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Two paternal mosaicism of mutation in *ELANE* causing severe congenital neutropenia exhibit normal neutrophil morphology and ROS production

Qiao Liu[a], Liang Zhang[a], Zhou Shu[b], Yuan Ding[b], Xue-Mei Tang[b], Xiao-Dong Zhao[a,b,c,*]

[a] Chong Qing Key Laboratory of Child Infection and Immunity, Children's Hospital of Chongqing Medical University, Chongqing 400014, China
[b] Division of Immunology, Children's Hospital of Chongqing Medical University, Chongqing 400014, China
[c] Ministry of Education Key Laboratory of Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Children's Hospital of Chongqing Medical University, Chongqing 400014, China

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ABSTRACT

Severe congenital neutropenia caused by *ELANE* gene mutation is a rare disease. To date, only four families were reported with mosaicism. Here we examined the morphology and function of granulocytes isolated from two patients and their mosaic fathers. Analysis of granulocytes isolated from the fathers revealed no genetic mutations. DNA extracted from fractionated peripheral blood mononuclear cells (PBMCs) and fingernails obtained from both fathers did harbor the mutation, suggesting mosaicism. Granulocytes isolated from the patients displayed significantly weaker ionomycin-induced intracellular reactive oxygen species (ROS) responses than those isolated from the fathers. Both patients showed increased expression of neutrophil elastase, whereas the mosaic fathers showed normal expression. Taken together, the results suggest that granulocytes from these SCN patients are immunocompromised, whereas those from the mosaic fathers are normal. These findings may provide new insight into disease diagnosis, prognosis, therapy and genetic counseling.

1. Introduction

Severe congenital neutropenia (SCN) encompasses a family of neutropenic disorders that can cause life-threatening pyogenic infections, acute gingivostomatitis, and chronic periodontal disease. Successive infections may result in permanent adverse sequelae. To date, a total of 24 genes have been linked to SCN [1], Mutations in the *ELANE* gene encoding neutrophil elastase are the most common cause. These mutations can cause two kinds of disease, which are categorized as two subtypes: SCN and cyclic neutropenia (CyN), which occur in about 40%–55% of patients with congenital neutropenia [2]. Although the same *ELANE* gene mutation can be responsible for both cyclic and permanent neutropenia [2], the treatment and prognosis are quite different. For CyN, the required dose of granulocyte colony stimulation factor (G-CSF) is generally below 5 μg/kg; injections can be given intermittently and the leukemic risk is very low or non-existent [3]. However, some patients with SCN do not respond to G-CSF [4]; the cumulative incidence of leukemia is about 15% [5]. The reasons for the different treatments and prognosis remain unknown. However, it is important to differentiate SCN from CyN. There are no absolute criteria by which to define CyN and SCN, although continuous and regular blood tests over a period of 6–8 weeks are helpful once congenital neutropenia is suspected. Somatic and germline mosaicism is a relatively common event in genetic diseases in which *de novo* mutations account for a substantial proportion of sporadic cases [6,7]. To date, more than 200 different *ELANE* mutations have been identified [8–10]; however, mosaicism of an *ELANE* mutation has been described in only four families with SCN [11–14]. In addition, no mosaicism of an *ELANE* mutation has been reported in a Chinese population. It has been reported phorbol myristate acetate (PMA) induced extracellular ROS is normal while the formyl-MLP peptide (fMLP) mediated extracellular ROS is severely reduced in *ELANE* patient after G-CSF treatment [15]. However, whether the ionomycin induced intracellular ROS in *ELANE* patient is impaired has not been studied before. Here, we describe in detail the clinical and laboratory findings of two Chinese patients harboring a novel mutation that extends the phenotype and functional spectrum of *ELANE* deficiency. These findings provide new insight into disease diagnosis, prognosis, therapy and genetic counseling.

* Corresponding author at: Chong Qing Key Laboratory of Child Infection and Immunity, Children's Hospital of Chongqing Medical University, Chongqing 400014, China.
E-mail address: zhaoxd530@aliyun.com (X.-D. Zhao).

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2. Methods

2.1. Patients and ethics statement

For patients, the diagnosis was based on clinical and hematologic data, taking into account the conventional criteria: persistent and severe neutropenia, severe bacterial and fungal infection, and arrest of bone marrow maturation at the promyelocyte/myelocyte stage [4]. Patient P1 (male) was born in August 2015 to healthy non-consanguineous parents. The child presented with otitis media and a perianal abscess during infancy. These infectious episodes were treated successfully with antibiotics without the need for hospitalization. After persistent fever lasting 1 month and repeated observation of neutropenia by staff at Children's Hospital of Chongqing Medical University, P1 was suspected as SCN. P2 (female) was aged 3 years and 2 months. She was referred to our hospital for genetic evaluation due to persistent severe neutropenia. She had a history of recurrent fever and hepatosplenomegaly. P3 was a boy aged 1 year and 5 months. He suffered from recurrent pneumonia and had a history of meningitis and sepsis during infancy. P1 received G-CSF treatment (8 μg/kg/d), while P2 received haploid bone marrow transplantation due to a poor response to G-CSF. P3 showed a satisfactory response to G-CSF treatment (5 μg/kg/d). For each experiment performed, at least one healthy control (HC) was included for comparison. The study was conducted according to the Helsinki Declaration. All study participants (or guardians) and HC provided written informed consent to participate in the study, which was approved by the Ethics Committee of children's hospital of Chong Qing medical University.

2.2. Molecular analysis of the ELANE gene

DNA was isolated from peripheral blood samples obtained from both patients using the QIAamp DNA Mini Kit (Qiagen Inc., Alameda, CA), according to the manufacturer's instructions. For the fathers, DNA was isolated from peripheral blood, peripheral blood mononuclear cells (PBMCs), granulocytes, and fingernails. Isolated DNA was subjected to polymerase chain reaction (PCR) to amplify all exons and flanking regions of the *ELANE* gene as described previously, with a few modifications [16]. In this case, the PCR conditions were as follows: denaturation at 95 °C for 5 min, followed by 39 cycles at 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 60 s. A final extension step was carried out at 72 °C for 5 min. The sequences of the primers targeting the *ELANE* gene are listed in Table 1. The PCR products were sequenced directly using the BigDye Terminator mix (Applied Biosystems Foster City, CA, USA) and oligonucleotide primers (Table 1). Clones were grown, selected, and purified according to the manufacturer's instructions (Zero TOPO-TA Cloning Kit, Yeasen, China).

2.3. Activity of intracellular NADPH oxidase in granulocytes

NADPH oxidase activity was measured by luminol-enhanced chemiluminescence (CL) [17] using a microplate reader (Synergy H1 Multi-Mode Reader, BioTek, U.S). In brief, granulocytes (before G-CSF administration) were isolated by centrifugation on Ficoll-Paque (GE Healthcare, Sweden) and then suspended in Krebs-Ringer phosphate buffer (KRG) at a final concentration of 3×10^6 cells/ml. Cells (20 μl) were then added to 160 μl KRG containing cell permeable luminol (final concentration, 2×10^{-5} M) and superoxide dismutase (SOD; final concentration, 50 units/ml) to scavenge extracellular superoxide. The reaction mixture was equilibrated for 5 min at 37 °C, after which 20 μl ionomycin was added (final concentration, 500 nM). CL was recorded continuously for 20–25 min.

Table 1
Primer sequences of *ELANE* gene.

ELANE	Forward primer	Reverse primer
Exon 1	CGAGCCAATCCAGCGTCTTGTC	TGGCTTCACCGCTCAGAACCTC
Exon 2	AGGTCTGTCTGTGCCTTGGAG	CTGAGGGCGAAGGTGCTC
Exon 3	CTCGAGCACCTTCGCCCTCAG	CCCGTTTCACAGAGGTGCAGAC
Exon 4	GAACCACAGTGGAACCTGAGATG	GTCTAGCCACGGTGCCTGTTG
Exon 5	CCCTAGGAGGGACTTCCCAACCCTG	CACCACGCCCGACCTACTGACC

2.4. Analysis of elastase expression

Granulocytes (after G-CSF administration for the patients) were counted and 1×10^6 cells were allowed to adhere to polylysine-coated glass slides for 30 min at 37 °C. The slides were then blocked for 30 min with 10% FBS and 2% BSA to block Fc receptors. Expression of neutrophil elastase was evaluated by staining cells with a mouse monoclonal antibody specific for human neutrophil elastase (1:50, Santa Cruz Biotechnology), followed by incubation at 4 °C overnight with a rabbit monoclonal antibody specific for myeloperoxidase (MPO) (1:100, Abcam). The slides were then washed and treated for 30 min at room temperature with AlexaFluor 488-congugated goat anti-mouse IgG (1:1000) and AlexaFluor 546-conjugated donkey anti-Rabbit IgG (H + L) (1:1000) cross adsorbed secondary antibodies (Thermo Fisher Scientific). Finally, slides were washed and mounted in Gold Anti-fade mounting media containing DAPI. Images were acquired under a confocal microscope (Nikon A1R, Japan). At least five visual fields per sample (magnification, ×20 and ×60) were selected at random and analyzed.

2.5. Immunoblotting

Total cell lysates were subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) on 12% gels and then transferred to PVDF membranes (Millipore). The blots were then rinsed in 0.05% PBS-T and blocked for 60 min in PBS-T containing 5% non-fat dry milk (pH 7.5). After overnight incubation (4 °C) with the following rabbit antibodies: anti-gp91phox, anti-p67phox, anti-p47phox, anti-p40phox, anti-p22phox (Abcam); The blots were incubated with horseradish peroxidase–conjugated anti–rabbit IgG (Cell Signaling Technology) for an hour at room temperature, Bound antibodies were detected by Clarity Western ECL substrate (Bio-Rad).

3. Results

3.1. Identification of a mutation in the ELANE gene, and identification of paternal mosaicism

Sequence analysis of all exons and flanking introns of the *ELANE* gene expressed by peripheral blood cells identified a heterozygous missense mutation (c.254G > T) in P1 (Fig. 1a), which result in G85 V amino acid substitution. A review of the database and the literature indicated that although a missense mutation (c.254G > A) was described before [18], c.254G > T was novel. Resulting in double nucleotide peaks on the chromatogram. Phylogenetic analysis indicated that the positions of the mutations are highly conserved across different species. Taken together with the clinical manifestations and laboratory findings, we concluded that the mutation is pathogenic. However, the mutation was also identified in peripheral blood cells from the asymptomatic father (Fig. 1b), although the peak height on the trace was lower than that for P1. To exclude CyN in the father, we performed sequential blood counts three times per week over a period of 1 month; the results showed no evidence of neutropenia.

P2 harbored a heterozygous missense mutation (c.164G > A) resulted in C55Y amino acid substitution (Fig. 2a) that has been reported before [18]. Sequencing of DNA derived from peripheral blood cells isolated from P2's father revealed a smaller peak corresponding to the c.164G > A mutation (Fig. 2b). However, sequential analyses of the father's blood (performed three times per week over a 1 month period)

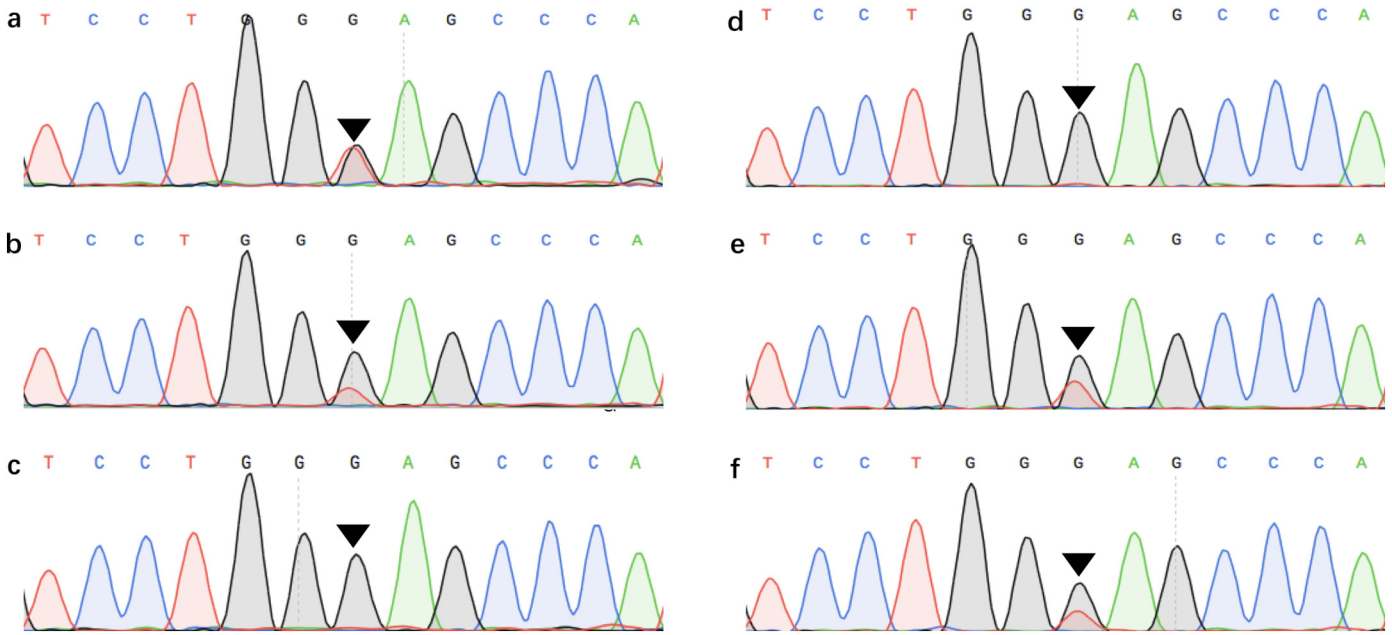

Fig. 1. Sequence analysis of *ELANE* in patient P1, his mother, and his father. (a) Identification of a heterozygous missense mutation in the *ELANE* gene in peripheral blood cells isolated from P1. (b) The same mutation was also identified, albeit with a smaller peak, in the asymptomatic father. (c) DNA from peripheral blood cells isolated from his mother. (d) DNA obtained from granulocytes isolated from the father. (e) DNA extracted from peripheral blood mononuclear cells isolated from the father. (f) DNA was isolated from fingernail clippings taken from the father and sequenced.

revealed a normal neutrophil count. P3 harbored a heterozygous missense mutation (c.337C > T) resulted S126 L amino acid substitution, which has been reported before [19]. Sequencing of DNA derived from peripheral blood cells isolated from his parent showed no mutation, suggesting that the mutation was sporadic. Because we suspected mosaicism on the side of P1'S and P2'S fathers, we isolated PBMCs and polymorphonuclear granulocytes from both fathers by Ficoll-Paque gradient centrifugation as previously described [15]. Fingernail clippings taken from both fathers were also examined. DNA was extracted and PCR products amplified from these samples were analyzed. The results showed that granulocytes isolated from both parents did not harbor the mutation (Figs. 1d and 2d); however, PCR products amplified from PBMCs and fingernails from both fathers also revealed heterozygous mutations as detected in peripheral blood cells (Figs. 1e, f and 2e, f). Since sanger sequencing can't quantificational detect the frequency of the mutant allele. *ELANE* DNA were cloned from both fathers' neutrophils, PBMC and fingernails, the mutant allele was identified in 15 of the 50 clones (30%) from the PBMC and 12 of the 50 clones (24%) from the fingernails of the asymptomatic P1's father. While the mutant allele was observed at a frequency of 18 of the 50

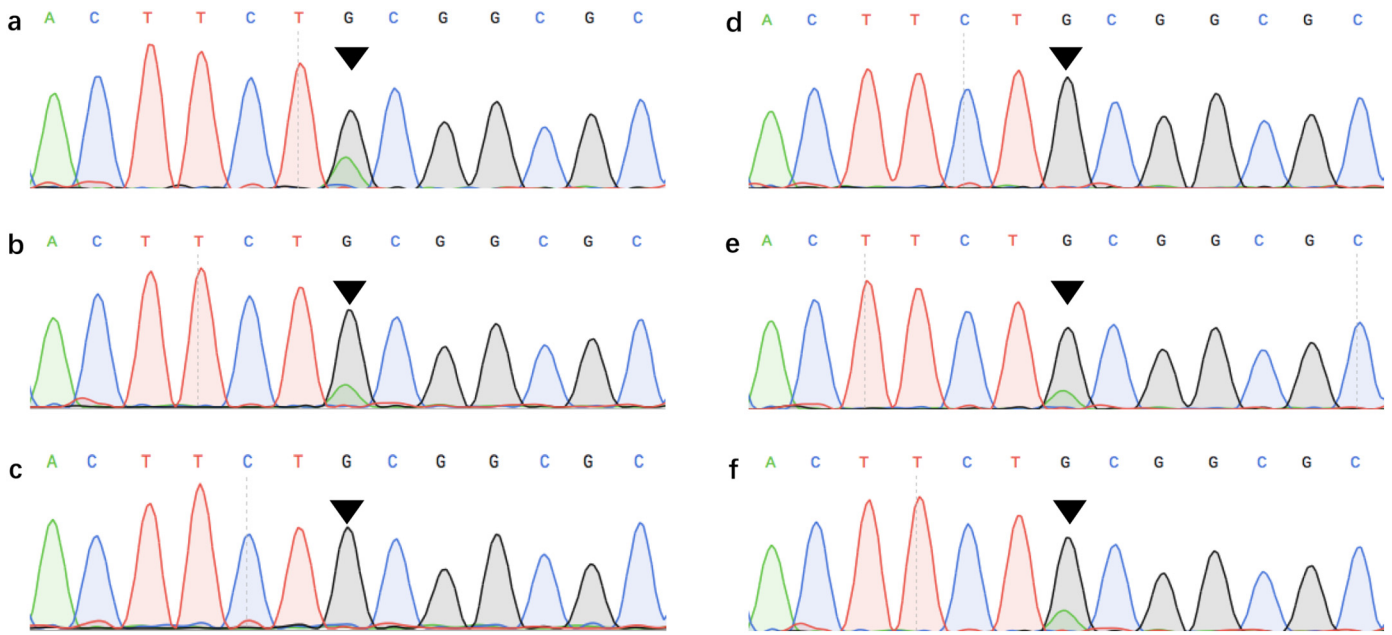

Fig. 2. Sequence analysis of *ELANE* in patient P2, her mother and her father. (a) Identification of a heterozygous missense mutation in the *ELANE* gene harbored by peripheral blood cells from P2. (b) The same mutation was also identified, albeit with a smaller peak, in the asymptomatic father. (c) DNA isolated from peripheral blood cells from her mother. (d) DNA was obtained from granulocytes isolated from the father and analyzed. (e) DNA obtained from peripheral blood mononuclear cells isolated from the father was analyzed. (f) DNA was obtained from fingernail clippings from the father and sequenced.

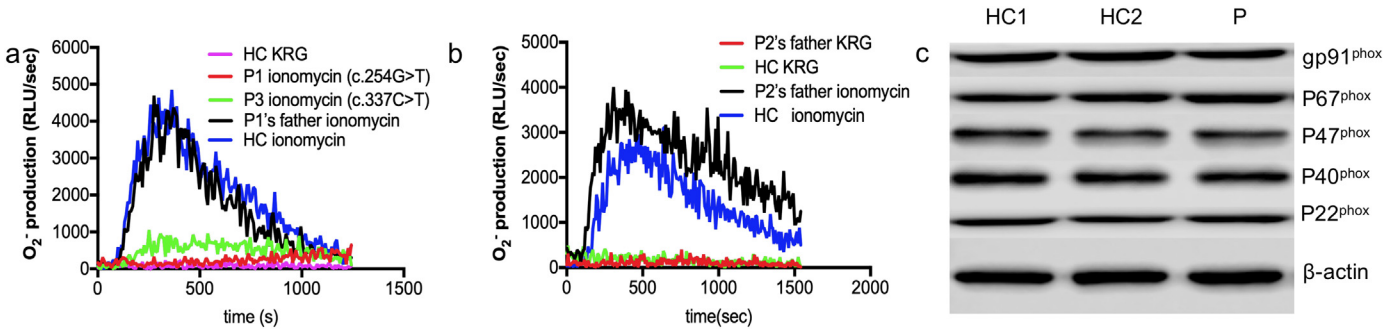

Fig. 3. NADPH oxidase component and Intracellular ROS production by granulocytes isolated from the patients and their mosaic fathers. (a) Granulocytes from P1 and P3 did not generate ionomycin-induced intracellular ROS in the absence of G-CSF; however, intracellular ROS production by granulocytes isolated from P1's father was comparable with that by cells isolated from healthy controls. (b) Granulocytes isolated from P2's father showed normal ionomycin-induced production of intracellular ROS. (c) Cell lysates of granulocytes purified P1 and 2 healthy subjects were subjected to Western blot analysis with anti-gp91phox, p47phox, p67phox, p40phox, or p22phox-specific antibodies.

clones (36%) from the PBMC and 10 of the 50 clones (20%) from the fingernails of the P2's father, however, 50/50 clones were wild-type in both fathers' neutrophils. These suggests that granulocytes expressing an elastase mutation were selectively lost at an early stage of myelopoiesis. The data also indicate mosaicism or reversion on the paternal side.

3.2. Intracellular assembly of NADPH oxidase in granulocytes isolated from the ELANE patients and their fathers

ROS production has not been extensively studied in *ELANE* patients, it is still unknown if intracellular ROS production is impaired in *ELANE* patients. Previous data show that ionomycin-induced neutrophil NADPH oxidase activity is inhibited by serine protease inhibitors [20]. Here, we examined intracellular ROS production by granulocytes (without G-CSF treatment) stimulated with ionomycin. We found that granulocytes from patients P1 and P3 lacked an ionomycin-induced intracellular ROS response; this response was intact in granulocytes from both fathers and two HC (Fig. 3a, b). Because P2 underwent hematopoietic cell transplantation shortly after diagnosis, we were unable to examine ROS production in granulocytes. Thereafter, we investigated the expression of NADPH oxidase, which plays a key role in ROS production. It showed that all the NADPH oxidase component were expressed at similar levels in control and SCN granulocytes (Fig. 3c).

3.3. Granulocytes from SCN patients show increased cytoplasmic accumulation of mutant elastase proteins whereas paternal granulocytes show normal expression patterns

Promyelocytes (the cell stage at which granulocytic differentiation is arrested in SCN) express extremely high levels of elastase [21]. Previous studies suggest that cytoplasmic accumulation of elastase protein in granulocytes, and induction of the unfolded protein response (UPR) triggered by mutant elastase, may contribute to the pathogenesis of SCN [22]. Therefore, we analyzed expression of elastase. Similar to previous reports, we noted greater elastase accumulation in *ELANE*-mutant SCN granulocytes (Fig. 4) than in HC cells. Expression of elastase by paternal granulocytes was comparable with that by granulocytes from HC. These data suggest that increased expression/accumulation of mutated elastase may be common phenomena in *ELANE* patient.

4. Discussion

SCN encompasses several life-threatening diseases characterized by recurrent fever, painful mouth ulcers, and skin and deep tissue infections; in severe cases infection or transformation to myeloid malignancies can be fatal. Here, we report two cases in which neutrophil elastase mutation acquired by the asymptomatic fathers was passed to the children; both children were heterozygous for the mutation and were diagnosed with classical SCN. The phenotype of these two children was severe because they suffered recurrent life-threatening infections and persistent neutropenia. P1 harbored a novel heterozygous missense mutation. His father also harbors this mutation, However, the frequency of the mutant allele in his father was much lower than expected for a typical heterozygous state. Further investigation of PBMCs, polymorphonuclear granulocytes, and fingernail clippings confirmed mosaicism in his father. Gene sequencing of P2 and her father identified a previous reported heterozygous missense mutation (c.164G > A) in the *ELANE* gene [18]. Polymorphonuclear granulocytes from her father did not harbor this mutation, although it was detected in his PBMCs and fingernails. This is presumably because mature granulocytes cannot be generated from stem cells expressing the *ELANE* mutation, or because the mutated cells have a growth disadvantage and are destroyed before entering the circulation. Mosaicism in the fathers explained their healthy status and normal cell counts. Previous reports also describe a hematologically normal phenotype in the carrier parent with mosaicism (summarized in Table 2) [11–14]. It is worth noting that the origin of the three mutations identified to date derived from mosaicism on the paternal side; only one CyN mutation has been reported on the maternal side [13]. This may infer that there is gender bias in *ELANE* patients. Our observation together with the previous data indicated that there is a selective advantage to the wild-type neutrophils. Paying careful attention to the sequence data of parents is crucial to diagnose mosaic parents, and it also have implications for genetic counseling.

Neutrophils are the first line of defense against bacterial and fungal infections. They execute their antimicrobial effects through oxygen-dependent and oxygen-independent mechanisms. Stimulated neutrophils activate NADPH oxidase to generate large amounts of superoxide. it has been reported that PMA induced superoxide production was normal, while the fMLP mediated ROS is severely reduced in *ELANE* patient after G-CSF treatment [15]. however, they use the cytochrome C reduction which can only detect the extracellular ROS [23]. It is still unknown whether the intracellular ROS is impaired in *ELANE* patients. Claes et al., showed that ionomycin-induced intracellular neutrophil NADPH oxidase activity is inhibited by serine protease inhibitors [20]. Indeed, we found that in the absence of G-CSF, no intracellular ROS was detected in the patients' granulocytes (granulocytes from the parents were normal). The biological relevance of ROS is revealed by the persistent bacteria and fungi infections associated with chronic granulomatous disease [24,25]. However, ROS production at vesicular or endosomal sites are unrelated to antimicrobial activity [26]. The biological relevance of non-phagosomal ROS is not

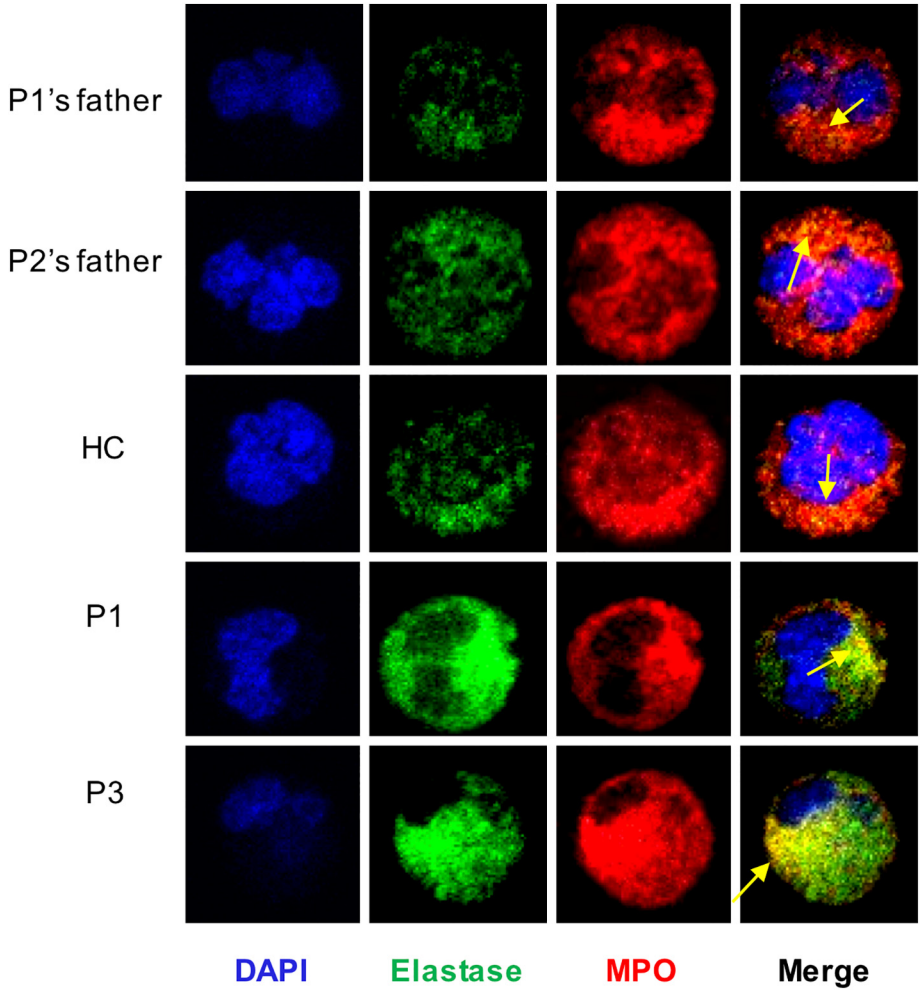

Fig. 4. Confocal microscopic analyses of neutrophil elastase expression by granulocytes from *ELANE* patients (stimulated with G-CSF) and the mosaic fathers. Cells were labeled with antibodies specific for elastase (green) and myeloperoxidase (red). Nuclei were counterstained with DAPI (blue). Yellow denotes colocalization of elastase and myeloperoxidase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

completely clear. Granulocytes from patients with synovitis, acne, pustulosis, hyperostosis, or osteitis (SAPHO) show severe suppression of intracellular ROS production [27,28]. These may, at least in part, explain why SCN patients are prone to cellulitis and osteoporosis [16]. The abolished ionomycin induced intracellular ROS is an interesting finding, it may be related to a defect in the signal transduction pathway in granulocytes from SCN patients triggered by ionomycin. The skewed gp91phox, p22phox expression may also contribute to it [15], however, we find the same expression of the NAPDH oxidase component. The different expression may relate to different point mutation and even the impact of a specific mutation may vary [19]. Here, normal intracellular ROS production by granulocytes isolated from the mosaic father confirmed that both the number and function of granulocytes were normal. This may explain why the parents are asymptomatic.

Immunofluorescence microscopy revealed that granulocytes isolated from the patients showed a higher expression of elastase, whereas that by granulocytes from the parents was comparable with that by cells from controls. This may be attributed to that intracellular processing of mutant elastase proteins is interrupted after exiting the ER, resulting in cytoplasmic accumulation [29]. The mutated elastase proteins can activate the unfolded protein response/ER stress (UPR/ER stress) which contribute to the pathobiology of SCN [30]. Normal expression of elastase by cells from both mosaic fathers is an interesting observation and, together with the intracellular ROS data, indicates that mature granulocytes from the mosaic fathers were morphologically and functionally normal. Clinical data about the fathers, particularly during childhood, are lacking and both refused to undergo bone marrow aspiration; therefore, we cannot exclude the possibility of reversion. Further studies are needed to fully identify the mechanism underlying mosaicism; the results may provide new insight into novel treatment strategies.

Table 2
summary of four-mosaic parents.

Family	Family 1	Family 2	Family 3	Family 4
Country	UK (Ancliff PJ, et al.[11])	Korea (Kim HJ, et al. [12])	Japan (Hirata O, et al. [13])	Germany (Germeshausen M, et al. [14])
Origin of mutation	Father	Father	Mother	Father
Subtypes	SCN	SCN	CyN	SCN
ELANE mutation	c.126C > T	c.658delC	IVS4 + 5SD G > T	c.254G > T
Numbers of patient	1	1	3	2

Conflicts of interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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References

[1] J. Donadieu, B. Beaupain, O. Fenneteau, Congenital neutropenia in the era of genomics: classification, diagnosis, and natural history, Br. J. Haematol. 179 (2017) 557.
[2] C. Bellanné-Chantelot, S. Clauin, T. Leblanc, B. Cassinat, F. Rodrigues-Lima, S. Beaufils, C. Vaury, M. Barkaoui, O. Fenneteau, M. Maier-Redelsperger, Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French neutropenia register, Blood 103 (2004) 4119–4125.
[3] D. Jean, F. Odile, B. Blandine, M. Nizar, C.C. Bellanné, Congenital neutropenia: diagnosis, molecular bases and patient management, Orphanet J. Rare Dis. 6 (2011) 1–28.
[4] D.C. Dale, T.E. Cottle, C.J. Fier, A.A. Bolyard, M.A. Bonilla, L.A. Boxer, B. Cham, M.H. Freedman, G. Kannourakis, S.E. Kinsey, Severe chronic neutropenia: treatment and follow-up of patients in the severe chronic neutropenia international registry, Am. J. Hematol. 72 (2003) 82.
[5] J. Donadieu, T. Leblanc, M.B. Bader, M. Barkaoui, O. Fenneteau, Y. Bertrand, M. Maierredelsperger, M. Micheau, J.L. Stephan, N. Phillipe, Analysis of risk factors for myelodysplasias, leukemias and death from infection among patients with congenital neutropenia. Experience of the French Severe Chronic Neutropenia Study Group, Haematologica 90 (2005) 45–53.
[6] H. Morita, I. Komuro, Somatic mutations in cerebral cortical malformations, N. Engl. J. Med. 371 (2014) 733–743.
[7] S.A. Huisman, E.J.W. Redeker, S.M. Maas, M.M. Mannens, R.C.M. Hennekam, High rate of mosaicism in individuals with Cornelia de Lange syndrome, J. Med. Genet. 50 (2013) 339–344.
[8] M.S. Horwitz, D. Zhijun, K. Brice, L. Hu-Hui, M.E. Mealiffe, S.J. Salipante, Neutrophil elastase in cyclic and severe congenital neutropenia, Blood 109 (2007) 1817.
[9] G. Manuela, D. Sabine, P. Yvonne, R. Christina, C.P. Kratz, B. Matthias, The spectrum of ELANE mutations and their implications in severe congenital and cyclic neutropenia, Hum. Mutat. 34 (2013) 905–914.
[10] M. Vahagn, Z. Cornelia, B. Audrey Anna, S. Julia, R. Elin, M.L. Kelley, L.A. Boxer, B. Mary Ann, P.E. Newburger, S. Akiko, The diversity of mutations and clinical outcomes for ELANE-associated neutropenia, Curr. Opin. Hematol. 22 (2015) 3–11.
[11] P.J. Ancliff, R.E. Gale, M.J. Watts, R. Liesner, I.M. Hann, S. Strobel, D.C. Linch, Paternal mosaicism proves the pathogenic nature of mutations in neutrophil elastase in severe congenital neutropenia, Blood 100 (2002) 707–709.
[12] H.-J. Kim, M.-J. Song, K.-O. Lee, S.-H. Kim, H.-J. Kim, Paternal somatic Mosaicism of a novel Frameshift mutation inELANECausing severe congenital neutropenia, Pediatr. Blood Cancer 62 (2015) 2229–2231.
[13] O. Hirata, S. Okada, M. Tsumura, S. Karakawa, I. Matsumura, Y. Kimura, T. Maihara, S. Yasunaga, Y. Takihara, O. Ohara, Mosaicism of an ELANE mutation in an asymptomatic mother in a familial case of cyclic neutropenia, J. Clin. Immunol. 35 (2015) 512.
[14] M. Germeshausen, H. Schulze, M. Ballmaier, C. Zeidler, K. Welte, Mutations in the gene encoding neutrophil elastase (ELA2) are not sufficient to cause the phenotype of congenital neutropenia, Br. J. Haematol. 115 (2001) 222–224.
[15] M. Donini, S. Fontana, G. Savoldi, W. Vermi, L. Tassone, F. Gentili, E. Zenaro, D. Ferrari, L.D. Notarangelo, F. Porta, F. Facchetti, L.D. Notarangelo, S. Dusi, R. Badolato, G-CSF treatment of severe congenital neutropenia reverses neutropenia but does not correct the underlying functional deficiency of the neutrophil in defending against microorganisms, Blood 109 (2007) 4716–4723.
[16] Z. Shu, X.H. Li, X.M. Bai, Z.Y. Zhang, L.P. Jiang, X.M. Tang, X.D. Zhao, Clinical characteristics of severe congenital neutropenia caused by novel ELANE gene mutations, Pediatr. Infect. Dis. J. 34 (2015) 203–207.
[17] J. Bylund, H. Björnsdottir, M. Sundqvist, A. Karlsson, C. Dahlgren, Measurement of Respiratory Burst Products, Released or Retained, During Activation of Professional Phagocytes, Humana Press, 2014.
[18] P.J. Ancliff, R.E. Gale, R. Liesner, I.M. Hann, D.C. Linch, Mutations in the ELA2 gene encoding neutrophil elastase are present in most patients with sporadic severe congenital neutropenia but only in some patients with the familial form of the disease, Blood 98 (2001) 2645–2650.
[19] D.C. Dale, R.E. Person, A.A. Bolyard, A.G. Aprikyan, C. Bos, M.A. Bonilla, L.A. Boxer, G. Kannourakis, C. Zeidler, K. Welte, Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia, Blood 96 (2000) 2317–2322.
[20] C. Dahlgren, A. Karlsson, Ionomycin-induced neutrophil NADPH oxidase activity is selectively inhibited by the serine protease inhibitor diisopropyl fluorophosphate, Antioxid. Redox Signal. 4 (2002) 17–25.
[21] N. Borregaard, J.B. Cowland, Granules of the human neutrophilic polymorphonuclear leukocyte, Blood 89 (1997) 3503–3521.
[22] I. Kollner, B. Sodeik, S. Schreek, H. Heyn, N. von Neuhoff, M. Germeshausen, C. Zeidler, M. Kruger, B. Schlegelberger, K. Welte, C. Beger, Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response, Blood 108 (2006) 493–500.
[23] W.M. Nauseef, Detection of superoxide anion and hydrogen peroxide production by cellular NADPH oxidases, Biochim. Biophys. Acta Gen. Subj. 1840 (2014) 757–767.
[24] B.H. Segal, T.L. Leto, J.I. Gallin, H.L. Malech, S.M. Holland, Genetic, biochemical, and clinical features of chronic granulomatous disease, Medicine 79 (2000) 170.
[25] K.M. Rigby, F.R. Deleo, Neutrophils in innate host defense against Staphylococcus aureus infections, Semin. Immunopathol. 34 (2012) 237–259.
[26] J.M. Robinson, Reactive oxygen species in phagocytic leukocytes, Histochem. Cell Biol. 130 (2008) 281–297.
[27] L. Björkman, C. Dahlgren, A. Karlsson, K.L. Brown, J. Bylund, Phagocyte-derived reactive oxygen species as suppressors of inflammatory disease, Arthritis Rheum. 58 (2008) 2931–2935.
[28] P.J. Ferguson, M.A. Lokuta, H.I. Elshanti, L. Muhle, X. Bing, A. Huttenlocher, Neutrophil dysfunction in a family with a SAPHO syndrome-like phenotype, Arthritis Rheum. 58 (2008) 3264–3269.
[29] D.S. Grenda, M. Murakami, J. Ghatak, J. Xia, L.A. Boxer, D. Dale, M.C. Dinauer, D.C. Link, Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis, Blood 110 (2007) 4179–4187.
[30] R.C. Nayak, L.R. Trump, B.J. Aronow, K. Myers, P. Mehta, T. Kalfa, A.M. Wellendorf, C.A. Valencia, P.J. Paddison, M.S. Horwitz, H.L. Grimes, C. Lutzko, J.A. Cancelas, Pathogenesis of ELANE-mutant severe neutropenia revealed by induced pluripotent stem cells, J. Clin. Investig. 125 (2015) 3103–3116.