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The diversity of mutations and clinical outcomes for ELANE-associated neutropenia

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Abstract

Purpose of review—Mutations in the gene for neutrophil elastase, *ELANE*, cause cyclic neutropenia (CyN) and severe congenital neutropenia (SCN). This study summarized data from the Severe Chronic Neutropenia International Registry (SCNIR) on genotype–phenotype relationships of *ELANE* mutations to important clinical outcomes. We also summarize findings for *ELANE* mutations not observed in SCNIR patients.

Recent findings—There were 307 SCNIR patients with 104 distinctive *ELANE* mutations who were followed longitudinally for up to 27 years. The *ELANE* mutations were diverse; there were

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Conflicts of interest

V.M., C.Z., A.A.B., J.S., E.R., M.L.K., M.A.B., P.E.N., A.S., B.Z., P.S.R., D.C.L., and K.W. have no conflicts to disclose. L.A.B. owns stock in Amgen, the manufacturer of G-CSF mentioned in the paper. D.C.D. is a consultant and receives research support from Amgen.

65 single amino acid substitutions; 61 of these mutations (94%) were ‘probably’ or ‘possibly damaging’ by PolyPhen-2 analysis, and one of the ‘benign’ mutations was associated with two cases of acute myeloid leukemia (AML). All frame-shift mutations (19/19) were associated with the SCN. The pattern of mutations in the SCN versus CyN was significantly different ($P < 10^{-4}$), but some mutations were observed in both groups (overlapping mutations). The cumulative incidence of severe adverse events, that is, myelodysplasia, AML, stem cell transplantation, or deaths was significantly greater for patients with SCN versus those with CyN or overlapping mutations. Specific mutations (i.e. G214R or C151Y) had a high risk for evolution to AML.

Summary—Sequencing is useful for predicting outcomes of *ELANE*-associated neutropenia.

Keywords

congenital neutropenia; cyclic neutropenia; *ELANE*; neutropenia; neutrophil elastase

INTRODUCTION

Cyclic neutropenia (CyN) and severe congenital neutropenia (SCN) are rare hematological disorders usually caused by heterozygous mutations in *ELANE*, the gene which encodes neutrophil elastase [1–3]. CyN is characterized by severe neutropenia [absolute neutrophil count (ANC) $< 0.2 \times 10^9/l$], fever, mouth ulcers, and infections recurring approximately every 21 days [4]. Patients with SCN usually have ANC less than $0.5 \times 10^9/l$ [5], more serious infections, and may develop acute myeloid leukemia (AML) [6–8]. In some cases, the diagnosis is difficult because there are too few serial ANCs for analysis [3,8].

Previous studies indicate that the diagnosis of CyN is associated somewhat selectively with mutations in exon 4, intron 4, and exon 5 [3,8,9,10,11], and that *ELANE* mutation G214R (also referred to as G185R) is associated with greater risk of myelodysplasia (MDS)/AML [10]. Most laboratory investigations indicate that expression on mutant *ELANE* initiates the unfolded protein response, accelerates apoptosis of developing myeloid cells, and results in ineffective myelopoiesis [12–15]. Mutations in *CSF3R*, *RAS*, and *RUNX1* are associated with evolution to MDS and AML [16,17,18], but the biological basis for the substantial difference in risk of leukemic evolution for CyN versus SCN is not known.

The study reviews data from the SCNIR on patients with *ELANE* mutations to assess the relationships of mutations and clinical outcomes, and summarizes data on *ELANE* mutations in other publications.

METHODS

This study reviews data from the Severe Chronic Neutropenia International Registry (SCNIR) on patients with *ELANE* mutations to assess the relationships of mutations and clinical outcomes, and summarizes data on *ELANE* mutations in other publications.

Literature survey

We reviewed PubMed for *ELANE*, *ELA-2*, neutrophil elastase, cyclic and congenital neutropenia, and related databases for this study.

SCNIR patients

The Institutional Review Boards at the University of Washington, Seattle, Washington, and Medizinische Hochschule, Hannover, Germany, approved the study protocol. Patients were referred to the SCNIR and diagnoses assigned based on clinical records, including family history, blood counts, and bone marrow reports. Patients with SCN had blood neutrophil counts usually below $0.5 \times 10^9/l$ without regular cyclic fluctuations and a bone marrow showing ‘maturation arrest’ of the myeloid cell lineage. The diagnosis of CyN was based on serial blood counts showing neutrophil fluctuations from near $2.0 \times 10^9/l$ to less than $0.2 \times 10^9/l$ at approximately 21-day intervals. The clinical status and treatment responses were reviewed annually using standard forms. Major events and illnesses were also reported to the SCNIR by the patients, families, or referring physicians when they occurred.

DNA procurement and *ELANE* mutational analysis

DNA sequencing was performed on biological samples utilizing standard genomic DNA extraction techniques. Mutational data for some of the SCNIR patients was previously reported [2,3,19]. *ELANE* mutations were categorized counting from the start codon. PolyPhen-2 predictions (HumDiv Model) were made as previously described [20].

Statistical methods

For genotype–phenotype associations, we compared the frequency distribution of mutations between groups using chi-square analysis. Because the numbers in some groups was small (less than 5), we assessed statistical significance based on the permutation distribution of the chi-square statistic estimated from 10 000 permutations [21]. Our primary analysis treated patients as independent; however, some patients were related, and therefore shared the same causative mutation. To examine whether associations were dependent on multiple cases in some families, we also calculated *P* values based on a random assignment of the same mutation to related individuals.

We used the nonparametric Wilcoxon rank-sum test to compare median values of granulocyte colony-stimulating factor (G-CSF) dose between groups, and the Kruskal–Wallis test to compare median dose values between three or more groups [22,23]. For the Wilcoxon and Kruskal–Wallis tests, to account for sparse data in some groups, we also determined statistical significance on the basis of the permutation distribution of the test statistics. For time-to-event analysis, we analyzed a composite outcome variable defined as the first occurrence of a severe adverse event. We estimated the cumulative incidence or proportion of patients experiencing a severe adverse event versus years on G-CSF therapy as the complement of the Kaplan–Meier curve using the 1-Kaplan–Meier estimator. We assessed statistical significance using the likelihood ratio test from the Cox proportional-hazards model, treating patients as independent. Because the numbers of adverse events were comparatively low, we also assessed statistical significance using the exact binomial test [24].

We examined associations between genotype and phenotype using a number of categorizations of mutations. These included distinct amino acid changes, mutation class, and disease associations. We also considered a restricted set of distinct amino acid changes,

with all mutations occurring at a frequency of 1% or less in the total group of 307 patients included in a single ‘other’ category.

For all analyses, *P* values less than 0.05 were considered statistically significant.

Observations

The SCNIR population included 40 families with two or more affected members (CyN 20 families; SCN 20 families). The diagnosis was made based on clinical data, and the *ELANE* mutations were identified later in all cases. The family members with neutropenia had *ELANE* mutations, and all members with *ELANE* mutations had neutropenia.

Patients, treatments, and outcomes

Table 1 summarizes mutations, treatments, and major events for the SCNIR population. The analysis of G-CSF treatment was limited to the 241 patients whose longitudinal treatments were followed by the SCNIR. Only 10 of the 307 patients were known to have not been exposed to G-CSF. The remaining 56 patients may have received G-CSF through their personal physicians and not reported this information. For the CyN patients, there were eight deaths, none due to MDS/AML. Three deaths were attributable to sepsis; two of these patients were known not to be taking G-CSF as recommended, and one other patient was never treated with G-CSF. One death followed hematopoietic transplantation (not for MDS/AML). The causes of the other four deaths were: cancer (one), heart failure (one), stroke (one), and unknown (one). For the SCN patients, there were 15 deaths attributable to MDS/AML and three transplant-related deaths in patients who failed to respond to G-CSF, and four others of unknown cause. In the 20 SCN families (two to seven affected members), there was only one family with more than one case of MDS/AML – a family with mutation C151Y.

Mutations and clinical diagnoses

The patients had 104 distinct *ELANE* mutations (Table 1 and Fig. 1). There were 187 SCN patients with 94 mutations, and 120 CyN patients with 22 mutations. The 65 missense mutations were analyzed by PolyPhen-2 (HumDiv Model). Fifty-four were predicted to be ‘probably damaging’, seven to be ‘possibly damaging’, and four to be ‘benign’. There were 27 patients with mutation S126L, an overlapping mutation, mutations observed in both CyN and SCN, predicted to be benign by PolyPhen-2; two of the SCN patients evolved to MDS/AML. The other three mutations predicted to be benign were all associated with SCN. Overall, there was a segregation of mutations for SCN versus CyN (Fig. 1), but 12 mutations were observed in both CyN and SCN patients – the overlapping mutations. Statistically, the distribution of mutations in CyN versus SCN was significantly different in permutation tests of specific mutations, mutation class, and mutation position (all *P* values less than 10^{-4} for patients or families).

Clinical outcomes

Infections were common in these patients before G-CSF, including mouth ulcers (80%), pneumonia (49%), abscesses (19%), sepsis (17%), cellulitis (12%), and peritonitis (3%). Mutations only seen in CyN or overlap mutations were strongly associated with mouth

ulcers (96 of 99 positive, 97%; Fig. 2a), versus patients with mutations only seen in SCN (61 of 97 positive, 63%, $P < 10^{-4}$; Fig. 2a). When analysis was restricted to patients with SCN risk of mouth ulcers also varied significantly by mutation ($P = 0.002$) and between overlapping versus unique-to-SCN mutation categories ($P = 0.01$) (data not shown).

Sixty-one of 97 patients (63%) with mutations unique to SCN reported pneumonia, compared to 31 of 73 patients (42%) with overlapping mutations and five of 26 patients (19%) with mutations only seen in CyN ($P = 3 \times 10^{-4}$; Fig. 2b). Abscesses occurred in all patient groups: overlapping mutations (four of 73, 5%), CyN (four of 26, 15%) or SCN (19 of 97, 20%; Fig. 2c).

With longitudinal follow-up, severe events (MDS/AML, stem cell transplant, or death) was far greater in SCN versus CyN patients (eight of 120 CyN versus 62 of 187 SCN, $P < 10^{-4}$; Table 1). The striking excess of severe events over time in SCN versus CyN and for mutation unique to SCN versus the overlapping mutations in patients on G-CSF was also highly statistically significant ($P < 10^{-4}$; Fig. 3).

Mutations and myelodysplasia/acute myeloid leukemia

The 104 different *ELANE* mutations were: 65 missense, 15 frameshift, eight termination, eight intronic, and seven in-frame deletion or insertion. There was a higher frequency of single base pair/ amino acid mutations in SCN versus CyN (134 of 187 SCN vs. 59 of 120 CyN; $P = 2 \times 10^{-4}$). All frame-shift mutations were associated with SCN.

Thirty of 187 SCN patients (16%) developed MDS/AML, associated with 25 different *ELANE* mutations. No CyN patients developed MDS/AML ($P < 0.0001$, Fisher's exact test). The MDS/AML cases in SCN patients were associated with mutations in all five exons and in introns III and IV. The mutations were: frameshift 6/19 (32%); termination 3/12 (25%); intronic 3/15 (20%); in-frame 1/7 (14%); and missense 17/134 (13%).

Figure 1 shows the specific mutations associated with MDS/AML. Clusters of MDS/AML cases were observed at two loci: three of four patients with mutation C151Y (two of these three were related), and three of nine patients with mutation G214R. Two of the 24 SCN patients with mutation S126L also developed MDS/AML. There was only one case of MDS/AML with each of the other mutations. There are 79/104 mutations which thus far have not been associated with MDS/AML.

G-CSF, mutations, and outcomes

There were 241 patients with longitudinal data on neutrophil counts (ANC) and G-CSF treatment (165 SCN, 76 CyN). The SCN patients' baseline ANC values were lower than those for the CyN group (0.10×10^{-9} versus 0.40×10^{-9} cells/l; $P = 5 \times 10^{-20}$) and G-CSF doses were higher (6.7 versus 2.1 $\mu\text{g/kg/day}$; $P = 2 \times 10^{-14}$). The median durations of G-CSF treatment overlapped: CyN, 13.1 years (range 0.6–24.6 years) and SCN, 10.6 years (range 0.4–23.7 years). Within the overall population of CyN and SCN, median baseline ANC values did not differ significantly according to specific mutation or mutation class or position. Within SCN, however, the fraction of patients with very low ANC (a total of 40 SCN patients had baseline ANC measured as 0) was significantly different across the

restricted set of specific mutations ($P = 0.002$), with very low ANC occurring with greater frequency in patients with A57T (four of four), C151Y (three of four), and G214R (three of seven).

Within CyN, G-CSF dose was quite uniform across mutations (data not shown). In contrast, in SCN there were highly statistically significant associations of median G-CSF dose with mutation position ($P = 0.001$; Fig. 4a) and specific mutation ($P = 0.001$; Fig. 4b). Higher doses were associated with the loci situated in proximity to the 5' and 3' regions of the gene versus the interior (Fig. 4a). Higher doses were also associated with mutations C151Y and G214R and lower doses with P139L and IVS4+5 G>A (Fig. 4b). For mutation G214R, five patients had stem cell transplants because of failure to respond to G-CSF (median dose = 80 $\mu\text{g/kg/day}$). There were also higher doses with mutations unique to SCN and lower doses with mutations observed in both SCN and CyN. Multivariate analysis of $\log_2$ G-CSF dose adjusted for age at start of therapy (quartiles) and registry (Seattle or Europe) suggested that these associations were not due to confounding by age or geography (data not shown).

In SCN, the rate of MDS/AML, transplant, or death was also significantly associated with mutation position ($P = 0.002$; Fig. 4b), specific mutation ($P = 0.003$; Fig. 4d), and diagnosis. After 20 years on G-CSF, the cumulative incidence or proportion of patients experiencing a severe event (MDS/AML, transplant, or death) was 53% for patients with mutations located from the 5' UTR region through exon 2, and 71% for patients with mutations located in exon 5, versus 35% for patients with mutations located in the interior of the gene from exon 3 through intron IV (Fig. 4c). After 20 years on G-CSF, all eight patients (100%) with mutation G214R and all four patients (100%) with mutation C151Y mutations experienced a severe event, as compared to zero of the 11 patients (0%) with P139L and IVS4+5 G>A mutations, and two of the 20 patients (10%) with the S126L mutation. After 20 years on G-CSF, the estimated cumulative incidence was 46% [95% confidence interval (CI) 35–55%] in SCN versus 7% (95% CI 0–17%) in CyN.

Update on mutations in *ELANE*-associated neutropenia

Table 2 lists new mutations in this study and in other recent studies. A comprehensive list of other *ELANE* mutations is presented in reference [11].

DISCUSSION

Mutations in *ELANE* were identified as the cause of CyN and autosomal dominant SCN through linkage analysis and sequencing of candidate genes [2,3,28,29]. *ELANE* encodes neutrophil elastase, a protease packaged in the primary granules of neutrophil precursors [30,31]. Mutant neutrophil elastase can trigger apoptosis of developing neutrophils via initiating the unfolded protein response [12–15,32]. 'Maturation arrest' in myeloid cell development in the bone marrow is probably the morphological correlate of these phenomena.

We conducted this review of the comprehensive database of the SCNIR to determine whether genotyping is useful for predicting severe adverse events, particularly the risk of MDS/AML. We observed that all exons as well as introns III and IV harbored mutations

associated with these complications. The highest proportion was in exon 1, with one of three patients evolving to MDS/AML.

We grouped mutations in five principal categories: missense, frameshift, termination, intronic, and deletion or insertion. The missense mutations were most common, and 94% were predicted to have a probable or possible damaging effect on the protein structure by Polyphen 2 analysis. This analysis was, however, imperfect because one of the three 'benign' mutations was associated with two cases of MDS/AML. The most striking finding was the association of frameshift mutations in congenital patients with a high risk of abscesses (8/17 evaluable patients, 47%) and evolution to MDS/AML (6/19 patients, 32%). It appears also that a higher proportion of termination mutations are associated with leukemic transformation, but the number of these patients is relatively small. Two mutations C151Y (3/4 patients) and G214R (3/9 patients) appear to confer a high risk of leukemia. The apparent high risk associated with these mutations may be attributable to major changes in protein structure due to size and charge/polarity differences of exchanged amino acids. C151Y disrupts the second disulfide bond and G214R replaces glycine (nonpolar, neutral) with the much larger arginine (polar, strongly basic). One recent publication indicates that mutations in the initiator methionine codon for *ELANE* may force translation from downstream internal initiation codons and produce a polypeptide truncated at the amino terminus that is subsequently mislocalized and accumulated in the cell's nucleus [32]. We believe frameshift and termination mutations may also have significant structural effects on the protein, but precisely how these mutations and categories of mutations increase the risk of leukemia is not yet known.

Our previous studies have focused on benefits and risks of G-CSF treatment and the relative risks of evolution specifically to MDS/AML or death from infections. The results of this genotype–phenotype study suggest that the *ELANE* genotype influences the risk of severe bacterial infections, the dose of G-CSF required to treat neutropenia, and the risk of evolution to MDS and AML. Other researchers have concluded that there are no associations of *ELANE* genotypes and clinical outcomes [11,33]. We believe that we reached different conclusions largely through the methods for assigning diagnosis at time of enrollment in the SCNIR and long-term follow-up of patients.

CONCLUSION

There are clinically important genotype–phenotype correlations of clinical outcomes and the results of sequencing of *ELANE* for patients with cyclic and congenital neutropenia. Some mutations appear to be linked to a relatively good prognosis, notably P139L, IVS4+5 G>A, and S126L, whereas other mutations, specifically C151Y and G214R, are associated with a notably poor prognosis. The results of this analysis provide useful information to guide clinical care and to focus research on the cellular and molecular mechanisms for neutropenia and leukemogenesis in these patients.

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■ of special interest

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KEY POINTS

- Cyclic and congenital neutropenia are most frequently caused by mutations in *ELANE* – the gene for neutrophil elastase.
- More than 120 distinct mutations are now known.
- This genotype–phenotype study points to certain mutations, for example, G214R and C151Y, and types of mutations, for example, frameshift mutations, which are intrinsically more risky than other mutations in this gene.
- The review serves to focus attention on the *ELANE* genotypes and outcomes in patients with cyclic and congenital neutropenia.

FIGURE 1. ELANE mutations in cyclic and congenital neutropenia. Linear representation of *ELANE* – five exons and four introns showing location and types of mutations observed in 307 patients. Mutations seen in SCN are listed above the gene map, and those seen in CyN are listed below. Mutations seen in both SCN and CyN are indicated in bold. Mutations with MDS/AML cases reported are italicized and the superscripts indicate the number of patients with that mutation who developed MDS/AML. AML, acute myeloid leukemia; CyN, cyclic neutropenia; MDS, myelodysplasia; SCN, severe congenital neutropenia.

FIGURE 2.

Associations between *ELANE* mutations and severe bacterial infections in patients with cyclic neutropenia (CyN) and severe congenital neutropenia (SCN) prior to any treatment with G-CSF therapy. (a) Percentage with a positive history of mouth ulcers (mo+) versus negative history (mo-); (b) history of pneumonia by disease association of mutation (pn+) versus negative (pn-); (c) history of deep-tissue abscess, positive (ab+) versus negative (ab-) amongst patients with mutations unique to CyN, overlapping mutations, and mutations unique to SCN. The number of patients is shown above each bar. The overlapping mutations are those listed in Fig. 1. *P* values are from permutation test with random assignment of genotype within families.

FIGURE 3.

Probability of adverse events in SCN vs. CyN and in SCN patients with unique vs. overlapping mutations. Cumulative incidence [cumulative proportion of patients experiencing a major event acute myeloid leukemia (AML) or myelodysplasia (MDS), hematopoietic stem cell transplantation, or death] (a) for patients with a diagnosis of cyclic neutropenia (CyN) or congenital neutropenia (SCN), or (b) by disease association of mutations in patients with SCN. Shaded areas represent 95% point-wise confidence bands. AML, acute myeloid leukemia; G-CSF, granulocyte colony-stimulating factor; MDS, myelodysplasia.

FIGURE 4.

Associations between *ELANE* mutations and phenotype in patients with severe congenital neutropenia (SCN) maintained on long-term G-CSF therapy. Left-hand panels: box-plots of dose versus (a) mutation position or (b) specific mutation. Central mark shows the median, whereas notches show 95% confidence limits for the median. Edges of boxes show 25th and 75th percentiles. Whiskers extend by 1.5 times the inter-quartile range. Dots show values for individual patients. Right-hand panels: cumulative incidence (cumulative proportion of patients experiencing MDS/AML, transplant, or death) versus years on G-CSF therapy by (c) mutation position or (d) specific mutation. Kaplan–Meier curves show grouped data for clarity. Shaded areas represent 95% point-wise confidence bands. AML, acute myeloid leukemia; G-CSF, granulocyte colony-stimulating factor; MDS, myelodysplasia.

Table 1

Patients, ELANE mutations, treatments and adverse events

Serious adverse event														
Subset	No. of patients	Age, median years (range)		Observation period, median years (range)		No. of mutations	ANC, before initial G-CSF treatment, median (range) ^a	G-CSF median dose, µg/kg/day (range)	MDS/AML (no. of deaths)	Transplant not attributed to MDS/AML (no. of deaths)	Deaths not attributed to MDS/AML or transplant			
		Male	Female		Total						Sepsis	Heart failure or stroke	Other	
All patients	307 ^b	18.1 (0.9–90.5)	153	153	18.1 (0.9–90.5)	104	0.18 (0.00–3.42)	NA	30 (15)	29 (4)	11	4	2	5
Patients treated with G-CSF ^c	241	17.2 (1.9–90.5)	115	126	11.6 ^d (0.4–24.6)	96	0.16 (0.00–3.42)	5.0 (0.2–183.2)	30 (15)	26 (3)	5	3	0	2
All CYN patients	120	28.9 (1.9–90.5)	61	59	28.9 (1.9–90.5)	22	0.59 (0.00–3.42)	NA	0 (0)	1 (1)	7	3	2	2
CyN patients treated with G-CSF ^c	76	24.0 (1.9–70.3)	36	40	13.1 ^d (0.6–24.6)	22	0.40 (0.00–3.42)	2.1 (0.2–45.0)	0 (0)	0 (0)	2	2	0	0
All SCN patients	187 ^b	13.1 (0.9–69.7)	92	94	13.1 (0.9–69.7)	94	0.10 (0.00–1.76)	NA	30 (15)	28 (3)	4	1	0	3
SCN patients treated with G-CSF ^c	165	14.1 (0.9–69.7)	79	56	10.6 ^d (0.4–23.7)	83	0.10 (0.00–1.76)	6.7 (0.4–183.2)	30 (15)	26 (3)	3	1	0	2

AML, acute myeloid leukemia; ANC, absolute neutrophil count; CyN, cyclic neutropenia; G-CSF, granulocyte colony-stimulating factor; MDS, myelodysplasia; SCN, severe congenital neutropenia.

^aMedian range of the blood neutrophil count before G-CSF treated was started.

^bSex not reported for one patient.

^cAll patients reported treated with G-CSF.

^dObservational period on G-CSF.

Table 2

Novel ELANE mutations

Mutations	References
In this study	
V98_Q102del; D117V; IVS3 +2100 C>T; IVS3 -2 A>C; M154R; W156G; Ins174D; IVS4 ins+6 gga; V207D; G226R; N240del	
In the literature	
c.-3 A>T	Tidwell <i>et al.</i> [32]
M66L	Horwitz <i>et al.</i> [9*]
S67P	Shu <i>et al.</i> [25]
Q97-V101del	Shu <i>et al.</i> [25]
I118N	Nayak <i>et al.</i> [26]
I120M	Horwitz <i>et al.</i> [9*]
Ins135DQLPA	Shu <i>et al.</i> [25]
R193Q	Xia <i>et al.</i> [19]
G203L	Horwitz <i>et al.</i> [9*]
G210RfsX	Horwitz <i>et al.</i> [9*]
W241L	Skokowa <i>et al.</i> [27]

Novel *ELANE* mutations reported in this study and in other recent publications. A comprehensive list of other *ELANE* mutations is listed in [11].