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Clinical and Molecular Assessment of Iranian Families with Severe Congenital Neutropenia, Identification of HYOU1 and SHOC2 as Potential Novel Gene Defects

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ABSTRACT

Neutropenia congenita grave (SCN) is a rare disease with a genetically and clinically heterogeneous nature, usually diagnosed in childhood, with an elevated risk of infections such as otitis, skin infections, pneumonia, deep abscesses, and septicemia. Patients with SCN also have an increased risk of leukemia, and mutations in the ELANE and the HAX1 genes have been observed in those patients.

This study was conducted to genetically screen six Iranian families with SCN who have at least one affected person. In the first step, all exons and intron boundaries of ELANE and HAX1 genes were sequenced in probands. Cases with no pathogenic mutations were tested through whole-exome sequencing (WES).

Analysis showed five different variants in *ELANE* (c.377 C>T), *HAX1* (c.130_131 insA), *HYOU1* (c.69 G>C and c.2744 G>A) and *SHOC2* (c.4 A>G) genes in four families. We found that two out of six families had mutations in *ELANE* and *HAX1* genes. Moreover, we found

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two novel mutations at the HYOU1 gene that had not previously been reported, as well as a pathogenic mutation at SHOC2 with multiple phenotypes, that will contribute to determining the genetic basis for SCN.

Our study revealed that WES could help diagnose SCN, improve the classification of neutropenia, and rule out other immunodeficiencies such as autoimmune neutropenia, primary immunodeficiency diseases, and inherited bone marrow failure syndromes.

Keywords: ELANE protein; HAX1 protein; HYOU1 protein; Severe congenital neutropenia; SHOC2 protein; Whole exome sequencing

INTRODUCTION

Severe congenital neutropenia (SCN) is a rare hematological disease considered a primary immunodeficiency (PID). This disease is clinically heterogeneous. However, a common characteristic of SCN is a selective decrease in circulating neutrophils with onset in childhood. The absolute number of blood neutrophils drops to less than 0.5×10^6 per ml in these patients.[1] Severity of neutropenia is usually measured against the number of neutrophil granulocytes in peripheral blood.[2] Patients with SCN usually display an arrest in the promyelocyte/ myelocyte stage of myelopoiesis, which results in reduced neutrophil counts. In addition, a conspicuous and possibly compensatory monocytosis can be seen in a significant number of the patients.[3]

This occurrence increases the risk of infections that may continue and have life-threatening consequences if the disease is left untreated.[4] The most prominent hallmarks of SCN are pneumonia, deep abscesses, otitis, skin infections, gingivitis, and septicemia, which begins in infancy.[5] Patients with SCN usually display recurrent bacterial infections, frequently observed in the oral cavity, skin, and mucous membranes. Still, diseases of the respiratory tract and otitis are not expected. Periodontitis, aphthous stomatitis, and abscesses are frequently seen as damage to teeth due to frequent gingivitis.[6,7] Some forms of congenital neutropenia are only associated with blood defects. In contrast, others are not limited to the blood and demonstrate syndromic states, which might affect other hematopoietic organs related to the pancreas, brain, heart, bone, and skin. Given the heterogeneity of SCN, identifying the underlying causes may shed new light on improving the management of this disease.

Most SCN cases show a monogenic inheritance, which may be autosomal dominant, autosomal recessive, or X-linked. Up to 50% of the forms of congenital neutropenia (including most of the patients with autosomal dominant inheritance) with normal adaptive immunity and absence of other hematopoietic manifestations are caused by mutations in the *ELANE* gene, which encodes neutrophil elastase. In the 1990s, mutations in this gene were reported as the first genetic cause of SCN.[8,9] Afterward, Horwitz et al found that some patients with cyclic neutropenia have mutations in the *ELANE* gene,[10,11] which pointed to a continuum between SCN and cyclin neutropenia and showed that both of them can be considered a congenital disability. It should be noted that patients with cyclic neutropenia have a lower risk for MDS/AML.[12] Further evaluations in patients with Kostmann's syndrome identified *HAX1* gene mutations. Kostmann's syndrome is usually considered a paradigm of congenital neutropenia, associated with profound neutropenia (<0.2 G/l) occurring during the first weeks of life.[13] The protein product of HAX1 is a ubiquitous mitochondrial protein. This gene mutation is linked to the patient's geographic origin (Kurdistan and Sweden).[14] Mutations in *ELANE* and *HAX1* genes have frequently been observed in SCN patients, whereas mutations in other genes, such as *GFI1*, *WAS*, *G6PC3*, and *VPS45*, have been reported in a lesser number of the patients.[15] In consanguineous pedigrees, homozygous pathogenic variants in the *HAX1* gene can cause autosomal recessive congenital neutropenia.[5] Diversities in relationship rates are probably the main reason explaining the observed differences in the prevalence of specific mutations associated with neutropenia between different ethnicities. Primary immunodeficiency disease (PID) is an uncommon cause of neutropenia in children is the primary immunodeficiency disease (PID). A significant

part of PID patients shows hematological manifestations such as neutropenia, which are inherent to a PID and result from a specific type of genetic background. This type of congenital neutropenia is quite heterogeneous and ranges from isolated congenital neutropenia to a complex group of hereditary diseases which, in addition to neutropenia, show other manifestations such as skin hypopigmentation, facial dysmorphias, and intellectual disability.[16,17]

Abnormalities in organs such as the heart, skeletal or urogenital system, and abnormal skin pigmentation or organomegaly may provide clues to the diagnosis of genetic neutropenia. Currently, genetic testing is increasingly playing a significant role in the diagnosis of SCN.[18] However, about one-third of patients with SCN is negative for reported pathogenic mutations.[19] Whole exome sequencing (WES) is a powerful tool for identifying underlying genetic causes in many diseases, especially in SCN patients with no pathogenic variants in *ELANE* and *HAX1* genes.[15,20] This method has intriguing potential for finding the other unidentified genetic changes involved in SCN. In the last decade, many novel neutrophil differentiation disorders have been identified; using the WES method.[21] In the present study, we implemented WES to find the genetic variants underlying the pathogenesis of SCN in patients who are negative for *ELANE* and *HAX1* mutations.

MATERIALS AND METHODS

Patients and Samples

Six Iranian families with at least one affected individual with severe congenital neutropenia were selected for this study and were referred to The Children's Medical Center of Tehran University of Medical Sciences is the immunogenetic clinic. After clinical evaluation by a relevant physician, the pedigree was drawn for each family. The study was carried out with the principles outlined in the Declaration of Helsinki. In addition, the whole protocol of this. the Ethics Committee approved the study of the Tehran University of Medical Sciences (ethics Code: IR.TUMS.MEDICINE.REC.1398.193). Written informed consent was obtained from all participants or their legal guardians (in case of individuals being minors).

Peripheral blood samples were taken from the patients and their parents and collected in EDTA tubes. According to the manufacturer's instructions, these samples were subjected to a DNA extraction kit (Parstous, Mashhad, Iran). The quality and concentration of the DNA samples were investigated by running on 1% agarose gel electrophoresis and a NanoDrop instrument (Eppendorf BioPhotometer, Germany).

Assessment of *ELANE* and *HAX1* Gene

All six probands were screened using specific primers for all exons and intron boundaries of *ELANE* and *HAX1* genes by Sanger sequencing. The sequences of the primers are shown in Supplementary Table 1.

Whole Exome Sequencing

Probands negative for pathogenic variants in *ELANE* and *HAX1* genes were further subjected to whole-exome sequencing. For this purpose, DNA was fragmented, and the targeted enrichment was done using the SureSelect Human All Exon V7 kit (Agilent Technologies, Santa Clara, CA, USA). Libraries were sequenced on the Illumine NovaSeq 6000 platform for 2*100 bp reads with an average coverage of 100X (Eurofins Genomic Services, India). The sequence read quality assessment performed quality control (QC) with FastQC. Burrows-Wheeler Alignment (BWA) algorithm was used with default parameters for alignment to human genome assembly GRCh37 (hg19). Picard-Tools was used to mark and remove the duplications, followed by variant calling with the HaplotypeCaller algorithm in Genome Analysis Toolkit version 4.0 (GATK4) package. Variants were annotated with ANNOVAR using various public and in-house databases.

In Silico Analyses

The allele frequency was searched in the 1000 Genomes Project (approximately 6500 exomes from the NHLBI), exome sequencing project (http://esp.gs.washington.edu), genome aggregation database (gnomAD,https://gnomad.broadinstitute.org/), and Iranome (http://www.iranome.ir/gene/ENSG00000196811, to exclude ethnic-specific variants). Furthermore, the functional analysis tools, MutationTaster, and genomic evolutionary rate profiling (GERP) scores were combined with functional effect predictions. The possible damaging effect of each variant on function and structure was estimated by SIFT (https://sift.bii.a-star.edu.sg/), PolyPhen2

(http://genetics.bwh.harvard.edu/pph2/) and CADD (https://cadd.gs.washington.edu/).

Sanger Sequencing and Co-segregation Analysis

Specific primers were designed for each candidate genetic variant using the Primer3 online software.[22] Polymerase chain reaction (PCR) was performed under standard conditions (for the list of primers, see Supplementary Table 2). PCR products were subjected to Sanger sequencing on an ABI 313XL genetic analyzer (Applied Biosystems, Waltham, MA, USA). Sequencing data were analyzed using the Chromas V.2.6.6 software and compared with wild-type samples and reference sequences from the NCBI database.

RESULTS

Clinical and Molecular Findings

An immunogenetic specialist evaluated all patients. Profound neutropenia was the common finding in all probands. All patients received G-CSF treatment after primary diagnosis, leading to a temporal improvement in their neutrophil count. The pedigrees of the patients are shown in Figure 1, and the clinical profiles and molecular findings are summarized in Table 1.

Table 1. Clinical and molecular findings

ID	Age (year)	Sex	ANC*	Clinical findings	Gene	Genetic variant	Zygosity	Pathogenicity	Reference
CN01	7	female	200	Anemia Recurrent severe bacterial infections	*ELANE*	c.377C>T (p. Ser126Leu)	Het	Pathogenic	ClinVar (ID:16745)
CN02	10	male	560	recurrent infections	*HAX1*	c.130_131insA (p. Trp44Ter)	Hom	Pathogenic	ClinVar (ID: 4651)
CN03	4	female	340	Chronic lung infection Refractory thrush	*HYOU1*	c.69G>C (p.Leu23Phe)	Het	VUS§	Novel
						c.2744G>A (p.Arg915Gln)	Het	VUS§	Novel
CN04	4	female	1500	Low -set ear Strabismus Atrial septal defect (ASD) Pulmonary Valve Stenosis (PS) Hematuria Inflamed and bleeding gums	*SHOC2*	c.4A>G (p.Ser2Gly)	Het	Pathogenic	ClinVar (ID: 6821)
CN05	5	male	210	Large aphthous ulcers Enlarged lymph nodes Fever Abdominal pain Diarrhea	-	-		-	-
CN06	5	male	370	Fever Rhinorrhea Shortness of breath Epilepsy Pneumonia Otitis media	-	-		-	-

*ANC: absolute neutrophil count before G-CSF therapy. VUS§: Variant of unknown significance

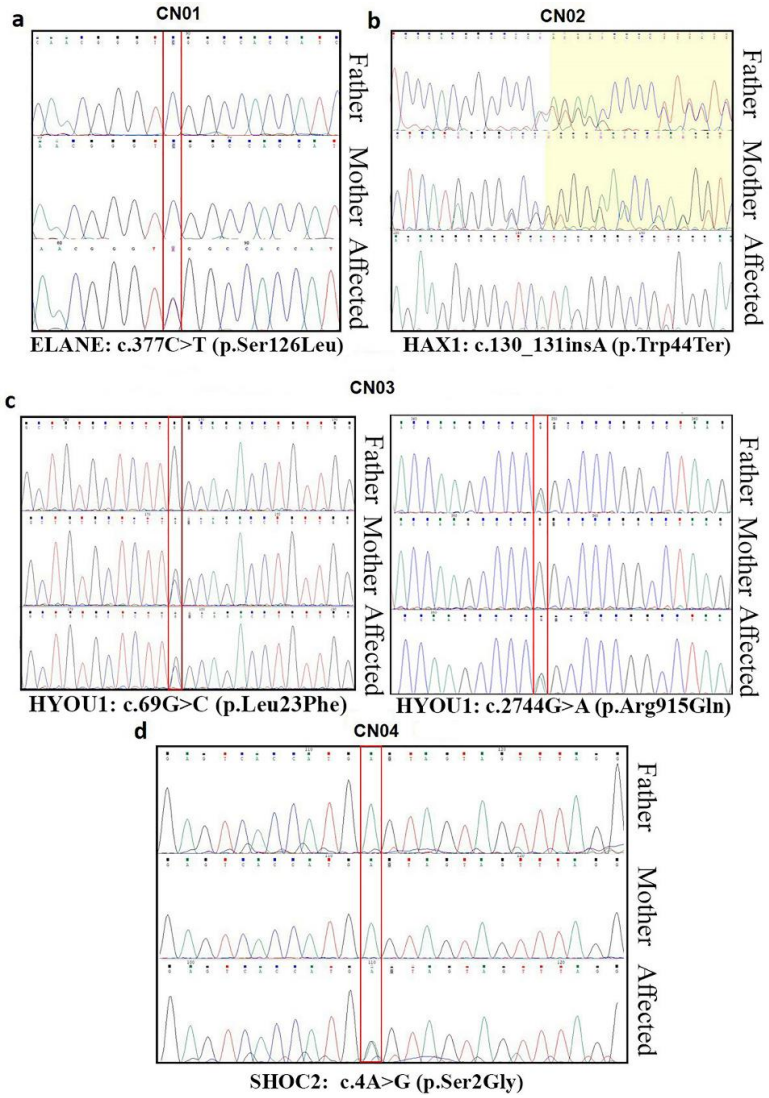

Figure 1. Pedigrees of the studied families. Black shapes represent the affected individuals. The arrows demonstrate the probands.

Probands of six families included in this study were screened for the genetic variants in all exons and intron boundaries of *ELANE* and *HAX1* genes. Based on PCR and Sanger sequencing results, the proband of family CN01 showed a heterozygote pathogenic variant c.377C>T (p.Ser126Leu) in the *ELANE* gene. Co-segregation analysis showed that this variant was not observed in the parents, demonstrating that this variant is a *de novo* mutation. Moreover, we identified a homozygote pathogenic variant, c.130_131insA (p.Trp44Ter), in the *HAX1* gene proband of family CN02. Sanger sequencing in the parents of this patient demonstrated that both of them are heterozygous for this single nucleotide insertion.

The other families (CN03, CN04, CN05, and CN06 families) did not show any pathogenic mutation in *ELANE* and *HAX1* genes and, therefore, were further investigated by the whole-exome sequencing technique. Interestingly, we found that the patient in the CN03 family is a compound heterozygote for two novel variants (c.69G>C and c.2744G>A) in the *HYOU1* gene (OMIM: 601746) with unknown pathogenic functions. According to the previous studies, mutations in this gene are related to immunodeficiency-59 and hypoglycemia with autosomal recessive inheritance.

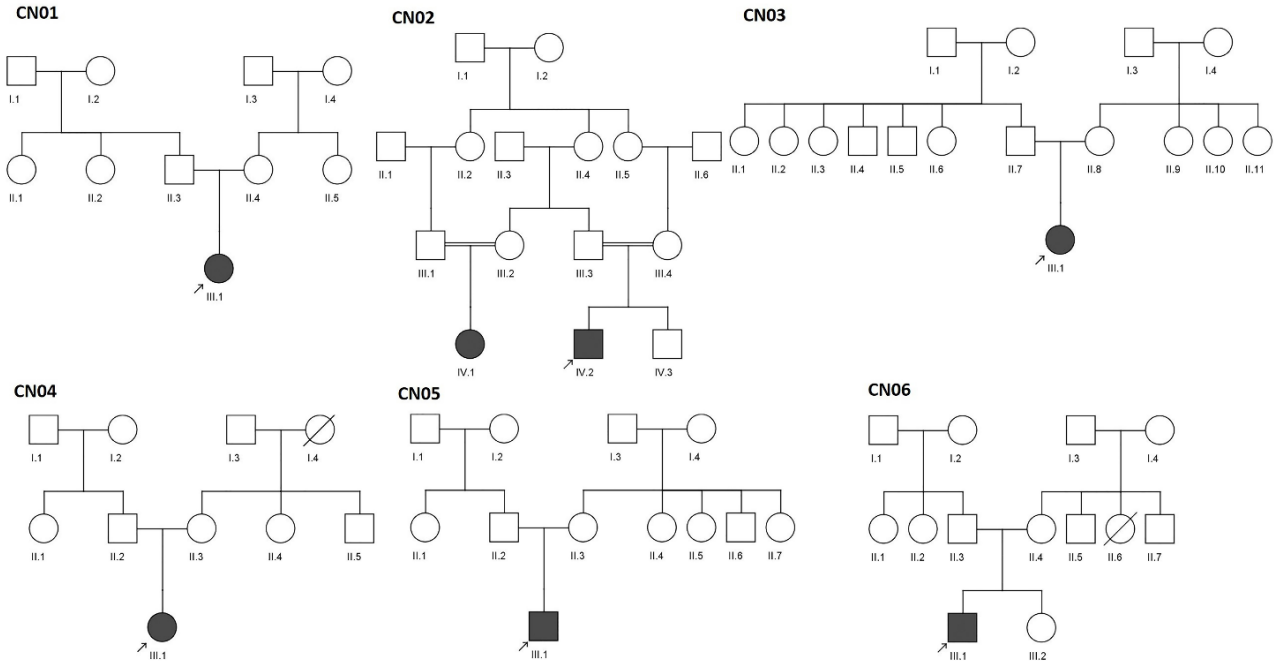

Figure 2. Chromatograms of Sanger sequencing results of *ELANE*, *HAX1*, *HYOU1* (variants c.69G>C and c.2744G>A in exons 2 and 23), and *SHOC2* genes in CN01(a), CN02 (b), CN03 (c) and CN04 (d) for probands and their parents.

The heterozygous variant c.69G>C located at exon 2 of the *HYOU1* gene has a CADD score of 25 and a DANN score of 0.998. The allelic frequency of this variant is not reported in the GnomAD database. Another heterozygous variant c.2744G>A (rs552980302), located at exon 23 of the *HYOU1* gene, has a CADD score of 23 and a DANN score of 0.999. The allelic frequency of this variant in the GnomAD database is reported to be about 0.0000954. Functional *in silico* prediction using MutationTaster, SIFT, and Polyphen2 described these two variants as disease-causing and damaging (Table 2). Neither of them has been reported in ClinVar and HGMD databases. According to the ACMG guidelines, these variants are classified as variants of unknown significance (VUS). Sanger analysis showed that variant c.2744G>A is the paternal allele, and variant c.69G>C is the maternal allele.

Analysis of WES data in CN04 proband indicated a heterozygote c.4A>G (p.Ser2Gly) variant in the *SHOC2* gene (OMIM: 602775). c.4A>G (p.Ser2Gly) results in a non-conservative amino acid substitution at the N-terminal region of the encoded protein. Most in-silico tools predicted this variant's damaging effect on protein function. The variant was proven *de novo* in multiple patients, strongly supporting its pathogenicity.

Pathogenic mutations in the *SHOC2* gene have been reported to be associated with Noonan-like syndrome with loose anagen hair (NSLH), an autosomal dominant condition. The c.4A>G variant we found in this study is located in exon 2 of the *SHOC2* gene and has previously been classified as a pathogenic variant in the ClinVar (VarID: 6821) and HGMD (CM095445) databases. The c.4A>G (p.Ser2Gly) variant in *SHOC2* has been confirmed as a *de novo* mutation in multiple patients with clinical features of a RASopathy.

Co-segregation analysis for all found mutations was performed by Sanger sequencing using specific primers (Figure 2). Despite the various signs of neutropenia and immunodeficiency, no pathogenic variant was found in CN05 and CN06 related to SCN. The list of variants found related to immunodeficiencies is given in Supplementary Tables 3 and 4.

Table 2. Analysis of mutations detected in SCN patients.

Gene/ transcript	Variant	dbSNP	Mutation Taster	Varsome	CADD	DANN	Polyphon	SIFT	GnomAD	1000 Genome	Iranome
ELANE NM_001972.4	c.377C>T p. Ser126Leu	rs137854450	Disease-causing	Pathogenic	15	0.990	benign	tolerated	0	0	-
HAX1 NM_006118.4	c.130_131insA p. Trp44Ter	rs1572018284	-	Pathogenic	-	-	-	-	0	0	-
SHOC2 NM_007373.3	c.4A>G p.Ser2Gly	rs267607048	Disease-causing	Pathogenic	25	0.996	damaging	damaging	0.0000319	0	-
HYOU1 NM_001130991.1	c.69G>C p.Leu23Phe	-	Disease-causing	VUS	25	0.998	damaging	damaging	0	0	-
	c.2744G>A p.Arg915Gln	rs552980302	Disease-causing	VUS	23	0.999	damaging	damaging	0.0000954	0	-

DISCUSSION

Severe congenital neutropenia (SCN) is a genetic disorder associated with three main characteristics: low neutrophil count and susceptibility to infection, abnormalities in various organs, and extremely high risk of leukemic transformation. Further complicating this disease is that a germline mutation in a gene may be responsible for an inherent defect, or it may be linked to leukemia through a sequential chain of somatic genetic events.[23] Due to the clinical and genetic heterogeneity of SCN, the diagnostic procedure for this disease is also complicated. In the last decade, the development of next-generation sequencing (NGS) has resulted in drastic advancements in identifying the genetic cause of SCN. In this regard, it has been reported that genetic testing based on the initial analysis of targeted panels and in the next step, analysis of all exons, is the most efficient strategy to identify the molecular etiology of SCN in the patients.[24]

The present study analyzed six unrelated patients using the abovementioned strategy. Four disease-causing mutations were found in them, which have been confirmed through co-segregation analysis. Molecular assessment of families showed pathogenic heterozygous missense variant, c.377C>T, in the *ELANE* gene, which is the most frequent molecular etiology of hereditary forms of SCN and periodic neutropenia with autosomal dominant inheritance pattern.[25] The patient was a 7-year-old girl from non-consanguineous parents who revealed severe neutropenia, which did not respond to treatment with G-CSF and is now a candidate for bone marrow transplantation. Co-segregation analysis revealed that this variant was not observed in the parents. Therefore, this nucleotide change occurred as a result of *de novo* mutation. It has been reported that about 60% of SCN cases in Europe and the Middle East have dominant mutations in the *ELANE* gene.[5] Ancliff et al reported two patients with c.377 C>T (S126L) in exon 4 of the *ELANE* gene, which resulted in the replacement of the serine with leucine at codon 126.[26] They declared that one patient responded well to G-CSF, while the other did not. The authors suggested that the difference in G-CSF responsiveness in these two patients with the same *ELANE* gene mutation may be the modifying effects of other interacting genes.[26] It should be noted that some mutations in the *ELANE* gene have been reported to be associated with a comparatively better prognosis, markedly S126L, P139L, and IVS4+5 G>A, while the others, notably C151Y and G214R, are linked to poor prognosis.[27]

We found homozygote variant c.130_131insA in *HAX1* gene in CN02 proband with heterozygous parents. This patient was a 10-year-old boy from consanguineous parents. A first cousin with a similar disease was observed in this pedigree, and both of these patients responded well to treatment with G-CSF.

Studies have shown that this variant leads to Kostmann syndrome, an autosomal recessive form of neutropenia.[28] The main reason for neutropenia in this group of patients is the deficiency in differentiation and maturation of neutrophils in bone marrow at the early differentiation stages. This group of patients usually responds well to G-CSF treatments.[13]

In 2007, Klein et al identified the c.130_131insA variant as homozygous in the *HAX1* gene in members of three unrelated Kurdish families, leading to a premature termination codon as Trp44Ter.[29] In a study of 27 Iranian patients with SCN, four mutations were found in the *ELANE* gene but 11 mutations were identified in the *HAX1* gene. These findings demonstrated that pathogenic mutations in *HAX1* and *ELANE* genes are the most frequent cause of SCN in Iranian populations.[30] The higher frequency of *HAX1* mutations in Iran compared to the global frequencies (about 10%) may be due to the relatively high rate of consanguineous marriage in Iran.

On the other hand, we found two novel missense variants, c.69G>C and c.2744G>A, in the *HYOU1* gene in the CN03 proband. The patient was a 4-year-old girl from unrelative parents who were first administrated to the hospital at the age of 6 months because of a lung infection. This patient was under treatment with G-CSF because of severe neutropenia. She was subjected to bone marrow aspiration twice to be investigated for her congenital neutropenia and the other immunodeficiencies, and the results were both averages. Also, the patient responded to G-CSF, but she represented neutropenia again after ending the treatment. Mutations in *HYOU1* are related to immunodeficiency 59 and hypoglycemia with autosomal recessive inheritance. Confirming this condition, the patient's unaffected parents were each heterozygous for one of the mutations.

The frequency of these variants was too low in the surveyed population databases such as GnomAD. Moreover, *in silico* analysis revealed that these mutations have a pathogenic effect on protein function. According to the ACMG guideline, these mutations were classified as part of the VUS category. In 2017, an interesting case reported by Haapaniemi et al. The proband was a 45-year-old woman with a combination of immunodeficiency symptoms and periods of hypoglycemia due to stress from birth. She suffered from numerous septic infections of the respiratory tract, skin, and mucous membranes from infancy to adulthood. Using the WES technique, two variants, c.1255G>C and c.691T>C were identified in this person as compound heterozygotes.[31]

As stated earlier, a less recognized and uncommon cause of neutropenia in children is PID, which is associated with hematological manifestations such as neutropenia, which are inherent to a PID and result from a specific type of genetic background. This group of congenital neutropenia is heterogeneous and shows other manifestations such as skin hypopigmentation, facial dysmorphias, and intellectual disability, in addition to neutropenia.[16,17] Given the heterogeneous causes of neutropenia from the isolated forms to those associated with immunodeficiency disorders, and because of the overlaps in their phenotypes, classification according to molecular status may help improve their diagnosis.

This is the first case of immunodeficiency with a mutation in the *HYOU1* gene, which is associated with severe congenital neutropenia. Therefore, to confirm this result, there should be further functional and population studies in the future. In addition, this patient is 4 years old, and consequently, the lack of stress-induced hypoglycemia in this patient may be due to her low age.

In this study, the proband of the CN04 family was heterozygote for pathogenic missense variant c.4A>G in the *SHOC2* gene, which leads to the substitution of serin at codon 2 to glycine. The patient was a 4-year-old girl with unrelative patients who caught attention because of persistent infection and fever and the presence of syndromic characteristics including short stature, developmental delay, low-set ears, heart defects, autistic spectrum disorder (ASD), pulmonic stenosis, strabismus, and visual defects. Clinical investigations revealed that the patient suffers from neutropenia, which was improved by G-CSF treatment. Subsequent follow-ups revealed mild cyclic neutropenia in that patient. Mutations in this gene are associated with NSLH, an autosomal dominant disease. In vitro, functional studies have shown that the p.Ser2Gly affects protein function. Pathogenic variant c.4A>G in the *SHOC2* gene has been reported in approximately 5% of patients with NSLH, which are harmful to pathogenic mutations in *PTPN11*, *SOS1*, *and RAF1*, *respectively*, and *KRAS* genes.[32]

Recently, several reports have confirmed the phenotype associated with the c.4A>G mutation and showed evidence for clinical variability of this

disorder.[33] Moreover, it is recommended that clinical manifestations in patients with *SHOC2* mutations may be similar to symptoms of patients with typical Noonan syndrome or Cardiofaciocutaneous syndrome.[34] Cordeddu et al. reported the c.4A> G (p.Ser2Gly) variant in the *SHOC2* gene as a newly confirmed event in several patients with clinical features of RASopathies.[35] The role of the *SHOC2* gene in neutropenia has been confirmed through functional studies in animal models, but our study is the first report related to congenital neutropenia in human subjects. In 2018, Jang et al showed that loss of SHOC2 protein reduces the number of circulating blood cells.[36] Results of this study indicated that loss of SHOC2 resulted in aberrant myelopoiesis and significant diminishment in neutrophil count compared to the control group.

"RASopathies" are an emerging group of clinically and genetically related disorders which affect development and growth. The shared pathogenic mechanism in this group of diseases is the dysregulation of the RAS signaling pathway.[37-39] Although exhibiting unique phenotypic features, they share overlapping characteristics, including cardiac malformations, craniofacial dysmorphology, and cutaneous, musculoskeletal, and ocular abnormalities. They also show neurocognitive impairment at various severity and, in some syndromes, an increased risk of developing malignancies.[37-39]

In this study, we identified several variants in the pathways related to immunodeficiency and blood disorders in CN05 and CN06 proband. However, none of these variants could alone explain the genetic cause of neutropenia and remained unsolved. As shown in Table 1, both CN05 and CN06 proband are five-year-old boys from non-relative parents. CN05 proband had inflammation, bleeding gums, large aphthous stomatitis, enlarged lymph nodes, fever, and abdominal pain. Before referral to the pediatrics center, it was written in her medical records that the patient was suspected of having a disease. However, Behcet's disease was ruled out after that, and he was supposed to have periodic neutropenia.[40,41] CN06 proband had a history of three hospitalizations, with diarrhea, fever, rhinorrhea, shortness of breath, epilepsy, prolonged coughs, pneumonia, and otitis media.

In conclusion, we found two novel variants in the *HYOU1* gene related to SCN, which have not been reported previously. Moreover, we identified three pathogenic reported variants in three unrelated patients. Exome sequencing focusing on the genes relating to the pathway of interest will help find disease-causing variants. The present study was an initial step in investigating the genetic causes of patients with SCN in the Iranian population. This study examined and classified the clinical information of affected families, identified responsible mutations in 2/3 of the studied families, and introduced new mutations in the Iranian population. Also, we demonstrated that the c.4A>G variant in the *SHOC2* gene could be related to periodic neutropenia, in addition to the broad phenotypes reported earlier.
The results would increase the knowledge about the genetic basis of SCN. However, in the case of the families in which WES could not identify a reliable variant, WGS may be an exciting alternative with the hope of finding a genetic factor anywhere outside the exonic regions.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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