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Clinical and Molecular Spectrum of Sarcoglycanopathy Related Genes in Iranian Families

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Abstract

Sarcoglycanopathies comprise four subtypes of autosomal recessive *Limb-Girdle Muscular Dystrophy* (LGMD2C, LGMD2D, LGMD2E, and LGMD2F) caused, respectively, by mutations in the SGCG, SGCA, SGCB, and SGCD genes. Knowledge about the clinical and genetic features of sarcoglycanopathies in Iranian patients is limited. This study aimed to investigate the clinical manifestations and gene mutations in Iranian patients with sarcoglycanopathies and identify possible correlations.

Studies on SGCs were performed on ten unrelated Iranian families referred to a genetic clinic for genetic diagnosis due to the initial diagnosis of muscular dystrophy. Based on clinical phenotype and genetic findings were observed, respectively: The ten unlinked LGMDs families originated from 9 different cities of Iran. Three patients were diagnosed with LGMD2D, four with LGMD2E, and three with LGMD2C. No patient was detected with LGMD2F. Ten mutations were detected in SGCA (n=3), SGCB (n=4), and SGCG (n=3); no SGCD mutation was found in these families. The finding will be beneficial for screening and genetic counseling of SGCs patients in Iran.

The present study demonstrates the clinical and molecular spectrum of Sarcoglycanopathy related-genes in the study families. Our finding provided additional insights into genotype and phenotype correlations in the Iranian population.

Keywords: Sarcoglycanopathy; Limb-girdle muscular dystrophy; WES

Introduction

The Limb Girdle Muscular Dystrophy (LGMD) includes many Mendelian disorders categorized by progressive degeneration of proximal limb muscles. The shoulders and hip region are the earliest affected muscles. Sarcoglycanopathies (SGCs) are subgroups of autosomal recessive LGMD that occur due to debilitating mutations in SGCA, SGCB, SGCG, and SGCD genes encoding the four types of the voluntary muscle sarcoglycan complex, alpha, beta, gamma, and delta-sarcoglycan sarcoglycan (transmembrane glycoproteins), which lead to LGMD2D, 2E, 2C, 2F, respectively [2]. These sarcoglycans form the stability of the dystrophin-dystroglycan complex and plasma membrane cytoskeleton [3]. The prevalence of sarcoglycanopathies differs between populations based on race and geographic region. LGMD2E and LGMD2D have the most and lowest frequency of sarcoglycanopathies in the Iranian, respectively [4]; while, LGMD2D is relatively prevalent in the US and Europe [5-6]. LGMD2C is the most common type of LGMD among Algerian and Indian populations [7-8]. Overall, 16 mutated genes have been reported to be involved with various forms of LGMD [6].

SGCs manifest different clinical features ranging from mild to severe [9]. The onset age of symptoms usually occurs between five and fifteen years old [10]. The primary symptoms of the diseases include weakness of the shoulder and pelvic girdle muscles; other manifestations may be revealed by calf

hypertrophy, scapular winging, lumbar hyperlordosis, and a higher plasma level of creatine kinase [10]. In the last stages of the disease, SGCSs often involve respiratory and cardiac systems [11]. SGCs resemble the Intermediate type of Duchenne and Becker Muscular Dystrophy [11-13]. Considering difficult differentiation among subtypes of SGCs depending on clinical manifestations, thus a combination of immunoblot, immunohistochemical analyses, and subsequent DNA sequencing have been proposed for accurately diagnosing the underlying pathology [14].

However, there is no definitive cure to attenuate muscle weakness and reverse mobility function of patients with limb-girdle muscular dystrophy; Supportive treatment should be adapted to each patient and each subtype of LGMD [15]. Accurate diagnosis of SGCs is a key factor in genetic counseling, preimplantation genetic diagnosis, and prospective therapies.

Lastly, studies on the SGCs have been conducted among Iranian patients by genetics analysis [16-19]. To the best of our knowledge, previously published studies about the clinical features and genotype of SGCs are limited in Iranian patients. We aimed to detail the clinical manifestations, pedigrees, genotype, and gene mutations in ten families of the Iranian population with sarcoglycanopathies.

Materials and Methods

Patients and samples

Probands were selected from ten unrelated families referred to the genetic clinic for genetic counseling due to a primary diagnosis of muscular dystrophy. Written informed consent was obtained from all the study participants or their parents. 5 ml of peripheral blood with EDTA were obtained from patients and other available family members. DNA was extracted using standards salting out protocol. The proband' DNA samples were sent to Macrogen Company (Korea) for the Whole-Exome Sequencing (WES) 100X.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Genetic testing and bio-informatics analysis

After data analysis, the Polymerase Chain Reaction (PCR) technique was performed using specific primers to confirm the candidate genetic variants, followed by Sanger sequencing. We interpreted and classified sequence variants through the Human Gene Mutation Database (HGMD) and ClinVar. In the case of a novel variant, it was classified as pathogenic, likely pathogenic, uncertain significance, likely benign, or benign according to the American College of Medical Genetics and Genomics (ACMG, 2015) guideline.

Results

Clinical phenotype

Table 1 summarizes the clinical features of patients with sarcoglycanopathies.

Table 1: Clinical characteristics of study patients.

ID	LGMD subtype	Age/sex	Onset age (years)	Disease duration years	Symptom(s) at onset	Current added symptoms	complications	AST	LDH	CK (IU/L)
F1	LGMD2D	10/F			Difficulties in standing and walking	Difficulties in standing and walking	NA	NA	NA	1200
F2	LGMD2D	13/M	6	7	Difficulties in climbing stairs post walking muscle pain upper limb weakness	Difficulties in standing and walking	Lordosis	-	-	11000
F3	LGMD2E	12/F	4	8	Difficulties in running, standing, and walking	Unable to stand(wheelchair)	Lordosis	-	-	1550

F4	LGMD2C	17/F (F4-P1)	9	8	Early fatigue calf stiffness difficulties in standing	Unable to stand(wheelchair) upper limb weakness	Lordosis	1110	-	16390
		9/F (F4-P2)	8	1	Calf stiffness	Difficulties in standing and running upper limb weakness	Lordosis	-	780	23040
F5	LGMD2C	17/M	6	11	Running difficulty	Difficulties in standing upper limb weakness	Cardiovascular Lordosis Scoliosis	-	99.8	4635
F6	LGMD2E	6/F	6		Proximal lower limb weakness, early fatigue, and calf hypertrophy	Thigh hypertrophy	-	437	5200	23665
F7	LGMD2E	7.7/M (F7-P1)	0.6	7.1	Difficulties in swallowing chronic diarrhea	Difficulties in standing, running and climbing upstairs Achilles tendinitis	Lordosis Respiratory disorder	323	-	23880
		3.7/F (F7-P2)	2.7	1	Asymptomatic hyper CKemia	Difficulties in climbing stairs, itching	-	226	-	14014
F8	LGMD2C	7/6/M	6.3	1.3	Myoglobinuria	Difficulties in standing post-walking muscle pain flank pain	Lordosis scoliosis respiratory disorder	-	-	21150
F9	LGMD2E	46/F	18	28	Early fatigue frequent fall proximal lower limb	Falling in walking lower limb atrophy	Lordosis	496	-	4342

					weakness foot pain lower back pain					
F10	LGMD2D	23/F (F10-P1)	2	21	Difficulties in running and standing. Falling upper limb weakness	Lower limb atrophy	Lordosis	-	-	1500
		2/M (F10-P2)	0.2	1.8	Drug-resistant epilepsy difficulties in holding head up	Difficulties in walking	-	-	-	1300

The ten unlinked LGMDs families originated from nine different cities of Iran, of which five were from nearby towns in a large province (Razavi Khorasan) in the northeast of Iran (Figure 1).

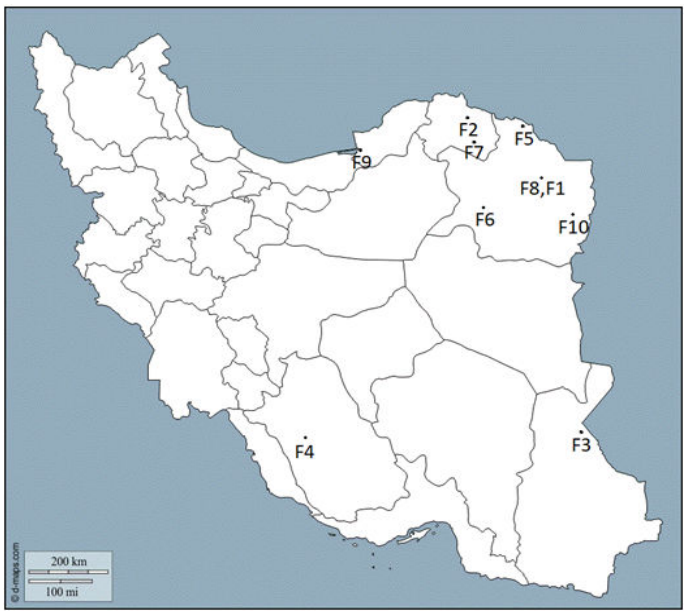

Figure 1: Map of Iran demonstrates the geographic origin of the families with SGCs.

Families 1 and 8 are from the same small community/village and presumably related. Each patient's city of residence has been shown with a number. (1) Mashhad, (2) Bojnurd, (3) Zabol, (4) Shiraz, (5) Dargaz, (6) Kashmar, (7) Esfarayen, (8) Mashhad, (9) Bandar Torkaman, (10) Torbat-e Jam.

Three patients were diagnosed with LGMD2D, four with LGMD2E, and three with LGMD2C. No patient was detected with LGMD2F. Three families reported a positive family history in the other offspring, which is confirmed by paraclinical evaluations. Thirteen affected subjects from ten families were assessed clinically in this study. There were five males (38%) and eight females (61%). The consanguinity rate was 80%; in seven families, the parents were first cousins (Figure 2).

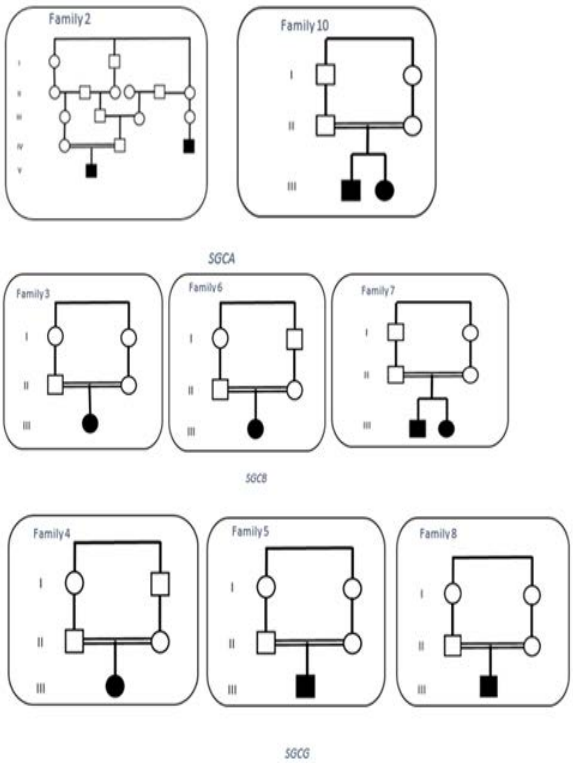

Figure 2: Pedigrees of four families affected by SGCA, SGCB, and SGCG mutations.

In three patients (33%), the symptoms at the onset were associated with difficulties in running and calf stiffness; in two patients (20%), the sign at onset was early fatigue. The clinical presentation of the affected individuals was varied, ranging from asymptomatic hyperkalemia to non-

ambulatory paraparesis. Difficulties in standing, walking, and climbing upstairs were the most common complaints among patients. Other clinical symptoms not related to motor included chronic diarrhea, Drug-resistant epilepsy, and myoglobinuria. Lordosis was observed as a common complication in almost all patients. Likewise, one patient with LGMD2C suffered a cardiac abnormality (F5), and two patients with LGMD2E and LGMD2C had respiratory complications (F7-P1 and F8 respectively). CK concentrations were elevated in all patients. One patient was diagnosed with sarcoglycanopathy after an incidental finding of hyper CKemia (F7-P2). Two patients were no longer capable of ambulating independently (F3 and F4-P1) (Figure 3).

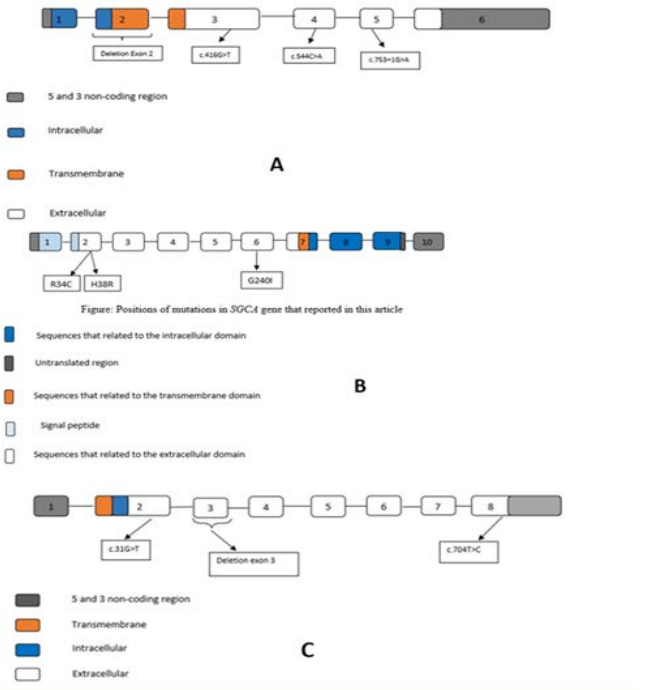

Figure 3: Schematic view o f gene structure a nd mutation sites; A: SGCB, B: SGCA, C: SGCG.

Genetic findings

According to Table 2 totally, ten mutations were detected in SGCA (n=3), SGCB (n=4), and SGCG (n=3), eight of them **have already been reported** pathogenic in genomic databases such as ClinVar and the remaining two variants were novel. No SGCD mutation was found in these families. All patients had a complete molecular diagnosis and were found to have only one mutation in SGCA, SGCB, or SGCG. The pathogenic mutations included six missense, two deletion, one-stop codon, and one splicing mutations. Except for the heterozygote mutations found in patient nine, other mutations were the homozygous state in different patients.

Table 2: Genetic analysis of patients with sarcoglycanopathy.

Family	Gene	c.DNA position	Exon	Effect on protien	Type of variants	Zygosity	Parental	Variants Pathogenicity
F1	SGCA	c.100C>T	Exon 2	p. Arg34Cys	missense	Hom	Proband	Pathogenic
F2	SGCA	c.113A>G	Exon 2/ CDS2	p. His38Arg	missense	Hom	Proband	VUS
F3	SGCB	Exon 2 deletion	Exon 2	-	deletion	Hom	Proband	Pathogenic
F4	SGCG	Exon 3 deletion	Exon 3/ CDS2	-	deletion	Hom	Proband	Pathogenic
F5	SGCG	c.704T>C	Exon 8/ CDS7	p. Leu235Pro	missense	Hom	Proband	Likely pathogenic
F6	SGCB	c.544A>C	Exon4	p. Thr182Pro	missense	Hom	Proband	VUS
F7	SGCB	c.753+1G>A	Exon 5	N/A	splicing	Hom	Proband	Likely pathogenic
F8	SGCG	c.31G>T	Exon 2	p. Glu11*	stop codon	Hom	Proband	VUS

F9	SGCB	c.416G>T	Exon 3	p. Gly139Val	missense	Het	mother	VUS
F10	SGCA	c.718T>A	Exon 3	p. Gly240Val	missense	Hom	Proband	VUS

† The variants have been previously reported as pathogenic; ‡, novel variants; Hom, homozygous; Het, heterozygous; VUS Variant of Undetermined Significance, N/A not available.

SGCA: We identified three missense mutations in SGCA, two of which have been reported previously [20,21], and one was novel (c.100C>T (p. Arg34Cys), c.718T>A (p.Gly91Ser), c.113A>G (p.His38Arg)). Two mutations were located in exon 2, and one was in exon 1. All mutations were homozygote in patients with LGMD2D. (Table 2)

SGCB: Four mutations were identified in SGCA, which all of them have been previously reported [22]. Including two missense mutations c.544A>C (p.Thr182Pro) and c.416G>T (p.Gly139Val)), one deletion mutation (Exon 2 deletion), and one splicing mutation (c.753+1G>A). Except for the heterozygote mutation found in patient nine, other mutations were the homozygote state in LGMD2E patients.

SGCG: Three homozygote mutations were detected in SGCG; two of them have been already reported as a deletion and a missense mutation in ClinVar database (Exon 3 deletion and c. 704T>C (p.Leu235Pro) respectively), and one was a novel stop codon mutation (c.31G>T (p.Glu11*)).

In 10 patients that we checked, there were no abnormalities on electrocardiographic or echocardiographic though generally, this group of genes is expressed in muscle and cardiac muscle.

Discussion

LGMD has been recognized as a broad and increasingly heterogeneous class of inherited muscle diseases [30]. With the increase in genetic discoveries of LGMD, it has become clear that clinical and histological manifestations and outcomes may vary widely between different subgroups and in affected individuals. However, these changes are not distinct and contradictory enough to enable physicians to identify new subtypes based on phenotype; hence, the diagnosis has always been challenging. With the appearance of NGS, the time and its cost required to sequence the entire human genome has reduced from years to days and from several billion dollars to several thousand dollars, respectively [31].

Today, due to the high number of genes involved in this disease, the approach of examining each gene by Sanger sequencing is not practical, and the next-generation sequencing, in which a set of genes can be examined simultaneously, is more cost-effective and time-consuming [32]. Currently, next-generation sequencing studies make it possible to analyze different types of genes simultaneously. However, these techniques must be performed carefully by adding clinical, radiological, and pathological data. The advent of next-generation sequencing approaches has accelerated the discovery of new LGMD genes. Ten years ago, the list included 16 loci, while today, the genes identified are thirty-one, with eight autosomal dominant and 23 autosomal recessive manners [32]. Conventional methods for identifying pathogenic mutations such as immunohistochemistry, Western blotting, and Sanger sequencing of selected genes can make a genetic diagnosis in 35% of families [33]. With the invention of Next Generation Sequencing (NGS), patients can now be screened through neuromuscular disease gene panels or by Whole Exome Sequencing (WES) [34,35].

Overall, it is reported that clinical exome sequencing has a 25% diagnosis rate [36]. In comparison, recent studies on exome sequencing for neuromuscular disease show a 46% diagnosis rate in the United States and 73% in the highly consanguineous population of Iran [37-38]. Although WES results require more validation for clinical diagnosis, in addition to the ability to screen all known disease-related genes, this method provides an additional opportunity to identify new genes associated with LGMD. Genetic diagnosis in LGMD and other genetic disorders will become increasingly important in the coming years as new molecular therapies target specific gene defects and even specific mutations. For example, several studies showed that LGMD2D is easily controllable by gene therapy approaches [39-41]. Any modifications that were found can disrupt the sarco glycan complex. All SG proteins are glycosylated membrane proteins with small intracellular and large extracellular domains. Four sarco glycan proteins form tetra meric membrane proteins fixed to the dystrophin axis by lateral association with the dystroglycan complex. Unlike dystroglycan, which is found in almost all cells, sarco glycans are predominantly found in muscle cells. Early studies in LGMD patients have shown that mutations in each of the four SG genes cause instability of the whole complex. However, further studies of patients and animal models have shown that not all sarco glycans are equally important for maintaining the stability of the complex.

In this study, we performed a comprehensive analysis of clinical phenotypes and genetic data in 10 sarco glycan-pathy-related patients from five separate provinces in Iran. Due to the locus heterogeneity in sarco glycanopathies, there is little information about its worldwide prevalence. However, reports that have the global prevalence of all forms of LGMD are estimated at one in 14,500 to 123,000 [30]. Based on the given information, a different subtype of SGCs shows various frequencies. Generally, the prevalence of different types of sarcoglycanopathy varies according to ethnicity and geographical area. For example, LGMD2D is relatively common in Europe and the United States while LGMD2C is more common in India and Algeria populations. In a report by Nilipour et al. in 100 muscle samples biopsy from Iranian patients, it was shown that SGP is the second most common muscular dystrophy among other muscular dystrophies (for example, Duchenne muscular dystrophy) in Iran and the types of sarcoglycanopathy α-SGPs are the most abundant form [43-47].

Clinical features associated with SGC mutations in the Iranian group, including the age of onset, disease progression, presence/absence of specific features, and severity of symptoms, are very heterogeneous, even in patients with the same mutation phenotypic variation intrafamilial and interfamilial. This diversity has been reported previously and suggests that other genetic and environmental factors may be involved [48-49].

The rare cause of sarcoglycanopathy is Mutation in SGCB, and the frequency of patients with SGCB mutation varies from 5.5% in India to 23% in Brazil. In this study, four mutations in SGCB were reported, that one of these is a deletion of exon 2 in the homozygous state. According to ACMG guidelines, this mutation is pathogenic and, with the elimination of the anchor domain's SGCB protein, has destructive effects on the assembly of the sarcoglycan complex. The family that showed this mutation was from the southeast of Iran and the Balouch ethnic group. In another study by Alavi. It was found that approximately 85% (12 out of 14) of their LGMD2E patients have been detected, which showed the complete deletion of exon 2 in the SGCB gene, and 10 out of 12 families was from southeast of Iran too. Haplotype analysis supported by three Single Nucleotide Polymorphism Markers (SNPs) and high frequency of this mutation in this region indicated the probably founder effect in this region of Iran could be helpful in screening for mutations in patients diagnosed with LGMD2 in this area. We need to further study with more sample size and additional markers [48]. Different alleles are proved by haplotype analysis that is identical with the deletion mutation in SGCB by descent. Since almost all patients from southeastern Iran had this mutation, it is suggested that deletion of exon 2 should be the first target screening for patients with LGMD2D in this region. All of the modifications in the SGCB gene reported in this article are located in the extracellular domain of the SGCB gene, like the SGCA gene. All of the three said left mutations are in the extracellular environment.

Sequence change c.A544C in SGCB gene replaces threonine with proline at the protein level. Although threonine residue is a high conservative amino acid at this position, there is little difference between threonine and proline in terms of physicochemically. This variant was reported in the population database (rs751427686, ExAC 0.01%), and its frequency is in the range of mutations frequency. In another publication, various changes at this codon (p.Thr182Ala) have been found in the compound heterozygous state in affected individuals with a pathogenic variant on the SGCB gene, too (PMID: 9032047). According to databases that can predict the function and structure of the proteins, this change is probably damaging, damaging, disease-causing on databases SIFT, PROVEAN, MutationTaster, respectively. Still, these disruptive functions have not been approved by published functional studies. Therefore, this variant (c.A544C) was classified as Likely Pathogenic in ACMG databases and variant of unknown significance on Clinvar. In summary, we do not have enough information to find out the function of this variant.

Alpha is the most common type of sarcoglycanopathy [32-34]. The important function of sarcoglycan complex was first indicated with α-sarcoglycan (adrenaline) deficiency in patients with autosomal recessive muscular dystrophy in Arabic countries. Defects in the SGCs destroy the sarcolemma back-bone so that the cell membrane is exposed to contractile muscle pressures, and as a result, rupture of the focal membrane occurs. Eventually, the dystrophic phenotype appears [35-38]. In this study, three mutations in the SGCA gene were seen. SGCA gene mutations may prevent the formation of sarcoglycan complexes or the binding of sarcoglycan complexes to DGC. All three of these mutations are located in the extracellular domain of the protein. One of these mutations is c.100C>T. With this mutation, the codon encoding the arginine amino acid is converted to cysteine, located in the protein's extracellular domain, the N terminal of the protein. This domain binds alpha sarcoglycans to the extracellular matrix. These mutations are found in the cadherin-like domain of the SGCA. This domain is a location for many missense mutations that cause LGMD2D. The cadherin-like domain is located in the extracellular environment and is involved in protein-protein interlinkage, cell polarization, and migration. It also plays an important role in interdependence with other dystrophin-glycoprotein complex molecules. All missense mutations reported in this article are located in the extracellular domain of α-sarcoglycan and have a strong quantitative effect on protein expression levels, indicating that mutated regions are critical for the formation and stability of the sarcoglycan complex.

In addition to c.101G>A (p.Arg34His), another nucleotide substitution at the same codon c.100C>T (p.Arg34Cys) has been proven to cause LGMD. Remarkably, the missense mutations that happened in these sites, R34, D97, and R98, were mapped to the putative Ca^{2+} binding site in the cadherin-like domain [39-42]. These approved that these residues are crucial, and any mutations cause LGMD. However, there is no significant difference in clinical phenotype among patients with various mutations Arg34His, Arg34Cys, and Arg34Leu in the SGCA gene. The two rest mutations in SGCA (c.113A>G, c.718T>A) are novel, and we do not find any information on databases. Both were found in the homozygous state on probands and were heterozygous on all healthy members, such as healthy parents and siblings. In addition to that, both of these are damaging, damaging, and disease-causing on SIFT, PROVEAN, Mutation Taster databases. The gamma gene mutation was first described in the Maghreb African country of North Africa [43].

The variant c.704T>C is a new variant, and this missense mutation caused to replacement of amino acid Leucine with Proline. According to databases SIFT, PROVEAN, Mutation Taster, this variant is damaging and disease-causing [44].

Best regards to all patients participating in this study; Finally, it is suggested that a comprehensive cohort study of all multiple geographical regions and ethnicities should be carried out to obtain complete and accurate information on the abundance of genes and mutations in Iranian sarcoglycanopathy patients. Also, larger cohorts of patients need to define genotype/phenotype correlations in SGCs. Further studies can help us determine the frequency of different SGCs and mutations in the Iranian population. In rare diseases, the small population of patients prevents access to accurate and comprehensive clinical, genetic,

and treatment information. To overcome this problem, it is recommended that reputable scientific centers worldwide work together and share their information, information that can be important and effective in clinical and genetic diagnosis and disease management and treatment [45-49].

Conclusion

In the present study, ten probands out of ten families suspected of being affected by LGMDs had a mutation (s) in SGCA, SGCG, and SGCB genes that four of them were novel. The most prevalent mutations of sarcoglycanopathy in our study were beta, and alpha and gamma were placed in the second stage. Missense's mutation was the most prevalent type, and in our research, no mutation was found in the SGCD gene.

Based on genetic classification four patients (%40) were affected with LGMD2E (SGCB mutations), three (%30) with LGMD2C (SGCG mutations), three (%30) with LGMD2D (SGCA mutations).

Our study could clarify the genetic cause of ten Iranian patients in ten unrelated families with ten different types of mutations. In this study, we found two new mutations that enrich human genetic mutation databases, and we were able to expand our knowledge about the genetic spectrum of LGMD in Iran. The present study expands the clinical and molecular spectrum of Sarcoglycanopathy related-genes in the study families. Our finding provided additional insights into genotype and phenotype correlations in the Iranian population.

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