Clinical and genetic spectrum in limb-girdle muscular dystrophy type 2E

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ABSTRACT

Objective: To determine the clinical spectrum of limb-girdle muscular dystrophy 2E (LGMD2E) and to investigate whether genetic or biochemical features can predict the phenotype of the disease.

Methods: All LGMD2E patients followed in participating centers were included. A specific clinical protocol was created, including quantitative evaluation of motor, respiratory, and cardiac function. Phenotype was defined as severe or mild if the age at loss of ambulation occurred before or after 18 years. Molecular analysis of *SGCB* gene and biochemical features of muscle biopsies were reviewed.

Results: Thirty-two patients were included (16 male, 16 female; age 7–67 years; 15 severe, 12 mild, and 5 unknown). Neurologic examination showed proximal muscle weakness in all patients, but distal involvement was also observed in patients with severe disease early in the disease course. Cardiac involvement was observed in 20 patients (63%) even before overt muscle involvement. Six patients had restrictive respiratory insufficiency requiring assisted ventilation (19%). Seventeen different mutations were identified, and 3 were recurrent. The c.377_384dup (13 alleles) was associated with the severe form, the c.-22_10dup (10) with the milder form, and the c.341C>T (9) with both. The entire sarcoglycan complex was undetectable by muscle immunohistochemistry or Western blot in 9/10 severe cases and reduced in 7/7 mild cases. The residual amount of sarcoglycan in muscle resulted a predictor of age at loss of ambulation.

Conclusions: This study expands the spectrum of phenotype in β -sarcoglycanopathy and provides strong evidence that severity of clinical involvement may be predicted by *SGCB* gene mutation and sarcoglycan protein expression. **Neurology® 2015;84:1772–1781**

GLOSSARY

ACE = angiotensin-converting-enzyme; **CK** = creatine kinase; **DG** = dystroglycanopathy; **FVC** = forced vital capacity; **IHC** = immunohistochemistry; **LGMD** = limb-girdle muscular dystrophy; **LoA** = loss of ambulation; **WB** = Western blot.

Sarcoglycanopathies are recessive limb-girdle muscular dystrophies (LGMDs) and represent 20%–25% of all LGMDs and 40%–65% of LGMD cases with infantile onset.^{1,2} Sarcoglycanopathies are caused by mutations in genes encoding 4 transmembrane glycoproteins, α -, β -, γ -, and δ -sarcoglycan, that form a tetrameric complex at the cell membrane of skeletal and cardiac muscle and play an important role in stabilizing the dystrophin-associated glycoprotein complex localized in the sarcolemma of muscle fibers.^{3–7}

Mutations in individual sarcoglycan genes are responsible for LGMD2C (γ -sarcoglycan, *SGCC* gene q12⁸), LGMD2D (α -sarcoglycan, *SGCA*^{9,10}), LGMD2E (β -sarcoglycan, *SGCB*^{11,12}), and LGMD2F (δ -sarcoglycan, *SGCD*¹³). LGMD2C and 2D are the most frequent and extensively studied of the 4 sarcoglycanopathies, with a clinical phenotype characterized by progressive skeletal muscle weakness ranging from a severe Duchenne-like dystrophy to a

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Table 1 Clinical features in limb-girdle muscular dystrophy 2E

	Total	Severe phenotype	Mild phenotype	Unknown phenotype
No. of patients	32	15	12	5
Sex, M/F	16/16	7/8	7/5	2/3
Age at onset, y, average $\pm$ SD (range)	9.3 $\pm$ 8.9 (1.5–40)	4.8 $\pm$ 2.4 (1.5–9)	16.4 $\pm$ 11.2 (1.5–40)	5.8 $\pm$ 2.4 (3–9)
Age at last evaluation, y, average $\pm$ SD (range)	32 $\pm$ 19.6 (7–67)	28 $\pm$ 17.9 (13–63)	45 $\pm$ 15.6 (19–67)	10.4 $\pm$ 3.4 (7–16)
Deceased patients, n (%)	2 (6)	1 (7)	1 (8)	0
Motor function, n (%)				
Signs and symptoms				
Loss of independent ambulation	20 (63)	15 (100)	5 (42)	0
Calf hypertrophy	25 (78)	11 (73)	10 (83)	4 (80)
Tendon contractures	22 (69)	13 (87)	6 (50)	3 (60)
Scoliosis	19 (59)	10 (67)	7 (58)	2 (40)
Scapular winging	19 (59)	8 (53)	9 (75)	2 (40)
Tiptoe gait pattern	18 (56)	12 (80)	6 (50)	2 (40)
Muscle pain	10 (31)	0	9 (75)	2 (40)
Macroglossia	10 (31)	6 (40)	3 (25)	1 (20)
Dysphagia	1 (3)	0	1 (8)	0
Osteoporosis	7 (22)	5 (33)	1 (8)	1 (20)
Muscle function treatment				
Physical therapy	17 (63)	9 (60)	8 (67)	3 (60)
Drug therapy: glucocorticoids	7 (22)	4 (27)	2 (17)	1 (20)
Creatine and vitamins	3 (9)	3 (20)	0	0
Cardiac function, n (%)				
Cardiac involvement	20 (63)	11 (73)	7 (58)	2 (40)
Dilated cardiomyopathy	6 (19)	5 (33)	1 (8)	0
Hypertrophic cardiomyopathy	2 (6)	1 (7)	1 (8)	0
Wall motion abnormalities	8 (25)	4 (27)	4 (33)	0
Other ^a	3 (9)	0	1 (8)	2 (40)
Heart rhythm abnormalities ^b	9 (28)	5 (33)	4 (33)	0
Signs and symptoms				
Palpitations	10 (31)	6 (40)	4 (33)	0
Edemas	6 (19)	6 (40)	0	0
Other ^c	8 (25)	3 (20)	5 (42)	0
Drug treatment for cardiac function (angiotensin-converting-enzyme inhibitor; β -blocker; digoxin; diuretic)				
One drug	10 (31)	2 (13)	6 (50)	2 (40)
Two drugs	3 (9)	2 (13)	1 (8)	0
Three or more drugs	3 (9)	3 (19)	0	0
Respiratory function, n (%)				
Respiratory support	6 (19)	4 (27)	2 (17)	0
Invasive	4 (13)	3 (19)	1 (8)	0
Noninvasive	2 (6)	1 (7)	1 (8)	0
Signs and symptoms				
Dyspnea at rest	5 (16)	4 (27)	1 (8)	0
Effort dyspnea	5 (16)	1 (7)	4 (33)	0

Continued

Table 1 Continued

	Total	Severe phenotype	Mild phenotype	Unknown phenotype
Morning headaches	3 (9)	2 (13)	1 (8)	0
Other ^d	4 (13)	1 (7)	3 (25)	0

^a Other: ischemic cardiopathy; asymptomatic pseudonecrosis signs at ECG.

^b Heart rhythm abnormalities: ventricular ectopic beats, left/right branch block; third degree atrioventricular block; extrasystolic arrhythmia; episodic ventricular tachycardia.

^c Other: syncope; chest pain; hypotension; cardiac dyspnea.

^d Other: recurrent pneumonias; orthopnea; ineffective cough; difficulty in singing.

relatively mild LGMD.^{14–19} LGMD2E is a rare cause of sarcoglycanopathy, with an estimated prevalence of 0.86×10^{-6} cases,²⁰ and it has generally been considered a severe muscular dystrophy often associated with cardiomyopathy.^{21–28} Knowledge about the specific features of this disease is scarce, because no large cohort has been reported. The aim of this study was to detail clinical, biochemical, and molecular data from a large population of patients with LGMD2E, to evaluate the specific clinical pattern of the disease, and to identify possible correlations among phenotype, genotype, and protein expression levels to recognize prognostic factors.

METHODS **Standard protocol approvals and patient consents.** The study was designed as an observational, cross-sectional study of natural course of LGMD2E. The study was approved by institutional review boards at participating centers. Written informed consent was obtained by patients or parents.

Study design and patient cohort. All patients diagnosed with LGMD2E underwent an extensive clinical evaluation or review of medical records. Clinical characteristics at time of diagnosis and progression were evaluated by review of medical records and specific questionnaire. Patients were divided into subgroups according to phenotype, defined as severe or mild if loss of ambulation (LoA) occurred before or after 18 years of age. The phenotype of patients younger than 18 years and still ambulant was defined as unknown.

Muscle function. Family and medical history were recorded. Patients were asked about first symptoms, age at onset, and age at loss of specific functions (running, climbing stairs, rising from a chair, walking without support). Pharmacologic treatments were recorded. Muscle weakness was evaluated by manual muscle testing, graded according to Medical Research Council score from 0 (no contraction) to 5 (normal). A cross-sectional analysis of creatine kinase (CK) values was performed using rank-transformed values in an analysis of covariance model with disease duration and phenotype.

Cardiac function. Symptoms and signs of cardiac involvement were noted. Patients underwent cardiac evaluation, including physical examination, electrocardiography, and echocardiography.

Respiratory function. The respiratory section of the questionnaire investigated signs and symptoms of chronic respiratory insufficiency (rest/exercise dyspnea, morning headache,

pneumonia, ineffective cough) and use of mechanical respiratory support. The respiratory evaluation included sitting forced vital capacity (FVC).

Sarcoglycan protein studies. At the time of diagnosis, an open skeletal muscle biopsy was obtained after informed consent from patients/parents. The level of sarcoglycans was assessed by Western blot (WB) or immunohistochemistry (IHC) by visual inspection using a panel of monoclonal antibodies (Novocastra, Newcastle-upon-Tyne, UK) and scored as normal, slightly reduced, markedly reduced, or absent.

SGCB gene analyses. The identification of *SGCB* gene mutations was obtained by single-strand conformation polymorphism/direct sequencing or direct sequencing of genomic DNA extracted either from muscle or blood leukocytes.

RESULTS **Patient cohort.** Thirty-two patients from 25 families (16 male, 16 female) were included. According to phenotype classification, 15 cases were classified as severe, 12 mild, and 5 had unknown phenotype. The age at last evaluation ranged from 7 to 67 years. Most patients were of Caucasian descent (15 from Italy, 7 from Denmark, 3 from France, 1 from Albania, and 1 from Greece); 3 unrelated patients originated from the Tunisian Sephardic Jewish community, 2 siblings were Jordanian, and 1 patient was from the Ivory Coast. Consanguinity was reported in 7 families. Two patients were deceased: a patient with severe disease at age 14 years due to cardiac failure despite pharmacologic treatment with diuretics, angiotensin-converting-enzyme (ACE) inhibitors, and β -blockers started at age 12 years when diagnosed with dilated cardiomyopathy, and a patient with mild disease at 60 years from pneumonia.

Muscle function. The main symptoms are listed in table 1 and table e-1 on the *Neurology*[®] Web site at Neurology.org. The age at onset ranged from 1.5 to 40 years. The first symptoms occurred before age 10 years in all patients with severe disease and in 4/12 patients with mild disease. At onset, symptoms were predominately in pelvic girdle muscles: difficulty in running (41%), gait abnormalities (34%), and difficulties in rising from the floor (22%). In 4 patients (12%), muscle weakness was associated at disease onset to muscle pain. Three patients had been diagnosed at a presymptomatic stage due to

incidental finding of increased CK levels. All patients with severe disease had lost independent ambulation, in accordance with the definition of severe phenotype, at a mean age of 13 years (range 9–17 years). Age at LoA was between 23 and 59 years in the 5 patients with mild disease (mean 40 years). The decline in muscle function was homogeneous and rapid in the patients with severe disease, while patients with mild disease showed a variable course of the disease (figure 1). Frequent clinical signs included calf hypertrophy (78% of patients), tiptoe gait pattern (56%), tendon contracture (69%), scapular winging (59%), and scoliosis (59%). Muscle pain was reported in 75% of mild, 40% of unknown phenotype, and no severe cases. No rhabdomyolysis events were reported. Macroglossia was found in 31% of patients, with almost double prevalence in severe vs mild cases. Neurologic examination showed proximal muscle weakness in all patients, predominantly in lower limb muscles (figure 2). Muscle weakness was more diffuse in patients with severe disease, involving both proximal and distal muscles. Distal muscles were severely affected only in patients with severe or unknown phenotype. Eight patients with severe disease presented distal weakness before age 18 years (5 of them before 14 years), while no patients with mild

disease did. Axial muscle weakness was found in 88% of examined patients.

CK levels were elevated in 17/22 patients. CK values did not differ significantly between phenotypes, but were inversely and significantly correlated with disease duration ($p < 0.0001$). CK levels were markedly elevated in patients with disease duration shorter than 5 years (median $30.5 \times$ normal value; range 8.2–209, $n = 12$) in comparison with those with disease duration longer than 5 years (median $2.75 \times$ normal value; range 0.6–44 $\times$ normal value; $n = 12$, $p = 0.01$).

Seven patients (4 severe, 2 mild, 1 unknown) had been treated with steroids, deflazacort (starting dose 0.9 mg/kg daily) or cloprednol (10 mg 20 days on/10 days off). The rationale was based on anecdotal reports of beneficial effect of steroids and pathophysiologic similarities with Duchenne muscular dystrophy. The treatment was started in childhood/early adulthood, at different stages of the disease (3–9 years after onset, age 7–25 years), and continued for 6 months to 6 years (table e-2). No clear benefits were reported by patients/parents and no quantitative analysis could be performed retrospectively; however, a trend toward a later LoA in patients with severe disease treated with steroids emerged (13.5 ± 2.7 in treated vs 12.9 ± 2.2 years in untreated patients).

Figure 1 Clinical progression in limb-girdle muscular dystrophy 2E

Age at loss of different clinical abilities and onset of cardiomyopathy or respiratory insufficiency in patients with severe and mild limb-girdle muscular dystrophy 2E. Bars represent median for severe and mild forms. A more rapid motor function deterioration and an earlier onset of cardiomyopathy or respiratory insufficiency in patients with severe disease compared to mild is observed, with a wider variability in the milder patients.

Figure 2 Muscle involvement in limb-girdle muscular dystrophy 2E

Medical Research Council (MRC) score was used to measure muscle strength in 15 muscle groups in the upper and lower limbs and in axial muscles. Bars represent the percent of patients with specific MRC score. All patients with limb-girdle muscular dystrophy 2E presented a significant weakness involving axial, pelvic, and shoulder girdle muscles. Distal muscles were significantly affected in the patients with severe disease. Dist = distal; LL = lower limbs; Prox = proximal; UL = upper limbs.

Cardiac function. Cardiac involvement was found in 20/32 patients (11/15 severe, 7/10 mild, and 2/5 unknown, table 1). Dilated cardiomyopathy was detected in 6 cases, localized wall motion abnormalities in 8, and hypertrophic cardiomyopathy in 2. Conduction or heart rhythm abnormalities were reported in 9 patients, including right/left bundle branch block, third-degree atrioventricular block, supraventricular arrhythmia, ventricular tachycardia, and ventricular ectopic beats (table 1). One patient required pacemaker implantation. One patient with dilated cardiomyopathy had experienced cardiac arrest at 54 years and was resuscitated. One patient presented symptomatic ventricular ectopic beats at age 16 years, without any further cardiologic alteration until the latest echocardiography evaluation age 34 years. Sixteen patients (7 severe, 7 mild, and 2 unknown phenotype) received cardiac treatments (ACE inhibitors, β -blockers, diuretics), most often with a combination of 2 or more drugs.

Respiratory function. Six patients (19%) presented respiratory insufficiency requiring invasive mechanical ventilation (3 severe, 1 mild) or noninvasive ventilation in 2 cases (1 severe and 1 mild) (table 1). Age at mechanical ventilation ranged between 22 and 45 years for patients with severe disease and 38 and 44 years for patients with mild disease (figure 1). Kaplan-Meier analysis of age at ventilation and muscle

phenotype (figure e-1) showed an earlier age at ventilation in patients with severe disease (median age 38; log-rank $p = 0.015$) vs mild. Spirometry data were available for 22 patients (9 severe, 18.9 ± 7.7 years; 10 mild, 40.7 ± 15.3 ; 3 unknown, 11.2 ± 4.4). FVC did not differ significantly among the different phenotypes ($60\% \pm 26\%$ of predicted value in severe, $71\% \pm 33\%$ in mild, and $67\% \pm 6\%$ in unknown cases).

Sarcoglycan protein studies. WB/IHC analyses of 1 or more sarcoglycans were available in 20 patients (table 2 and table e-1). Deficiency of 1 or more sarcoglycans was obvious in all patients. In 11 patients (9 severe, 2 unknown), the deficiency of all sarcoglycans analyzed was complete. No patients presenting with a mild phenotype had a complete deficiency of sarcoglycans. Among milder cases, a variable reduction of sarcoglycans was observed.

To test the hypothesis that a complete vs partial reduction of β -sarcoglycan level may be associated with a more or less severe phenotype, a time-to-event analysis of LoA was carried out comparing patients with absence ($n = 9$) or partial ($n = 9$) expression of β -sarcoglycan protein. The Kaplan-Meier survival curve is shown in figure 3. Median ages at LoA were significantly lower in patients with absence of protein compared to residual protein expression (log-rank $p = 0.0005$). Age at diagnosis

Table 2 SGCB gene mutation and sarcoglycan protein expression studies in limb-girdle muscular dystrophy 2E

Mutation	Exon	Protein change	Type of mutation	Ethnicity	No. of mutated alleles	Sarcoglycan expression	Phenotype
c.-22_10dup	1	p.Ala8Glyfs*22	Frameshift	Tunisia, Ivory Coast, Italy	10: 5 homozygous	↓ or ↓↓	Mild ± CM
c.1A>G ^a	1	p.Met1 ?	Nonsense	Jordan	4: 2 homozygous	0	Unknown + CM
c.33+1 G>C	Intron 1	p.(?)	Splicing	Italy	2: 1 homozygous	NA ^b	Unknown
c.36_37delinsG ^a	2	p.Ser13Valfs*6	Frameshift	Italy	4: 2 homozygous	NA ^b	Mild + CM
c.85A>T	2	p.Arg29*	Nonsense	Italy	1: 1 heterozygous with c.377_384dup	NA ^{b,26}	Severe
c.212T>G	3	p.Leu71Arg	Missense	France	1: 1 heterozygous with c.334_337del	↓	Mild
c.275T>C	3	p.Ile92Thr	Missense	Greece	2: 1 homozygous	NA ^{b,27}	Severe + CM
c.341C>T	3	p.Ser114Phe	Missense	Denmark, France	9: 3 homozygous	↓ or ↓↓	Severe or mild ± CM
					2 heterozygous with c.543C>G	Normal or ↓	Mild, no CM
					1 heterozygous with c.499 G>A	0	Severe + CM
c.334_337del	3	p.Gln112Tyrfs*9	Frameshift	France	1: 1 heterozygous with c.212T>G	↓	Mild
c.377_384dup	3	p.Gly129Glnfs*2	Frameshift	Italy	13: 5 homozygous	0	Severe + CM
					1 heterozygous with c.699_702del	0	Severe + CM
					1 heterozygous with c.552T>G	0	Severe + CM
					1 heterozygous with c.85A>T	0	Severe
c.499 G>A	4	p.Gly167Ser	Missense	Denmark	1: 1 heterozygous with c.341C>T	0	Severe + CM
c.552T>G	4	p.Tyr184*	Nonsense	Italy	3: 1 homozygous	0	Unknown
					1 heterozygous with c.377_384dup	0	Severe + CM
c.518_519insC ^a	4	p.Pro173Profs*12	Frameshift	Albania	2: 1 homozygous	0	Severe + CM
c.543C>G ^a	4	p.Ser181Arg	Missense	Denmark	2: 2 heterozygous with c.341C>T	Normal or ↓	Mild, no CM
c.544A>G	4	p.Thr182Ala	Missense	Italy	2: 1 homozygous	NA ^{c,24}	Severe + CM
c.699_702del	5	p.Phe233Leufs*16	Frameshift	Denmark, Italy	5: 2 homozygous	0	Severe + CM
					1 heterozygous with c.377_384dup	0	Severe + CM
c.754_957del ^a	6	p.Glu252*	Nonsense	Italy	2: 1 homozygous	0	Severe

Abbreviations: ↓ = Slightly reduced; ↓↓ = markedly reduced; 0 = absent; CM = cardiomyopathy; NA = not available.

^a Novel mutation.

^b Predicted absent.

^c Predicted reduced.

of cardiac involvement was independent from residual sarcoglycan expression (figure e-2).

SGCB gene analyses. Both *SGCB* mutated alleles were identified in all patients. Seventeen different mutations were found: 7 missense, 2 nonsense, 1 splice site, and 7 frameshift insertion/duplication or deletion (table 2). Five mutations were novel: c.518_519insC, c.543C>G, c.754_957del, c.36_37delinsG, c.1A>G. Three mutations were recurrent. The 8-bp duplication in exon 3 (c.377_384dup; p.Gly129Glnfs*2; 20% of mutated alleles) was detected only in patients with severe disease

(5 homozygous and 3 compound heterozygous with other null mutations). The c.341C>T (p.Ser114Phe) was observed in 6 cases (14% of mutated alleles; 3 homozygous and 3 compound heterozygous) that presented with a clinical phenotype classified either as severe or mild. The 32-bp duplication in exon 1 (c.-22_10dup; p.Ala8Glyfs*22; 16% of the mutated alleles) predicted a mild phenotype being found only in patients with mild disease (3 from Tunisia, 1 from Italy, and 1 from Ivory Coast). This mutation encompasses the first ATG codon (start codon) and it is predicted to be frameshifting with premature stop codon 22 amino acid

Figure 3 Time-to-event analysis of loss of ambulation comparing presence or absence of sarcoglycan proteins

In case of absence of the protein, median age at loss of ambulation (LoA) is predicted to be 13 years, while if a residual level of protein is present, the LoA is predicted to occur later than 60 years ($p < 10^{-4}$).

downstream. To reconcile the discrepancy among frameshift mutation, biochemical data of residual sarcoglycan expression in skeletal muscle, and mild phenotype, we hypothesize that the c.-22_10dup alters the Kozak consensus sequence, diminishing the amount of protein translated from the first AUG codon and allowing the recognition of the second AUG, predicted to drive the translation of a full-length protein (figures e-3 and e-4). DNA TIS Miner and ATGpr algorithms (http://www.hri.co.jp/atgpr/ATGpr_sim.html)²⁹ confirmed this hypothesis.

DISCUSSION For rare diseases, small patient populations limit the ability to collect valid data, which inhibits progress in research and care. This limitation can be overcome by collaborative efforts among specialized centers sharing experience in clinical evaluation and management of such patients. The aim of this study was to collect accurate clinical, genetic, and protein expression data in a large number of LGMD2E patients and thereby learn whether there exists any genotype/phenotype correlation, and whether prognostic factors can be identified. Four tertiary referral centers contributed to the data collection

and we report the largest series of LGMD2E patients to date.

Muscle weakness in LGMD2E predominates in proximal muscles, with a more severe involvement of lower limbs, similar to other LGMDs. Axial and distal involvement is frequent and prominent in severe forms even at the early stages of disease. This distal pattern of muscle involvement never preceded or surpassed proximal involvement, and did not raise issues of differential diagnosis with distal myopathies, but could help in differentiation between a severe or mild phenotype. Our retrospective study did not allow us to determine the precise age at distal weakness onset and thus we cannot determine its prognostic value in predicting disease course in very early stages of the disease (<10 years of age). Calf and tongue hypertrophy is frequent. LGMD2E patients often present tiptoe walking due to Achilles tendon contractures appearing early in the disease, and subsequently develop contractures in elbows and knees.

The phenotypic spectrum of LGMD2E is broader than previously described, ranging from the severe Duchenne-like to a milder LGMD phenotype. Differential diagnosis among the different sarcoglycanopathies is not feasible on clinical grounds only and molecular analysis of the 4 sarcoglycan genes is mandatory to achieve a definitive diagnosis. More challenging may be differential diagnosis between LGMD2E and dystroglycanopathies (DGs); however, neonatal-onset DGs are characterized by severe phenotype with CNS involvement whereas the later-onset forms are milder and may present only with muscle weakness. The clue to the diagnosis is the study of sarcoglycans and glycosylated-DG on muscle biopsy, where no secondary sarcoglycan deficiency has been reported in DGs.

Cardiac involvement affects more than 50% of patients with LGMD2E^{22–24,28} independently from skeletal muscle phenotype and may occur at all stages of the disease, even before skeletal muscle involvement. This discrepancy is surprising, but has been reported for related muscular dystrophies with cardiac involvement.³⁰ It is usually characterized by dilated cardiomyopathy preceded by ECG or localized wall motion abnormalities or hypertrophic cardiomyopathy. In our cohort, 2 patients had hypertrophic cardiomyopathy, but the correlation with β -sarcoglycanopathy is uncertain because of concomitant hypertension in 1 case and lack of follow-up in the other. Heart rhythm and conduction abnormalities were frequent (28% of patients). Heart conduction disturbance in LGMD2E has been reported before, but after diffuse myocardial involvement.^{21–24} In our series, 2 patients presented symptomatic ventricular ectopic beats or right bundle branch block before the onset of overt cardiac involvement, suggesting the need

for close monitoring of cardiac function. Cardiac muscle involvement did not correlate with residual sarcoglycan expression (figure e-2).

Severe respiratory involvement requiring mechanical ventilation (6/32; 19%) is less frequent than cardiac involvement and occurs later in disease course, often in patients who had lost ambulation. It appears significantly earlier in severe vs mild cases even if FVC did not differ among phenotypes probably because ventilated patients could not perform spirometry and were lost in the analyses. Respiratory muscle involvement never preceded limb muscle involvement as in other LGMDs except LGMD2I, characterized by early and prominent respiratory involvement.³¹

Three mutations were recurrent. The frameshift mutation c.377_384dup was found in 8 patients from Northern Italy. This mutation was found to be in linkage disequilibrium with neighboring polymorphisms in Northern Italy, suggesting a founder effect combined with a relative genetic isolation,³² and our new cases add to this conclusion. All these patients presented with a severe phenotype and showed absence of the 4 sarcoglycan proteins in skeletal muscle. The c.377_384dup is a truncating mutation, and it has been shown that truncated β -sarcoglycan, missing the extracellular domain, accumulate around the nucleus and are not properly localized at the plasma membrane, thus disrupting the sarcoglycan complex assembly.³³ Moreover, β -sarcoglycan is essential for the formation of the β/δ -sarcoglycan core structure, and disruption of this core affects targeting of the sarcoglycan complex to the plasma membrane, which explains the lack of the complex in muscle biopsies.³⁴

The c.-22_10dup homozygous mutation was found in 5 patients presenting mild phenotype and cardiac abnormalities. All patients presented a variable residual level of sarcoglycans according to the hypothesized shift of the initiation codon. The c.341C>T missense mutation, found in 6 patients, is located a few amino acids downstream of the transmembrane domain in the C-terminal extracellular domain. The N-terminal half extracellular domain is important for sarcoglycan interaction and disruption of the β/δ -sarcoglycan interaction will affect membrane localization.³⁵ Immunofluorescence analyses in our patients further confirm these findings. Patients carrying this mutation present variable phenotypes probably due to still unknown molecular mechanisms (i.e., unrecognized polymorphisms in sarcoglycans or related proteins that modify the stability of the complex).

As previously described in smaller cohorts of sarcoglycanopathies, the level of protein expression predicts disease severity.²⁶ In our cohort, absence of

sarcoglycan complex is common in patients with severe disease, and never detected in patients with milder disease. LoA occurred earlier in patients with absence of protein compared to those with residual protein expression. As expected, there was a correlation between the predicted effect of mutations on protein sequence and WB/IHC results. All mutations predicted to cause frameshift or premature termination codon resulted in absent labeling for sarcoglycans, and missense mutations showed variable sarcoglycan labeling, reflecting different impact of individual amino acid changes on sarcoglycan assembly. In patients with absence of β -sarcoglycan, WB and IHC results were comparable, while in patients with mild reduction of sarcoglycans at WB, a normal IHC pattern may be detected, likely due to the non-quantitative nature of IHC (table e-1). In LGMD2E, absence of the entire sarcoglycan complex or partial residual sarcoglycan expression may be detected, as we and others previously reported.^{24,36} Sarcoglycan expression in muscle is a strong predictor of age at LoA and may be used as a prognostic factor in patient stratification for future clinical studies.

There is no primary medical treatment for LGMD2E.³⁷ Several studies are currently ongoing, including gene therapy and approaches aimed at rescuing misfolded sarcoglycans at the cell membrane, by assisting their maturation along the ER secretory pathway.^{3,38} An accurate knowledge of clinical features and natural history of each sarcoglycanopathy is therefore crucial to determine inclusion criteria and outcome measures in future clinical trials.

This study expands the spectrum of phenotype in β -sarcoglycanopathy and provides strong evidence that severity of clinical involvement may be predicted by *SGCB* gene mutation and sarcoglycan protein expression.

AUTHOR CONTRIBUTIONS

Claudio Semplicini contributed to the design and conceptualization of the study, selection and clinical evaluation of patients, and analysis and interpretation of data. John Vissing contributed to the design and conceptualization of the study, selection and clinical evaluation of patients, and analysis and interpretation of data. Julia R. Dahlqvist contributed to the selection and clinical evaluation of patients. Tanya Stojkovic contributed to the selection and clinical evaluation of patients. Luca Bello contributed to the selection and clinical evaluation of patients and analysis and interpretation of data. Nanna Witting contributed to the selection and clinical evaluation of patients. Morten Duno contributed to the molecular analyses. France Leturcq contributed to the molecular analyses. Cinzia Bertolin contributed to the molecular analyses. Paola D'Ambrosio contributed to the selection and clinical evaluation of patients. Bruno Eymard contributed to the selection and clinical evaluation of patients. Corrado Angelini contributed to the selection and clinical evaluation of patients. Luisa Politano contributed to the selection and clinical evaluation of patients. Pascal Laforêt contributed to the design and conceptualization of the study, selection and clinical evaluation of patients, and analysis and interpretation of data. Elena Pegoraro contributed to the design and conceptualization of the study, selection and clinical evaluation of patients, and analysis and interpretation of data.

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