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Histopathological and Genetic Features of Patients with Limb Girdle Muscular Dystrophy Type 2C

Kavşak Tipi Müsküler Distrofi Tip 2C Hastalarının Histopatolojik ve Genetik Özellikleri

Gülden DİNİZ[1], Filiz HAZAN[2], Hülya TOSUN YILDIRIM[3], Aycan ÜNALP[4], Muzaffer POLAT[5], Gül SERDAROĞLU[6], Orkide GÜZEL[4], Özlem BAĞ[4], Yaprak SEÇİL[7], Figen ÖZGÖNÜL[1], Sabiha TÜRE[7], Galip AKHAN[7], Ajlan TÜKÜN[8]

[1]Tepecik Education and Research Hospital, Neuromuscular Disease Center, İZMİR, TURKEY
Department of [2]Medical Genetics, [3]Pathology and [4]Pediatric Neurology, Dr. Behçet Uz Children's Hospital, İZMİR, TURKEY
Department of Pediatric Neurology [5]Celal Bayar University, Faculty of Medicine, MANİSA, TURKEY and [6]Ege University, Faculty of Medicine, İZMİR, TURKEY
[7]Department of Neurology, Katip Çelebi University, Faculty of Medicine, İZMİR, TURKEY
[8]Department of Medical Genetics, Düzen Laboratory, ANKARA, TURKEY

ABSTRACT

Objective: In this study, it was aimed to describe the clinical, histopathological and genetic features of 20 patients with gamma sarcoglycanopathy confirmed by muscle biopsies and genetic analysis.

Material and Method: We retrospectively reviewed 20 patients from whom muscle biopsy specimens were obtained between 2007 and 2012. All patients were clinically diagnosed as muscular dystrophy and biopsy materials were collected from five different centers of neurological disorders. All DNAs were extracted from muscle tissues or blood samples of patients and genetic tests (mutation analyses for gamma sarcoglycan gene and deletion-duplication analyses for all 4 sarcoglycan genes) were performed.

Results: The mean age of the patients was 7.6 years (2 -21 years). Only one case (5%) was older than 14 years. The mean CPK level was 10311 U/L (1311 – 35000 U/L). There were 4 siblings in these series. Expression defects of gamma sarcoglycan staining were determined in (15 males, and 5 females) all patients with muscle biopsy specimens. But only in 9 of them, disease-causing defects could be determined with genetic analyses.

Conclusion: The present study has demonstrated that both examination of muscle biopsy specimens and DNA analysis remain important methods in the differential diagnosis of muscular dystrophies. Because dystrophinopathies and sarcoglycanopathies have similar clinical manifestation.

Key Words: Dystrophin, Gamma sarcoglycan, Genetic testing, Muscular dystrophy, Limb-Girdle

ÖZ

Amaç: Bu çalışmada, kas biyopsi incelemesi ve genetik analizle tanısı doğrulanan gama sarkoglikanopatili 20 olgunun klinik, histopatolojik ve genetik özelliklerinin sunulması amaçlandı.

Gereç ve Yöntem: Patoloji laboratuvarımızda, 2007 ile 2012 yılları arasında kas biyopsisi incelenen 20 hasta retrospektif olarak değerlendirildi. Tüm hastalar klinik olarak müsküler distrofi tanısı almış olup, biyopsiler 5 farklı nöroloji kliniğinde alındı. Hasta DNA'ları kas dokusu veya periferik kandan çıkartıldı ve genetik testler (gama sarkoglikan geni için mutasyon analizleri ve tüm 4 sarkoglikan için delesyon duplikasyon testleri) yapıldı.

Bulgular: Hastaların ortalama yaşı 7,6 yıl (2- 21 yıl) idi. Yalnızca tek olgu (%5) 14 yaşın üzerindeydi. Ortalama CPK düzeyleri 10311 U/L (1311- 35000 U/L) bulundu. Grupta 4 kardeş hasta (n=8) vardı. Kas biyopsisinde tüm hastalarda (15 erkek ve 5 kadın) gama sarkoglikan boyanma defekti saptandı. Fakat genetik incelemede sadece 9 hastada gama sarkoglikan geninde hastalığa yol açacak defekt vardı.

Sonuç: Çalışmamız, hem kas biyopsi incelemesi, hem de genetik analizin müsküler distrofilerin ayırıcı tanısında çok önemli olduğunu göstermiştir. Çünkü distrofinopati ve sarkoglikanopatiler benzer klinik bulgulara sahiptirler.

Anahtar Sözcükler: Distrofin, Gama sarkoglikan, Genetik analiz, Müsküler distrofi, Uzuv-Kemer

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Correspondence: Gülden DİNİZ
Tepecik Eğitim ve Araştırma Hastanesi, Nöromuskuler Hastalıklar Merkezi, İZMİR, TURKEY
E-mail: agdiniz@gmail.com Phone: +90 232 469 69 69

INTRODUCTION

Gamma sarcoglycan (γ-SGC) is one of the four sarcoglycans (SGCs) found at the cell membrane of skeletal muscle. The SGCs form a subcomplex closely linked to the dystrophin-associated glycoprotein complex (DAG). Proper presence of SGCs is essential for membrane integrity during muscle contraction. Limb girdle muscular dystrophy type 2C (LGMD-2C) is an autosomal recessive muscle-wasting disorder caused by genetic defects in the sarcoglycan gamma (SGCG) gene. It is also known as the childhood severe muscular dystrophy and clinically resembles the dystrophinopathies which are the most common muscular dystrophies (1-4).

No definitive treatments for the LGMD-2C and the other muscular dystrophies exist. Management to prolong survival and improve quality of life includes physical therapy, and stretching exercises to promote mobility and prevent contractures, weight control to avoid obesity, surgery for orthopedic complications, use of mechanical and respiratory aids to help ambulation, mobility and respiration. Monitoring cardiomyopathy for cardiac involvement and emotional support are also required (5-9).

Differential diagnosis of LGMD-2C is made in consideration of Duchenne and Becker muscular dystrophies (DMD/BMD) and it is impossible to differentiate between these conditions solely on clinical grounds. Therefore immunohistochemical staining of muscle biopsy specimens and molecular genetic analysis are mandatory for correct diagnosis (10-12). In this study, we aimed to determine the spectrum of genetic defects in immunohistochemically proven cases of LGMD-2C, to correlate the findings with clinical phenotypes and to display the regional differences as for the clinical, histopathological, and genetic characteristics of gamma sarcoglycanopathies.

MATERIAL and METHODS

Histopatological examinations of muscle biopsies were performed at Pathology Laboratory of Izmir Dr. Behçet Uz Children's Hospital. Genetic analyses were performed at Ankara Düzen Laboratory from January 2007 through December 2012. Twenty patients with defective gamma sarcoglycan expressions found on the muscle biopsy specimens, and clinically diagnosed as muscular dystrophy were included in this study. Immunohistochemical analysis (IHC) was repeated to confirm the diagnosis. Individual patient database was reviewed in all cases, and clinical information of patients was recorded including age, gender, detailed family history and consanguinity. Neurological examination and laboratory findings were also evaluated. Laboratory evaluation included serum creatine kinase (CK), serum aspartate aminotransferase (AST) analyses, and nerve conduction and electromyographic (EMG) studies. All muscle biopsies were obtained from the gastrocnemius muscle.

Samples were frozen in isopentane cooled in liquid nitrogen and 8- to 12- micron sections were cut using the cryostat. Slides were stained with hematoxylin-eosin (H&E), as well as with several histochemical and enzymatic stains. Cryosections were immunostained for dystrophin using a polyclonal antibody (Neomarkers) with a monoclonal spectrin antibody (Novocastra) as a control. SGCs were detected with anti alpha (α-), beta (β-), delta (δ-) and γ-SGC antibodies (Novocastra).

Genomic DNAs were extracted from the remnant muscle tissues or blood samples using available DNA extraction kits (QiaGen, US) following the manufacturer's standard protocol. The exon regions and flanking short intronic sequences of the SGCG gene were amplified using the polymerase chain reaction (PCR), followed by direct sequencing of the PCR products (ABI, US). In addition, the multiplex ligation-dependent probe amplification (MLPA) technique was used for deletion and duplication analysis for all 4 SGCs.

Frequencies and descriptive analyses were performed using the statistical software SPSS 9.05 for Windows.

RESULTS

Twenty patients with severe muscle disease were evaluated respectively. All of them had been diagnosed as muscular dystrophy on the basis of muscle biopsy findings. Severe alterations of myofiber size and shape, splitting, increase in the number of internal nuclei, fiber type disproportions; necrosis, myophagocytosis, regeneration and fibrosis were simply classified as muscular dystrophy (Figure 1).

The mean age of the patients was 7.6 years (2 to 21 years). There were 4 siblings (n=8) in these series. Expression defects of gamma sarcoglycan staining were determined in (15 male and 5 female) all patients with available muscle biopsy specimens. However, disease-causing defects could be determined with genetic analyses in only 9 of them. The mean age of the patients was 7.6 years (± 4.11), ranging from 2 to 21 years. Only one case (5%) was older than 14 years. The detailed clinical characteristics of the patients were presented in Table I. All patients presented some degree of muscle weakness. All of them had high creatine kinase (CPK) levels. The mean CPK level was 10311 U/L (1311 – 35000 U/L). Ten patients (50%) had similarly affected family

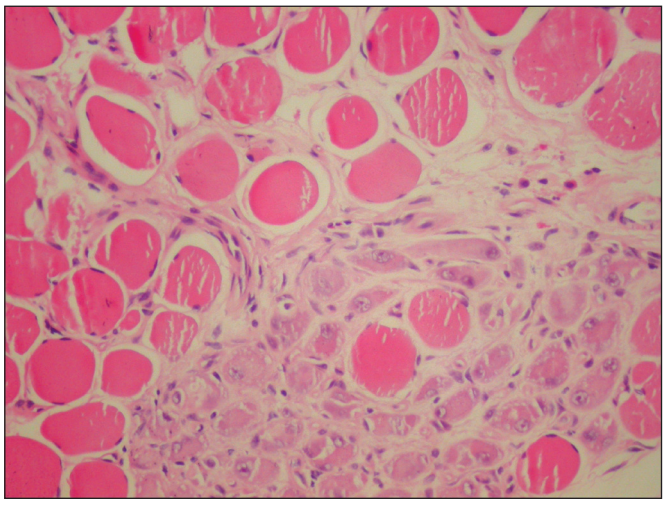

Figure 1: Note the regenerated muscle fibers which are specific for muscular dystrophies (H&E, x100).

members. The consanguinity rate was 45% (n=9). Physical examination at the time of diagnosis revealed weakness in proximal limb muscles. Needle electromyogram was performed and revealed myopathy in all patients. All patients were ambulatory at the time of diagnosis.

The final diagnosis was made on the basis of muscle biopsy findings. All twenty cases showed staining defects for gamma sarcoglycan with the presence of staining for other sarcoglycans and dystrophin (Figure 2A-D). Similarly there were no defects in the dystrophin genes. Although there were defective expressions of gamma sarcoglycan protein in all biopsy specimens, the disease-causing genetic defects could be determined in only nine of them. Most cases had silent homozygous or heterozygous mutations. The detailed genetic defects of the patients are presented in Table II.

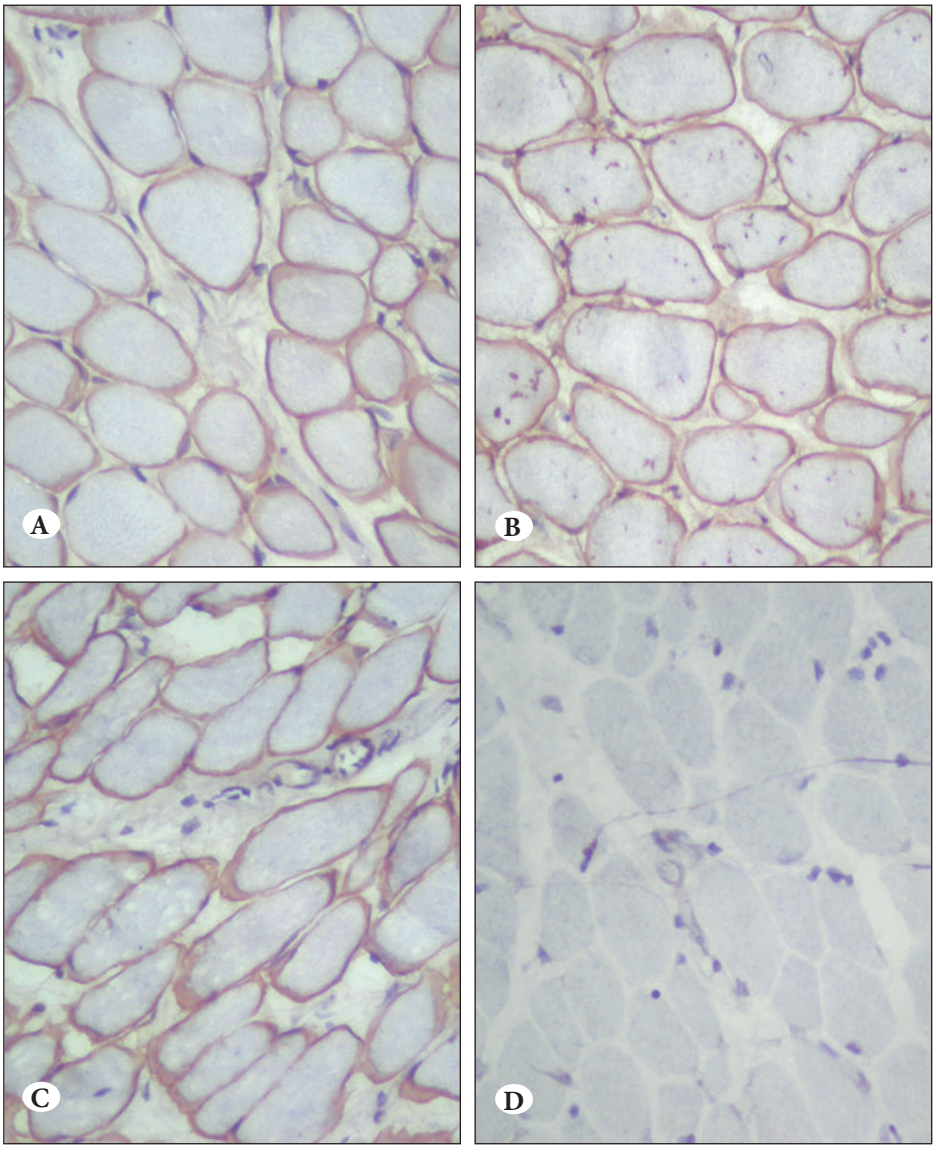

Figure 2: A) Nearly normal expressions of α-SGC, **B)** β-SGC, **C)** δ-SGC and **D)** Defective expression of sarcolemmal γ-SGC (DAB, x100).

Table I: Clinical and genetic features of patients

Case	Sex	Age	CPK level	Family history	Consanguinity	Starting age of symptoms	Gowers sign	Calf pseudo hypertrophy	Mutations of SGCG gene	Deletion-Duplication of all 4 sarcoglycan genes
1	F	21	5300	-	-	8	+	-	R116H(G347A), S287N(G860A), I218T(T654C) Compound heterozygosity	-
2	M	6	4500	-	-	2.5	+	+	R116H(G347A), S287N(G860A), Compound heterozygosity	-
3	M	9	15000	+	+	7	-	-	-	-
4	M	11	17500	+	-	6	+	+	S287N(G860A)	-
5	M	11	5000	-	-	8	+	+	R116H(G347A)	Duplication of exon 10 in SGCA gene
6 sibling1	M	8	2200	+	-	5	-	+	R116H(G347A)	-
7 sibling1	M	5	6100	+	-	4	+	+	R116H(G347A)	-
8 sibling2	M	6	5000	+	-	5	+	+	808-801DELGT	SGCG.Exon8 LOH
9 sibling2	M	8	13000	+	-	4	+	+	808-801DELGT	SGCG.Exon8 LOH
10	F	7	10000	-	+	5	-	-	R116H(G347A), S287N(G860A),	Duplication of exons 1-10 in SGCA gene
11	M	10	7500	-	+	7	+	+	-	İntragenic amplification in SGCA gene
12	M	6	11000	-	+	9	+	+	-	-
13	M	10	7500	-	-	9	-	-	T312G, T705C	-
14	M	2	1312	-	-	2	-	-	R116H(G347A)	-
15	M	7	11000	-	+	6	+	+	T312G, T705C	-
16 sibling3	F	2	7541	+	+	2	-	-	525delT homozygous	-
17 sibling3	M	6	15784	+	+	5	+	-	525delT homozygous	-
18	F	3	13000	-	-	2	-	-	T312G, T705C	-
19 sibling4	F	6	35000	+	+	4	+	+	T228C, T312G, T705C	-
20 sibling4	M	8	13000	+	+	4	+	+	T228C, T312G, T705C	-

DISCUSSION

The human SGCG gene is located on chromosome 13q12. It consists of 8 exons. The sequence of SGCG is composed of 291 amino acids. Three portions of the gamma sarcoglycan extracellular domain display possible critical function, two for the assembly with either beta or alpha sarcoglycan, and the putative EGF-like domain. Hitherto forty mutations have been described in the gamma sarcoglycan gene (2). The homozygous del525T mutation generates a truncated gamma sarcoglycan protein without EGF-like domain, which is able to assemble with the other sarcoglycans (1,2,13-15). This mutation is commonly found in North Africa. The C283Y mutation in the cystein-rich domain could be functionally relevant, because this cysteine is crucial for the EGF-like domain. The C283Y mutation can cause severe LGMD and it is the most common mutation in the Gypsy ethnic groups of the Europe (1,2,15-17). In the present study, we have determined homozygous del525T mutation in a sibling, but we could not find a C283Y mutation.

Table II: Nucleotide and amino acid sequences of SGCG gene

1 1	ATG Met	GTG Val	CGT Arg	GAG Glu	CAG Gln	TAC Tyr	ACT Thr	ACA Thr	GCC Ala	ACA Thr	GAA Glu	GGC Gly	ATC Ile	TGC Cys	ATA Ile	45 15
46 16	GAG Glu	AGG Arg	CCA Pro	GAG Glu	AAT Asn	CAG Gln	TAT Tyr	GTC Val	TAC Tyr	AAA Lys	ATT Ile	GGC Gly	ATT Ile	TAT Tyr	GGC Gly	90 30
91 31	TGG Trp	AGA Arg	AAG Lys	CGC Arg	TGT Cys	CTC Leu	TAC Tyr	TTG Leu	TTT Phe	GTT Val	CTT Leu	CTT Leu	TTA Leu	CTC Leu	ATC Ile	135 45
136 46	ATC Ile	CTC Leu	GTT Val	GTG Val	AAT Asn	TTA Leu	GCT Ala	CTT Leu	ACA Thr	ATT Ile	TGG Trp	ATT Ile	CTT Leu	AAA Lys	GTG Val	180 60
181 61	ATG Met	TGG Trp	TTT Phe	TCT Ser	CCA Pro	GCA Ala	GGA Gly	ATG Met	GGC Gly	CAC His	TTG Leu	TGT Cys	GTA Val	ACA Thr	AAA Lys	225 75
226 76	GAT Asp	GGA Gly	CTG Leu	CGC Arg	TTG Leu	GAA Glu	GGG Gly	GAA Glu	TCA Ser	GAA Glu	TTT Phe	TTA Leu	TTC Phe	CCA Pro	TTG Leu	270 90
271 91	TAT Tyr	GCC Ala	AAA Lys	GAA Glu	ATA Ile	CAC His	TCC Ser	AGA Arg	GTG Val	GAC Asp	TCA Ser	TCT Ser	CTG Leu	CTT Leu	CTA Leu	315 105
316 106	CAA Gln	TCA Ser	ACC Thr	CAG Gln	AAT Asn	GTG Val	ACT Thr	GTA Val	AAT Asn	GCG Ala	CGC Arg	AAC Asn	TCA Ser	GAA Glu	GGG Gly	360 120
361 121	GAG Glu	GTC Val	ACA Thr	GGC Gly	AGG Arg	TTA Leu	AAA Lys	GTC Val	GGT Gly	CCC Pro	AAA Lys	ATG Met	GTA Val	GAA Glu	GTC Val	405 135
406 136	CAG Gln	AAT Asn	CAA Gln	CAG Gln	TTT Phe	CAG Gln	ATC Ile	AAC Asn	TCC Ser	AAC Asn	GAC Asp	GGC Gly	AAG Lys	CCA Pro	CTA Leu	450 150
451 151	TTT Phe	ACT Thr	GTA Val	GAT Asp	GAG Glu	AAG Lys	GAA Glu	GTT Val	GTG Val	GTT Val	GGT Gly	ACA Thr	GAT Asp	AAA Lys	CTT Leu	495 165
496 166	CGA Arg	GTA Val	ACT Thr	GGG Gly	CCT Pro	GAA Glu	GGG Gly	GCT Ala	CTT Leu	TTT Phe	GAA Glu	CAT His	TCA Ser	GTG Val	GAG Glu	540 180
541 181	ACA Thr	CCC Pro	CTT Leu	GTC Val	AGA Arg	GCC Ala	GAC Asp	CCG Pro	TTT Phe	CAA Gln	GAC Asp	CTT Leu	AGA Arg	TTA Leu	GAA Glu	585 195
586 196	TCC Ser	CCC Pro	ACT Thr	CGG Arg	AGT Ser	CTA Leu	AGC Ser	ATG Met	GAT Asp	GCC Ala	CCA Pro	AGG Arg	GGT Gly	GTG Val	CAT His	630 210
631 211	ATT Ile	CAA Gln	GCT Ala	CAC His	GCT Ala	GGG Gly	AAA Lys	ATT Ile	GAG Glu	GCG Ala	CTT Leu	TCT Ser	CAA Gln	ATG Met	GAT Asp	675 225
676 226	ATT Ile	CTT Leu	TTT Phe	CAT His	AGT Ser	AGT Ser	GAT Asp	GGA Gly	ATG Met	CTT Leu	GTG Val	CTT Leu	GAT Asp	GCT Ala	GAA Glu	720 240
721 241	ACT Thr	GTG Val	TGC Cys	TTA Leu	CCC Pro	AAG Lys	CTG Leu	GTG Val	CAG Gln	GGG Gly	ACG Thr	TGG Trp	GGT Gly	CCC Pro	TCT Ser	765 255
766 256	GGC Gly	AGC Ser	TCA Ser	CAG Gln	AGC Ser	CTC Leu	TAC Tyr	GAA Glu	ATC Ile	TGT Cys	GTG Val	TGT Cys	CCA Pro	GAT Asp	GGG Gly	810 270
811 271	AAG Lys	CTG Leu	TAC Tyr	CTG Leu	TCT Ser	GTG Val	GCC Ala	GGT Gly	GTG Val	AGC Ser	ACC Thr	ACG Thr	TGC Cys	CAG Gln	GAG Glu	855 285
856 286	CAC His	AGC Ser	CAC His	ATC Ile	TGC Cys	CTC Leu	TGA Ter									

(*) Note the previously determined missense mutations listed in bold in the gray cells.
The homozygous mutations in these patients were highlighted with dark cells and white letters.
The silent mutations was underlined.

Immunohistochemical analysis of the sarcolemmal proteins such as dystrophin, SGCs, merosin, and dysferlin is an important part of the diagnostic evaluation of muscle biopsies in patients with muscular dystrophy. Reduced or absent sarcolemmal expression of one of the 4 SGCs can be found in patients with any LGMDs and also in patients with dystrophinopathies. It has been previously suggested that different patterns of SGC expression could predict the primary genetic defect, and that genetic analysis could be directed by these patterns (1,12). However Klinge et al. (10) reported that residual SGC expression could be highly variable and an accurate prediction of the genotype could not be achieved. Therefore they recommended using antibodies against all four SGCs for immunoanalysis of skeletal muscle sections. Similarly, a concomitant reduction of dystrophin and any one of SGCs may have a crucial importance in the differential diagnosis of dystrophinopathies for sarcoglycan deficient LGMD (1-5). For this reason, it is not easy to decide whether the disease is a dystrophinopathy with defective expressions of SGCs or a LGMD with defective expression of dystrophin. Since in the cases of this series, the sarcolemmal dystrophin staining and dystrophin gene were not abnormal, Duchenne Muscular Dystrophy (DMD) or Becker Muscular Dystrophy (BMD) were not considered for differential diagnosis.

Patients with any LGMD may be clinically indistinguishable from those with primary dystrophinopathies. Probably, the diagnosis of LGMD has been underestimated and a number of male patients were diagnosed as DMD or BMD (1,3,7). If a definitive diagnosis can be made based on appropriate immunohistochemical examinations and molecular analysis performed in those patients, a normal staining pattern of dystrophin and an autosomal recessive mode of inheritance can be determined. On the contrary, patients with dystrophinopathy may show variable findings from normal to regional absence or mosaic pattern of sarcolemmal staining with anti-SGCs antibodies which signify different presentation of abnormal organization of the cell-membrane associated dystrophin glycoprotein complex. Therefore careful examination of immunohistochemical staining with genetic study is necessary to make an accurate diagnosis (1,2).

In summary, this study adds different mutations to the growing list of defects that can be associated with LGMD-2C and further emphasizes the importance of systematic analysis of all related genes, instead of analyzing only the primarily deficient SGCs gene. In this study, we have also highlighted the patterns of genetic complexity associated with LGMD-2C encountered during the process of differential diagnosis of muscular dystrophies (18).

CONFLICT of INTEREST

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