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Combined use of genetic and immunohistochemical analysis is a critical step in differential diagnosis of sarcoglycanopathies

Genetik ve immünohistokimyasal incelemelerin birlikte kullanımı sarkoglikanopatilerin ayırıcı tanısında önemli bir basamaktır

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Dear Editors,

As essential components of the membrane integrity during muscular contraction, sarcoglycan (SGC) proteins at the cell membrane form a subcomplex closely linked with the dystrophin- associated glycoprotein (DAG) complex [(1)]. Deficiencies of alpha-, beta-, delta-, and gamma- sarcoglycans cause autosomal recessive (type 2) limb- girdle muscular dystrophies (LGMDs) and no definitive treatment exists for LGMDs [(1,2)]. Physical therapy, stretching exercises which increase range of motion of affected structures, and also prevent contractures, anti-obesity measures, surgical treatment of orthopedic complications, mechanical and/or respiratory armamentorium to ease ambulation, mobility, and respiration are among some of the therapeutic approaches used to prolong survival and improve quality of life. The patient should be closely observed for the development of cardiac involvement such as cardiomyopathy, and also emotional support should be provided [(2)]. In the differential diagnosis of LGMDs most commonly seen Duchenne and Becker muscular dystrophies (DMD/BMD) should be considered. However differential diagnosis can not be made among these entities based solely on clinical information. Therefore immunohistochemical staining of muscle biopsy specimens is mandatory for accurate diagnosis [(1-3)].

In our earlier study, we reported 20 clinically diagnosed muscular dystrophy patients (4 siblings) with defective gamma- sarcoglycan expressions found in the histopathological examination of their muscle biopsy specimens [(4)]. Although there were defective expressions of gamma-sarcoglycan protein in all biopsy specimens, the culprit genetic defects could be determined in only nine of them. Most cases had silent homozygous or heterozygous mutations [(4-7)]. After this report, we have also diagnosed 19 new patients (3 siblings) with LGMD. Interestingly some of these patients presented with multiple sarcoglycan defects [(8-10)]. In addition, dystrophin gene mutations were determined in a sibling who demonstrated permanent gamma-sarcoglycan deficiency with normal dystrophin expression as detected on the histopathological examination of the muscle biopsy specimen (Table 1).

All histopatological examinations of muscle biopsies were performed at Pathology Laboratories of Behcet Uz Children's Hospital and Tepecik Hospital. Genetic analyses were performed at Duzen Laboratory

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Table 1. Histopathologic and genetic features of patients.

Patient	Age	Sex	CK	Defectiive SGC (IHC)	Defective gene	Genetic pathology	Gene defects
GK	21	F	5300	gamma	gamma	compound heterozygosity	R116H(c.G347A) maternal S287N(c.G860A) paternal I218T(c.T653T) maternal
MY	6	M	4500	gamma	gamma	compound heterozygosity	R116H(c.G347A) paternal S287N(c.G860A) paternal+ maternal
ŞD	9	M	15000	gamma	-	-	-
DAÇ	11	M	17500	gamma	gamma	heterozygot	c.G860A (S287N)
FK	11	M	5000	gamma	gamma and alpha	heterozygot	c.G347A (R116H) SGCG/ duplication of exon 10 SGCA
KŞ/KŞ	8/5	M/M	2200/6100	gamma	gamma	heterozygot	c.G347A (R116H)
MK/İK	6/8	M/M	5000/13000	gamma	gamma	compound heterozygosity	c.808-801DELGT Exon 8 LOH
NU	7	F	10000	gamma	gamma	compound heterozygosity	R116H(c.G347A) S287N(c.G860A)/duplication of exon 1-10 SGCA
OH	10	M	7500	gamma	alpha	-	İntragenic amplification SGCA
İT	6	M	11000	gamma	-	-	-
ARU	10	M	7500	gamma	gamma	silent mutation	c.T312G c.T705C
YÖ	2	M	1312	gamma	gamma	heterozygot and silent mutation	c.G347A (R116H) c.T705C
NSÖ	7	F	11000	gamma	gamma	silent mutation	c.T312G c.T705C
AT/NT	2/6	F/M	7500/15700	gamma	gamma	homozygot	c.525delT
EK	3	F	13000	gamma	gamma	Silent mutation	c.T312G c.T705C
AE/AE	6/8	M	35000/13000	gamma	gamma	Silent mutations	c.T312G c.T705C(L235L) c.T228C
EG/OG	11/7	M/M	9000/7000	gamma	dystrophin	Homozygot deletion	Deletions of exon 45- 47
ÇG	1	M	11000	All SGCs	beta	homozigot gene deletion	Total gene deletion SGCB
TA	5	M	15000	alpha and gamma	alpha	homozygot novel deletion	Deletion of 3. Exon, SGCA
FÇ/SÇ	8/6	M/M	16000	alpha and gamma	alpha	homozygot novel mutation	c.226 C > T (p.L76 F) SGCA
İY	12	M	1700	gamma	gamma	heterozygot.	c.T312G(L104L)
MMK	0	F	200	beta	beta	heterozigot intronic	c.244-21 T/C d (rs225170)
ID/RD	7	F	24068	alpha and gamma	alpha	Homozygot mutation	c.G101A (R34H)
Aİ	44	M	1189	alpha	alpha	Homozygot mutation	c.C850T (R284C)
NM	9	F	5323	gamma	gamma	Homozygot mutation	c.G848A (C283Y)
ZG	5	F	250	All SGCs	gamma	Heterozygot mutation	c.G347A (R116H)
BE	3	F	5313	All SGCs	gamma and beta	compound heterozygosity (gamma) and homozygot mutations (beta)	c.T312G (L104L)rs1800351 c.G860A (S287N)/ c. G265A (V89M) SGCB
EB	14	F	250	beta	beta	İntronic difference	c.T244-21C (rs225170)
HK	1	F	7000	alfa and gamma	alfa	heterozygot and silent mutation	c.G347A (R116H) c.T705C(L235L)
LC	21	F	600	gamma	beta	Homozigot. mutation	C.265.G/A (V89M) SGCB
SB	26	F	800	Alpha and gamma	beta	İntronic difference	c.244-21 T/C i (rs225170)
ZS	6	M	500	beta	gamma	compound heterozygosity (gamma)	c.G347A (R116H) c.G860A (S287N)/

from January 2007 through December 2014. 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 exa-

mination and laboratory findings were also evaluated. We tried to determine the spectrum of genetic defects in immunohistochemically proven cases of sarcoglycanopathies, to correlate the findings with clinical phenotypes and to display the regional differences as for the clinical, histopathological, and genetic characteristics of sarcoglycanopathies [11,12].

As a known fact because of the potential variations in residual SGC expressions, genotyoe of sarcoglycanopathies can not be predicted accurately based solely on variable SGC expressions. Therefore, immunoanalysis of skeletal muscle sections for four SGCs must be performed [5-7]. Besides, concomitant reduction in the levels of dystrophin, and any SGC may play a critical role in the differential diagnosis of dystrophinopathies among sarcoglycan-deficient LGMDs. Therefore, it is a challenging issue to discriminate between an entity of dystrophinopathy caused by defective expressions of SGCs and a LGMD induced by defective expression of dystrophin. Since, siblings of this series demonstrated dystrophin gene deletions, despite normal sarcolemmal dystrophin staining pattern, dystrophinopathies were also considered in the differential diagnosis.

Patients with LGMD or primary dystrophinopathies may not be differentiated based on clinical evidence. Probably, many male patients with LGMD might be underestimated, and these patients might be misdiagnosed as having DMD or BMD [8-10]. If these patients can be subjected to appropriate immunohistochemical examinations and molecular analysis, then normal staining pattern of dystrophin can be revealed, and an autosomal recessive mode of inheritance can be disclosed. On the contrary, patients with dystrophinopathy may demonstrate normal - regional absence or mosaic pattern of sarcolemmal staining patterns with anti-SGC antibodies. These variations in staining may signify that cell membrane- associated dystrophin glycoprotein complex may have different abnormal presentations [4]. Therefore, accurate diagnosis requires careful analysis using immunohistochemical staining in combination with genetic study [1,2]. In addition, we must keep on mind that, there can be some neuromuscular diseases presented with focal sarcoglycan defects as detected during immunohistochemical analysis of muscle biopsy specimen [13].

In summary, as an important corollary not only the primarily deficient SGC gene, but also all related genes should be analyzed. Besides, patterns of genetic complexity associated with LGMDs should be considered in the differential diagnosis of muscular dystrophies [2,4,8-10,13].

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