To the editor:

Expression of granule-associated proteins in neutrophils from patients with severe congenital neutropenia

In a recent publication in Blood, Donini et al conclude that granulocyte colony-stimulating factor (G-CSF) treatment of severe congenital neutropenia (SCN) results in abnormal expression of granule-associated proteins. We have previously published this concept, and showed that neutrophils from patients with SCN lack the antimicrobial peptide LL-37 and have reduced levels of defensins (HNP1–3), but are functionally capable of both phagocytosis and generating oxidative radicals. Our recent results (Figure 1) differ in part to those of Donini et al.

SCN is a heterogeneous disease that is characterized by bone marrow failure to produce normal numbers of mature neutrophils. The mechanisms behind this arrest involve apoptosis and 2 genes that are frequently mutated, HAX1 and ELA2, in autosomal recessive and autosomal dominant/sporadic SCN, respectively (reviewed in Skokowa et al). Without correction of neutrophil levels, the patients are at risk for life-threatening infections. Despite treatment with G-CSF that normally reverses neutropenia, many patients still are at risk for infections, notably in the oral cavity.

We have recently analyzed circulating neutrophils from 4 patients with SCN and compared with 3 control participants and 1 patient who underwent bone marrow transplantation. Lysozyme, myeloperoxidase (MPO), and lactoferrin are produced at similar levels, while neutrophils from some but not all patients with SCN have lower levels of gelatinase and HNP1–3 (Figure 1). Compared with the results of Donini et al, our patients are not deficient in lactoferrin, HNP1–3 (mature), or MPO. In addition, Donini et al noted normal levels of ELA2 mRNA, while Skokowa et al present patients with reduced ELA2 mRNA levels.

What could be the reason for this variation in expression, and is it of functional significance? One important issue may be the responsiveness to G-CSF treatment. Although the authors state that G-CSF reverses neutropenia (which commonly is the case), 2 of their patients in fact respond poorly to the treatment, and thus a proper increase of neutrophil protein synthesis may not occur.

A second issue is the cohort chosen. It is estimated that about 60% of patients with SCN harbor mutations in ELA2. However, more than 40 different sites for mutations are known, and thus the impact of a specific mutation may vary. Donini et al investigated 3 patients with 2 different ELA2 mutations (G185R, P176fsX182), while our study included 2 patients with ELA2 mutations at other locations (L92H, C265). Besides abnormal protein expression, neutrophils from patients with SCN are known to respond poorly to IMLP with diminished chemotaxis and O2− generation, the latter in accordance to findings by Donini et al. A previous report concludes that neutrophils from patients with SCN kill Staphylococcus aureus, while Donini et al reported reduced killing of Escherichia coli and Candida albicans.

Taken together, the results presented by Donini et al, interesting as they are, may be restricted to certain ELA2 phenotypes, especially G185R, and not a general phenomenon of patients with SCN. Additional clinical details of the patients presented by Donini et al would have been useful for comparison to earlier work, but also to understand the pathophysiology of SCN.

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References
It is conceivable that association of a specific genotype with the levels of ELA2 mRNA.5 For differences due to posttranslational processing of the protein. In any case, we believe that analysis of ELA2 mRNA may not be required for higher G-CSF dosage, and show higher risk of developing myelodysplastic syndromes or acute myeloid leukemia4; this unfavorable outcome is observed in children presenting a poor response to the treatment and/or receiving large doses of G-CSF (higher than 8 µg/kg per day).9 Accordingly, in the 2 SCN patients with the ELA2 mutation 4942G>A, one presented with omphalitis and one, with severe pneumonia. These patients required prolonged intravenous antibiotic therapy and large G-CSF dosage. Because of the unsatisfactory hematologic response to this treatment, and despite the large G-CSF doses used, the patients were subjected to bone marrow transplantation. The patient bearing 4899 dele mutation, who also displayed an early onset of bacterial infections and absolute neutrophil counts at the same levels as the other 2 patients, had a more favorable outcome and is still under treatment with G-CSF. These opposite clinical outcomes may depict 2 extremes of the same disease, characterized by distinct patterns of antimicrobial peptide expression in granulocytes, and possibly, by diverse prognosis. Therefore, our study supports the hypothesis of a genotype-phenotype correlation, and we believe that when other ELA2 genotypes will be investigated at the protein level, the study of neutrophil antimicrobial peptides will probably gain clinical significance.

**Response:**

**ELA2 genotype-phenotype correlation to be expected in patients with severe congenital neutropenia**

We appreciate the letter by Andersson and colleagues regarding our recent report on granulocyte colony-stimulating factor (G-CSF) response in children with severe congenital neutropenia (SCN). Although there are several causes of SCN, including **ELA2, HAX1,** and **Gfi1** mutations, our study was designed to define the effects of **ELA2** mutations on antibacterial machinery of neutrophils isolated from children under treatment with G-CSF.1-3 We showed abnormal elastase, myeloperoxidase, cathepsin-G, and human neutrophil peptide expression, as well as antimicrobial deficiency in neutrophils bearing 4924G>A or 4899 dele (Pro 176 Pro fs 7) mutation of **ELA2.**

We agree with Andersson et al that different mutations could lead to distinctive cellular features. In our report, neutrophils from 2 SCN patients with the 4942G>A mutation display altered electrophoretic mobility of 60 kDa myeloperoxidase heavy chain and complete absence of cathepsin-G and lactoferrin, whereas cells of a patient with 4899 dele mutation show an expression pattern characterized by reduced—but still detectable—myeloperoxidase, cathepsin-G, and lactoferrin. Of note, this latter pattern resembles those observed by Andersson et al in 2 SCN patients with 92L>H and 26C>S mutations of **ELA2.**

Andersson et al point out that, in contrast to our report, Skokowa et al describe reduced ELA2 mRNA levels in SCN patients. This discrepancy could be due to the fact that we measured ELA2 mRNA in total hematopoietic cells, whereas the other authors analyzed the expression in isolated myeloid cells.5,6 In any case, we believe that analysis of ELA2 mRNA may not be adequate to characterize SCN patients because it does not account for differences due to posttranslational processing of the protein. In accord with our hypothesis, Skokowa et al failed to demonstrate an association of a specific genotype with the levels of ELA2 mRNA.5 It is conceivable that **ELA2** mutations might instead influence intracellular accumulation and trafficking of the protein.7

Patients with 4942G>A mutation display severe neutropenia, require higher G-CSF dosage, and show higher risk of developing...
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