Kostmann disease and other forms of severe congenital neutropenia

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Abstract
Congenital neutropenia with autosomal recessive inheritance was first described by the Swedish paediatrician Rolf Kostmann who coined the term 'infantile genetic agranulocytosis'. The condition is now commonly referred to as Kostmann disease. These patients display a maturation arrest of the myelopoiesis in the bone marrow and reduced neutrophil numbers and suffer from recurrent, often life-threatening infections. The molecular mechanism underlying congenital neutropenia has been intensively investigated, and mutations in genes that impinge on programmed cell death have been identified. The present review provides an overview of these studies.

KEYWORDS
apoptosis, bone marrow failure, leukaemia, necroptosis, neutropenia

1 | PIONEERING STUDIES OF CHILDREN WITH CONGENITAL NEUTROPENIA

Rolf Kostmann, a Swedish paediatrician working in the north of Sweden, reported his first observations of children with neutropenia in 1950 in the official journal of the Swedish Medical Association.¹ The report was based on a seminar held in 1949. Rolf Kostmann referred to this previously unknown condition as 'hereditary reticulosis.'¹ In his subsequent doctoral thesis, published in 1956 as a supplement to Acta Paediatrica, he introduced the term 'infantile genetic agranulocytosis'.² Kostmann was thus the first person to describe congenital neutropenia with autosomal recessive inheritance. He described a total of 14 children, and detailed clinical and morphological data were provided for six of them. In a follow-up review in 1975, at which time about 30 patients with similar features had been identified, he provided further clinical details on

Abbreviations: AML, acute myeloid leukaemia; BMF, bone marrow failure; CLPB, caseinolytic peptidase B; ELANE, elastase, neutrophil expressed; ER, endoplasmic reticulum; IMLP, N-formyl-methionyl-leucyl-phenylalanine; G-CSF, granulocyte colony-stimulating factor; GFI1, growth factor independent-1 transcriptional repressor; GTP, guanosine triphosphate; HAX-1, HS-1 associated protein X; HCSL1 (short name: HS-1), haematopoietic cell-specific Lyn substrate 1; IDAX, inhibition of the Dvl and Axin complex; IUIS, International Union of Immunological Societies; JAGN1, jagunal homolog 1; MDS, myelodysplastic syndrome; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; OMIM, Online Mendelian Inheritance in Man; PARL, presenilin-associated, rhomboid-like; SCN, severe congenital neutropenia; Skd3, suppressor of potassium transport defect 3; SRPS4, signal recognition particle 54; UPR, unfolded protein response; VPS45, vacuolar protein sorting 45; WHIM, warts, hypogammaglobulinemia, infections and myelokathexis.

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affected patients belonging to the original family residing in northern Sweden. Today, the condition discovered by Rolf Kostmann is commonly referred to as Kostmann disease. Carlsson and Fasth provided an update on the only long-term survivor described in the 1975 report along with a presentation of five additional patients from the original ‘Kostmann family’ who were born after 1975. The latter report was published twenty years ago in *Acta Paediatrica*.

The clinical features of Kostmann disease, along with an overview of clinical and basic research conducted on patients belonging to the original Kostmann family in northern Sweden, have been previously reviewed in this journal and in the official journal of the Swedish Medical Association. Additionally, Rolf Kostmann’s colleagues recently provided a glimpse of the man behind the disease. The purpose of the present review is to discuss recent findings pertaining to the molecular aetiology of Kostmann disease and other forms of severe congenital neutropenia (SCN).

2 | SEVERE CONGENITAL NEUTROPENIA: CLINICOPATHOLOGICAL FEATURES

Patients with SCN including patients with Kostmann syndrome often display a typical arrest in the myelopoiesis at the promyelocyte/myelocyte stage leading to reduced neutrophil counts. Moreover, in many cases, a conspicuous and possibly compensatory monocytosis can be seen. In Kostmann’s doctoral thesis, the morphology of the cells was described as larger than normal with abnormal nuclei displaying protrusions and coarse clefts. Furthermore, in advanced disease, small myeloblasts appeared next to the erythroid precursors with an increasing number of atypical monocytoid cells. The latter were so exuberant in Kostmann’s first patient that he referred to the disease as a reticuloysis. The term was commonly used at the time to denote the abnormal proliferation of histiocytes, monocytes or other reticuloendothelial elements. The conclusion was strengthened by the finding of similar cells in a lymph node and in pulmonary lesions. Kostmann noted the same findings in his thesis, with a predominance of atypical monocytoid cells at the end stage of the disease. This morphology is amply illustrated in the black-and-white photographs shown in the thesis, and it is readily understood how difficult it can be to distinguish immature, abnormal myelocytic cells from reactive or atypical monocytes. We re-photographed the bone marrow smears from the original patient from Kostmann’s own collection, and in these photographs, we noted mainly immature myelocytes and monocytes (Figure 1). Notwithstanding, there is a considerable phenotypic overlap among the inherited bone marrow failure syndromes, a heterogeneous group of genetic disorders that includes Shwachman-Diamond syndrome, Diamond-Blackfan anaemia, Fanconi anaemia, dyskeratosis congenita and severe congenital neutropenia (SCN). Thus, a molecular diagnosis is needed.

It is notable that even though patients with Kostmann disease who are treated granulocyte colony-stimulating factor (G-CSF) are spared from life-threatening bacterial infections, they frequently suffer from recurrent gingivitis and severe periodontitis. The antibacterial peptides, such as defensins and LL-37 (a 37 amino acid cationic peptide that is also named FALL-39 after the first four amino acids), are natural bactericidal components similar in potency to conventional antibiotics. LL-37 in the oral cavity is produced mainly by infiltrating neutrophils. Boman and colleagues reported two decades ago that neutrophils from patients with Kostmann disease are deficient in the pro-form of LL-37. Subsequent work has shown that patients with SCN are deficient for pro-LL-37 whereas patients with autoimmune or idiopathic neutropenia exhibit normal pro-LL-37 levels in plasma. Plasma levels of pro-LL-37 may thus prove useful for differential diagnosis of chronic neutropenia. Furthermore, neutrophils also help shape adaptive immune responses, in addition to their role in host defence against microbes. Recent work has shown that LL-37 potentiates the IL-17-producing T helper 17 (Th17) subset of CD4-positive T cells. Further studies in SCN patients lacking LL-37 may thus be of interest.

The prevalence of SCN has previously been estimated to be 1–2 per million individuals. However, our population-based study published in 2012 revealed a total incidence of 1 per 100 000 live births of verified or probable SCN. In total, 32 patients were diagnosed

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**FIGURE 1** Rolf Kostmann’s index case. The photograph shows a May-Grünwald-Giemsa-stained bone marrow smear taken from Kostmann’s index patient in 1949. From below, one myeloblast, one granulated myelocyte, two monocytoid cells and probably a myelocyte. We were unable to extract nucleic acids for molecular analysis, but studies of individuals from the same kindred have revealed HAX1 mutations.
with congenital neutropenia during a 20-year period (1987–2006), of whom 9 children (43%) carried heterozygous ELANE mutations, 4 children (19%) had homozygous or compound heterozygous HAX1 mutations, and 8 children (38%) had no known mutations, including two children who had not been tested for mutations. SCN is known to be a pre-malignant condition, even though none of the patients described by Rolf Kostmann developed malignancies: most of the patients in the pre-G-CSF era died before one year of age from bacterial infections. In our nation-wide study, 4 of 21 patients with SCN (19%) developed myelodysplastic syndrome (MDS)/acute myeloid leukaemia (AML); two had ELANE mutations, one had HAX1 mutations, and one had never been tested for disease-causing mutations. The cumulative incidence of MDS/AML after 15 years on G-CSF therapy and supportive care was 31%. However, it is important to point out that a prospective study of 374 SCN patients enrolled in the Severe Chronic Neutropenia International Registry showed that the annual risk of MDS/AML attained a plateau (2.3% per year after 10 years). Long-term, the risk thus appears similar to the risk of AML in Fanconi anaemia and dyskeratosis congenita. Nonetheless, as emphasised by the authors, it is important that patients continue to be monitored for signs of evolution to leukaemia.

One of the major drivers of the evolution of secondary malignancies in SCN patients is the presence of somatic or acquired mutations in the CSF3R gene encoding the G-CSF receptor. Additionally, by applying next-generation sequencing of haematopoietic stem cell clones, Skokowa et al. could show that 20 of 31 patients (64.5%) with SCN who had progressed to MDS or AML had clones with mutations in RUNX1, and these mutations were frequently found in clones that had already acquired CSF3R mutations. Thus, it appears that RUNX1 and CSF3R mutations may cooperatively promote leukaemogenesis in SCN patients. Indeed, the progression from SCN to AML is a multi-step process, with distinct mutations arising early in SCN and others later during progression to AML. Using a mouse model of leukaemic progression, Olofsen et al. investigated how the combination of G-CSF therapy and CSF3R and RUNX1 mutations contributed to AML development. The authors found that an additional acquired ‘driver’ mutation in Cxxc4 (encoding IDAX, an inhibitor of Wnt signalling) was involved in AML development in mice. Moreover, the authors identified CXXC4 mutations in a few patients with de novo AML. However, it remains to be understood whether CXXC4 mutations play a role for secondary leukaemia in SCN patients.

More generally, next-generation sequencing approaches have unearthed a complex genetic landscape in inherited bone marrow failure (BMF) syndromes, and such approaches could be of potential clinical utility, as a powerful diagnostic tool while also pointing to individually tailored or personalised therapies for these patients.

3 | SEVERE CONGENITAL NEUTROPENIA: NOSOLOGICAL CONSIDERATIONS

The classification of congenital neutropenia is an important topic, and many experts have weighed in on this subject. According to the Severe Chronic Neutropenia International Registry, congenital neutropenia is a term used to describe ‘a group of serious but rare inherited hematological disorders which cause continuous neutropenia for many months or years’, while cyclic neutropenia, in turn, is characterised by recurrent episodes of neutropenia that last 3–5 days typically every 21 days. This is perfectly sound, though the fact that all three terms (chronic neutropenia, congenital neutropenia and cyclic neutropenia) share the same two-letter abbreviation is potentially ambiguous. For the purpose of the present review, we shall use the term severe congenital neutropenia (SCN). It is important to acknowledge that SCN is a genetically heterogeneous group of disorders of haematopoesis encompassing conditions with autosomal dominant and autosomal recessive inheritance (discussed below). Furthermore, it may be useful to put SCN in the context of other inborn errors of immunity (also referred to as primary immunodeficiencies). Indeed, the International Union of Immunological Societies (IUIS) has compiled a compendium of 430 human genes and genetic disorders, and it states that SCN can be divided into several subcategories on the basis of the underlying gene defect. Hence, patients with ELANE mutations are categorised as SCN1, patients with GFI1 deficiency as SCN2, patients with HAX1 deficiency as SCN3, patients with G6PC3 deficiency as SCN4, patients with VPS45 deficiency as SCN5, patients with JAGN1 deficiency as SCN6, patients with CSF3R deficiency as SCN7, patients with SRRP4 deficiency as SCN8 and patients with WAS deficiency as SCN9 (as the latter condition is an X-linked condition; SCN1, SCN2 and SCN8 are autosomal dominant conditions, and SCN3–SCN7 are all autosomal recessive conditions). We agree that a molecular disease classification is important in that it may increase awareness and facilitate the clinical management of patients and their families, but the clinical presentation should always remain in focus. Hence, it is noted that the IUIS expert committee proposed that Kostmann disease equals ‘HAX1 deficiency’ whereas children with JAGN1 mutations were classified as having ‘JAGN1 deficiency’, but this is a circular argument that is of little use (clinically). In fact, patients with Kostmann disease may harbour homozygous mutations in HAX1 or JAGN1, or in other yet unknown disease-causing genes, as we will discuss in the present review. Boztug et al. reported that patients with JAGN1 mutations (classified as SCN6 according to OMIM) display clinical features that resemble those of patients with HAX1 mutations (classified as SCN3 according to OMIM), though the authors noted that the response to recombinant G-CSF treatment was often poor in patients with JAGN1 mutations. Nevertheless, from a clinical perspective, we consider both conditions, that is autosomal recessive SCN with homozygous HAX1 mutations or JAGN1 mutations, as Kostmann disease (Figure 2). From a molecular perspective, the
OMIM classification and the partially overlapping IUIS classification may certainly be of use.

4 | SEVERE CONGENITAL NEUTROPENIA: DISEASE-CAUSING MUTATIONS

Rolf Kostmann speculated that the maturation defect in the myeloid lineage in the bone marrow might be due to a deficiency of a ‘serum factor’. However, contemporary research, performed in part on cells from patients belonging to the original ‘Kostmann family’, has shown that the disease is caused by a cell autonomous deficiency in myeloid progenitors in the bone marrow. In the following section, we will briefly discuss disease-causing mutations in patients with SCN.

More than 20 years ago, Dale and co-workers identified mutations in ELANE (though the gene was known as ELA2 at the time) encoding neutrophil elastase (NE) in patients with cyclic neutropenia. Mutations in ELANE were subsequently identified in patients with other types of SCN. In fact, the majority of patients with autosomal dominant SCN are now known to harbour mutations of the ELANE gene. It is notable that patients with cyclic neutropenia have a lower risk of evolution to MDS/AML. With regard to the mechanism, it has been suggested that the intracellular accumulation and mislocalisation of misfolded NE induces endoplasmic reticulum (ER) stress leading to activation of the so-called unfolded protein response (UPR) which in turn triggers apoptosis. However, using human and murine myeloid cell lines with inducible expression of human ELANE, it was recently shown that disease-associated ELANE mutations do not necessarily trigger the UPR. Furthermore, it is not clear why the same ELANE mutations result in cyclic neutropenia in some patients and the non-cyclic form of SCN in other patients. This puts a spotlight on the limitations of current in vitro and in vivo models of human disease. Using primary bone narrow cells, Welte and co-workers tested the hypothesis that the cyclic haematopoiesis may be explained by a ‘tug-of-war’ between UPR-induced ER stress and a compensatory G-CSF-stimulated proliferation of haematopoietic progenitor cells. The authors argued that UPR-escaping cells might be able respond to G-CSF.

Mutations in GFI1 encoding the growth factor independent-1 (GF11) transcription factor are also associated with SCN, and GFI1 was suggested to repress ELA2 (ELANE), potentially linking these two genes in a common pathway of myeloid differentiation. The combined neutropenia and immunodeficiency in patients with GFI1 mutations is reminiscent of the warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome. However, as previously noted by Person et al., the undifferentiated appearance of neutrophils in patients with GFI1 mutations differs from the hyper-segmented mature neutrophils present in the bone marrow in WHIM syndrome. Muench et al. provided evidence, by introducing patient-derived GFI1 mutations into one Gfi1 allele in mice, that the cellular ‘state’ plays a major role in determining the outcome of neutropenia-associated mutations. Hence, the authors revealed cell state-specific effects of Gfi1 mutations, and they reasoned that this could be related to the expression of transcription factors and chromatin accessibility of their respective target genes, which is dynamically altered along the trajectory of granulopoiesis. Hence, this elegant molecular study demonstrated that mutations matter, but so does the cellular context in which the mutations are manifested.

In addition to mutations in ELANE and GFI1 that give rise to autosomal dominant SCN, a number of studies have reported mutations in other genes in SCN of autosomal recessive inheritance, including HAX1 and JAGN1 (which we will discuss in the next section). Klein and co-workers identified mutations in G6Pc3 (encoding glucose-6-phosphatase, catalytic subunit 3) in patients with neutropenia associated with cardiac and urogenital malformations. Neutrophils and fibroblasts from these patients displayed increased susceptibility to apoptosis. This was followed by the discovery (in 2013) of mutations in VPS45 (vacuolar protein sorting 45) in families afflicted with neutropenia combined with myelofibrosis. VPS45 encodes a protein that regulates membrane trafficking through the endosomal system, and it is worth noting that loss-of-function mutations of genes implicated in protein trafficking to endosomes are also associated with other conditions with inherited neutropenia, including Chediak-Higashi syndrome, Griscelli syndrome and Hermansky-Pudlak syndrome.
type 2. Notably, we tor cells from the same individuals display excessive mitochondrial depolarisation. The signal recognition particle (SRP) pathway is a conserved pathway that delivers membrane and secretory proteins to the ER. Recently, mutations in SRPS4 (encoding signal recognition particle 54, the catalytic GTPase of the SRP) were identified in patients with SCN with Shwachman-Diamond syndrome-like features, including neurodevelopmental defects and pancreatic insufficiency. Knock-down of SRPS4 in zebrasfish was shown to recapitulate the human disease. However, these findings imply a blurring of diagnostic boundaries between bone marrow failure syndromes. Indeed, as pointed out in a recent commentary: should one 'lump or split' genetic diseases?

5 | DANCE MACABRE: THE MOLECULAR AETIOLOGY OF KOSTMANN DISEASE

Fifty years after the publication of Kostmann’s doctoral thesis, we and others identified mutations in HAX1 encoding HS-1-associated protein X (HAX-1), a protein that is located in mitochondria as well as in the ER. It was noted that HAX-1 protects mitochondria from depolarisation. Hence, the observation that patients with Kostmann disease (including patients belonging to the original ‘Kostmann family’ in northern Sweden) harbour mutations leading to a deficiency of HAX-1 aligned well with our observation that bone marrow progenitor cells from the same individuals display excessive mitochondrial release of cytochrome c, a key instigator of apoptosis. Notably, we also found that recombinant G-CSF reversed cytochrome c release and improved the survival of myeloid progenitor cells. G-CSF also prevents cytochrome c release from haematopoietic progenitor cells obtained from MDS patients. Other investigators have confirmed that HAX-1 plays a key role in protecting mitochondria from depolarisation. Furthermore, we generated Epstein-Barr virus (EBV)-transformed B cell lines from patients with Kostmann disease and could show that these cells were more susceptible to mitochondria-dependent apoptosis when compared to cells from healthy controls. Dale and co-workers reported that accelerated apoptosis of bone marrow myeloid progenitors is also a prominent feature of myelokathexis, a rare form of SCN that is attributed to the retention of neutrophils in the bone marrow.

Some authors have speculated that neutropenia in SCN patients harbouring HAX1 mutations is due to the lack of HAX-1-driven activation of HCSL1 (haematopoietic cell-specific Lyn substrate 1), one of the many binding partners of HAX-1. In fact, HAX1 appears to sit at the nexus of a large network of genes in myeloid progenitor cells. Indeed, Pittermann et al. could show, using patient-derived induced pluripotent stem cells (iPSCs) carrying the homozygous W44X HAX1 mutation, that targeted correction of the HAX1 mutation re-established a HAX1 and HCSL1 associated gene transcription network in myeloid progenitors. The authors performed pathway analysis of the 533 myeloid-specific genes that were downregulated in cells harbouring W44X HAX1 mutations and identified nodes or clusters of interactions that could be attributed to gene ontology categories related to mitochondrial homeostasis (apoptotic mitochondrial changes, regulation of mitochondrial membrane potential) and the activation of apoptotic signalling pathways (regulation of apoptotic signalling pathway, regulation of leucocytic apoptosis, apoptotic mitochondrial changes). These studies corroborated our observations using primary CD33-positive and CD34-positive bone marrow progenitor cells from patients belonging to the original ‘Kostmann family’ insofar as they highlighted a role for apoptosis deregulation in the pathogenesis of Kostmann disease. Moreover, Pittermann et al. showed that a Kostmann disease-specific gene signature exists in which HAX1 and HCSL1 act in concert as part of a gene transcription network involved in the regulation of apoptosis and myeloid differentiation. However, every model has its limitations. Thus, while iPSCs have the potential to differentiate into all cell types, it remains to be understood whether these in vitro-derived cells are true equivalents of their primary counterparts.

Carlsson and Fasth observed that some patients from the ‘Kostmann family’ developed neurological symptoms including epilepsy and neuropsychological deficits. In a follow-up study by Henter and co-workers, three patients with Kostmann disease harbouring Q190X HAX1 mutations who were alive and available for evaluation were found to display neurological disease with decreased cognitive function, and three of four patients who reached 10 years of age developed epilepsy. In contrast, in one Swedish patient of Kurdish extraction who carried the alternative W44X HAX1 mutation, no neurological abnormalities were found. Moreover, two alternative splice variants of HAX1 were identified in normal human tissues (including the brain). Both transcripts contained exon 5, which is affected by the Q190X mutation, whereas the 5′ end of exon 2 containing the W44X mutation is spliced out from the second transcript. On the basis of these findings, we postulated that HAX1 mutations affecting both splice variants I and II (Q190X) versus splice variant I only (W44X) could result in divergent outcomes in terms of protein expression in different tissues, and hence in terms of phenotypic manifestations in these patients. It is notable that other investigators reported in the same year (2008), using a HAX1 knockout mouse model, that loss of HAX-1 is associated with neuronal apoptosis in the striatum and the cerebellum. More recently, Bidwell et al. reported, using a cardiac-specific, inducible knockout mouse model, that HAX-1 is a critical regulator of sarcoplasmic reticulum calcium handling and heart muscle contractility. However, patients with Kostmann syndrome do not suffer from cardiac disease, though the fact that these patients receive life-long treatment with recombinant G-CSF makes it difficult to address this question (as it is conceivable that growth factor treatment could also be cardioprotective).
Nonetheless, the latter observations are of interest as they suggest that HAX-1 may exert a role at the level of the ER as well as the mitochondrion, though this could turn out to be cell type specific.

During the preparation of this review, heterozygous missense mutations in the gene encoding CLPB (caseinolytic peptidase B) were identified in patients with SCN. CLPB (also known as Skd3, for suppressor of potassium transport defect 3) is a mitochondrial protein disaggregate meaning that it is essential for maintaining the solubility of mitochondrial inner membrane and intermembrane space protein complexes. Intriguingly, Skd3 (CLPB) was shown to maintain solubility of HAX-1 in mitochondria in human cells. Additionally, the mitochondrial inner membrane protease, PARL (presenilin-associated, rhomboid-like), was found to remove an autoinhibitory peptide from Skd3 to enhance its disaggregate activity. This is notable as Chao et al. have previously shown that Hax-1 is required to suppress neuronal apoptosis in mice and this apoptosis inhibition was found to require the interaction of Hax-1 with the mitochondrial proteases Parl and HtrA2 (high-temperature-regulated A2). These interactions allowed Hax1 to present HtrA2 to Parl, thereby facilitating the activation of HtrA2. Taken together, one may speculate that defects in Skd3 (CLPB) could lead to a loss of functional HAX-1, which may drive neutropenia in SCN patients. However, further studies are required to disclose the precise mechanism of regulation between HAX-1 and other mitochondrial proteins.

Mutations in JAGN1 (encoding jagunal homolog 1) have also been associated with Kostmann disease. Hence, Boztug et al. identified 9 distinct homozygous mutations in the JAGN1 gene in 14 individuals with SCN. The authors did not find any obvious differences between individuals with SCN carrying JAGN1 mutations and those with HAX1 mutations. However, the response to recombinant human G-CSF treatment was poor in several JAGN1-deficient patients. In a companion paper, the authors reported that mice carrying a haematopoietic lineage-specific deletion of Jag1 were unable to mount an efficient neutrophil-dependent immune response to the human fungal pathogen Candida albicans. Treatment of isolated bone marrow neutrophils from these mice with GM-CSF but not G-CSF restored their ability to kill C. albicans. We could confirm, in a recent study, that human neutrophil-differentiated cells in which JAGN1 expression had been silenced were deficient for neutrophil extracellular trap (NET) dependent killing of C. albicans. NETs are required for extracellular killing of pathogens. Specifically, we noted that even though JAGN1-deficient cells remained capable of emitting NETs, myeloperoxidase (MPO) expression in NETs was severely reduced. Notably, the administration of recombinant GM-CSF upregulated the expression of MPO and calprotectin. Hence, while mutations in JAGN1 are associated with SCN (i.e., a lack of neutrophils), a lack of JAGN1 is associated with a deficiency in anti-fungal activity of neutrophils. These studies are relevant as they show that while treatment with recombinant G-CSF may restore neutrophil numbers in SCN patients, these cells may nonetheless display functional abnormalities when compared to normal neutrophils. This is in accordance with a previous study in which neutrophils from patients with ELANE mutations failed to respond appropriately to stimulation with N-formyl-methionyl-leucyl-phenylalanine (fMLP) despite ongoing G-CSF treatment.

Does JAGN1 control apoptosis as shown previously for HAX1? Boztug et al. suggested as much, using peripheral blood neutrophils as a model, though the evidence for apoptosis was sparse and the underlying mechanism was not disclosed. We recently identified two different homozygous JAGN1 mutations in three patients with Kostmann disease, thus confirming the attribution of JAGN1 mutations to SCN. We examined peripheral blood neutrophils from one of our patients (who responded poorly to recombinant G-CSF treatment) and observed ultrastructural changes suggestive of aberrant granule exocytosis, but no signs of apoptosis (Figure 3). To further study the impact of these JAGN1 mutations, we expressed both mutations in the human myeloid HL-60 cell line, a commonly used model of myeloid cells. We found that overexpression of the patient-derived JAGN1 mutations augmented cell death in response to classical pro-apoptotic stimuli, including the chemotherapeutic agent, etoposide and thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca2+-ATPase that triggers calcium-dependent cell death. Furthermore, we observed that cells expressing disease-associated JAGN1 mutations were susceptible to agonists that trigger degranulation in neutrophils (i.e., the chemottractant, fMLP, combined with the priming agent, cytochalasin D) and succumbed to a calcium-dependent cell death programme. This was completely prevented by pharmacological inhibition of calpain but remained unaffected upon caspase inhibition, suggesting that this cell death was non-apoptotic. We also noted that bongkrekic acid, a specific inhibitor of mitochondrial permeability transition pore opening, partially rescued the cells from calpain-dependent cell death, pointing to a crosstalk between the ER and mitochondria. Finally, we conducted a yeast two-hybrid screen and found that JAGN1 interacts with Grp78, a known regulator of the UPR as well as a regulator of calcium homeostasis in the ER. In conclusion, our results showed that SCN associated JAGN1 mutations unleashed a calcium- and calpain-dependent cell death in a human myeloid cell model. Further investigations are required to understand the role of calpain-mediated cell death in the pathogenesis of JAGN1-associated SCN. Nevertheless, our findings underscore the importance of other forms of regulated or programmed cell death. For comparison, a recent study disclosed that Ripk1-mediated necroptosis leads to bone marrow failure in mice, resembling myelodysplastic syndrome (MDS). Moreover, signs of autophagy were noted in cells from patients with ‘congenital dysgranulopoietic neutropenia’, prompting the question whether autophagy is a non-specific response to impending cell death, or whether autophagy plays a role in neutrophil maturation or survival. Thus, when we address the mechanism of cell death and its deregulation in SCN, we need to cast a wider net and consider not only apoptosis (the most famous and well-studied mode of cell death), but also other forms of cell death, such as necroptosis, as well as autophagy or autophagic cell death.
Careful investigations conducted in multiple laboratories have disclosed a role for deregulated cell death in Kostmann disease and other forms of severe congenital neutropenia (SCN). Moreover, evidence for organelle crosstalk in the regulation of the survival and death of myeloid progenitor cells has been provided. Hence, mitochondria as well as the endoplasmic reticulum (ER) have been shown to play key roles in cell death, and calcium has emerged as one of the conduits between these organelles. We have also learned that mouse models of SCN do not necessarily recapitulate the human disease. Indeed, none of the mouse models in which genes corresponding to ELANE, HAX1 or JAGN1 were deleted displayed neutropenia. The real ‘model’ of a rare disease is the patient, and close cooperation between paediatricians and other clinicians with preclinical scientists is of utmost importance if we are to unravel the mechanism of disease. Furthermore, patient-derived induced pluripotent stem cells (iPSCs) harbouring disease-causing mutations may also serve as useful (human) models with which to explore the underlying pathophysiology of SCN.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.
11. Karlsson J, Carlsson G, Ramme KG, et al. Low plasma levels of the protein pro-LL-37 as an early indication of severe disease in patients with chronic neutropenia. Br J Haematol. 2007;137(2):166-169.

12. Mantovani A, Cassatella MA, Costantini C, Jaillon S. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol. 2011;11(8):519-531.

13. Minns D, Smith KJ, Alessandrin V, et al. The neutrophil antimicrobial peptide cathelicidin promotes Th17 differentiation. Nat Commun. 2021;12(1):1285.

14. Karlsson G, Fath A, Berglöf E, et al. Incidence of severe congenital neutropenia in Sweden and risk of evolution to myelodysplastic syndrome/leukaemia. Br J Haematol. 2012;158(3):363-369.

15. Rosenberg PS, Zeidler C, Bolyard AA, et al. Stable long-term risk of leukemia in patients with severe congenital neutropenia maintained on G-CSF therapy. Br J Haematol. 2010;150(2):196-199.

16. Skowoka J, Steinen D, Katsman-Kuipers JE, et al. Cooperativity of RUNX1 and CSF3R mutations in severe congenital neutropenia: a unique pathway in myeloid leukaemogenesis. Blood. 2014;123(14):2229-2237.

17. Beekman R, Valkhof MG, Sanders MA, et al. Sequential gain of mutations in severe congenital neutropenia progressing to acute myeloid leukemia. Blood. 2012;119(22):5071-5077.

18. Olofsen PA, Fatrai S, van Strien PMH, et al. Malignant transformation involving CXXC4 mutations identified in a leukemic progression model of severe congenital neutropenia. Cell Rep Med. 2020;15(5):100074.

19. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. Blood. 2018;131(7):717-732.

20. Rio-Machin A, Vulliamy T, Hug N, et al. The complex genetic landscape of familial MDS and AML reveals pathogenic germline variants. Nat Commun. 2020;11(1):1044.

21. Wlodarski MW. The arrival of personalized genomics in bone marrow failure. Haematologica. 2021;106(1):11-13.

22. Boxer LA, Newburger PE. A molecular classification of congenital neutropenia syndromes. Pediatr Blood Cancer. 2007;49(5):609-614.

23. Oyarbide U, Corey SJ. SRP54 and a need for a new neutropenia row failure. Haematologica. 2021;106(1):11-13.

24. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene ELANE, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Commun. 2021;12(1):1285.

25. Dale DC, Person RE, Bolyard AA, et al. Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. J Clin Invest. 2015;125(8):3103-3116.

26. Al Ustwani O, Kurzrock R, Wetzler M. Genetics on a WHIM. Br J Haematol. 2014;164(1):15-23.

27. Skokowa J, Klimiankou M, Klimenkoza O, et al. Interactions among HCLS1, HAX1 and LEF-1 proteins are essential for G-CSF-triggered granulopoiesis. Nat Med. 2012;18(10):1550-1559.

28. Pitterman E, Lachmann N, MacLean G, et al. Gene correction of VPS45 gene mutation imitates severe neutropenia associated with defective expression of Bcl-2, constitutive mitochondrial release of cytochrome c, and excessive apoptosis of myeloid progenitor cells. Blood. 2004;103(9):3355-3361.

29. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet. 1999;23(4):433-436.

30. Dale DC, Person RE, Bolyard AA, et al. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. Blood. 2000;96:2317-2322.

31. Nayak RC, Trump LR, Aronow BJ, et al. Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells. J Biol Chem. 2020;295(21):7492-7500.
activity on calcium cycling and contractility in the heart. J Biol Chem. 2018;293(1):359-367.

52. Warren JT, Cupo RR, Wattanasirakul P, et al. Heterozygous variants of CLPB are a cause of severe congenital neutropenia. Blood. 2021;blood.2021010762. https://doi.org/10.1182/blood.2021010762. [Epub ahead of print].

53. 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:e55279.

54. Wirnsberger G, Zwolanek F, Stadlmann J, et al. Jagunal homolog 1 is a critical regulator of neutrophil function in fungal host defense. Nat Genet. 2014;46(9):1028-1033.

55. Khandagale A, Lazzaretto B, Carlsson G, et al. JAGN1 is required for fungal killing in neutrophil extracellular traps: implications for severe congenital neutropenia. J Leukoc Biol. 2018;104(6):1199-1213.

56. Donini M, Fontana S, Savoldi G, et al. G-CSF treatment of severe congenital neutropenia reverses neutropenia but does not correct the underlying functional deficiency of the neutrophil in defending against microorganisms. Blood. 2007;109(11):4716-4723.

57. Khandagale A, Holmlund T, Entesarian M, et al. Severe congenital neutropenia-associated JAGN1 mutations unleash a calpain-dependent cell death programme in myeloid cells. Br J Haematol. 2021;192(1):200-211.

58. Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol. 2021;18(5):1106-1121.

59. Wagner PN, Shi Q, Salisbury-Ruf CT, et al. Increased Ripk1-mediated bone marrow necroptosis leads to myelodysplasia and bone marrow failure in mice. Blood. 2019;133(2):107-120.

60. Parmley RT, Crist WM, Ragab AH, et al. Congenital dysgranulopoietic neutropenia: clinical, serologic, ultrastructural, and in vitro proliferative characteristics. Blood. 1980;56(3):465-475.

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