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PREVALENCE OF MUTATIONS IN *ELANE*, *GFI1*, *HAX1*, *SBDS*, *WAS*, AND *G6PC3* IN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA

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SUMMARY

Severe congenital neutropenia (SCN) is a genetically heterogeneous syndrome associated with mutations of *ELANE* (*ELA2*), *HAX1*, *GFI1*, *WAS*, *CSF3R or G6PC3*. We investigated the prevalence of mutations of *ELANE* in a cohort of 162 SCN patients for whom blood or bone marrow samples were submitted to the North American Severe Chronic Neutropenia Tissue Repository. Mutations of *ELANE* were found in 90 of 162 patients (55.6%). Subsequently, we conducted an analysis of a subset of 73 of these cases utilizing a high throughput sequencing approach to determine the prevalence of other mutations associated with SCN. Among the 73 patients, mutations of *ELANE* were detected in 28. In the remaining 45 patients with wild type *ELANE* alleles, 5 patients had mutations: *GFI1* (1), *SBDS* (1), *WAS* (1) and *G6PC3* (2); no mutations of *HAX1* were detected. In approximately 40% of our cases, the genetic basis of SCN remains unknown. These data suggest that for genetic diagnosis of SCN, *ELANE* genotyping should first be performed. In patients without *ELANE* mutations, other known SCN-associated gene mutations will be found rarely and genotyping can be guided by the clinical features of each patient.

Keywords

bone marrow failure; chronic neutropenia; DNA mutation

INTRODUCTION

Severe congenital neutropenia (SCN) is a bone marrow failure syndrome characterized by severe neutropenia present from birth, an arrest of neutrophil differentiation at the promyelocyte/myelocyte stage, and a marked propensity to develop myelodysplasia and acute myeloid leukemia. SCN demonstrates multiple modes of inheritance including autosomal recessive, autosomal dominant, X-linked, and sporadic patterns. Accordingly, recent genetic studies have identified multiple gene mutations in SCN. By far the most common mutations affect the *ELANE* (*ELA2*) gene encoding neutrophil elastase (all in autosomal dominant or sporadic SCN) (Ancliff, *et al* 2001, Ancliff, *et al* 2002, Bellanne-Chantelot, *et al* 2004, Dale, *et al* 2000, Germeshausen, *et al* 2001, Horwitz, *et al* 1999).

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Recent studies suggest that mutations of *HAX1* and *G6PC3* are responsible for cases of autosomal recessive SCN (Carlsson, *et al* 2008, Germeshausen, *et al* 2008, Ishikawa, *et al* 2008, Klein, *et al* 2007). Mutations of *WAS*, encoding the Wiskott-Aldrich syndrome protein, are associated with X-linked inherited SCN (Ancliff, *et al* 2006, Devriendt, *et al* 2001). There are also case reports of mutations of *GFI1* (Person, *et al* 2003), and CSF3R (Dror, et al 2000, Druhan, et al 2005, Sinha, et al 2003) (encoding the granulocyte colony-stimulating factor (G-SCF) receptor) in SCN.

In this study, we first investigated the frequency of *ELANE* mutations in a cohort of 162 SCN patients for whom bone marrow samples were available in the North American Severe Chronic Neutropenia Tissue Repository. In a subset of 73 cases, we subsequently screened for mutations in *ELANE, HAX1, WAS, SBDS, GFI1, and G6PC3* (Figure 1). *SBDS* was included, because mutations of this gene have been associated with severe neutropenia (Boocock, *et al* 2003,Calado, *et al* 2007). A high-throughput sequencing pipeline using exon-based re-sequencing of whole-genome-amplified genomic DNA and a semi-automated method was used to detect mutations. Our goal was to define the incidence of mutations in these genes in a North American population of patients with clinically defined SCN.

MATERIALS AND METHODS

Human subjects

A total of 162 patients diagnosed with SCN were included in this study. All of these patients had a bone marrow sample suitable for genotyping available in the North American Severe Chronic Neutropenia Tissue Repository. This study was conducted with approval of the Human Subjects Committee, University of Washington, and the institutional review boards of other participating institutions for the study. Informed consent was obtained in accordance with the Declaration of Helsinki. All patients met clinical criteria for SCN, including a minimum of 3 documented absolute neutrophil counts (ANCs) < 0.5×10^9/l over a 3-month period and onset of neutropenia within the first few months of life. Patients with cyclic neutropenia and syndromic neutropenia (e.g., Shwachman-Diamond syndrome and Barth syndrome) were specifically excluded from this study.

ELANE genotyping was initially performed in all patients as previously described and reported for some of these patients (Dale, *et al* 2000, Rosenberg, *et al* 2008). To investigate the frequency of mutations in genes other than *ELANE*, a subset of 73 patients with SCN were chosen for further sequencing based on the following criteria: 1) Informed consent allowing for resequencing at Washington University was obtained; 2) Adequate genomic DNA for resequencing was available. Priority was given to those cases with wild type *ELANE* alleles; of the 73 cases, no *ELANE* variants were detected in 45. Genomic DNA was prepared from patients' blood or bone marrow using standard protocols.

Sequencing

Initially, Phi 29-based whole-genome amplification was performed on genomic DNA samples using the Qiagen Repli-G service (Qiagen, Valencia, CA). Coding exons, including the exon/intron boundaries, were amplified by polymerase chain reaction (PCR) to generate amplicons for resequencing, because of the limited starting material available for many of the samples. Universal tails were added to the 5′ ends of amplification primers to serve as the sequencing primer sites. Amplicons were purified by Exonuclease/SAP treatment and then directly sequenced using Big Dye Terminator chemistry on an ABI3730 automated sequencer (Applied Biosystems, Foster City, CA). All coding exons of *ELANE, HAX1, SBDS, GFI1,* and *G6PC3* were sequenced. Due to its large size, only those exons previously shown to harbour mutations were sequenced for *WAS* (exons 7–12). All primer sequences

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are provided in Table S1 and the nucleotide sequence of all novel genetic variants is provided in Table S2. The semiautomated sequence analysis was performed as previously described (Link, *et al* 2007).

RESULTS

ELANE genotyping

ELANE genotyping data was obtained on a total of 162 patients with SCN; results for some of these patients have been previously reported (Dale, *et al* 2000, Rosenberg *et al* 2008). *ELANE* sequence variants were observed in 90 (55.6%) of cases of SCN. A total of 43 distinct *ELANE* variants were identified (Figure 2). Consistent with previous reports, these putative pathogenic mutations were distributed throughout the entire gene, most of which were missense, with a few nonsense, indel, and splice variants (Ancliff, *et al* 2001, Ancliff, *et al* 2002, Bellanne-Chantelot, *et al* 2004, Dale, *et al* 2000, Germeshausen, *et al* 2001, Horwitz, *et al* 1999). Seventeen of the missense and nonsense variants have not been reported previously, including G27R, I31M, A32G, M37R, V54D, G56R, R74P, I91N, L92F, I100del, L143P, V157I, R162S, R164Q, D172TfsX10, C179X, G181W. Together with previous studies, this brings the total number of reported *ELANE* putative mutations in SCN to 73 (Ancliff, *et al* 2001, Bellanne-Chantelot, *et al* 2004, Carlsson, *et al* 2006, Dale, *et al* 2000, Germeshausen, *et al* 2001, Horwitz, *et al* 1999, Ishikawa, *et al* 2008, Kawaguchi, *et al* 2003, Salipante, *et al* 2007, Sera, *et al* 2005).

Selection of samples for high-throughput sequencing

To investigate the relative frequency of other SCN-associated gene mutations, a high-throughput sequencing strategy was employed to screen for mutations in *HAX1, WAS, SBDS, GFI1, G6PC3* and *ELANE* (Figure 1). In the cohort of 162 patients with SCN, no sequence variants of *ELANE* were detected in 72. Of these, adequate genomic DNA enabling sequencing at Washington University was available for 45. We also sequenced 28 cases with known *ELANE* variants to assess the fidelity of our sequencing pipeline and to determine whether *ELANE* variants co-existed with other SCN-associated gene mutations. The clinical characteristics of the 28 patients with *ELANE* variants and 45 with normal *ELANE* alleles are summarized in Tables 1 and 2, respectively.

Sequencing strategy

A high-throughput exon-based sequencing strategy utilizing whole-genome amplified genomic DNA isolated from bone marrow or blood leucocytes was used (Link, *et al* 2007). A semi-automated method to assess sequence quality and coverage was applied to each gene. With the exception of exon 1 of *SBDS*, sequence quality was very high, with an average of 95 ± 3.0% of samples having adequate coverage (range 81–98%). For a few patient samples, sequence quality/coverage for specific genes was inadequate (white boxes in Figure 3). These samples were eliminated from the final analysis of that gene.

GFI1

Heterozygous germline mutations of *GFI1* (N382S and K403R) each have been reported in a family with SCN (Person, *et al* 2003). These mutations are thought to act in a dominant-negative fashion to inhibit granulocytic differentiation. A single patient with the *GFI1* N382S mutation was detected in our series; no other mutation was detected in this patient (Figure 3). Though the small numbers preclude definitive genotype-phenotype correlations, it is interesting to note that this patient, similar to the previously published case (Person, *et al* 2003), had a high basal level of circulating monocytes. Moreover, a striking monocytosis was noted after treatment with G-CSF (Table 2). Three other novel sequence variants of

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GFI1 not in public single nucleotide polymorphism (SNP) databases were detected: R412X, L400F, and P107A. Two of these, L400F and P107A, were seen in patients who also had an *ELANE* variant.

WAS

Mutations of the *WAS* gene, encoding the Wiskott-Aldrich syndrome protein (*WAS*P), are associated with X-linked SCN. Three mutations, L270P, I294T, and S270P have been described in patients with SCN (Ancliff, *et al* 2006, Devriendt, *et al* 2001). All are thought to disrupt an auto-inhibitory domain in the *WAS*P protein. In the present study, the L270P mutation was identified in a single male patient with SCN (Figure 3). Of note, monocytopenia was noted in our and the previously reported patients with the L270P *WAS* mutation (Table 2) (Devriendt, *et al* 2001). A novel *WAS* sequence variant, P460S, was identified in a female patient with SCN who had no other SCN-associated gene mutation.

SBDS

Compound heterozygous mutations of *SBDS* (most commonly K62X and 84Cfs3) are present in the majority of cases of SDS (Boocock, *et al* 2003). These mutations are thought to result in loss-of-function alleles. In the present study, two patients with SCN were found to have heterozygous mutations of *SBDS* (Figure 3). In addition to the C84YfsX3 mutation, a novel *SBDS* variant, Q94X, was detected in a single patient with SCN who also carried an *ELANE* variant.

HAX1 and G6PC3

Recent studies suggest the homozygous or compound heterozygous loss-of-function germline mutations of *HAX1* or *G6PC3* are responsible for most cases of autosomal recessive inherited SCN (Boztug, *et al* 2009, Carlsson, *et al* 2008, Germeshausen, *et al* 2008, Ishikawa, *et al* 2008, Klein, *et al* 2007). Of note, SCN caused by *G6PC3* mutations is associated with cardiac and urogenital abnormalities and thrombocytopenia (Boztug, *et al* 2009). Surprisingly, in our series of patients with SCN, no *HAX1* mutations were detected (Figure 3). However, two of our cases were associated with genetic variants of *G6PC3*. In one patient, the previously described *G6PC3* G260R mutation was coupled with the novel genetic variant T118R. This patient has a secundum atrial septal defect, intermittent thrombocytopenia (median 162×10^9/l, range 56–574), but no urogenital abnormality. A second patient carried a homozygous single nucleotide deletion in codon 70 of *G6PC3*, resulting in a frameshift followed by premature stop codon. This patient also has cardiac abnormalities (atrial septal defect and coronary aneurysm), intermittent thrombocytopenia (median 113×10^9/l, range 42–474), but no urogenital abnormality.

DISSCUSSION

SCN is genetically heterogeneous with multiple genes reported to be associated with this disease, including *ELANE, HAX1, WAS, SBDS, GFI1, and G6PC3.* There is evidence that the frequency of gene mutations in SCN may depend upon the ethnic composition of the patient population. For example, Rosenberg *et al* (2008) reported that in 82 North American patients with SCN the frequency of *ELANE* mutations was 63%. In contrast, a study of 54 patients with SCN from the French Neutropenia Register reported *ELANE* mutations in only 35% of cases (Bellanne-Chantelot, *et al* 2004). In the present study, a two-tiered sequencing approach was used to define the mutation frequency of *ELANE, HAX1, WAS, SBDS, GFI1, and G6PC3* in a relatively large cohort of patients with SCN. The genotyping data is summarized in Figure 4. Of note, consistent with previous reports (Ancliff, *et al* 2001, Bellanne-Chantelot, *et al* 2004, Germeshausen, *et al* 2001, Rosenberg, *et al* 2008), we considered all novel *ELANE* sequence variants as putative pathogenic mutations. It is

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possible that some of these *ELANE* variants may represent rare SNPs. Likewise, we considered the biallelic sequence variants of *G6PC3* to be likely pathogenic mutations. A more conservative approach was taken for the other SCN-associated genes; for *WAS*, *SBDS*, and *GFI1* only those variants previously reported in the literature were considered pathogenic mutations. Based on these considerations, and consistent with previous reports (Ancliff, *et al* 2001, Bellanne-Chantelot, *et al* 2004, Germeshausen, *et al* 2001, Rosenberg, *et al* 2008), *ELANE* was found to be the most commonly mutated gene in SCN, with a frequency of 55.6% (90 of 162 patients). A total of 5 mutations in the other SCN-associated genes (*HAX1, WAS, SBDS, GFI1, and G6PC3*) were detected in the 45 evaluable patients with normal *ELANE* alleles, yielding a combined mutation frequency of 11.1%. However, since only 44.4% of patients with SCN in this study had normal ELA2 alleles, it follows that the combined frequency of *HAX1, WAS, SBDS, GFI1, and G6PC3* mutations in our SCN patient population is 4.9% (11.1% × 44.4% = 4.9%). Thus, in nearly 40% of our cases, the genetic basis of SCN remains unknown.

A number of novel genetic variants were detected in *GFI1, WAS,* and *SBDS* (Figure 3, black type). SIFT (Sorting Intolerant From Tolerant) analysis indicated that the *GFI1* R412X, *GFI1* L400F, and *SBDS* Q94X variants were likely to result in functionally deleterious changes (Ng and Henikoff 2003). Interestingly, similar to the *GFI* N382S mutant, the R412X and L400F variants are predicted to disrupt the final zinc finger of GFI1. Whether these *GFI1* variants act as dominant-negative mutants will require further study. Two patients with SCN in this study had heterozygous *SBDS* mutations. Similar to *SBDS* C84YfsX3, it is likely that the *SBDS* Q94X variant results in a loss-of-function allele. Interestingly, a recent report linked heterozygous *SBDS* C84YfsX3 mutations to aplastic anemia (Calado, *et al* 2007). On the other hand, neutropenia has not been reported in family members of patients with SDS who are heterozygous for *SBDS* mutations (Boocock, *et al* 2003). It is possible that genetic variants of, as yet undefined, genes cooperate with heterozygous *SBDS* mutations to induce neutropenia. Analysis of a larger series of individuals will be required to define the role of heterozygous *SBDS* mutations in the pathogenesis of SCN.

Based on this data, we recommend that *ELANE* genotyping be performed in all patients with suspected SCN. For those patients with *ELANE* mutations, no further genotyping studies are needed. For those without *ELANE* mutations, a careful search for associated clinical features and review of the family history to ascertain the pattern of inheritance should be performed. Genotyping of *G6PC3* and *HAX1* should be performed in all patients with autosomal recessive inherited SCN, especially if associated with cardiac (*G6PC3*) or neuropsychological abnormalities (*HAX1*). *WAS* genotyping should be performed in patients with an X-linked pattern of inheritance, especially if associated with monocytopenia. Finally, *GFI1* genotyping should be considered in patients with SCN who exhibit extreme monocytosis.

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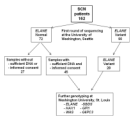

Figure 1. Study design

A total of 162 individuals with SCN and DNA samples in the Severe Chronic Neutropenia International Registry (SCNIR) tissue repository underwent *ELANE* genotyping. *ELANE* sequence variants were detected in 90 (55.6%). Of the 72 SCN samples with normal *ELANE*, 45 had sufficient DNA and appropriate informed consent to enable additional genotyping at Washington University. These 45 samples, along with 28 of the *ELANE* variant samples, underwent sequencing of their *ELANE*, *HAX1*, *WAS*, *SBDS*, *GFI1*, and *G6PC3* genes.

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Figure 2. Summary of ELANE sequence variants

Sequence variants detected by genotyping 162 patients with SCN in the North American SCNIR are summarized. Three cases with putative splice mutants [IVS4+5 G>A (2) and IVS3-8 (Exon4) C>A] are not included in this figure, because their effect on neutrophil elastase (NE) protein has not been experimentally validated. Multiple patients with the same mutation are indicated in parentheses. Novel variants are bolded.

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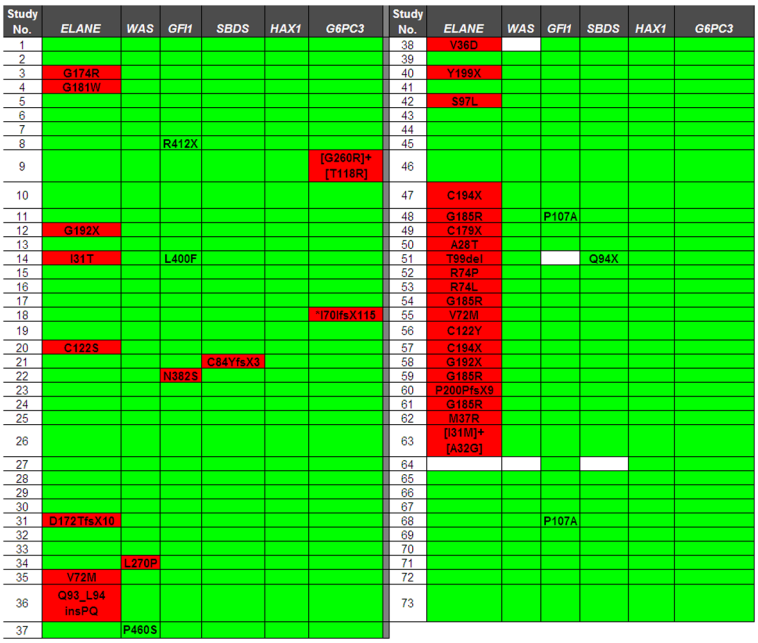

Study No.	ELANE	WAS	GFI1	SBDS	HAX1	G6PC3	Study No.	ELANE	WAS	GFI1	SBDS	HAX1	G6PC3
1							38	V36D					
2							39						
3	G174R						40	Y199X					
4	G181W						41						
5							42	S97L					
6							43						
7							44						
8			R412X				45						
9						[G260R]+ [T118R]	46						
10							47	C194X					
11							48	G185R		P107A			
12	G192X						49	C179X					
13							50	A28T					
14	I31T		L400F				51	T99del			Q94X		
15							52	R74P					
16							53	R74L					
17							54	G185R					
18						*I70IfsX115	55	V72M					
19							56	C122Y					
20	C122S						57	C194X					
21				C84YfsX3			58	G192X					
22			N382S				59	G185R					
23							60	P200PfsX9					
24							61	G185R					
25							62	M37R					
26							63	[I31M]+ [A32G]					
27							64						
28							65						
29							66						
30							67						
31	D172TfsX10						68			P107A			
32							69						
33							70						
34		L270P					71						
35	V72M						72						
36	Q93_L94 insPQ						73						
37		P460S											

Figure 3. Summary of genotypes

Nonsynonymous nucleotide changes and gene deletions or insertions are shown. Known single nucleotide polymorphisms were excluded. White boxes indicate missing sequence data. Sequence variants previously implicated in SCN are highlighted in red. *Homozygous variant.

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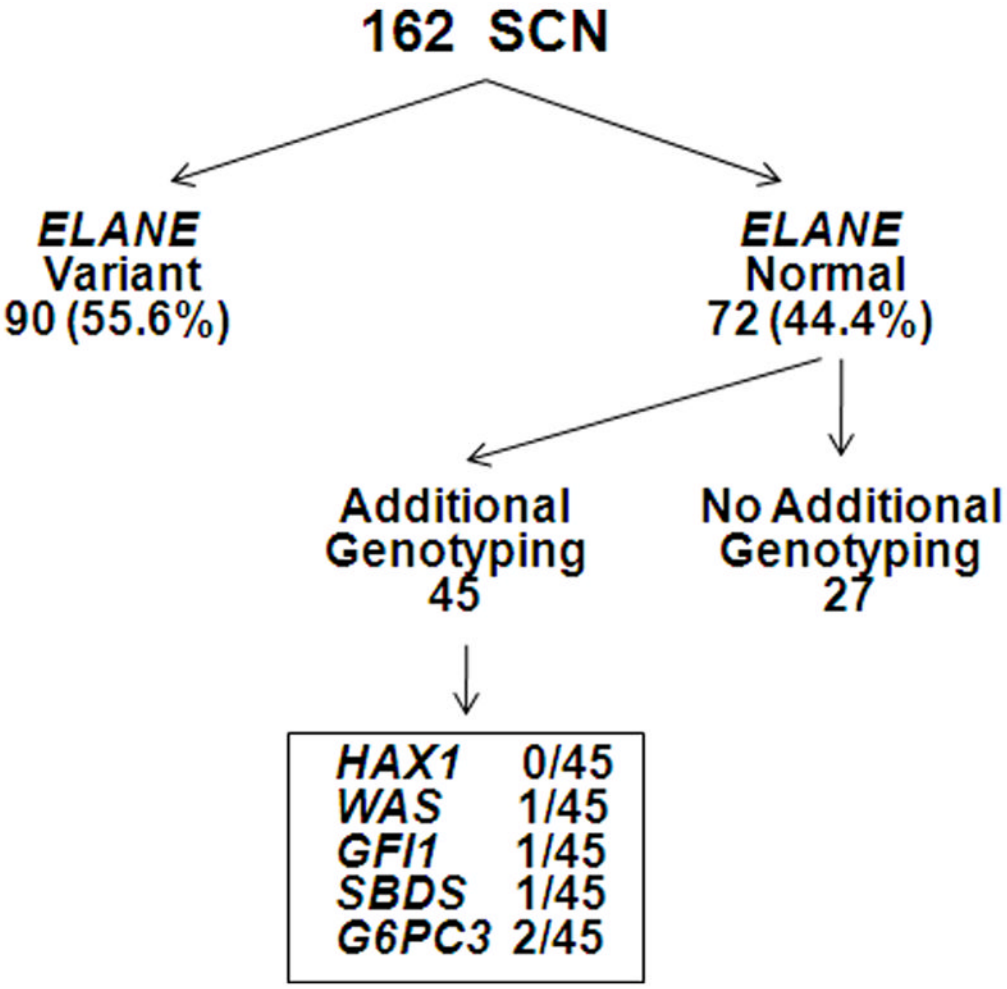

Figure 4. Genotyping summary

A total of 162 patients with SCN enrolled in the North American SCNIR underwent *ELANE* genotyping. *ELANE* sequence variants were detected in 90 (55.6%) of patients. In the subgroup of 45 patients with SCN with normal *ELANE* that underwent additional genotyping, putative mutations of *HAX1*, *WAS*, *GFI1*, *SBDS*, and *G6PC3* were collectively detected in 5.

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Table 1

Clinical characteristics for 28 SCN patients with mutant *ELANE* allele

			Pre-G-CSF Treatment								
Patient	**Gender**	**AML/MDS**	**WBC ×10^9/l**	**RBC ×10^{12}/l**	**MCV fl**	**Platelets ×10^9/l**	**ANC ×10^9/l**	**AMC ×10^9/l**	**ALC ×10^9/l**	**Post- G-CSF ANC**	**Post- G-CSF AMC**
3	F		10.1	4.3	79	464	0.0	2.42	6.26	0.78	0.7
4	M		8.5	5.0	78	275	0.09	3.42	4.60	2.24	1.4
12	F		12.5	4.5	71	798	0.00	1.41	8.52	1.44	4.65
14	M		NA	NA	NA	NA	NA	NA	NA	1.17	3.70
20	M		15.5	3.4	92	658	0.08	5.84	8.51	1.50	1.55
31	M	AML	5.5	4.5	80	425	0.10	1.90	3.96	2.26	1.53
35	F	AML	7.8	4.1	79	411	0.05	0.15	7.04	1.61	1.31
36	M	MDS	5.4	4.0	94	375	0.27	1.12	2.67	0.00	1.76
38	M	MDS	2.7	4.5	NA	589	0.00	0.54	1.65	3.40	0.81
40	M	AML	13.6	4.0	64	722	0.08	3.51	8.63	0.28	0.68
42	M		2.7	4.9	75	331	0.09	0.67	1.11	1.51	1.19
47	F	AML	11.3	4.0	72	328	0.05	3.97	5.89	0.88	2.21
48	F	AML	7.2	3.9	73	580	0.00	1.59	5.20	0.57	1.04
49	M	AML	9.1	4.1	79	423	0.26	3.37	4.60	1.13	4.53
50	M		5.4	4.7	NA	304	0.00	1.40	3.90	0.78	1.47
51	F		14.9	3.0	91	369	0.00	4.47	7.15	2.25	2.97
52	F		6.5	3.2	NA	235	0.00	2.02	4.49	0.80	3.97
53	M		8.0	4.7	78	444	0.19	1.33	6.14	1.62	1.20
54	M		NA	NA	NA	NA	NA	NA	NA	0.10	1.90
55	F		9.7	3.7	81	547	0.09	1.38	8.15	0.81	1.78
56	F		10.2	5.2	96	537	0.00	2.02	6.09	1.19	2.03
57	F		5.6	4.5	NA	434	0.06	1.12	2.03	1.59	0.90
58	M		10.0	3.2	NA	350	0.07	2.62	6.34	0.71	2.74
59	M		13.4	3.2	91	656	0.07	2.33	10.43	1.67	8.70
60	F		12.0	3.1	85	704	0.12	1.99	9.96	1.79	0.90
61	F		15.8	3.3	92	739	0.09	2.49	10.04	1.23	1.72

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			Pre-G-CSF Treatment								
Patient	**Gender**	**AML/MDS**	**WBC ×10^9/l**	**RBC ×10^{12}/l**	**MCV fl**	**Platelets ×10^9/l**	**ANC ×10^9/l**	**AMC ×10^9/l**	**ALC ×10^9/l**	**Post- G-CSF ANC**	**Post- G-CSF AMC**
62	F		18.0	3.7	90	277	0.18	1.62	16.11	2.05	2.17
63	M		8.2	3.7	84	459	0.00	2.95	5.03	2.06	1.91

ANC: absolute neutrophil count; AMC: absolute monocyte count; ALC: absolute lymphocyte count

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Table 2

Clinical characteristics for 45 SCN patients with wile type *ELANE* allele

			Pre-G-CSF Treatment								
Patient	**Gender**	**AML/MDS**	**WBC ×10^9/l**	**RBC ×10^{12}/l**	**MCV fl**	**Platelets ×10^9/l**	**ANC ×10^9/l**	**AMC ×10^9/l**	**ALC ×10^9/l**	**Post- G-CSF ANC**	**Post- G-CSF AMC**
1	M		5.4	5.2	NA	389	0.36	0.77	4.33	NA	NA
2	M		3.3	4.6	NA	270	0.18	0.99	2.18	1.40	1.2
5	F		4.9	4.3	76	404	0.28	0.65	3.50	1.22	0.50
6	M		4.2	4.3	99	370	0.14	0.85	2.80	0.91	0.60
7	F		3.1	3.9	82	247	0.09	0.30	2.55	2.09	0.48
8	F		5.7	NA	NA	108	0.68	0.32	4.50	NA	NA
9	F		4.3	4.6	100	188	0.04	1.28	3.24	3.51	0.59
10	M		6.1	3.3	85	319	0.25	0.59	5.13	NA	NA
11	M		3.3	NA	NA	949	0.12	0.17	2.84	1.24	0.31
13	F	AML	6.1	4.4	74	201	NA	NA	NA	NA	NA
15	M		NA	NA	NA	NA	NA	NA	NA	0.24	1.65
16	M		3.9	3.8	79	656	0.08	0.34	3.08	2.57	0.60
17	M		4.1	4.5	76	376	0.12	1.86	1.79	1.12	1.74
18	M		3.6	4.0	NA	336	0.43	0.50	0.00	2.02	0.19
19	F		2.5	5.2	NA	240	0.28	0.31	1.75	NA	NA
21	F		6.1	3.9	79	534	0.04	1.72	4.41	0.09	1.03
22	M		11.9	3.5	85	532	0.57	4.69	6.19	2.42	10.71
23	F		2.3	4.4	91	211	0.32	0.31	0.88	1.66	0.46
24	M		6.4	3.7	86	NA	0.00	1.85	4.74	0.51	2.84
25	M		NA	NA	NA	NA	NA	NA	NA	NA	NA
26	M		2.8	3.7	90	243	0.23	0.26	2.41	0.33	0.45
27	F		3.0	3.7	84	241	0.16	0.81	1.73	4.66	2.16
28	F		2.6	2.9	111	263	0.41	0.31	1.69	0.06	0.45
29	F		4.0	4.3	82	296	0.34	0.41	2.69	NA	NA
30	F		4.3	4.8	81	379	0.22	0.85	3.11	0.54	0.75
32	M		1.5	4.1	82	368	0.14	0.15	1.05	0.14	0.06

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			Pre-G-CSF Treatment								
Patient	**Gender**	**AML/MDS**	**WBC ×10^9/l**	**RBC ×10^{12}/l**	**MCV fl**	**Platelets ×10^9/l**	**ANC ×10^9/l**	**AMC ×10^9/l**	**ALC ×10^9/l**	**Post- G-CSF ANC**	**Post- G-CSF AMC**
33	F	AML	6.6	3.9	77	328	0.80	0.60	4.20	6.00	1.10
34	M	AML	1.9	4.7	89	148	0.22	0.04	1.46	1.98	0.08
37	F	AML	3.5	4.9	77	255	0.07	0.82	2.14	2.03	0.77
39	M	AML	6.4	4.8	NA	464	0.00	0.77	5.18	4.42	1.20
41	M	MDS	10.6	3.8	68	564	0.18	2.65	6.89	3.44	0.68
43	M		11.8	4.3	80	351	0.16	2.13	8.75	0.17	1.49
44	M		6.9	4.7	72	390	0.15	1.40	4.46	0.07	1.15
45	M		3.2	4.4	81	298	0.18	0.37	2.29	1.93	0.38
46	F		4.1	5.0	NA	376	0.04	1.39	2.01	1.48	0.99
64	M		7.9	4.3	73	349	0.28	1.48	5.97	4.28	1.10
65	M		6.7	3.9	73	463	0.08	1.23	4.15	2.05	0.62
66	F		3.0	4.5	76	238	0.59	0.47	2.15	1.05	0.38
67	M		7.4	4.2	75	390	0.18	1.61	5.06	3.70	2.39
68	M		4.1	4.0	76	230	0.11	0.45	3.16	11.78	0.43
69	M		12.9	3.2	94	524	0.00	3.15	8.39	3.67	0.23
70	F		7.2	4.2	78	389	0.09	1.74	5.24	0.37	0.42
71	M		5.5	4.8	107	252	0.18	0.48	4.52	0.37	0.24
72	M		5.1	4.4	74	417	0.09	1.08	3.38	5.77	1.73
73	M		11.0	3.98	79	556	0.00	3.30	7.15	1.17	0.36

ANC: absolute neutrophil count; AMC: absolute monocyte count; ALC: absolute lymphocyte count