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DE NOVO MUTATIONS OF *ELANE* GENE IN THREE VIETNAMESE PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA

Duong Anh Linh[1,3], Nguyen Thi Van Anh[2], Nguyen Van Tung[1,3], Nguyen Huy Hoang[1,3], Ngo Diem Ngoc[4], Nguyen Thi Phuong Mai[4], Ngo Manh Tien[4], Nguyen Thi Kim Lien[1,*]

[1]Institute of Genome Research, VAST, Vietnam
[2]Immunology and Rheumatology Department, Vietnam National Hospital Pediatrics, Vietnam
[3]Graduate University of Science and Technology, VAST, Vietnam
[4]Human Genetics Department, Vietnam National Hospital Pediatrics, Vietnam

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ABSTRACT

Severe congenital neutropenia (SCN) is a congenital condition in which granulocytes mature abnormally owing to a variety of genetic defects, resulting in immunodeficiency. Among the several genetic variations related to SCN, heterozygous mutations in the *ELANE* gene encoding neutrophil elastase account for approximately 60% of the genetic causes. Here, we present three patients from different Vietnamese families who were susceptible to infectious diseases such as lung abscesses, sepsis, cellulitis, and septicemia. Moreover, their hematological and immunological parameters were below the reference range. Whole exome sequencing (WES) analysis was performed in all cases harboring three previously described disease-causing mutations, including p.Arg103Pro, p.Trp156Arg, and p.Arg81Pro in the *ELANE* gene (NM_001972.4). These mutations were confirmed by the Sanger sequencing method in the patients, helping to identify *de novo* mutations in all cases. Our data increase more evidence for the function of *ELANE* in SCN, as well as raise awareness of this rare disease in the context of frequent infections in Vietnam.

Keywords: De novo, *ELANE*, severe congenital neutropenia, Vietnamese, whole-exome sequencing.

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[*]*Corresponding author email*: ntkimlienibt@gmail.com

INTRODUCTION

Severe congenital neutropenia (SCN; OMIM # 202700) is a rare heterogeneous disorder initially described in 1956. Affected individuals with SCN are usually children who have an absolute neutrophil count, often less than 0.2 × 10 g/L. Prior to the availability of myeloid growth factors, these patients usually died from severe bacterial infections in early childhood (Kostman, 1975). Congenital neutropenia follows approximately 60% of cases and is inherited through autosomal dominant, autosomal recessive, or X-linked recessive patterns (Donadieu et al., 2017; Furutani et al., 2019). Mutations cause most autosomal dominant cases of SCN in *ELANE* (*ELA2*), a gene encoding a cytolytic granule-associated serine protease produced in response to bacteria's neutrophil activation factors (Dale et al., 2000; Grenda et al., 2007). In growing myeloid cells, mutations in this gene affect protein folding; misfolding begins the unfolded protein response and induces apoptosis (Grenda et al., 2007). Over 200 different *ELANE* mutations have been found, spanning five coding exons and four introns of the *ELANE* gene (Makaryan et al., 2015). It has been proposed that the *ELANE* mutations may predispose patients with SCN to leukemogenesis because myelodysplastic syndrome or acute myeloid leukemia primarily arises in the subset of severe congenital neutropenia patients whose illness is associated with the *ELANE* mutation (Bellanne-Chantelot et al., 2004).

With the progress of gene sequencing technologies, increasing numbers of SCN-causing genes are being diagnosed. Whole exome sequencing (WES) has proven an exceptionally useful tool as the method of choice for diagnosing various SCNs (Gauthier-Vasserot et al., 2017; McNulty et al., 2021; Zhang et al., 2016). The early identification of SCN in the patients increases the effectiveness of treatment and improves patients' quality of life. Besides, exome sequencing reduces cost and increases speed and accuracy, improving the genetic diagnostic yield. The aim of the study was to conduct WES sequencing in SCN patients to identify disease-related genetic variations. The results of the study will contribute to a general understanding of the etiology of the disease and supports clinicians in genetic counseling for patients' families.

MATERIALS AND METHODS

Three patients from three unrelated families with full-term pregnancies, girls ages less than one to five years, were identified and treated for SCN at the Vietnam National Hospital Pediatrics. All experiments performed by relevant guidelines and regulations based on the experimental protocol on human subjects which was approved by the Scientific Committee of the Institute of Genome Research, Vietnam Academy of Science and Technology under reference number 01/QD-NCHG on January 10, 2020. The hematological and immunological indices of their blood samples were assessed according to routine methods at the hospital. Their hematological and serum immunological evaluation did not reach the normal parameters for healthy people (Table 1). On August 29, 2016, a 1-day-old baby came to the hospital for the first time with a neonatal sepsis sign. In addition, her umbilical cord separation time was recorded for one month. At one year old, the patient had a lung infection and dental caries, and the blood cell count showed no neutrophils per microliter (0.00×10^9/L) besides low levels of monocytes hemoglobin, and platelets. Up to now, she still meets aphthous ulcers, lung infection, and infectious diarrhea two to three times every year. In patient 2, the child had a lobectomy at the end of month three. From ages 3 to 5 years, she had upper respiratory tract infection, molluscum contagiosum, and acute otitis media. At five years old, the clinical examination found that her white blood cell, neutrophil, and hemoglobulin count were low at 1.93, 0.05, and 75.0×10^9/L, respectively. Moreover, she got hepatosplenomegaly, leading to her bone marrow biopsy with a negative effect on delayed maturation of granulocytes. Patient 3 was fistula operated on at 2-month-old. Under one year old, the patient

was admitted with aphthous ulcers, acute otitis media, cellulitis, and septicemia. She tolerated each symptom at least once or twice per year. Furthermore, her neutrophil cell count was in danger (0.01×10^9/L, reference range 1.0–8.5 $\times$ 10^9/L) while her mental and motor development was normal.

Peripheral blood from patients, their parents, and person healthy for control were collected and stored at -20 ^oC for extracting genomic DNA using the QIAamp DNA blood mini kit (Qiagen, Germany). Agilent SureSelect Human All Exon v6 Kit (Agilent Technologies, Santa Clara, CA, USA) was used for WES with the patients, and sequencing was conducted using the Illumina platform sequencer (San Diego, CA, USA). Briefly, the sequencing reads were aligned to GRCh37/hg19 human genome reference using Burrows-Wheeler Aligner v0.7.12 (BWA) (Li & Durbin, 2010). The GATK Software v3.4.0 (https://www.broadinstitute.org/gatk/) was used for base quality score recalibration and calling variants (McKenna et al., 2010), resulting in a variant call format (VCF) computer file.

Table 1. Hematological and immunological evaluation and phenotype in *ELANE* patients

		Patient		
		1	2	3
	Ethnicity	Vietnamese	Vietnamese	Vietnamese
	Sex	Female	Female	Female
	Age at onset	1 day	18 months	2 months
	Age at diagnosis (months)	12	60	5
Blood cell count	White blood cells ($5.0–17.0 \times 10^9$/L)	14.13	1.93	10.39
	Neutrophils ($1.0–8.5 \times 10^9$/L)	0.00	0.05	0.1
	Lymphocytes ($1.5–9.5 \times 10^9$/L)	6.24	1.13	6.37
	Monocytes ($0.2–1.0 \times 10^9$/L)	7.6	0.12	3.4
	Eosinophils ($< 0.81 \times 10^9$/L)	0.29	0.02	0.49
	Basophils ($<0.21 \times 10^9$/L)	0.00	0.01	0.02
	Hemoglobin (115–140 g/L)	78	75	91
	Platelet count ($150–400 \times 10^9$/L)	893	257	514
Serum Immunoglobulin concentrations	IgG (635–1,284 mg/dL)	1,269	406	309
	IgA (32–191 mg/dL)	148	303	22
	IgM (44–190 mg/dL)	189	145	44

Sanger sequencing was applied to amplify the *ELANE* gene (NM_001972.4) to confirm nucleotide changes. Two primer pairs (ELANE_1F 5'-CGGAGGGGCAGAGACCCCGGA-3', ELANE_1R 5'-AGACCGGGACGCGGGGTCCGA-3' and ELANE_2F 5'-CTCGAGCACCTTCGCCCTCAG-3', ELANE_2R 5'-TCAACGGCCCATGGCGGGTAT-3') were used to amplify and Sanger sequencing the fragments that contain nucleotide changes in patients, their parents, and control sample. PCR amplification was performed at an optimum thermal of 95 ^oC in 12 min; after that was 35 cycles with 95 ^oC in 45 s; 60 ^oC in the 30 s; 72 ^oC in 45 s and final at 72 ^oC in 8 minutes. The purified PCR products were sequenced on ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, USA). Then, sequencing data were analysed using BioEdit (Ibis Biosciences, USA). To investigate putative links between genetic mutation and phenotypic variance, we employed *in silico* analysis to prioritize high-risk nonsynonymous mutations in *ELANE* coding

areas that are likely to affect the protein's structure and function, including Sorting Intolerant From Tolerant (SIFT, http://provean.jcvi.org/seq_submit.php), Polymorphism Phenotyping 2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/), Pmut (http://mmb.irbbarcelona.org/PMut/) and SNAP 2 (https://www.rostlab.org/services/snap/). To assess the effect of mutations on protein structure, the three-dimensional structure of the wild type and mutant type was constructed using Swiss-PDB Viewer v4.1 with PDB: 4NZL as a template.

RESULTS

WES results met sufficient quality standards, with 90.5–99.4% bases at sequencing depth ≥ 20X. Over 11,300 missense variants were identified (Table S1). After evaluation and filtration, we found three missense mutations, including c.308G>C (p.Arg103Pro), c.406T>C (p.Trp156Arg), and c.242G>C (p.Arg81Pro) in the *ELANE* gene (NM_001972.4) in patient 1, patient 2, and patient 3, respectively. These mutations have been reported as pathogenic variants in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) (Table 2). Moreover, p.Arg103Pro was previously reported in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) with the identity VCV000842953.3. Three mutations were sorted as "probably damaging" with a PolyPhen-2 score over 0.998. Both mutations in patient 1 and patient 2 were classified as "deleterious" due to their SIFT score in the range of 0.0 to 0.05. In patient 3, the mutation was predicted "tolerated" with SIFT score of 0.072 (Table 2). Using Pmut and SNAP software, three mutations were predicted "Disease" and "Effect", respectively. Sanger sequencing analysis confirmed three heterozygous *de novo* mutations in our patients (Fig. 1).

In addition, the 3D structures of wild-type and mutant protein indicated changes in the total number of hydrogen bond establishments of patient 2 and patient 3, except for patient 1 (Fig. 2). To be more specific, Arg103 was predicted to have a hydrogen-bonding interaction with Gln122 in patient 1. However, replacing arginine (a branched-chain amino acid) with proline (acyclic amino acid) at codon 103 did not alter hydrogen bonding with glutamine (Fig. 2A). In patient 2, Trp156 was predicted to have a hydrogen-bonding interaction with Ala166, while p.Trp156Arg introduced a new hydrogen bond with Ser167 (Fig. 2B). In patient 3, the wildtype Arg81 formed five hydrogen bonds with Gln48, Arg78, Val80, and a ligand FUL4. The mutated Pro81 kept only two hydrogen bonds and caused physical clashes with Gln48 (Fig. 2C).

Table 2. The variants detected on the *ELANE* gene and effect prediction score

Patient	P1	P2	P3
Nucleotide change Zygosity	c.308G>C Heterozygous	c.406T>C Heterozygous	c.242T>C Heterozygous
Amino acid change	p.Arg103Pro	p.Trp156Arg	p.Arg81Pro
SIFT	Deleterious (0.038)	Deleterious (0.011)	Tolerated (0.072)
PolyPhen-2	Probably Damaging (0.999)	Probably Damaging (1.00)	Probably Damaging (0.998)
Pmut	Disease (0.81)	Disease (0.90)	Disease (0.84)
SNAP2	Effect (77)	Effect (96)	Effect (63)
dbSNP 155	rs745455816	-	-
ClinVar	VCV 000842953.3	-	-
Human gene mutation database	CM097551	CM135441	CM041333

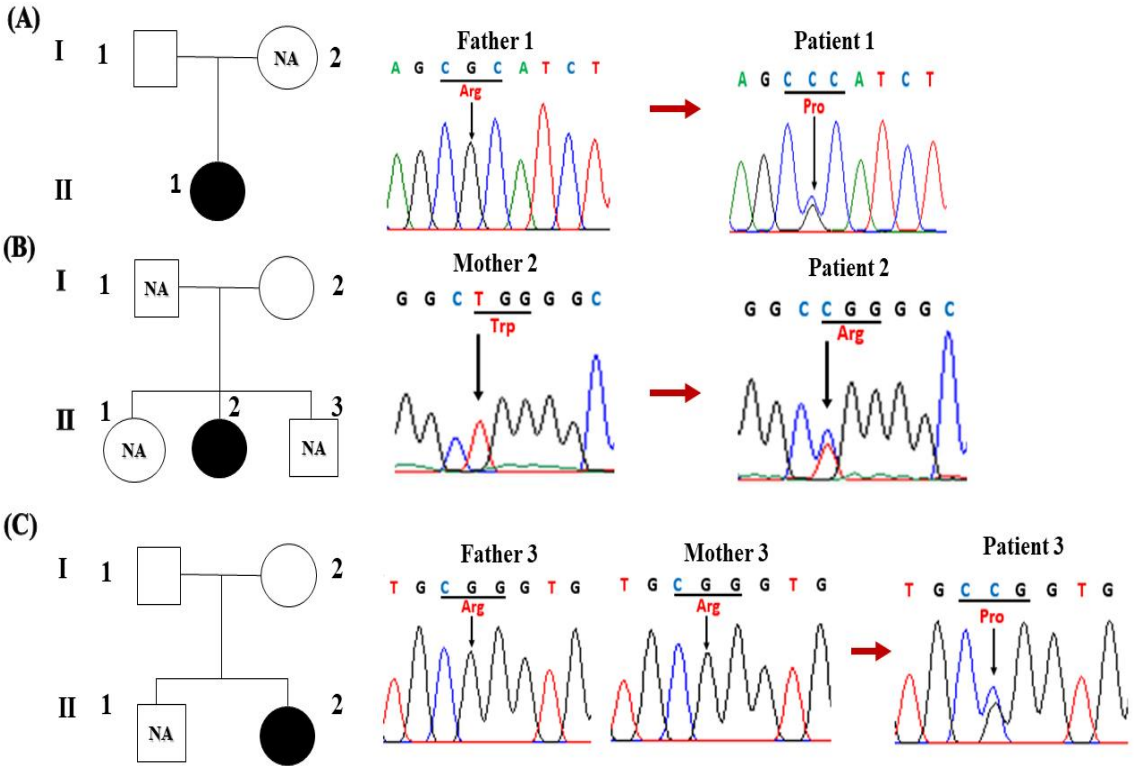

Figure 1. Genetic examination of the affected patients with severe congenital neutropenia. **(A)** Family 1 (c.308G>C), **(B)** Family 2 (c.406T>C) **(C)** Family 3 (c.242G>C). The upper panels are pedigrees of the patient families, and the below panels are chromatograms of Sanger demonstrating heterozygous for the missense mutations of affected patients in each family. Squares present males and circles denote females. The full filled symbol is the carrier individual; the empty symbol is unaffected; NA is Not Available
(No samples for genetic testing)

DISCUSSION

In this study, we describe three unrelated patients with severe congenital neutropenia. The girls, whose median age at diagnosis was approximately 22 months (range 5–60 months), developed SCN that manifested as typical symptoms, including severe neutropenia and infectious diseases such as recurrent pneumonia, mucositis, gingivitis, skin abscesses, and otitis media. Applying WES, all patients were harboring heterozygous mutations in the *ELANE* gene. Most mutations were located in exon 3, were associated with variable phenotypes, and might be a life-threatening condition (Rydzynska et al., 2021); moreover, SCN due to *ELANE* mutations cause neutropenia starting at birth like in patient 1 and patient 3. Meanwhile, patient 2 had a late onset disease than others because the disease phenotype is not determined by mutation alone; it can be influenced by different genetic, epigenetic, and environmental levels (Makaryan et al., 2015; Nayak et al., 2015; Nustede et al., 2016). Mutations in the *ELANE* gene are dominant inheritance, so the carrier of the mutant gene in the heterozygous state will show the disease. In addition, we performed Sanger sequencing on the samples of the patient's parents, the results showed that the patient's parents did not carry the mutation and the patient's family history did not have the disease. Thus, these mutations are *de novo* mutations in three cases.

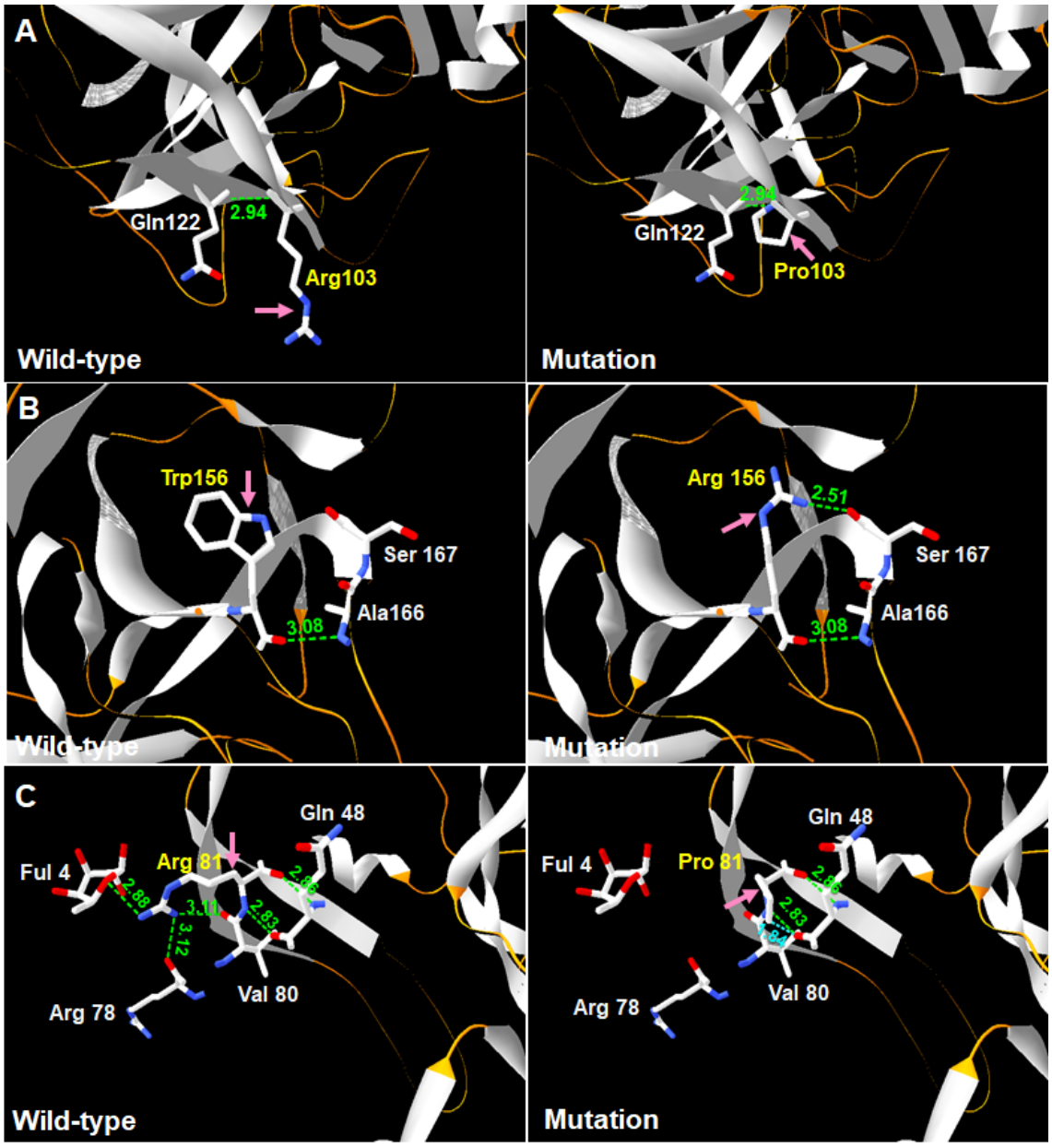

Figure 2. Ribbon diagrams of neutrophil elastase. **(A)** p.Arg103Pro. **(B)** p.Trp156Arg **(C)** p.Arg81Pro. Comparing wild-type (left) and mutant (right) residues pairwise for expected alterations in local interactions with other amino acids. The ribbon diagrams deleted partial tertiary structures to show mutation variants and adjacent residues. Colour codes for molecules and bonds were as follows: carbon, white; oxygen, red; nitrogen, blue; strong H- bond, green discontinuous line; distance making clashes between adjacent side chains, light blue discontinuous line

To support the accuracy of *in silico* results, the 3D structures were constructed for three cases. In patient 1, the mutation does not appear to generate any visible alterations in the structural interface. Compared to the previous study, our patient had SCN

symptoms earlier than the child having the same mutation in the report of Ittiwut et al. (2020). The girl in our research is six years old at the time of writing and still experiencing disease symptoms in the last year. By combining this point with the evaluation of four computational methods, we estimate that our patient will be experienced SCN until 22 years old as patient 1 in the prior study (Ittiwut et al., 2020).

As we know, hydrogen bonds are not the primary force responsible for peptide and protein folding, and they contribute considerably to peptide folding preservation (Scheike et al., 2007). Missense mutations that affect hydrogen bonds significantly affect protein stability, DNA binding specificity, protein expression, and interactions. Therefore, patient 2 may expose to more severe symptoms, as evidenced by her having to return to the hospital every year with lung abscesses despite taking medical treatment. Especially, p.Trp156Arg also appeared on SCN female children in the study of Deordieva et al. (2021). The previous patient met SCN symptoms sooner than patient 2 in our study (6 months and 18 months, respectively). Further functional analysis of this mutation is needed to reveal the molecular mechanism of its action.

In patient 3, the mutated Pro81 clashed with Gln48 due to the substituting residue did not fit in the protein structure, and a drastic conformation change occurred, leading to local or global structural alteration in the protein (Calyseva & Vihinen, 2017). Therefore, p.Arg81Pro appeared to have a functional and structural impact on the encoded proteins. Some previous studies proved p.Arg81Pro was detected in both congenital neutropenia and cyclic neutropenia (Bellanne-Chantelot et al., 2004; Germeshausen et al., 2013).

CONCLUSION

In this study, we have described three Vietnamese patients who suffered from severe congenital neutropenia (SCN). The *de novo* mutations p.Arg103Pro, p.Trp156Arg, and p.Arg81Pro were detected by whole-exome sequencing in the patients. Our study also supports the promising potential of WES in the genetic diagnosis of SCN. The case report may contribute to accumulating the number of SCN cases in Vietnam, which may help explore this rare disease's pathogenesis.

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REFERENCES

Bellanne-Chantelot C., Clauin S., Leblanc T., Cassinat B., Rodrigues-Lima F., Beaufils S., Vaury C., Barkaoui M., Fenneteau O., Maier-Redelsperger M., Chomienne C., Donadieu J., 2004. Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French Neutropenia Register. *Blood*, 103(11): 4119–4125. https://doi.org/10.1182/blood-2003-10-3518

Calyseva J., Vihinen M., 2017. PON-SC - program for identifying steric clashes caused by amino acid substitutions. *BMC Bioinformatics*, 18(1): 531. https://doi.org/10.1186/s12859-017-1947-7

Dale D.C., Person R.E., Bolyard A.A., Aprikyan A.G., Bos C., Bonilla M.A., Boxer L.A., Kannourakis G., Zeidler C., Welte K., Benson K.F., Horwitz M., 2000. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood*, 96(7): 2317–2322. https://doi.org/10.1182/blood.V96.7.2317.h8002317_2317_2322

Deordieva E., Shvets O., Voronin K., Maschan A., Welte K., Skokowa J., Novichkova G., Shcherbina A., 2021. Nicotinamide (vitamin B3) treatment improves response to G-CSF in severe congenital neutropenia patients. *Br J*

Haematol, 192(4): 788–792. https://doi.org/10.1111/bjh.17313

Donadieu J., Beaupain B., Fenneteau O., Bellanne-Chantelot C., 2017. Congenital neutropenia in the era of genomics: classification, diagnosis, and natural history. *Br J Haematol*, 179(4): 557–574. https://doi.org/10.1111/bjh.14887

Furutani E., Newburger P.E., Shimamura A., 2019. Neutropenia in the age of genetic testing: Advances and challenges. *Am J Hematol*, 94(3): 384–393. https://doi.org/10.1002/ajh.25374

Gauthier-Vasserot A., Thauvin-Robinet C., Bruel A. L., Duffourd Y., St-Onge J., Jouan T., Riviere J. B., Heron D., Donadieu J., Bellanne-Chantelot C., Briandet C., Huet F., Kuentz P., Lehalle D., Duplomb-Jego L., Gautier E., Maystadt I., Pinson L., Amram D., El Chehadeh S., Melki J., Julia S., Faivre L., Thevenon J., 2017. Application of whole-exome sequencing to unravel the molecular basis of undiagnosed syndromic congenital neutropenia with intellectual disability. *Am J Med Genet A*, 173(1): 62–71. https://doi.org/10.1002/ajmg.a.37969

Germeshausen M., Deerberg S., Peter Y., Reimer C., Kratz C. P., Ballmaier M., 2013. The spectrum of ELANE mutations and their implications in severe congenital and cyclic neutropenia. *Hum Mutat*, 34(6): 905–914. https://doi.org/10.1002/humu.22308

Grenda D. S., Murakami M., Ghatak J., Xia J., Boxer L.A., Dale D., Dinauer M. C., Link D. C., 2007. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. *Blood*, 110(13): 4179–4187. https://doi.org/10.1182/blood-2006-11-057299

Ittiwut R., Sengpanich K., Lauhasurayotin S., Ittiwut C., Shotelersuk V., Sosothikul D., Suphapeetiporn K., 2020. Clinical and molecular characteristics of Thai patients with ELANE-related neutropaenia. *J Clin Pathol*, 75(2): 99–103. https://doi.org/10.1136/jclinpath-2020-207139.

Kostman R., 1975 Mar. Infantile genetic agranulocytosis. A review with presentation of ten new cases. *Acta Paediatr Scand*, 362–368. https://doi.org/10.1111/j.1651-2227.1975.tb03847.x

Li H., Durbin R., 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, 26(5): 589–595. https://doi.org/10.1093/bioinformatics/btp698

Makaryan V., Zeidler C., Bolyard A. A., Skokowa J., Rodger E., Kelley M. L., Boxer L. A., Bonilla M.A., Newburger P.E., Shimamura A., Zhu B., Rosenberg P.S., Link D.C., Welte K., Dale D.C., 2015. The diversity of mutations and clinical outcomes for ELANE-associated neutropenia. *Curr Opin Hematol*, 22(1): 3–11. https://doi.org/10.1097/MOH.0000000000000105

McKenna A., Hanna M., Banks E., Sivachenko A., Cibulskis K., Kernytsky A., Garimella K., Altshuler D., Gabriel S., Daly M., DePristo M.A., 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*, 20(9): 1297–1303. https://doi.org/10.1101/gr.107524.110

McNulty S. N., Evenson M. J., Riley M., Yoest J. M., Corliss M. M., Heusel J. W., Duncavage E.J., Pfeifer J.D., 2021. A Next-Generation Sequencing Test for Severe Congenital Neutropenia: Utility in a Broader Clinicopathologic Spectrum of Disease. *J Mol Diagn*, 23(2): 200−211. https://doi.org/10.1016/j.jmoldx.2020.10.014

Nayak R. C., Trump L. R., Aronow B. J., Myers K., Mehta P., Kalfa T., Wellendorf A. M., Valencia C. A., Paddison P. J., Horwitz M. S., Grimes H. L., Lutzko C., Cancelas J. A., 2015. Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem

cells. *J Clin Invest*, 125(8): 3103–3116. https://doi.org/10.1172/JCI80924

Nustede R., Klimiankou M., Klimenkova O., Kuznetsova I., Zeidler C., Welte K., Skokowa J., 2016. ELANE mutant-specific activation of different UPR pathways in congenital neutropenia. *Br J Haematol*, 172(2): 219−227. https://doi.org/10.1111/bjh.13823

Rydzynska Z., Pawlik B., Krzyzanowski D., Mlynarski W., Madzio J., 2021. Neutrophil Elastase Defects in Congenital Neutropenia. *Front Immunol*, 12(653932. https://doi.org/10.3389/fimmu.2021.653932

Scheike J. A., Baldauf C., Spengler J., Albericio F., Pisabarro M.T., Koksch B., 2007. Amide-to-ester substitution in coiled coils: the effect of removing hydrogen bonds on protein structure. *Angew Chem Int Ed Engl*, 46(41): 7766–7769. https://doi.org/10.1002/anie.200702218

Zhang J., Barbaro P., Guo Y., Alodaib A., Li J., Gold W., Ades L., Keating B. J., Xu X., Teo J., Hakonarson H., Christodoulou J., 2016. Utility of next-generation sequencing technologies for the efficient genetic resolution of haematological disorders. *Clin Genet*, 89(2): 163–172. https://doi.org/10.1111/cge.12573

Table S1. Data summary of exome sequencing in each patient.

Patient	Patient 1	Patient 2	Patient 3
Total number of reads (Million)	49.23	43.53	42.87
Total mappable reads (Million)	49.19	43.49	42.83
% mappable reads	99.9	99.9	99.9
% Coverage			
1X (%)	99.4	99.2	99.3
10X (%)	97.0	96.4	96.2
20X (%)	92.5	91.1	90.5
Number of SNP	80.674	79.124	78.778
Synonymous Variant	11.976	11.810	11.968
Missense Variant	11.503	11.329	11.444