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**Clinical correlations and long-term follow-up in 100 patients with sarcoglycanopathies**

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**Running head:** Long-term follow-up in sarcoglycanopathies

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## ABSTRACT

**Background:** To describe a large series of patients with  $\alpha$ ,  $\beta$  and  $\gamma$  sarcoglycanopathies (LGMD-R3, R4 and R5) and study phenotypic correlations and disease progression.

**Methods:** A multicentric retrospective study in 4 centers in the Paris area collecting neuromuscular, respiratory, cardiac, histologic and genetic data. The primary outcome of progression was age of loss of ambulation (LoA); disease severity was established according to LoA before or after 18 years of age. Time-to-event analysis was performed.

**Results:** 100 patients (54  $\gamma$ -SG; 41  $\alpha$ -SG; 5  $\beta$ -SG) from 80 families were included.  $\gamma$ -SG patients had earlier disease onset than  $\alpha$ -SG patients (5.5 vs 8 years,  $p=0.022$ ) and  $\beta$ -SG patients (24.4 years). Axial muscle weakness and joint contractures were frequent and exercise intolerance was observed. At mean follow-up of 22.9 years, 65.3% of patients were wheelchair-bound (66.7%  $\alpha$ -SG, 67.3%  $\gamma$ -SG, 40%  $\beta$ -SG). Dilated cardiomyopathy occurred in all sarcoglycanopathy subtypes, especially in  $\gamma$ -SG patients ( $p=0.01$ ). Thirty patients were ventilated and 6 died. Absent sarcoglycan protein expression on muscle biopsy and younger age at onset were associated with earlier time-to-LoA ( $p=0.021$  and  $p=0.002$ ). Age at onset was an independent predictor of both severity and time-to-LoA ( $p=0.0004$  and  $p=0.009$ ).  $\alpha$ -SG patients showed genetic heterogeneity, while >90% of  $\gamma$ -SG patients carried the homozygous p.Phe175Leufs\*20 frameshift variant. Five new mutations were identified.

**Conclusion:** This large multicentric series delineates the clinical spectrum of patients with sarcoglycanopathies. Age at disease onset is an independent predictor of severity of disease and LoA and should be taken into account in future clinical trials.

## INTRODUCTION

Sarcoglycanopathies are recessive limb-girdle muscular dystrophies (LGMD) caused by mutations in transmembrane glycoproteins known as sarcoglycans (SG).<sup>1</sup> A tetrameric subcomplex of SG ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ -SG) forms part of the dystrophin-associated glycoprotein complex (DGC) which confers stability to the sarcolemma and protects striated muscle fibers from contraction-induced damage.<sup>2,3</sup>

The prevalence of the different sarcoglycans varies across the world. Mutations in  $\alpha$ -SG (LGMDR3) are most prevalent in Europe,<sup>4</sup> whereas mutations in  $\gamma$ -SG (LGMDR5) are most common in the North African population.<sup>5</sup> Mutations in  $\beta$ -SG (LGMDR4) are represented in different proportions around the world,<sup>4,6,7</sup> while mutations in  $\delta$ -SG (LGMDR6) are almost exclusive to the Brazilian population.<sup>8</sup> Many pathogenic mutations have been reported, and some particular hotspots have been described, such as the c.525delT mutation in Northern African patients with  $\gamma$ -SG and the c.229C>T mutation in  $\alpha$ -SG patients.<sup>1,4,9,10</sup>

The clinical presentation of sarcoglycanopathies is that of an LGMD characterized by symmetric involvement of the trunk and proximal limb muscles of pelvic and shoulder girdles, with diverse degrees of cardiorespiratory involvement.<sup>11-16</sup> However, little is known about the progression of the disease over the patient's lifespan. Although diagnosis is genetically determined, absent sarcoglycan immunostaining in muscle biopsies increases the chance of reaching the genetic diagnosis<sup>4,9</sup> and may be correlated to disease severity.<sup>16-18</sup>

Despite the emergence of a number of gene therapy trials in this disease (NCT03652259, NCT01976091, NCT01344798), predictors of disease progression are lacking.

The aim of this study is to present a large series of patients with sarcoglycan mutations in order to provide information on the long-term follow-up of the disease and search for



correlations with clinical, molecular and histologic data that could improve our understanding of the natural history of the disease and assist clinical trial development.

## **METHODS**

### **Study design**

We retrospectively reviewed the medical records of genetically confirmed sarcoglycan patients followed-up between 1999 and 2015 at Pitié-Salpêtrière, Raymond Poincaré, Necker and Armand-Trousseau Hospitals' Nord-Est/Ile-de-France neuromuscular centers, and the Cardiology Department of Cochin Hospital in the Paris region.

Neurologic, cardiac and respiratory assessments at first presentation and at last annual or biannual follow-up visit were collected. Where available, data concerning muscle biopsies were recovered. The population included both pediatric and adult patients.

As required under French legislation, the study was approved by the local Ethics Committee.

### **Clinical definitions**

The 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, and a mean MRC score was calculated from proximal and distal muscles in upper and lower limbs.

Skeletal muscle disease severity was defined by the age at loss of ambulation (LoA):<sup>12</sup> the patients were categorized as severe or “Duchenne-like” if ambulation was lost at or before 18 years of age and mild or “Becker-like” if LoA was after 18 years. Patients still ambulant and younger than 18 years of age were considered as having an “undefined phenotype”.

Respiratory insufficiency was considered when forced vital capacity (FVC) was less than 70% of its predicted value. Spirometry was performed in the upright position.

Cardiomyopathy was defined by a left ventricular ejection fraction (LVEF) below 50% on transthoracic echocardiography (TTE).

### **Sarcoglycan gene analysis**

Genomic DNA was extracted from patient's whole blood using standard procedures. Exons and flanking intronic sequences of the four SG genes were amplified by PCR using primers and sequenced using the Big Dye Terminator-1 Applied Biosystems kit and the Applied Biosystems 3130XL sequencer (Applied Biosystems, Foster City, CA) as previously described.<sup>4</sup> Quantitative fluorescent PCR analysis and RNA extraction and RT-PCR analysis were also performed.

### **Biopsy immunohistochemistry (IHC) and western blot (WB) analysis**

IHC was performed on muscle surgical biopsy samples for dystrophin, dysferlin and sarcoglycans according to standard procedures. Muscle protein analysis was performed by multiplex WB method using a previously described procedure.<sup>19,20</sup> Monoclonal antibodies used for multiplex WB were from Novocastra (Newcastle, UK; [www.novocastra.co.uk](http://www.novocastra.co.uk)).

### **Statistical analysis**

Data analysis was carried out using SPSS 26 (IBM). Mean, standard deviation and range of quantitative variables and absolute or relative frequencies of categorical variables were reported. Association was studied using the Student's t-test (continuous dependent variables), Chi-squared test (categorical dependent variables) and one-way ANOVA (categorical dependent variables for more than two groups) after verifying normality using the Shapiro-Wilk test. Regression analyses were performed to search for predictive variables for severity (logistic) and LoA (linear).  $\beta$ -SG cases were excluded from associative and predictive analysis and were only described given the low number of patients (n=5). A time-to-event analysis for LoA was undertaken, using Kaplan-Meier survival curves and log-rank analysis. Statistical significance was established at  $p \leq 0.05$ .

### **Data availability statement**

The anonymized dataset for this series of patients will be shared by the authors upon request from any qualified investigator.

## RESULTS

### Clinical and morphological skeletal muscle involvement

Among the 100 patients included in the study (females=54%),  $\gamma$ -SG patients accounted for 54% of the cohort while 41% were  $\alpha$ -SG and 5%  $\beta$ -SG patients. The majority (88.9%) of  $\gamma$ -SG patients were of North African origin (Algeria, Morocco or Tunisia) while  $\alpha$ -SG patients were mainly of European origin (70.7%). Of the 5  $\beta$ -SG patients, one patient was European, one was from Guadeloupe and three were from Tunisia. Consanguinity was observed in 44% of all patients, been highest in  $\gamma$ -SG patients (72.3% vs 33.3% in  $\alpha$ -SG;  $p=0.001$ ).

Mean age at symptom onset in the global cohort was  $7.6\pm 6.9$  years (range=0-42). Symptom onset was earlier in  $\gamma$ -SG patients than in  $\alpha$ -SG patients ( $5.5\pm 3.3$  years vs  $8\pm 5.7$  years;  $p=0.022$ ) and was  $24.4\pm 14.3$  years for  $\beta$ -SG patients (table 1). The first symptom was difficulty walking in 54% of the patients whereas the rest of the patients presented with other, less frequent symptoms. Isolated CK elevation without weakness was detected in only 4% patients at onset of the disease, while pseudo-metabolic symptoms, such as exercise intolerance and exercise-induced myalgia, were present in 11% of patients.

Muscle biopsy was performed for diagnostic purposes in 58 patients (31  $\alpha$ -SG, 22  $\gamma$ -SG, 5  $\beta$ -SG) and IHC or WB results were available in 42 patients (25  $\alpha$ -SG, 13  $\gamma$ -SG, 4  $\beta$ -SG).

Expression of one or more sarcoglycans was reduced in 47.6% of biopsies (IHC or WB), while it was absent in 52.4% (12  $\alpha$ -SG, 9  $\gamma$ -SG, 1  $\beta$ -SG) (table 2).

Mean age at last examination was  $30.8\pm 14.7$  years (range=4-70) and  $\gamma$ -SG patients were younger compared to other SG groups ( $\gamma$ -SG  $25.5\pm 12.8$ ,  $\alpha$ -SG  $35.5\pm 13$ ,  $\beta$ -SG  $47.6\pm 21.9$ ;  $p=0.001$ ). All patients with sarcoglycanopathies showed a predominantly proximal muscle weakness of all four limbs (figure 1), and axial weakness was almost invariably present (91.5%). Joint contractures, when recorded on clinical reports (85 patients), were present in 81.2% of patients and across all sarcoglycan groups (table 1). Considering only patients still

ambulant at last evaluation (33 in total; 13  $\alpha$ -SG, 17  $\gamma$ -SG and 3  $\beta$ -SG), 17 patients presented joint contractures: 8 with isolated Achilles involvement (1  $\alpha$ -SG, 6  $\gamma$ -SG and 1  $\beta$ -SG); 5 involving the knees (3  $\alpha$ -SG, 2  $\gamma$ -SG) and 4 involving also the hips (2  $\alpha$ -SG, 2  $\gamma$ -SG). Other frequent clinical findings were calf pseudohypertrophy (78.7%), scapular winging (64.7%) and scoliosis (63.3%). These features did not differ significantly between sarcoglycan groups. Among infrequent findings in sarcoglycanopathies, swallowing difficulties were referred to by 18.3% of patients and cognitive or behavioral disturbances were found in 4  $\gamma$ -SG patients. At mean follow-up of  $22.9 \pm 13.5$  years (range=2-65), six deaths had been notified, all involving  $\gamma$ -SG patients. Overall, 63% of patients were wheelchair-bound (66.67% of  $\alpha$ -SG patients, 67.3% of  $\gamma$ -SG patients, 40% of  $\beta$ -SG patients). LoA generally occurred in the first 2 decades of life (mean age of  $17.8 \pm 10.2$  years, range=8-60) and did not differ between  $\alpha$ -SG and  $\gamma$ -SG patients ( $17.6 \pm 10.5$  vs  $17.1 \pm 7.5$ ) (table 1). Two out of five  $\beta$ -SG patients had lost ambulation at last evaluation, respectively at 8 and 59 years of age. Disease severity was considered mild (LoA > 18 years of age) in 41.8% of patients and severe (LoA  $\leq$  18 years of age) in 43.9% of patients. The remaining patients (14.3%), all  $\gamma$ -SG, were considered as having an “undefined phenotype” (meaning still ambulatory but younger than 18 years).

### **Respiratory involvement**

FVC was low (less than 70% of the predicted value) in 74.2% of patients. Low FVC was slightly more frequent in  $\gamma$ -SG than in  $\alpha$ -SG patients (80.4% vs 71.1%) and was present in 2  $\beta$ -SG patients. The mean percent of theoretical FVC was  $52.4\% \pm 31.7$  in  $\alpha$ -SG,  $52\% \pm 27.41$  in  $\gamma$ -SG and  $91.3\% \pm 25.45$  in  $\beta$ -SG patients. There was a non-significant trend towards greater use of non-invasive ventilation (NIV) among  $\alpha$ -SG patients (47.1% vs 31.8% in  $\gamma$ -SG, and none in  $\beta$ -SG). Two patients, one  $\alpha$ -SG and one  $\gamma$ -SG, had been tracheotomized (figure 2).

### **Cardiac involvement**

Cardiomyopathy, specifically a dilated cardiomyopathy (DCM), was present in 25% of patients and was more frequent in  $\gamma$ -SG than in  $\alpha$ -SG patients after matching patients for age and sex (37.2% vs 11.1% respectively,  $p=0.01$ ). One patient with  $\beta$ -SG developed a DCM (figure 2). Eleven patients had severe cardiomyopathy with an LVEF  $\leq 35\%$  and were all in the  $\gamma$ -SG group. In this group, 54.8% of patients were receiving cardiotropic treatment (vs 23.7% of  $\alpha$ -SG patients;  $p=0.005$ ); this mainly consisted in angiotensin-converting-enzyme inhibitor and/or angiotensin II antagonists and beta-blockers, respectively prescribed in 26% and 17% of all patients. One patient with  $\gamma$ -SG was implanted with a cardioverter defibrillator in primary prevention for sudden death as he had an LVEF below 30% and associated conduction defects on ECG.

Electrical abnormalities were diagnosed in 41.5% of patients, including sinus tachycardia in 10% of patients, premature ventricular contractions in 7% and left and right bundle branch blocks in 5% and 10% of patients, respectively. None of the patients developed atrial fibrillation, high-degree blocks or ventricular tachyarrhythmias.

### **Genetic results**

Results are represented in figure 3. The  $\alpha$ -SG group showed genetic heterogeneity; however, 18 patients (43.9%) presented at least one c.229C>T (p.Arg77Cys) missense variant, 5 of them in homozygous state. Two variants have not previously been reported in the Leiden Open Variation Database (LOVD) and ClinVar database: an exon 5 c.434C>A (p.Ala545Glu) and an exon 7 c.793G>A (Gly265Ser) missense variant.

Among the  $\gamma$ -SG group, the c.525delT (p.Phe175Leufs\*20) frameshift variant was present in 49 patients (90.7%) at homozygous state and a further two patients carried this variant in heterozygous state. We report a new exon 8 c.818\_821delACCT (p.Tyr273Lysfs\*6) frameshift homozygote mutation in two siblings from consanguineous parents.

In the  $\beta$ -SG group, three patients carried the c.-22\_10dup32 (p.Ala8Glyfs\*22) frameshift variant in homozygous state. We report two new variants: an exon 5 c.654\_655DelAA (p.Tyr219Serfs\*2) frameshift variant and an exon 3 c.290G>A (p.Cys97Tyr) missense variant.

### **Clinical predictors of loss of ambulation and other association studies**

A time-to-LoA analysis was performed, both since birth and since the onset of disease, to account for different durations of the disease. The sarcoglycan group (excluding  $\beta$ -SG patients) ( $p=0.05$ ), sarcoglycan protein expression on muscle IHC and WB ( $p=0.022$ ), and age at onset (<10 years vs  $\geq 10$  years) ( $p=0.000$ ), but not sex ( $p=0.139$ ) were associated with time-to-LoA since birth. Sarcoglycan protein expression ( $p=0.021$ ) and age at onset before or after 10 years ( $p=0.002$ ) were also associated with time-to-LoA since the onset of the disease (figure 4). When subdividing age at onset into the categories <5, 5-9, 10-15 and >15 years, we found that the biggest difference in time-to-LoA was when onset of disease occurred before or after 10 years of age.

Furthermore, a younger age at onset was found to be an independent predictor for a severe phenotype ( $p=0.0004$ ) and for earlier LoA ( $p=0.009$ ) on multivariate regression.

With regard to other association studies, the percent of severe phenotypes was significantly higher in  $\gamma$ -SG than in  $\alpha$ -SG patients (60% vs 46.3%;  $p=0.001$ ), although this difference was lost after matching patients based on age and sex. Overall, male patients showed a non-significant higher frequency of severe phenotypes than female patients ( $p=0.077$ ). Patients with absent protein expression more frequently showed a severe phenotype (absent-SG: 62.5% vs reduced-SG: 27.8%  $p=0.042$ ) and a tendency towards an earlier onset of symptoms (absent:  $6\pm 6.1$  vs reduced:  $11.5\pm 10.8$ ;  $p=0.071$ ) and a low FVC (absent: 80% vs reduced: 50%,  $p=0.052$ ). With regard to genotype-phenotype associations, the presence of two, one or none missense variants was not associated with phenotype severity or age of LoA ( $p=0.196$

and  $p=0.967$ , respectively). Finally, LoA was associated with low FVC ( $p<0.001$ ) but not with cardiomyopathy on multivariate analysis ( $p=0.559$ ).

## DISCUSSION

We have described a large multicentric series of patients with  $\alpha$ - ,  $\beta$ - and  $\gamma$ -sarcoglycan mutations in the Paris region and have presented important correlations, including SG protein expression and age at symptom onset, with the ambulatory prognosis of patients with the disease.

Contrary to previous European and American studies,<sup>4,7</sup> half of our patients carried  $\gamma$ -SG mutations, followed closely by patients carrying  $\alpha$ -SG mutations, in accordance with the high prevalence of citizens in France of North African descent (1.846 million according to INSEE [Institut national de la Statistique et des Etudes économiques, France]).<sup>21</sup> As previously reported, most patients with  $\alpha$ -SG and  $\gamma$ -SG had an onset of disease before 10 years of age.<sup>16,17</sup> In our series, patients with  $\beta$ -SG had a mean onset of disease at almost 25 years, which is considerably later than that described in a previous but larger LGMDR4 cohort.<sup>12</sup> Given the low number of  $\beta$ -SG patients in our study, the results for LGMD2R4 should be interpreted with caution. Nevertheless, regardless of the specific subgroup of sarcoglycanopathies, walking disturbances and recurrent falls were the most common symptom at onset, and an insidious onset, such as isolated CK elevation, remained extremely rare in the disease (4%). Interestingly, 11% of patients presented with pseudometabolic symptoms, which had only rarely been reported previously.<sup>16</sup> Pseudometabolic phenotype is a rare but possible presentation of LGMD caused by mutations in sarcolemmal proteins, such as dystrophin (*DMD*), anoctamine 5, dysferlin, and proteins associated with  $\alpha$ -dystroglycan deficiency (fukutin-related protein, GDP-mannose pyrophosphorylase B);<sup>22-26</sup> therefore, mutations in these sarcolemmal proteins, including sarcoglycans, should be considered in undiagnosed patients with pseudometabolic symptoms.

A predominantly proximal muscle weakness of upper and lower limbs associating calf pseudohypertrophy resembling a Duchenne muscular dystrophy (DMD) was similarly observed across all sarcoglycanopathy groups. The almost systematic presence of axial weakness and high frequency of joint contractures, even in ambulant patients, should be considered when establishing rehabilitation protocols and adaptation techniques and could impact the clinical outcomes of any future targeted therapy. Almost two thirds of the  $\alpha$ -SG and  $\gamma$ -SG patients were wheelchair bound at 23 years of mean follow-up. Although the age at LoA, as well as the time-to-LoA when adjusting for the duration of the disease was similar for  $\alpha$ -SG and  $\gamma$ -SG patients, severe forms were significantly more frequent amongst  $\gamma$ -SG patients than amongst  $\alpha$ -SG patients. Therefore, the presence of defects in  $\gamma$ -SG could entail a poorer functional prognosis.

The relationship between sex and phenotype has previously been reported in prevalent myopathies, such as myotonic dystrophy type 1,<sup>27</sup> as well as a sexual dimorphism of skeletal muscle metabolic and gene expression profiles.<sup>28</sup> Although a tendency for male patients to present an earlier age at LoA and severe phenotype was observed in our series of sarcoglycan patients, no definitive conclusion can be drawn from our results.

Another important clinical aspect to consider in sarcoglycanopathies is that one quarter of our patients developed a cardiomyopathy, a finding in line with previous reports.<sup>13,29</sup> Patients with  $\gamma$ -SG showed a significantly higher cardiac burden, with a 3-fold higher risk of cardiomyopathy when compared to  $\alpha$ -SG patients. Nevertheless, 11% of the  $\alpha$ -SG patients presented cardiomyopathy and therefore the known weak expression of  $\alpha$ -SG in heart muscle does not fully protect this subgroup of patients from cardiomyopathy.<sup>29,30</sup> One patient with  $\beta$ -SG also had a DCM; thus, cardiac function should be carefully monitored in all patients with sarcoglycanopathy.



Restrictive respiratory insufficiency is also common in this group of muscular dystrophies, as 75% of all patients had a low FVC, in line with previous reports.<sup>29</sup> The relationship between motor and respiratory decline has previously been described in DMD,<sup>31</sup> and we have shown that LoA is independently associated with low FVC but not with DCM. The fact that respiratory and motor function could show a parallel progression while DCM could progress independently should be taken into account in future muscle-targeted therapies.

Cognitive or behavioral disturbances were found in four  $\gamma$ -SG patients, although  $\gamma$ -SG is expressed only in striated and cardiac muscles.<sup>30</sup> The fact that all four patients are homozygous, three with clear evidence of parental consanguinity, could increase the possibility of other genetic contributors to these behavioral disturbances.

Regarding the IHC and WB muscle biopsy analyses, the significant association of absent sarcoglycan expression on muscle biopsy with time-to-LoA (even after accounting for disease duration) and with a severe phenotype, in conjunction with a non-significant trend towards earlier onset of symptoms and low FVC, underscores the relevance of sarcoglycan muscle IHC and WB in disease prognosis, as previously hypothesized.<sup>16,17</sup> However, in the present study, sarcoglycan expression on muscle biopsy was not an independent predictor of age at LoA and severity of the disease. Muscle morphological data were available for a limited number of patients (42 of the 58 biopsies performed) and could have limited our capacity to obtain a significant result.

Finally, although there is a broad spectrum of mutations among LGMDR3, LGMDR4 and LGMDR5, there were mutational hotspots in each gene (the p.Arg77Cys in  $\alpha$ -SG, the p.Phe175Leufs\*20 in  $\gamma$ -SG and the p.Ala8Glyfs\*22 in  $\beta$ -SG). The number of missense variants did not predict a milder phenotype or later onset of symptoms, possibly because some variants could be located at crucial sites, thus determining the disruption of the whole sarcoglycan complex by affecting interaction between sarcoglycans.

The innate limitation to this retrospective study is that it does not provide as accurate information as a prospective natural history, however, it does show important long-term milestones in the progression of sarcoglycan skeletal myopathy, cardiomyopathy and respiratory failure.

In conclusion, we report a large series of sarcoglycanopathies of multiethnic background where long-term follow-up data show that early age at onset of symptoms, notably before 10 years of age, is an independent predictor of an earlier loss of ambulation. The frequency of joint contractures and axial muscle weakness, the fact that motor and respiratory function may progress in parallel, and especially sarcoglycan expression on muscle biopsy, should also be taken into account in the development of future clinical trials. Finally, although the incidence is higher in  $\gamma$ -SG patients, all sarcoglycan subtypes are at risk of developing a DCM and should therefore be carefully monitored.

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Raquel Guimarães-Costa MD, analysis of medical and records (neurologic, cardiologic, respiratory, histopathologic and genetic data), neurologic evaluation of patients, writing of manuscript. Gorka Fernández-Eulate MD, revision of clinical data and interpretation of genetic data, neurologic evaluation of patients, statistical analysis and writing of the manuscript. Karim Wahbi, MD, PhD, cardiologic evaluation of patients, statistical analysis, revision of manuscript. France Leturcq, MD, genetic analysis and interpretation. Edoardo Malfatti, MD, revision of histopathologic data. Anthony Behin, MD, neurologic evaluation of patients. Sarah Leonard-Louis, MD, neurologic evaluation of patients. Isabelle Desguerre, MD, PhD, neurologic evaluation of patients. Christine Barnerias, MD, neurologic evaluation

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## **DISCLOSURES**

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## FIGURE LEGENDS

### **Figure 1 Manual muscle testing (Medical Research Council [MRC] score) in patients with sarcoglycanopathies at last evaluation**

Bars represent the percent of patients with specific MRC scores. MRC score data were available in 39  $\alpha$ -SG, 43  $\gamma$ -SG and 5  $\beta$ -SG patients. Abbreviations: Prox = proximal; Dist = distal; UL= upper limbs; LL= lower limbs.

### **Figure 2 Respiratory and cardiac assessments in patients with sarcoglycanopathies at last evaluation**

**A)** Number of patients under mechanical ventilation (invasive or non-invasive); Boxplot of predicted forced vital capacity (FVC). **B)** Cardiac electrical abnormalities; **C)** Cardiomyopathy; **D)** Left ventricular ejection fraction (LVEF) on transthoracic echocardiography

### **Figure 3 Spectrum of variants in the alpha (SGCA), gamma (SGCG) and beta (SGCB) sarcoglycan genes**

To accommodate the number and distribution of mutations, the size of exons is not shown to scale. Gray exonic regions represent the 5' and 3' non-coding sequences, whereas dark green exonic regions represent sequences coding for transmembrane domains. Numerals within parentheses indicate, for each mutation, the number of mutated alleles. Variants in orange have not previously been described in ClinVar or LOVD databases.



**Figure 4 Loss of ambulation (LoA) over duration of disease according to several parameters**

**A)** Sarcoglycan group ( $\alpha$ -SG or  $\gamma$ -SG); **B)** Sex; **C)** Sarcoglycan expression measured by IHC or WB (absent vs reduced) on muscle biopsy; **D)** Age at disease onset (<10 years vs  $\geq$ 10 years); **E)** Age at disease onset subgrouped (<5, 5-9, 10-15 and >15 years). Censored patients (had not lost ambulation at last evaluation) are mark as “+”.

**Table 1 Patients' characteristics over follow-up**

	<b>TOTAL (n=100)</b>	<b>ALPHA (n=41)</b>	<b>GAMMA (n=54)</b>	<b>BETA (n=5)</b>	<b>P-value</b>
Probands	80	33	42	5	
Female	54	21 (51.2)	29 (53.7)	4 (80)	0.81
Age at onset of disease	7.6±6.9	8±5.7	5.5±3.3	24.4±14.3	0.022
Age at last evaluation	30.8±14.7	35.5±13	25.5±12.8	47.6±21.9	0.001
<b>Ethnic origin</b>					
Europe	33	29 (70.7)	3 (5.6)	1 (20.0)	
North Africa	60	9 (21.9)	48 (88.9)	3 (60.0)	
Other	7	3 (7.3)	3 (5.6)	1 (20.0)	
Consanguinity	44	10 (33.3)	34 (72.3)	0	0.001
<b>Symptom(s) at onset</b>					
Walking difficulties or recurrent falls	54	19 (46.3)	31 (57.4)	4 (80)	
Pseudometabolic	11	4 (0.1)	6 (0.11)	1 (20)	
Isolated CK	4	1 (0.02)	3 (0.06)	0	
Others	31	17 (41.5)	14 (25.9)	0	
<b>Neuromuscular pattern</b>					
Calf hypertrophy	48	23 (74.2)	21 (80.8)	4 (100)	0.556
Scapular winging	44	24 (70.6)	16 (55.2)	4 (80)	0.205
Joint contractures	69	28 (80)	38 (82.6)	3 (75)	0.765
Scoliosis	31	16 (72.7)	14 (56)	1 (50)	0.234
Cognitive	4	0	4 (8.7)	0	0.59
Swallowing problems	11	6 (24)	4 (13.3)	1 (20)	0.307
Axial weakness	65	33 (91.7)	27 (90)	5 (100)	0.815
<b>Loss of ambulation (LoA)</b>					
Age at LoA	17.8±10.2	17.6±10.5	17.1±7.5	33.5±36.1	
<b>Severity</b>					
Severe (LoA ≤ 18 years old)	43	18 (46.2)	24 (46.2)	1 (20)	0.001
Mild (LoA >18 years old)	41	21 (53.8)	16 (30.8)	4 (80)	
Undefined or still ambulant and <18 years at last evaluation	12	0	12 (23.1)	0	
<b>Respiratory involvement</b>					
Low FVC	66	27 (71.1)	37 (80.4)	2 (40)	0.315
FVC % of theoretical value	(54.4±30.3)	(52.4±31.7)	(52±27.4)	(91.3±25.5)	0.252
Non-invasive ventilation (NIV)	28	15 (47.1)	13 (31.8)	0	0.17
Invasive ventilation (tracheostomy)	2	1 (2.9)	1 (2.3)	0	
<b>Cardiac involvement</b>					
Cardiomyopathy	21	4 (11.1)	16 (37.2)	1 (20)	0.008
ECG abnormalities	34	12 (33.3)	22 (51.2)	0	
Cardiac treatment	35	9 (23.7)	23 (54.8)	3 (60)	0.005

For categorical variables: absolute number of patients with the percent in parenthesis (divided by the number of cases where information was available).

For continuous variables: mean and standard deviation.

Abbreviations: CK = creatine kinase; FVC = forced vital capacity; ECG = electrocardiogram.

**Table 2: Association of muscle biopsy with important clinical parameters in patients with sarcoglycanopathies**

	Sarcoglycan protein expression on Immunohistochemistry or Western blot		P-value
	Reduced (n=20)	Absent (n=22)	
<b>Group</b>			0.238
Alpha	13	12	
Gamma	4	9	
Beta	3	1	
<b>Sex</b>			0.746
Male	9	11	
Female	11	11	
<b>Cardiomyopathy</b>	2	2	0.855
<b>Low FVC</b>	9	16	0.052
<b>Severity</b>			0.042
Mild	13	5	
Severe	6	10	

Abbreviations: FVC = forced vital capacity

Severity: patients were categorized as “severe” if ambulation was lost at or before 18 years of age and “mild” if LoA was after 18 years.