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Case Report

Concomitant Alpha- and Gamma-Sarcoglycan Deficiencies in a Turkish Boy with a Novel Deletion in the Alpha-Sarcoglycan Gene

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Limb-girdle muscular dystrophy type 2D (LGMD-2D) is caused by autosomal recessive defects in the alpha-sarcoglycan gene located on chromosome 17q21. In this study, we present a child with alpha-sarcoglycanopathy and describe a novel deletion in the alpha-sarcoglycan gene. A 5-year-old boy had a very high serum creatinine phosphokinase level, which was determined incidentally, and a negative molecular test for the dystrophin gene. Muscle biopsy showed dystrophic features. Immunohistochemistry showed that there was diminished expression of alpha- and gamma-sarcoglycans. DNA analysis revealed a novel 7 bp homozygous deletion in exon 3 of the alpha-sarcoglycan gene. His parents were consanguineous heterozygous carriers of the same deletion. We believe this is the first confirmed case of primary alpha-sarcoglycanopathy with a novel deletion in Turkey. In addition, this study demonstrated that both muscle biopsy and DNA analysis remain important methods for the differential diagnosis of muscular dystrophies because dystrophinopathies and sarcoglycanopathies are so similar.

1. Introduction

Limb girdle muscular dystrophy type 2D (LGMD-2D) is an autosomal recessive muscular disease caused by genetic defects in sarcolemmal alpha sarcoglycan (α-SGC) glycoprotein. Alpha-SGC or adhalin, one of the four sarcoglycans (SGCs), is essential for membrane integrity during muscle contraction and provides a scaffold for important signaling molecules [1–3]. Alpha-SGC is encoded by the sarcoglycan alpha gene (SGCA) located on chromosome 17q21 [1, 4]. LGMD-2D predominantly affects proximal muscles around the scapular and the pelvic girdles. LGMD-2D has a very heterogeneous phenotype. The age of onset, rate of progression, and the severity of disease can vary between and also within affected families. The most clinically severe course is generally observed when the sarcolemmal α-SGC is totally absent whereas milder phenotypes are observed when residual proteins are present [1–4]. Interestingly, a mutation in any SGC gene can lead to a reduction or absence of the other SGCs [4–7]. It was previously reported that the SGCA gene must be evaluated first if there is a concomitant absence of α-SGC and gamma- (γ-) SGC [4].

The differential diagnosis for LGMD-2D includes Duchenne and Becker muscular dystrophies (DMD/BMD) and it is impossible to reach a diagnosis on clinical grounds alone. Therefore, immunohistochemical staining of a muscle biopsy and molecular genetic analysis are mandatory for the correct diagnosis [3, 5, 8, 9]. In this report, the patient's

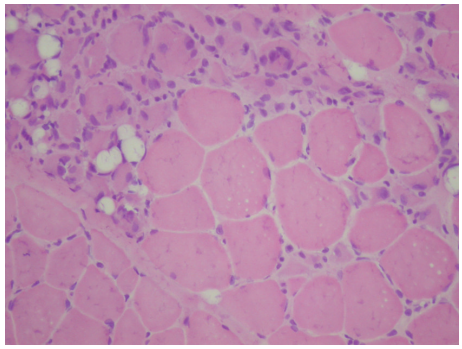


Figure 1: Differences in the size and shape of myofibers as well as regeneration (HEx 200).

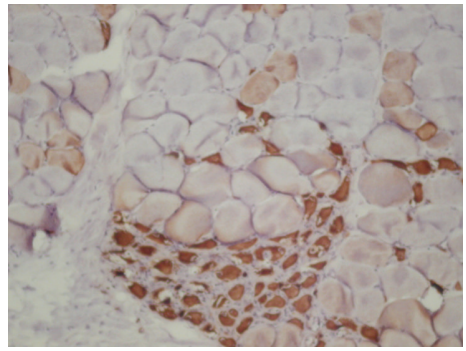


Figure 2: Immature pathological fibers visualized with anti-neonatal myosin antibody staining (DABx 100).

genotype was a previously unknown 7 bp deletion in exon 3. This finding adds to the growing spectrum of mutations in the alpha-sarcoglycan gene. Finally, we also discuss important considerations in the differential diagnosis of the muscular dystrophies.

2. Case Report

A 5-year-old boy had second degree consanguineous parents from Turkey without an ancestral history of neuromuscular disorders. There were no complications during pregnancy, and antenatal signs of muscular disorders such as polyhydramnios and reduced fetal movements were not noted. Cognitive and motor development was normal. At the time of presentation, his previously undetected mild muscle weakness was predominantly proximal. Deep tendon reflexes were present and he had no contractures. He was walking normally but he had mild difficulty when climbing stairs and running. Pulmonary function tests were normal. His creatinine phosphokinase (CPK) levels were between 9000 and 15000 units per liter (normal < 250 U/L), and there were myopathic changes on electromyography. Because of the very high CPK level, muscular dystrophy was suspected and, after informed consent, samples were obtained for histopathology, immunohistochemistry, and molecular genetics testing.

A muscle biopsy specimen from the left gastrocnemius muscle of the patient was frozen in isopentane that was precooled to −160°C in liquid nitrogen. Cryosections were immunostained for dystrophin using a polyclonal antibody (Neomarkers), with a monoclonal spectrin antibody (Novocastra) as a control. A neonatal myosin heavy chain (Neonatal myosin, Novocastra) antibody was used for the identification of pathological immature myofibers. SGCs were detected with anti-α-, -β-, -δ-, and -γ-SGC antibodies (Novocastra).

Peripheral blood specimens were collected from the proband and parents. Genomic DNA was extracted from whole blood using a commercial DNA extraction kit (QiaGen, USA) following the standard manufacturer's protocol. The concentration of sample DNA was determined by a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). The exon regions and flanking short intronic sequences of the SGCA gene were amplified using polymerase chain reaction (PCR), followed by direct sequencing of the PCR products (ABI, US) (NCBI Reference Sequence: NG_008889.1). Hitherto reported genetic abnormalities in LGMD-2D are listed in Table 1.

3. Results

The muscle biopsy showed dystrophic changes like contraction, regeneration (Figure 1), degeneration, necrosis, nuclear internalization, and fibrosis. In addition, many pathological immature myofibers were visualized using the neonatal myosin staining (Figure 2). Based on immunostaining, dystrophin and spectrin expressions were normal. Except for isolated deficient fibers, beta (β) sarcoglycan and delta (δ) sarcoglycan were present at normal levels, whereas α-SGC and γ-SGC were diffusely absent (Figure 3).

Based on analysis of the proband, we have identified a previously undetermined homozygous 7 bp deletion in exon 3 (Figure 4). A similar heterozygous deletion was found in both parents (Figures 5 and 6). Location of this deletion was also indicated in Table 1. In addition, there were no abnormalities in the dystrophin gene and the other sarcoglycan genes (SGCB, SGCD, and SGCG) in the patient and his parents.

4. Discussion

Human SGCA cDNA from a human skeletal muscle library was isolated and sequenced in 1993. This gene consisted of 10 exons. The protein product of SGCA gene consisted of 387 amino acids with an extracellular N-terminus, a transmembrane domain, and an intracellular C-terminus. Northern blot analysis showed that human adhalin mRNA was expressed at the highest levels in skeletal muscle. It was also expressed in cardiac muscle and in the lung, but at much lower levels. On the contrary, adhalin mRNA was not detected in the brain. It was also reported that the adhalin mRNA from cardiac muscle was shorter relative to skeletal muscle and that the base sequence encoding the transmembrane domain was absent. It is known that LGMD-2D primarily affects skeletal muscles while brain and peripheral nerve functions are largely preserved. Briefly, the less severe cardiac dysfunction and lack of mental retardation

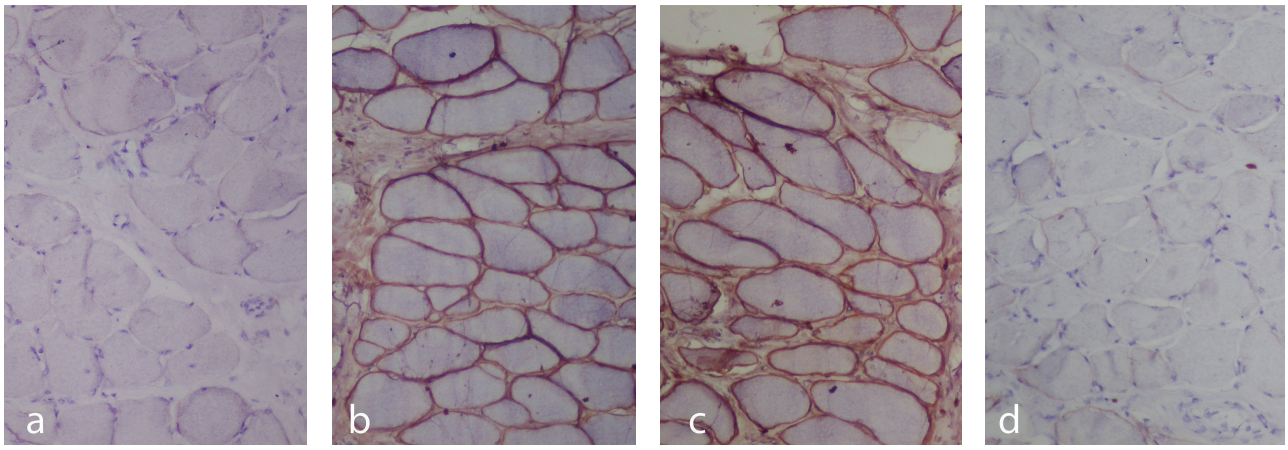
Table 1: Nucleotide and amino acid sequences of α-SGC gene.

1	ATG	GCT	GAG	ACA	CTC	TTC	TGG	ACT	CCT	CTC	CTC	GTG	GTT	CTC	CTG
1	Met	Ala	Glu	Thr	Leu	Phe	Trp	Thr	Pro	Leu	Leu	Val	Val	Leu	Leu
46	GCA	GGG	CTG	GGG	GAC	ACC	GAG	GCC	CAG	CAG	ACC	ACG	CTA	CAC	CCA
16	Ala	Gly	Leu	Gly	Asp	Thr	Glu	Ala	Gln	Gln	Thr	Thr	Leu	His	Pro[#]
91	CTT	GTG	GGC	CGT	GTC	TTT	GTG	CAC	ACC	TTG	GAC	CAT	GAG	ACG	TTT
31	Leu[#]	Val	Gly	Arg[#]	Val	Phe	Val	His	Thr	Leu	Asp	His	Glu	Thr	Phe
136	CTG	AGC	CTT	CCT	GAG	CAT	GTC	GCT	GTC	CCA	CCC	GCT	GTC	CAC	ATC
46	Leu	Ser	Leu	Pro	Glu	His	Val	Ala[#]	Val	Pro	Pro	Ala	Val	His	Ile
181	ACC	TAC	CAC	GCC	CAC	CTC	CAG	GGA	CAC	CCA	GAC	CTG	CCC	CGG	TGG
61	Thr	Tyr[#]	His	Ala	His	Leu	Gln	Gly[#]	His	Pro	Asp	Leu	Pro	Arg[#]	Trp
226	CTC	CGC	**TAC**	**ACC**	**C**AG	CGC	AGC	CCC	CAC	CAC	CCT	GGC	TTC	CTC	TAC
76	Leu	Arg[#]	Tyr[##]	Thr[##]	Gln[##]	Arg[#]	Ser	Pro	His	His	Pro	Gly	Phe	Leu[#]	Tyr[#]
271	GGC	TCT	GCC	ACC	CCA	GAA	GAT	CGT	GGG	CTC	CAG	GTC	ATT	GAG	GTC
91	Gly[#]	Ser	Ala[#]	Thr	Pro	Glu	Asp[#]	Arg[#]	Gly	Leu	Gln	Val	Ile[#]	Glu	Val
316	ACA	GCC	TAC	AAT	CGG	GAC	AGC	TTT	GAT	ACC	ACT	CGG	CAG	AGG	CTG
106	Thr	Ala	Tyr	Asn	Arg[#]	Asp	Ser	Phe	Asp	Thr	Thr	Arg	Gln	Arg	Leu
361	GTG	CTG	GAG	ATT	GGG	GAC	CCA	GAA	GGC	CCC	CTG	CTG	CCA	TAC	CAA
121	Val	Leu	Glu	Ile[#]	Gly	Asp	Pro	Glu	Gly	Pro	Leu	Leu	Pro	Tyr	Gln
406	GCC	GAG	TTC	CTG	GTG	CGC	AGC	CAC	GAT	GCG	GAG	GAG	GTG	CTG	CCC
136	Ala	Glu[#]	Phe	Leu[#]	Val	Arg[#]	Ser	His	Asp	Ala	Glu	Glu	Val	Leu	Pro
451	TCA	ACA	CCT	GCC	AGC	CGC	TTC	CTC	TCA	GCC	TTG	GGG	GGA	CTC	TGG
151	Ser	Thr	Pro	Ala	Ser	Arg	Phe	Leu[#]	Ser	Ala	Leu	Gly	Gly	Leu	Trp
496	GAG	CCC	GGA	GAG	CTT	CAG	CTG	CTC	AAC	GTC	ACC	TCT	GCC	TTG	GAC
166	Glu	Pro	Gly	Glu	Leu	Gln	Leu	Leu[#]	Asn	Val[#]	Thr	Ser	Ala	Leu	Asp
541	CGT	GGG	GGC	CGT	GTC	CCC	CTT	CCC	ATT	GAG	GGC	CGA	AAA	GAA	GGG
181	Arg[#]	Gly	Gly	Arg	Val	Pro	Leu	Pro	Ile	Glu	Gly	Arg	Lys	Glu	Gly[#]
586	GTA	TAC	ATT	AAG	GTG	GGT	TCT	GCC	TCA	CCT	TTT	TCT	ACT	TGC	CTG
196	Val[#]	Tyr	Ile	Lys	Val	Gly	Ser	Ala	Ser	Pro[#]	Phe	Ser	Thr[#]	Cys	Leu
631	AAG	ATG	GTG	GCA	TCC	CCC	GAT	AGC	CAC	GCC	CGC	TGT	GCC	CAG	GGC
211	Lys	Met	Val	Ala	Ser[#]	Pro	Asp	Ser	His	Ala	Arg[#]	Cys	Ala	Gln[#]	Gly
676	CAG	CCT	CCA	CTT	CTG	TCT	TGC	TAC	GAC	ACC	TTG	GCA	CCC	CAC	TTC
226	Gln	Pro[#]	Pro[#]	Leu	Leu	Ser	Cys[#]	Tyr	Asp	Thr	Leu	Ala	Pro[#]	His	Phe
721	CGC	GTT	GAC	TGG	TGC	AAT	GTG	ACC	CTG	GTG	GAT	AAG	TCA	GTG	CCG
241	Arg	Val[#]	Asp	Trp	Cys	Asn	Val[#]	Thr	Leu	Val	Asp	Lys	Ser	Val	Pro
766	GAG	CCT	GCA	GAT	GAG	GTG	CCC	ACC	CCA	GGT	GAT	GGG	ATC	CTG	GAG
256	Glu	Pro	Ala	Asp	Glu	Val	Pro[#]	Thr	Pro	Gly	Asp	Gly	Ile	Leu	Glu
811	CAT	GAC	CCG	TTC	TTC	TGC	CCA	CCC	ACT	GAG	GCC	CCA	GAC	CGT	GAC
271	His	Asp[#]	Pro	Phe	Phe	Cys	Pro	Pro	Thr	Glu	Ala	Pro	Asp	Arg[#]	Asp
856	TTC	TTG	GTG	GAT	GCT	CTG	GTC	ACC	CTC	CTG	GTG	CCC	CTG	CTG	GTG
286	Phe	Leu	Val	Asp	Ala	Leu	Val	Thr	Leu	Leu	Val	Pro	Leu	Leu	Val
901	GCC	CTG	CTT	CTC	ACC	TTG	CTG	CTG	GCC	TAT	GTC	ATG	TGC	TGC	CGG
301	Ala	Leu	Leu	Leu	Thr	Leu	Leu	Leu	Ala	Tyr	Val	Met[#]	Cys	Cys	Arg
946	CGG	GAG	GGA	AGG	CTG	AAG	AGA	GAC	CTG	GCT	ACC	TCC	GAC	ATC	CAG
316	Arg	Glu	Gly	Arg	Leu	Lys	Arg	Asp	Leu	Ala	Thr	Ser	Asp	Ile	Gln

Table 1: Continued.

991	ATG	GTC	CAC	CAC	TGC	ACC	ATC	CAC	GGG	AAC	ACA	GAG	GAG	CTG	CGG
331	Met	Val	His	His	Cys	Thr	Ile	His	Gly	Asn	Thr	Glu	Glu	Leu	Arg
1036	CAG	ATG	GCG	GCC	AGC	CGC	GAG	GTG	CCC	CGG	CCA	CTC	TCC	ACC	CTG
346	Gln	Met	Ala	Ala	Ser	Arg	Glu	Val	Pro	Arg	Pro	Leu	Ser	Thr	Leu
1081	CCC	ATG	TTC	AAT	GTG	CAC	ACA	GGT	GAG	CGG	CTG	CCT	CCC	CGC	GTG
361	Pro	Met	Phe	Asn	Val	His	Thr	Gly	Glu	Arg	Leu	Pro	Pro	Arg	Val
1126	GAC	AGC	GCC	CAG	GTG	CCC	CTC	ATT	CTG	GAC	CAG	CAC	TGA		
376	Asp	Ser	Ala	Gln	Val	Pro	Leu	Ile	Leu	Asp	Gln	His	Ter		

*Note the previously determined missense mutations marked with #. The present deletion was marked with ## and bold letters.



(a) (b) (c) (d)

Figure 3: Diffuse absence of sarcolemmal α-SGC (a) and γ-SGC (d) expression and normal β-SGC (b) and δ-SGC (c) expression (DABx 200).

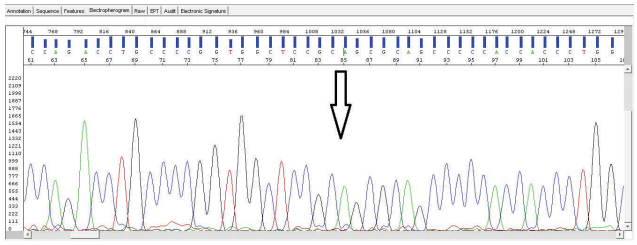


Figure 4: Proband exon 3 homozygous del TACACCC site.

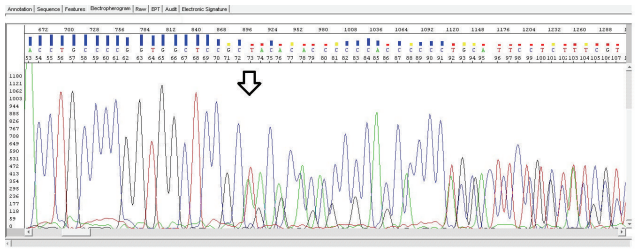


Figure 5: Maternal heterozygous del TACACCC site.

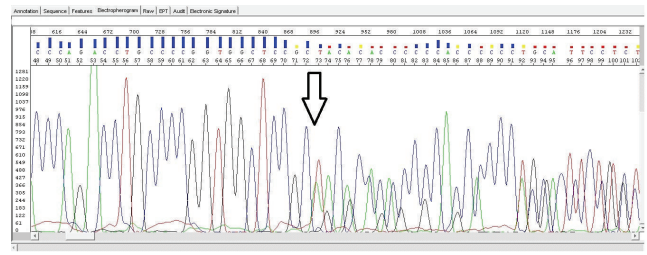


Figure 6: Paternal heterozygous del TACACCC site.

in patients with LGMD-2D may be explained by the lower expression of α-SGC in cardiac muscle and the absence of adhalin expression in the brain [1, 3, 10]. In the patient described in this report, we did not find clinical evidence of cardiac involvement, decreased intellectual capacity, or denervation (as demonstrated by electromyography). The course of the disease in this case suggests that this novel deletion may cause a milder phenotype of LGMD-2D despite the diffuse absence of α-SGC and γ-SGC.

Immunohistochemical analysis of sarcolemmal proteins in muscle biopsies like dystrophin, SGCs, merosin, and dysferlin is an important part of the diagnostic evaluation of patients with muscular dystrophy. Reduced or absent sarcolemmal expression of one of the 4 SGCs can be found in patients with any type of LGMDs and also in patients with dystrophinopathies. It has previously been suggested that different patterns of SGC expression could predict the primary genetic defect and that genetic analysis could be directed by these patterns [5–8]. However, Klinge et al. [9] reported that residual SGC expression could be highly variable and an accurate prediction of the genotype could not be achieved. Babameto-Laku et al. [4] also determined that the concomitant absence of α-SGC and γ-SGC expression was caused by defects in the SGCA gene. Therefore, they recommended using antibodies against all four SGCs for immunoanalysis of skeletal muscle sections. Similarly, a concomitant reduction in dystrophin and any of the SGCs may illustrate the importance of considering coexisting dystrophinopathies in patients with sarcoglycan-deficient LGMD [9–13]. 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. However, in the patient described in this report, dystrophinopathies, such as DMD and BMD, were ruled out because the expression of sarcolemmal dystrophin was diffusely present and molecular tests for dystrophin gene were normal.

At present, more than 70 mutations have been reported in the SGCA gene that cause changes in the α-SGC glycoprotein. Approximately a two-thirds of mutations are missense mutations that generate a complete protein with a single

residue substitution, whereas other mutations like nucleotide replacements, duplications, deletions, or insertions produce truncated, incomplete, or anomalous proteins. Almost all missense mutations map to the extracellular domain which is a critical region for the organization of SGCs and their association with dystroglycan. Only a single missense mutation maps to the intracellular domain and causes LGMD-2D in homozygous cases. Similarly, two mutations caused by deletions generate a normal extracellular portion of α-SGC and truncated intracellular tails. At present, there is no data about the intracellular tail of the α-SGC protein and its function [1, 10–14]. In the family described in this report, we discovered a novel deletion in the TACACCC site of exon 3 that would cause a frame-shift mutation. The past literature highlights that the prediction of pathological consequences associated with different mutations of SGCA gene is very complex. It is not clear whether this novel deletion generates a severe disease phenotype or whether it also has additional, undetermined consequences.

Patients with any of the LGMDs may be clinically indistinguishable from those with the primary dystrophinopathies. It is likely that the prevalence of LGMD is underestimated and a number of male patients are incorrectly diagnosed with DMD or BMD [13]. A definitive diagnosis rests on performing the appropriate immunohistochemical examination as well as doing a molecular analysis. A normal dystrophin staining pattern should be seen as well as an autosomal recessive mode of inheritance. In contrast, the patients with dystrophinopathies may show variable findings from a normal to a regional absence or a mosaic pattern of sarcolemmal staining with anti-SGCs antibodies which correspond to an abnormal organization of the cell-membrane-associated dystrophin glycoprotein complex. Therefore, it is necessary to perform a careful examination of the immunohistochemical staining as well as a genetic study in order to make the correct diagnosis.

In summary, this report describes a novel deletion that adds to the growing list of defects associated with LGMD-2D and further emphasizes the importance of systematic analysis of all related genes, instead of limiting the analysis to the one SGC gene that is hypothesized to be the cause of the abnormalities. In this study, we also highlight the complexity of staining patterns associated with sarcolemmal proteins and the importance of careful analysis of this staining pattern in order to narrow the differential diagnosis of muscular dystrophies.

Conflict of Interests

The authors declare that there is no conflict of interests.

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