Description of an *ELANE* Mutation in a Girl with Severe Congenital Neutropenia: A Paradigm of Targeted Genetic Screening Based on Clinical Findings

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Abstract

We describe the case of a 5-year-old girl with severe congenital neutropenia presenting with recurrent skin and respiratory infections. Sequence analysis of *ELANE* and *HAX1* genes identified a mutation in heterozygous state in exon 2 of the *ELANE* gene: c.157C > G (p.His53Asp), not previously described in the literature at the exon coding level. Given the autosomal dominant inheritance and the location of the mutation within a “hotspot,” this mutation was considered as clinically relevant. *ELANE* should be screened in patients with congenital neutropenia of no obvious etiology. A detailed medical history and clinical evaluation can prevent unnecessary investigations allowing for a targeted diagnostic strategy.

Keywords

- *ELANE*
- congenital neutropenia
- pediatric hematology

Introduction

The “many genetic faces” of severe congenital neutropenia present a challenge for clinicians. A series of genes have been found to be involved in the pathogenesis of this entity: *ELANE*, *HAX1*, *G6PC3*, *WAS*, *GCSF3R*, and *GFI1* (→ **Table 1**). The common endpoint of changes in these genes is the apoptosis of neutrophils and their precursors through various mechanisms (loss of survival signals, accumulation of proapoptotic factors, misfolding of neutrophil elastase, endoplasmic reticulum stress, disturbed glucose metabolism).¹

Case Description

A 5-year-old girl with congenital neutropenia was referred to our department with left lobar pneumonia. Symptoms started 7 days before and the girl received antibiotic treatment per os with no improvement. A chest X-ray was taken 2 days before and showed left lower lobe consolidation.

Our patient is the first child of healthy and nonconsanguineous parents and was born at term after an uneventful gestation. At 40 days of age she developed a skin abscess, which was surgically drained. During her hospitalization, severe neutropenia was first assessed. Since then the girl exhibits recurrent skin and respiratory infections.

A computed tomography chest scan was performed and revealed an area of low attenuation with decreased parenchymal enhancement in the left low lobe with air–fluid levels within this area. Intravenous antibiotic administration was initiated (cefepime and linezolid). Blood cultures were negative. Our patient also responded positively to subcutaneous granulocyte colony-stimulating factor (G-CSF) administration. The child got afebrile 3 days later.

With regards to congenital neutropenia investigation, our laboratory tests revealed severe neutropenia (absolute neutrophils count: 0–100 cells/μL) with normal hemoglobin and normal lymphocyte, monocytes, and platelet counts.

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Table 1 Most frequent genes associated with severe congenital neutropenia

Gene	Chromosome	Protein	Useful clues
<i>ELANE</i>	19p	Neutrophil elastase	Often without additional signs/symptoms, autosomal dominant inheritance
<i>HAX1</i>	1p	HCLS1-associated protein X-1	Neurodevelopmental disorders probably, autosomal recessive inheritance
<i>G6PC3</i>	17q	Glucose-6-phosphatase 3,	Frequent cardiac abnormalities, thrombocytopenia, autosomal recessive inheritance
<i>CSF3R</i>	17q	GCSF receptor	Resistance to GCSF administration, autosomal dominant inheritance
<i>WAS</i>	Xp	Wiskott–Aldrich syndrome protein	Rare, thrombocytopenia, X-linked inheritance
<i>GFI1</i>	1p	Zinc finger protein Gfi-1	Rare, autosomal dominant inheritance

Abbreviations: GCSF, granulocyte colony-stimulating factor; HCLS, hematopoietic lineage cell-specific.

Distribution of lymphocyte subsets, serum immunoglobulins, protective antibody response to previous immunizations, complement, and dihydrorhodamine test were normal too. Abdominal ultrasound and echocardiography had no pathologic findings. No antineutrophil antibodies were found in the patient’s serum, while screening for inborn errors of metabolism had normal findings. Bone marrow aspiration showed paucity of mature neutrophils.

Upon informed consent, sequence analysis of the *ELANE* and *HAX1* gene was performed. Additional genes associated with congenital neutropenia were excluded from our analysis in the view of our patient’s clinical traits and inheritance patterns of these genes (►Table 1, ►Fig. 1). Genetic screening revealed a mutation in exon 2 of the *ELANE* gene in the heterozygous state: c.157C > G (p.His53Asp). To our knowledge, this mutation has not been previously described in the literature at the exon coding level, although a description at the genomic level was provided by Fioredda et al.² Furthermore, this genetic change was not described in genetic databases and no data about the minor allele frequency was available in the literature. Parents were recommended to undergo testing for the identified mutation to determine if it is de novo or inherited, but they have not, yet, performed it.

Treatment lasted for 3 months, including intravenous and oral antibiotic treatment. The girl is now under regular follow-up, while she receives oral prophylactic antibiotic treatment. A new computed tomography chest scan was performed 3 months later showing significant improvement. Hematopoietic cells transplantation is also under consideration in our patient as a permanent therapy.

Discussion

According to the current literature data, the *ELANE* gene encoding for neutrophil elastase is the most frequent genetic cause in patients with severe congenital neutropenia. To date, more than 100 mutations in the *ELANE* gene have been described in patients with severe congenital neutropenia. Mutations in this gene are generally spread over the entire sequence and mainly consist of missense amino acids substitutions, while their inheritance pattern is the

autosomal dominant.¹ On the other hand, it should be noted that in countries with high rates of consanguinity congenital neutropenia is more likely to be due to mutations in genes with recessive patterns of inheritance.^{3,4} In general, *ELANE* mutation carriers exhibit a more severe phenotype of congenital neutropenia, compared with noncarriers, in terms of recurrent bacterial infections, younger onset age, and need for more intensive GCSF treatment schedule.⁵ On the other hand, cases of patients showing congenital neutropenia of different severity even with the same mutation of the *ELANE*, as well as cases of asymptomatic mutation carriers, have also been described. Besides, there is a genetic overlap between severe congenital neutropenia and the more benign condition of cyclic neutropenia, thus implying a potential role of additional modifying genes in the determination of the specific phenotype within the spectrum of the same disease process.^{5–9}

Although the identified mutation in the *ELANE* (c.157C > G) found in our case has not been previously described in the literature at the exon coding level,² it seems to affect a “hotspot” codon, as additional pathogenic missense mutations have been previously documented at this codon (p.His53Gln, p.His53Leu, p.His53Tyr).⁷ Moreover, the affected amino acid position 53 has been found to be highly conserved among species, while the physicochemical differences between histidine and aspartic acid (molecular weight, polarity, composition) are classified as moderate, according to the Grantham score (distance: 81).¹⁰ To further confirm the pathogenicity of the identified mutation, we used online prediction programs (POLYPHEN2, SIFT). According to their results, the mutation was predicted to be “probably damaging.” Under this view and given the autosomal dominant inheritance pattern associated with the *ELANE* gene, we strongly believe that the specific amino acid replacement has a significant impact on the functionality of the neutrophil elastase and the identified mutation is pathogenic, although due to technical limitations we could not perform segregation analysis or accurately measure neutrophil elastase activity (►Fig. 2).

Despite progress in the molecular analysis techniques, it is estimated that approximately 40% of patients with severe congenital neutropenia demonstrate no mutations in any of

Fig. 1 Differential diagnosis of genetic causes of congenital neutropenia in our patient. Clinical features associated with each genetic mutation are described in ► **Table 1**.

the aforementioned genes.¹¹ Diagnostic approach should be primarily based on medical history and on findings of physical evaluation, as nonhematologic parameters may provide useful clues, appropriately guide diagnostic investigations and permit a more targeted genetic testing. For instance, the coexistence of cardiac abnormalities is associated with changes in the *G6PC3* gene, while resistance to G-CSF treatment strongly implies mutations in the G-CSF receptor gene¹² (► **Table 1**). Conversely, severe neutropenia is frequently the only finding in patients with *ELANE* gene mutations.¹¹ In this way, Dale and Link suggest *ELANE* genotyping in all patients with severe congenital neutropenia and selective testing of other genes based on clinical findings. In any case, bone marrow examination is the most determinant step of the diagnostic process.¹¹

It should be highlighted that genetic findings in these patients cannot be directly translated into new treatment options and gene therapy is still in an experimental stage. Nevertheless, specific mutations have clinical significance. More specifically, patients with severe congenital neutropenia due to *ELANE* mutations compared with those without *ELANE* mutations have been found to exhibit an earlier median age of diagnosis, lower neutrophil counts, increased frequency of recurrent infections, a stronger dependence from G-CSF administration, and a higher risk for progression to myelodysplasia/acute myelogenous leukemia after G-CSF treatment.^{5,13} In this way, the *ELANE* gene could be used to individualize treatment options and minimize adverse events (e.g., more regular follow-up, prophylactic treatments). In future, the systematic record of new mutations

Fig. 2 Taking into account data from genetic databases, results from prediction programs, and patient's medical history, we can assume that the identified mutation is clinically relevant. Abbreviation: CN, congenital neutropenia.

could permit a more effective classification of clinical features and complications (e.g., per codon affected).

Conclusion

Future genetic studies are anticipated to shed light into the molecular background of congenital neutropenia and permit better genotype–phenotype association, as well as more effective genetic counseling and treatment options. *ELANE* sequencing should be considered in any patient with severe congenital neutropenia of no obvious cause. A detailed medical history and clinical evaluation can prevent from unnecessary investigations and appropriately guide genetic testing in patients with congenital neutropenia.

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Conflict of Interest
None declared.

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