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Case report

SEVERE CONGENITAL NEUTROPENIA CAUSED BY THE ELANE GENE MUTATION IN A 4-YEAR-OLD VIETNAMESE GIRL

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ABSTRACT

Severe congenital neutropenia (SCN) is an exceptionally rare genetic disorder associated with life-threatening bacterial infections. Among the several genetic variations related to SCN, heterozygous mutations in the ELANE gene encoding neutrophil elastase account for approximately 40 - 55% of the genetic causes. Herein, we present the first documented case of SCN in a Vietnamese girl from the Central region of Vietnam. The diagnosis was confirmed through genetic analysis of the ELANE gene, a known causative gene in SCN. The patient exhibited severe neutropenia and a history of recurrent infections that did not respond well to treatment. Treatment involved the administration of granulocyte-stimulating factor (G-CSF) and antibiotics, resulting in a successful increase in neutrophil counts. This report contributes to the understanding of SCN's clinical presentation, diagnosis, and management, particularly in regions with limited documented cases.

Keywords: *Neutropenia, severe congenital neutropenia, ELANE mutation.*

I. INTRODUCTION

Congenital neutropenia, a rare genetic disorder, can be categorized into two primary subtypes: Severe congenital neutropenia (SCN) and Cyclic neutropenia (CN) [1]. Typically, severe congenital neutropenia (SCN) is defined by extremely low absolute neutrophil count (ANC) (< 0.5 x 109/L for at least three months) and recurrent life-threatening bacterial infections [2]. The incidence of SCN is estimated to be 1 in 200,000 individuals [3].

Among several associated genetic mutations, heterogeneous mutations of the ELANE gene have been associated with both SCN and CN. ELANE mutations are known to correlate with more severe neutropenia and serious manifestations in SCN [4, 5]. In fact, ELANE mutations are the most recognized cause of congenital neutropenia and account for 40 - 55% of cases [1]. ELANE encodes a cytotoxic serine protease called neutrophil elastase, and over 200 ELANE mutations have been identified [3]. Most of the mutations (~80%) are missense mutations, although mutations that lead to splicing defects (~10%) and premature stop codons (~10%) also have been observed [6].

Reports of SCN originating from developing countries remain scarce, with few documented cases. The first reported case of SCN in Vietnam dates back to 2015 [7], followed by sporadic reports from various regions in the country. Additionally, clinical signs of this rare condition frequently overlap with those of other infectious diseases, leading to delayed or missed diagnoses. This report documents the first known case of SCN in a girl from Central Vietnam treated at Hue Central Hospital. The diagnosis was confirmed through mutation analysis of the ELANE gene, with the goal of improving SCN diagnosis and management through this report.

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II. CASE PRESENTATION

A 4-year-old girl was admitted to Hue Central Hospital because of a cough and high fever (39 - 40^0C). She had been treated at home with oral Cefuroxime (a 2nd Cephalosporin) for 4 days, but her symptoms did not improve. Upon clinical examination, she exhibited pale skin, rapid breathing, and moist crackles in both lungs. Her liver and spleen were not enlarged, but there was noticeable enlargement of neck lymph nodes.

Laboratory tests: Hemoglobin level of 9.5g/dL, white blood cell count of 5.3k/µL, neutrophil count of 0.0k/µL, lymphocyte count of 2.9k/µL, monocyte count of 2.2k/ µL, C-reactive protein (CRP) level of 83.1mg/L. The x-ray showed bilateral lung infection and moderate right pleural effusion (Figure 1). Because of the severe infection and neutropenia, the patient's medical history was collected and analyzed to evaluate. Her previous history of neutropenia or neutrophil count was unknown. However, she had experienced recurrent respiratory tract infections, otitis media, and gastrointestinal infections that did not respond well to anti-infection therapy. Specifically, she had otitis media at 9 months, followed by severe community-acquired pneumonia when she was 12 months old. Afterward, she experienced frequent episodes of respiratory infections and oral fungal infections, averaging around 7 to 8 episodes per year.

Two months prior to this admission, she had another episode of severe pneumonia with complications, including pleural effusion, and she received treatment for one month. Approximately one month before hospitalization, she had chickenpox and has since recovered. She had not been vaccinated against influenza and pneumococcal disease and was not taking any medications or toxins. Her family had no recorded history of allergies, asthma, blood diseases, or recurrent infection conditions.

Numerous tests were performed to determine whether her neutropenia was congenital or acquired. Tests for infection-related neutropenia were mostly negative. Viral serologies for CMV, HSV, EBV, and HIV were negative. Both blood culture and fungal culture also yielded negative results.

The hemogram results indicated several noteworthy findings: hypochromic anemia, a decreased white blood cell count, and an increase in two peripheral blood cell lines - platelets and monocytes. Bone marrow aspiration yielded no evidence of malignant cells and revealed reduced granulocyte proliferation. Further examinations, including tests for tuberculosis, hemophagocytosis, autoimmune diseases, and cancer, all returned normal results. Consequently, the possibility of acquired neutropenia was ruled out, leading us to focus on hereditary causes.

Given that certain primary immunodeficiencies can manifest as neutropenia, such as hyper IgM syndrome or Wiskott Aldrich syndrome, we conducted additional tests to exclude these disorders. Subsequent results indicated an elevated IgG level, specifically by 25.6 g/L. Serum titers of IgA and IgM, as well as the percentage of CD+ cells in flow cytometry, remained within normal ranges. Therefore, other immunological causes were entirely ruled out.

She received a regimen of broad-spectrum antibiotics, combining a 3rd cephalosporin with aminoglycoside, alongside antifungal prophylaxis. Additionally, subcutaneous injections of granulocyte-stimulating factor (G-CSF) were administered. Given the patient's medical history, it was deemed reasonable to initiate G-CSF treatment while awaiting genetic testing.

The initial G-CSF dosage was 5µg/kg/day for three days, followed by an increase to 6µg/kg/day for the subsequent five days. Unfortunately, there was minimal improvement in neutrophil levels compared to the first days of her admission, as depicted in Table 1, which illustrated the pre-and post-treatment changes in complete blood count.

The G-CSF dosage was incrementally raised every three days. Eventually, her neutrophil levels reached 3.47k/µL on the third day with around 8µg/kg/day of G-CSF. After a month of intensive treatment for severe neutropenia, during which she continued to receive G-CSF at a dose of 8µg/kg/day, the patient was discharged from the hospital in a healthy condition.

Table 1: Changes in the patient's complete blood count over the course of treatment.
[a] Before taking G-CSF. [b] After taking G-CSF.

Date	WBC (k/µL)	NEU (k/µL)	Hb (g/dL)	PLT (k/µL)
12/06/2023 [a]	5.3	0.0	9.5	280
13/06/2023 [a]	4.5	0.0	10.0	280
20/06/2023 [a]	6.2	0.1	10.1	572
26/06/2023 [a]	7.7	0.1	11.2	435
29/06/2023 [b]	4.9	0.0	11.9	289
30/06/2023 [b]	7.2	0.0	11.3	237
03/07/2023 [b]	7.4	0.2	10.5	178
06/07/2023 [b]	5.6	0.8	10.8	213
10/07/2023 [b]	9.45	3.47	10.8	319
27/07/2023 [b]	8.78	1.18	11.4	268

Genetic mutation testing results revealed that the patient's severe neutropenia resulted from a heterozygous mutation in the neutrophil elastase ELANE gene located on chromosome 19: c.242G > C (p.Arg81Pro). This mutation involved a change from G to C in the 242nd base, resulting in a codon alteration from Arginine to Proline. Consequently, the patient was diagnosed with severe congenital neutropenia (SCN).

Since her discharge, the patient has remained free of fever or signs of infection. G-CSF treatment continues, and she undergoes regular follow-up appointments to adjust the G-CSF dosage with the goal of maintaining an ANC within the range of 1.0 to 1.5x109/L.

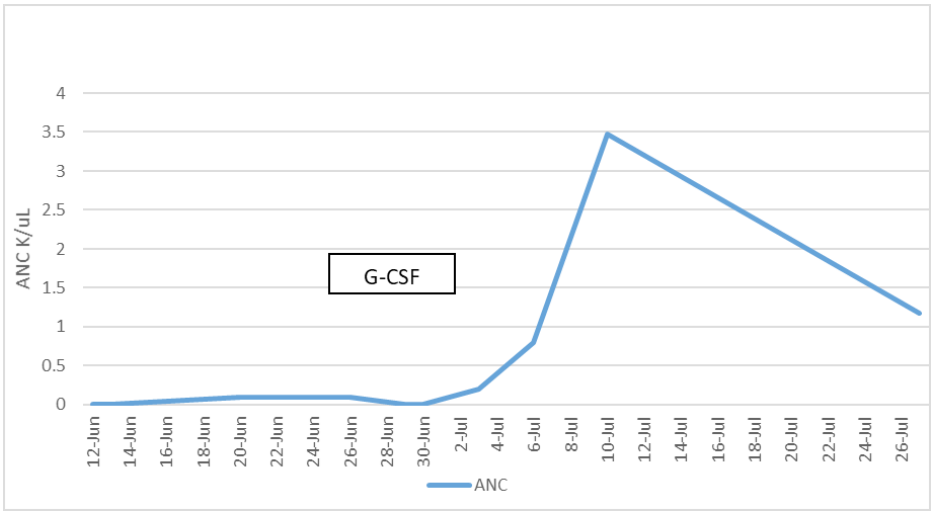

Figure 1: Absolute neutrophil count (ANC) during the first 2 months following the diagnosis of SCN, as influenced by G-CSF administration.

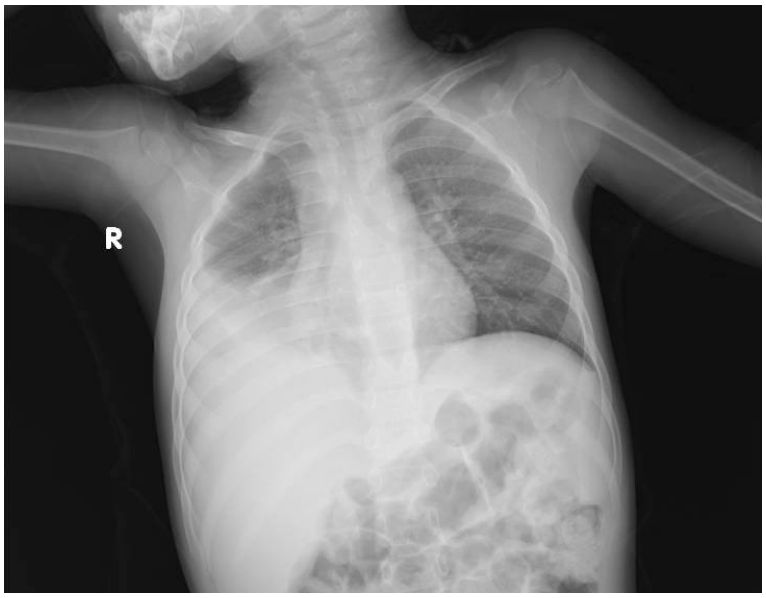

Figure 2: Chest X-ray at the time of admission

III. DISCUSSION

Severe congenital neutropenia (SCN), initially described in 1956, is a rare heterogeneous disorder characterized by an exceptionally low ANC (< 0.5x109/L for at least three months) associated with recurrent, life-threatening bacterial infections [2]. SCN encompasses a group of rare diseases that result from diverse defects in myeloid cell proliferation and maturation [4]. Cyclic neutropenia (CN) is another subtype of congenital neutropenia, characterized by neutropenia that typically recurs in a 21-day periodicity. Compared to CN, SCN is associated with more severe neutropenia and more serious clinical manifestations, and it confers an additional risk of developing acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) [1, 3].

While the classical form of both disorders may be easily identifiable, a continuum of phenotypes occurs (i.e. variable length of neutropenia in CN or oscillating neutropenia in SCN), because the pathological mechanisms which result in the neutropenia affect both the degree of the nadir and the interval of fluctuations [4, 8]. If an ELANE gene mutation is present, the designation ELANE-related neutropenia or ELANE-associated neutropenia has been used, regardless of the pattern of variation in neutrophils [8]. It is unclear which factors determine whether a patient with an ELANE mutation will develop an SCN or CN phenotype, however, host factors and modifying genes have been proposed to play a role [4, 9].

Neutrophil elastase (NE) is a serine protease stored in the azurophilic granules of neutrophils along with other bactericidal proteins, and it plays a key role in host defense against bacteria [10]. NE is encoded by the ELANE gene on chromosome 19 and mutations in this gene have been implicated in both CN and SCN, with ELANE mutations seen in a majority of SCN and almost all CN cases [11]. Nowadays, more than 200 ELANE mutations have been identified [3].

Several hypotheses have been proposed to explain the pathogenic effects of NE mutations, one of which posits that the neutropenia is attributed to the accelerated apoptosis of neutrophil precursors secondary to mutant neutrophil elastase not being processed and packaged normally in the cell primary granules. The mutant enzyme impairs the survival of myeloid precursors through the initiation of the unfolded protein response, leading to programmed cell death and early-stage maturation arrest of myelopoiesis [9]

Horwitz et al. also proposes the mislocalisation hypothesis after demonstrating that mutant NE in neutrophils from patients with ELANE mutations

was mislocalised within the cell. They postulate this mislocation results in neutropenia as a result of induced endoplasmic reticulum stress which activates the unfolded protein syndrome with resultant apoptosis [11].

Diagnosis of ELANE-related neutropenia relies on the clinical presentation and companying history, serial measurements of ANC over a period of weeks, and demonstration of an ELANE gene mutation by molecular testing. A bone marrow biopsy is useful in assessing abnormalities in myelopoiesis and may be helpful in the demonstration maturation arrest. Karyotyping using conventional cytogenetics will help in demonstration of any chromosomal lesions that may be associated with specific syndromes if present. Additionally, it is crucial to rule out congenital malformations and investigate for extra haematopoietic system involvement to exclude other syndromes that are associated with neutropenia [8].

Management is multi-dimensional and includes treatment of manifestations of neutropenia (e.g. infections, fever, etc.), prevention of primary manifestations and secondary complications, as well as surveillance for malignant transformation. The introduction of G-CSF was a breakthrough in the treatment of congenital neutropenias and before its widespread use, patients regularly succumbed to life-threatening bacterial infections. The mainstay of therapy is daily subcutaneous G-CSF and vigorous treatment of infections. The goal is to maintain ANC > 1x109/L. It is recommended to start with a low dose of G-CSF (1-3 micrograms/kg/day). If the patient is not responding the dose can be increased. The majority respond well to G-CSF. Overall survival of individuals with SCN is estimated to exceed 80% [3]. However, since its introduction in 1998, G-CSF has attracted considerable attention owing to its potential risk of malignancy [12]. Recently, a strong relationship between G-CSF treatment and malignant transformation in SCN has also been reported [13]. Therefore, the dose, timing, and duration of G-CSF therapy should be very carefully determined in patients with congenital neutropenia, such as SCN and CN [3, 13]. Approximately five percent of patients do not respond even to higher doses of G-CSF. Haemopoietic stem cell transplantation should be considered in patients with refractory disease.

The girl we herein report showed typical manifestations of SCN including severe neutropenia and serious infection. ANCs have ranged from 0 to 3.47 k/µL due to the response of G-CSF. After excluding other secondary and primary causes of neutropenia, we thought much of this patient's severe neutropenia was due to genetic abnormalities. After the G-CSF (with the dose of G-CSF gradually increasing to 8 µg/kg/day) treatment, her neutrophil counts grew up, making mutations of the patient's G-CSF receptor gene less likely. To confirm the diagnosis of SCN, we sent out blood specimen to Gene laboratory for the Congenital Neutropenia Panel. The genes tested in the panel include ACTB, CSF2RA, CSF3R, CTSC, ELANE, G6PC3, GATA2, GFI1, HAX1, IFNGR2, LAMTOR2, LYST, RAC2, SLC37A4, SRP72, VPS13B, WAS. The ELANE gene is the most common gene alteration in SCN [4, 8, 14]. Finally, the gene result showed that in chromosome 19, there was a heterozygous mutation in the ELANE gene: c.242G > C 9p.Arg81Pro), with the change of G to C in the 242nd base, resulting in a change of the 81st codon (Arginine to Proline). This mutation has been reported to cause severe congenital neutropenia and cyclic neutropenia. So, this gene result is suitable for clinical manifestations.

IV. CONCLUSION

In summary, we report a child genetically diagnosed with Severe congenital neutropenia (SCN) due to ELANE mutation. This is the first case diagnosed in Hue Central Hospital and Central region of Vietnam. The case report may contribute to accumulating the number of SCN cases, which will help to expand the knowledge on clinical presentation, diagnosis, and management on this rare disease.

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