Clinical and molecular characteristics of Thai patients with *ELANE*-related neutropaenia

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ABSTRACT

Aims Congenital neutropaenia is a rare inherited disorder that mainly affects neutrophils causing severe infection. Mutations in several genes have been implicated in the disease pathogenesis. The genetic defects may vary in different populations, influenced by ethnicity and geographical location. Here we describe the clinical and genotypic characteristics of seven unrelated Thai cases with congenital neutropaenia.

Methods Seven unrelated patients with congenital neutropaenia were enrolled (5 female and 2 male) at King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Clinical and laboratory data were collected. Whole exome sequencing (WES) analysis was performed in all cases.

Results WES successfully identified disease-causing mutations in the *ELANE* gene in all cases, including two novel ones: a heterozygous 12 base pair (bp) inframe insertion (c.289_300dupCAGGTGTTCGCC; p.Q97_A100dup) and a heterozygous 18 bp inframe deletion (c.698_715delCCCCGGTGGCACAGTTTG; p.A233_F238delAPVAQF). Five other previously described *ELANE* mutations (p.Arg103Pro, p.Gly214Arg, p.Trp241X, p.Ser126Leu and p.Leu47Arg) were also detected.

Conclusions All Thai patients with congenital neutropaenia in this study harboured causative mutations in the *ELANE* gene, suggesting it the most common associated with the disease. Two novel mutations were also identified, expanding the genotypic spectrum of *ELANE*.

INTRODUCTION

Congenital neutropaenia is a rare inherited haematological disorder caused by defects in several genes. It is characterised by peripheral blood absolute neutrophil counts (ANC) below 0.5×10^9/L, maturation arrest of myelopoiesis at the level of promyelocytes, and early-onset life-threatening bacterial infections. The prevalence is around 3–8.5 cases per million births.[1 2] Majority of patients respond to treatment with granulocyte-colony stimulating factor (G-CSF) by having lower frequencies and lesser severity of infections.[3]

There have been a number of genes identified to be associated with congenital neutropaenia. Mutations in the encoding neutrophil elastase (*ELANE*) gene are mostly found in patients with autosomal dominant severe congenital neutropaenia (SCN; MIM #202700) and cyclic neutropaenia (CN; MIM #162800).[4] Other genes responsible for SCN include HCLS-1-associated protein X1 (*HAX1*),[5] growth factor independent 1 transcription repressor (*GFI1*),[6] glucose 6 phosphatase catalytic subunit 3 (*G6PC3*),[7] colony-stimulating factor 3 receptor (*CSF3R*)[8]and Wiskott-Aldrich syndrome (*WAS*).[9] The prevalence of specific genetic defects among patients with SCN appears to be associated with the degree of consanguinity. In populations with high rate of consanguineous marriage, *HAX1* and *G6PC3* mutations were found more common in patients with autosomal recessive SCN.[10–13]

Here, we report on the clinical and molecular characteristics of seven unrelated Thai patients with congenital neutropaenia. The response to G-CSF treatment and the clinical outcomes were also investigated. Whole exome sequencing (WES) successfully identified causative mutations in *ELANE* in all cases, with two being novel, expanding the genotypic spectrum of *ELANE*.

MATERIALS AND METHODS

Patients and families

The medical records of patients with clinical and haematological findings suspected of congenital neutropaenia who were followed up at King Chulalongkorn Memorial Hospital from January 1994 to January 2019 were reviewed. A total of seven unrelated patients were recruited in the study. They were diagnosed with congenital neutropaenia if they had severe neutropaenia (ANC $<0.5\times10^9$/L), maturation arrest at the promyelocyte/myelocyte stage in the bone marrow and recurrent bacterial infections.

After informed consent, blood samples from the patients and their available parents were collected. The clinical manifestations and haematological findings are summarised in table 1.

Whole exome sequencing

After informed consent, 3 mL of peripheral blood was taken from the patients and their available parents. DNA was extracted from peripheral blood leucocytes using the Puregene Blood Kit (Qiagen, Hilden, Germany). WES was done by Macrogen (Seoul, Korea). The sequencing libraries were enriched by SureSelect Human All Exon V5 kits. The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer. Sequence reads were mapped against University of California Santa Cruz (UCSC) hg19 using Burrows-Wheeler Alignment software (http://bio-bwa.source forge.net/).

Table 1 Clinical and molecular characteristics of Thai patients with *ELANE*-related neutropaenia

Patient	Diagnosis	Sex	Age at onset	Age at diagnosis	Other features	ANC before G-CSF, median (range)	G-CSF dose (µg/kg/day)	ANC after G-CSF, median (range)	Mutation	Age at last follow-up (years)
1	SCN	F	2 weeks	7 months	None	175±18	6.5 µg/kg twice a week	968±125	Exon 3 c.308G>C (p.Arg103Pro) Known	22 years
2	SCN	F	1 month	10 months	Imperforate anus, rectovaginal fistula	345±14	5 µg/kg 4 times per week	1786±59	Exon 3 c.289_300dupCAGGTGTTCGCC (p.Q97_A100dup) Novel	3 years and 1 month
3	SCN	F	2 months	7 months	Strabismus, congenital ptosis	190±67	24.3 µg/kg once daily	1140±54	Exon 5 c.640G>A (p.Gly214Arg) Known	3 years and 1 month
4	SCN	M	7 months	5 years	None	149±48	6 µg/kg 3 times per week	1670±147	Exon 5 c.723G>A (p.Trp241X) Known	7 years and 10 months
5	SCN	F	2 months	1 year and 4 months	None	281±62	10 µg/kg once daily	1210±37	Exon 5 c.698_715delCCCCGGTGGCACAGTTTG (p.A233_F238delAPVAQF) Novel	2 years and 8 months
6	CN	M	8 months	6 years	None	625±53	None	NA	Exon 4 c.377C>T (p.Ser126Leu) Known	6 years and 5 months
7	CN	F	1 year and 6 months	1 year and 9 months	None	429±128	5 µg/kg/day × 4 days every 4 weeks	2200±98	Exon 2 c.140T>G (p.Leu47Arg) Known	8 years

ANC, absolute neutrophil count; CN, cyclic neutropaenia; F, female; G-CSF, granulocyte-colony stimulating factor; M, male; NA, not applicable; SCN, severe congenital neutropaenia.

The single-nucleotide polymorphisms and indels were detected by SAMTOOLS (http://samtools.source forge.net/) and annotated by dbSNP and 1000G. The variants were subsequently filtered out if they were present in our inhouse database of 2166 unrelated Thai exomes. The variants would be called novel if they were not listed in the ClinVar Miner database (https://clinvarminer.genetics.utah.edu/) and the Exome Aggregation Consortium database (exac.broadinstitute.org/). The identified novel variants were verified by PCR-Sanger sequencing. Prediction software, PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (Sorting Intolerant From Tolerant; http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html), were used to analyse the potential pathogenicity of the missense variants. In addition, for insertion and deletion variants, PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org) was used for protein function prediction.

RESULTS

A total of seven unrelated patients with congenital neutropaenia were included. Five were female and two were male. Most of the patients were noted to have bacterial infections within 1 year of age. The median age of disease onset was 2 months (range, 0.5–18 months) and the median age at diagnosis was 16 months (range, 7–72 months). Five patients had no physical or organ anomalies. Patient 2 had imperforate anus with rectovaginal fistula. Patient 3 had strabismus and congenital ptosis. All had attained normal developmental milestones for age.

All patients had an increase in ANC and a decrease in life-threatening infections after receiving various doses of G-CSF. The highest dose, 24.3 µ/kg/day, was required to raise ANC in patient 3 to above 1.5×10^9 cells/µL (table 1). Although her ANC was increased, she developed two episodes of severe infection and subsequently needed to receive a bone marrow transplant. The longest follow-up was 22 years in patient 1 (table 1), and no one developed myelodysplastic syndrome or haematological malignancy.

WES was performed to investigate the genetic defects in all cases. The clinical manifestations, genetic findings and information on treatment are summarised in table 1.

We found different mutations in the *ELANE* gene in all seven unrelated patients. Two have not been previously described. These novel heterozygous mutations, 12 bp inframe insertion (c.289_300dupCAGGTGTTCGCC; p.Q97_A100dup) and 18 bp inframe deletion (c.698_715delCCCCGGTGGCACAGTTTG; p.A233_F238delAPVAQF), were found in patients 2 and 5, respectively (table 1, figure 1). They were located in the peptidase S1 domain. The previously reported mutations in *ELANE*, with four being missense and one nonsense, were also identified in our cohort. All *ELANE* mutations occurred de novo. In addition, we identified a novel heterozygous *TCIRG1* missense variant (c.1604T>C; p.Met535Thr) in patient 2 (figure 2), which was inherited from her mother. No other pathogenic or likely pathogenic variants in the genes related to SCN were detected.

DISCUSSION

In this study, we characterised seven unrelated patients with congenital neutropaenia. Using WES, all patients were found to harbour heterozygous mutations in the *ELANE* gene. Of these seven different mutations, two have not yet been described. The majority of mutations are located in exons 3 and 5. All occurred de novo. Two newly identified mutations, c.289_300dupCAGGTGTTCGCC; p.Q97_A100dup and c.698_715delCCCCGGTGGCACAGTTTG; p.A233_F238delAPVAQF, were found in patients 2 and 5, respectively. The other five mutations (p.Arg103Pro, p.Gly214Arg, p.Trp241X, p.Ser126Leu and p.Leu47Arg identified in patients 1, 3, 4, 6 and 7, respectively) were previously reported.[4 14–17] The longest follow-up period was 22 years in patient 1 with favourable clinical outcome. Patient 3 required a high dose of G-CSF. None of our patients with *ELANE*-related neutropaenia developed haematological malignancies. Patient 3 harboured the previously reported mutation c.640G>A (p.Gly214Arg).[18] Previous studies revealed that patients with this particular mutation had a severe phenotype with a poor response to G-CSF and an increased risk of developing leukaemia.[19–21] However, a previous study showed that only two out of seven patients with p.Gly214Arg developed leukaemia.[4] Our patient (patient 3) had a partial response to

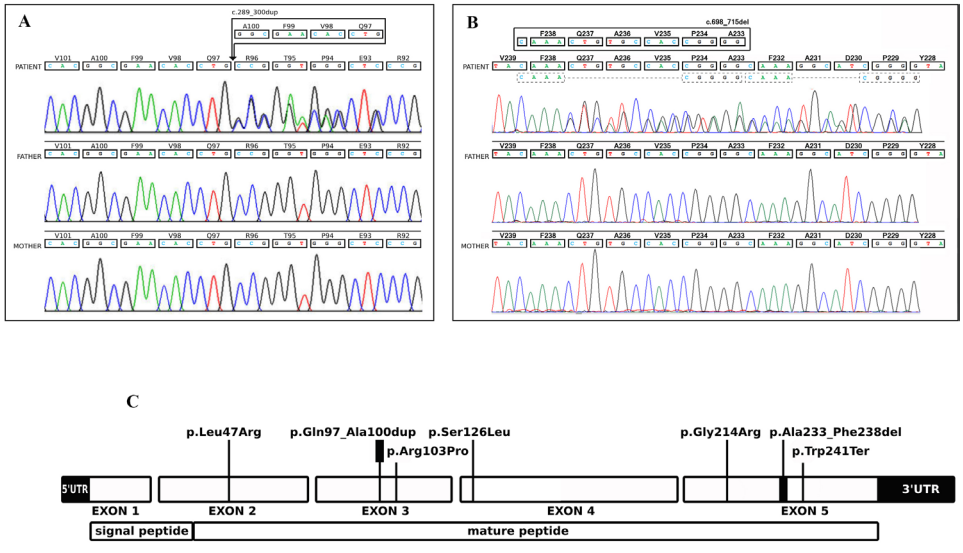

Figure 1 (A) Electropherograms showing the heterozygous 12 bp inframe insertion (NM_001972.3, c.289_300dupCAGGTGTTCGCC; p.Q97_A100dup: chr19:g.853326_853337dup) in the *ELANE* gene present in patient 2. (B) Electropherograms showing the heterozygous 18 bp inframe deletion (NM_001972.3, c.698_715delCCCCGGTGGCACAGTTTG; p.A233_F238delAPVAQF: chr19:g.856058_856075del) found in patient 5. (C) The location of all seven *ELANE* mutations identified. bp, base pair.

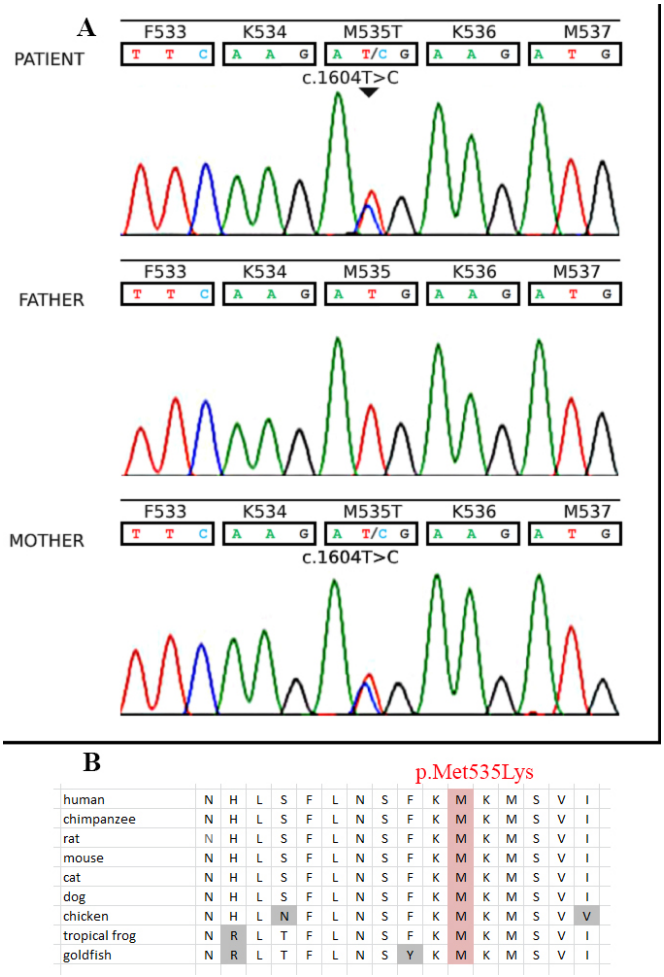

Figure 2 (A) Electropherograms showing the c.1604T>C (p.Met535Thr) variant in the *TCIRG1* gene found in patient 2 and her mother. (B) Sequence alignment of partial amino acid sequence of *TCIRG1* from various species showing that the p.Met at 535 is highly conserved.

high-dose G-CSF. She had recurrent infections despite increasing the dose of G-CSF to 24.3 µ/kg/day.

The *ELANE* p.Ser126Leu mutation found in patient 6 has been predicted to be correlated with low-risk leukaemia.[19] Our patient with this particular mutation was diagnosed with CN.

Patients 2 and 5 had novel inframe mutations in *ELANE*, located in exons 3 and 5, respectively. It is not in the gnomAD or Human Gene Mutation Database (HGMD) database and predicted to have deleterious impact on the biological function of an ELA2 protein by PROVEAN (p.Q97_A100dup with a score of −10.96 and p.A233_F238delAPVAQF with a score of −23.96). PCR-Sanger sequencing confirmed the presence of the mutation in the proband and showed that both parents harboured only the wild-type allele (figure 1). Previous studies have demonstrated small *ELANE* inframe insertions and deletions in patients with SCN.[22–24] Patient 2 harboured a 12 bp insertion in exon 3 leading to the insertion of four amino acids (c.289_300dupCAGGTGTTCGCC; p.Q97_A100dup). It is located close to the 15 bp inframe deletion (p.Q97-V101del) identified previously in one patient with SCN.[24]

Combinations of mutations or digenic mutations of genes responsible for congenital neutropaenia have been described previously,[13] for example, a heterozygous *ELANE* mutation combined with a homozygous mutation in *G6PC3* or *HAX1* genes. In this study, we found a heterozygous *ELANE* 12 bp inframe insertion with a heterozygous *TCIRG1* mutation (c.1604T>C; p.Met535Thr; SIFT prediction damaging with a score of 0.000) in patient 2. *TCIRG1* has been proposed to be another gene associated with congenital neutropaenia as the *TCIRG1* variant was found to cosegregate with the disease in a five-generation family. In addition, two unrelated patients with SCN were found to be heterozygous for likely pathogenic *TCIRG1* variants.[25] How *TCIRG1* dysfunction leads to SCN requires further studies.[26]

Our study describes Thai cases with molecularly confirmed congenital neutropaenia. All seven unrelated patients harboured causative mutations in the *ELANE* gene. These data suggest that heterozygous mutations in the *ELANE* gene are the most common genetic abnormality in the Thai population. We also identified two novel inframe mutations, expanding the genotypic spectrum of *ELANE*.

Take home messages

- The genes contributing to congenital neutropaenia (CN) may vary in different populations, influenced by ethnicity and geographical location.
- Mutations in genes responsible for CN are identified by whole exome sequencing analysis.
- All Thai patients with CN in this study harboured causative mutations in the *ELANE* gene.
- Two inframe mutations have not been previously described, expanding the genotypic spectrum of *ELANE*.

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Competing interests None declared.

Patient consent for publication Parental/guardian consent obtained.

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