Genetic etiologies of severe congenital neutropenia

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Current Opinion in Pediatrics 2011, 23:21–26

Purpose of review
To review recent advances in severe congenital neutropenia (SCN) syndromes.
Recent findings
The majority of patients with SCN bear monoallelic mutations in the *neutrophil* elastase (*ELANE*) gene. Biallelic mutations in the antiapoptotic gene *HAX1* were identified in patients with autosomal recessive SCN. G6PC3 deficiency is a syndromic variant of SCN associating congenital neutropenia with various developmental defects including cardiac or urogenital malformations. The pathophysiology of these distinct genetic variants of SCN is complex. Increased apoptosis of neutrophil granulocytes may be caused by various molecular mechanisms including destabilization of the mitochondrial membrane potential and/or activation of the so-called 'unfolded protein response'.
Summary
SCN represents a heterogenous group of disorders that may be caused by genetic defects in *ELANE*, *GFI1*, *HAX1, G6PC3* or activating mutations in the *Wiskott–Aldrich syndrome (WAS)* gene. Ongoing research will uncover additional genetic defects in SCN patients.

Keywords
congenital, *G6PC3*, *HAX1*, neutropenia

Curr Opin Pediatr 23:21–26

1040-8703

Introduction

Children presenting with severe or recurrent bacterial infections should be worked up for quantitative and qualitative deficiencies of neutrophil granulocytes. Although children with autoimmune neutropenia or benign ethnic neutropenia usually do not develop severe infections, patients with congenital neutropenia are at high risk for life-threatening bacterial infections. In recent years, several novel genetic defects causing congenital neutropenia have been elucidated. Knowledge of the underlying genetic defects is not only relevant with respect to risk assessment of affected patients but also highlights general biological principles governing life and death of neutrophil granulocytes.

Clinical features and molecular subtypes

Neutropenia is a condition defined by low absolute numbers of peripheral neutrophil granulocytes (i.e., fewer than 1500 neutrophil granulocytes/microliter blood). Severe neutropenia refers to counts less than 500/μl peripheral blood. As a consequence, patients develop severe bacterial infections affecting lungs, skin, and deep tissues. A characteristic feature is the absence of pus. In addition, patients often show chronic gingivitis and osteopenia leading to early loss of teeth (Fig. 1a and b).

Neutropenia is mostly an acquired condition. Autoimmune and alloimmune neutropenia account for the majority of pediatric patients referred to assess low neutrophil counts.

During the last few years, several genetic causes of neutropenia have been elucidated, highlighting the heterogeneity of these disorders. The most common genetic variant associated with low neutrophil counts is found in geographic areas where *Plasmodium vivax* is endemic. A polymorphism in *DARC* (Duffy null) confers protection against *Plasmodium vivax* and has recently been associated with ethnic neutropenia [1•]. Despite low neutrophil counts, individuals with ethnic neutropenia usually do not develop severe infections.

The first genetic defects identified in patients with SCN1 involved the gene encoding neutrophil elastase, *ELANE* (formerly referred to as *ELA2*) [2,3]. *ELANE* mutations were initially discovered in patients suffering from a cyclic variant of congenital neutropenia with oscillating neutrophil counts at 21-day intervals, cyclic neutropenia (CyN) [3]. Monoallelic mutations in *ELANE* either are

 DOI:10.1097/MOP.0b013e32834262f8

inherited in an autosomal dominant pattern or occur sporadically. To date, more than 50 distinct *ELANE* mutations have been described in CyN and severe congenital neutropenia (SCN) patients (reviewed in [4]). Interestingly, there does not appear to be a clear genotype–phenotype correlation, as several *ELANE* mutations have been found in CyN and SCN patients, respectively [4].

The pathophysiology of SCN related to mutations in *ELANE* implicates an aberrant stress response in the endoplasmic reticulum. Misfolded proteins, such as mutated neutrophil elastase, evoke cellular responses aiming to avoid toxic effects to the cell [5]. When these signals are unable to reconstitute a normal endoplasmic reticulum homeostasis, the cells undergo apoptosis. Biochemical markers of premature apoptosis and increased endoplasmic reticulum stress are evident in myeloid cells from patients with mutations in *ELANE* (reviewed in [6]). However, other mechanisms may also be of relevance and are currently under investigation.

GFI1 is a transcriptional repressor and splicing control factor with eminent roles in controlling normal hematopoietic cell differentiation [7,8]. In a few patients, monoallelic mutations have been described leading to SCN2 [9,10]. In addition, mutations in *GFI1* are associated with aberrations in lymphoid and myeloid cells. Not surprisingly, a defect of an important transcriptional master switch factor leads to dysregulation of multiple pathways that may contribute to aberrant differentiation of neutrophil granulocytes.

Using genome-wide linkage analysis and candidate gene sequencing in three unrelated Turkish families, we have discovered loss-of-function mutations in *HAX1* (SCN3) [11]. *HAX1* mutations may account for approximately 15% of patients with SCN (SCN International Registry, unpublished results). Patients present either with isolated SCN or with SCN and associated neurological problems such as cognitive impairment, epilepsy, or developmental delay. This striking clinical dichotomy corresponds to differential function of two HAX1 isoforms. Patients bearing mutations which affect exclusively isoform A suffer from SCN without additional neuronal impairment, whereas mutations affecting both isoforms A and B are associated with neurological aberrations including developmental delay or epilepsy [12]. In line with these observations, targeted deletion of *Hax1* in a murine knockout mouse model leads to increased neuronal apoptosis and premature death [13]. HAX1 has pleiotropic functions, and the detailed mechanisms in HAX1 deficiency are incompletely understood. HAX1 controls the inner mitochondrial membrane potential [11,14] but is also a cytoplasmic protein with multiple interaction partners. For example, HAX1 binds to several viral proteins, suggesting that various viruses may have developed strategies to interfere with apoptosis programs in the cell.

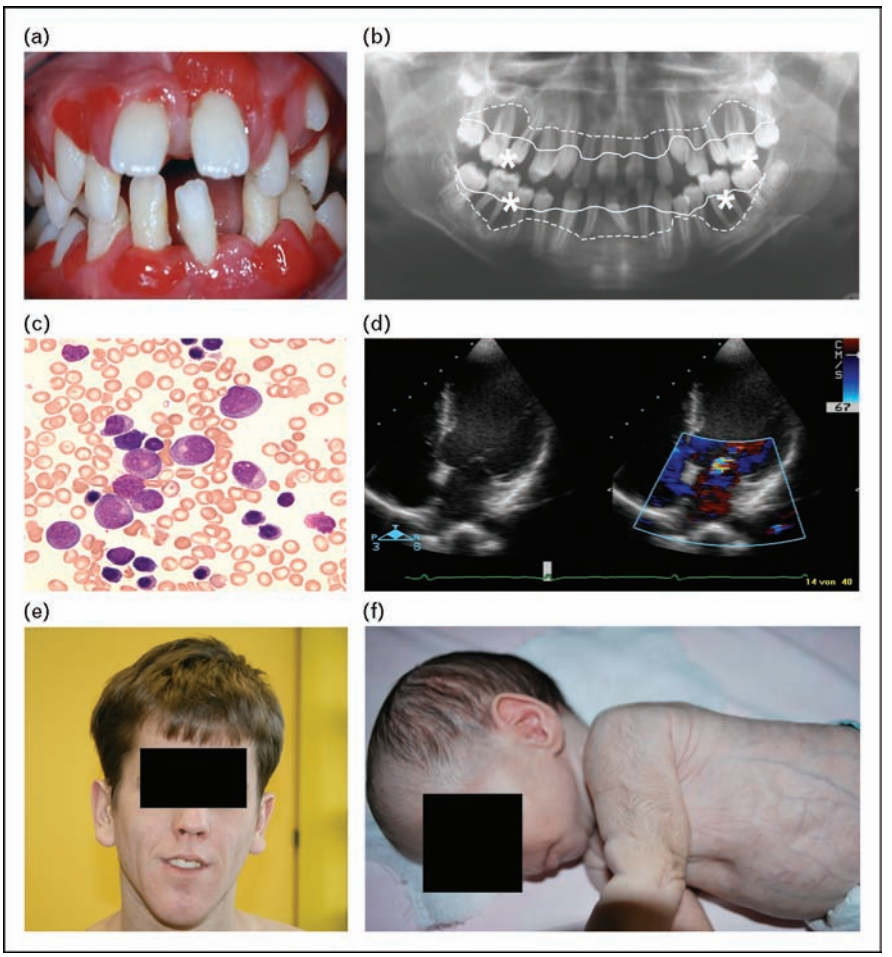
Key points

- Severe congenital neutropenia (SCN) comprises a heterogenous group of disorders commonly characterized by low numbers of absolute neutrophil counts (<500/μl) and an increased susceptibility to bacterial and fungal infections.
- Known genetic variants include defects in the genes encoding neutrophil elastase (ELANE), GFI1, WAS, and G6PC3.
- In one-third to half of all patients with SCN, none of these genes is mutated, warranting further research to discover novel genetic defects.
- The molecular pathophysiology of SCN is complex and only partially understood. For example, increased neutrophil apoptosis can be associated with mitochondrial dysfunction and/or aberrant signaling in the endoplasmic reticulum.

Neutrophil granulocytes are particularly vulnerable to defects in glucose metabolism. It has been known for a long time that patients with glycogen storage disease type Ib, caused by mutations in the gene encoding the glucose-6-phosphate-transporter protein [15], suffer not only from symptoms related to hypoglycemia and glycogen storage but also from congenital neutropenia. A related disorder, G6PC3 deficiency, affects a ubiquitously expressed homologue of glucose-6-phosphate named G6PC3 (SCN4) [16••]. Patients with mutations in *G6PC3* suffer from a syndromic variant of congenital neutropenia with typical myeloid maturation arrest (Fig. 1c) and various other congenital aberrations. In addition to cardiac (Fig. 1d) and urogenital defects, patients may show facial dysmorphia (Fig. 1e), increased visibility of superficial veins (Fig. 1f), inner ear hearing loss, endocrine abnormalities, or myopathy [16••,17] (and Boztug K *et al.*, unpublished observations). The molecular pathophysiology of G6PC3 deficiency involves activation of the unfolded protein response and increased endoplasmic reticulum stress [16••,18], associated with the disturbed intracellular glucose homeostasis [16••,19].

Deleterious mutations in *WAS* usually cause Wiskott–Aldrich syndrome, an X-linked immunodeficiency disorder characterized by recurrent infections, eczema, and bleeding [20]. Wiskott–Aldrich syndrome protein (WASP) is an adaptor protein orchestrating polymerization of actin filaments [21•]. WASP-deficient cells show defects in cellular locomotion and receptor relocalization. In contrast to WASP deficiency, rare mutations in *WAS* cause a constitutive activation of WASP, leading to increased formation of actin polymers and consecutively

Figure 1 Clinical findings in severe congenital neutropenia



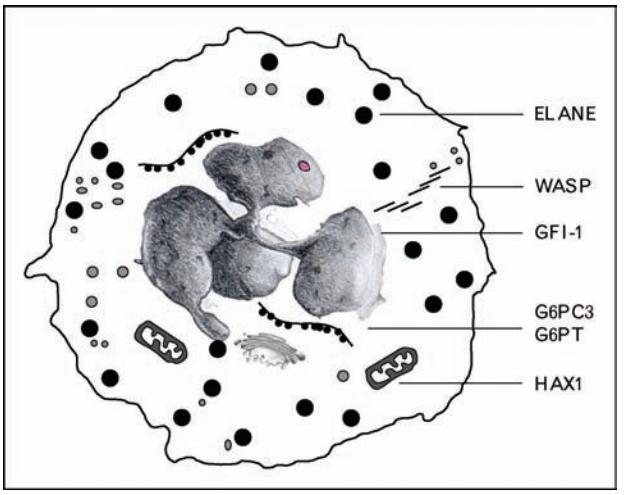
(a) Gingivitis in a 12-year-old patient suffering from severe congenital neutropenia (SCN) prior to initiation of treatment using recombinant human granulocyte colony-stimulating factor (rh-G-CSF). The gingiva is fiery red and oedematous enlarged around some teeth. As a result of the periodontal attachment loss, the teeth are spaced and showed an increased mobility. (b) Panoramic radiograph of the dental status from the same patient. The difference between the expected physiological alveolar bone level (continuous line) and the actual alveolar bone margin (dotted line) shows the extensive loss of periodontal attachment. (c) Bone marrow smear in a patient with G6PC3-deficient SCN showing the classical picture of the so-called 'maturation arrest' at the promyelocyte/myelocyte stage. The appearance of bone marrow smears in other genetic subtypes of SCN is indistinguishable from the picture seen in G6PC3 deficiency. (d–f) G6PC3 deficiency represents a syndromic variant of SCN. Patients often have congenital heart defects such as atrial septal defects (ASD; d), facial dysmorphy (e) or an increased superficial venous marking, as shown in a mature newborn baby (f). Part (d) is reproduced with permission from [16••]; the remaining images are original.

aberrant cell divisions [22,23]. These patients do not have the classical features of Wiskott–Aldrich syndrome but rather show congenital neutropenia [24–26] associated with myelodysplasia [24,25], increased myeloid cell apoptosis [22,25], and lymphoid cell abnormalities [23,24].

The prevalence of these defined genetic subgroups depends on the ethnic background. An analysis of patients by the North American Severe Chronic Neutropenia Registry showed that the majority of patients had mutations in *ELANE* (90 of 162 patients, 55.6%) [27]. In patients with wild-type *ELANE* alleles, only five patients had a mutation in either *GFI1*, *WAS*, *SBDS*, or *G6PC3* [27]. In contrast, mutations in *HAX1* or *G6PC3* appear to be more frequent in Turkish or Middle Eastern patients. In many patients, no genetic defect can be identified as yet. Genome-wide studies are ongoing to discover novel genetic defects in patients with SCN.

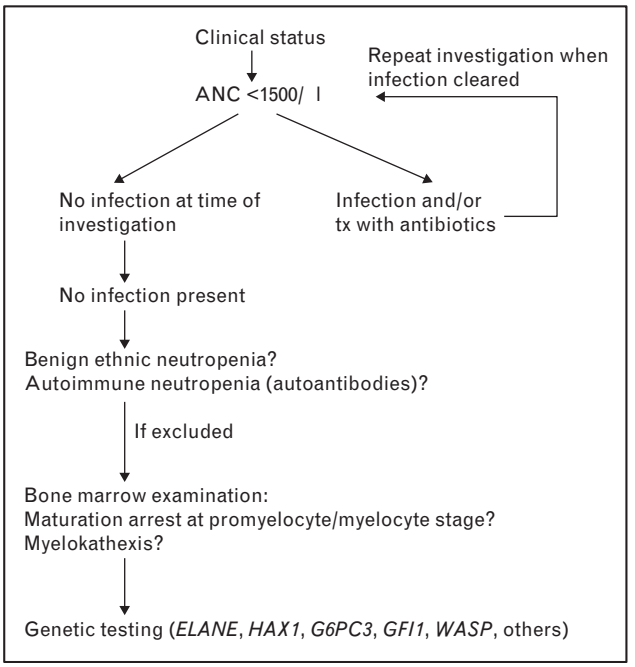
In addition to the genetic defects causing SCN described above (summarized schematically in Fig. 2), congenital neutropenia can be seen in several syndromic disorders. For a detailed summary of these multiorgan diseases, the reader is referred to recently published reviews [28,29]. Genetic testing for SCN ought to be performed by specialized laboratories with expertise in the genetic analysis of these disorders. Figure 3 shows a simplified flowchart for diagnostics in nonneonatal patients suspected to suffer from SCN.

Figure 2 Schematic drawing illustrating the cellular localization of the different genetic defects identified in severe congenital neutropenia until present



As shown, the gene products may involve proteins with predominant localization in the neutrophil granules (e.g., ELANE), cytoplasm (WASP), endoplasmic reticulum (G6PC3, G6PT), mitochondria (HAX1) or nucleus (GFI-1).

Figure 3 Proposed flowchart for diagnostic evaluation of pediatric patients with neutropenia exluding patients with syndromic features and/or neonates



If congenital neutropenia is suspected, a specialized reference center ought to be contacted regarding genetic testing and treatment. ANC, absolute neutrophil count; tx, therapy.

Recent data indicate that patients with SCN may not only have decreased numbers of neutrophil granulocytes, but may also have defective function. This is illustrated by defective expression of proteins stored in specific or azurophilic granules, such as myeloperoxidase, lactoferrin, cathepsin G, and human-neutrophil-peptide [30]. Furthermore, ELANE expression levels are significantly lower in myeloid cells from SCN patients harboring *ELANE* or *HAX1* mutations [31]. Recent studies have highlighted the role of lymphoid enhancer-binding factor 1 (LEF-1) for neutrophil granulopoiesis, and have demonstrated reduced expression of LEF-1 in myeloid progenitor cells in SCN patients irrespective of the genetic cause [32].

Natural history and therapeutic interventions

Although the treatment of patients suffering from SCN has improved significantly due to the availability of recombinant human granulocyte colony-stimulating factor (rh-G-CSF) [33], patients with SCN still have limited life expectancy and impaired quality-of-life. The standard therapy consists of daily subcutaneous injections of rh-G-CSF (standard dose 3–5 μg/kg per day) [34]. The use of pegylated G-CSF has been assessed in patients with SCN, yet this drug may lead to less efficient control of infections and increased risk of therapy-associated side-effects such as bone pain or allergic reactions [35]. Even though most patients respond to therapy using rh-G-CSF by increased counts of neutrophil granulocytes, they continue to be at risk for osteopenia, gingivitis, and sometimes even severe infections. Infectious episodes in SCN patients require prompt clinical attention and broad-spectrum antibiotic therapy.

Importantly, many genetic subgroups of SCN are at risk for myelodysplastic syndromes (MDS) or acute leukemia [36–38]. Data from international registries suggest that 20–30% of patients develop such a clonal hematopoietic disorder. At risk are patients with mutations in *ELANE*, *HAX1* or *WAS* [24,25,36–38], whereas the risk of MDS/acute myeloid leukemia (AML) transition may be lower in patients with G6PC3 deficiency (Boztug K *et al.*, unpublished). Patients with insufficient response to rh-G-CSF treatment or development of MDS/AML are candidates for allogeneic hematopoietic stem cell transplantation (HSCT). A recent study from Japan reported the experience with 18 SCN patients refractory to rh-G-CSF therapy undergoing HSCT [39•]. Nine patients received stem cells from an HLA-identical sibling donor and nine from an alternative donor using various conditioning regimens. Sixteen patients are reported to be alive and in complete remission with a median follow-up of 6.5 years. Several centers have established protocols for allogeneic hematopoietic stem cell transplantation. Optimized risk-based preparative regimens and

transplant protocols are being developed. In addition, preclinical gene therapy investigations may serve as a basis for the development of hematopoietic stem cell gene therapy for genetically defined variants of SCN in the future.

Conclusion

Remarkable progress has been made in the elucidation of the various genetic causes of congenital neutropenia syndromes. Nonetheless, a significant proportion of patients remain genetically unclassified, and we expect that state-of-the art genomic technologies will further enhance discovery of the genetic causes of these disorders. An in-depth understanding of the molecular pathophysiology of granulopoiesis will be necessary to enable the development of molecular therapies in the future.

Acknowledgements

The authors would like to thank the members of the International Chronic Neutropenia Registries for ongoing support, the Bundesministerium für Bildung und Forschung and Deutsche Forschungsgemeinschaft for financial support, and Dr Reinhard Schilke (Hannover Medical School) for his generous sharing of photographs. K.B. is recipient of a fellowship by the Else-Kröner-Fresenius-Stiftung.

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