

## Case Report

# A Chinese Girl with ELANE-related Severe Congenital Neutropenia

SB CHENG, PPW LEE, TL KU, CH LI

**Abstract** ELANE-related severe congenital neutropenia is a very rare genetic disorder characterised by very low absolute neutrophil count at early age. Affected individuals suffer from recurrent infections and have potential of malignant transformation. This paper describes a case report of a Chinese girl who had severe neutropenia since neonatal age with recurrent perianal and oral infection. Clinical features, diagnosis and management of ELANE-related severe congenital neutropenia are discussed. Clinicians are alerted how to differentiate severe congenital neutropenia from usually benign chronic neutropenia.

**Key words** Children; Congenital neutropenia; ELANE-related neutropenia; Neutropenia

### Case Report

A Chinese girl born to non-consanguineous parents was referred to haematology clinic for evaluation of persistent neutropenia at the age of 5 months. She was born with prematurity of 30 weeks and her birth weight was 1.54 kg. She was the first child in the family with unremarkable family history. During neonatal period, she had mild respiratory distress syndrome, grade 1 necrotising enterocolitis resolved by intravenous antibiotics and supportive management. She did not have omphalitis or delayed umbilical cord detachment. She was discharged

from the neonatal unit on day 59 of life. There was no syndromal feature or signs of exocrine pancreatic insufficiency.

Her total white cell count, haemoglobin and platelet counts were normal. The absolute neutrophil count (ANC) was  $9.22 \times 10^9/L$  (N:  $6-23.5 \times 10^9/L$ ) at birth and it dropped to  $1.4 \times 10^9/L$  on day 10 of life. Since 51-day-old, the ANCs were noted persistently less than  $0.2 \times 10^9/L$  (Figure 1). No cyclic pattern of ANC or abnormal cells was noted. IgA, IgG and IgM levels were unremarkable. The anti-neutrophil antibody was negative. The girl had repeated infections involving oral mucosa and skin since three months of age. Her infection history was summarised in Table 1.

At 13-month-old, bone marrow examination showed granulocytic hypoplasia with markedly reduced in granulopoiesis and neutrophilic series was virtually absent. Only monocytic and eosinophilic series were evident. Erythropoiesis was active and megakaryocytes were adequate. There was no cellular infiltration or overt dysplastic features. In view of persistent severe neutropenia and repeated infection, whole exome sequencing was performed and she was found to have a heterozygous nucleotide deletion in exon 5 causing frameshift and premature stop mutation in the *ELANE* gene ( $5:c.618_627del:p.L206fs$ ), which was confirmed by Sanger sequencing. Hence, the patient was diagnosed to have ELANE associated severe congenital neutropenia (SCN). Sanger sequencing of the *ELANE* gene was

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Received March 18, 2020

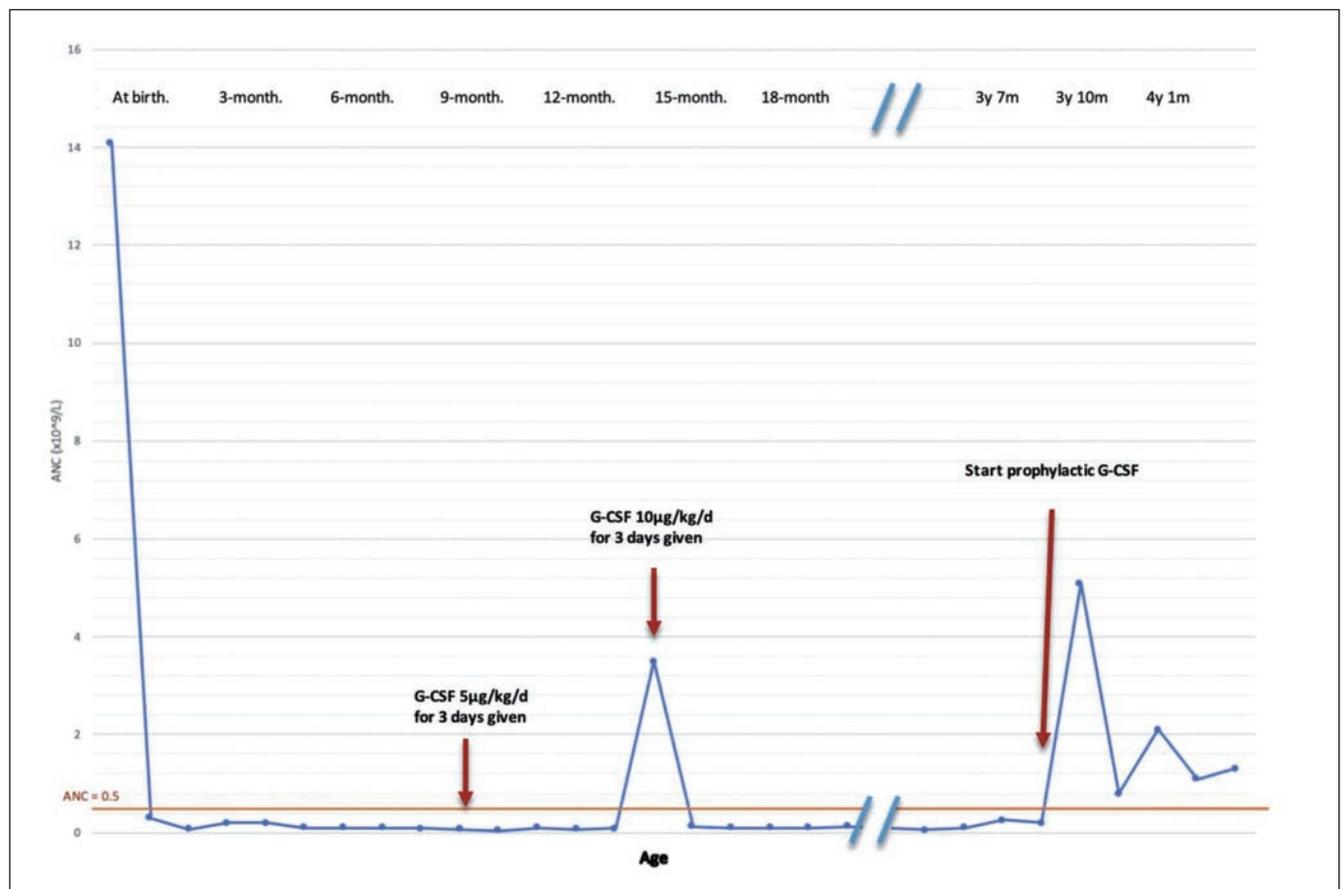
performed for her parents and both were not found to be carriers of the mutation.

Granulocyte colony stimulation factor (G-CSF) treatment (5 microgram/kg/day for 3 days) was tried when she was 9-month old during an episode of vulval abscess. However, there was no response with ANC remained  $0.07 - 0.09 \times 10^9/L$  after the treatment. During an episode of severe oral infection at 13-month old, she was given high dose of G-CSF treatment at 10 microgram/kg/day for three days. Her ANC responded and rose from  $0.06 \times 10^9/L$  to the peak of  $3.5 \times 10^9/L$ . Prophylactic G-CSF was initially declined by parent. At 3-year-old, the child still suffered from recurrent infection with repeated hospital admission once a month. The weight gain was not satisfactory and dropped to 10th percentile. After further discussion, parent agreed to start regular G-CSF 10 microgram/kg/day at cycle of three consecutive days and rest two days. Parents were educated and empowered to do subcutaneous G-CSF injection at home. The neutrophil could be maintained

above  $0.5 \times 10^9/L$  (Figure 1) with no more hospitalisation for infection. The family was referred to university center for consideration of haematopoietic stem cell transplantation. There was no suitable donor identified yet. Six months after starting regular G-CSF therapy, the patient showed catch-up in body weight back to 25th percentile and enjoyed normal development at the age of 4 years.

## Discussion

Neutropenia is a disorder of abnormally low absolute neutrophil count (ANC) in the blood. It is classified as severe when ANC is less than  $0.5 \times 10^9/L$ . Diagnosis of congenital neutropenia requires three episodes of ANCs lower than  $0.5 \times 10^9/L$  for at least three months after birth. Patients with SCN usually have ANC less than  $0.2 \times 10^9/L$  since infancy.<sup>1</sup>



**Figure 1** Absolute neutrophil count of the patient.

It is not uncommon for children to have acquired neutropenia after viral infection or intake of drugs such as sulfonamides, anticonvulsants, phenothiazines, chemotherapy agents, anti-thyroid drugs etc..<sup>2</sup> In such cases, the children have normal ANC before and their ANC level return to normal after the infection subsided or the causative drug is removed. In children with chronic neutropenia which defined as neutropenia on at least three occasions over 3 months, it is important to differentiate them from chronic benign neutropenia and severe congenital neutropenia. Chronic benign neutropenia includes two groups of disorder, autoimmune neutropenia (AIN) and chronic idiopathic neutropenia (CIN). AIN and CIN share a similar clinical course with the difference only in the presence of anti-neutrophil antibody in AIN but absent in CIN. The majority of children with chronic benign neutropenia do not have life threatening infection.<sup>3</sup>

SCN patients usually suffer from recurrent episodes of significant or life-threatening infections. The most frequent sites of infection are skin and mucosa, ear, nose and throat and the lungs. Diffuse mucosal lesion could involve digestive tract leading to abdominal pain and diarrhoea.<sup>1</sup> For our patient, she was found persist severe neutropenia (ANC < 0.2x10<sup>9</sup>/L) since 1 month of age, repeated episodes of febrile illness and infection over skin and oral mucosa

required multiple hospital admissions and antibiotic treatment.

Kostmann syndrome, first described by Rolf Kostmann in a Swedish publication in 1950 was considered as the paradigm of congenital neutropenia.<sup>4</sup> Kostmann reported on 6 children with severe neutropenia (ANC < 0.2x10<sup>9</sup>/L) at first week of life with arrest of granulocytic differentiation at the promyelocytic stage. Without treatment, affected children suffered from recurrent fever, skin infections, oral ulcers and even died from severe infections. Fifty years later, homozygous mutations in the gene encoding the mitochondrial protein HCLS1-associated X1 (HAX1) were identified in these patients.<sup>5</sup> With the advancement of molecular science, SCN is now deemed a genetically heterogenous group of related disorder. The discovery of most forms of autosomal-dominant SCN, and virtually all forms of cyclic neutropenia, are due to mutations in the coding region of ELANE (previously known as ELA2), the gene for neutrophil elastase.<sup>6</sup> Nowadays, many congenital syndromes with genetic mutations are also known to be associated with congenital neutropenia, e.g. Kostmann syndrome (HAX1), glycogen storage disease type 1b (SLC37A4), Shwachman-Diamond syndrome (SDBS gene), Chediak-Higashi syndrome (LYST), Barth syndrome (TAZ),

**Table 1** Summary of infection in the patient

Age	Presenting symptom	ANC (10 <sup>9</sup> /L)	Culture result	Treatment	Outcome
3 months	Fever and cough	0.2	No organism found	IV Cefotaxime	Full recovery
7 months	Chickenpox	0.1	Not done	IV Acyclovir	Full recovery
9 months	Vulval abscess	0.08	Abscess swab: Pseudomonas Aeruginosa, Acinetobacter species	IV Amoxicillin-Clavulanate G-GSF 5 microgram/kg/day for 3 days	Abscess resolved ANC after G-CSF: 0.09x10 <sup>9</sup> /L
11 months	Perianal ulcer and abscess	0.1	Abscess swab: Enterobacter, Citrobacter	IV Vancomycin & Timentin followed by oral Levofloxacin & Linezolid	Full recovery
12 months	Perianal abscess	0.07	Abscess swab: E. coli, Aeromonas Streptococcus oralis	IV Vancomycin & Timentin, followed by oral Augmentin & Linezolid plus topical fusidic acid	Abscess resolved
13 months	Lower lip ulcers and abscess	0.1	No organism found	IV Ceftriaxone & Meropenam IV Acyclovir IV & oral Fluconazole G-CSF 10 microgram/kg/day for 3 days	Full recovery ANC after G-CSF: 3.5x10 <sup>9</sup> /L
16 months	Fever, oral thrush and cellulitis of nose	0.0	No organism found	IV Tazocin & oral fluconazole, acyclovir	Full recovery

ANC=absolute neutrophil count; G-CSF=granulocyte colony stimulation factor

dyskeratosis congenita (various genes associated including *DKC1*, *TERC*, *TERT*, *CTCT*) etc.

ELANE-related neutropenia includes SCN and cyclic neutropenia (CyN). Mutations in *ELANE* were found in 80-100% of individuals with well-documented cyclic neutropenia and 35-63% of individuals with congenital neutropenia.<sup>6</sup> In a review by Markaryan et al. with the data from the Severe Chronic Neutropenia International Registry (SCNIR) on genotype-phenotype relationships of *ELANE* mutations, there were 187 SCN patients with 94 mutations and 120 CyN patients with 22 mutations. Twelve overlapping mutations were observed in both CyN and SCN patients. Although the distribution of mutations in CyN versus SCN was statistically significantly different in premutation tests of specific mutations, mutation class and mutation position, individual mutation may not be strictly correlating with the phenotype. However, in the cohort all frameshift *ELANE* gene mutations were associated with severe congenital neutropenia.<sup>7</sup> The genetic variant 5:c.618\_627del:p.L206fs in our case was not reported before in other literature.

Bone marrow examination is often helpful to confirm the diagnosis of SCN and to rule out other disorders such as myelodysplasia or leukaemia. Bone marrow in SCN patients typically shows maturation arrest at the promyelocyte stage of neutrophil formation and cytogenetic analysis is normal.<sup>1</sup>

CyN is distinguished from SCN by regular oscillation of neutrophil counts. In most cases of CyN, neutropenia recurs on an average of every 21 days while SCN patients shows no cyclic pattern. Infectious complications in CyN patients are generally milder and it is not associated with an increased risk of malignancy or conversion to leukaemia.<sup>6</sup>

Individuals diagnosed with *ELANE*-related SCN, regular dental examination for gingival and periodontal disease, prompt treatment of fever and infection with antibiotics, and careful evaluation by an otolaryngologist and pulmonologist for chronic sinopulmonary inflammation and deep abscess are recommended. The administration of prophylactic antibiotics e.g. Cotrimoxazole was commonly used. Treatment with granulocyte colony-stimulating factor (G-CSF) is effective in preventing infections, alleviating symptoms in almost all affected individuals by raising blood ANC and reducing mortality from sepsis. The required doses are usually daily or alternate-day injections of 5-10 microgram/kg/day.<sup>8</sup> Our patient only responded to high dose G-CSF (10 microgram/kg/day). Regular evaluation for evidence of myelodysplasia (MDS) or acute myelogenous leukaemia

(AML) is recommended. Individuals with congenital neutropenia with or without an *ELANE* variant have cumulative incidences of 21% for MDS or AML, after 10 years of started treatment with G-CSF.<sup>9</sup>

Haematopoietic stem cell transplantation (HSCT) is the curative treatment for *ELANE*-related congenital neutropenia.<sup>10</sup> For individuals with SCN who are refractory to high-dose G-CSF or who undergo malignant transformation, HSCT is the only alternative treatment.

In summary, we report a case of *ELANE*-related congenital neutropenia in a Chinese girl. The severe neutropenia may result in life-threatening pyogenic infections, acute or chronic gingivostomatitis, sinusitis or skin infection. It can be differentiated from benign chronic neutropenia by its more serious clinical course with repeated significant infections. Clinicians should be alerted and further investigated with bone marrow and genetic tests. Aggressive antibiotic treatment for infections, use of G-CSF and HSCT have significantly improved the outcome of this group of patients.

## Acknowledgement

We thank Prof. Yu-Lung Lau and Dr. Wanling Yang from Department of Paediatrics and Adolescent Medicine the University of Hong Kong for performing whole exome sequencing and genetic confirmation for this patient and Hong Kong Society for Relief of Disabled Children for funding the genetic testing. We also thank the family for consent to report this case.

## Declaration of Interest

None

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