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SARCOGLYCANOPATHIES DIAGNOSED AT NEUROLOGY DEPARTMENT OF CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

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SARCOGLYCANOPATHIES DIAGNOSED AT NEUROLOGY DEPARTMENT OF CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

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SARCOGLYCANOPATHIES DIAGNOSED AT NEUROLOGY DEPARTMENT OF CENTRO HOSPITALAR E UNIVERSITÁRIO DE COIMBRA

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Abstract

Introduction: Sarcoglycanopathies are muscle dystrophies with autossomic recessive transmission that result from mutations in the sarcoglycans genes. Sarcoglycans α, β, γ and δ, are structural proteins of the muscle fiber, with transmembrane location, that form an anatomo-functional unit, the sarcoglycan complex, that integrates the dystrophin-glycoprotein complex. According to the location of the mutation in the individual sarcoglycan genes, it is possible to identify four sarcoglycanopathies, α-sarcoglycanopathy (Cr.17q21), β-sarcoglycanopathy (Cr.4q12), γ-sarcoglycanopathy (Cr.13q12) and δ-sarcoglycanopathy (Cr.5q33), classified as LGMD2D, 2E, 2C, and 2F, respectively, based on the proposed classification of the European Neuromuscular Centre. Diagnosis is based on the clinical history, neurological examination, CK levels, muscle pathology (muscle biopsy with immunohistochemistry study), and molecular studies (for detection of mutations in the sarcoglycans genes).

Objective: The aim of this work is to describe the clinical, laboratory, muscle pathology and molecular results of a group of nine patients diagnosed with sarcoglycanopathies and their relative distribution among the limb-girdle muscular dystrophy patients diagnosed at the Neuromuscular Outpatient Clinic of the Neurology Department – Centro Hospitalar e Universitário de Coimbra (CHUC).

Material and Methods: The individual clinical files of all patients were analyzed and demographic and historical data, physical evaluation and functional tests recorded. The results of manual muscle testing of the upper and lower limbs were graded according the MRC scale with an expected global score of 160. The muscle biopsies with a descriptive report available were analyzed and the most prominent features were recorded and graded. A molecular result was available in eight patients.

Results: Nine patients from eight unrelated families were studied, six females and three males. Familial consanguinity was reported by three of them. Their actual mean age is 32,78 years and the mean age of first symptoms was 10,22 years. Mean time from first symptoms to molecular diagnosis was 13,63 years. Eight patients needed support to walk at the mean age of 18,71 years and five of them were wheelchair bound at mean age of 15,60 years. The manual muscle testing scored below 160 and CK values were elevated in all patients. Immunohistochemic study was abnormal and suggested the specific diagnosis and the molecular study performed in eight patients showed homozygous mutations in four patients with γ-sarcoglycanopathy and one patient with α-sarcoglycanopathy and compound heterozygous mutations in one patient with γ-sarcoglycanopathy and in two patients with α-sarcoglycanopathy.

Conclusion: This study shows that γ-sarcoglycanopathy is the most frequent sarcoglycanopathy diagnosed at the Neuromuscular Outpatient Clinic of the Neurology Department – CHUC.

Key-Words: Limb-Girdle muscular dystrophies (LGMD); Sarcoglycanopathies; α, β, γ, and δ-sarcoglycan; physical evaluation; biopsy; mutation; molecular diagnosis; dystrophin-glycoprotein complex (DGC).

Resumo

Introdução: Sarcoglicanopatias são distrofias musculares com transmissão autossómica recessiva que resultam de mutações nos genes sarcoglicanos. Sarcoglicans α, β, γ e δ, são proteínas estruturais da fibra muscular, com localização transmembranar, que formam uma unidade anatomo-funcional, o complexo sarcoglicano, que integra o complexo distrofina - glicoproteína. De acordo com a localização da mutação em cada gene sarcoglicano, é possível identificar quatro sarcoglicanopatias, α-sarcoglicanopatia (Cr.17q21), β-sarcoglicanopatia (Cr.4q12), γ-sarcoglicanopatia (Cr.13q12) e δ-sarcoglicanopatia (Cr.5q33), classificadas como DMC2D, 2E, 2C, e 2F, respetivamente, baseado na classificação proposta pelo European Neuromuscular Centre. O diagnóstico é feito com base na história clínica, no exame neurológico, níveis de creatina cinase, patologia muscular (biópsia muscular com estudo imuno-histoquímico), e estudo molecular (para deteção de mutações nos genes sarcoglicanos).

Objectivo: O objetivo deste trabalho é descrever os resultados clínicos, laboratoriais, histopatológicos e moleculares de nove doentes diagnosticados com sarcoglicanopatias e a sua distribuição relativa entre os diferentes subtipos de distrofias musculares das cinturas autossómicas recessivas diagnosticadas no Serviço de Neurologia – Centro Hospitalar e Universitário de Coimbra (CHUC).

Material e Métodos: Os processos clínicos individuais de todos os doentes foram analisados e registaram-se os dados clínicos e os resultados dos testes funcionais e de avaliação física. Os resultados do *manual muscle testing* dos membros superiores e inferiores foram quantificados de acordo com a escala MRC com um resultado global máximo de 160. As biópsias musculares, com relatório descritivo disponível, foram analisadas e as

características mais proeminentes foram registadas e quantificadas. O resultado do estudo molecular estava disponível em oito doentes.

Resultados: Foram estudados nove doentes de oito famílias não relacionadas, seis mulheres e três homens. Foi descrita consanguinidade em três deles. A idade média atual destes doentes é de 32,78 anos e a idade média de aparecimento dos primeiros sintomas de 10,22 anos. O tempo médio decorrido desde o aparecimento dos primeiros sintomas até à realização do estudo molecular foi 13,63 anos. Oito doentes necessitaram de apoio para andar com a idade média de 18,71 anos e cinco deles necessitaram de cadeira de rodas com a idade média de 15,60 anos. O *manual muscle testing* registou resultados abaixo de 160 e os valores de creatina cinase estavam elevados em todos os doentes. O estudo imuno-histoquímico foi anormal e sugeriu um diagnóstico específico e o estudo molecular realizado em oito doentes revelou mutações homozigóticas em quatro doentes com γ-sarcoglicanopatia e um doente com α-sarcoglicanopatia e mutações heterozigóticas compostas num doente com γ-sarcoglicanopatia e em dois doentes com α-sarcoglicanopatia.

Conclusão: Este estudo demonstra que a γ-sarcoglicanopatia é a sarcoglicanopatia mais frequentemente diagnosticada no Serviço de Neurologia do Centro Hospitalar e Universitário de Coimbra (CHUC).

Palavras-chave: Distrofias Musculares das Cinturas (DMC); Sarcoglicanopatias; α, β, γ, e δ-sarcoglicano; avaliação física; biópsia; mutação; estudo molecular; complexo distrofina - glicoproteína (CDG).

Introduction

Sarcoglycanopathies are subtypes of autosomal-recessive limb-girdle muscular dystrophies and are caused by pathogenic mutations in the genes encoding the α, β, δ and γ sarcoglycans. Sarcoglycans are glycoproteins of the sarcoglycan tetrameric complex that contributes to the stability of the plasma membrane cytoskeleton and facilitates the association of dystrophin with the dystroglycans.[1-6]

The sarcoglycans α, β, γ and δ are related to each other structurally and functionally, but each has a discrete chromosomal location, namely 17q21, 4q12, 13q12 and 5q33, respectively. According to the proposed classification of the European Neuromuscular Centre they are classified as LGMD 2D, 2E, 2C, and 2F, when the pathogenic mutation is located in the α, β, γ,and δ genes, respectively[5].

The majority of sarcoglycanopathies are caused by missense mutations that generate substitution of single residues that could lead to a misfolded protein.[3] A primary mutation in any one of the sarcoglycan genes can result in total or partial loss of that specific sarcoglycan, secondary deficiencies of the other sarcoglycans, presence of only trace amounts of the protein in the cell membrane and the occasional reduction of dystrophin labeling in muscle tissue.[6] α-sarcoglycanopathy is the most common worldwide sarcoglycanopathy, whereas δ-sarcoglycanopathy is the rarest.[3-4,7]

These limb-girdle muscular dystrophies are characterized, like most others LGMDs, by a slowly progressive proximal muscle weakness. Sarcoglycanopathies are frequently found among the more severe LGMDs forms with onset in childhood, but some cases might have a delayed onset into adulthood.[8-10] The clinical course of sarcoglycanopathies is invariably progressive, leading to loss of ambulation in adolescence in most patients. Patients with later onset may have preserved ambulation into adulthood. As a consequence of increasing muscle

weakness, reduced joint mobility and fibrotic degeneration of skeletal muscles, contractures and scoliosis commonly develop and might be severe. With progression of the disease, muscle weakness may involve the respiratory muscles and often necessitates the initiation of respiratory support.[4]

The sarcoglycans are found almost exclusively in skeletal and cardiac muscle, so it is expected that these patients may develop a cardiomyopathy. Reports have suggested that cardiac involvement might represent an important and sometimes life-threatening clinical complication in these disorders. It has been reported in LGMD2C, 2E and 2F and rarely in LGMD2D.[10-11] The same study concluded that the severity of the skeletal muscle disease matches the severity of the cardiomyopathy: cardiac muscle involvement was asymptomatic until late childhood, progressed with the skeletal muscle disease, becoming symptomatic when the patients became wheelchair bound, or remaining stable with the patients in a steady-stage of the disease for several years.[10]

There appears to be no cognitive involvement in patients with sarcoglycanopathies.[4]

The diagnostic workup of patients presenting with proximal muscle weakness will always include serum levels of creatine kinase, which are invariably significantly elevated.[4] Finding reduced or absence of sarcoglycan immunolabeling or reduced amounts of sarcoglycans by immunobloting analysis was shown to be a useful indicator justifying subsequent molecular studies to establish a correct diagnosis.[11]

The aim of this work is to describe the clinical, laboratory, muscle pathology and molecular results of a group of nine patients diagnosed with sarcoglycanopathies and their relative distribution at the Neuromuscular Outpatient Clinic of the Neurology Department of the CHUC.

The Outpatient Neuromuscular Clinic is the reference center for the study of adult neuromuscular diseases in the central region of Portugal, a geographical area with a population of about 1.5 million people.[1]

Material and Methods

Patients

At the Outpatient Neuromuscular Clinic were found nine patients from eight unrelated families diagnosed with sarcoglycanopathies. They are part of larger group of 42 patients with a diagnosis of autosomal-recessive limb-girdle muscular dystrophies, actually attending the Neuromuscular Clinic.

The requirements to be included in the study were: weakness of the limb-girdle muscles, a muscular biopsy of the dystrophic type and reduced or absence of immunolabeling of one or all sarcoglycan and/or a molecular study confirming the diagnosis of sarcoglycanopathy.

The nine patients had a muscle biopsy and/or a molecular result confirming the diagnosis, except for a female patient of gypsy descendent which declined the molecular study after the result of a muscle biopsy with a suspected γ-sarcoglycanopathy. Three patients had their muscle biopsy performed in another centre and the details of the pathologic data were not available.

Clinical Evaluation

The study protocol included: 1- demographic and historical features: age, date of birth, gender, race, ethnicity, birthplace, parents´ birthplace, consanguinity, family history of similar diseases, associated medical diseases, current medication, age of first symptoms, initial site of first symptoms, age of walking with support and age of wheelchair bound. 2- Physical

evaluation: eight, weight, state of respiratory function and the need of respiratory ventilator support, state of cardiac function, description of muscular hypertrophy and atrophy, description of contractures, facial weakness and weakness in the flexion of the neck. 3- Functional tests: walking, grade as without support, with support or impossible; ability to climb stairs, characterized as normal, with support or impossible; rising from a chair, described as normal, with support or impossible; raising of the arms, characterized as normal and above head, until shoulder level and bellow shoulder level and Gowers' maneuver, positive or negative. 4- Manual muscle testing (MMT) was done and graded according to the MRC scale, where 5 is normal and 0 is absence of any voluntary muscle activation. The following movements were evaluated bilaterally: upper limbs - arm abduction, flexion and extension of the arm, flexion and extension of the hand and fingers and finger abduction; lower limbs - flexion and extension of the thigh, flexion and extension of the leg and dorsiflexion and plantar flexion of the foot. A global score of 160 will be obtained in a normal person - upper limbs partial score (ULPS) of 80 and lower limbs partial score (LLPS) of 80. A global score below 160 will reflect muscle weakness, after exclusion of non muscular causes preventing adequate development of muscle strength.

Besides the muscle biopsy and molecular study, another laboratory feature was included in the study, the highest serum creatine kinase value present on the medical file of each patient.

Muscle biopsy evaluation

There were six muscles biopsies available to analysis which were performed and processed by the Neuropathology Unit of the Neurology Department of the CHUC. Three patients (B, C and I) had their muscle biopsies performed elsewhere and were not available for analysis.

The muscle fragments were frozen in isopentane chilled in liquid nitrogen and kept ate -70ºC. The transverse and longitudinal cryostat sections were cut 8µ thick, and stained by histochemical (H/E, PAS, Red-oil and Trichrome Gomori) and histoenzimatic routine methods (NADH-TR, SDH, ATPase pH4,35 and pH9,4) and cut 4µ thick for immunohistochemistry study with antibodies against dystrophin (*dys* 1, *dys* 2,*dys* 3), α, β, δ and γ sarcoglycans, dysferlin, α-dystroglycan, merosin and emerin (all from Novocastra). The intensity of the staining with each antibody was graded from zero (absent) to 3+ (normal expression). Control human skeletal muscle was included with patient material in each glass slide immunostained in the study.

The reports of the muscle biopsies were evaluated and graded, when present, the most prominent features: variability of fiber diameter (normal or increased), internal nuclei (absent or increased), necrotic fibers (absent or present: rare or frequent), basophilic fibers (absent or present), predominance of fiber type, connective tissue (normal, increased: focal or generalized), fat infiltration (absent, present: focal or generalized), inflammatory infiltrates (absent or present), vascular abnormalities and the presence of special histopathologic features such as lobulated fibers and rimmed vacuoles.

Molecular Studies

The molecular genetics studies were requested after the informative results of muscle biopsy protein findings and were performed at the Molecular Genetics Unit of the Institute of Jacinto Magalhães, Porto.

gDNA analysis

Genomic DNA was extracted from peripheral blood by the salting-out method.[12] Normal or M13-tailed primers udes to amplify all the coding exons and directly flanking intronic sequences, were designed with aid of Primer Express (Applied Biosystems, Foster City, CA). Amplicons were purified using ExoSAP-IT (USB Corporation, Cleveland, OH) and sequenced with the respective normal or M13 universal primers, using the Big-DyeTM Terminator Cycle Sequencing Kit V1.1. The products were resolved on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Mutation analysis was aided by SeqScape V2.5 software (Applied Biosystems, Foster City, CA) and Alamut V2.1 (Interactive Biosoftware, Rouen).

Transcript analysis

Total RNA was extracted from peripheral blood and/or muscle biopsies of patients and controls using TRIzol isolation reagent (Invitrogen, CA), and reverse transcribed using either Superscript One-Step RT-PCR with Platinum Taq (Invitrogen, CA) or the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA). Primers were designed according to case-specific interrogations. Amplicons resolved on 1% w/v agarose gels were eluted and sequenced in both directions, as described above.

Mutation characterization

Sequences variants in each gene were described according to the mutation nomenclature recommendations of the Human Gene Variation Society (HGVS)[13], using c-DNA reference sequences filed under the following respective accession numbers: SGCA – NM_000023.1+L34355.1; SGCB – NM_000232.3+CN483961.1; SGCG – NM_000231.2 and SCGD – NM_000337.4.

Results

Clinical evaluation

Historical features (Table 1)

There were nine patients, 6 females and 3 males, from eight unrelated families. All the patients were caucasian and three of them (patients A, B, C) were of gypsy descent, two of them male brothers (patients B and C). These three patients (A, B and C) had an intricate, proximal and remote, parental and familial consanguinity.

None of the patients had other medical conditions that could be responsible for the weakness in the limb girdle muscles.

The mean age of the patients at the moment of the study was 32,78 years and the mean age of first symptoms was 10,22 years. Seven patients reported the first symptoms of weakness in the first decade of life, another in the second decade and the last one in the third decade. The patient presenting the youngest age of first symptoms had a LGMD2C and the patient presenting the oldest age a LGMD2D.

The mean time from first symptoms to muscle biopsy was 13,22 years. Eight patients had a molecular study, with the mean age of and time from first symptoms to molecular study 24,12 and 13,63 years, respectively.

All patients mention the site of first symptoms in the lower limbs, with increasing difficulties in running, climbing stairs and frequent falls.

Table 1 – Historical features

Patient	Sex F/M	Parental Consanguinity	Age (years)		Age (years) of molecular diagnosis	From first symptoms - Time to (years)	
			Actual	First symptoms		Biopsy	Molecular Diagnosis
A	F	First degree	23	6	NP	6	NP
B	M	Remote	21	6	6	0	0
C	M	Remote	24	5	9	4	4
D	F	Negative	42	19	41	28	29
E	F	Negative	50	27	46	20	20
F	F	Negative	23	9	20	4	6
G	M	Negative	37	3	36	33	33
H	F	Negative	38	9	27	16	17
I	F	Negative	37	8	8	8	0
Ratio/ Mean	6F:3M	3P:5N	32,78 ± 10,32	10,22 ± 7,76	24,12 ± 15,80	13,22 ± 11,66	13,63 ± 13,02

F: female; M: male; P: positive; N: negative; NP: not performed; ±: standard deviation.

Physical evaluation (Table 2)

Calf hypertrophy was identified in four patients and five patients presented joint contractures with significant reduced joint mobility (elbow, knee and ankle). Facial weakness was absent in all of the patients.

Generalized muscle atrophy was present in five patients, focal atrophy of the arm in patient F and proximal atrophy of both upper and lower limbs was present in two patients (H and I). Only one patient (patient E) had no apparent clinical muscle atrophy, despite significant muscle weakness and probably overshadowed by obesity.

There were five patients with severe respiratory syndrome, four with LGMD2C (patients A-C, G) and one with LGMD2D (patient E). Two of them required intermittent ventilator support (patients A and E).

Only one patient presented combined symptomatic dilated cardiomyopathy and severe respiratory syndrome (patient A). Four patients had no respiratory or cardiac malfunction.

The CK levels were elevated in all patients. The highest and the lowest values were of 7376 UI/L and 328 UI/L, respectively, both in patients with LGMD2C.

Table 2 – Physical evaluation

Patient	Calf hypertrophy	Muscle contractures	Muscle atrophy	DCMP	SRS	CK (UI/L)
A	No	Yes	Generalized	Yes	Yes	3367
B	No	Yes	Generalized	No	Yes	973
C	No	Yes	Generalized	No	Yes	1479
D	Yes	Yes	Generalized	No	No	745
E	Yes	No	No	No	Yes	450
F	Yes	No	Biceps	No	No	7376
G	No	Yes	Generalized	No	Yes	2923
H	Yes	No	Proximal Upper and Lower limb	No	No	1740
I	No	No	Proximal Upper and Lower limb	No	No	328
Ratio/ Mean	4Y:5N	5Y:4N	8Y:1N	1Y:8N	5Y:4N	2153,4 ± 2224,2

Y: Yes; N: No; DCMP: dilated cardiomyopathy; SRS: severe respiratory syndrome; ±: standard deviation

Functional Tests (Table 3)

Five patients, wheelchair bound (patients A-D and G), lost the ability to walk at mean age of 15,60 years and the mean age to walk with support was 18,71 years (n=8). Only one patient kept the ability to walk without support (patient F). From the group of patients with retained ability to walk, one could not rise from the chair even with support (patient E) and the other three were able to do it, but with support (patients F, H and I).

Only two patients retained ability to climb stairs (patients F and I), but with support and only one patient was able to raise the arms above the head (patient F) and the other eight patients were unable to do it even up to the shoulder level. When it was possible to perform the Gowers' maneuver (patients E, F, H, I), it was always positive.

Table 3 – Functional tests

Patient	Walking			Rising From a Chair			Climbing Stairs			Raising Arms			Gowers Maneuver	
	N	WS	I	N	WS	I	N	WS	I	AH	SL	BSL	P	N
A			X			X			X			X	X	
B			X			X			X			X	X	
C			X			X			X			X	X	
D			X			X			X			X	X	
E		X				X			X			X	X	
F	X				X			X		X			X	
G			X			X			X			X	X	
H		X			X				X			X	X	
I		X			X			X				X	X	
Total	**1**	**3**	**5**	**0**	**3**	**6**	**0**	**2**	**7**	**1**	**0**	**8**	**9**	**0**

N – without support; WS – with support; I – impossible; AH – normal/above head; SL – shoulder level; BSL – bellow shoulder level; P- positive; N-negative

Manual Muscle Testing (Tables 4, 5)

Muscle strength, as evaluated by MMT, was reduced in all patents. The highest global score was 130 (patient F) and the lowest 18 (patient B). The global score was below 50% of the normal value in five patients (A, B, C, D and G) (55.5%) and the LLPS was below 50% of the normal partial score in all patients except in patients E and F (77.7%), while the ULPS

was below 50% of the normal partial score in five patients (55.5%). The lowest global scores were present in 57% of the patients with their first symptoms present in the first decade of life.

As expected, the functional tests reflected a more serious motor handicap than the manual muscle test could suggest.

Table 4 – Manual muscle testing: upper limbs

Patient	Arm						Finger						Hand				Total score
	AB		F		E		AB		F		E		E		F		
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	
A	1	1	1	1	1	1	1	1	2	2	1	1	1	1	2	2	**20**
B	0	0	0	0	0	0	0	0	3	3	0	0	0	0	3	3	**12**
C	0	0	0	0	0	0	0	0	3	3	0	0	0	0	3	3	**12**
D	2	2	1	1	3	3	1	1	2	2	2	2	2	2	2	2	**30**
E	2	2	2	2	5	5	5	5	5	5	5	5	5	5	5	5	**68**
F	4+	4+	2	2	5	5	5	5	5	5	5	5	5	5	5	5	**72**
G	0	0	0	0	0	0	4	4	0	0	2-	2-	2-	2-	0	0	**16**
H	1	2	0	0	5	5	4-	4-	4-	4-	5	5	5	5	5	5	**59**
I	2	2	2	2	5	5	5	5	5	5	4-	4-	4	4	5	5	**64**

AB: abduction; F: flexion; E: extension; R: right; L: left.

Table 5 – Manual muscle testing: lower limbs

Patient	Thigh								Leg				Foot				Total score
	F		E		AB		AD		E		F		DF		PF		
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	
A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	**6**
B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	**6**
C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	**6**
D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	**6**
E	0	0	0	0	4	4	0	0	3	3	5	5	5	5	5	5	**44**
F	4-	4-	0	0	4-	4-	4	4	2	2	5	5	5	5	5	5	**58**
G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	**10**
H	0	0	0	0	0	0	1	2	2	2	1	1	1	1	5	5	**21**
I	1	1	0	0	0	0	1	1	2	2	2	2	3	3	5	5	**28**

AB: abduction; AD: adduction; F: flexion; E: extension; DF: dorsiflexion; PF: plantar flexion; R: right; L: left.

Histopathology and Immunophenotypes (Table 6)

Six biopsies performed and processed in the Neuropathology Unit showed severe lesions of dystrophic type and abnormal immunostaining of the sarcoglycans was present in all of them. The most common pathologic findings were increased fiber variability, central nuclei and connective tissue. Necrotic fibers were also a common finding. Inflammatory infiltrates, vascular lesions and rimmed vacuoles were never found in the six muscle specimens.

The immunohistochemistry study showed isolated absence of γ-sarcoglycan labeling in three patient with normal presence of the other three sarcoglycans (patient A, F, G), patients D and E showed absence of labeling of δ-sarcoglycan with irregular (patient D) or normal presence (patient E) of the other sarcoglycans and patient H with isolated absence of labeling of α-sarcoglycan.

Reports of the six biopsies suggested that three patients had α-sarcoglycanopathy and six patients a γ-sarcoglycanopathy.

Table 6 – Histopathologic and Immunohistochemic findings

Patient	Muscle/ Year of biopsy	Fibre variability	Central nuclei	Necrotic fibers	Rimmed vacuoles	Basophilic fibers	Connective tissue/fat infiltration	Level of inflammation	Inflammatory infiltrates	Vascular lesions	Sarcoglycans Immuno histochemistry
A	Deltoid/2001	+++	++	++	-	±	+++	-	-	-	absent γ normal α, β, δ
B	NA										
C	NA										
D	Deltoid/2010	+++	+++	-	-	-	+++	-	-	-	absent δ irregular α, β, γ
E	Deltoid/2008	+++	++	++	-	±	+++	-	-	-	absent δ normal α, β, γ
F	Deltoid/2001	+++	++	++	-	-	+++	-	-	-	absent γ normal α, β, δ
G	Deltoid/2011	+++	++	++	-	-	+++	-	-	-	absent γ normal α, β, δ
H	Deltoid/1998	+++	++	++	-	-	---	-	-	-	absent α normal β, δ, γ
I	NA										

NA: not available; +++: markedly increased; ++: moderately increased; + slight increased; ±: occasional; -: absent.

Molecular Study (Table 7)

All patients, except patient A, had a positive molecular study confirming the clinical and the histopathological data (five γ-sarcoglycanopathies and three α-sarcoglycanopathies). Five patients had a homozygous mutation (four γ-sarcoglycanopathies and one α-sarcoglycanopathy) and the remaining three were compound heterozygous (two α-sarcoglycanopathy and one γ-sarcoglycanopathy). It was identified only two types of mutations, frameshift (patients G and I) and seven missense, both present in γ and α-sarcoglycanopathies. The two male brothers (patients B, C) presented the same type of mutation in exon 9 of the γ-sarcoglycan gene (missense in homozygous state), the mutation c.629A>G in exon 7 of the γ-sarcoglycan gene was found in two patients (patients F and G), in a homozygous state in patient F and as second mutation in patient G, which presented a frameshift mutation in exon 6 (c.525delT), identical to the mutation found in patient I, but in a homozygous state.

The mutations were randomly distributed over the different exons [(exon 3 (n=3), 6 (n=3), 7 (n=3) and 9 (n=2)], without predominance of any of them.

Table 7 – Molecular data: Location of mutations and consequences at protein level

Patient	Location of the mutation	Consequences at protein level
A	Not available	
B	γ sarcoglycan gene exon 9 homozygous c.848G>A	p.Cys283Tyr
C	γ sarcoglycan gene exon 9 homozygous c.848G>A	p.Cys283Tyr
D	α sarcoglycan gene exon 3 homozygous c.229C>T	p.Arg77Cys
E	α sarcoglycan gene exon 3 c.229C>T exon 6 c.739G>A	p.Arg77Cys p.Val247Met
F	γ sarcoglycan gene exon 7 homozygous c.629A>G	p.His210Arg
G	γ sarcoglycan gene exon 6 c.525delT exon 7 c.629A>G	p.Phe175LeufsX20 p.His210Arg
H	α sarcoglycan gene exon 3 c.229C>T exon 7 c.850C>T	p.Arg77Cys p.Arg284Cys
I	γ sarcoglycan gene exon 6 homozygous c.525delT	p.Phe175LeufsX20

Discussion and Conclusion

The limb-girdle muscular dystrophies (LGMD) include a heterogeneous group of progressive disorders mainly affecting the pelvic and shoulder girdle musculature, ranging from severe phenotypes with onset in the first decade of life and rapid progression to milder phenotypes of later onset and slower progression.[14-16] Classified in two forms according to the mode of heredity, currently they comprise twenty-one subtypes, seven autosomal-dominant (LGMD1A to G subtypes) and fourteen autosomal-recessive subtypes (LGMD2A to N subtypes), with seventeen of them having their protein products identified. The LGMD1 is relatively rare and represents probably less than 10% of all LGMD cases.[1] Variable relative distribution of the different subtypes of the LGMD2 form have been presented, probably reflecting different ethnic backgrounds and geographic origins.[17-18] The prevalence of LGMD2 is extremely variable according to the country or region in which it was determined but the overall incidence is 1 for 15000 habitants. Some studies have found that the subtypes LGMD2A, 2C-F and 2I were the most common in each of the different countries where the studies were conducted, with small differences among them.[19]

Sarcoglycans are thought to provide structural support to the plasma membrane and to protect it from the mechanical stress of contractile activity by transmitting the lateral tension generated by muscle contraction to the extracellular matrix.[20] Dystrophin is composed of four distinct functional domains: the N-terminal actin-binding domain, a long central domain containing 24 spectrin-like repeats, a cysteine-rich domain and the C-terminal domain.[21] The critical protective role of dystrophin and of the entire membrane-associated DGC is demonstrated by the fact that genetic defects of dystrophin are responsible for the most common type of muscular dystrophy, the severe Duchenne muscular dystrophy and the milder Becker muscular dystrophy. With a defective DGC, the backbone structure is dismantled so that the cell membrane becomes exposed to muscle contraction stresses. As a consequence,

cell membrane focal ruptures might occur, leading to transient intracellular calcium influx, which triggers a series of pathogenic events that result in muscle degeneration and the dystrophic phenotype.[22-23]

In striated muscle, the complex includes dystroglycans, sarcoglycans and syntrophins. The dystroglycan sub-complex is composed of α-dystroglycan and β-dystroglycan: α-dystroglycan is extracellular and binds to merosin, a laminin sub-unit in the basal lamina, while β-dystroglycan is a transmembrane protein that binds to α-dystroglycan extracellularly and to the cysteine-rich and C-terminal domains of dystrophin within the cell.[24]

The role of the four sarcoglycans in the molecular organization of the DGC is not yet well defined. The sarcoglycan complex is known to form a tight side-association with dystroglycan, but is also involved in composite molecular relationships with other constitutive DGC elements such as α-dystrobevin and syntrophin, neuronal nitric oxide synthase (nNOS) and sarcospan, enforcing the view of its crucial role in stabilizing the whole DGC structure.[3-6] Sarcoglycans are single-pass transmembrane proteins, with a short intracellular tail and a large extracellular glycosylated portion that is rich in conserved cysteine residues. Six sarcoglycans have been cloned so far: α, β, γ, δ, ε, and ζ-sarcoglycan. The α and ε-sarcoglycans, are type I membrane proteins with an extracellular N-terminal domain, whereas β, γ, δ, ζ-sarcoglycan are type II membrane proteins, with an extracellular C-terminal domain. The sarcoglycan complex, excluding γ-sarcoglycan, is thought to form an association with α-dystroglycan via an extracellular proteoglycan, biglycan. The intracellular tail of β and δ-sarcoglycan seems to associate directly with the C-terminus of dystrophin, whereas the N-terminal region of α-dystrobrevin secures sarcoglycans to dystrophin. Two hybrid screens identified γ-filamin as a γ and δ-sarcoglycan interacting protein, and as a result, γ-filamin is reduced in LGMD-2C (γ-sarcoglycan) but not in LGMD-2D (α-sarcoglycan). Since γ-filamin

is an actin-binding protein, it provides, trough the interaction with the sarcoglycan complex, additional structural linkages between the DGC and the actin cytoskeleton.[3]

Occasionally, defective proteins may pass the quality control and reach the cell membrane, where, since they are nonfunctional and thus unstable, they are dismantled and degraded. Defects in each sarcoglycan have destabilizing consequences on the entire sarcoglycan complex.[2-4]

The LGMD2 subtypes C to F, collectively designated as sarcoglycanopathies are the main objective of this study. The present study gives the relative distribution of the different sarcoglycanopathies diagnosed in the Neuromuscular Clinic of the CHUC. They are the second most common AR-LGMD, being LGMD2B the most common diagnosed AR-LGMD.[1]

Sarcoglycanopathies are more common in the pediatric than in adult population, which forms the population of the study. [1] Only two types of sarcoglycanopathies were identified – three patients presented a α-sarcoglycanopathy and six a γ-sarcoglycanopathy. The last one was the more prevalent in this study which goes against most reports that suggest that α-sarcoglycanopathy is usually more common[3-4,7,26,27]; γ-sarcoglycanopathy is diagnosed more frequently in gipsy populations[11] and three patients were of gipsy descent what could explain the higher prevalence of this subtype of sarcoglycanopathy in this patient population.

Four of the six patients with γ-sarcoglycanopathy had a rapidly progressive course, as it has been reported by others.[25] Two of the three patients with α- sarcoglycanopathy had their first symptoms on the second and third decade which goes against studies that report age of onset on the first decade.[26] Patient F, despite the early onset of the disease (9 years of age), shows a less severe progression.

The mean time until biopsy was considerably high which might be explained by the insidious nature of most LGMDs and consequent delay of the patient and family in searching

for medical care.[1] This delay is also responsible for the high mean time from first symptoms to molecular diagnosis. As the natural history of the disease is becoming well known, it is possible, that in near future, this orderly sequence of investigation, muscle biopsy followed by molecular studies, will be any more necessary and it should be possible to proceed immediately to molecular studies.

Pathologic data showed abnormalities of the dystrophic type, as usual. The suspicious of muscle disease caused by pathologic mutations of any of the sarcoglycans genes is provided by immunohistochemic, as it happened in all the cases studied in our centre, and/or immunoblot studies (not available in our centre). It is important to notice that the most abnormal sarcoglycan in the immunohistochemic study may not be the specific sarcoglycan responsible for the muscle disease.

Patients with the youngest age of first symptoms are also those who presented more severe disability at the second decade. Four of the five patients with involvement of the respiratory system, including the patient with cardiac insufficiency, belong to this last group, confirming the impression that the youngest age of onset of first symptoms is associated with a more severe and rapid progression of the disease.

These same patients also developed joint contractures as consequence of increasing weakness and reduced joint mobility. On manual muscle testing all patients had a total score below 160, reflecting the severe muscle weakness characteristic of sarcoglycanopathy. Muscles of lower limbs and proximal segments were the most affected.

Molecular study showed, in the two brothers of gipsy descent, a mutation in exon 9, coding c.848G>A, which has been reported as a founder mutation among this population.[11]

It has been reported that the majority of sarcoglycanopathies are associated with missense mutations that generate substitution of single residues that could lead to a misfolded protein.[16] This molecular aspect is present in six of the eight patients with molecular study.

The molecular study showed that mutations in exons 3, 6 and 7 were the most common but no conclusions can be drawn from the type and location in the gene of the mutation and the severity of clinical phenotype, due to the reduced number of patients. The direct consequence of the defective protein over the integrity of the sarcolemma, the destabilization of the dystrophin-glycoprotein complex and the efficacy of the repair mechanisms of the injured sarcolemma are probably more important in determining the severity and progression of the muscular dystrophy then the mutation itself.[1]

Although different research groups are working on causative strategies for sarcoglycanopathies, currently none of these therapies has reached application in clinical practice.[3] Specific molecular therapeutic modalities in investigation are direct correction of the genetic defect, correction of the pathogenic effect of the genetic defect at primary transcript level, interference in the process of traduction and upper-regulation of analogous molecules.[19] A pilot study using direct injection of adeno-associated virus carrying an α-sarcoglycanopathy cDNA into a test muscle in α-sarcoglycanopathy patients has yielded inconsistent results and has not been pursued further.[28] Therefore treatment is symptomatic and aims at amelioration of locomotor, respiratory and cardiac manifestation of the disease.[4]

Patients with sarcoglycanopathies must be subject of a multidisciplinary approach and an individualized plan of therapeutic intervention must be established.[19]

The gold of the therapeutic approach is to give the patient quality of life and reduce the impact of the disease. This includes a multidisciplinary approach with the contribution of different medical specialties, such as physiotherapy, to preserve locomotor autonomy, optimize the present muscle force and to prevent and correct musculoskeletal deformities[19], cardiology and pneumology, since the involvement of the cardiac and respiratory system is part of the evolution of the disease, orthopedic surgery, to correct scoliosis of over 30 degrees to maintain posture, maximize pulmonary function and minimize musculoskeletal pain[29], and

psychological and psychiatric support to help the adequate insertion of the patient in the society at a familiar and professional level, and to help him to accept the impact that the progression of the disease is going to have in his life.

Several genetic therapeutic trials are being conducted and it is expected that with new technologies the natural history of sarcoglycanopathies can be altered in the next few years.

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