

Mutations in *ELANE* and *COH1* (*VPS13B*) Genes Cause Severe Neutropenia in a Patient with Cohen Syndrome

Lauren Beene^{1,2}, Baozhong Xin¹, Claudia Lukas³ and Heng Wang^{1,2*}

¹DDC Clinic -- Center for Special Needs Children, Middlefield, Ohio, USA

²Department of Pediatrics, Case Western Reserve University School of Medicine, Rainbow Babies and Children's Hospital, Cleveland, Ohio, USA

³Department of Hematology-Oncology, All Children's Hospital, St. Petersburg, Florida, USA

*Corresponding author: Heng Wang, MD, PhD, DDC Clinic -- Center for Special Needs Children, 14567 Madison Road, Middlefield, OH 44062, USA, Tel: 440-632-1668; Fax: 440-632-1697; E-mail: wang@ddcclinic.org

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Abstract

In this case report we describe a patient with Cohen syndrome and severe neutropenia. The patient was found to have a mutation of previously undetermined significance in the *ELANE* gene and compound heterozygous mutations in the *COH1* gene causing Cohen syndrome. While the mutation in *ELANE* may not have led to clinically significant neutropenia independently, the presence of this mutation in conjunction with mutations in *COH1* led to neutropenia that was more severe than what is typically seen in Cohen syndrome. This case report suggests that the combination of mutations in *ELANE* and *COH1*, both impacting similar intracellular trafficking mechanisms, led to an exaggerated clinical phenotype. Based on this case presentation, we encourage consideration of additional candidate genes when an identified genetic mutation cannot fully explain the scope or severity of the clinical picture, as recognition of all mutations impacting a clinical phenotype will help achieve a more comprehensive diagnosis.

Keywords: ELANE; ELA2; COH1; Severe congenital neutropenia; Cohen syndrome; Synergistic effect

Introduction

Congenital neutropenia is characterized by chronic neutropenia presenting early in life and is attributed to numerous genetic causes, either isolated or as part of a syndrome [1]. The most common cause of isolated congenital neutropenia is *ELANE*-related severe congenital neutropenia (SCN) [2-4]. Additional cases of isolated congenital neutropenia have been attributed to mutations in other genes such as *CSF3R*, *GFI1*, *WAS*, and *CXCR4* [1]. Congenital neutropenia is also a feature of numerous genetic syndromes, including Kostmann disease (*HAXI*), SCN type 4 (*G6PC3*), Hermansky-Pudlak syndrome type 2 (*AP3B1*), Cohen syndrome (CS, *COH1*), and others [1].

Neutropenia in *ELANE*-related SCN is characteristically severe (absolute neutrophil count [ANC] <500 cells/ μ l) and is associated with significant bacterial infections in childhood, and affected individuals almost universally require treatment with granulocyte colony stimulating factor (G-CSF) [1,4]. The neutropenia of CS is described as non-cyclic, mild-to-moderate (ANC usually ranging from 500-1200 cells/ μ l), and not typically requiring treatment with G-CSF [5-8]. The extra-hematopoietic features of CS are broad and include facial dysmorphism, microcephaly, developmental delay, hypotonia, joint hypermobility, retinal dystrophy, progressive high myopia, and truncal obesity (OMIM #216550) [8-12].

In this case report we describe a patient with severe neutropenia who was found to have mutations in both *ELANE* and *COH1*.

Case

A 14-year-old girl with complex medical history had been evaluated for severe chronic neutropenia over the course of four years. She was initially referred to hematology-oncology at age 10, and at that time her neutropenia was non-cyclical and ranged from mild-to-severe (ANC 200-1100 cells/ μ l). Her neutropenia continued to dip into the severe range, and at age 14 she experienced recurrent infections of Streptococcal pharyngitis, otitis media, and methicillin resistant *Staphylococcus aureus* skin lesions. She had a history of mouth ulcers and gingivitis, but no other significant infections.

Of note, the patient also had multiple congenital clinical findings including hypotonia, joint laxity, microcephaly, facial dysmorphism, myopia, and severe developmental delay. Previous evaluations to explain her neutropenia and syndromic features were unrevealing. There was no family history of clinically significant neutropenia or recurrent infections. There was no family history of consanguinity.

Physical exam was notable for microcephaly, short stature, and truncal obesity. Abnormal facial features included prominent eyebrows, prominent nasal tip, full lips with wide philtrum, and posteriorly rotated ears with simple helices. She had increased extensibility of the elbows and small joints, 2,3 toe syndactyly, and long and slender fingers and toes. She had one café-au-lait lesion on the abdomen.

Her neutropenia was then investigated more closely. Immunological causes of neutropenia were largely excluded. ANA and granulocyte antibody were negative. Immunoglobulins were within normal limits. Coombs test was weakly positive, which was suspected to be secondary to drug-effect as reticulocyte count was not elevated and she had no history of anemia. Her bone marrow biopsy was normal.

Genetic testing was performed for mutations in the *ELANE* gene. Results revealed a heterozygous c.770C>T variant in exon 5 of *ELANE* in the patient (Figure 1), resulting in replacement of a proline with a leucine residue at codon 257 of the *ELANE* protein (p.Pro257Leu). The patient's father was heterozygous for this mutation, while he did not have a history of clinically significant neutropenia, he was later found to have borderline neutropenia with ANC 1400 cells/ μ l. The patient's mother was wild type for this variant.

As the *ELANE* mutation alone could not explain her extra-hematopoietic features, a *COH1* (*VPS13B*) gene test was also performed and revealed 2 heterozygous mutations, a deletion of 4 nucleotides in exon 11 leading to frameshift (c.1495_1498delTTTG, p.Phe499Ilefs*28), and a C to T nucleotide substitution in exon 14 leading to a stop codon (c.1915C>T, p.Arg639Ter) (Figure 1). While

not previously reported, these mutations are predicted to result in truncated protein. Further, none of them has been observed in the 1000 Genomes Project or the NHLBI Exome Sequencing Project databases, indicating they are not common benign variants in the general population. Thus the detection of these two mutations was consistent with a diagnosis of CS. The patient's father was found to be a carrier of the c.1915C>T mutation (Figure 1). The patient's mother did not carry a *COH1* mutation in her blood DNA, and thus the patient's second mutation could be explained by maternal mosaicism or *de novo* mutation.

Over the next few months the patient's ANC levels continued to decrease, with her lowest ANC 90 cells/ μ l. She was ultimately started on G-CSF with immediate improvement of her neutropenia.

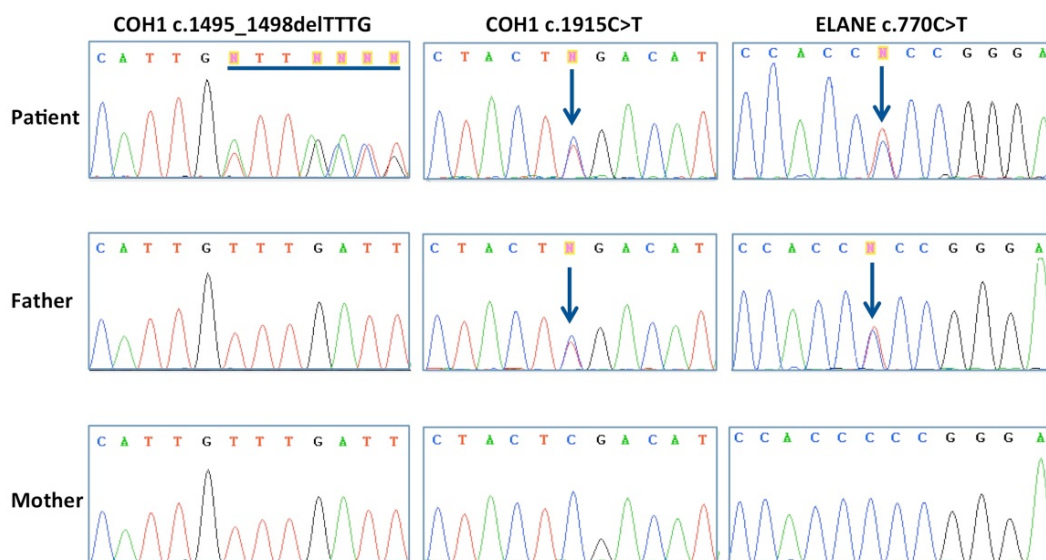


Figure 1: Sequencing results for *COH1* and *ELANE* genes in the patient and both parents. Sanger sequencing was performed using genomic DNA isolated from whole blood. The base-pair number of the mutation site was determined according to the GenBank mRNA reference sequence NM_001972.2 and NM_017890.4 for *ELANE* and *COH1*, respectively. The electropherograms show the two heterozygous mutations (c.1495_1498delTTTG and c.1915C>T) in *COH1*, and the heterozygous c.770C>T mutation in *ELANE*. Affected residuals are underlined or indicated by an arrow.

Discussion

Here we describe the atypical presentation of severe neutropenia in a girl with Cohen syndrome who also harbors a mutation of previously undetermined significance in *ELANE*, the gene most often associated with SCN [3]. This case brings to light the potential role of synergy in genetic disorders, as well as the importance of considering a second mutation when clinical and laboratory features cannot be explained by mutation in a single gene.

In this patient case, a heterozygous missense mutation was identified in the carboxyl terminus of *ELANE* (Pro257Leu). Multiple mutations associated with SCN have been discovered in this region, however Pro257Leu was previously described as having undetermined significance [13-16]. Specifically, Germeshausen et al. detected Pro257Leu in 2% of individuals with SCN or cyclic neutropenia (CN)

and in 1.2% of controls, and while this mutation was predicted to be deleterious, it was ultimately classified as a non-disease-causing low frequency polymorphism [15]. Similar variants of undetermined significance have also been found in nearby DNA regions such as Pro228Leu and Pro233Leu in individuals with SCN and CN [16,17].

Given these prior reports, as well as the status of the patient's father as carrying the Pro257Leu mutation, this patient's *ELANE* mutation could have been dismissed. However, we suspect the combination of mutations in *ELANE* and *COH1*, both impacting similar intracellular trafficking mechanisms, is what explains our patient's exaggerated phenotype. Indeed, *COH1* (or *VPS13B*, vacuolar protein sorting 13B) encodes a matrix protein required for maintained integrity of the peripheral Golgi membrane [18]. A recent study detected an abnormal glycosylation pattern in serum proteins of individuals with CS, and found early endosomes to be nearly absent in CS fibroblasts [19]. These

findings, along with the known role of its *Saccharomyces cerevisiae* homologue *VPS13B* in trafficking of membrane proteins from the trans-Golgi network to the prevacuolar compartment, suggest *COH1* plays a role in endosomal-lysosomal trafficking [11].

Mutations in *ELANE* may lead to dysfunctional intracellular trafficking of the protein it encodes, neutrophil elastase [1,13,20-22]. Upon exposure to inflammatory stimuli, bactericidal neutrophil elastase is released to the extracellular environment [1,20]. It is suspected that mutated *ELANE* is not properly shuttled to azurophilic granules, and is instead sent in excess to the plasma membrane where it causes damage [21,22]. This theory is strengthened by the finding that mutated *ELANE* has impaired binding to AP3, an adaptor protein that shuttles cargo proteins from the trans-Golgi network to lysosomes [21,23]. Mutations in AP3 causes canine CN and Hermansky-Pudlak syndrome type 2, a genetic disorder of humans associated with neutropenia and partial albinism [14,21].

While SCN is traditionally considered a monogenetic disorder, the current case presentation adds support for the potential of more than one mutation to impact the phenotype of inherited neutropenia. Previous studies have also presented this possibility. In particular, Germeshausen et al. discussed a potential role for digenicity in the phenotypic spectrum of SCN in their report of four individuals each with mutations in 2 separate candidate genes [24]. Mutations in additional candidate genes such as *CSF3R*, *GFI1*, *WAS*, and *CXCR4* may also potentially contribute to the phenotypic spectrum, however these genes were not specifically examined in this case report [1]. The potential for modifier genes such as the transcription factors *GFI1* and *LEF-1* to impact clinical variability has also been proposed due their known ability upregulate expression of *ELANE* [25,26].

Conclusion

In conclusion, this patient's mutation in *ELANE* may not have led to clinically significant neutropenia. However, in conjunction with the compound heterozygous *COH1* mutations, the patient developed neutropenia that was more severe than what is typically seen in Cohen syndrome. Based on this we encourage consideration of additional candidate genes when an identified genetic mutation cannot fully explain the scope or severity of the clinical picture. Recognition of all mutations impacting a clinical phenotype will not only help achieve a more comprehensive diagnosis, but it will also help us gain a deeper understanding of the condition at hand.

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Disclosure of conflicts of interest

The authors have no conflicts of interest.

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