

Severe congenital neutropenia, a genetically heterogeneous disease group with an increased risk of AML/MDS

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Abstract

Over the past decade, enormous progress has been made in the understanding of severe congenital neutropenia (SCN), by identification of several causal gene mutations: in *ELANE*, *GFI1*, *HAX1*, *WAS* and *G6PC3*. SCN is a preleukemic condition, independent of the genetic subtype. Acquired CSF3R mutations are specific for SCN and are strongly associated with malignant progression. In this review, we describe the known genetic subtypes of SCN, their molecular basis and clinical presentation and summarize the available evidence on CSF3R mutations and monosomy 7 in malignant conversion.

Introduction

Severe congenital neutropenia (SCN) was first described in 1956 by Rolf Kostmann as infantile genetic agranulocytosis.¹ Since then, almost half a century passed before *ELANE* mutations were discovered as the first genetic cause underlying SCN.² Two years later, a new SCN subtype, named X-linked neutropenia, was identified, caused by gain-of-function *WAS* mutations.³ 6 years later, *HAX1* mutations, underlying Kostmann syndrome or autosomal recessive SCN, were discovered.⁴ *GFI1* mutations and *G6PC3* mutations, both extremely rare, complete the list of causal genetic mutations in SCN known today.^{5,6} SCN is a rare disease, with an incidence of 1 in 200 000 births.⁷ SCN represents a heterogeneous group of inherited disorders, characterized by absolute neutrophil counts below $0.5 \times 10^9/L$ in the peripheral blood on 3 separate occasions during 6 months of observation, early onset of severe bacterial infections and a maturation arrest of granulopoiesis at the promyelocyte/myelocyte stage.^{8,9} The clinical diagnosis is usually made at birth or during the first months of life, based on recurrent severe infections -most commonly muco-cutaneous,

ear-nose-throat and pulmonary and on severely reduced neutrophil counts.¹⁰ Much has been learned from two large registration networks: the Severe Chronic Neutropenia International Registry (SCNIR), in Seattle (USA) and Hannover (Germany) and the French SCN registry. The genes most commonly involved are *ELANE* (in 50-60% of cases) and *HAX1* (in 4-30% of cases), while mutations in *GFI1*, *WAS* and *G6PC3* have been described in smaller numbers of patients.^{3-6,9} Recently, four patients with concurrent mutations in 2 of these genes have been described, (*ELANE+HAX1*, *ELANE+G6PC3* and 2 with *HAX1+G6PC3*), challenging mutation analysis algorithms in SCN.¹¹ As of today, nearly 40% of SCN cases remain unexplained from a genetic point of view.^{12,13} For most subtypes of SCN, increased apoptosis of neutrophils and neutrophil precursors is considered the main underlying mechanism.^{12,14}

Genetic subtypes

ELANE

In 1999 *ELANE* mutations were discovered in cyclic neutropenia (OMIM #162800).² Subsequent studies revealed that heterozygous mutations in the *ELANE* gene were found in approximately 50% of SCN patients of Central European and Northern American ancestry and are the most common cause of familial and sporadic SCN (SCN1, OMIM #202700).⁴

ELANE encodes the serine protease neutrophil elastase, in azurophil granules in the cytoplasm of promyelocytes and promonocytes, where it contributes to proteolytic and antibacterial properties. SCN patients with an *ELANE* mutation have isolated neutropenia and define a subset of SCN with more severe disease, lower neutrophil counts and only modest responses to rG-CSF. According to some, *ELANE* mutant SCN patients may also have a higher incidence of AML/MDS.¹⁵⁻¹⁷

For a long time, it remained ill-understood how loss-of-function *ELANE* mutations can cause a hereditary disease with dominant inheritance.¹⁸ An elegant new hypothesis was proposed, implying the unfolded protein response (UPR) in SCN with *ELANE* mutations.^{19,20} Most secreted proteins in eukaryotes fold and mature in the endoplasmic reticulum (ER). Imbalances in the load of unfolded proteins versus the handling capacity of the ER will lead to ER stress. The UPR refers to a coordinated adaptive program protecting the cell from ER stress, by attenuating protein synthesis, by increasing ER handling capacity of unfolded proteins and by degrading misfolded proteins.²⁰ In case of overwhelming ER stress, apoptosis will be triggered.²¹ Mild UPR activation would thus lead to the less severe phenotype of cyclic neutropenia and increasing UPR

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activation to the more severe SCN phenotype.²⁰

GFI1

An extremely rare subtype of SCN is caused by autosomal dominant mutations in *GFI1* (growth factor independent 1) (SCN2, OMIM #600871). Here, a striking monocytosis and mild lymphopenia accompany the neutropenia. So far, only one sporadic¹³ and 3 familial cases^{5,18} have been described. *GFI1* represents a new link in the *ELANE* pathway, as it encodes a transcriptional repressor for *ELANE*.¹⁰ *GFI1* mutations abolish binding of *GFI1* to *ELANE* in a dominant negative fashion, leading to upregulation of *ELANE* expression, postulated to induce the UPR, although this has not been proved.²⁰

HAX1

More than 50 years after Kostmann's original report, Klein *et al.* discovered *HAX1* mutations as the genetic cause of Kostmann's disease, the autosomal recessive form of SCN.⁴ *HAX1* mutations cause premature stop codons and are loss-of-function mutations, explaining the autosomal recessive inheritance pattern. *HAX1* is ubiquitously expressed, maintains the inner mitochondrial membrane potential and protects myeloid cells from apoptosis. Absence of *HAX1* leads to increased apoptosis. Patients with Kostmann's disease have very low neutrophil counts, usually around $0.2 \times 10^9/L$. Intriguingly, *HAX1* mutations affecting only the full length *HAX1* isoform are associated with neutropenia only, whereas mutations affecting the full length isoform and a short isoform are associated with neutropenia and neurological defects, ranging from mild cognitive impairment to severe developmental delay and/or epilepsy, usually from the second decade. This suggests that the shorter isoform has a function in neurological tissue.^{22,23} The frequency of *HAX1* mutations varies widely depending on

the ethnic composition of the tested SCN cohort. Most reported patients (>22 cases) are from consanguineous marriages from Middle-Eastern (Kurdish) descent^{4,22,24} while Kostmann first described the disease in a consanguineous Norwegian family.¹

G6PC3

In 2008, a new subtype of SCN (SCN4, OMIM #612541), was identified, caused by biallelic missense mutations in *G6PC3*.⁶ As *HAX1*, these mutations have been discovered in the German SCN Registry, containing a relatively high number of intermarried Kurdish immigrant families. In addition, these patients had cardiac malformations, prominent subcutaneous veins or venous angiectasia, urogenital malformations, inner-ear hearing loss or delayed growth. Increased stress on the ER is the proposed cause of the increased apoptosis. Two new cases were recently reported.¹³

XLN

In 2001, we reported our discovery of X-linked neutropenia (XLN).³ This rare subtype of SCN with X-linked inheritance is caused by gain-of-function mutations in the Wiskott-Aldrich syndrome gene.³ These mutations are essentially different from the loss-of-function mutations in the classical Wiskott-Aldrich syndrome, which cause a triad of immunodeficiency, thrombocytopenia and eczema. In the original report, 5 XLN cases were described. Since, 13 additional cases were described^{13,14,25} while we have 3 additional unpublished cases. Infectious mortality is limited in the antibiotic era. Non-haematological manifestations have not yet been observed. Distinguishing features of XLN are monocytopenia and very low NK cell numbers. Other features are low B-cell counts, platelet counts in the low-normal range, inversion of the CD4/CD8 ratio and IgA levels in the low-normal range. Not all cases with XLN require hematopoietic growth factor support, but if needed, gratifying responses are usually observed to low-doses. B- and T- cell function do not seem to be grossly abnormal.

WAS mutations reported in XLN are missense mutations, all positioned in exon 9 encoding part of the GTP-ase binding domain (GBD) of the WASP protein.^{3,14,25} They destabilize hydrophobic interactions, essential for the auto-inhibited structure of WASP. As a result, WAS becomes constitutively active, independently of Cdc42 and will – via Arp2/3 – induce increased actin polymerization *in vitro* and in cell-based assays.^{3,25,26}

Two patients of the original Belgian XLN family developed a myeloid malignancy. In both, we found *CSF3R* mutations in leukemic phase. Thus, XLN also encompasses an increased risk of conversion to a myeloid malignancy, as does classical, autosomal SCN.

Activating *WAS* mutations strongly disturb

the cytoskeletal organisation in different *in vitro* cell models. Cell lines transfected with mutant *WASP* have a diffuse F-actin pattern and are significantly smaller than cells expressing WT *WASP*. Finally, mutant *WASP* transfected cells exhibit altered mobility patterns.²⁶ These data support the notion that an increased actin polymerization disturbs the function of the actin cytoskeleton, leading to a loss of the capacity to coordinate logical and directional cellular movement and cell migration. The mechanism by which activating *WASP* mutations cause XLN may lie in this loss of migrational capacity of progenitor cells, who fail to encounter the adequate environment and cell-cell contacts for the development into normal granulocytes. In addition, the altered cytoskeleton organisation may lead to genomic instability.^{26,27}

G-CSF, friend or foe?

Approximately 95% of SCN patients benefit from recombinant G-CSF (rG-CSF) treatment and morbidity and mortality have decreased dramatically since the availability of recombinant rG-CSF.^{28,30} Before, 42% of the patients died before the age of 2 years with a median survival of only 3 years.³¹ Since rG-CSF, sepsis mortality dropped to 0.9% per year.³² However, with longer survival of these patients, a substantial risk for leukemic conversion has emerged, and is an increasing reason for concern.³³

Even before rG-CSF, surviving patients with SCN and with Shwachman-Diamond syndrome were known to run an increased risk of myeloid malignancies,^{34,35} although the true risk was never defined. On one hand, prolonged patient survival might merely unveil an intrinsic increased risk of leukemic conversion.³⁵ On the other hand, rG-CSF might be more directly involved and act as a promoter carcinogen, by increasing myeloid mitotic activity or by protecting myeloid progenitors with mutations against apoptosis.^{9,37,38} Even if the role of rG-CSF remains unclear to date, it seems cautious to use it sparingly, and to titer the dosage individually to achieve the minimal ANC required to prevent or battle major infections.

In 2006, the cumulative incidence of MDS/AML in 374 patients of the SCNIR was reported 21% after 10 years and 34% after 15 years of rG-CSF treatment.^{17,32} In a more re-

cent report, a cumulative incidence of malignant transformation in SCN of more than 25% after 20 years of observation was reported. The malignancies were predominantly AML, but ALL, CMML and MDS have also been observed. A subgroup of patients was defined with apparently more severe disease and with poor responsiveness to rG-CSF, despite a rG-CSF dose of more than 8 µg/kg/day.³² This subgroup had a 2-fold increase in risk of death from sepsis and a cumulative incidence of malignant transformation as high as 40% after 10 years.³²

Mutations in the part of the G-CSF receptor gene (*CSF3R*), encoding the intracellular domain of the G-CSFR were first reported in SCN in 1994, and then incorrectly considered the cause of SCN.^{39,40} Later it became clear that these mutations are not inherited but acquired, in patients defining a subgroup at high risk for leukemic conversion.^{37,41,42} The frequency of *CSF3R* mutations in SCN patients with leukemic transformation is 78% in both *ELANE* and *HAX1* positive patients, whereas in SCN patients without leukemia, the frequency of *CSF3R* mutations is only 30%.⁹

CSF3R mutations in SCN are truncating mutations, leading to the loss of regulators in the carboxyterminal part of the intracellular domain of the receptor, while the N-terminal region is important for granulocyte proliferation, associated with loss of maturation and differentiation signals. As in many types of cancer, STAT5 plays an important role in this proliferative dominance.

CSF3R mutations have been described in SCN patients without progression to leukemia and some SCN patients develop leukaemia without *CSF3R* mutations. Therefore, *CSF3R* mutations are not sufficient nor required for malignant conversion.³⁷ The exact role of rG-CSF in the development of *CSF3R* mutations and in leukemic progression remains unknown. In SCN, the acquisition of a *CSF3R* mutation in SCN mostly occurs prior to malignant transformation.³⁷ Therefore, *CSF3R* mutations are considered promoter mutations, giving a growth advantage to a premalignant clone, especially in the presence of rG-CSF treatment.^{43,44}

A directly mutagenic effect seems unlikely, as *CSF3R* mutations can occur in the absence of prior rG-CSF treatment. Moreover, malignant conversion and *CSF3R* mutations are not seen with prolonged rG-CSF use in cyclic neu-

Table 1. Genetic subtypes of SCN.

Genetic subtypes of SCN				
inheritance	gene	frequency	associated features	trunc. CSF3R mutations
autosomal dominant	ELA2 (ELANE)	50%	none	yes
	GFI1	rare	monocytosis, lymphopenia	unknown
	extracellular CSF3R	rare	resistance to G-CSF	yes
autosomal recessive	HAX1	0-30%	neuropsychological defects	yes
	G6PC3	rare	cardiac and urogenital malformations	unknown
X-linked	WAS	rare	monocytopenia, very low NK cells	yes

tropenia and patients with SDS, despite treatment with rG-CSF. Eight cases with *HAX1* mutations and with *CSF3R* mutations have been described, one of whom developed myelodysplasia.^{4,9} We have described two XLN cases with *CSF3R* mutations and myeloid malignancy.^{4,5}

Chromosome 7

Monosomy of chromosome 7(q) is among the most common cytogenetic aberrations in de novo and secondary AML and MDS and is associated with an unfavourable prognosis. Loss of 7(q) is also associated with an array of congenital bone marrow failure syndromes, that carry an intrinsic high risk of myeloid leukemia, including Fanconi anemia, Shwachman-Diamond syndrome, neurofibromatosis and SCN. In different subtypes of SCN, patients were reported with *CSF3R* mutations and monosomy 7.⁴⁴⁻⁴⁷ A possible link between *CSF3R* mutations and monosomy 7(q) was suggested by the fact that monosomy 7 cells are reportedly more sensitive to high doses of rG-CSF and that rG-CSF therapy likely promotes selective expansion of a pre-existing monosomy 7 clone.⁴⁸ The acquisition of monosomy 7 is a reason to proceed to urgent stem cell transplantation.⁹

Conclusions

Major strides in the insights of SCN have been made in the last decade. However, 40% of SCN patients still have unknown mutations.^{12,13} Identification of new mutations is expected in the near future, with increasing use of high-throughput parallel sequencing.

The risk of MDS/AML is increased in SCN, independent of the genetic background and leukemic conversion in all subtypes of SCN seems associated with *CSF3R* mutations and monosomy 7(q), in a final common pathway. Although the exact sequence of events in the development of leukemia in SCN patients remains unclear, these genetic aberrations are diagnostically useful to identify a subgroup of patients with a highly increased risk of AML/MDS and in need of urgent therapeutic intervention. It remains prudent to use rG-CSF at the minimal dosage required to reach a safe absolute neutrophil count.

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