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RESEARCH ARTICLE

Homozygous c.130–131 ins A (pW44X) mutation in the HAX1 gene as the most common cause of congenital neutropenia in Turkey: Report from the Turkish Severe Congenital Neutropenia Registry

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Abbreviations: AML, acute myeloblastic leukemia; ANC, absolute neutrophil count; CN, congenital neutropenia; CSF3R, colony-stimulating factor 3 receptor; ELANE, neutrophil elastase; G6PC3, glucose 6 phosphatase catalytic subunit 3; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; GSD1b, glycogen storage disease type 1b; HAX1, HSLC-1–associated protein X-1; HSCT, hematopoietic Stem Cell Transplantation; JAGN1, Jagunal homolog 1; MAS, macrophage activation syndrome; MDS, myelodysplastic syndrome; NGS, next-generation sequencing; SBDS, Shwachman–Bodian Diamond syndrome; SCN, severe congenital neutropenia; SLC37A4, solute carrier family 37 member 4.

*Turkish SCNR GroupTurkish Severe Congenital Neutropenia (SCN) Group

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Abstract

Background: Severe congenital neutropenia is a rare disease, and autosomal dominantly inherited ELANE mutation is the most frequently observed genetic defect in the registries from North America and Western Europe. However, in eastern countries where consanguineous marriages are common, autosomal recessive forms might be more frequent.

Method: Two hundred and sixteen patients with severe congenital neutropenia from 28 different pediatric centers in Turkey were registered.

Results: The most frequently observed mutation was *HAX1* mutation ($n = 78$, 36.1%). A heterozygous *ELANE* mutation was detected in 29 patients (13.4%) in our cohort. Biallelic mutations of *G6PC3* ($n = 9$, 4.3%), *CSF3R* ($n = 6$, 2.9%), and *JAGN1* ($n = 2$, 1%) were also observed. Granulocyte colony-stimulating factor treatment was given to 174 patients (80.6%). Two patients died with infectious complications, and five patients developed myelodysplastic syndrome/acute myeloblastic leukemia. The mean (± mean standard error) follow-up period was 129.7 ± 76.3 months, and overall survival was 96.8% (CI, 94.4–99.1%) at the age of 15 years. In Turkey, severe congenital neutropenia mostly resulted from the p W44X mutation in the *HAX1* gene.

Conclusion: In Turkey, mutation analysis should be started with *HAX1*, and if this is negative, *ELANE* and *G6PC3* should be checked. Because of the very high percentage of consanguineous marriage, rare mutations should be tested in patients with a negative mutation screen.

KEYWORDS
CSF3R, *ELANE*, *HAX1*, SCN registry, severe congenital neutropenia

Congenital neutropenia (CN) is a rare disorder characterized by very low neutrophil counts, which can subsequently result in life-threatening bacterial infections.[1–6] Over the last 30 years, granulocyte colony-stimulating factor (G-CSF) has been successfully used to increase the absolute neutrophil count (ANC) in patients with CN, with a corresponding decrease in the number of infectious complications.[1,7,8]

Additionally, the prompt initiation of antibiotic treatment and improvements in hospital care has led to a marked increase in the life expectancy of patients with CN, although the risk of myelodysplastic syndrome or acute myeloblastic leukemia (MDS/AML) development remains high.[7–12]

There are several underlying genetic defects that result in the same clinical CN phenotype. In European and North American registries, the most commonly observed mutated gene is the neutrophil elastase (*ELANE*) gene, with an autosomal dominant inheritance pattern. Other mutations, including those in HSLC-1–associated protein X-1 (*HAX1*), glucose 6 phosphatase catalytic subunit 3 (*G6PC3*), and colony-stimulating factor 3 receptor (*CSF3R*), are inherited recessively and are reported much less frequently.[1,7,9,13] However, in the registries from countries where consanguineous marriage is common, such as Iran and Israel, *HAX1* and *G6PC3* gene mutations are reported more frequently.[14,15] Both of these series have limited patient numbers.

It should be expected that different populations have different frequencies of severe congenital neutropenia (SCN)-associated genes, with both ethnicity and consanguineous marriages influencing the results. We, therefore, believe that national registries are important

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to determine the genetic mutations causing SCN in different parts of the world, to determine the outcomes of certain groups of neutropenia in different countries, and to understand the mechanisms behind this heterogeneity.

It is important for all countries to determine whether the approach to genetic testing should be the same as in Western Europe or whether the diagnostic algorithm should be adapted to the patient's descent. In this context, we planned to establish a national neutropenia registry in Turkey, a country with an extraordinary mixed population of Caucasian and Asian descent and a higher proportion of consanguineous families than in most other parts of Europe.

1 | METHOD

1.1 | Patients

SCN was defined as having an ANC below 500/mm^3 at least three times in 3 months. Patients with a known mutation related to CN and patients with glycogen storage disease type 1b (GSD1b) were also included the registry, even if they had ANC between 500 and 1000/mm^3.

Exclusion criteria were drug-induced neutropenia, human immunodeficiency virus seropositivity, known hematologic malignancy, neutropenia associated with immune deficiency syndromes, thrombocytopenia of less than 50 000 platelets/mm^3, and anemia with hemoglobin levels of less than 8 g/dL without any explainable reason, except for patients with GSD1b or Shwachman–Bodian Diamond syndrome (SBDS). If the neutropenia is resolved and remained in the normal ranges in the follow-up period, they were excluded from the analysis.

Antineutrophil antibodies were screened in these patients. Even in the presence of positive results for antineutrophil antibody test, patients with SCN were included if at least one of the criteria reported below was positive: (1) patients with any known mutation that cause SCN; (2) decreased myeloid cell activity and maturation arrest at early myeloid lineages in bone marrow aspiration; (3) no available normal ANC since birth; (4) persistence of SCN in the follow-up period; or (5) life-threatening, severe, recurrent infectious complications, particularly started at early ages.

Patients with antineutrophil antibodies were excluded from the registry if they fulfill all these conditions: (1) ANC increased and persistently stayed in the normal ranges, (2) no decreased myeloid cell activity and no maturation arrest at early myeloid lineages in bone marrow aspiration, and (3) no typical cyclic neutropenia pattern.

1.2 | Registry

An online registry was established by the Bone Marrow Failure Subcommittee of the Turkish Pediatric Hematology Association in 2010. A total of 32 pediatric hematology centers were invited to share their data in this system. All centers were given a user ID and password to enter their data. Data were checked by the study coordinator, and, if necessary, the responsible person of each center was called, and the data were discussed. The registry was planned as prospective data collection of the registered patients. All centers were invited to register all CN patients into the registry. Every other year, registered patients' data would be prospectively updated. Patients' biological material was collected if the patients/or their parents, and the responsible center, gave informed consent. The patients who were born before October 2018 entered into the study, and the last follow-up time until February 2019 was evaluated; the cutoff date was February 2019.

The local ethics committee approved the study, and the study conforms to recognized standards of the Helsinki Declaration.

1.3 | Genetic analyses

The genetic analyses of the patients had been done across different laboratories in Europe before they were performed in Turkey. *ELANE* and *HAX1* mutation analyses have been performed at the Ege University Faculty of Medicine, Medical Genetic Laboratory in Turkey since 2012. *G6PC3*, *CSF3R*, Jagunal homolog 1 (*JAGN1*), and *SBDS* genes were added to the genetic test in 2016. In 2017, solute carrier family 37 member 4 (*SLC37A4*) and other rare mutations causing severe neutropenia were added to the screening list for CN.

HAX1, *ELANE*, *G6PC3*, *CSF3R*, *SBDS*, *JAGN1*, and *SLC37A4* gene mutation analyses were performed by sequencing of the coding exons and the exon–intron boundaries of the genes. Genomic DNA was isolated from peripheral blood cells with the QIAGEN DNA Blood Mini Kit (Germany) according to the manufacturer's instructions.

Sequencing was performed by Sanger sequencing or next-generation sequencing (NGS). For Sanger sequencing, PCR products were sequenced by the dye termination method using a DNA sequencing kit (Perkin-Elmer, Foster California, USA) and analyzed using The ABI Prism 3100 sequence analyzer (Applied Biosystems, Foster, California, USA). NGS sequencing was performed with Miseq V2 chemistry on a MiSeq instrument (Illumina California, USA). The analysis was performed with IGV software.

Statistics: Survival was described using Kaplan–Meier analysis. Prevalence and incidence of SCN were described using the data from the Turkish National Statistics Association.[16]

2 | RESULTS

2.1 | Participants

Twenty-eight out of 32 centers entered their data into the system. A total of 264 patients' data were entered. Patients with immune deficiency syndromes ($n = 15$) were excluded from the study.

Patients with positive results ($n = 126$) after genetic analyses and five patients with a diagnosis of GSD type 1b entered into the registry.

Thirty-three out of 98 patients with negative results for mutation analysis were excluded the registry. Fifteen of those patients developed other cytopenias ($n = 2$ with pancytopenia, $n = 11$ with

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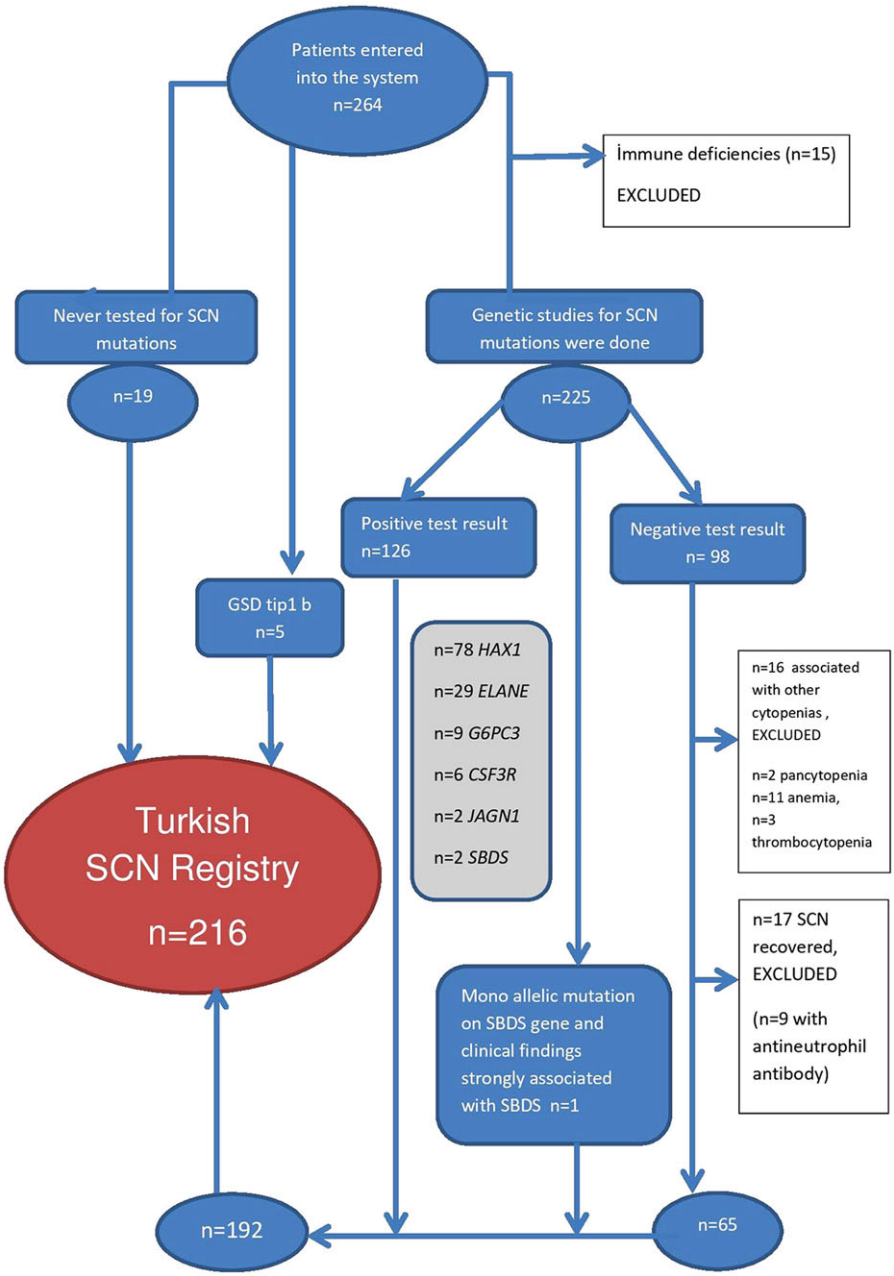


FIGURE 1 Inclusion and exclusion of patients to the Turkish Severe Chronic Neutropenia Registry

thrombocytopenia, and $n = 3$ with severe anemia) and other 17 patients, including nine patients with positive antineutrophil antibody test results, showed recovery of ANC. The remaining 65 patients without any SCN mutation and 19 patients who had never been tested for SCN have been entered into the registry.

One patient with a monoallelic mutation on the *SBDS* gene and clinical findings strongly associated with SBDS was entered into the registry. Therefore, a total of 216 patients were registered, and they constituted our cohort. Patient exclusions and inclusions are shown in Figure 1.

The antineutrophil antibody had been screened in 112 patients, and 13 of them had positive results, including two patients with CN mutation (one patient with biallelic CSF3R mutation and one patient with homozygous HAX1 mutation). Although nine out of these 13 patients showed normalization of ANC with a median of 14 months (range, 7 months–4 years) and were excluded from the study, two patients without any genetic mutation for SCN did not show recovery in ANC and stayed in the registry. Two patients with genetic mutation for SCN and antineutrophil antibody had already been entered the registry.

2.2 | Demographic data

The median age of the patients was 12.9 years (range, 5 months–36 years) at the cutoff dates. There were 115 girls and 101 boys (ratio:

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TABLE 1 CN mutations and their frequencies in the Turkish Severe Congenital Neutropenia Registry

Mutation analyses	*n* (%)
HAX1 (+)	78 (36.1)
ELANE (+)	29 (13.4)
G6PC3 (+)	9 (4.2)
CSF3R (+)	6 (2.8)
JAGN1 (+)	2 (1)
ELANE$^-$/ HAX1$^-$	23 (10.6)
ELANE$^-$/HAX1$^-$/G6PC3$^-$	20 (9.3)
ELANE$^-$/ HAX1$^-$/G6PC3$^-$/JAGN1$^-$/CSF3R$^-$	22 (10.2)
GSD type 1b	5 (2.3)
SBDS	3 (1.4)
No genetic testing performed	19 (8.8)

1.14) in the registry. The median age of the diagnosis of neutropenia was 16.5 months (range, 1 month–28 years) for this cohort.

There were 216 patients in this registry. They were the children of 205 parents from 201 families. Consanguinity was reported between 99 (48.2%) of the parents.

In 17 out of the 201 families, more than one affected member was present. In one of the families, there were three affected children (two sisters and one cousin), and the responsible gene was *HAX1*. There were two affected patients in the remaining 16 families (mother and daughter [$n = 1$], siblings [$n = 10$], and cousins [$n = 2$]). *HAX1* ($n = 8$), *G6PC3* ($n = 2$), *CSF3R* ($n = 1$), *JAGN1* ($n = 1$), and *ELANE* ($n = 1$) mutations were the responsible mutations in those families. No underlying genetic defect was found in three out of 17 families, although they were screened for *ELANE* and *HAX1* mutations. All affected members of the families were registered into the study.

2.3 | Distribution of genetic defects

The most frequently observed mutation was a homozygous *HAX1* mutation in 36.1% of all patients (78 patients). A heterozygous *ELANE* mutation was detected in 29 patients (13.4%). The biallelic mutation of *G6PC3* ($n = 9$, 4.2%), *CSF3R* ($n = 6$, 2.8%), and *JAGN1* ($n = 2$, 1%) was also observed. In this registry, SBDS was reported in three (1.4%) patients. The biallelic mutation could not be found in a patient with SBDS, and the diagnosis was confirmed according to the clinical features. In addition, there were five patients (2.3%) with GSD1b.

The underlying genetic defect was not identified in 65 (30.1%) of the registered patients despite several genetic analyses. All 65 patients were screened for *ELANE* and *HAX1* mutations, and all were found to be negative. Additionally, a *G6PC3* mutation was screened for in 42 patients, *CSF3R*, *JAGN1*, and *SBDS* mutations were screened for in 22 patients, and all were negative. In this registry, 19 patients (8.8%) were not screened for any of the genetic causes of SCN. All of the mutations are summarized in Table 1.

2.3.1 | HAX1 mutations

Patients with *HAX1* mutations made up the major subgroup of all patients. Seventy-seven of these patients with *HAX1* had a homozygous mutation, and one had a double heterozygous mutation for the *HAX1* defect. Interestingly, 89.7% of the patients ($n = 70$) with a *HAX1* mutation had the same defect: homozygous c.130–131 ins A (pW44X) and the one patient who had the double heterozygous mutation also had this mutation in one allele. All of the patients with this mutation were of Kurdish origin. Among the remaining eight patients with *HAX1* mutation, four previously undefined mutations were detected as being responsible for SCN. The *HAX1* gene and its frequency are reported in Table 2. Seventy-eight patients with HAX1 mutation were from 70 families, of which 52 (74.3%) were defined as parental consanguinity.

2.3.2 | ELANE mutations

A heterozygous *ELANE* mutation was detected in 29 of our patients with SCN. In our cohort, seven new *ELANE* mutations were defined in patients. The *ELANE* mutations and their frequencies are shown in Table 2.

2.3.3 | Patients with other mutations

A *biallelic CSF3R* mutation was detected in six patients from five different families, with each family showing a different mutation. In four of the families (five of the patients) homozygous *CSF3R* mutations were defined, and the sibling pair had the same mutation. There were consanguineous marriages between the parents of each of these five patients. The remaining patient, with double heterozygous biallelic mutation, has no consanguinity between her parents. Overall, two of the five *CSF3R* mutations identified were first described in their respective patients.

One patient showed a monoallelic *CSF3R* mutation (G5895) alongside her homozygous *HAX1* mutation [c.130–131ins A (pW44X)]. Her father only carried a heterozygous HAX1 mutation, while her mother was carrying both mutations heterozygously without showing any clinical phenotype.

A homozygous *G6PC3* mutation was seen in nine patients; two were siblings. All patients had characteristic abnormal physical findings attributed to this mutation. Interestingly, four out of these mutations were described for the first time in these patients.

In two patients, the *SBDS* gene was mutated, and they had the clinical findings of SBDS. One patient showed a homozygous mutation for IVS2+2T>C, and the other a heterozygous positivity for IVS2+2T>C (g.1439T>C) and IVS3-1G>A (g.4347G>A).

All these other mutations and their frequencies are shown in Table 2.

2.4 | Hematological findings

Seven out of 29 patients with an *ELANE* mutation (24.1%) had cyclic neutropenia, with the remaining majority presenting with persistent neutropenia. Cyclic neutropenia was defined in three patients with a *HAX1* mutation (3.8%) and in three patients who never underwent

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TABLE 2 Distribution of mutations

Mutations	*n* (%)	Novelty
Biallelic *HAX1* mutations	**78 (62.9)**	
Hom c.130_131insA (PW44X)	68 (%87.2)	Known
Hom c.180delA (E60DfsX25)	2 (%2.6)	Known
Hom c.53+1G>A	1 (%1.3)	**Novel**
Hom c.376delC (R126GfsX88)	1 (%1.3)	**Novel**
Hom c.430dupG (V144GfsX5)	4 (%5.1)	Known
Hom c.415dupA (R139KfsX10)	1 (%1.3)	**Novel**
Het c.130_131insA (PW44X) / Het c.505-1G>C	1 (%1.3)	Known/**novel**
ELANE	**29 (23.4)**	
Het c.253G>A (G85R)	2 (6.9)	Known
Het c.128T>G (F43C)	1 (3.4)	**Novel**
Het c.295_300dupTTCGCC (F99_A100dup)	1 (3.4)	**Novel**
Het c.137C>G (S46C)	1 (3.4)	Known
Het c.182C>T (A61V)	2 (3.4)	Known
Het c.598-2_598-1delAG	2 (6.9)	**Novel**
Het c.416C>T (P139L)	2 (6.9)	Known
Het c.137_138delCCinsTG (S46L)	1 (3.4)	**Novel**
Het c.330delC (V112X)	2 (6.9)	**Novel**
Het c.607G>C (G203R)	1 (3.4)	Known
Het c.225-6C>A	1 (3.4)	**Novel**
Het c.597+5G>A	2 (6.9)	Known
Het c.362T>C (L121P)	1 (3.4)	Known
Het c.170C>T (A57V)	2 (6.9)	Known
Het c.371A>T (N124I)	1(3.4)	Known
Het c.377C>T (S126L)	1(3.4)	Known
Het c.247_270del (V83_S90del)	1(3.4)	**Novel**
Het c.556G>A (V186I)	1(3.4)	Known
Het c.200C>G (S67W)	1(3.4)	Known
Het c.290A>T (Q97L)	1(3.4)	Known
Het c.416C>T (P139L)	1(3.4)	Known
Het c.254G>A (G85E)	1(3.4)	Known
Biallelic *CSF3R* mutations	**6 (4.8)**	
Hom c.1765G>A (G589S)	2 (33.3)	**Novel**
Hom c.611A>G (Q204R)	1 (16.5)	**Novel**
Hom c.922C>T (R308C)	2 (33.3)	Known
Het c.569G>A (R190H)/Het c.266T>C (L89P)	1 (16.5)	Known/known
Biallelic *G6PC3* mutations	**9 (7.3)**	
Hom c.326-1G>C	1 (11.1)	**Novel**
Hom c.141C>G (Y47X)	1 (11.1)	Known
Hom c.535+1G>A	1 (11.1)	**Novel**
Hom c.394C>T (Q132X)	1 (11.1)	**Novel**
Hom c.935dupT (N313Qfs*74)	1 (11.1)	Known
Hom c.765_766 delAG (A257Cfs*129)	1 (11.1)	Known
Hom c.131C>T (P44L)	1 (11.1)	Known
Hom c.194A>C (E65A)	2 (22.2)	**Novel**
Biallelic *SBDS* mutation (*n* = 2)	**2 (1.6)**	
Hom c.258+2T>C	1 (50)	Known
Het c.258+2T>C / Het c.460-1G>A	**1 (50)**	Known/known

genetic tests for SCN. An additional two patients with cyclic neutropenia were defined among the patients who were tested and found to be negative for *ELANE* and *HAX1* mutations.

In 50.4% ($n = 109$) of the patients, the ANC was below 200/mm^3. Bone marrow aspiration findings were reported in 191 out of 216 patients (88.4%). In patients with *ELANE* and *HAX1* mutations, myeloid maturation arrest was seen at the promyelocytic/myelocytic stage. Increased eosinophil and monocyte count, and a decreased number of myeloid cells with normal cellularity, were also reported. No maturation arrest was described in patients with *G6PC3* or *biallelic CSF3R* mutations, except one patient with *biallelic CSF3R* with an end-stage maturation arrest in the myeloid lineage without a decrease in myeloid cells.

A bone marrow cytogenetic evaluation was done in 152 patients (70.4%) and, in 39 patients (18.1%), it has been repeated annually for a median of 4 years (range, 2–8 years).

An evaluation of an acquired *CSF3R* mutation was made in 29 patients (13.4%) regularly for a median of 3 years (range, 2–5 years). No abnormal findings were observed.

In this cohort, four patients with a homozygous c.130–131 ins A HAX1 mutation developed Macrophage Activation Syndrome (MAS) prior to their SCN diagnosis. Their median age was 8 months (7–19 months), and three were male. No mutation was detected in perforin, syntaxin, or *UNC* genes, and no abnormal findings attributed to Griscelli and Chediak Higashi syndrome were seen. Two of the patients received the HLH 2004 treatment protocol, and one of them died during this treatment. In the three surviving patients, a persistent low ANC continued, and following their screening for genes responsible for CN, they were all found to be positive for a *HAX1* mutation. A previously reported infection was the underlying cause of MAS in these patients.[17]

2.5 | Physical findings

Abnormal physical findings were seen in 28 patients (12.9%).

2.5.1 | *HAX1*-mutated patients

In patients with a *HAX1* mutation ($n = 78$), growth retardation ($n = 1$), delayed puberty ($n = 2$), polycystic over syndrome ($n = 1$), urogenital abnormality ($n = 1$), convulsions ($n = 4$), and mild mental retardation ($n = 2$) were reported. One patient with a pW44X *HAX1* mutation, who

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had lost four siblings due to infectious complications, was diagnosed and treated for Gaucher disease, but his ANC did not improve, and he was subsequently diagnosed as SCN. He had mutant genes for both conditions. This patient was reported before.[18]

2.5.2 | *ELANE*-mutated patients

In patients with an *ELANE* mutation ($n = 29$), growth retardation ($n = 3$), mental retardation ($n = 1$), hypospadias ($n = 1$), and extremity abnormalities ($n = 1$) were observed.

2.5.3 | *G6PC3*-mutated patients

In all of the nine patients with biallelic *G6PC3* mutation, visible cutaneous veins were seen. Cardiac defects (atrial septal defect [$n = 1$], patent ductus arteriosus [$n = 1$], and pulmonary stenosis [$n = 1$]), rectovaginal fistula ($n = 1$), hypospadias ($n = 1$), and undescended testis ($n = 1$) were also observed.

2.5.4 | *CSF3R*-mutated patients

Five of the six patients with *CSF3R* had no abnormal physical or neurological findings. Situs inversus totalis and ciliary dyskinesia were reported in the other patient, who had consanguineous parents. Most of the patients had tooth decay due to recurrent stomatological infections.

2.6 | Infectious complications

The most commonly reported infections were recurrent upper airway infections ($n = 148$, 68.5%) and mouth ulcers ($n = 135$, 62.5%). Others included skin infections ($n = 117$, 54.2%), gingivitis periodontitis ($n = 102$, 47.2%), recurrent acute otitis media ($n = 29$, 13.4%), mastoiditis ($n = 5$, 2.3%), gastroenteritis ($n = 39$, 18.1%), and recurrent pulmonary infections ($n = 29$, 13.4%). Sepsis was seen in 17 patients (7.9%).

There were 19 patients (8.8%) with oral candidiasis. Other than those patients, no fungal infection was described in our series.

Mouth ulcers and gingivitis were the main infections seen before G-CSF treatment, and dose or injection frequency changes solved the problem.

2.7 | Treatment

The main aim of treatment was to maintain the patients' ANC levels above 500/mm^3. G-CSF treatment was given to 174 patients (80.6%). This was started according to the institutional or physicians' personal preferences. The dose ranged from 3 μg/kg once every 10 days to 25 μg/kg/day, with a median dose of 5 μg/kg 3 days a week (2.1 μg/kg/day). G-CSF doses given were shown in Table 3. In this cohort, physicians preferred to give G-CSF every day when infections/febrile neutropenia occurred. Fifty-two percent of the patients were on regular G-CSF treatment. Six patients, five of which had a HAX1 mutation, only received G-CSF during periods of infection. Otherwise, the dose and frequency of G-CSF treatment were kept the same whether or not an infection was present. Two patients from the same center with G6PC3 mutation were given pegylated filgrastim (pegylated G-CSF) at the dose of 100 μg/kg every 7 days.

TABLE 3 ANC and G-CSF dose in patients with different genetic mutations

Mutation	*n*	ANC [median (range)] mm^3/L	G-CSF dose μg/kg/day [median (range)]
ELANE$^+$	29	112 (6–420)	5 (4.3–25)
HAX1$^+$	78	150 (10–500)	2.9 (1.3–10)
GSD type 1b	5	540 (130–890)	5 (5–7)
ELANE$^-$/HAX1$^-$	65	324 (64–470)	1.6 (0.03–10)
G6PC3	9	240 (80–460)	10 (2.9–20)
CSF3R	6	210 (30–550)	7[a]
JAGN1	2	130–230	4.3
SBDS	3	140 (50–310)	8 (4–11)

[a]GM-CSF dose was given only for one patient.

One patient with a biallelic CSF3R mutation responded well to G-CSF, which has not been previously reported in children with this mutation, and accordingly, the other patients with this biallelic CSF3R mutation did not respond to G-CSF treatment.[19] However, one of these children was treated with granulocyte macrophage colony-stimulating factor (GM-CSF), which increased her ANC to above 1000/mm^3. This case was reported before.[20]

Prophylactic oral antibiotic treatment was given to 35 patients (18.2%), who were not receiving G-CSF treatment. The most frequently used antibiotic was trimethoprim-sulfamethoxazole ($n = 27$, 12.5%), followed by cefixime ($n = 8$, 3.7%). Additional antifungal prophylaxis with fluconazole was given to three patients (1.4%).

Hospitalization because of febrile neutropenia was required in 73.6% ($n = 159$) of all patients.

Twenty patients (9.3%) underwent hematopoietic stem cell transplantation (HSCT). All were on G-CSF treatment. The main reason for HSCT was an inadequate clinical response to G-CSF treatment. Among these patients, the median G-CSF dose was 10 μg/kg/day (range, 5–25 μg/kg/day). The median age of the patient was 45 months (range, 11 months–13 years) at the time of HSCT. These patients had been receiving G-CSF for a median of 26.5 months (range, 7 months–12 years). *ELANE* ($n = 2$), *HAX1* ($n = 10$), and *G6PC3* ($n = 2$) mutations were detected in 14 patients. Six other patients were screened for *ELANE* and *HAX1* mutations and found to be negative. One patient underwent HSCT three times in a five-month period because of graft failure and survived. However, one patient died as a result of severe graft-versus-host disease.

2.8 | Outcome

The mean (± mean standard error) follow-up period was 129.7 ± 78.6 months. The overall survival of this cohort was 97.7% (CI, 95.8%–99.5%) at the age of 10 years, and 96.8% (CI, 94.4%–99.1%) at the age of 15 years.

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TABLE 4 Clinical features and characteristics of the patients who developed MDS/AML

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Male	Male	Male	Female	Male
Age at diagnosis	4 months	2 months	1 year	1 year	1.5 years
ANC	50/mm^3	110/mm^3	210/ mm^3	120/mm^3	150/mm^3
Molecular diagnosis	HAX1$^+$	ELANE-HAX1$^-$	ELANE-HAX1$^-$	Not done	Not done
Consanguinity between parents	Yes	No	No	Yes	Yes
Malign disease	AML	AML	MDS	MDS	AML
Age at diagnosis of MDS/AML	12 years	3.5 years	9.5 years	9 years	11 years
G-CSF dose	8 μg/kg/d	11 μg/kg/d	Only during infectious period 5 μg/kg/d	14 μg/kg/ d	15 μg/kg/d
Chemotherapy	Yes	Yes	No	No	Yes
Transplantation	No	Yes	Yes	Yes	No
Survival	Died	Died	Survived	Died	Died
Age at exitus	12 years	4 years 2 months		9.5 years	11.5 years

Two patients, who were not receiving G-CSF treatment, died of infectious complications.

Five patients (2.3%) developed MDS/AML. One patient had *HAX1* mutation. Two of them had been screened for *ELANE* and *HAX1* mutations, but both were found to be negative. Three patients with AML received intensive chemotherapy, and two of them died during treatment. Two patients with MDS and one patient with AML underwent an HSCT. Two of them died of transplant-related complications. Table 4 shows the characteristics and outcome of the patients who developed MDS/AML.

3 | DISCUSSION

SCN registries provide vital information on the prevalence, frequencies, and outcomes of specific neutropenia groups. The accurate recording and registering of data without any missing information is crucial.[1–6] In the present study, 87.5% of the centers in Turkey which take care of the 80%–85% of the SCN patients participated in the registry. However, this registry is the first national registry for SCN patients in Turkey, and all efforts will be shown to complete and continue to update it.

In our registry, the most commonly seen mutation was *HAX1* mutation. In general, *HAX1* mutation is very rare in other registries.[4,7,13,15] It has, however, been shown at a high percentage in both the Iranian and Swedish CN registries, but both registries have very limited patient numbers.[9,14]

The Turkish SCN Registry, therefore, represents a large *HAX1*-mutated cohort. However, the variety of the mutation is very limited, with more than 80% of patients having same point mutation: homozygous c.130–131 ins A (*pW44X*). This is not a common point mutation in other registries. In our cohort, all patients with this mutation were from the Kurdish population. In Turkey, the Kurdish population has a very high rate (30%–40%) of consanguineous marriages, much higher than the national average.

However, a very high prevalence of *HAX1* mutations in this cohort cannot be explained by only the high consanguinity rate between the parents. There are several recessive SCN such as SBDS, which was not found in high frequencies in this cohort. Geographical and ethnical polymorphisms are probably the main cause, as well as a founder effect, which is revealed by consanguinity.

There are two *HAX1* splice isoforms, and genotype–phenotype studies have shown that *HAX1* mutations affecting just isoform A lead to SCN without any neurological involvement, whereas mutations that affect both isoforms cause SCN and neurological disorders.[21,22] *pW44X* mutation affects just isoform A. Accordingly, only four patients in our cohort showed mild neurological symptoms.

Other mutations in this registry, including *CSF3R*, *G6PC3*, and *JAGN1*, are mostly autosomal recessive inherited mutations and have been previously described in a few patients.[23–26] In our cohort, *G6PC3* was the third common cause, after *ELANE* and *HAX1* mutations.

Patients with a biallelic *CSF3R* mutation are very rare, and clinical management is not easy due to their general lack of response to G-CSF.[11,26] Although GM-CSF treatment is not generally effective in patients with SCN, it has been reported to be beneficial in patients with biallelic *CSF3R* mutations,[27,28] and, accordingly, one of our patients responded very well to GM-CSF treatment.[20] Another patient in our cohort with hypomorphic biallelic *CSF3R* mutation responded well to G-CSF.[19]

Transformation to MDS/AML in our registry is 2.3%, which is lower than that is observed in most of the studies.[1,8,10,13,14] Data from SCNIR, for example, showed that at a 15-year follow-up, the risk for MDS/AML is 22%. However, in the French Neutropenia registry, the cumulative incidence of MDS and AML was 2.7% at 10 years, which is similar to our results.[7]

In this registry, a relatively high percentage of the patients (9.3%) underwent HSCT at earlier ages. Therefore, the possibility of the clonal diseases might have been eliminated in those patients. Lack of prospective registration and follow-up of the patients could have caused some missing data for example, early death due to infections before the

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diagnosis of SCN, clonal diseases secondary to SCN mistakenly considered as primary AML/MDS, might have masked the real rate of the patients with MDS/AML. These issues (at least in part) might have contributed to lower MDS/AML development and in our series.

Although the median dose of G-CSF in this series was lower than that of other registries, our mortality rate from infectious complications is not high.[1,7,8,10,15,29,30] In our registry, the most frequent SCN mutation is the *HAX1*, notably at pW44X. Some *ELANE* mutations have a very dismal prognosis, whereas some patients with other mutations have a very good clinical outcome,[29,31] which could explain our low mortality rate.

There are some limitations to this study. The lack of coverage of the whole population is significant. Another issue is the relatively short follow-up period. Also, all of the rare genetic defects were not screened in all patients without *HAX1* and *ELANE* mutation, and this is a shortcoming of this study. Therefore, this study provides—at least—a preliminary estimation of the epidemiology of SCN in the Turkish population. The plan is to expand our registry and to include all Turkish patients with SCN into the registry, and to study all rare mutations in patients without known mutations, and to prolong the follow-up period.

In conclusion, we have established the Turkish National SCN Registry for the first time. There are notable differences between the Turkish SCN Registry and those from other countries in the genes responsible for SCN. Notably, the high frequency of the pW44X mutation on the *HAX1* gene, which should be the first gene screened for patients with consanguineous parents, particularly in those of Kurdish origin. Outside of the Kurdish population, *ELANE* mutation screening, followed by that of other autosomal recessive genes, should be recommended. The rare mutations that were observed in a small number of patients in this registry will now be studied in all patients with SCN but without known mutations, thereby identifying the underlying genetic defects in more patients.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Each of the authors reported how they were involved in the design of the study, data collection, analysis, and editing.

DYK, TP, AM, ÜÇ, TC, BY, ZK, THK, BA, FÖ, HO, MAY, and the Turkish SCNR Group.

Turkish Severe Congenital Neutropenia (SCN) Group: HHA, GT, HT, GNÖ, AAK, SK, YK, YO, AK, LO, ZKY, GA, MG, Tİ, YZA, AB, BA, ZK, MS, NÖK, UÖ, BÖS, HHÖ, VU, GTK, ŞY.

DYK: Design of the study, patient care, data collection, analysis the results and writing the manuscript; TP, AM, ÜÇ, TC, BY, ZK, THK, BA, HHA, GT, HT, GNÖ, AAK, SK, YK, YO, AK, LO, ZKY, GA, MG, Tİ, YZA, AB, BA, ZK, MS, NÖK, HHÖ, VU: Patient care, data collection; HO, FÖ: Mutational analysis, editing the manuscript; UÖ, BÖS: Mutational analysis, GTK, ŞY: Design of the study, MAY: Design of the study, editing the manuscript.

DATA AVAILABILITY STATEMENT

The research data and other artifacts supporting the results reported in the paper can be found in archive of Ege University Faculty of Medicine Pediatric Hematology Department.

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