first months of life. Molecular abnormalities in 10 genes have been identified that are responsible for SCNs. The pathophysiological mechanisms of SCNs are the subject of extensive investigation and are not fully known. The current review aims to summarize the studies exploring the biological role of SCN-associated genes and the effects of mutant genes in neutropenia pathogenesis. We mainly focus on the genetic mutations that lead to SCN1 to SCN9 and X-linked SCN (XSCN) to shed more light on the pathophysiology of these diseases.

Please cite this article as: Younesian S, Yousefi AM, Eshghi P, Faranoush M, Faranoush P, Keikhaei B, Eghbali A, Shamsian BS, Abdolkarimi B, Haghi S, Abolghasemi H, Bashash D. Genetics and pathophysiology of severe congenital neutropenias (SCNs). Iranian Journal of Blood and Cancer. 2023; 15(2): 146-171.

## 1. Introduction

Severe congenital neutropenias (SCNs) are the rare heterogenous group of preleukemia bone marrow failure syndromes characterized by impaired differentiation of neutrophilic granulocytes and, as a result, severe chronic neutropenia (blood absolute neutrophil counts (ANCs) of $<0.5 \times 10^9/\mu L$). Patients with SCN are predisposed to recurrent, often life-threatening bacterial and/or fungal infections beginning in the first months of life (1). In most affected individuals, bone marrow (BM) examination revealed a maturation arrest of granulopoiesis at the level of promyelocytes, leading to decreased neutrophil counts but increased

numbers of promyelocytes. SCNs can manifest as isolated neutropenia, neutropenia with other hematologic abnormalities, or syndromic SCN with extrahematopoietic organ dysfunctions. Some patients with SCN are at high risk of developing acute myeloid leukemia (AML) or myelodysplastic neoplasms (MDS) in the long term (2, 3). SCNs are categorized under myeloid neoplasms with germline predisposition and potential organ dysfunction in the 5th edition of the World Health Organization (WHO) classification of hematolymphoid tumors (4).

In recent years, Online Mendelian Inheritance in Man (OMIM) has categorized SCNs into several subtypes (SCN1-9 and XSCN) based on genetic defects. Furthermore, Human Inborn Errors of Immunity (HIEI) have incorporated the majority of SCNs as subtypes under congenital neutropenias (CNs). HIEI have proposed that elastase-, GFI1-, HAX1- (Kostmann disease), G6PC3-, and VPS45-deficiency equal SCN1, SCN2, SCN3, SCN4, and SCN5, respectively. While patients with mutations in genes JAGN1, CSF3R, SRP54, CLPB, or WAS are classified as JAGN1 deficiency, CSF3 receptor deficiency, SRP54 deficiency, 3-Methylglutaconic aciduria, or X-linked neutropenia/myelodysplasia, respectively (**Table 1**) (5).

The dysregulated molecular pathways linked to the genetic defects of SCNs include the unfolded protein response (ELANE and G6PC3), endoplasmic reticulum (ER) stress (ELANE, G6PC3, JAGN1, and SRP54), defective endosome trafficking (VPS45), intracellular glucose homeostasis (G6PC3), $Ca^{2+}$ homeostasis (JAGN1), CSF3R signal transduction (CSF3R and HAX-1), mitochondrial function (HAX1 and CLPB), transcription factor (GFI1) and cytoskeleton polymerization (WAS) (**Figure 1**). Of note, these different pathways result in increased apoptosis of neutrophil precursors and the arrest of granulopoiesis at the promyelocyte stage. In this review, we focus on the genetic mutations that lead to SCN1 to 9 and X-linked SCN (XSCN) and discuss recent advances in understanding the genetics and pathophysiology of these SCNs.

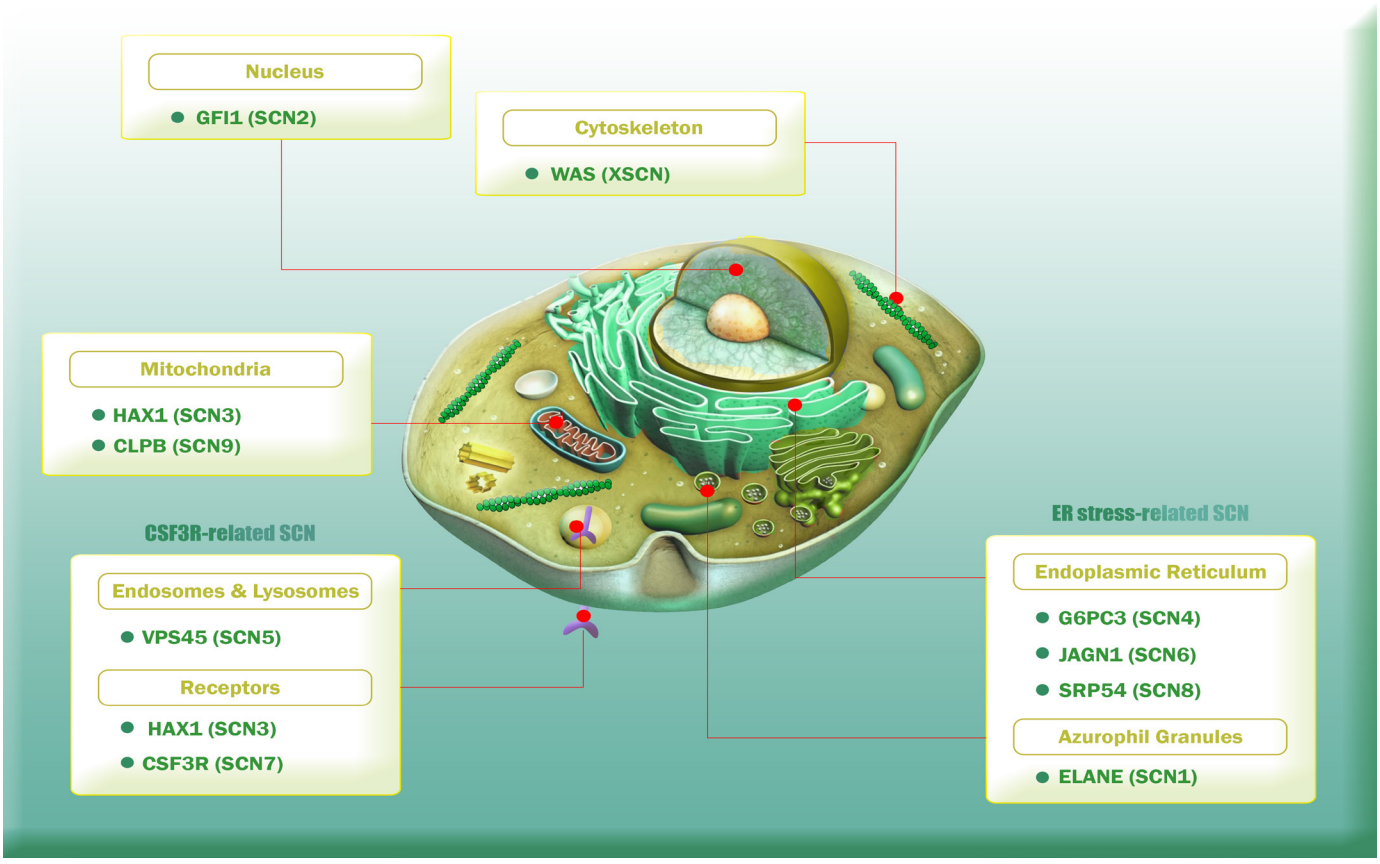



**Figure 1.** The dysregulated molecular pathways linked to the genetic defects of severe congenital neutropenias (SCNs). Abbreviations: ELANE: elastase, neutrophil expressed; ER: endoplasmic reticulum; GFI1: growth factor independent 1 transcriptional repressor; HAX1: HCLS1 associated protein X-1; G6PC3: glucose-6-phosphatase catalytic subunit 3; VPS45: vacuolar protein sorting 45 homolog; JAGN1: jagunal homolog 1; CSF3R: colony stimulating factor 3 receptor; SRP54: signal recognition particle 54; CLPB: ClpB family mitochondrial disaggregase; WAS: WASP actin nucleation promoting factor.

## 2. Mechanisms/pathophysiology

In recent decades, with the improvement of sequencing technologies and the establishment of the Severe Chronic Neutropenia International Registry (SCNIR), molecular abnormalities in 10 genes have been identified that are responsible for SCNs (**Table 1**). However, approximately one-third of patients with a clinical history suggestive of severe neutropenia have no identifiable genetic cause. The most frequent reasons for SCNs are autosomal dominant (AD) mutations in ELANE, which encodes neutrophil elastase, and autosomal recessive (AR) mutations in HAX1, encoding a multifunctional protein associated with mitochondrial integrity, and the CSF3 signaling pathway (6). Interestingly, digenic mutations (combinations of HAX1 and ELANE or G6PC3 and ELANE mutations) have been reported in some patients with SCN (7).

SCN-associated proteins have critical roles in different cellular compartments, including azurophil granule (elastase neutrophil), endoplasmic reticulum (G6PC3, JAGN1, and SRP54), endosome trafficking (VPS45), intracellular glucose homeostasis (G6PC3), CSF3R signal transduction (CSF3R and HAX-1), mitochondrial function (HAX1 and CLPB), transcription factor (GFI1) and cytoskeleton polymerization (WAS).

The dysregulated molecular pathways that underlie a maturation arrest of granulopoiesis at the level of promyelocyte can differ according to the various mutations that cause SCN (**Figure 1**). However, the molecular abnormalities in genes ELANE, G6PC3, JAGN1, and SRP54 lead to the endoplasmic reticulum (ER) stress, increased apoptosis of neutrophil precursors and the arrest of granulopoiesis at the promyelocyte stage. In this section, we focus our discussion on the pathomechanism of SCNs based on the different genetic etiologies.

### 2.1. SCN1: Defect in elastase, neutrophil expressed (ELANE) gene

Autosomal-dominant mutations in the elastase, neutrophil expressed (ELANE), the gene encoding neutrophil elastase (NE), are found in about half of the cases of SCNs and most cases of cyclic neutropenia (**Tables 1 and 2**) (6). NE, a serine protease, is predominantly synthesized at the promyelocytic stage and is stored in azurophil granules (8). Soluble serine proteases of azurophil granules, especially NE, fuse to the phagolysosome during inflammation and directly participate in the intracellular microbial killing. NE is also involved in the function of neutrophil extracellular traps (NETs), a network of fibers of DNA, histones, serin proteases, and antimicrobial molecules that bind to pathogenic microbes. Moreover, NE can cleave a great variety of different proteins such as fibronectin, elastin, proteoglycans, collagens, coagulation factors, CSF3, CSF3R, KIT proto-oncogene, receptor tyrosine kinase (KIT), vascular cell adhesion molecule (VCAM), and C-X-C motif chemokine receptor 4 (CXCR4). (9, 10).

So far, more than 200 different mutations have been detected in the ELANE gene, distributed in all five exons and introns 3 and 4. However, how these mutations cause neutropenia has not yet been fully elucidated. The ELANE mutations include missense, nonsense, frameshifts, inframe indels, and splice junction loss. These mutations lead to the production of a mutant polypeptide but do not affect biochemical properties such as proteolytic activity, serpin inhibition, and glycosylation. (8, 11, 12). Interestingly, the mutations predicting an absence of NE protein have not yet been reported in SCNs. Individuals with ELANE whole gene deletion mutation produce functional neutrophils with normal complete blood counts (13).

Two prevailing hypotheses on how mutations affect NE have been proposed. In one, ELANE mutations disrupt intracellular transport of NE, known as mistrafficking NE mutations. In the other, ELANE mutations disrupt the proper folding, processing, and secretion of NE, known as misfolding NE mutations. In addition, some ELANE mutations in the first codon and the Kozak sequence cause N-terminally truncated NE lacking ER-localizing (pre) and zymogen-maintaining (pro) sequences, which cannot be targeted toward ER and are probably mislocalized to the nucleus (14). A recent study has reported that inducible expression of a disease-associated ELANE mutation (p.Gly185Arg) reduced NE enzymatic activity and granulocyte differentiation without evoking an unfolded protein response (15).

#### 2.1.1. Mistrafficking NE mutations

NE is produced as an inactive form of pre-pro-serine protease (zymogen) of 267 amino acids, containing an N-terminal signal peptide, an N-terminal pro-dipeptide, and a C-terminal pro-peptide. It is converted to the active serine protease through four consecutive

Table 1. Gene mutations in severe congenital neutropenias (SCNs).

| Disease (OMIM) | HIEI | Gene | Location/ Inheritance | Functions of coding protein | CSF3 therapy |
|---|---|---|---|---|---|
| SCN1 (202700) | SCN1 | ELANE | 19p13.3/AD | Elastase, neutrophil expressed; primary or azurophilic granule protease | Responded to CSF3 |
| SCN2 (613107) | SCN2 | GFI1 | 1p22.1/AD | Growth factor independent 1 transcriptional repressor; interacts with myeloid-specific transcription factors CEBPA, CEBPB, and SPI1 | Responded to CSF3 |
| SCN3 (Kostmann disease) (610738) | SCN3 | HAX1 | 1q21.3/AR | HCLS1 associated protein X-1; activation of HCLS1 adaptor protein essential for CSF3R signaling, antiapoptotic protein in mitochondria | Responded to CSF3 |
| SCN4 (612541) | SCN4 | G6PC3 | 17q21.31/AR | Glucose-6- phosphatase catalytic subunits 3; hydrolyzes glucose-6-phosphate to glucose and phosphate in the ER | Responded to CSF3 |
| SCN5 (615285) | SCN5 | VPS45 | 1q21.2/AR | Vacuolar protein sorting 45 homolog; converts early endosomes to late endosomes, regulating cargo trafficking and the intracellular organization of endolysosomal vesicles, regulation of the assembly of the SNARE complex, which plays an essential part in the trafficking and recycling of proteins through lysosomes, other endosomes and trans-Golgi complex | Refractory to high-dose CSF3 |
| SCN6 (616022) | JAGN1 deficiency | JAGN1 | 3p25.3/AR | Jagunal homolog 1; an ER-resident protein, is involved in the early secretory pathway, it regulates the proper glycosylation of proteins involved in cell adhesion and cytotoxicity | A poor or no response to CSF3 |
| SCN7 (617014) | CSF3 receptor deficiency | CSF3R | 1p34.3/AR or AD | Colony-stimulating factor 3 receptor; activation of the intracellular signaling responsible for hematopoietic and myeloid differentiation of HSCs upon CSF3 binding | No response to CSF3 (See **Table 3**) |
| SCN-8 (618752) | SRP54 deficiency | SRP54 | 14q13.2/AD, de novo or unknown | Signal recognition particle 54; a key component of the ribonucleoprotein complex that mediates the cotranslational targeting and the translocation of secretory and membrane proteins to the ER | Responded to CSF3 (See **Table 4**) |
| SCN9 (619813) | 3-Methylglutaconic aciduria | CLPB | 11q13.4/AD or de novo | ClpB family mitochondrial disaggregase; mitochondrial ATPase chaperonin associated with DNA replication, protein degradation, and reactivation of misfolded proteins | Responded to CSF3 (See **Table 5**) |
| XSCN (300299) | X-linked neutropenia/ myelodysplasia | WAS | Xp11.23/ X-linked | WASP actin nucleation promoting factor; WAS protein is an essential regulator of actin rearrangement in association with Arp2/3 and WAVE complexes | Not need permanent CSF3 support |

AD: autosomal dominant; AR: autosomal recessive; Arp2/3 complex: Actin Related Protein 2/3 complex; CEBPA: CCAAT enhancer binding protein alpha; CEBPB: CCAAT enhancer binding protein beta; ER: endoplasmic reticulum; HCLS1: hematopoietic cell-specific Lyn substrate 1; HIEI: Human Inborn Errors of Immunity; HSCs: hematopoietic stem cells; OMIM: Online Mendelian Inheritance in Man; SNARE: soluble N-ethylmaleimide-sensitive factor activating protein receptor; SPI1: Spi-1 proto-oncogene; WAVE complex: WRC, SCAR complex.

post-translational modifications. The signal peptide (N-terminus) is removed during translation to form pro-NE, which is then glycosylated and transported to the Golgi apparatus for trafficking to the plasma membrane or granules. Inside the secretory vesicle, the C-terminal pro-peptide is cleaved by an as-yet unidentified protease. C-terminal processing creates a binding site for AP3B1 (tyrosine-based μ3a sorting signal), which is involved in the intracellular trafficking of NE (16). Then pro-dipeptide is removed from the amino terminus by cathepsin C, encoding by the CTSC (AR mutation in CTSC causes skin and periodontal diseases known as Papillon-Lefevre syndrome) (12). Finally, fully processed mature NE contains 218 amino acid residues sorted in the azurophil granules. In addition to being located within azurophil granules, NE has been found on the cell surface and within the nucleus. Under normal conditions, NE forms a ternary complex with GFI1 and N4BP2L2 in the nucleus, which enhances the inhibitory effect of GFI1 on its promoter. Consequently, NE regulates its transcription by a feedback circuit (**Figure 2A**) (17).

The C-terminal processing of the NE is critical for the interaction between NE and AP3B1 (AR mutation in AP3B1 causes Hermansky Pudlak syndrome type 2). Therefore, loss of the sorting signal or failure to remove the 20-residue C-terminal pro-peptide disrupts the interaction between NE and AP3B1. In most cases of SCN, chain-terminating mutations within ELANE (nonsense or frameshift mutations) occur in exons 4$^{th}$ and 5$^{th}$, leading to NE truncated at the carboxyl terminus and deletion of the sorting signal from NE. These mutations disrupt the interaction between NE and AP3B1, causing mistrafficking and mislocalization of the mutant NE to the plasma membrane and the nucleus (12, 16, 18). It appears that mutant NE does not entirely lose its proteolytic activity and, through mislocalization, proteolytically degrades CSF3 and CSF3R, which are essential for granulopoiesis. As a result, it reduces the biological activity of the CSF3 and signaling transduction of the CSF3R because of decreased CSF3R surface expression, leading to impaired differentiation of neutrophilic granulocytes and severe chronic neutropenia (19). Moreover, the mislocalized NE cannot bind to intranuclear N4BP2L2 due to the loss of the C-terminus required for binding to N4BP2L2, failing to form a feedback circuit. ELANE overexpression exacerbates the effects of mutant NE on granulocytic differentiation, reducing response to CSF3 treatment (**Figure 2B**) (17).

### 2.1.2. Misfolding NE mutations

Some mutations in the ELANE gene disrupt the proper folding, processing, secretion, or degradation of NE in myeloid cells from patients with SCN. The misfolded NE accumulation in ER causes ER stress and activation of a regulatory pathway called the unfolded protein response (UPR) (18, 20, 21). Early UPR events induce dissociation of the master regulator, HSPA5, from ATF6, and then ATF6 translocates to the Golgi apparatus, where it is cleaved into the active form. The active form of ATF6 migrates to the nucleus, inducing the transcription of target genes that mediate protein folding and promote the degradation of misfolded proteins (22). Due to excessive accumulation of misfolded NE, the UPR fails to degrade unfolded NE in promyelocytes and initiates ER-associated apoptosis (**Figure 2C**). In myeloid cells from SCN patients, the ATF6 activity and ATF6 target genes HSPA5, XBP1 mRNA splicing, PPP1R15A, and DDIT3 are increased; while levels of secretory leukocyte peptidase inhibitor (SLPI), a natural NE inhibitor, are decreased. In addition, the active form of ATF6 induces calreticulin (CALR) expression, a chaperone in the lumen of the ER in the context of the cellular stress response and UPR (21). It has been reported that CALR suppresses granulopoiesis-activating protein CCAAT enhancer binding protein alpha (CEBPA) in AML (23). Interestingly, the expression of the CEBPA is severely diminished, while the CEBPB expression and activated CALR are increased in arrested promyelocytes of patients with misfolding NE mutations (21). Perhaps the increase in CEBPA in response to CSF3 treatment explains how CSF3 treatment induces granulocytic differentiation of apoptosis-prone myeloid progenitors from SCN patients in whom the UPR is activated.

A recent study reported no increase in UPR-related genes in hematopoietic progenitor cells (HPCs) derived from SCN patients with misfolding NE mutations. However, the levels of reactive oxygen species (ROS) and promyelocytic nuclear bodies (PML-NBs) were increased in HPCs from these patients. This evidence leads to an alternative hypothesis for the pathogenic effects of misfolding NE mutations. Intracellular accumulation of misfolded NE increases the levels of ROS that induce the formation of PML-NBs. PML-NBs target misfolded NE for ubiquitination and further proteasomal degradation. On the other hand, PML-NBs bind to the ELANE promoter, increasing the production of misfolded NE and providing a feed-

forward mechanism. Moreover, PML-NBs decrease the response to CSF3 treatment in an SCN patient with ELANE mutation (**Figure 2D**) (24).

## 2.2. SCN2: Defect in growth factor independent 1 transcriptional repressor (GFI1) gene

Autosomal dominant negative mutations in GFI1, the gene encoding growth factor-independent transcriptional repressor 1 (GFI1), have been identified in rare cases of SCNs, known as SCN2 (**Tables 1 and 2**) (25). GFI1 is an essential transcription factor involved in various stages of hematopoiesis in the myeloid and lymphoid lineages. In the myeloid compartment, GFI1 is part of a regulatory network that determines the commitment of myeloid progenitors to the granulo-

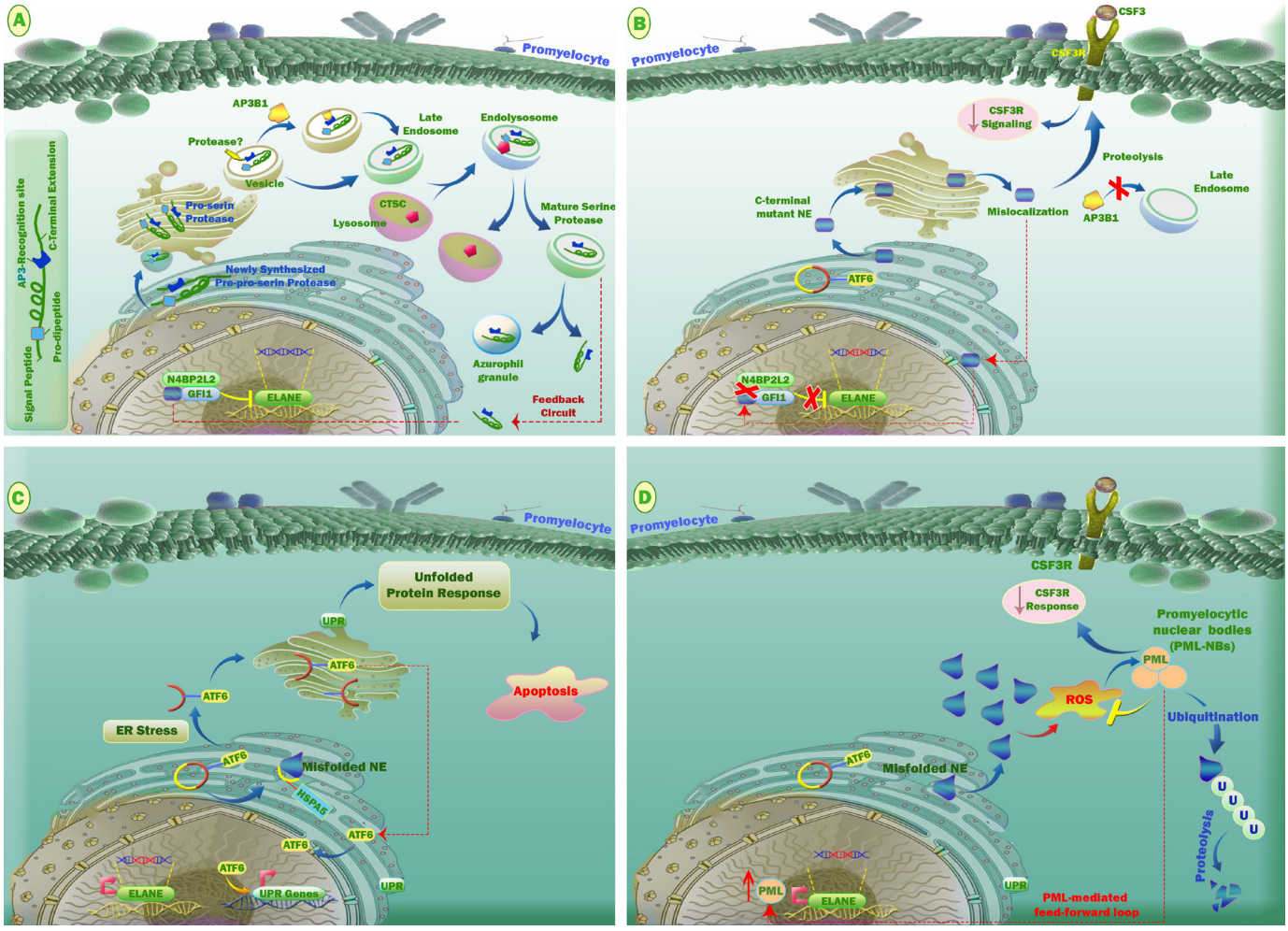



**Figure 2.** A schematic representation of dysregulated pathway involved in the pathogenesis of SCN1. A) Neutrophil elastase is trafficked from Golgi to the azurophil granules. B) Mistrafficking. ELANE mutations causing loss of the sorting signal or failure to remove the 20-residue C-terminal pro-peptide disrupts the interaction between NE and AP3B1, leading to mistrafficking and mislocalization of the mutant NE to the plasma membrane and the nucleus. C) Unfolded protein response. The misfolded NE accumulation in ER causes ER stress and activation of the UPR pathway. Due to excessive accumulation of misfolded NE, the UPR fails to degrade unfolded NE in promyelocytes and initiates ER-associated apoptosis. D) PML-mediated feed-forward loop. Intracellular accumulation of misfolded NE increases the levels of ROS that induce the formation of PML-NBs. In addition to ubiquitination and further proteasomal degradation of NE, PML-NBs bind to the ELANE promoter, increasing the production of misfolded NE and providing a feed-forward mechanism. Abbreviations: AP3B1: adaptor-related protein complex 3 subunit beta 1; ATF6: activating transcription factor 6; CTSC: cathepsin C; CSF3R: colony-stimulating factor 3 receptor; CSF3: colony-stimulating factor 3; ER: endoplasmic reticulum; ELANE: elastase, neutrophil expressed; GFI1: growth factor independent 1 transcriptional repressor; HSPA5: heat shock protein family A (Hsp70) member 5; NE: neutrophil elastase; N4BP2L2: NEDD4 binding protein 2 like 2; PML-NBs: promyelocytic nuclear bodies; ROS: reactive oxygen species; UPR: unfolded protein response.

cytic or monocytic lineage. GFI1 antagonizes the function of the SPI1 transcription factor. SPI1 enhances monocyte differentiation, while GFI1 promotes granulocyte differentiation. Therefore, loss of GFI1 function may shift the balance toward SPI1-mediated monocyte development, which may be an explanation for neutropenia in patients with SCN2 (26).

GFI1, a dual-function transcription factor, regulates myeloid cell maturation by transcriptional repression of target genes, such as ELANE, CSF1, SOCS3, miR-21, and miR-196b. While GFI1 actively promotes granulocytic differentiation by increasing RASGRP1 expression (27). It has been reported that the expression and activity of NE are increased in GFI1-deficient patients and mice. However, it is unclear whether increased wild-type NE, resulting from the loss of GFI1 function, contributes to neutropenia (25). Apart from the ELANE gene, the expression of the genes involved in myelopoiesis, such as CSF1 and RASGRP1, is dysregulated by the loss of GFI1 function (27, 28). Wild-type GFI1 promotes granulocytic differentiation by maintaining low levels of CSF1. While the SCN-associated GFI1N382S (c.1145A>G/p.Asn382Ser) mutation inhibits the normal function of GFI1 and leads to increased monocyte differentiation and decreased granulocyte differentiation (**Figure 3**) (28). A recent study has shown that GFI1 promotes CSF3-dependent neutrophil differentiation from HPCs by inducing the expression of RASGRP1, a critical regulator of CSF3/CSF3R–induced Ras activation. The RASGRP1 expression is reduced in GFI1-deficient mice (27). Therefore, GFI1 enhances the expression of genes that promotes granulocytic differentiation while it represses genes that favor monocytic differentiation. These findings explain the cause of monocytosis in patients with SCN2.

### 2.3. SCN3: Defect in HCLS1-associated protein X-1 (HAX1) gene

Autosomal recessive mutations in HAX1, the gene encoding HCLS1-associated protein X-1 (HAX1),

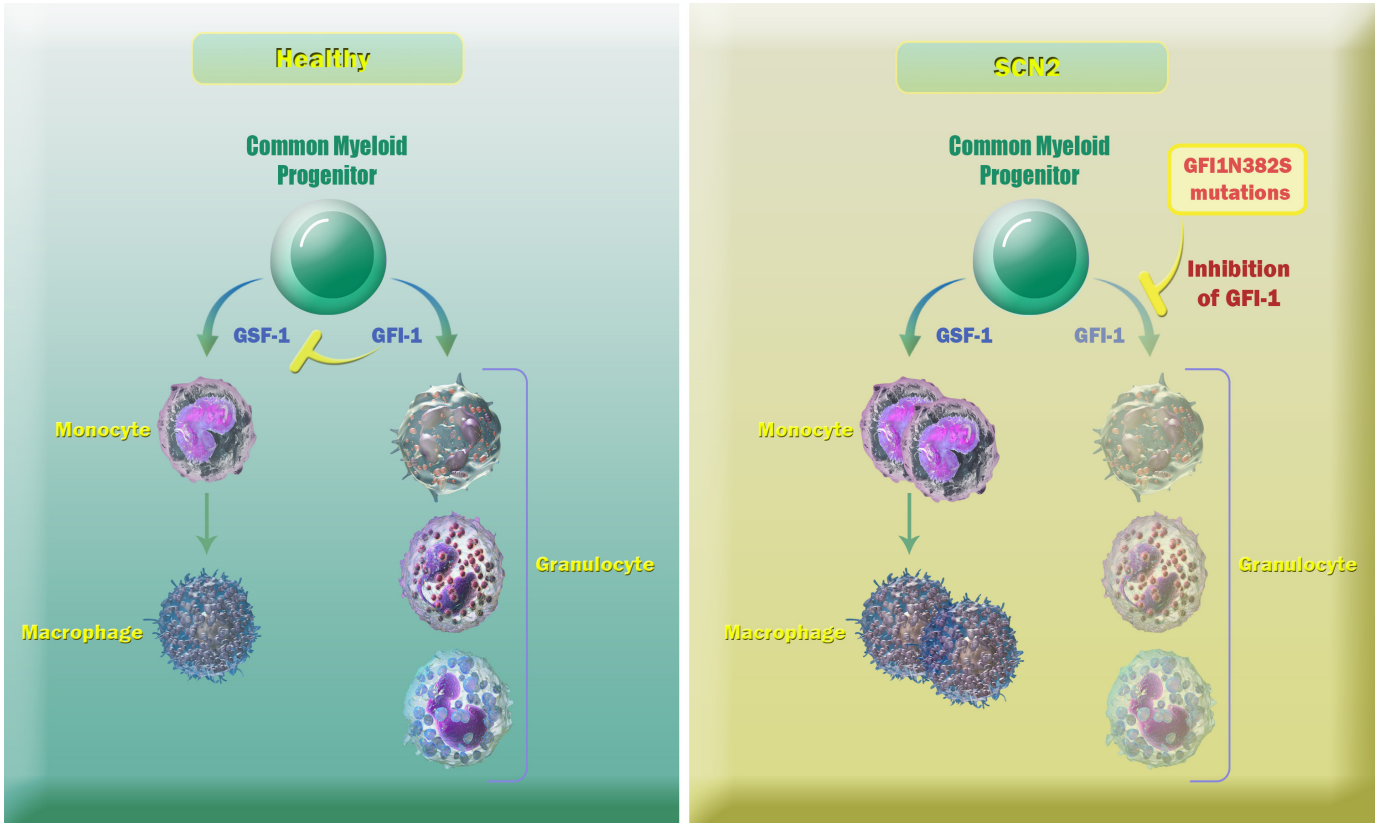



Figure 3. A schematic representation of dysregulated pathway involved in the pathogenesis of SCN2. In healthy individuals, wild-type GFI1 promotes granulocytic differentiation by maintaining low levels of CSF1. In SCN2 patients, GFI1N382S mutation inhibits the normal function of GFI1 and leads to increased monocyte differentiation and decreased granulocyte differentiation. Abbreviations: CMP: common myeloid progenitor; CSF1: colony-stimulating factor 1; GFI1: growth factor independent 1 transcriptional repressor.

were first described in the original affected family in Northern Sweden and later in other patients, known as infantile genetic agranulocytosis (**Table 1**). The disease was found by Rolf Kostmann in 1956, and later, the term 'Kostmann syndrome' was used for many years for patients with SCNs (29, 30). Of note, the homozygous germline mutation in HAX1 was identified about 50 years after the introduction of Kostmann disease (30). The onset of neurological symptoms during childhood may be associated with SCN3 (**Table 2**).

There are two alternatively spliced isoforms of HAX1 in humans, and mutations affecting these isoforms have different clinical presentations (31). Patients with HAX1 mutations affecting the splice variant I or the full-length transcript (p.Trp44X) develop SCN without neurological involvement (30). While patients with mutations affecting splice isoforms I and II (p.Gln190X and p.Arg86X) develop SCN with neurological abnormalities (32-34). The isoform II, a shortened exon 2 transcript, is markedly expressed in human brain tissue (33). Therefore, HAX1 isoforms may play a distinctive role in the neuronal system.

HAX1 is a ubiquitously expressed protein that acts as a binding partner for several proteins and is involved in various cellular processes and signaling pathways (35-39). In hematopoietic progenitors, upon activation of the CSF3 receptor, HCLS1, an essential adapter protein of CSF3R signaling, is phosphorylated by LYN and SYK. HCLS1 and cytoplasmic HAX1 bind to LEF1, transporting LEF1 into the nucleus. LEF1 activates target genes of the CSF3 signaling pathway (for example, LEF1, CEBPA, and HCLS1) and promotes granulocytic differentiation. In patients with SCN3, HAX1 mutations annul the activation of phosphorylated HCLS1 and its downstream signaling, which is essential for granulocytic differentiation. Indeed, HCLS1-deficient mice are neutropenic (**Figure 4**) (39).

Additionally, HAX1 is a mitochondrial protein that interacts with the mitochondrial proteases HTRA2 and PARL and is involved in the anti-apoptotic pathway (37). In neural cells, loss of HAX1 function may reduce HTRA2 activity, leading to the accumulation of sufficient amounts of activated BAX to induce cytochrome c release and apoptosis (37). HAX1 also maintains the mitochondrial inner membrane potential and rescues myeloid cells from apoptosis. The loss of HAX1 function reduces mitochondrial inner membrane potential, increasing the release of cytochrome c, as a result, accelerates apoptosis of myeloid cells (**Figure 4**) (30). These findings may explain why treatment with CSF3, a cytokine with known anti-apoptotic functions, mitigates the neutropenia phenotype in SCN3 patients.

### 2.4. SCN4: Defect in glucose-6-phosphatase catalytic subunit 3 (G6PC3) gene

The autosomal recessive mutations in G6PC3, the gene encoding the ER enzyme glucose-6-phosphatase catalytic subunit 3 (G6PC3), cause SCN with extra-hematopoietic features, known as SCN4 (40). SCN4 is associated with considerable clinical heterogeneity. Affected individuals have neutropenia, neutrophil dysfunction, thrombocytopenia, and other syndromic features (40-42). Furthermore, evolution to AML or MDS has been reported in patients with SCN4 (**Tables 1 and 2**)(41).

Glucose-6-phosphatase (G6PC) catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and phosphate when coupled with the SLC37A4 that transports G6P from the cytoplasm into the lumen of the ER. In humans, there are three G6PC enzymes (G6PC1–3). G6PC1 (liver, kidney, and small intestine) and G6PC2 (pancreatic islet cells) are exclusively expressed in specific tissues, while G6PC3 is expressed ubiquitously (43).

Recent studies have demonstrated the disturbance of intracellular glucose homeostasis, such as reduced glucose uptake, cytosolic G6P, lactate, and ATP, as well as the reduction of expression and activation of NADPH oxidase in the neutrophils of G6PC3-deficient murine and humans (44, 45). Moreover, the myeloid cells from G6PC3-deficient patients showed increased ER stress, UPR activation, and elevated apoptosis (44). Consistent with this, expression of ER stress-related proteins (HSPA5) and GSK3B activity increased in myeloid cells of patients with SCN4. GSK3B, a key enzyme regulating cellular differentiation and apoptosis, phosphorylates the anti-apoptotic molecule MCL1 and promotes the degradation of MCL1 (40). Therefore, these findings support the hypothesis that the underlying mechanism of increased apoptosis and neutrophil dysfunction in G6PC3 deficiency is the disruption of intracellular glucose homeostasis resulting from the loss of the endogenous glucose/G6P reservoir in neutrophils.

A recent study reported defects in chemotaxis and the killing activity of neutrophils in a patient with G6PC3 deficiency. The neutrophils of this patient showed a

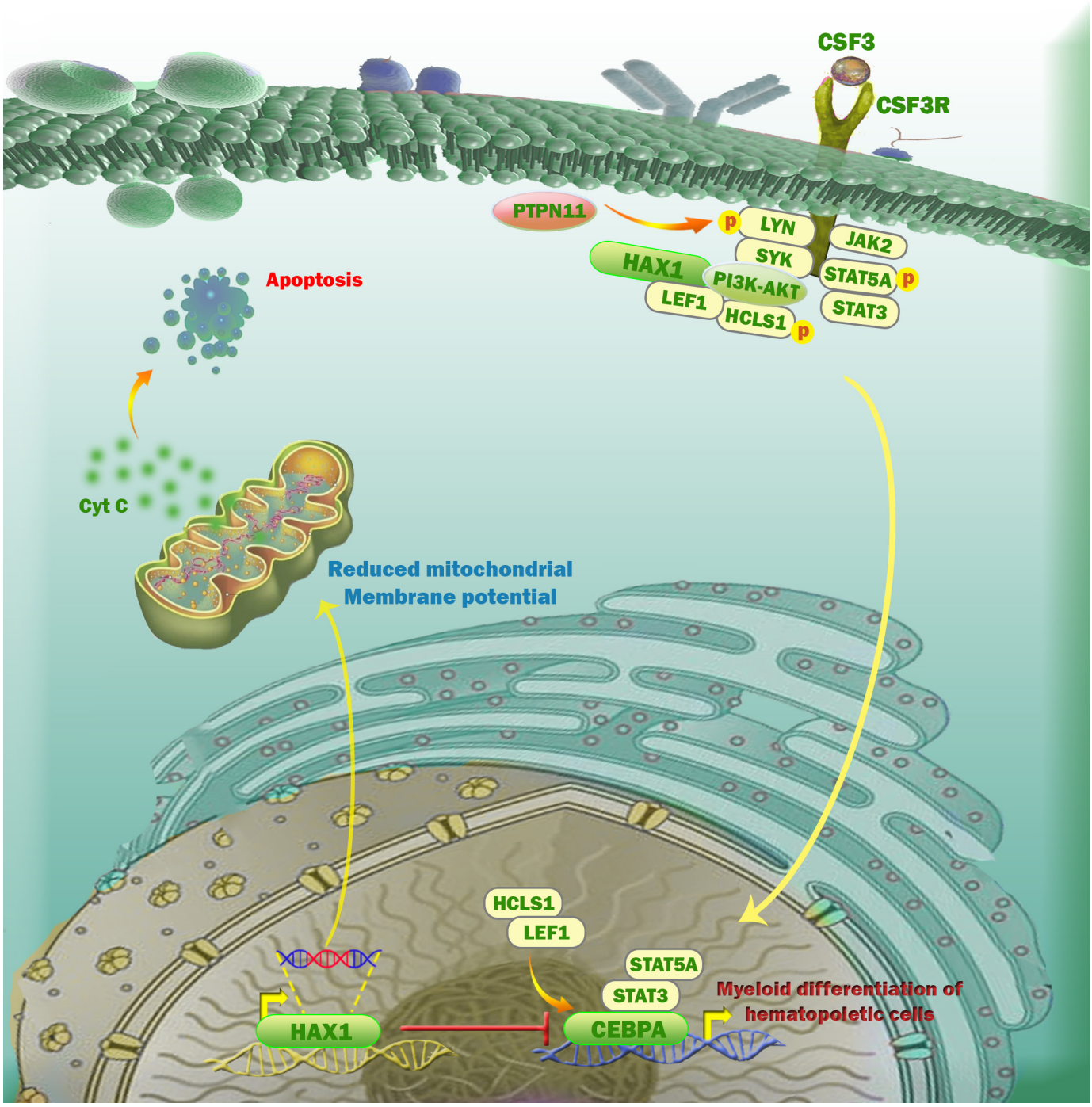



Figure 4. A schematic representation of dysregulated pathway involved in the pathogenesis of SCN3. Upon activation of the CSF3R, HAX1 activates phosphorylated HCLS1. HCLS1 and HAX1 bind to LEF1, transporting LEF1 into the nucleus. LEF1 activates target genes of the CSF3R signaling pathway and promotes granulocytic differentiation. In SCN3 patients, HAX1 mutations annul the activation of phosphorylated HCLS1 and its downstream signaling. As a result, it reduces the activation of CSF3R signaling. The loss of HAX1 function reduces mitochondrial inner membrane potential, increasing the release of cytochrome c, as a result, accelerates apoptosis of myeloid cells. Abbreviations: CEBPA: CCAAT enhancer binding protein alpha; CSF3R: colony-stimulating factor 3 receptor; Cyt C: cytochrome c; HCLS1: hematopoietic cell-specific Lyn substrate 1; JAK2: janus kinase 2; LEF1: lymphoid enhancer binding factor 1; LYN: LYN proto-oncogene, Src family tyrosine kinase; PI3K/AKT: phosphoinositide 3-kinase (PI3K)/Protein kinase B; PTPN11: protein tyrosine phosphatase non-receptor type 11; STAT: signal transducer and activator of transcription; SYK: spleen associated tyrosine kinase.

disruption in glycolysis, hexose monophosphate shunt, glutaminolysis, and redox pathways (46). Interestingly, the expression of CXCR4, a known neutrophil retention factor in BM, is increased in neutrophils of G6PC3-deficient murine and humans (47). Retention of these neutrophils in the BM, similar to WHIM syndrome (caused by gain-of-function mutations in CXCR4), may lead to neutropenia in G6PC3 deficiency and respond well to CSF3 therapy. Furthermore, CSF3 treatment appears to improve the function of mouse G6PC3-deficient neutrophils by modulating apoptosis and enhancing energy homeostasis (48). A schematic representation of dysregulated pathway involved in the pathogenesis of SCN4 is demonstrated in **Figure 5**.

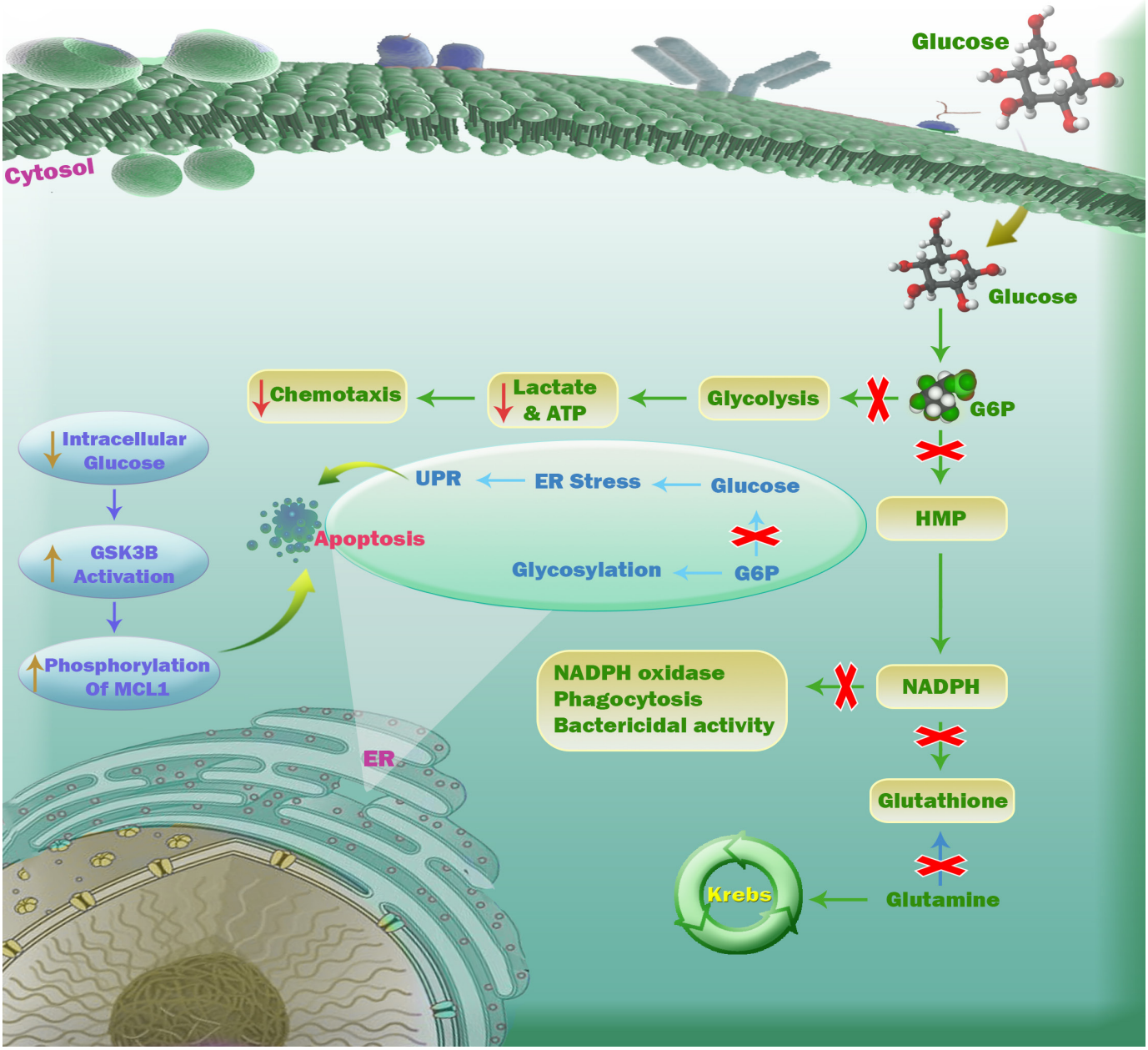



Figure 5. A schematic representation of dysregulated pathway involved in the pathogenesis of SCN4. G6PC3 deficiency triggers metabolic impairments in glycolysis, hexose monophosphate shunt (HMP), glutaminolysis, and redox pathways, increasing apoptosis and neutrophil dysfunction. Abbreviations: ATP: adenosine triphosphate; ER: endoplasmic reticulum; G6P: glucose 6-phosphate; GSK3B: glycogen synthase kinase 3 beta; MCL1: MCL1 apoptosis regulator, BCL2 family member; NADPH: nicotinamide adenine dinucleotide phosphate; UPR: unfolded protein response.

### 2.5. SCN5: Defect in vacuolar protein sorting 45 homolog (VPS45) gene

The autosomal recessive mutations in VPS45, the gene encoding the vacuolar protein sorting 45 homolog (VPS45), have been identified in rare cases of SCN with extra-hematopoietic features, known as SCN5. VPS45, a Sec1/Munc18 (SM) protein, is involved in endosomal trafficking. Interestingly, the lack of VPS45 in mice causes embryonic lethality (49). The patients with VPS45 deficiency manifest severe neutropenia, progressive BM fibrosis, hypergammaglobulinemia, renal extramedullary hematopoiesis, and other syndromic features. Of note, children with SCN5 are refractory to the treatment with CSF3, even in high doses (50, 51). Hematopoietic stem cell transplantation (HSCT) is a curative option for these children, although some may experience post-HSCT behavioral regression (**Tables 1 and 2**) (50).

In children with SCN5, the protein levels of VPS45 and its binding partners Rabenosyn, RAB effector (RBSN), and Syntaxin 16 (STX16) are decreased in fibroblasts and neutrophils (51). Depletion of integrin subunit beta 1 (ITGB1) on the plasma membrane impairs the migration of neutrophils and fibroblasts, possibly caused by impaired ITGB1 recycling in VPS45 deficiency (51, 52). VPS45 deficiency triggers increased apoptosis of neutrophils, BM myeloid cells, and fibroblasts (50, 51). Induction of wild-type VPS45 expression in patient cells corrected the migration defect and reduced apoptosis (51). In addition, the neutrophils of these children defect in killing activity and cannot produce superoxide ions (51). The lack of lysosomes in fibroblasts and reduced abundance of granules α in platelets of children with SCN5 suggest a defect in the endosomal-lysosomal pathway (50). Therefore, BM fibrosis may result from the failure of megakaryocytes to retain synthesized transforming growth factor beta (TGFB), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF).

The exact disease mechanism is entirely unclear, but the reduced level of the VPS45 protein causes a defect in the conversion of early endosomal (RAB5A-positive) to late endosomal (RAB7A-positive). As a result, it triggers the trapping of cargos in early endosomes, cargo mistrafficking, and impairs the intracellular organization of endolysosomal vesicles. Furthermore, the loss of VPS45 perturbs the efficient CSF3R recycling and delivery to late endosomes, but CSF3R-mediated signaling remains intact (49). However, how CSF3R mistrafficking affects resistance to CSF3 therapy in children with SCN5 requires further investigation. **Figure 6** provides a schematic representation of dysregulated pathway involved in the pathogenesis of SCN5.

### 2.6. SCN6: Defect in jagunal homolog 1 (JAGN1) gene

In 2013, Boztug et al. described for the first time the autosomal recessive mutations in JAGN1, the gene encoding the jagunal homolog 1 (JAGN1), in 14 patients with SCN, known as SCN6 (53). SCN6 is associated with considerable clinical heterogeneity (**Tables 1 and 2**) (54). In affected individuals, the BM examination revealed a maturation arrest of granulopoiesis at the level of promyelocytes. The JAGN1-deficient granulocytes showed ultrastructural defects, reduced granules, abnormal protein N-linked glycosylation, and elevated apoptosis. JAGN1 deficiency triggers a poor or no response to CSF3 treatment in SCN6 patients. Of note, a child with SCN6 progressed to AML (54).

JAGN1, an ER-resident protein, interacts with the COP1 complex (COPA and COPB2) and is involved in vesicular trafficking from the Golgi complex to the ER (early secretory pathway) (54). JAGN1 was initially identified in Drosophila and facilitated vesicular trafficking by ER reorganization during oogenesis (55). The loss of JAGN1 function impairs neutrophil migration in response to fungal infection in JAGN1-deficient mice. It appears that JAGN1 is involved in the proper glycosylation of proteins involved in cell adhesion and cytotoxicity of mice neutrophils (56). However, the underlying mechanism of increased apoptosis and neutropenia in JAGN1-deficient humans is not fully understood. In human JAGN1-deficient granulocytes, the expression of ER stress-related proteins HSPA5 is increased and may activate the UPR pathway and induce apoptosis in granulocyte precursors (54). Notably, HSPA5 is a multifunctional protein that also plays a role in regulating $Ca^{2+}$ homeostasis in ER.

A recent study has shown that JAGN1 interacts with HSPA5 and regulates $Ca^{2+}$ homeostasis in the ER. Mutant JAGN1 protein expression disrupts $Ca^{2+}$ homeostasis and increases $Ca^{2+}$ efflux from the ER to the cytosol in cells expressing mutant JAGN1 protein. An increase in intracellular $Ca^{2+}$ activates calpains, serine proteases sensitive to $Ca^{2+}$ concentration. In

the ER, the inactive calpains are heterodimers composed of a regulatory subunit and a proteolytic subunit and are associated with calpastatin, an endogenous calpain inhibitor. $Ca^{2+}$ stimulates the dissociation of the regulatory subunit and release of of calpastatin, leading to the activation of the calpain enzymes. Activation of calpains decreases the mitochondrial membrane potential, resulting in calcium- and calpain-dependent cell death in cells expressing mutant JAGN1 protein (57). Calpains cleave the

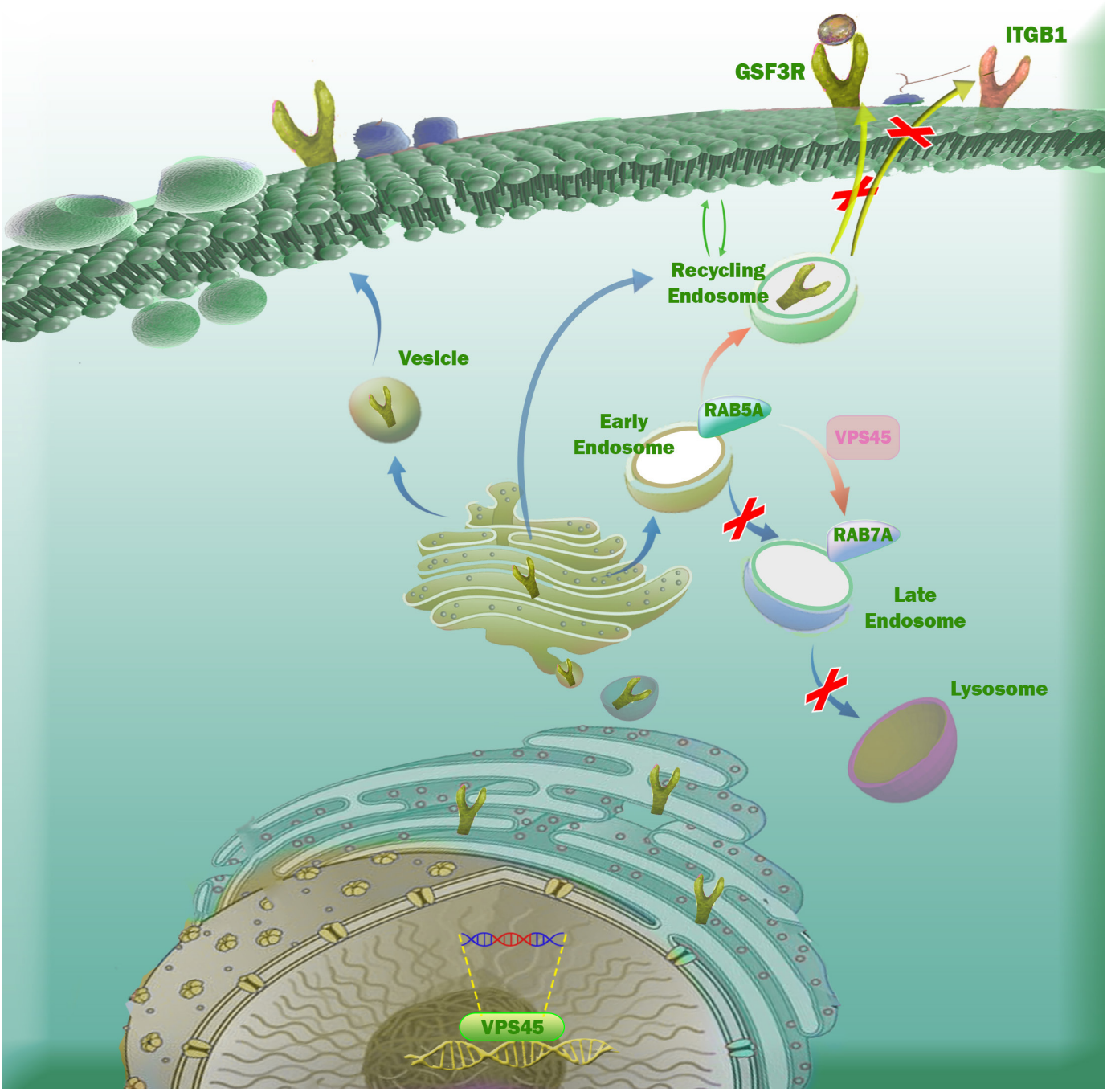



**Figure 6.** A schematic representation of dysregulated pathway involved in the pathogenesis of SCN5. The reduced level of the VPS45 protein impaired the conversion of early endosomal (RAB5A-positive) to late endosomal (RAB7A-positive) and recycling of ITGB1 and CSF3R. Abbreviations: CSF3R: colony-stimulating factor 3 receptor; ITGB1: integrin subunit beta 1; RAB5A: RAB5A, member RAS oncogene family; RAB7A: RAB7A, member RAS oncogene family; VPS45: vacuolar protein sorting 45 homolog.

apoptosis-inducing factor mitochondria associated 1 (AIFM1) and BAX. AIFM1 exits from mitochondrial intermembrane space through BAX-formed pores. AIFM1 translocates to the nucleus, inducing chromatin fragmentation that culminates in cell death. However, how JAGN1 mutations cause severe neutropenia and the poor response to CSF3 therapy in children with SCN6 requires further investigation. A schematic representation of dysregulated pathway involved in the pathogenesis of SCN6 is demonstrated in **Figure 7**.

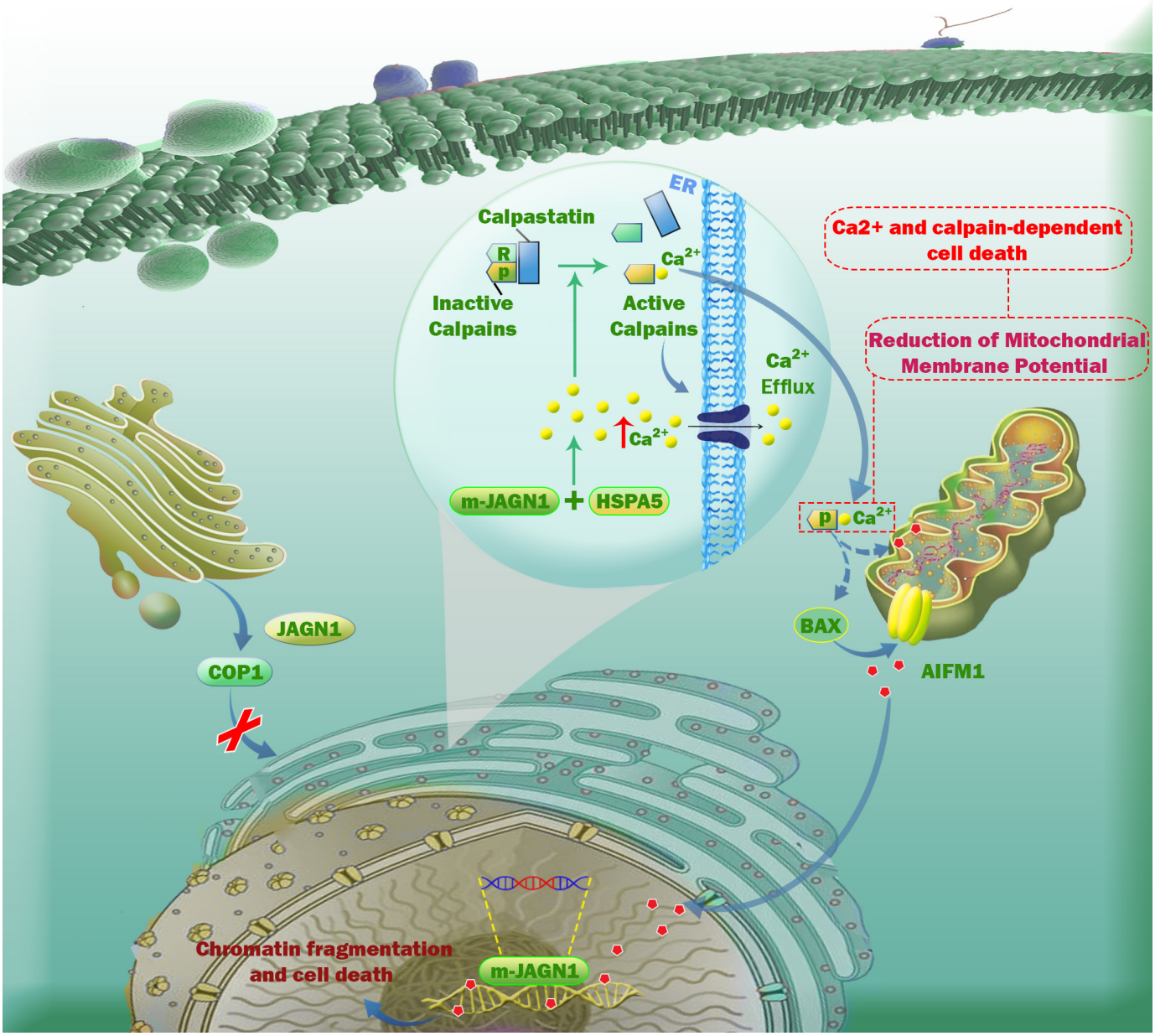



Figure 7. A schematic representation of dysregulated pathway involved in the pathogenesis of SCN6. The mutant-JAGN1 impairs the early secretory pathway and $Ca^{2+}$ homeostasis. The increased intracellular $Ca^{2+}$ stimulates the dissociation of the regulatory subunit and release of calpastatin, leading to the activation of the calpain enzymes. Activated calpains decrease the mitochondrial membrane potential, resulting in calcium- and calpain-dependent cell death in cells expressing mutant JAGN1 protein. Calpains may cleave the AIFM1 and BAX. AIFM1 exits from mitochondrial intermembrane space through BAX-formed pores. AIFM1 translocates to the nucleus, inducing chromatin fragmentation that culminates in cell death. Abbreviations: AIFM1: apoptosis inducing factor mitochondria associated 1; BAX: BCL2-associated X, apoptosis regulator; COP1: COP1 E3 ubiquitin ligase; HSPA5: heat shock protein family A (Hsp70) member 5; m-JAGN1: mutant-jagunal homolog 1; P: proteolytic subunit; R: regulatory subunit.

### 2.7. SCN7: Defect in colony-stimulating factor 3 receptor (CSF3R) gene

Germline mutations in the extracellular domain of CSF3R, the gene encoding the colony-stimulating factor 3 receptor (CSF3R), have been identified in rare cases of isolated severe neutropenia, known as SCN7 (**Tables 1 and 2**). The first report of a homozygous mutation in the cytokine receptor homologous (CHR) region in the extracellular domain of CSF3R was by Ward et al., who identified the p.Pro206His (P206H) mutation.

**Table 2.** Hematologic and non-hematologic abnormalities in severe congenital neutropenias (SCNs).

| Disease | Other hematologic abnormalities | Non-hematologic abnormalities |
|---|---|---|
| SCN1 | Monocytosis, eosinophilia, and evolution to AML or MDS | Osteopenia |
| SCN2 | Lymphopenia, increased numbers of immature myeloid cells in the PB, and evolution to AML or MDS | No |
| SCN3 | Evolution to AML or MDS | Neurologic phenotype (Developmental delay, epileptic seizures, and cognitive defects) in patients with mutations affecting both isoforms of HAX1 |
| SCN4 | Thrombocytopenia, neutrophil dysfunction, and evolution to AML or MDS | Cardiac defects, prominent superficial vasculature, urogenital abnormalities, intellectual disability, endocrine abnormalities, and skin hyper-elasticity |
| SCN5 | Anisocytosis and poikilocytosis, progressive anemia and thrombocytopenia, reduced abundance of granules α in platelets, neutrophil dysfunction, BM fibrosis, renal extramedullary hematopoiesis, hypergammaglobulinemia | Nephromegaly, splenomegaly, osteosclerosis, and neurological abnormalities such as delayed development, cortical blindness, hearing loss, and thin corpus callosum |
| SCN6 | Ultrastructural defects, reduced granules, abnormal protein N-linked glycosylation, and elevated apoptosis were reported in JAGN1-deficient granulocytes, impaired neutrophil migration, extramedullary hematopoiesis with thickening of skull bones, and hypogammaglobulinemia | Short stature, bone and teeth abnormalities, severe osteoporosis and repeated bone fractures (continuing post-HSCT), pyloric stenosis, febrile convulsion, focal epilepsy, learning disability, neurodevelopmental delay, failure to thrive, dysmorphic and triangular face, extrovert ears, pancreatic insufficiency, lipomatosis, coarctation of the aorta, hypothyroidism, hypospadias and left undescended testis, and gingival hypertrophy |
| SCN7 | No | No |
| SCN8 | BM examination revealed granulopoietic hypoplasia with a reduction of all myeloid stages, a maturation arrest at the level of promyelocytes, and a prominent dysgranulopoiesis; reduced migration capacity of neutrophils | Shwachman-Diamond (SDS)-like features, including exocrine pancreatic insufficiency, neurodevelopmental delay, and skeletal dysplasia (Non-hematologic abnormalities are fully presented in **Table 3**) |
| SCN9 | Evolution to AML | Developmental delays, epilepsy, and cataracts (Non-hematologic abnormalities are fully presented in **Table 4**) |
| XSCN | The normal numbers of neutrophils are present in saliva, reduced NK cells, lymphopenia, reduced CD4/CD8 ratio, macrothrombocytopenia, and reduced IgA (Other hematologic abnormalities are fully presented in **Table 5**) | No |

AML: acute myeloid leukemia; BM: bone marrow; HAX1: HCLS1 associated protein X-1; HSCT: hematopoietic stem cell transplantation; IgA: immunoglobulin A; JAGN1: jagunal homolog 1; MDS: myelodysplastic neoplasms; NK cell: natural killer cell; PB: peripheral Blood; SCN: severe congenital neutropenia; XSCN: X-linked SCN.

BM examination of the P206H patient shows a maturation arrest at the promyelocyte/myelocyte stage. The P206H mutation reduces the number of ligand binding sites and thus alters ligand-receptor complex formation and signal transduction, leading to resistance to CSF3 therapy (58). However, combination therapy with CSF3 and corticosteroids causes a sustained increase in neutrophil counts and the cessation of infections in this patient. The P206H patient was successfully treated with HSCT (59). In addition, the dominant-negative mutations in the extracellular domain identified that form truncated/wild-type CSF3R heterodimers independent of ligand binding, reducing the surface expression of the wild-type receptor. The affected individuals demonstrate BM maturation arrest at the promyelocyte/myelocyte stage and refractory to CSF3 therapy (60, 61).

However, subsequent studies reported homozygous and biallelic mutations in different regions of the extracellular domain, which caused a hypo/normocellular condition without any maturation arrest of the myeloid lineage in the BM (62-66). These studies suggest that CSF3/CSF3R signaling is essential for sufficient production but not terminal maturation of granulocytic lineage. The CSF3/CSF3R signaling also plays a role in the egress of neutrophils from BM. Therefore, the impaired CSF3/CSF3R signaling may cause the retention of neutrophils in the BM of some patients with SCN7. The patients with mutant CSF3R were unresponsive to CSF3 therapy, While CSF2 therapy was effective in some patients (63-65). CSF2 and HSCT are the most promising therapies for patients with SCN7. Hence, the evaluation of CSF3R mutation can consider for SCN patients unresponsive to CSF3 therapy. **Table 3** provides the molecular pathological mechanisms in SCN7.

### 2.8. SCN8: Defect in signal recognition particle 54 (SRP54) gene

Germline mutations in the central GTPase (G) domain of SRP54, the gene encoding the signal recognition particle 54 (SRP54), occur predominantly as a sporadic disease but have familial forms, known as SCN8. In affected individuals, BM examination revealed granulopoietic hypoplasia with a reduction of all myeloid stages, a maturation arrest at the level of promyelocytes, and a prominent dysgranulopoiesis (67-69). Increased apoptosis has also been reported in BM myeloid progenitor cells from patients with SRP54 mutations (68). SCN8 can manifest as isolated neutropenia or syndromic SCN with Shwachman-Diamond (SDS)-like features, including exocrine pancreatic insufficiency, neurodevelopmental delay, and skeletal dysplasia (**Tables 1 and 2**) (67-69). In addition to decreases in neutrophil numbers, the migration capacity of neutrophils reduces in some affected individuals (67). SRP54 deficiency triggers a poor or no response to CSF3 therapy in cases of SCN8, which were eventually successfully treated with HSCT (67, 68). Of note, two patients with SCN8 progressed to AML (70) and B-acute lymphoblastic leukemia (B-ALL) (**Table 4**) (71).

SRP54, a core component of the cytosolic ribonucleoprotein complex, consists of 3 functional domains, the N-terminal domain (N-domain), the central GTPase domain (G domain), and the M domain. The M domain binds to the signal sequence of newly synthesized proteins from the ribosome. The G domain is involved in GTP-dependent interaction with the SRP receptor subunit alpha (SRPRA) in ER membrane and forms the functional NG domain. The G domain contains G1 to G5 elements that have a role in nucleotide binding and hydrolysis (72).

The cytosolic ribonucleoprotein complex consists of 6 signal recognition particles (SRPs) (SRP9, SRP14, SRP19, SRP54, SRP68, and SRP72) and a single 7S RNA. SRPs recognize and bind to the N-terminal signal sequence of newly synthesized proteins from the ribosome. Subsequently, the ribonucleoprotein/ribosome complex translocates to the ER membrane. The G domain of SRP54 interacts with SRPRA in a GTP-regulated manner, resulting in the delivery of secretory and membrane proteins to ER for posttranslational modification.

The different mutations in the central G domain of SRP54 impair the GTPase activity or forming of the functional NG domain. A recent study demonstrated that SRP54 mutations destabilize the G domain and abolish the binding of SRP54 to the SRPRA, thereby negatively affecting protein secretion by the SRP pathway. Mutant SRP54 protein inhibits the function of wild-type SRP54 in a dominant-negative manner (73). Interestingly, the patients with SRP54 mutations interfering with the G1 region presented only a hematological phenotype. In contrast, the patients with SRP54 mutations located within the G4 or G5 region manifested severe neutropenia with SDS-like features (68).

Table 3. Molecular pathological mechanisms in severe congenital neutropenia 7 (SCN7).

| Mutations | Pathomechanism | Therapy | Ref |
|---|---|---|---|
| **Mutations with maturation arrest at the promyelocyte/myelocyte stage in BM** | | | |
| Homozygous mutation p.Pro206His in of the CRH domain region of the CSF3R extracellular domain | Reduced the number of ligand binding sites; Altered ligand-receptor complex formation and signal transduction; Diminished activation of CSF3R signaling | No CSF3 Response; Responded to combination CSF3/corticosteroids therapy; Treated with HSCT | (58) |
| A 182-bp deletion in the extracellular domain with dominant-negative phenotype | The out-of-frame deletion causes a premature termination codon and produces a truncated protein of 351 amino acids; Formed truncated/wild-type CSF3R heterodimers independent of ligand binding; Reduced surface expression of the wild-type receptor | No CSF3 Response; Treated with HSCT | (60) |
| A 191-bp deletion in the extracellular domain with dominant-negative phenotype | The out-of-frame deletion causes a premature termination codon and produces a truncated protein of 319 amino acids; Formed Δ319/wild-type CSF3R heterodimers independent of ligand binding; Sequestered intracellularly in Δ319/wild-type CSF3R heterodimeric receptor complexes | No CSF3 Response; Treated with HSCT | (61) |
| **Mutations without maturation arrest at the promyelocyte/myelocyte stage in BM** | | | |
| Homozygous missense mutation p.Arg308Cys identified in 3 affected children of a family; Compound heterozygous deletions p.Gly316fsTer322 and p.Gly415fsTer432 in 1 affected child | p.Arg308Cys resulted in impaired N-glycosylation and aberrant localization of CSF3R to the cell surface; Biallelic mutation causes frameshifts and premature stop codon; Abrogated downstream signaling; Full myeloid cell maturation in the BM but neutropenia in peripheral blood | No CSF3 Response | (62) |
| Compound heterozygous mutation c.998-2A>T and p.Trp547* | Biallelic CSF3R mutations cause a premature stop-codon or by shifting the codon frame and introducing spurious amino acids, resulting in the absence of CSF3R surface expression; Granulopoietic hypoplasia with reduction of all stages but no maturation arrest or increase in blasts | No CSF3 Response; Responded to CSF2 therapy | (63) |
| Homozygous c.610-611 del ins AG (p.Gln204Arg) mutation | Hypocellular BM without any maturation arrest of the myeloid lineage | No CSF3 Response; Responded to CSF2 therapy | (64) |
| Compound heterozygous mutation c.690delC (p.Met231Cysfs*32) and c.64+5G>A | BM examination showed normal cellularity in the proportion of cells in each granulocyte stage and cell morphology | No CSF3 Response; Responded to CSF2 therapy | (65) |
| Compound heterozygous mutation p.Arg190His (c.569G>A) and p.Leu89Pro (c.266T>C) | Hypocellular BM without any maturation arrest of the myeloid lineage | Responded to CSF3 therapy | (66) |

BM: bone marrow; CRH: cytokine receptor homologous; CSF2: colony-stimulating factor 2; CSF3: colony-stimulating factor 3; CSF3R: colony-stimulating factor 3 receptor; HSCT: hematopoietic stem cell transplantation.

Of note, SRP54 mainly expresses in granulocytic lineage, especially in the late stages of differentiation, from promyelocytes to neutrophils. The production of azurophilic granules containing a lot of proteases begins in the promyelocytes stage and needs the ribonucleoprotein complex to follow the secretory pathway. Therefore, the secretory pathway of azurophilic granules disrupts in promyelocytes of patients with mutant SRP54. As a result, these mislocalized proteins may cause ER stress and the activation of the UPR pathway, inducing apoptosis in granulocyte precursors similar to ELANE mutation. Consistent with this, expression of ER stress-related proteins, ATF4, DDIT3, and spliced XBP1 increased in myeloid cells from patients with SCN8. The ER stress causes increased phosphorylation of ULK1, an autophagy marker, and increased expression of BAX1 and NOXA1, indicating p53-dependent apoptosis in SRP54 mutant cells (68).

A recent study reported that unconventional splicing of XBP1 is disrupted in SRP54-mutant zebrafish and human cells. The impaired XBP1 splicing causes the SDS-like phenotype in zebrafish embryos with SRP54-deficiency. XBP1 is an essential transcription factor that regulates the UPR by the endoplasmic reticulum to nucleus signaling 1 (ERN1) pathway (79).

XBP1 is activated only when unspliced XBP1 mRNA (XBP1u) is cleaved by an endoribonuclease ERN1 located in the ER membrane during an unconventional splicing process. In conditions of ER stress, SRP54 recognizes and binds to the N-terminal signal sequence of newly synthesized XBP1 from the ribosome and transports the ribonucleoprotein/ribosome complex to the ER membrane. Then, XBP1u is spliced by ERN1, and spliced XBP1 mRNA (XBP1s) is translated to an active protein. The active transcription factor enters the nucleus and binds to the promoter regions of UPR-related genes (80). Disruption of SRP54-SRP receptor binding hampers the translocate of XBP1u mRNA close to the ER membrane resident endonuclease ERN1, resulting in XBP1u mRNA is not spliced by ERN1. The absence of the UPR mediator XBP1s leads to unresolved ER stress in patients with SRP54 mutations (79). Interestingly, ERN1/XBP1 activation is essential for the differentiation of neutrophils in a stage-specific manner; disruption of the ERN1/XBP1 pathway may reduce granulocyte differentiation (81).

Apart from SRP54, the germline variants of SRP19, SRP68, and SRPRA have been reported in patients with SCN (82, 83). Therefore, these findings support the hypothesis that the disruption in cotranslational targeting machinery may cause unresolved ER stress, increased P53-dependent apoptosis, and autophagy of granulocytic precursors and neutrophils. However, the exact mechanism of how the SRP54 mutations cause the SDS-like features is not fully understood. **Figure 8** provides a schematic representation of dysregulated pathway involved in the pathogenesis of SCN8.

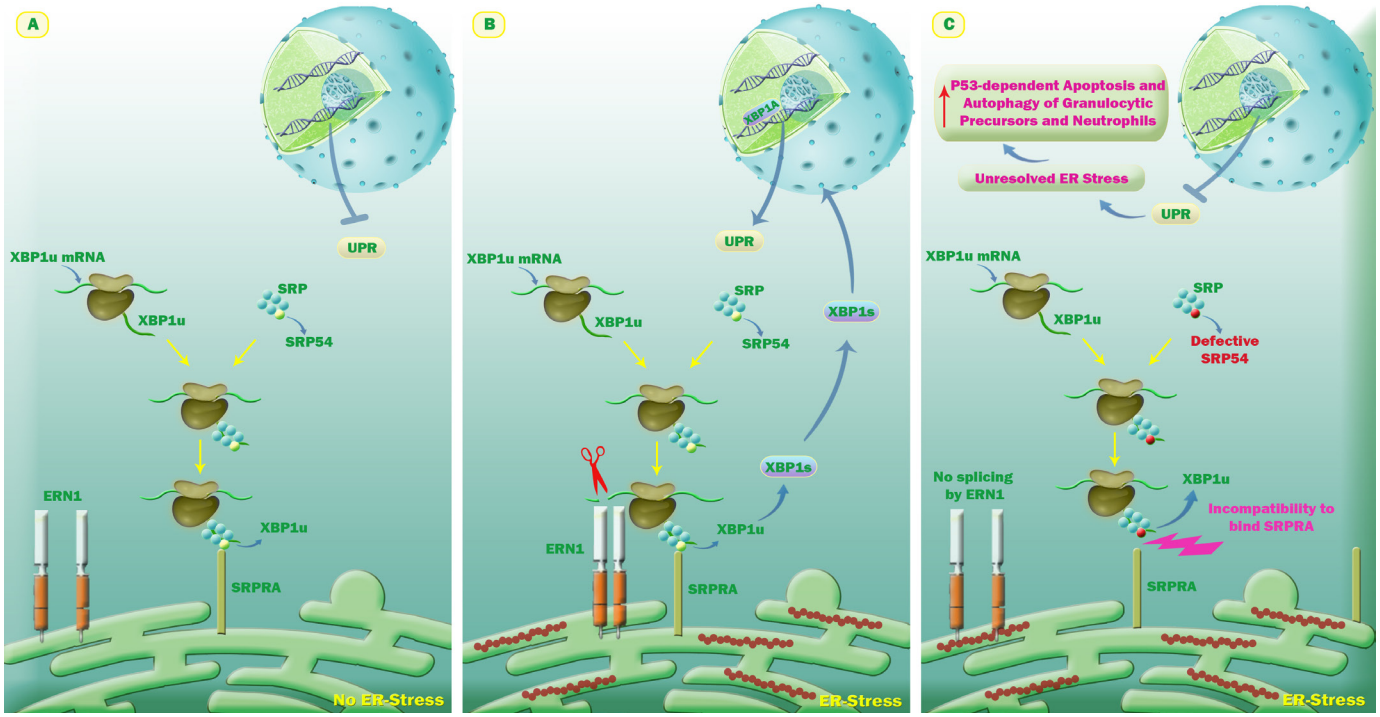


**Figure 8.** A schematic representation of dysregulated pathway involved in the pathogenesis of SCN8. A) SRP54 recognizes and binds to the N-terminal signal sequence of newly synthesized XBP1 from the ribosome and transports the ribonucleoprotein/ribosome complex to the ER membrane. In cells without or with moderate ER stress, ERN1 monomers cannot splice the unspliced XBP1 mRNA (XBP1u). B) Upon ER stress, ERN1 monomers dimerized in ER membrane and spliced XBP1u. The spliced XBP1 mRNA (XBP1s) is translated to an active protein. The active transcription factor enters the nucleus and binds to the promoter regions of UPR-related genes. C) SRP54 mutations destabilize the binding of the SRP54 to the SRPRA. This disruption hampers the translocate of XBP1u mRNA close to the ER membrane resident endonuclease ERN1, resulting in XBP1u mRNA is not spliced by ERN1. The absence of the UPR mediator XBP1s leads to unresolved ER stress that may increase P53-dependent apoptosis and autophagy of granulocytic precursors and neutrophils. Abbreviations: ER: endoplasmic reticulum; ERN1: endoplasmic reticulum to nucleus signaling 1; SRP54: signal recognition particle 54; SRPRA: SRP receptor subunit alpha; UPR: unfolded protein response; XBP1: X-box binding protein 1; XBP1s: spliced XBP1 mRNA; XBP1u: unspliced XBP1 mRNA.

### 2.9. SCN9: Defect in CLpB family mitochondrial disaggregase (CLPB) gene

Heterozygous missense mutations in the ATP-binding pocket of CLPB, the gene encoding ClpB family mitochondrial disaggregase (CLPB), have been identified in rare cases of sporadic and familial SCN, known as SCN9. BM examination revealed the maturation arrest at the promyelocyte/myelocyte stage and increased apoptosis in myeloid progenitor cells from patients with CLPB mutations. SCN9 can manifest as isolated neutropenia or syndromic SCN with developmental delays, epilepsy, and cataracts. Affected individuals typically respond well to CSF3 therapy. However, one patient with SCN9 progressed to AML, indicating the need for careful monitoring and follow-up of individuals with this condition (**Tables 1 and 2**) (84).

The exact disease mechanism is entirely unclear, but the different mutations in the ATP-binding pocket of CLPB impair mitochondrial respiration, resulting in reduced ATP production. The mutant CLPB protein inhibits the ATPase and disaggregase activity of wild-type CLPB in a dominant-negative manner. As a result, it disrupts granulocytic differentiation and increases apoptosis without affecting the cell cycle or causing ER stress (84).

Human CLPB located between the mitochondrial membrane has ATPase and refoldase activity. CLPB, the first mitochondrial protein refoldase associated with human disease, is involved in mitochondrial-mediated antiviral innate immunity, mitochondrial crista formation, and regulation of apoptosis (85). ATP-dependent protein disaggregase activity of CLPB is required to retain the solubility of essential mitochondrial proteins, such as HAX1 (86). The mutant CLPB protein may disrupt the function of HAX1, associated with a loss of mitochondrial integrity and increasing the release of cytochrome c, as a result, accelerates apoptosis of myeloid cells.

Of note, de novo monoallelic missense and biallelic mutations of CLPB are associated with a syndrome of 3-methylglutaconic aciduria, neurologic disease, cataracts, and variable neutropenia. Unlike the CLPB mutations in SCN9, these mutations mainly disrupt the refoldase activity of CLPB. A longer-lasting interaction between mutant CLPB protein and HAX1 was observed in affected individuals. Consequently, decreased HAX1 monomers and function may be responsible for neutropenia, seizures, and neurodevelopmental delay (87). The exact mechanism of how CLPB mutations cause the SCN9 or 3-Methylglutaconic aciduria syndrome requires further investigation. However, genetic tests for CLPB mutation can consider for patients presenting with congenital neutropenia. **Table 5** provides the CLPB mutations involved in patients with SCN9.

### 2.10. XSCN: Defect in WASP actin nucleation promoting factor (WAS) gene

The gain-of-function (GoF) mutations in the GTPase-binding domain (GBD) of WAS, the gene encoding the WASP actin nucleation promoting factor (WAS), have been identified in rare cases of X-linked SCN (XSCN), formerly known as X-linked neutropenia. XSCN can manifest as severe neutropenia with other hematologic abnormalities. The marked decrease in neutrophils and natural killer (NK) cells is a consistent finding in XSCN patients (**Tables 1 and 2**). Despite profound neutropenia, XSCN patients are typically not at high risk of infection and thus do not need permanent CSF3 support (88-93).

The WAS protein (WASp) is uniquely expressed in all hematopoietic cells, especially during neutrophil maturation. It is involved in signal transmission from the

Table 4. SRP54 mutations in severe congenital neutropenia 8 (SCN8).

| SRP54 variant | Inheritance | Pts (N) | Symptoms | | | | Therapy | ref |
|---|---|---|---|---|---|---|---|---|
| | | | Severe or recurrent infections | Pancreatic insufficiency | Psychomotor, developmental delay, or autistic behaviors | Other symptoms | | |
| c.677G>A (p.Gly226Glu) | De novo | 2 | Yes | Yes /no | Yes | ASD/VSD, dysmorphic features (Ref.67) | HSCT | (67), (68) |
| c.343A>G (p.Thr115Ala) | De novo | 1 | Yes | Yes | No | No | HSCT at 1 y, died at 16 m of age from VOD | (67) |
| c.349_351del (p.Thr117del) | De novo | 15 | Yes /no | Yes /no | Yes /no | DiGeorge, VSD/IAA, FTT, osteoporosis and type 2 diabetes | Responded to CSF3; HSCT; AML dx at 15 y in a patient | (67), (68), (70), (74), (75), (76) |
| c.349_351del (p.Thr117del) | Autosomal dominant | 3 | Yes /no | No | No | No | Responded to CSF3 (yes/no) | (68) |
| c.349_351del (p.Thr117del) | Unknown | 2 | Yes /no | No | Yes /no | IUGR, GH deficiency | HSCT | (68) |
| c.337G>C (p.Gly113Arg) | De novo | 1 | Yes | No | No | No | Responded to CSF3 | (68) |
| c.353G>A (p.Cys118Tyr) | Autosomal dominant | 3 | Yes /no | No | Yes /no | No | Responded to CSF3 (yes/no) | (68) |
| c.407G>A (p.Cys136Tyr) | De novo | 1 | Yes | No | Yes | No | Responded to CSF3; B-ALL dx at 10 y and in remission post-HSCT | (68) |
| c.407G>A (p.Cys136Tyr) | Unknown | 1 | Yes | No | Yes | Short stature, obesity | Responded to CSF3 | (68) |
| c.668C>A (p.Ala223Asp) | De novo | 1 | Yes | Yes | Yes | IUGR | Responded to CSF3 | (68) |
| c.821G>A (p.Gly274Asp) | De novo | 1 | Yes | Yes | Yes | IUGR, short stature, bone dysplasia | Responded to CSF3 | (68) |
| c.342_344del (p.Thr115del) | De novo | 1 | Yes | No | No | No | Responded to CSF3 | (69) |
| c.342_344del (p.Thr115del) | Autosomal dominant | 4 | Yes /no | No | No | No | Responded to CSF3 | (69) |
| c.674G>A (p.Gly225Asp) c.821G>A (p.Gly274Asp) | De novo | 1 | Yes | No | Yes | No | Responded to CSF3; HSCT | (77) |
| c.331G>T (p.Gly111Trp) | De novo | 1 | Yes | Yes | No | Short stature, congenital vertebral abnormalities | 1st HSCT at 1 y of age, 2nd HSCT 13 m later | (78) |

AML: acute myeloid leukemia; ASD: atrial septal defect; B-ALL: B-acute lymphoblastic leukemia; dx: diagnosis; FTT: failure to thrive; CSF3: colony-stimulating factor 3; GH: growth hormone; HSCT: hematopoietic stem cell transplantation; IAA: interrupted aortic arch; IUGR: intrauterine growth restriction; m: months; VOD: veno-occlusive disease; VSD: ventricular septal defect; y: years; Pts: patients.

Table 5. CLPB mutations in severe congenital neutropenia 9 (SCN9) (84).

| CLPB variant | Inheritance | Pts (N) | Symptoms | | | | | Therapy |
|---|---|---|---|---|---|---|---|---|
| | | | Infections | Neurological | Cataracts | 3-MGA | Other symptoms | |
| 1163C>A (p.Thr388Lys) | De novo | 1 | Yes | Negative | Yes | None | Azoospermia neurinoma (age 41) | Responded to CSF3 |
| 1488T>A (p.Asn496Lys) | Autosomal dominant | 1 | Yes | Epilepsy | No | N/A | Splenomegaly Evolution to AML | Responded to high dose CSF3 |
| 1669G>A (p.Glu557Lys) | Autosomal dominant | 1 | Yes | Developmental delays | No | N/A | None | Responded to CSF3 |
| 1681C>G (p.Arg561Gly) | Autosomal dominant | 1 | Yes | Negative | No | None | Splenomegaly | Responded to CSF3 |
| 1682G>A (p.Arg561Gln) | De novo | 4 | Yes /no | Negative/ Epilepsy | No | None | IUGR, GH deficiency, POF, Learning difficulties | Responded to CSF3 |
| 1858C>T (p.Arg620Cys) | Autosomal dominant | 2 | Yes /no | Negative | No | None | None | Responded to CSF3 |

AML: acute myeloid leukemia; CSF3: colony-stimulating factor 3; CLPB: ClpB family mitochondrial disaggregase; GH: growth hormone; IUGR: intrauterine growth restriction; N: number; N/A: not available; POF: premature ovarian failure; 3-MGA: 3-methylglutaconic aciduria; Pts: patients.

surface of blood cells to the actin cytoskeleton, a collection of actin filaments that form the cell's structural framework. This signaling causes the cell to move and attach to other cells (adhesion) and eventually migrate to tissues (94). Interestingly, B-cell affinity maturation in the germinal center depended on regulating actin dynamics for cell migration and cell-to-cell interaction (88).

The activity of the WASp strongly relies on its structure conformation. The WASp resides passively in the cytoplasm when the C-terminal verprolin-cofilin-acidic (VCA) domain interacts with the GBD, forming an autoinhibited conformation. The binding of CDC42 and phosphatidyl 4,5-bisphosphate (PIP2) to the WASp disrupts this interaction, releasing the VCA domain to bind to the Arp2/3 complex, which induces actin polymerization (94). In addition, phosphorylation of tyrosine Y291 in GBD is involved in forming open conformation even upon separation from CDC42 (95).

The importance of the WAS gene was determined by identifying Wiskott-Aldrich syndrome (WAS), X-linked thrombocytopenia (XLT), and XSCN. Mutations leading to reduced expression of WASp are frequently reported in XLT patients, sometimes in patients with only intermittent thrombocytopenia (96). XSCN patients with GoF mutations in WASp have clinical manifestations vastly different from WAS. The WAS patients with loss-of-function mutations in WASp suffer from microthrombocytopenia, severe immunodeficiency, and eczema (97).

So far, the six XSCN mutations in the GBD region of WAS gene have been described. These various mutations impair the auto-inhibitory function of GBD, resulting in enhanced WASp activation and actin polymerization (**Table 6**) (89, 93, 98). Mouse and human XSCN neutrophils show a hyperactive phenotype with increased actin polymerization and migration into tissues, which may compensate for the decreased myelopoiesis in XSCN patients. Consistent with this, XSCN patients are typically not at high risk of infection and can categorize as atypical SCN (98).

A recent study reported that the overactivation of WASp triggers aberrant B-cell division with decreased Ig class switching and premature differentiation of B cells into plasma cells in mouse models (88). Likewise, decreased IgA levels were reported in cases of patients with XSCN (88, 93). Although the WASp is expressed in all blood cells, the exact mechanism of how its

overactivation causes a marked decrease in neutrophils and NK cells in XSCN patients is not fully understood and requires further investigation. **Figure 9** provides a schematic representation of dysregulated pathway involved in the pathogenesis of XSCN.

## 3. Leukemic progression

MDS or leukemic transformation has been reported in SCN patients, particularly those treated with high-dose CSF3. The US FDA approved CSF3 for the treatment of congenital neutropenias in 1993, and the life-long administration of CSF3 alleviates the numbers and function of neutrophils in most types of SCN (99). However, prolonged exposure to high-dose CSF3 might trigger the acquired (somatic) mutations in the cytoplasmic domain of CSF3R that generate truncated CSF3R. The truncated CSF3R becomes highly sensitive to the CSF3 treatment, inducing defective CSF3R signal transduction and clonal proliferation. Subsequently, the additional mutations in RUNX1 are acquired during the leukemic transformation of SCNs and occur predominantly in clones that already harbor somatic CSF3R mutations (100). In addition, the acquisition of mutations in SUZ12, ASXL1, EP300, NPM1, and FIZ1 internal tandem duplication, as well as chromosomal aberrations, such as monosomy 7 and trisomy 21, have also been found in SCN patients who have transformed into MDS or leukemia (100, 101). In CSF3R/RUNX1 mutant mice model, the acquisition of CXXC4 mutation triggers increased CXXC4, decreased TET2 protein, enhanced inflammatory signaling, and leukemic transformation (102).

It's worth noting that specific mutations in the ELANE gene, such as p.Cys151Tyr, p.Gly214Arg, and p.Gly815Arg, have been associated with a poor response to CSF3 treatment and an increased risk of developing AML or MDS (11, 12). These mutations can be used as prognostic factors to identify patients at high risk of developing leukemia. Moreover, screening for acquired mutations, such as CSF3R, RUNX1, ASXL1, SUZ12, EP300, NPM1, and FIZ1, can help identify high-risk patients who should be transplanted to minimize the risk of MDS or AML occurrence.

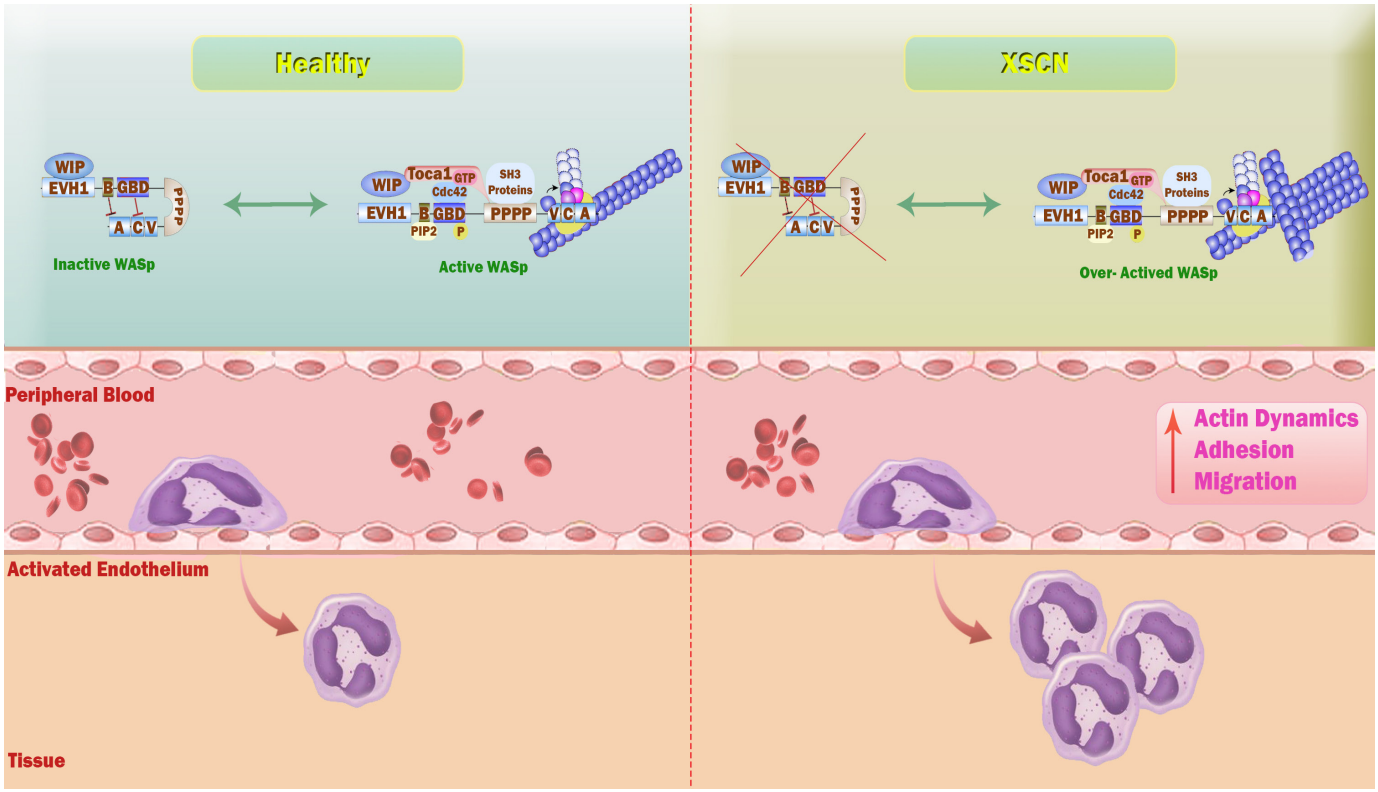



Figure 9. A schematic representation of dysregulated pathway involved in the pathogenesis of X-linked SCN. The WAS mutations enhance WASp activation and actin polymerization, leading to the increased adhesion and migration of neutrophils into tissues. As a result, it may compensate for the decreased myelopoiesis in XSCN patients. Abbreviations: WAS: WASP actin nucleation promoting factor; WASp: WAS protein.

Table 6. WAS germline mutations in X-linked severe congenital neutropenia (XSCN).

| Mutations | BM findings | Other findings | Ref |
|---|---|---|---|
| c.802C>T (p.Arg268Trp) | Not determined | Neutropenia, reduced NK cells | (88) |
| c.809T>C (p.Lue270Pro) | The reduction of granulocyte lineage and the maturation arrest at the promyelocyte/myelocyte stage | Neutropenia, but their neutrophils had a hyperactive phenotype and were present in normal numbers in saliva, monocytopenia, reduced NK cells; reduced B lymphocytes, reduced CD4/CD8 ratio | (89), (98) |
| c.812T>C (p.Phe271Ser) | The reduction of granulocyte lineage, BM blast cells: 3% | Neutropenia, monocytopenia, reduced B lymphocytes, reduced NK cells, reduced IgA | (88) |
| c.814T>C (p.Ser272Pro) | The maturation arrest at the promyelocyte stage | Neutropenia, reduced NK cells, impaired phagocytosis | (90) |
| c.869T>C (p.Ile290Thr) | BM blast cells: 5% | Neutropenia, but normal numbers of neutrophil is present in saliva, reduced NK cells, lymphopenia, reduced CD4/CD8 ratio, macrothrombocytopenia | (91), (92) |
| c.881T>C (p.Ile294Thr) | The reduction of granulocyte lineage and a maturation delay in the promyelocyte/ metamyelocyte stage, BM blast cells: 4.5-9%, Trilineage dysplasia; Beel et al. reported no dysplasia in patients with the p.Ile294Thr mutation (93). | Neutropenia, but normal numbers of neutrophil is present in saliva, monocytopenia, reduced NK cells, low or low-normal numbers of lymphocytes, reduced IgA | (88), (90), (93) |

BM: bone marrow; IgA: immunoglobulin A; NK cell: natural killer cell; WAS: WASP actin nucleation promoting factor; XSCN: X-linked severe congenital neutropenia.

## 4. Conclusion and Future Perspectives

Improvement in sequencing technologies led to identifying molecular abnormalities in 10 genes responsible for SCNs. However, about one-third of patients with a clinical history suggestive of severe neutropenia have no identifiable genetic cause. The dysregulated molecular pathways that underlie a maturation arrest of granulopoiesis at the level of promyelocyte differ according to the various mutations that cause SCNs. However, two critical organelles in regulating the survival and death of myeloid progenitor cells, mitochondria, and ER, play a fundamental role in the pathogenesis of most SCNs. It is interesting to understand that mouse models of SCNs, especially ELANE, HAX1, or JAGN1, did not necessarily recapitulate the human disease. Therefore, the pathophysiological mechanisms of SCNs are still the subject of extensive investigation and are not fully known. Induced pluripotent stem cells (iPSCs) produced from somatic cells of patients with ELANE or HAX1 mutations may be used as an experimental model to explore the underlying pathophysiology of SCNs (103-105). Lentivirus-based or CRISPR–Cas9-based correction of mutated ELANE or HAX1 in iPSCs successfully restores defective granulopoiesis in vitro. Therefore, correcting the underlying gene mutation ex vivo with CRISPR-Cas9 technology followed by autologous HSCT is a promising therapeutic option in the future.

## Conflicts of interest

The authors declare that they have no conflict of interest.

## Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

## Acknowledgment

The authors would like to express their gratitude to Shahid Beheshti University of Medical Sciences (Tehran, Iran) for supporting this study.

## References


1. Welte K, Zeidler C, Dale DC. Severe congenital neutropenia. Seminars in hematology. 2006;43(3):189-95.
2. Rosenberg PS, Alter BP, Bolyard AA, Bonilla MA, Boxer LA, Cham B, et al. The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood. 2006;107(12):4628-35.
3. Rosenberg PS, Zeidler C, Bolyard AA, Alter BP, Bonilla MA, Boxer LA, et al. Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. British journal of haematology. 2010;150(2):196-9.
4. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720-48.
5. Tangye SG, Al-Herz W, Bousfiha A, Cunningham-Rundles C, Franco JL, Holland SM, et al. Human Inborn Errors of Immunity: 2022 Update on the Classification from the International Union of Immunological Societies Expert Committee. Journal of clinical immunology. 2022;42(7):1473-507.
6. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K. Severe congenital neutropenias. Nature Reviews Disease Primers. 2017;3(1):17032.
7. Germeshausen M, Zeidler C, Stuhrmann M, Lanciotti M, Ballmaier M, Welte K. Digenic mutations in severe congenital neutropenia. Haematologica. 2010;95(7):1207-10.
8. Germeshausen M, Deerberg S, Peter Y, Reimer C, Kratz CP, Ballmaier M. The spectrum of ELANE mutations and their implications in severe congenital and cyclic neutropenia. Human mutation. 2013;34(6):905-14.
9. Horwitz MS, Duan Z, Korkmaz B, Lee HH, Mealiffe ME, Salipante SJ. Neutrophil elastase in cyclic and severe congenital neutropenia. Blood. 2007;109(5):1817-24.
10. Korkmaz B, Horwitz MS, Jenne DE, Gauthier F. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacological reviews. 2010;62(4):726-59.
11. Makaryan V, Zeidler C, Bolyard AA, Skokowa J, Rodger E, Kelley ML, et al. The diversity of mutations and clinical outcomes for ELANE-associated neutropenia. Current opinion in hematology. 2015;22(1):3-11.
12. Horwitz MS, Corey SJ, Grimes HL, Tidwell T. ELANE mutations in cyclic and severe congenital neutropenia: genetics and pathophysiology. Hematology/oncology clinics of North America. 2013;27(1):19-41, vii.
13. Horwitz MS, Laurino MY, Keel SB. Normal peripheral blood neutrophil numbers accompanying ELANE whole gene deletion mutation. Blood advances. 2019;3(16):2470-3.
14. Tidwell T, Wechsler J, Nayak RC, Trump L, Salipante SJ, Cheng JC, et al. Neutropenia-associated ELANE mutations disrupting translation initiation produce novel neutrophil elastase isoforms. Blood. 2014;123(4):562-9.
15. Garg B, Mehta HM, Wang B, Kamel R, Horwitz MS, Corey SJ. Inducible expression of a disease-associated ELANE mutation impairs granulocytic differentiation, without eliciting an unfolded protein response. The Journal of biological chemistry. 2020;295(21):7492-500.
16. Benson KF, Li FQ, Person RE, Albani D, Duan Z, Wechsler J, et al. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. Nature genetics. 2003;35(1):90-6.
17. Salipante SJ, Rojas ME, Korkmaz B, Duan Z, Wechsler J, Benson KF, et al. Contributions to neutropenia from PFAAP5 (N4BP2L2), a novel protein mediating transcriptional repressor cooperation between Gfi1 and neutrophil elastase. Molecular and cellular biology. 2009;29(16):4394-405.
18. Massullo P, Druhan LJ, Bunnell BA, Hunter MG, Robinson JM, Marsh CB, et al. Aberrant subcellular targeting of the G185R neutrophil elastase mutant associated with severe congenital neutropenia induces premature apoptosis of differentiating promyelocytes. Blood. 2005;105(9):3397-404.
19. Hunter MG, Druhan LJ, Massullo PR, Avalos BR. Proteolytic cleavage of granulocyte colony-stimulating factor and its receptor by neutrophil elastase induces growth inhibition and decreased cell surface expression of the granulocyte colony-stimulating factor receptor. American journal of hematology. 2003;74(3):149-55.
20. Köllner I, Sodeik B, Schreek S, Heyn H, von Neuhoff N, Germeshausen M, et al. Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. Blood. 2006;108(2):493-500.
21. Nustede R, Klimiankou M, Klimenkova O, Kuznetsova I, Zeidler C, Welte K, et al. ELANE mutant-specific activation of different UPR pathways in congenital neutropenia. British journal of haematology. 2016;172(2):219-27.
22. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nature Reviews Molecular Cell Biology. 2012;13(2):89-102.
23. Schardt JA, Eyholzer M, Timchenko NA, Mueller BU, Pabst T. Unfolded protein response suppresses CEBPA by induction of calreticulin in acute myeloid leukaemia. Journal of cellular and molecular medicine. 2010;14(6b):1509-19.
24. Olofsen PA, Bosch DA, Roovers O, van Strien PMH, de Looper HWJ, Hoogenboezem RM, et al. PML-controlled responses in severe congenital neutropenia with ELANE-misfolding mutations. Blood advances. 2021;5(3):775-86.
25. Person RE, Li FQ, Duan Z, Benson KF, Wechsler J, Papadaki HA, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. Nature genetics. 2003;34(3):308-12.
26. van der Meer LT, Jansen JH, van der Reijden BA. Gfi1 and Gfi1b: key regulators of hematopoiesis. Leukemia. 2010;24(11):1834-43.
27. de la Luz Sierra M, Sakakibara S, Gasperini P, Salvucci O, Jiang K, McCormick PJ, et al. The transcription factor Gfi1 regulates G-CSF signaling and neutrophil development through the Ras activator RasGRP1. Blood. 2010;115(19):3970-9.
28. Halene S, Krause D. Gfi1: Dual Functions as Oncogene and Inducer of Differentiation. The Hematologist. 2009;6(1).
29. Kostmann R. Infantile genetic agranulocytosis (agranulocytosis infantilis hereditaria) a new recessive lethal disease in man. Acta paediatrica. 1956;45(3):309-10.
30. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nature genetics. 2007;39(1):86-92.
31. Fadeel B, Grzybowska E. HAX-1: A multifunctional protein with emerging roles in human disease. Biochimica et Biophysica Acta (BBA) - General Subjects. 2009;1790(10):1139-48.
32. Germeshausen M, Grudzien M, Zeidler C, Abdollahpour H, Yetgin S, Rezaei N, et al. Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. Blood. 2008;111(10):4954-7.

33. Carlsson G, Van't Hooft I, Melin M, Entesarian M, Laurencikas E, Nennesmo I, et al. Central nervous system involvement in severe congenital neutropenia: neurological and neuropsychological abnormalities associated with specific HAX1 mutations. Journal of internal medicine. 2008;264(4):388-400.
34. Matsubara K, Imai K, Okada S, Miki M, Ishikawa N, Tsumura M, et al. Severe developmental delay and epilepsy in a Japanese patient with severe congenital neutropenia due to HAX1 deficiency. Haematologica. 2007;92(12):e123-e5.
35. Suzuki Y, Demoliere C, Kitamura D, Takeshita H, Deuschle U, Watanabe T. HAX-1, a novel intracellular protein, localized on mitochondria, directly associates with HS1, a substrate of Src family tyrosine kinases. Journal of immunology (Baltimore, Md : 1950). 1997;158(6):2736-44.
36. Yap SV, Vafiadaki E, Strong J, Kontrogianni-Konstantopoulos A. HAX-1: a multifaceted antiapoptotic protein localizing in the mitochondria and the sarcoplasmic reticulum of striated muscle cells. Journal of molecular and cellular cardiology. 2010;48(6):1266-79.
37. Chao JR, Parganas E, Boyd K, Hong CY, Opferman JT, Ihle JN. Hax1-mediated processing of HtrA2 by Parl allows survival of lymphocytes and neurons. Nature. 2008;452(7183):98-102.
38. Gallagher AR, Cedzich A, Gretz N, Somlo S, Witzgall R. The polycystic kidney disease protein PKD2 interacts with Hax-1, a protein associated with the actin cytoskeleton. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(8):4017-22.
39. Skokowa J, Klimiankou M, Klimenkova O, Lan D, Gupta K, Hussein K, et al. Interactions among HCLS1, HAX1 and LEF-1 proteins are essential for G-CSF-triggered granulopoiesis. Nature medicine. 2012;18(10):1550-9.
40. Boztug K, Appaswamy G, Ashikov A, Schäffer AA, Salzer U, Diestelhorst J, et al. A syndrome with congenital neutropenia and mutations in G6PC3. The New England journal of medicine. 2009;360(1):32-43.
41. Desplantes C, Fremond ML, Beaupain B, Harousseau JL, Buzyn A, Pellier I, et al. Clinical spectrum and long-term follow-up of 14 cases with G6PC3 mutations from the French severe congenital neutropenia registry. Orphanet Journal of Rare Diseases. 2014;9(1):183.
42. Al Sharie AH, Al Zu'bi YO, Abu Mousa BM, Al Khatib S, Al-Sheyyab M, Altamimi E. Severe congenital neutropenia type 4 with early-onset inflammatory bowel disease attributed to a G6PC3 variant (not previously associated with disease): A case report and a literature review. Human Gene. 2023;36:201171.
43. Banka S, Newman WG. A clinical and molecular review of ubiquitous glucose-6-phosphatase deficiency caused by G6PC3 mutations. Orphanet J Rare Dis. 2013;8:84.
44. Jun HS, Lee YM, Cheung YY, McDermott DH, Murphy PM, De Ravin SS, et al. Lack of glucose recycling between endoplasmic reticulum and cytoplasm underlies cellular dysfunction in glucose-6-phosphatase-beta-deficient neutrophils in a congenital neutropenia syndrome. Blood. 2010;116(15):2783-92.
45. Hayee B, Antonopoulos A, Murphy EJ, Rahman FZ, Sewell G, Smith BN, et al. G6PC3 mutations are associated with a major defect of glycosylation: a novel mechanism for neutrophil dysfunction. Glycobiology. 2011;21(7):914-24.
46. McKinney C, Ellison M, Briones NJ, Baroffio A, Murphy J, Tran AD, et al. Metabolic abnormalities in G6PC3-deficient human neutrophils result in severe functional defects. Blood advances. 2020;4(23):5888-901.
47. McDermott DH, De Ravin SS, Jun HS, Liu Q, Priel DA, Noel P, et al. Severe congenital neutropenia resulting from G6PC3 deficiency with increased neutrophil CXCR4 expression and myelokathexis. Blood. 2010;116(15):2793-802.
48. Jun HS, Lee YM, Song KD, Mansfield BC, Chou JY. G-CSF improves murine G6PC3-deficient neutrophil function by modulating apoptosis and energy homeostasis. Blood. 2011;117(14):3881-92.
49. Frey L, Ziętara N, Łyszkiewicz M, Marquardt B, Mizoguchi Y, Linder MI, et al. Mammalian VPS45 orchestrates trafficking through the endosomal system. Blood. 2021;137(14):1932-44.
50. Stepensky P, Saada A, Cowan M, Tabib A, Fischer U, Berkun Y, et al. The Thr224Asn mutation in the VPS45 gene is associated with the congenital neutropenia and primary myelofibrosis of infancy. Blood. 2013;121(25):5078-87.
51. Vilboux T, Lev A, Malicdan MC, Simon AJ, Järvinen P, Racek T, et al. A congenital neutrophil defect syndrome associated with mutations in VPS45. The New England journal of medicine. 2013;369(1):54-65.
52. Rahajeng J, Caplan S, Naslavsky N. Common and distinct roles for the binding partners Rabenosyn-5 and Vps45 in the regulation of endocytic trafficking in mammalian cells. Experimental cell research. 2010;316(5):859-74.
53. Boztug K, Järvinen PM, Salzer E, Racek T, Mönch S, Garncarz W, et al. Deficiency Of JAGN1 Causes Severe Congenital Neutropenia Associated With Defective Secretory Pathway and Aberrant Myeloid Cell Homeostasis. Blood. 2013;122(21):439.
54. Boztug K, Järvinen PM, Salzer E, Racek T, Mönch S, Garncarz W, et al. JAGN1 deficiency causes aberrant myeloid cell homeostasis and congenital neutropenia. Nature genetics. 2014;46(9):1021-7.
55. Lee S, Cooley L. Jagunal is required for reorganizing the endoplasmic reticulum during Drosophila oogenesis. The Journal of cell biology. 2007;176(7):941-52.
56. Wirnsberger G, Zwolanek F, Stadlmann J, Tortola L, Liu SW, Perlot T, et al. Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nature genetics. 2014;46(9):1028-33.
57. Khandagale A, Holmlund T, Entesarian M, Nilsson D, Kalwak K, Klaudel-Dreszler M, et al. Severe congenital neutropenia-associated JAGN1 mutations unleash a calpain-dependent cell death programme in myeloid cells. British journal of haematology. 2021;192(1):200-11.
58. Ward AC, van Aesch YM, Gits J, Schelen AM, de Koning JP, van Leeuwen D, et al. Novel point mutation in the extracellular domain of the granulocyte colony-stimulating factor (G-CSF) receptor in a case of severe congenital neutropenia hyporesponsive to G-CSF treatment. The Journal of experimental medicine. 1999;190(4):497-507.
59. Dror Y, Ward AC, Touw IP, Freedman MH. Combined corticosteroid/granulocyte colony-stimulating factor (G-CSF) therapy in the treatment of severe congenital neutropenia unresponsive to G-CSF: Activated glucocorticoid receptors synergize with G-CSF signals. Experimental Hematology. 2000;28(12):1381-9.
60. Sinha S, Zhu QS, Romero G, Corey SJ. Deletional mutation of the external domain of the human granulocyte colony-stimulating factor receptor in a patient with severe chronic neutropenia refractory to granulocyte colony-stimulating factor. J Pediatr Hematol Oncol. 2003;25(10):791-6.
61. Druhan LJ, Ai J, Massullo P, Kindwall-Keller T, Ranalli MA, Avalos BR. Novel mechanism of G-CSF refractoriness in patients

with severe congenital neutropenia. Blood. 2005;105(2):584-91.
genetics. 2003;35(1):90-6.
62. Triot A, Järvinen PM, Arostegui JI, Murugan D, Kohistani N, Dapena Díaz JL, et al. Inherited biallelic CSF3R mutations in severe congenital neutropenia. Blood. 2014;123(24):3811-7.
63. Klimiankou M, Klimenkova O, Uenalan M, Zeidler A, Mellor-Heineke S, Kandabarau S, et al. GM-CSF stimulates granulopoiesis in a congenital neutropenia patient with loss-of-function biallelic heterozygous CSF3R mutations. Blood. 2015;126(15):1865-7.
64. Karapinar DY, Özdemir HH, Akinci B, Yasar AS, Sivis ZÖ, Onay H, et al. Management of a patient with congenital biallelic CSF3R mutation with GM-CSF. Journal of Pediatric Hematology/Oncology. 2020;42(3):e164-e6.
65. Zhou J, Sun C, Huang H, Zhu Q, Wen F, Dong Y, et al. Efficacy of Low-Dose rhGM-CSF Treatment in a Patient With Severe Congenital Neutropenia Due to CSF3R Deficiency: Case Report of a Novel Biallelic CSF3R Mutation and Literature Review. Frontiers in Pediatrics. 2021;9:746159.
66. Yilmaz Karapinar D, Akinci B, Şahin Yaşar A, Hekimci Özdemir H, Önder Siviş Z, Onay H, et al. Congenital neutropenia patient with hypomorphic biallelic CSF3R mutation responding to GCSF. Journal of Pediatric Hematology/Oncology. 2019;41(3):e190-e2.
67. Carapito R, Konantz M, Paillard C, Miao Z, Pichot A, Leduc MS, et al. Mutations in signal recognition particle SRP54 cause syndromic neutropenia with Shwachman-Diamond-like features. The Journal of clinical investigation. 2017;127(11):4090-103.
68. Bellanné-Chantelot C, Schmaltz-Panneau B, Marty C, Fenneteau O, Callebaut I, Clauin S, et al. Mutations in the SRP54 gene cause severe congenital neutropenia as well as Shwachman-Diamond-like syndrome. Blood. 2018;132(12):1318-31.
69. Goldberg L, Simon AJ, Rechavi G, Lev A, Barel O, Kunik V, et al. Congenital neutropenia with variable clinical presentation in novel mutation of the SRP54 gene. Pediatr Blood Cancer. 2020;67(6):e28237.
70. Sabulski A, Grier DD, Myers KC, Davies SM, Rubinstein JD. Acute myeloid leukemia in SRP54-mutated congenital neutropenia. EJHaem. 2022;3(2):521-5.
71. Calvo C, Lainey E, Caye A, Cuccuini W, Fenneteau O, Yakouben K, et al. Leukaemic transformation in a 10-year-old girl with SRP54 congenital neutropenia. British journal of haematology. 2022;198(6):1069-72.
72. Wild K, Bange G, Motiejunas D, Kribelbauer J, Hendricks A, Segnitz B, et al. Structural Basis for Conserved Regulation and Adaptation of the Signal Recognition Particle Targeting Complex. Journal of molecular biology. 2016;428(14):2880-97.
73. Juaire KD, Lapouge K, Becker MMM, Kotova I, Michelhans M, Carapito R, et al. Structural and Functional Impact of SRP54 Mutations Causing Severe Congenital Neutropenia. Structure. 2021;29(1):15-28.e7.
74. Saettini F, Cattoni A, D'Angio M, Corti P, Maitz S, Pagni F, et al. Intermittent granulocyte maturation arrest, hypocellular bone marrow, and episodic normal neutrophil count can be associated with SRP54 mutations causing Shwachman-Diamond-like syndrome. British journal of haematology. 2020;189(4):e171-e4.
75. Manabe T, Taku K, Hoshina T, Higuchi N, Karakawa S, Kusuhara K. A pediatric case of congenital neutropenia with SRP54 gene mutation in which monocytosis and gingival swelling were useful in differentiating from autoimmune neutropenia. Pediatr Blood Cancer. 2022;69(9):e29648.
76. Fan EM, Vagher J, Meznarich JA, Ubico EM, Goteti S, Peterson D, et al. Severe congenital neutropenia, SRP54 pathogenicity, and a framework for surveillance. American journal of medical genetics Part A. 2023;191(5):1434-41.
77. Tamura T, Yagasaki H, Nakahara E, Ito M, Ueno M, Kanezawa K, et al. A Filipino infant with severe neutropenia owing to SRP54 mutations was successfully treated with ethnically mismatched cord blood transplantation from a Japanese cord blood bank. Annals of hematology. 2021;100(11):2859-60.
78. McCarthy P, Cotter M, Smith OP. Autosomal dominant Shwachman-Diamond syndrome with a novel heterozygous missense variant in the SRP54 gene causing severe phenotypic features. British journal of haematology. 2022;196(3):e39-e42.
79. Schürch C, Schaefer T, Müller JS, Hanns P, Arnone M, Dumlin A, et al. SRP54 mutations induce congenital neutropenia via dominant-negative effects on XBP1 splicing. Blood. 2021;137(10):1340-52.
80. Uemura A, Oku M, Mori K, Yoshida H. Unconventional splicing of XBP1 mRNA occurs in the cytoplasm during the mammalian unfolded protein response. Journal of cell science. 2009;122(Pt 16):2877-86.
81. Tanimura A, Miyoshi K, Horiguchi T, Hagita H, Fujisawa K, Noma T. Mitochondrial Activity and Unfolded Protein Response are Required for Neutrophil Differentiation. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2018;47(5):1936-50.
82. Linder MI, Mizoguchi Y, Hesse S, Csaba G, Tatematsu M, Łyszkiewicz M, et al. Human genetic defects in SRP19 and SRPRA cause severe congenital neutropenia with distinctive proteome changes. Blood. 2023;141(6):645-58.
83. Schmaltz-Panneau B, Pagnier A, Clauin S, Buratti J, Marty C, Fenneteau O, et al. Identification of biallelic germline variants of SRP68 in a sporadic case with severe congenital neutropenia. Haematologica. 2021;106(4):1216-9.
84. Warren JT, Cupo RR, Wattanasirakul P, Spencer DH, Locke AE, Makaryan V, et al. Heterozygous variants of CLPB are a cause of severe congenital neutropenia. Blood. 2022;139(5):779-91.
85. Yoshinaka T, Kosako H, Yoshizumi T, Furukawa R, Hirano Y, Kuge O, et al. Structural Basis of Mitochondrial Scaffolds by Prohibitin Complexes: Insight into a Role of the Coiled-Coil Region. iScience. 2019;19:1065-78.
86. Cupo RR, Shorter J. Skd3 (human ClpB) is a potent mitochondrial protein disaggregase that is inactivated by 3-methylglutaconic aciduria-linked mutations. eLife. 2020;9.
87. Wortmann SB, Ziętkiewicz S, Guerrero-Castillo S, Feichtinger RG, Wagner M, Russell J, et al. Neutropenia and intellectual disability are hallmarks of biallelic and de novo CLPB deficiency. Genetics in Medicine. 2021;23(9):1705-14.
88. He M, Saeed MB, Record J, Keszei M, Gonçalves Pinho L, Vasconcelos-Fontes L, et al. Overactive WASp in X-linked neutropenia leads to aberrant B-cell division and accelerated plasma cell generation. The Journal of allergy and clinical immunology. 2022;149(3):1069-84.
89. Devriendt K, Kim AS, Mathijs G, Frints SG, Schwartz M, Van Den Oord JJ, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nature genetics. 2001;27(3):313-7.
90. Ancliff PJ, Blundell MP, Cory GO, Calle Y, Worth A, Kempski H, et al. Two novel activating mutations in the Wiskott-Aldrich syndrome protein result in congenital neutropenia. Blood. 2006;108(7):2182-9.
91. Kobayashi M, Chanda B, Futami M, Yamashita M, Hirano M, Yokoyama K, et al. A Novel Mutant Wasp in X-Linked Neutropenia

Partially Recapitulates Defective Neutrophil Production through Its Expression in WASP-Deficient NB4 Cells. Blood. 2017;130:3560.
92. Arwani M, Lee D, Haddad A, Mewawalla P. A novel mutation in Wiskott-Aldrich gene manifesting as macrothrombocytopenia and neutropenia. BMJ Case Reports. 2018;2018:bcr-2018-225123.
93. Beel K, Cotter MM, Blatny J, Bond J, Lucas G, Green F, et al. A large kindred with X-linked neutropenia with an I294T mutation of the Wiskott-Aldrich syndrome gene. British journal of haematology. 2009;144(1):120-6.
94. Kim AS, Kakalis LT, Abdul-Manan N, Liu GA, Rosen MK. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. Nature. 2000;404(6774):151-8.
95. Cory GO, Garg R, Cramer R, Ridley AJ. Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein. The Journal of biological chemistry. 2002;277(47):45115-21.
96. Albert MH, Bittner TC, Nonoyama S, Notarangelo LD, Burns S, Imai K, et al. X-linked thrombocytopenia (XLT) due to WAS mutations: clinical characteristics, long-term outcome, and treatment options. Blood. 2010;115(16):3231-8.
97. Bosticardo M, Marangoni F, Aiuti A, Villa A, Grazia Roncarolo M. Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome. Blood, The Journal of the American Society of Hematology. 2009;113(25):6288-95.
98. Keszei M, Record J, Kritikou JS, Wurzer H, Geyer C, Thiemann M, et al. Constitutive activation of WASp in X-linked neutropenia renders neutrophils hyperactive. The Journal of clinical investigation. 2018;128(9):4115-31.
99. Dale DC, Bonilla MA, Davis MW, Nakanishi AM, Hammond WP, Kurtzberg J, et al. A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. Blood. 1993;81(10):2496-502.
100. Skokowa J, Steinemann D, Katsman-Kuipers JE, Zeidler C, Klimenkova O, Klimiankou M, et al. Cooperativity of RUNX1 and CSF3R mutations in severe congenital neutropenia: a unique pathway in myeloid leukemogenesis. Blood. 2014;123(14):2229-37.
101. Beekman R, Valkhof MG, Sanders MA, van Strien PM, Haanstra JR, Broeders L, et al. Sequential gain of mutations in severe congenital neutropenia progressing to acute myeloid leukemia. Blood. 2012;119(22):5071-7.
102. Olofsen PA, Fatrai S, van Strien PMH, Obenauer JC, de Looper HWJ, Hoogenboezem RM, et al. Malignant Transformation Involving CXXC4 Mutations Identified in a Leukemic Progression Model of Severe Congenital Neutropenia. Cell reports Medicine. 2020;1(5):100074.
103. Nayak RC, Trump LR, Aronow BJ, Myers K, Mehta P, Kalfa T, et al. Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. The Journal of clinical investigation. 2015;125(8):3103-16.
104. Morishima T, Watanabe K, Niwa A, Hirai H, Saida S, Tanaka T, et al. Genetic correction of HAX1 in induced pluripotent stem cells from a patient with severe congenital neutropenia improves defective granulopoiesis. Haematologica. 2014;99(1):19-27.
105. Hiramoto T, Ebihara Y, Mizoguchi Y, Nakamura K, Yamaguchi K, Ueno K, et al. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(8):3023-8.