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 infanticile 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 Approximately 6 years later, HAX1 mutations, underlying Kostmann syndrome or autosomal recessive SCN, were discovered.4 GWAS mutations and G6PC3 mutations, both extremely rare, complete the list of causal gene mutations in SCN known today.5-8 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 European and Northern American ancestry and are the most common cause of familial and sporadic SCN (SCN, OMIM #202700).6 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 reduction of AML/MDS.9,10 For a long time, it remained ill-understood how loss-of-function ELANE mutations can cause a hereditary disease with dominant inheritance.11 An elegant new hypothesis was proposed, implying the unfolded protein response (UPR) in SCN with ELANE mutations.12,13 Most secreted proteins in eukaryotes fold and mature in the endoplasmic reticulum (ER). 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.14 In case of overwhelming ER stress, apoptosis will be triggered.15 Mild UPR activation would thus lead to the less severe phenotype of cyclic neutropenia and increasing UPR activation to the more severe SCN phenotype.16

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 (SCN, OMIM #202700).6 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 reduction of AML/MDS.9,10 For a long time, it remained ill-understood how loss-of-function ELANE mutations can cause a hereditary disease with dominant inheritance.11 An elegant new hypothesis was proposed, implying the unfolded protein response (UPR) in SCN with ELANE mutations.12,13 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.14 In case of overwhelming ER stress, apoptosis will be triggered.15 Mild UPR activation would thus lead to the less severe phenotype of cyclic neutropenia and increasing UPR activation to the more severe SCN phenotype.16

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 cases5-8 have been described. GFI1 represents a new link in the SCN pathway, as it encodes a transcriptional repressor for ELANE.17 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.18

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 0.2x109/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.19,20 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 \(^2^{2,24}\) while Kostmann first described the disease in a consanguineous Norwegian family.\(^1\)

**G6PC3**

In 2003, a new subtype of SCN (SCN4, OMIM #612541), was identified, caused by biallelic missense mutations in **G6PC3**.\(^3\) 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.\(^1\)

**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\(^11,14,25\) 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 monocytes 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, grafting 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 on exon 9 encoding part of the GTPase 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 **WAS** 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.\(^29,30\) 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.\(^3,37,38\) Even if the role of rG-CSF remains unclear until 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.\(^32\)

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.\(^37,32\) In a more recent 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.\(^41,44\)

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-

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**Table 1. Genetic subtypes of SCN.**

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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.\textsuperscript{4,3} We have described two XLN cases with CSF3R mutations and myeloid malignancy.\textsuperscript{45}

**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,\textsuperscript{46-47} 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.\textsuperscript{48} The acquisition of monosomy 7 is a reason to proceed to urgent stem cell transplantation.\textsuperscript{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.\textsuperscript{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.

**References**

1. Kostmann R. Infantile genetic agranulocytosis: agranulocytosis infantilis hereditaria. Acta Paediatr Suppl 1956;45:1-78.
2. Horwitz M, Benson RF, Person RE, et al. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet 1999;23: 433-6.
3. Devriendt K, Kim AS, Mathijs G, et al. Constitutively activating mutation in WASP causes X-linked severe congenital neutropenia. Nat Genet 2001;27:313-7.
4. Klein C, Grudzien M, Appasswamy G, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet 2007;39:86-92.
5. Person RE, Li FQ, Duan Z, et al. Mutations in proto-oncogene GFI1 cause human neutropenia and target ELA2. Nat Genet 2003;34:308-12.
6. Boztug K, Appasswamy G, Ashikov A, et al. A syndrome with congenital neutropenia and mutations in G6PC3. N Engl J Med 2009;360;362-7.
7. Skokova J, Welte K. LEP-1 is a decisive transcription factor in neutrophil granulopoiesis. Ann N Y Acad Sci 2007;1106:143-51.
8. Zeidler C, Welte K. Kostmann syndrome and severe congenital neutropenia. Semin Hematol 2002;39:82-8.
9. Zeidler C, Gernsheimen M, Klein C, Welte K. Clinical implications of ELA2-, HAX1-, and G-CSF-receptor (CSF3R) mutations in severe congenital neutropenia. Br J Haematol 2009;144:459-67.
10. Badolato R, Fontana S, Notarangelo LD, Savoldi G. Congenital neutropenia: advances in diagnosis and treatment. Curr Opin Allergy Clin Immunol 2004;4:513-21.
11. Gernsheimen M, Zeidler C, Stuhrmann M, et al. Digenic mutations in severe congenital neutropenia. Haematologica 2010;95:1207-10.
12. Dale DC, Link DC. The many causes of severe congenital neutropenia. N Engl J Med 2009;360:3-5.
13. Xia J, Boyard AA, Rodger E, et al. Prevalence of mutations in ELANE, GFI1, HAX1, SBDS, WAS and G6PC3 in patients with severe congenital neutropenia. Br J Haematol 2009;147:335-42.
14. Ancliff PJ, Blundell MP, Cory GO, et al. Two novel activating mutations in the Wiskott-Aldrich syndrome protein result in congenital neutropenia. Blood 2006;108:2182-9.
15. Bellanne-Chantelot C, Clauin S, Leblanc T, et al. Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French Neutropenia Register. Blood 2004; 103:4119-25.
16. Donadieu J, Leblanc T, Bader Meunier B, et al. Analysis of risk factors for myelodysplasias, leukemias and death from infection among patients with congenital neutropenia. Experience of the French Severe Chronic Neutropenia Study Group. Haematologica 2005;90:45-53.
17. Rosenberg PS, Alter BP, Link DC, et al. Neutrophil elastase mutations and risk of leukemias in severe congenital neutropenia. Br J Haematol 2008;140:210-3.
18. Horwitz MS, Duan Z, Korkmaz B, et al. Neutrophil elastase in cyclic and severe congenital neutropenia. Blood 2007;109: 1817-24.
19. Kolliner I, Sodeik B, Schreek S, et al. Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. Blood 2006; 108:493-500.
20. Grenda DS, Murakami M, Ghatkar J, et al. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. Blood 2007;110:4179-87.
21. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007;8: 519-29.
22. Gernsheimen M, Grudzien M, Zeidler C, et al. Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. Blood 2008;111:4954-7.
23. Ishikawa N, Okada S, Miki M, et al. Neurodevelopmental abnormalities associated with severe congenital neutropenia due to the R86X mutation in the HAX1 gene. J Med Genet 2008;45:802-7.
24. Smith BN, Ancliff PJ, Path MRC, et al. Identification of Two Novel Homozygous HAX1 Mutations in an Autosomal Recessive Jewish and Two Unrelated Sporadic British Families with Severe Congenital Neutropenia. Blood 2007;110.
25. Beel K, Cotter MM, Blatny J, et al. A large kindred with X-linked neutropenia with an I294T mutation of the Wiskott-Aldrich Syndrome Gene. Br J Haematol 2009;144: 129-6.
26. Moulding DA, Blundell MP, Spiller DG, et al. Unregulated actin polymerization by WASp causes defects of mitosis and cytokinesis in X-linked neutropenia. J Exp Med 2007;204:2213-24.
27. Westerberg L, Larsson M, Hardy SJ, et al. Wiskott-Aldrich syndrome protein deficiency leads to reduced B-cell adhesion, migration, and homing, and a delayed humoral immune response. Blood 2005;105: 1144-52.
28. Boxer LA, Hutchinson R, Emerson S. Recombinant human granulocyte-colony-stimulating factor in the treatment of patients with neutropenia. Clin Immunol Immunopathol 1992;62:S39-46.
29. Dale DC, Bonilla MA, Davis MW, et al. A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. Blood
1993;81:2496-502.

30. Welte K, Gabrilove J, Bronchud MH, et al. Filgrastim (r-metHuG-CSF): the first 10 years. Blood 1996;88:1907-29.

31. Ancliff PJ. Congenital neutropenia. Blood Rev 2003;17:209-16.

32. Rosenberg PS, Alter BP, Bolyard AA, et al. The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood 2006;107:4628-35.

33. Ward AC, Dale DC. Genetic and molecular diagnosis of severe congenital neutropenia. Curr Opin Hematol 2009;16:9-13.

34. Gilman PA, Jackson DP, Guild HG. Congenital agranulocytosis: prolonged survival and terminal acute leukemia. Blood 1970;36:576-85.

35. Mack DR, Forstner GG, Wilschanski M, et al. Shwachman syndrome: exocrine pancreatic dysfunction and variable phenotypic expression. Gastroenterology 1996;111:1593-602.

36. Dale DC, Cottle TE, Fier CJ, et al. Severe chronic neutropenia: treatment and follow-up of patients in the Severe Chronic Neutropenia International Registry. Am J Hematol 2003;72:82-93.

37. Germeshausen M, Ballmaier M, Welte K. Incidence of CSF3R mutations in severe congenital neutropenia and relevance for leukemogenesis: Results of a long-term survey. Blood 2007;109:93-9.

38. Germeshausen M, Welte K, Ballmaier M. In vivo expansion of cells expressing acquired CSF3R mutations in patients with severe congenital neutropenia. Blood 2009;113:668-70.

39. Dong F, Hoefsloot LH, Schelen AM, et al. Identification of a nonsense mutation in the granulocyte-colony-stimulating factor receptor in severe congenital neutropenia. Proc Natl Acad Sci U S A 1994;91:4480-4.

40. Dong F, Brynes RK, Tidow N, et al. Mutations in the gene for the granulocyte colony-stimulating-factor receptor in patients with acute myeloid leukemia preceded by severe congenital neutropenia. N Engl J Med 1995;333:487-93.

41. Dong F, Dale DC, Bonilla MA, et al. Mutations in the granulocyte colony-stimulating factor receptor gene in patients with severe congenital neutropenia. Leukemia 1997;11:120-5.

42. Tidow N, Pilz C, Teichmann B, et al. Clinical relevance of point mutations in the cytoplasmic domain of the granulocyte colony-stimulating factor receptor gene in patients with severe congenital neutropenia. Blood 1997;89:2369-75.

43. Bernard T, Gale RE, Evans JP, Linch DC. Mutations of the granulocyte-colony-stimulating factor receptor in patients with severe congenital neutropenia are not required for transformation to acute myeloid leukemia and may be a bystander phenomenon. Br J Haematol 1998;101:141-9.

44. Freedman MH, Bonilla MA, Fier C, et al. Myelodysplasia syndrome and acute myeloid leukemia in patients with congenital neutropenia receiving G-CSF therapy. Blood 2000;96:429-36.

45. Beel K, Vandenberghe P. G-CSF receptor (CSF3R) mutations in X-linked neutropenia evolving to acute myeloid leukemia or myelodysplasia. Haematologica 2009;94:1449-52.

46. Imashuku S, Hibi S, Kataoka-Morimoto Y, et al. Myelodysplasia and acute myeloid leukemia in cases of aplastic anaemia and congenital neutropenia following G-CSF administration. Br J Haematol 1995;89:188-90.

47. Kalra R, Dale D, Freedman M, et al. Monosomy 7 and activating RAS mutations accompany malignant transformation in patients with congenital neutropenia. Blood 1995;86:4579-86.

48. Sloand EM, Yong AS, Ramkissoon S, et al. Granulocyte colony-stimulating factor preferentially stimulates proliferation of monosomy 7 cells bearing the isoform IV receptor. Proc Natl Acad Sci U S A 2006;103:14483-8.