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ORIGINAL PAPER

HAX1-related congenital neutropenia: Long-term observation in paediatric and adult patients enrolled in the European branch of the Severe Chronic Neutropenia International Registry (SCNIR)

Denys Pogozhykh[1] | **Deniz Yilmaz Karapinar**[2] | **Maksim Klimiankou**[3] | **Natali Gerschmann**[1] | **Georg Ebetsberger-Dachs**[4] | **Jan Palmblad**[5] | **Göran Carlsson**[6] | **Tania Masmas**[7] | **Sally Kinsey**[8] | **Marije Bartels**[9] | **Sabine Mellor-Heineke**[3] | **Karl Welte**[10] | **Julia Skokowa**[3] | **Cornelia Zeidler**[1]

[1]Clinic for Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

[2]Department of Pediatric Hematology, Children's Hospital, Izmir, Turkey

[3]Department of Hematology, Oncology, Clinical Immunology, and Rheumatology, University Hospital Tübingen, Tübingen, Germany

[4]Department of Paediatrics and Adolescent Medicine, Kepler University Hospital, Linz, Austria

[5]Departments of Medicine and Hematology, The Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

[6]Childhood Cancer Research Unit, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

[7]Pediatric Hematopoietic Stem Cell Transplantation and Immunodeficiency, The Child and Adolescent Clinic, Copenhagen University Hospital, Copenhagen, Denmark

[8]Leeds Institute for Medical Research, University of Leeds, Leeds, UK

[9]Department of Paediatric Haematology, University Medical Centre Utrecht, Utrecht, The Netherlands

[10]University Children's Hospital Tübingen, Tübingen, Germany

Correspondence
Denys Pogozhykh, Clinic for Hematology, Hemostaseology, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany.
Email: pogozhykh.denys@mh-hannover.de

Summary

HAX1-related congenital neutropenia (*HAX1*-CN) is a rare autosomal recessive disorder caused by pathogenic variants in the *HAX1* gene. *HAX1*-CN patients suffer from bone marrow failure as assessed by a maturation arrest of the myelopoiesis revealing persistent severe neutropenia from birth. The disorder is strongly associated with severe bacterial infections and a high risk of developing myelodysplastic syndrome or acute myeloid leukaemia. This study aimed to describe the long-term course of the disease, the treatment, outcome and quality of life in patients with homozygous *HAX1* mutations reported to the European branch of the Severe Chronic Neutropenia International Registry. We have analysed a total of 72 patients with different types of homozygous ($n = 68$), compound heterozygous ($n = 3$), and digenic ($n = 1$) *HAX1* mutations. The cohort includes 56 paediatric (<18 years) and 16 adult patients. All patients were initially treated with G-CSF with a sufficient increase in absolute neutrophil counts. Twelve patients required haematopoietic stem cell transplantation for leukaemia ($n = 8$) and non-leukaemic indications ($n = 4$). While previous genotype–phenotype reports documented a striking correlation between two main transcript variants and clinical neurological phenotypes, our current analysis reveals novel mutation subtypes and clinical overlaps between all genotypes including severe secondary manifestations, e.g., high incidence of secondary ovarian insufficiency.

KEYWORDS
acute leukaemia, bone marrow failure, *HAX1* mutation, MDS, neutropenia

INTRODUCTION

Up to date mutations in more than 25 genes have been identified as causative for severe congenital neutropenia (CN), including *ELANE, GFI1, GATA2, TCIRG1, CXCR4, HAX1, JAGN1, G6PC3, SLC37A4, SBDS, STK4, CLPB, AP3B1, LAMTOR2, USB1, VPS13B, VPS45, CXCR2, EIF2AK3, LYST, RAB27A, AK2, RMRP, TCN2, CSF3R, WAS, TAZ and CD40LG*.[1,2] Severe CN is a common name designated to a group of conditions, primarily characterized by abnormally low neutrophil counts (typically less than 500 neutrophils in one μL peripheral blood), which significantly increases the susceptibility to bacterial infections. Some CN subtypes are restricted to a haematological phenotype only, whereas others are accompanied by developmental abnormalities and syndromic conditions, including systemic organ dysfunction, malformations of the skeleton, pancreas, heart, liver and neurological complications.[1–7]

In former years, the rate of mortality from severe bacterial infections in all subtypes of CN was over 80% even after the introduction of antibiotics in the 1950s, and over 90% prior to this period.[8,9] A drastic change in survival prognosis could be achieved in the last decade of the 20th century with the availability of treatment with recombinant human granulocyte-colony-stimulating factor (G-CSF).[10–14] Under G-CSF therapy survival rates exceed more than 80%, even considering those patients who develop malignant complications.[1,15] Statistically, around 10% of patients still die from sepsis, but these are mainly the non-responders to G-CSF or non-compliant patients.[1,15,16]

In 2007, we discovered that homozygous mutations in the *HAX1* gene, which encodes HCLS1-associated protein X-1, lead to an autosomal recessive bone marrow failure syndrome, characterized by a maturation arrest in granulopoiesis at the stage of promyelocyte to myelocyte transition (Figure 1A), leading to absolute neutrophil counts below 0.5×10^9/L in the peripheral blood.[1,17] *HAX1*-CN belongs to a group of rare inborn genetic defects with an increased risk for evolution to myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML), similar to a number of other forms of CN.[1,16–20] Decreased production or compromised maturation of functional neutrophils results in life-threatening severe recurrent bacterial infections from the first weeks of life of the affected individuals.[1,7] Infections are predominantly affecting the oral cavity (gingivitis and periodontitis), respiratory tract (bronchitis and pneumonias) and skin (omphalitis and abscesses). Furthermore, some patients with *HAX1*-related neutropenia develop neurological pathologies, including neurodevelopmental delay, cognitive impairment and epileptic seizures.[3,4,6,7,19–22] Analysis of the pedigree of patients initially described by Dr. Rolf Kostmann in the 1950s revealed that these patients also harboured mutations in the *HAX1* gene.[17,23–26]

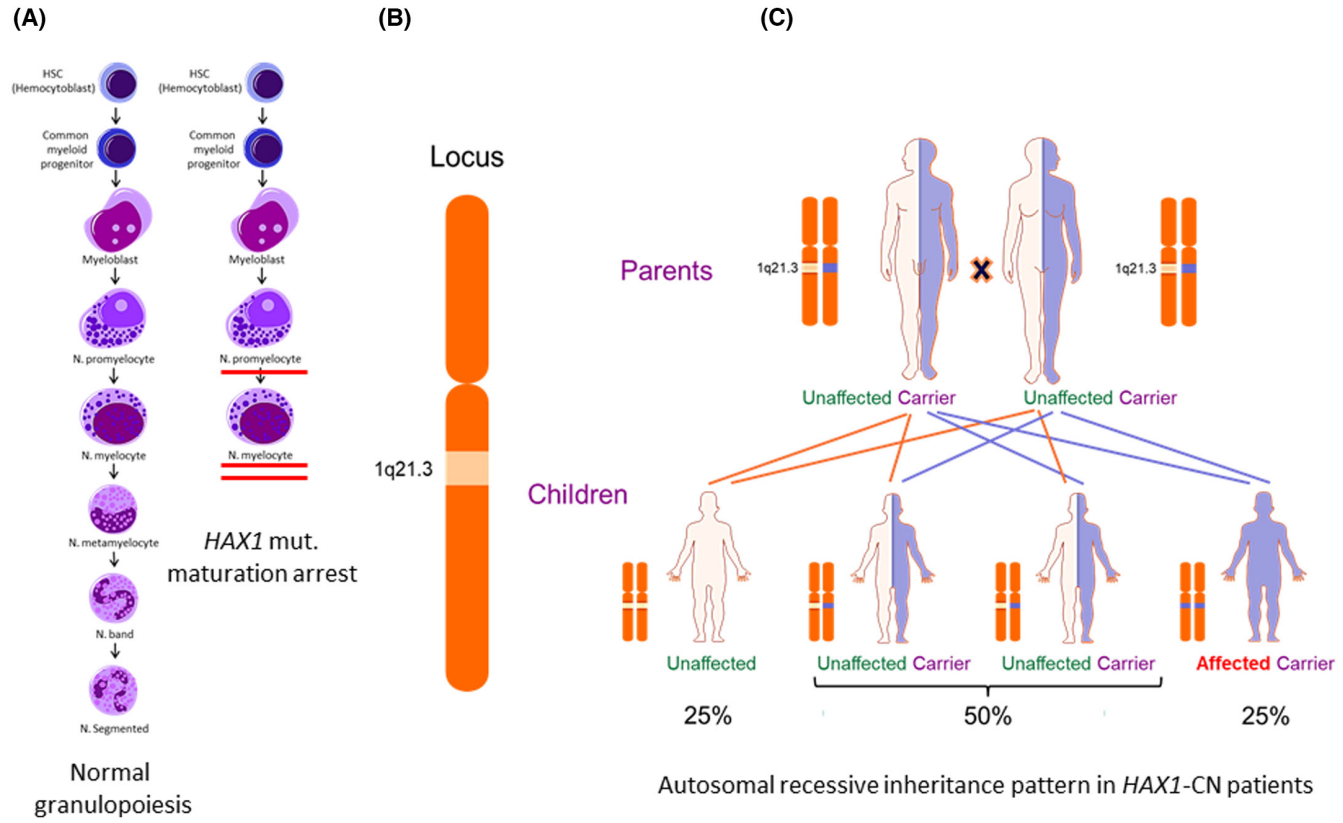


FIGURE 1 (A) Normal granulopoiesis and maturation arrest of granulopoiesis in *HAX1* mutated bone marrow. (B) *HAX1* gene locus on chromosome 1, NG_007369.1 RefSeqGene. (C) The autosomal recessive inheritance pattern of *HAX1*-related congenital neutropenia.

HAX1 gene is located at position 1q21.3 of the long arm of chromosome 1 (Figure 1B). Although HAX1 is a ubiquitously expressed protein, its binding partner, haematopoietic lineage cell-specific protein (HCLS1), is an essential signal transducing adaptor protein of the granulocyte colony-stimulating factor receptor (G-CSF-R) signalling, an important component of granulopoiesis.[27,28] In addition, HAX1 is critical for maintaining the inner mitochondrial membrane potential.[17] Recently, Fan et al. identified Caseinolytic peptidase B protein homologue (CLPB) isoform 2 as a critical interaction partner of HAX1 in human cells and demonstrated that the CLPB/HAX1 axis controls the balance of mitochondrial protein synthesis being crucial for proper mitochondrial function.[29] Intriguingly, a subset of patients with mutations in CLPB is characterized by brain dysfunction and congenital neutropenia.[30] Previously, two main *HAX1* splice isoforms have been identified. Mutations affecting one isoform only (p.W44X) are primarily found in families of Middle East origin, whereas, mutations affecting both isoforms are found in the originally discovered pedigree from Sweden (p.Q190X) and in Japanese patients (p.R86X).[17,18,21,23–26,31,32] At present, the broader availability of powerful molecular diagnostics methods, in particular, targeted next-generation sequencing (NGS) and exome sequencing, resulted in the continuous identification of numerous additional *HAX1* mutations in CN patients.[1,4,5,7,33,34] While the autosomal recessive inheritance pattern implies 25% of the statistical probability of having an affected child by carrier parents, it still leaves the probability of 75% (50% unaffected carriers plus 25% affected) to carry the mutation further (Figure 1C). This contributes to the high incidence in certain populations, as the majority of *HAX1*-CN patients come from families with strong traditional backgrounds of consanguine marriages and/or reside in isolated rural areas for many generations.

Here, we analyse the genotype–phenotype correlation, describe the course of the disease, response to treatment and long-term outcome of 16 adults and 56 minor patients ($N=72$) with various types of homozygous, compound heterozygous, as well as digenic (combined with a mutation in another CN-causative gene) *HAX1* mutations enrolled by the SCNIR in Europe. Additionally, we performed a survey on family background and various aspects of quality of life, such as overall development, presence of a degree of

TABLE 1 General characteristics of the *HAX1*-CN cohort.

Variable	Value
Number of patients, *N*	72
Gender	
Male, *N* (%)	39 (54.2)
Female, *N* (%)	33 (45.8)
Ratio, ♂: ♀	1.18: 1
Age at last follow-up, years	
Median (range)	10.4 (1.0–40.8)
Minor patients (<18 years old)	
0–5 years old, *N* (%)	16 (22.2)
6–11 years old, *N* (%)	24 (33.3)
12–17 years old, *N* (%)	16 (22.2)
Adult patients (>18 years old)	
18–25 years old, *N* (%)	6 (8.3)
>25 years old, *N* (%)	10 (13.9)
Age at confirmed neutropenia onset, years	
Median (range)	0.2 (0–5.7[a])
Age at diagnosis, years	
Median (range)	0.9 (0–19.9)
G-CSF dose (μg/kg/day)	
Median (range)	3.59 (0.24–21.29)
Age at G-CSF Start, years	
Median (range)	1.12 (0.02–19.87)
Patient status	
Total alive patients, *N* (%)	69 (95.8)
Total expired patients, *N* (%)	3 (4.1)

[a]One patient was reported to the registry with the age of onset of neutropenia at 5.7 years, which is due to the late diagnosis establishment rather than the actual late onset.

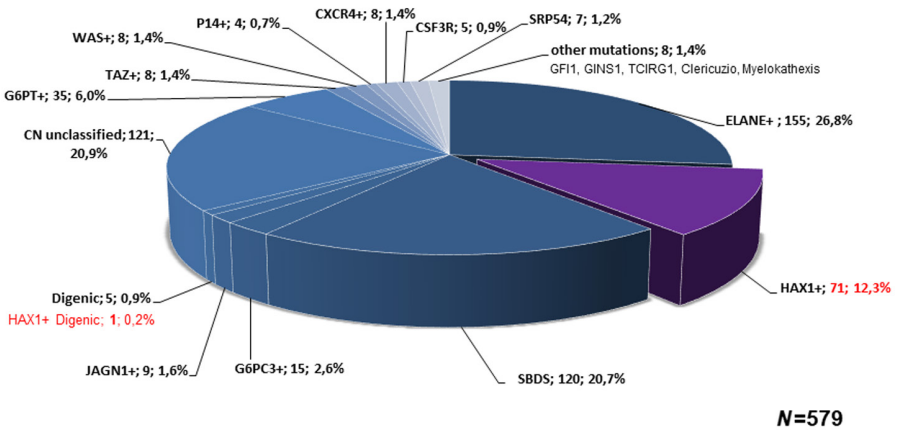


FIGURE 2 Distribution of disease-causing mutations in CN patients registered in the European branch of the SCNIR ($N=579$). The contribution of *HAX1* mutation is displayed in red font.

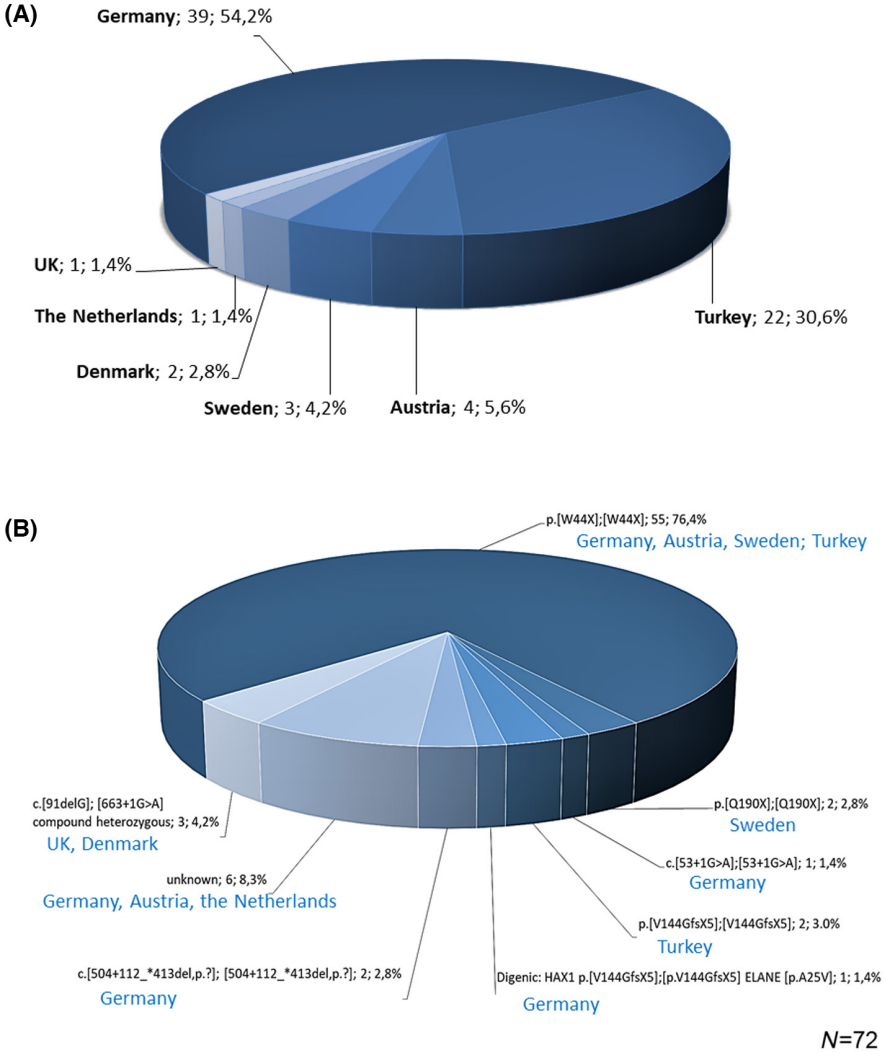


FIGURE 3 Diagram of the distribution of CN patients with *HAX1* mutations by country of registration (A) and mutation position (B).

disability, family status, education, professional status, living conditions, requirement for additional support, overall satisfaction with life and well-being in a cohort of 17 patients registered in Germany and 22 registered in Turkey.

METHODS

Severe chronic neutropenia international registry, European branch

A total of 1003 patients with different subtypes of severe chronic neutropenia were enrolled in the European branch of the SCNIR as of January 2023. Here, we analysed all 72 available neutropenia patients with a variety of homozygous and compound heterozygous *HAX1* mutations including one patient with a combined homozygous *HAX1* plus heterozygous *ELANE* mutation. Median follow-up time was 4.68 years (0–30.34 years) with 867.37 patient years under observation (Table 1).

Ethical approval for this study was obtained from the Ethic Committee of Hannover Medical School (APPROVAL Nr. 2993). Patients were enrolled on referral from cooperating haematologists, paediatricians and other treating physicians in Europe using standard enrolment procedures of the registry (www.severe-chronic-neutropenia.org). At the time of enrolment, clinical and laboratory information was reviewed by an expert clinician associated with the SCNIR (see protocol). Informed written consents from the patients themselves or the legal guardians of minor patients were obtained according to the Declaration of Helsinki. The biological material of the patients (blood, bone marrow and DNA samples) was declared in compliance with current legislation. Hematologic parameters at diagnosis and during annual follow-up, bone marrow cytogenetics and morphology, genetic analysis, infectious episodes, dose and frequency of granulocyte colony-stimulating factor (G-CSF) treatment, clinical symptoms and family history were entered into a standardized database of the registry. Data on the health-related quality of life has been obtained by the SCNIR from

TABLE 2 Phenotype and pathology in correlation with genotype variations of HAX1 mutation.

Genotype		Total *N* Gender, *N* [%]	Associated phenotype and pathology (occurrence, *N*)	
Homozygous	*Short annotation*: p.(Trp44*)(;)(Trp44*) NC_000001.11:g.154273412_154273413insA *HAX1*(NM_006118.4):c.130_131insA(;)(130_131insA), p.(Trp44*)(;)(Trp44*) Variant is predicted to affect only NM_006118.4—long isoform ACMG classification: *pathogenic*	**55** Male, 29 [52.7%]; Female, 26 [47.3%]	***Congenital anomalies***	
			Cardiac	Heart valve insufficiency (3), ventricular septal defect (1)
			Musculoskeletal	Talipes calcaneus (1), hip dysplasia (1)
			Urological	Undescended testicles (2), degenerated testicles (1), hypospadia (1)
			Ears, Nose, Throat	Hypakusis with conductive deafness (1)
			Haematological	anaemia (4), β-thalassemia minor (5)
			Others	Gaucher disease (1)
			Acquired in the course of the disease	
			Endocrinological	Secondary hyperparathyroidism (1), hypothyroidism (2), oligomenorrhea (1), growth hormone deficiency (2), Hashimoto's thyroiditis (3), polycystic ovary syndrome (1), hypogonadism (8), primary ovarian insufficiency (1)
			Neurological	Epilepsy (4), mental retardation (4)
			Hematologic	Thrombocytopenia (1), MDS/AML (6)
			Other	Splenomegaly (3), chronic hepatitis (1) osteoporosis (2), diabetes mellitus type III (1), osteopenia (2), parodontitis (1), pancreatitis (1), dwarfism (4)
	Short annotation: p.(Gln190*)(;)(Gln190*) NC_000001.11:g.154275165C>T *HAX1*(NM_006118.4):c.568C>T(;)(568C>T), p.(Gln190*)(;)(Gln190*) Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform ACMG classification: *pathogenic*	**2** Male, 2 [100%]	***Acquired in the course of the disease***	
			Neurological	Epilepsy (2)
			Haematological	MDS/AML (1)
	Short annotation: c.[53+1G>A];[53+1G>A] NC_000001.11:g.154272777G>A NC_000001.11(NM_006118.3):c.[53+1G>A];[53+1G>A], p.[?];[?] Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform ACMG classification: *pathogenic*	**1** Female, 1 [100%]	***Congenital anomalies***	
			CNS	Microcephalia (1)
	Short annotation: p.(Val144Glyfs*5)(;)(Val144Glyfs*5) NC_000001.11:g.154273887dup *HAX1*(NM_006118.4):c.430dup(;)(430dup), p.(Val144Glyfs*5)(;)(Val144Glyfs*5) Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform ACMG classification: *pathogenic*	**2** Male, 1 [50%]; Female, 1 [50%]	–	
	Short annotation: c.[504+112_*413del];[504+112_*413del] or homozygous deletion of exons 4–7 of *HAX1* *HAX1*(NM_006118.4):c.[504+112_*413del];[504+112_*413del], p.[(?)]; [(?)] Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform. ACMG classification: *pathogenic*	**2** Male, 1 [50%]; Female, 1 [50%]	***Acquired in the course of the disease***	
			Haematological	Leukopenia (2), anaemia (1)
			Others	Eustachian tube dysfunction (1)

(Continues)

TABLE 2 (Continued)

Genotype		Total *N* Gender, *N* [%]	Associated phenotype and pathology (occurrence, *N*)	
Compound heterozygous	*Short annotation*: c.91del(;)663+1G>A NC_000001.11:g.154273373del(;)154275261G>A NC_000001.11(NM_006118.4):c.91del(;)663+1G>A, p.(Glu31Lysfs*54)(;)(?) Deletion c.91del: Variant is predicted to affect only NM_006118.4—long isoform Splice site variant c.663+1G>A Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform ACMG classification for both variants is *pathogenic*	**3** Male, 2 [66.7%]; Female, 1 [33.3%]	***Acquired in the course of the disease***	
			Endocrinological	Primary ovarian insufficiency (1)
Digenic	*Short annotation*: p.(Val144Glyfs*5)(;)(Val144Glyfs*5) NC_000001.11:g.154273887dup *HAX1*(NM_006118.4):c.430dup(;)(430dup), p.(Val144Glyfs*5)(;)(Val144Glyfs*5) Variant is predicted to affect both isoforms, NM_006118.4—long isoform and NM_001018837.2—short isoform ACMG classification—pathogenic ELANE (p.Ala25Val) NC_000019.10:g.852882C>T *ELANE*(NM_001972.4):c.74C>T, p.(Ala25Val) ACMG classification: *uncertain significance*	**1** Female, 1 [100%]	***Congenital anomalies***	
			CNS	Microcephalia (1)
			Ears, nose, throat (ENT)	Hypoacusis (1), absence of ossicles (1)
			Acquired in the course of the disease	
			Musculoskeletal	Dwarfism (1)
			Neurological/psychiatry	Developmental disorder (1)

a survey on *HAX1*-CN patients enrolled in Germany and Turkey using the age group-specific questionnaires that correspond to the first column of Tables 3–6. The questionnaire was conducted in the native language of the patients and evaluated background, social status, global health status, physical functioning, role functioning, emotional functioning, cognitive functioning and social functioning.

Statistical analysis was performed using the SPSS V. 9.0 statistical package (SPSS). Each analysis was carried out by using all subjects with available data.

RESULTS AND DISCUSSION

Among the large variety of genetic abnormalities contributing to presentation with severe CN in patients registered in the European branch of the SCNIR as of January 2023 ($N = 579$), *HAX1-associated* mutations contributed to 12.3% of the cases, being the third largest group of genetically classified cases after *ELANE*- and *SBDS*-associated neutropenias (Figure 2).

Patient characteristics

Table 1 presents the general characteristics of a cohort of studied *HAX1*-CN patients.

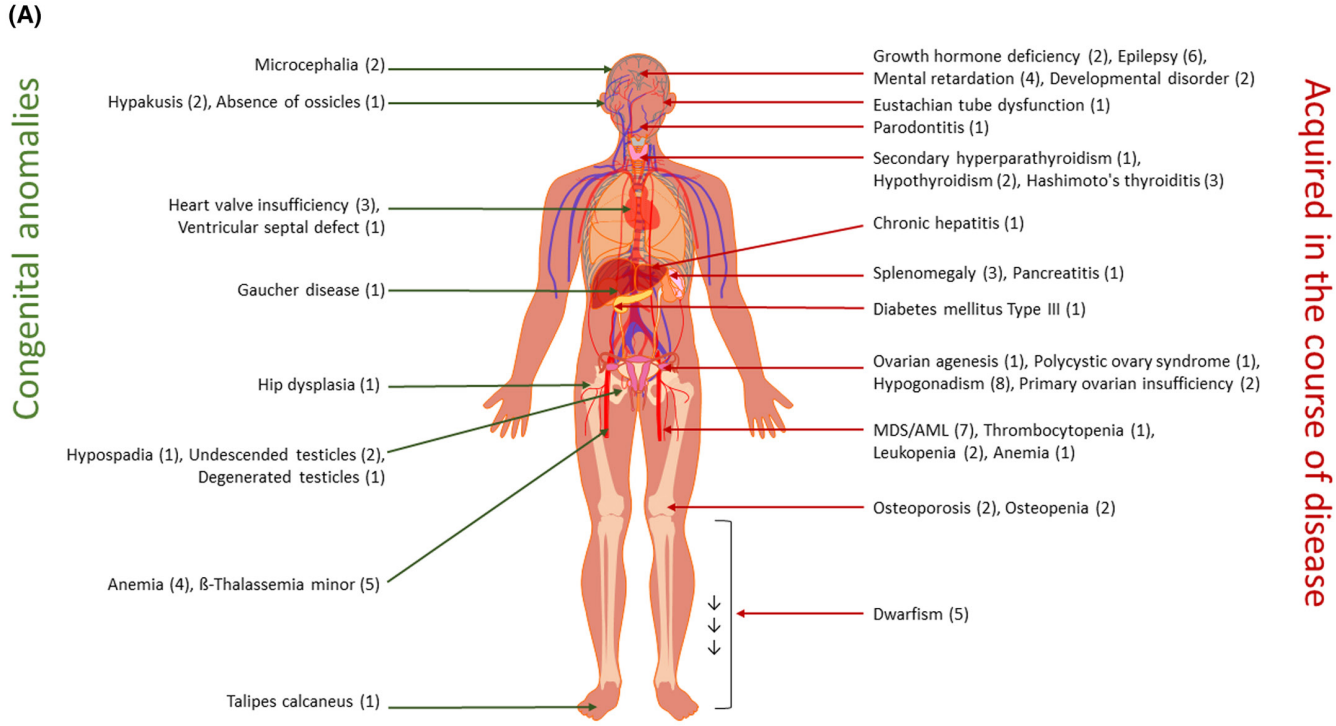
Out of 72 patients with *HAX1*-associated CN, 39 patients are male and 33 patients are female. The median age of the cohort was 10.4 years (1.0–40.8). 56 patients were below 18 years of age, while 16 patients have reached adulthood. 69 patients were alive, and 3 patients were expired. All patients have received G-CSF treatment post-diagnosis.

Country of registration

The majority of the *HAX1*-CN patients submitted to the European branch of the SCNIR were referred from Germany (40 patients), and Turkey (22 patients), comprising 54.2% and 30.6% of the studied cohort, respectively (Figure 3A). However, it has to be noted, that most of the patients submitted from Germany, or their parents, belong to ethnic groups from the Middle East. Additionally, 4 patients were registered from Austria (5.6% of the studied cohort), 3 from Sweden (4.2%), 2 from Denmark (2,8%), 1 from the Netherlands (1.4%) and 1 from the UK (1.4%) (Figure 3A).

Genetics

Analysis of different positions of the *HAX1* gene mutations showed that homozygous *HAX1* (p.W44X) was the major disease-causing mutation (76.4%) in the studied



(B)

Patient 1 **Minor ♀**	Patient 2 **Minor ♀**	Patient 3 **Adult ♀**	Patient 4 **Adult ♀**	Patient 5 **Minor ♂**	Patient 6 **Minor ♂**	Patient 7 **Adult ♂**	Patient 8 **Adult ♂**
Transient anemia	Sickle cell anemia	Hypothyroidism	β thalassemia	β thalassemia	β thalassemia	Osteopenia	Hypothyroidism
	Heart valve insufficiency	Primary ovarian insufficiency	Epileptic seizures	Hypospadia	Heart valve insufficiency		
	Lack of pubertal development	Hypogonadism	Mild mental retardation	Undescended testicles			
		Hip dysplasia	Low language ability	Spenomegaly			
				Dwarfism			

FIGURE 4 (A) Congenital anomalies and medical comorbidities in the course of disease in the studied cohort of *HAX1*-CN patients, $N = 72$. (B) Examples of inborn and acquired pathologies and abnormalities accumulated in individual patients (2 minor females, 2 adult females, 2 minor males and 2 adult males) within p.W44X *HAX1* mutation subtype, $N = 8$.

cohort (Figure 3B). This mutation was present in patients registered from Germany, Turkey, Austria and Sweden. Other homozygous mutations were found in 2 patients from Sweden (p.Q190X), in 2 siblings from Turkey (HAX1 p.(Val144GlyfsX5)) and one patient from Germany (c.[53+1G>A]; [53+1G>A]). One novel type of homozygous mutation (c.[504+112_*413del,p.?]) was found in two siblings registered from Germany. Compound heterozygous mutations (c.[91delG]; [663+1G>A]) were found in two siblings from Denmark and one patient from the UK. In addition, one digenic patient was identified, as being affected by homozygous *HAX1* p.[V144GfsX5] and heterozygous *ELANE* (p.A25V) mutation. Six of the studied patients (8.3%) harboured *HAX1* mutations, but their exact position was not reported to the SCNIR. 57 out of 72 studied patients (79.2%) revealed confirmed consanguinity of their parents. Although 8 patients (11.1%) declared no consanguinity of their parents and 7 (9.7%) did not provide information about consanguinity, the absence of consanguinity in the families was not confirmed and may still be the case.

Secondary congenital abnormalities and development of medical comorbidities during the course of disease in relation to genetic subtypes of *HAX1* mutation

At the onset, 39 patients (54%) presented with isolated neutropenia without any inborn conditions. Yet, our data show that besides severe neutropenia, *HAX1* mutations appear to be associated with a wide range of other congenital

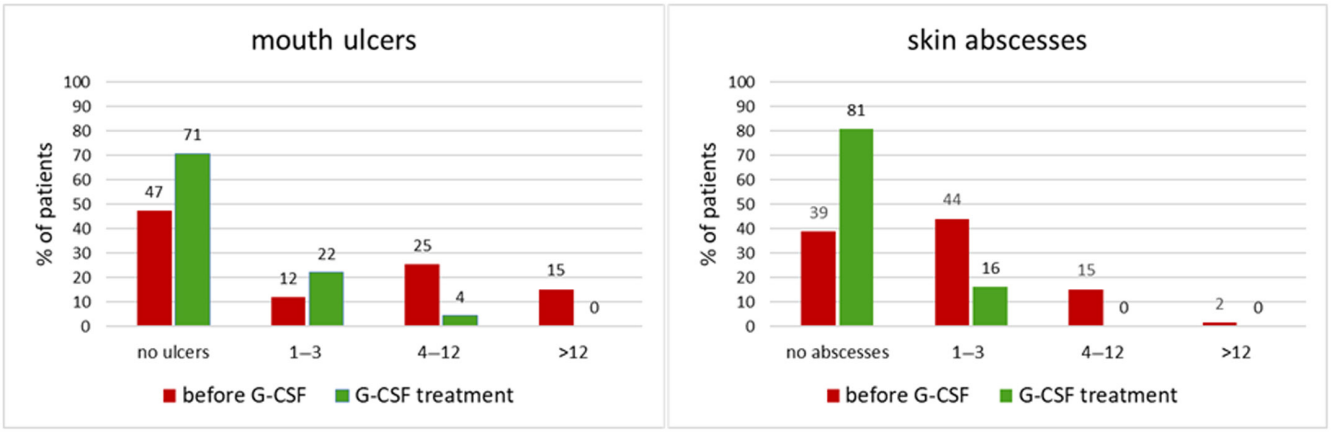


FIGURE 5 Infections in *HAX1*-CN patients before and after the initiation of G-CSF treatment. Clustered columns represent the percentage of patients with no infectious events per year, 1–3 infectious events per year, 4–12 infectious events per year and over 12 infectious events per year.

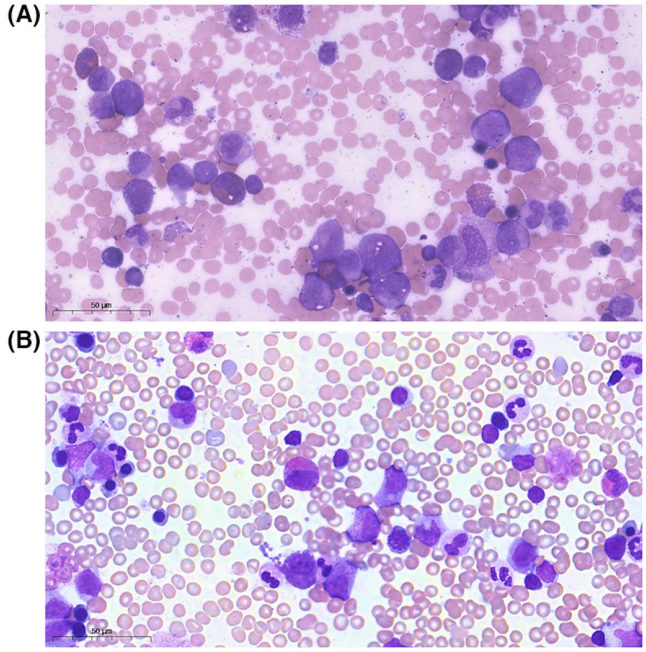


FIGURE 6 Cytological characteristics of bone marrow granulocytic cells in *HAX1*-CN patients. (A) at diagnosis, before the start of G-CSF therapy; (B) under the therapy with adjusted G-CSF doses. Cytology analysis after May-Grünwald Giemsa staining. Images represent granulocytic precursors and mature neutrophils (original magnification ×63).

abnormalities, as well as the development of medical comorbidities in the course of the disease. Both inborn and acquired conditions in the studied cohort of *HAX1*-CN patients are presented in Table 2 in correlation with the genotype. Figure 4A displayed both congenital anomalies and medical comorbidities in the course of disease for the whole studied cohort of *HAX1*-CN patients in general. It is shown that inborn abnormalities involve nearly all organ systems, including cardiovascular, musculoskeletal, urogenital, haematological, ENT and CNS entities (Table 2; Figure 4A). Associated pathologies also developed in the course of the disease (Table 2; Figure 4). The data in Figure 4B represent examples of inborn and acquired pathologies and abnormalities accumulated in eight individual patients within the most represented *HAX1* mutation subtype (p.W44X) of the studied cohort, including 2 minor and 2 adult patients of both sexes. Within the same mutation subtype, there is a broad heterogeneity in the clinical presentation. Additionally, it should be noted that ß-Thalassemia minor was present in 5 of the 55 studied patients with the p.W44X mutation subtype. ß-Thalassemia is another inherited blood disorder often found in the population of the Mediterranean area. In multiple cases, both male and female *HAX1*-CN patients have a tendency to have abnormalities in the reproductive system.

Response of patients to G-CSF treatment

All 72 patients received G-CSF treatment with a median dose of 3.59 µg/kg/day (0.24–21.29).

Before the start of G-CSF treatment, the vast majority of patients with *HAX1* mutations had suffered from mouth ulcers, skin abscesses and other infections of bacterial origin (Figure 5). Initiation of G-CSF treatment and adjustment of the dose resulted in the increase of neutrophils to adequate numbers, significant reduction of such infections, and improvement of quality of life.

Cytologic examination of bone marrow aspirates of *HAX1*-CN patients at diagnosis revealed a maturation arrest of the granulopoiesis at the promyelocytes and myelocytes stage of differentiation (Figure 6A). After the start of G-CSF therapy, the granulopoiesis was partially restored, enabling differentiation towards the band and segmented neutrophils (Figure 6B). The level of absolute neutrophil counts could be generally recovered in *HAX1*-CN patients towards adequate values, however, the bone marrow still presents with maturation arrest in follow-up bone marrow samples.

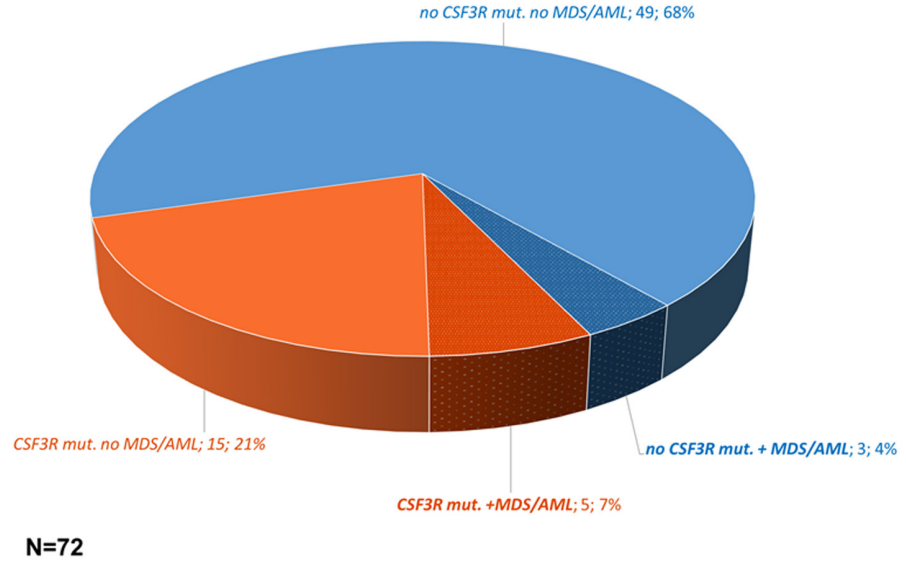


FIGURE 7 Acquired *CSF3R* mutations as a pre-leukaemic condition in *HAX1*-CN patients. Orange sections represent the patients with somatic *CSF3R* mutations, including the patients with MDS/AML transformation. Blue sections represent the patients without somatic *CSF3R* mutations, including the patients with MDS/AML transformation. The data is presented in absolute values of the number of patients and percentage of the number of patients of each section to the entire *HAX1*-CN cohort.

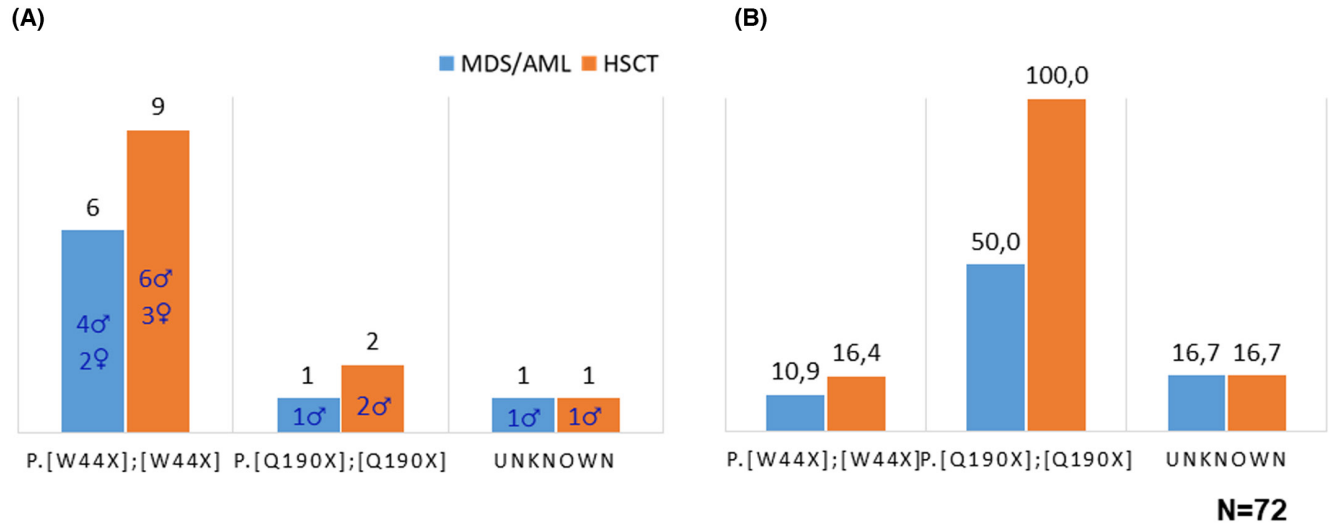


FIGURE 8 Patients with secondary MSD/AML and undertaken HSCT in correlation with genotype variations of *HAX1* mutation. Absolute (A) and relative values, calculated in percentage from the total number of patients with corresponding mutation (B) are presented.

Acquired *CSF3R* mutations associated with leukaemic development potential

Mutations in the *CSF3R* gene encoding the G-CSF receptor protein are a major risk factor for leukemogenesis in patients with CN.[1,28,32,35,36] Twenty patients of the studied *HAX1* cohort (28%) presented with somatic (acquired) mutations of the *CSF3R* gene at the last follow-up (Figure 7). Out of these patients, 7 were female (35%) and 13 were male (65%). Eight patients of the entire *HAX1* cohort have developed MDS/AML, of whom 5 (i.e., 63% of all leukaemic patients in the cohort) presented with somatic *CSFR3* mutations at the time of MDS/AML transformation. Three patients with MDS/AML (37% of all leukaemic patients in the cohort) did not have *CFSF3R* mutations at the time of transformation. Somatic *CSF3R* mutations may be detected several years prior to leukaemia development.[1] Up to now, 25% of *HAX1*-CN patients with acquired *CSF3R* mutations have already transformed. It remains unclear whether finally, all *HAX1*-CN patients with somatic *CSF3R* mutations end up in leukaemic transformation. Therefore, such patients have to be especially closely monitored by the treating physicians. One of the patients with *CSF3R* mutation and subsequent leukaemia development has expired

TABLE 3 Background and quality of life of a cohort of adult *HAX1*-CN patients from Germany registered in the SCNIR ($n = 9$).

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Marital status							
Single	6 (66.7)	—	—	—	—	—	—
Married	3 (33.3)						
Living together with a partner							
Yes	3 (33.3)	—	—	—	—	—	—
No	6 (66.7)						
Total number of persons in household	—	4	3.78	1.48	2	6	4
Number of persons in household under 14 years old	—	0	0.44	0.53	0	1	1
Country of birth							
Germany	8 (88.9)	—	—	—	—	—	—
Others	1 (11.1)						
Citizenship							
German	7 (77.8)	—	—	—	—	—	—
Others	2 (22.2)						
At least one parent born outside of Germany							
Yes	9 (100.0)	—	—	—	—	—	—
No	0 (0.0)						
Highest school degree		3	—	—	1	4	—
No qualification = 1	1 (11.1)						
Elementary school or secondary school qualification = 2	2 (22.2)						
Secondary school qualification/ middle school leaving certificate = 3	4 (44.4)						
Technical college qualification or university qualification = 4	2 (22.2)						
Vocational training		4	—	—	1	5	—
Still student = 1	1 (11.1)						
Still in vocational training = 2	2 (22.2)						
No professional qualification and not in vocational training = 3	1 (11.1)						
Vocational training completed = 4	4 (44.4)						
Higher education completed = 5	1 (11.1)						
Occupational group		2	—	—	1	3	—
None = 1	2 (22.2)						
Employee with simple duties = 2	5 (55.6)						
Qualified employee = 3	2 (22.2)						
Employment status		3	—	—	1	3	—
Unemployed = 1	1 (11.1)						
Still student/still in vocational training = 2	3 (33.3)						
Full-time employment = 3	5 (55.6)						
Currently on sick leave							
Yes	1 (11.1)	—	—	—	—	—	—
No	8 (88.9)						

TABLE 3 (Continued)

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Leaving area of residence							
Rural	6 (66.7)	—	—	—	—	—	—
City	3 (33.3)						
Disability degree (%)		3	—	—	1	4	—
No disability (0%) = 1	3 (33.3)						
30% = 2	1 (11.1)						
40% = 3	2 (22.2)						
50% = 4	3 (33.3)						
Necessity for assistance with activities of daily living		—	—	—	—	—	—
Yes	1 (11.1)						
No	8 (88.9)						
Overall satisfaction with life (0–10, where 0 = not satisfied, 10 = completely satisfied)	—	8	7.56	2.13	3	10	7

post haematopoietic stem cell transplantation (HSCT) due to the malignancy relapse.

Leukaemia secondary to CN and haematopoietic stem cell transplantation

MDS/AML is secondary to *HAX1*-CN and has developed in 8 patients (11%) from the studied cohort of 72 patients with all varieties of *HAX1* gene mutations (Figure 8). In terms of correlation with the particular mutation, 6 cases of MDS/AML were reported in patients with homozygous p.[W44X];[W44X] mutation (10.9% from all studied patients with this mutation); one case occurred in a patient with homozygous p.[Q190X];[Q190X] mutation (50% from all studied patients with this mutation); and in a patient with a confirmed *HAX1* mutation, but of unspecified subtype in the records of the Registry (16.7% from all unspecified cases of the cohort). The latter case of unknown specification, however, may have contributed to any of the studied mutation subtypes.

HSCT was performed in all patients who developed MDS/AML. Additionally, HSCT was carried out in 4 patients without overt leukaemia, making it a total of 12 patients (17%) from the studied cohort of 72. In terms of correlation with particular *HAX1* mutation variations, 9 HSCTs were performed in patients with homozygous p.[W44X];[W44X] mutation (16.4% from all studied patients with this mutation); 2 HSCTs were performed in patients with homozygous p.[Q190X];[Q190X] mutation (all 100% from the studied patients with this mutation); and 1 HSCT in a patient with unspecified *HAX1* mutation (16.7% from all unspecified cases of the cohort), which may contribute to any of the studied mutation subtypes. Besides MDS/AML, the indications for HSCT in *HAX1*-CN patients included repeated severe infections in 3 patients with homozygous p.[W44X];[W44X] mutation, with one of them also having an acquired stop codon mutation in the *CSF3R* gene. Additionally, there was one patient with homozygous p.[Q190X];[Q190X] mutation with no access to the G-CSF therapy and family history of numerous relatives decreasing from complications related to severe neutropenia. All HSCTs in MDS/AML patients have been performed during the first 18 years of life. MDS/AML affected 6 male patients and 2 female patients suggesting a higher probability of developing MDS/AML in male patients (Figure 8A). Due to the higher occurrence of p.[W44X-];[W44X] *HAX1* mutation in comparison to other subtypes, we present the frequency of MDS/AML and HSCT events both in absolute values and in percentages from the total number of each mutation position subtype (Figure 8A,B). The majority of the patients responded well to HSCT, with 10 transplanted patients (83.3%) having >5-year survival post-HSCT, including 7 (58.3%) being long-term survivors (>10 years). Two patients (16.7% from all transplanted from the studied cohort) have expired post-HSCT.

Quality of life

The questionnaire survey related to the quality of life, social status and family background has been performed for 9 adults and 8 minor *HAX1*-CN patients registered in the SCNIR from Germany (Tables 3 and 4), as well as for 3 adults and 19 minor patients registered from Turkey (Tables 5 and 6) as these are the two most represented countries in the entire studied cohort. It was found that the whole range of the analysed parameters was satisfactory in both adult and minor patients. The results strongly correlated between German and Turkish patients. At the same time, it has to be noted, that the vast majority of the patients or their parents registered in the SCNIR from Germany are of Turkish origin with associated cultural and traditional backgrounds. As all patients received G-CSF treatment, it can be concluded that this therapy allows patients to have a normal quality of life.

TABLE 4 Background and quality of life of a cohort of minor *HAX1*-CN patients from Germany registered in the SCNIR ($n = 8$).

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Living together with parents							
Yes	8 (100.0)	—	—	—	—	—	—
No	0 (0.0)						
Total number of persons in household	—	5	5.25	1.39	3	7	4
Number of persons in household under 14 years old	—	1.50	1.75	1.28	0	4	4
Country of birth							
Germany	5 (62.5)	—	—	—	—	—	—
Others	3 (37.5)						
Citizenship							
German	6 (75.0)	—	—	—	—	—	—
Others	2 (25.0)						
At least one parent born outside of Germany							
Yes	7 (87.5)	—	—	—	—	—	—
No	1 (12.5)						
Highest requested or achieved school degree		1	—	—	1	2	—
Still pupil = 1	7 (87.5)						
Still in vocational training = 2	1 (12.5)						
Patient feels fit and well		5	4.38	0.91	3	5	2
Not at all = 1	0 (0.0)						
Not well = 2	0 (0.0)						
Mediocre = 3	2 (25.0)						
Fairly well = 4	1 (12.5)						
Very well = 5	5 (62.5)						
Vital energy		5	4.25	1.16	2	5	3
Not at all = 1	0 (0.0)						
Low = 2	1 (12.5)						
Mediocre = 3	1 (12.5)						
Acceptable = 4	1 (12.5)						
Very good = 5	5 (62.5)						
Sadness		1	1.88	1.46	1	5	4
Not at all = 1	5 (62.5)						
Slightly sad = 2	1 (12.5)						
Mediocre = 3	1 (12.5)						
Sad = 4	0 (0.0)						
Very sad = 5	1 (12.5)						
Feeling of loneliness		1	1.5	1.41	1	5	4
Not at all = 1	7 (87.5)						
Slightly = 2	0 (0.0)						
Mediocre = 3	0 (0.0)						
Lonely = 4	0 (0.0)						
Very lonely = 5	1 (12.5)						
Does patient have enough time for oneself?		4.5	4.38	0.74	3	5	2
Not at all = 1	0 (0.0)						
Very little time = 2	0 (0.0)						

TABLE 4 (Continued)

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Mediocre = 3	1 (12.5)						
Almost enough = 4	3 (37.5)						
Enough = 5	4 (50.0)						
Individually planned free time		5	4.5	1.07	2	5	3
Not at all = 1	0 (0.0)						
Very basic = 2	1 (12.5)						
Mediocre = 3	0 (0.0)						
Good planned = 4	1 (12.5)						
Very well planned = 5	6 (75.0)						
Well treated by parents		5	4.88	0.38	4	5	1
Not at all = 1	0 (0.0)						
Not very well = 2	0 (0.0)						
Mediocre = 3	0 (0.0)						
Well = 4	1 (12.5)						
Very well = 5	7 (87.5)						
Patient has good time with friends		5	4.38	1.06	2	5	3
Not at all = 1	0 (0.0)						
Not so good = 2	1 (12.5)						
Mediocre = 3	0 (0.0)						
Good time = 4	2 (25.0)						
Excellent time = 5	5 (62.5)						
Patient gets along well with school		4.5	4.25	0.87	3	5	2
Not at all = 1	0 (0.0)						
Not so well = 2	0 (0.0)						
Mediocre = 3	2 (25.0)						
Well = 4	2 (25.0)						
Very well = 5	4 (50.0)						
Patient pays attention in school		5	4.5	0.93	3	5	2
Not at all = 1	0 (0.0)						
Not so well = 2	0 (0.0)						
Mediocre = 3	2 (25.0)						
Well = 4	0 (0.0)						
Very well = 5	6 (75.0)						
General health status		2.5	2.75	1.04	2	5	3
Excellent = 1	0 (0.0)						
Good = 2	4 (50.0)						
Average = 3	3 (37.5)						
Not good = 4	0 (0.0)						
Bad = 5	1 (12.5)						
Overall satisfaction with life (0–10, where 0 = not satisfied, 10 = completely satisfied)	—	8	8.63	1.19	7	10	3

Puberty development and pregnancy

Gonadal insufficiency and delays of puberty development have been regularly observed in *HAX1*-CN patients according to previous reports.[37,38] Associated abnormalities have been also found in both male and female patients in the studied cohort. In particular, various hypogonadism forms have been found in 7 female patients (53.8% from all adult *HAX1*-CN female patients) and one male patient (7.1% from all adult *HAX1*-CN male patients). One male presented

TABLE 5 Background and quality of life of a cohort of adult *HAX1*-CN patients from Turkey registered in the SCNIR ($n = 3$).

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Marital status							
Single	2 (66.7)	—	—	—	—	—	—
Married	1 (33.3)						
Living together with a partner							
Yes	1 (33.3)	—	—	—	—	—	—
No	2 (66.7)						
Total number of persons in household	—	4	5.33	3.21	3	9	6
Number of persons in household under 14 years old	—	1	2.00	1.73	1	4	3
Country of birth							
Turkey	3 (100.0)	—	—	—	—	—	—
Others	0 (0.0)						
Citizenship							
Turkey	3 (100.0)	—	—	—	—	—	—
Others	0 (0.0)						
At least one parent born outside of Turkey							
Yes	0 (0.0)	—	—	—	—	—	—
No	3 (100.0)						
Highest school degree		3	—	—	2	4	—
No qualification = 1	0 (0.0)						
Elementary school or secondary school qualification = 2	1 (33.3)						
Secondary school qualification/ middle School leaving certificate = 3	1 (33.3)						
Technical college qualification or University qualification = 4	1 (33.3)						
Vocational training		3	—	—	3	5	—
Still student = 1	0 (0.0)						
Still in vocational training = 2	0 (0.0)						
No professional qualification and not in Vocational training = 3	2 (66.7)						
Vocational training completed = 4	0 (0.0)						
Higher education completed = 5	1 (33.3)						
Occupational group		1	—	—	1	3	—
None = 1	2 (66.7)						
Employee with simple duties = 2	0 (0.0)						
Qualified employee = 3	1 (33.3)						
Employment status		1	—	—	1	3	—
Unemployed = 1	2 (66.7)						
Still student/still in vocational training = 2	0 (0.0)						
Full-time employment = 3	1 (33.3)						
Currently on sick leave							
Yes	0 (0.0)	—	—	—	—	—	—
No	3 (100.0)						

TABLE 5 (Continued)

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Leaving area of residence							
Rural	1 (33.3)	—	—	—	—	—	—
City	2 (66.7)						
Disability degree (%)		1	—	—	1	1	—
No disability (0%) = 1	3 (100.0)						
30% = 2	0 (0.0)						
40% = 3	0 (0.0)						
50% = 4	0 (0.0)						
Necessity for assistance with activities of daily living							
Yes	0 (0.0)	—	—	—	—	—	—
No	3 (100.0)						
Overall satisfaction with life (0–10, where 0 = not satisfied, 10 = completely satisfied)	—	7	7.33	2.52	5	10	5

TABLE 6 Background and quality of life of a cohort of minor *HAX1*-CN patients from Turkey registered in the SCNIR (*n* = 19).

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Living together with parents							
Yes	19 (100.0)	—	—	—	—	—	—
No	0 (0.0)						
Total number of persons in household	—	5	6.16	2.29	3	10	7
Number of persons in household under 14 years old	no data on the cohort of minor Turkish patients						
Country of birth							
Turkey	19 (100.0)	—	—	—	—	—	—
Others	0 (0.0)						
Citizenship							
Turkey	19 (100.0)	—	—	—	—	—	—
Others	0 (0.0)						
At least one parent born outside of Turkey							
Yes	0 (0.0)	—	—	—	—	—	—
No	19 (100.0)						
Highest requested or achieved school degree		1	—	—	1	1	—
Still pupil = 1	19 (100.0)						
Still in vocational training = 2	0 (0.0)						
Patient feels fit and well		5	3.95	1.27	1	5	4
Not at all = 1	1 (5.3)						
Not well = 2	1 (5.3)						
Mediocre = 3	6 (31.6)						
Fairly well = 4	1 (5.3)						
Very well = 5	10 (52.6)						
Vital energy		5	4.32	0.95	3	5	2
Not at all = 1	0 (0.0)						

(Continues)

TABLE 6 (Continued)

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Low = 2	0 (0.0)						
Mediocre = 3	6 (31.6)						
Acceptable = 4	1 (5.3)						
Very good = 5	12 (63.2)						
Sadness		1	1.89	0.99	1	3	2
Not at all = 1	10 (52.6)						
Slightly sad = 2	1 (5.3)						
Mediocre = 3	8 (42.1)						
Sad = 4	0 (0.0)						
Very sad = 5	0 (0.0)						
Feeling of loneliness		1	1.47	0.90	1	4	3
Not at all = 1	14 (73.7)						
Slightly = 2	2 (10.5)						
Mediocre = 3	2 (10.5)						
Lonely = 4	1 (5.3)						
Very lonely = 5	0 (0.0)						
Does patient have enough time for oneself?		5	4.16	1.26	1	5	4
Not at all = 1	1 (5.3)						
Very little time = 2	1 (5.3)						
Mediocre = 3	4 (21.1)						
Almost enough = 4	1 (5.3)						
Enough = 5	12 (63.2)						
Individually planned free time		5	4.21	1.51	1	5	4
Not at all = 1	3 (15.8)						
Very basic = 2	0 (0.0)						
Mediocre = 3	1 (5.3)						
Good planned = 4	1 (5.3)						
Very well planned = 5	14 (73.7)						
Well treated by parents		5	4.74	0.93	1	5	4
Not at all = 1	1 (5.3)						
Not very well = 2	0 (0.0)						
Mediocre = 3	0 (0.0)						
Well = 4	1 (5.3)						
Very well = 5	17 (89.5)						
Patient has good time with friends		5	4.05	1.54	1	5	4
Not at all = 1	3 (15.8)						
Not so good = 2	0 (0.0)						
Mediocre = 3	3 (15.8)						
Good time = 4	0 (0.0)						
Excellent time = 5	13 (68.4)						
Patient gets along well with school		3	2.89	1.76	1	5	4
Not at all = 1	7 (36.8)						
Not so well = 2	2 (10.5)						
Mediocre = 3	2 (10.5)						
Well = 4	2 (10.5)						
Very well = 5	6 (31.6)						

TABLE 6 (Continued)

Parameter	*N* (% from *n*)	Median	Mean	SD	Min	Max	Range
Patient pays attention in school		3	2.74	1.59	1	5	4
Not at all = 1	7 (36.8)						
Not so well = 2	1 (5.3)						
Mediocre = 3	5 (26.3)						
Well = 4	2 (10.5)						
Very well = 5	4 (21.1)						
General health status		3	2.63	1.07	1	5	4
Excellent = 1	4 (21.1)						
Good = 2	2 (10.5)						
Average = 3	11 (57.9)						
Not good = 4	1 (5.3)						
Bad = 5	1 (5.3)						
Overall satisfaction with life (0–10, where 0 = not satisfied, 10 = completely satisfied)	—	8	7.84	1.89	5	10	5

with cryptorchidism and degenerated testicles and another male patient with cryptorchidism and hypospadias. In addition, three female patients (23.1% of all adult *HAX1*-CN female patients) presented with polycystic ovary syndrome and primary ovarian insufficiency. Furthermore, one female patient, with pending registration at the SCNIR, presented with ovarian agenesis. As abnormalities of the development of the reproductive system appear to be of particularly high frequency in female *HAX1*-CN patients,[37,38] the female part of the studied cohort will be further evaluated for the relevant parameters with the additional long-term clinical survey.

Previously, there were no known cases of successful pregnancies in the female patients registered at the SCNIR with *HAX1*-CN. However, lately, one successful case was reported in a patient from Turkey, although only after the application of assisted reproductive technology. The pregnancy occurred after the second IVF-ET (in vitro fertilization and embryo transfer) attempt in the third year of marriage and no insemination or other technics have been used in this couple prior to the IVF-ET procedure. At the same time, it seems that *HAX1* mutation has a less pronounced influence on puberty development and fertility in males. At least two male patients from the studied cohort became fathers of their own biological children, one of whom even twice. Both male patients have not used any assisted reproduction procedures to facilitate fertilization.

Deaths

Three patients from the studied cohort have expired. Two of the expired patients underwent HSCT prior to death, both because of leukaemia. One death was transplant-related, and the other patient died of severe alveolar haemorrhage, associated with a relapse of leukaemia. The third patient died at the age of 2 years from infection with the following sepsis, having a secondary diagnosis of familial hemophagocytic lymphohistiocytosis.

CONCLUSION

Patients with all known types of *HAX1* mutations present with severe congenital neutropenia. At the same time, all studied patients respond well to long-term G-CSF treatment. CN patients with *HAX1* mutations required HSCT mainly due to secondary leukaemia. Nearly 1/3 of the patients presented with somatic (acquired) *CSF3R* mutations, which is among the highest rates in all known subtypes of CN. Such mutations are strongly associated with the risk of leukaemia development. The specific *HAX1* genotype affecting mutations in transcript variant 2 is often associated with neurologic symptoms. Abnormalities in the development of the reproductive system are frequently observed in *HAX1*-CN female patients and should be closely monitored during the course of the disease. At the same time, we report the first known case of successful pregnancy outcome after assisted reproduction in *HAX1*-CN female patients, as well as three normal pregnancies in couples with *HAX1*-CN males. Timely and regular supportive G-CSF treatment allows for maintaining satisfactory quality of life for both adult and minor *HAX1* patients. Long-term follow-up and monitoring of the patients through the Severe Chronic Neutropenia International Registry and collection of the CN patient's samples in the biobank are of the highest priority for understanding the mechanisms and consequences of the rare cases of congenital neutropenia, as well as for the development of adequate and efficient diagnostic and treatment strategies.

AUTHOR CONTRIBUTIONS

Denys Pogozhykh analysed the data and wrote the manuscript, Deniz Yilmaz Karapinar, Georg Ebetsberger-Dachs, Jan Palmblad, Göran Carlsson, Tania Masmas, Sally Kinsey, Marije Bartels, Sabine Mellor-Heineke, Karl Welte, Julia Skokowa and Cornelia Zeidler obtained the primary clinical data and enrolled patients of the studied cohort in the SCNIR, Natali Gerschmann performed the primary data retrieval and summary, Maksim Klimiankou and Julia Skokowa performed research and genetic analysis, Cornelia Zeidler supervised the study.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

All relevant data are provided in the manuscript. All information arising from the database of the European Branch of the SCNIR is disseminated among the members of this network in order to provide an update of the professional skills of the European network partners, which in turn is passed on to other physicians in the participating countries. The SCNIR manages patient data in accordance with the applicable data protection regulations, in particular the General Data Protection Regulation (GDPR) of 25 May 2018.

ORCID

Denys Pogozhykh https://orcid.org/0000-0001-7298-5425
Julia Skokowa https://orcid.org/0000-0002-4399-2324

REFERENCES

1. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K. Severe congenital neutropenias. Nat Rev Dis Primers. 2017;3:17032.
2. Bartels M, Murphy K, Rieter E, Bruin M. Understanding chronic neutropenia: life is short. Br J Haematol. 2016;172(2):157–69.
3. Roques G, Munzer M, Barthez MA, Beaufils S, Beaupain B, Flood T, et al. Neurological findings and genetic alterations in patients with Kostmann syndrome and HAX1 mutations. Pediatr Blood Cancer. 2014;61(6):1041–8.
4. Lanciotti M, Indaco S, Bonanomi S, Coliva T, Mastrodicasa E, Caridi G, et al. Novel HAX1 gene mutations associated to neurodevelopment abnormalities in two Italian patients with severe congenital neutropenia. Haematologica. 2010;95(1):168–9.
5. Aydogmus C, Cipe F, Tas M, Akinel A, Öner Ö, Keskindemirci G, et al. HAX-1 deficiency: characteristics of five cases including an asymptomatic patient. Asian Pac J Allergy Immunol. 2016;34(1):73–6.
6. Carlsson G, van't Hooft I, Melin M, Entesarian M, Laurencikas E, Nennesmo I, et al. Central nervous system involvement in severe congenital neutropenia: neurological and neuropsychological abnormalities associated with specific HAX1 mutations. J Intern Med. 2008;264(4):388–400.
7. Lyu B, Lyu W, Zhang X. Kostmann syndrome with neurological abnormalities: a case report and literature review. Front Pediatr. 2020;8:586859.
8. Chown G, Gelfand AS. Agranulocytosis. Can Med Assoc J. 1933;29(2):128–34.
9. Gilman PA, Jackson DP, Guild HG. Congenital agranulocytosis: prolonged survival and terminal acute leukemia. Blood. 1970;36(5):576–85.
10. Schroten H, Wendel U, Burdach S, Roesler J, Breidenbach T, Schweitzer S, et al. Colony-stimulating factors for neutropenia in glycogen storage disease Ib. Lancet. 1991;337(8743):736–7.
11. Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science. 1986;232(4746):61–5.
12. Dale DC, Bonilla MA, Davis MW, Nakanishi AM, Hammond WP, Kurtzberg J, et al. A randomized controlled phase III trial of recombinant human granulocyte colony-stimulating factor (filgrastim) for treatment of severe chronic neutropenia. Blood. 1993;81(10):2496–502.
13. Cottle TE, Fier CJ, Donadieu J, Kinsey SE. Risk and benefit of treatment of severe chronic neutropenia with granulocyte colony-stimulating factor. Semin Hematol. 2002;39(2):134–40.
14. Dale DC, Bolyard AA, Schwinzer BG, Pracht G, Bonilla MA, Boxer L, et al. The severe chronic neutropenia international registry: 10-year follow-up report. Support Cancer Ther. 2006;3(4):220–31.
15. Rosenberg PS, Zeidler C, Bolyard AA, Alter BP, Bonilla MA, Boxer LA, et al. Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. Br J Haematol. 2010;150(2):196–9.
16. Rosenberg PS, Alter BP, Bolyard AA, Bonilla MA, Boxer LA, Cham B, et al. The incidence of leukemia and mortality from sepsis in patients with severe congenital neutropenia receiving long-term G-CSF therapy. Blood. 2006;107(12):4628–35.
17. Klein C, Grudzien M, Appaswamy G, Germeshausen M, Sandrock I, Schäffer AA, et al. HAX1 deficiency causes autosomal recessive severe congenital neutropenia (Kostmann disease). Nat Genet. 2007;39(1):86–92.
18. Germeshausen M, Grudzien M, Zeidler C, Abdollahpour H, Yetgin S, Rezaei N, et al. Novel HAX1 mutations in patients with severe congenital neutropenia reveal isoform-dependent genotype-phenotype associations. Blood. 2008;111(10):4954–7.
19. Matsubara K, Imai K, Okada S, Miki M, Ishikawa N, Tsumura M, et al. Severe developmental delay and epilepsy in a Japanese patient with severe congenital neutropenia due to HAX1 deficiency. Haematologica. 2007;92(12):e123–5.
20. Xue SL, Li JL, Zou JY, Su J, Chen SN, Wu DP. A novel compound heterozygous HAX1 mutation in a Chinese patient with severe congenital neutropenia and chronic myelomonocytic leukemia transformation but without neurodevelopmental abnormalities. Haematologica. 2012;97(2):318–20.
21. Ishikawa N, Okada S, Miki M, Shirao K, Kihara H, Tsumura M, et al. Neurodevelopmental abnormalities associated with severe congenital neutropenia due to the R86X mutation in the HAX1 gene. J Med Genet. 2008;45(12):802–7.
22. Rezaei N, Chavoshzadeh Z, R Alaei O, Sandrock I, Klein C. Association of HAX1 deficiency with neurological disorder. Neuropediatrics. 2007;38(5):261–3.
23. Klein C. Kostmann's disease and HCLS1-associated protein X-1 (HAX1). J Clin Immunol. 2017;37(2):117–22.

24. Kostmann R. Infantile genetic agranulocytosis; agranulocytosis infantilis hereditaria. Acta Paediatr Suppl. 1956;45(Suppl 105):1–78.
25. Kostman R. Infantile genetic agranulocytosis. A review with presentation of ten new cases. Acta Paediatr Scand. 1975;64(2):362–8.
26. Bohn G, Welte K, Klein C. Severe congenital neutropenia: new genes explain an old disease. Curr Opin Rheumatol. 2007;19(6):644–50.
27. Skokowa J, Klimiankou M, Klimenkova O, Lan D, Gupta K, Hussein K, et al. Interactions among HCLS1, HAX1 and LEF-1 proteins are essential for G-CSF-triggered granulopoiesis. Nat Med. 2012;18(10):1550–9.
28. Skokowa J, Welte K. Defective G-CSFR signaling pathways in congenital neutropenia. Hematol Oncol Clin North Am. 2013;27(1):75–88. viii.
29. Fan Y, Murgia M, Linder MI, Mizoguchi Y, Wang C, Łyszkiewicz M, et al. HAX1-dependent control of mitochondrial proteostasis governs neutrophil granulocyte differentiation. J Clin Invest. 2022;132(9):e153153.
30. Wortmann SB, Ziętkiewicz S, Kousi M, Szklarczyk R, Haack TB, Gersting SW, et al. CLPB mutations cause 3-methylglutaconic aciduria, progressive brain atrophy, intellectual disability, congenital neutropenia, cataracts, movement disorder. Am J Hum Genet. 2015;96(2):245–57.
31. Yılmaz Karapınar D, Patıroğlu T, Metin A, Çalışkan Ü, Celkan T, Yılmaz B, et al. 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. Pediatr Blood Cancer. 2019;66(10):e27923.
32. Carlsson G, Aprikyan AA, Ericson KG, Stein S, Makaryan V, Dale DC, et al. Neutrophil elastase and granulocyte colony-stimulating factor receptor mutation analyses and leukemia evolution in severe congenital neutropenia patients belonging to the original Kostmann family in northern Sweden. Haematologica. 2006;91(5):589–95.
33. Goktas S, Azizoglu ZB, Petersheim D, Erdogan M, Gungor HE, Bisgin A, et al. A novel intronic mutation reduces HAX1 level and is associated with severe congenital neutropenia. J Pediatr Hematol Oncol. 2021;44(1):e62–7.
34. Karapınar TH, Yılmaz Karapinar D, Oymak Y, Ay Y, Demirağ B, Aykut A, et al. HAX1 mutation positive children presenting with haemophagocytic lymphohistiocytosis. Br J Haematol. 2017;177(4):597–600.
35. Klimiankou M, Mellor-Heineke S, Zeidler C, Welte K, Skokowa J. Role of CSF3R mutations in the pathomechanism of congenital neutropenia and secondary acute myeloid leukemia. Ann N Y Acad Sci. 2016;1370(1):119–25.
36. Klimiankou M, Mellor-Heineke S, Klimenkova O, Reinel E, Uenalan M, Kandabarau S, et al. Two cases of cyclic neutropenia with acquired CSF3R mutations, with 1 developing AML. Blood. 2016;127(21):2638–41.
37. Cekic S, Saglam H, Gorukmez O, Yakut T, Tarim O, Kilic SS. Delayed puberty and gonadal failure in patients with HAX1 mutation. J Clin Immunol. 2017;37(6):524–8.
38. Carlsson G, Kristrom B, Nordenskjold M, Henter JI, Fadeel B. Ovarian failure in HAX1-deficient patients: is there a gender-specific difference in pubertal development in severe congenital neutropenia or Kostmann disease? Acta Paediatr. 2013;102(1):78–82.

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