SHORT REPORT

Further evidence for the organisation of the four sarcoglycans proteins within the dystrophin–glycoprotein complex

M Vainzof^{1,2}, ES Moreira², G Ferraz³, MR Passos-Bueno², SK Marie¹ and M Zatz²

¹*Departamento de Neurologia, FMUSP, São Paulo*

²*Departamento de Biologia, IB-USP, São Paulo*

³*Departamento de Genética, UFPE, Recife, PE, Brazil*

Based on the pattern of distribution of the SG proteins in patients with LGMD2C and 2D, and on the observed decreased abundance of dystrophin through WB in some sarcoglycans (SG) patients, we have recently suggested that α , β and δ subunits of sarcoglycan complex might be more closely associated and that γ -SG might interact more directly with dystrophin. Two additional SG patients here reported give further support to these suggestions: an LGMD2F patient showed patchy labelling for γ -SG, despite the lack of staining of the other three SG proteins; an LGMD2C boy showed deficiency in dystrophin by means of WB and IF, comparable with an DMD manifesting carrier. These two patients represent further evidence of a closer relation of α , β and δ -SG than of γ -SG and of the possible association of γ -SG with dystrophin. In addition the LGMD2C patient illustrates the potential risk of misdiagnosis using only dystrophin analysis, in cases with no positive family history, or when DNA analysis is not informative.

Keywords: sarcoglycans; muscular dystrophy; limb-girdle MD

Introduction

The dystrophin–glycoprotein complex (DGC), composed by the dystroglycan and the sarcoglycan sub-complexes, forms an important bridge across the muscle membrane, between dystrophin, in the inner cytoskeleton and merosin, in the basal lamina.^{1,2} The DGC may stabilise the sarcolemma and protect muscle fibres from long-term contraction-induced damage and necrosis.

A primary deficiency of dystrophin in Duchenne muscular dystrophy (DMD) leads to a secondary deficiency of members of the DGC, implying that dystrophin is necessary for the maintenance of the complex.^{3,4} Among the seven mapped autosomal recessive limb-girdle muscular dystrophies, the sarcoglycanopathies (SGpathies) or LGMD2D, 2E, 2C and 2F are caused by a primary lack of one of the sarcoglycan glycoproteins (α -SG, β -SG, γ -SG or δ -SG), resulting in the deficiency of the whole complex.^{5–11}

In a recent study we observed that the primary complete lack of α -SG staining in LGMD2D patients may be associated with a complete lack of β and δ -SG but not of γ -SG staining. In addition, the primary lack of staining for γ -SG was associated with only a partial deficiency of α , β and δ -SG proteins in some LGMD2C

Correspondence: Dr Mariz Vainzof, Depto. de Biologia - IB-USP, R. do Matão, 277, São Paulo, SP - CEP 05508-900, Brazil. Tel: 55 11 3061 4036; Fax: 55 11 818 7419; E-mail: mvainzof@usp.br

Received 24 February 1998; revised 8 July 1998; accepted 18 August 1998

patients. Furthermore, a secondary reduction in dystrophin amount was seen in patients with primary sarcoglycan mutations, which was most marked in patients with primary γ -SG deficiency. Based on the above findings, we have suggested that α , β and δ subunits of sarcoglycan might be more closely associated, whilst γ -SG might interact more directly with dystrophin.¹²

Here we report two additional SGpathy patients with a 'DMD-like presentation' which gives further support to our previous study. In addition, one of them illustrates the potential risk of misdiagnosis in cases with a patchy pattern of dystrophin by means of IF methodology.

Patients and Methods

Patient 1 is a 15-year-old girl, from a Caucasian consanguineous kindred who has three normal sibs. She was wheelchair bound at age 12 and currently shows severe weakness and is unable to raise her arms. DNA analysis showed a novel homozygous missense mutation (E262K) in exon 8 of the δ -SG gene.¹³

Patient 2 is a 9-year-old boy, who has difficulty in running and cannot climb stairs unassisted. He belongs to a highly consanguineous family with 19 additional affected individuals: ten males and nine females. DNA analysis confirmed that he has the 521-T mutation in the γ -SG gene.

Dystrophin was analysed by immunofluorescence (IF) and western blot (WB) with the rabbit polyclonal N-terminal 303-8 (kindly provided by J Chamberlain) and C-terminal monoclonal Dy8/6C5 antibodies (kindly provided by LVB Anderson), as described elsewhere.^{14,12} Sarcoglycans proteins were studied by means of IF, using the following antibodies: α -SG (Ad1/20A6, from Dr LVB Anderson), β -SG,⁶ γ -SG⁹ and δ -SG.¹¹

Results

In patient 1, IF analysis of the sarcoglycan proteins revealed a total absence of δ -SG, as expected, accompanied by the total absence of α -SG and β -SG, but only a partial deficiency of γ -SG (Figure 1).

In patient 2, IF analysis showed the complete absence of the four sarcoglycan glycoproteins from muscle membranes (Figure 1). However, it differed from the previous 5LGMD2C patients we had reported, who showed a positive and apparently normal pattern of dystrophin distribution on muscle fibres. In this patient, we observed a weak IF pattern of dystrophin, with some clusters of strongly positive fibres, which is comparable with the pattern we observed in some DMD manifesting carriers (Figure 1). In addition, a very weak dystrophin band (+/- 10% of normal) was detected using western blot methodology, with antibody directed against the rod domain of dystrophin (Figure 2), thus confirming a dystrophin deficiency.

Discussion

In our previous report, all patients affected by LGMD2E and 2F were severely affected; their muscles were highly degenerate and showed a total absence of the four SG glycoproteins on muscle fibres. Therefore our previous suggestion of the probable association of the SG proteins was indirectly based on observations in six LGMD2D patients who showed a primary deficiency of α -SG and a total deficiency of β - and δ -SG, but not γ -SG, as well as in two LGMD2C patients, who

Figure 1 IF pattern of dystrophin (DYS) and the four sarcoglycans proteins in a control muscle (CON), in the patient with LGMD2F and 2C.

Figure 2 Dystrophin western blot, showing the dystrophin band (D) in each patient (P), as compared with a normal control (C), using the N-terminal and C-terminal antibodies for dystrophin, M = myosin content in muscle extract, as shown by Ponceau pre-stained blot.

had a total absence of γ -SG, but only a partial deficiency of α , β and δ -SG.¹²

The LGMD2F patient here reported also showed patchy labelling for γ -SG, despite the absence of the other three SG proteins and therefore represents another evidence of a closer relation to α , β and δ -SG than to γ -SG.

Moreover, the patchy dystrophin pattern observed in the LGMD2C (γ -SG deficient) patient gives further support to the hypothesis of a closer association between γ -SG and dystrophin. However, since all our patients studied had the same mutation, we cannot rule out the possibility that this specific mutation affects the protein interaction within the sarcoglycan complex. In this respect it will be interesting to verify if patients with different mutations in the γ -SG gene also have the same complex disarrangement.

We have already shown that many SG patients had a very weak band of dystrophin using WB methodology, as a secondary effect of SG deficiency. However, this was not evident using the IF technique, which usually presents a positive pattern.¹² A possible explanation is that, as reported previously for DMD heterozygotes (and mdx carriers), dystrophin present in a mosaic pattern in young individuals and manifesting carriers tends to show a more uniform arrangement on fibre sarcolemma with aging.^{15,16} Consequently, a deficiency in dystrophin quantity, which can occur as a patchy

pattern of distribution, might be transformed to a positive pattern as dystrophin is redistributed on sarcolemma.

Finally, it is important to point out that some LGMD patients can present a reduction in the amount of dystrophin¹² whilst some BMD patients can have normal dystrophin.¹⁷ Therefore, based on the low dystrophin quantity from WB and the patchy pattern from IF analysis, this LGMD2C boy would have been diagnosed as having Becker muscular dystrophy, or as a manifesting DMD carrier if he had been a girl. So in the absence of a positive family history, or when DNA analysis is not informative, results concerning dystrophin must be interpreted with caution.

Acknowledgements

Antibodies were kindly given by Dr Louise Anderson (α -SG, γ -SG and dystrophin), Dr Carsten Bonnemann and Dr LM Kunkel (β -SG), Dr Elizabeth McNally (γ -SG), Dr Vincenzo Nigro (δ -SG) and Dr J Chamberlain (dystrophin, 303-8), to whom we are very grateful. We also wish to thank Marta Canovas for excellent technical assistance. The collaboration of the following persons is also gratefully acknowledged: Dr Rita CM Pavanello, Dr Ivo Pavanello Filho, Cleber Costa, Antonia MP Cerqueira and Constância G Urbani. This work was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), PRONEX, FINEP, PADCT, IAEA, and Associação Brasileira de Distrofia Muscular (ABDIM).

References

- 1 Yoshida M, Ozawa E: Glycoprotein complex anchoring dystrophin to sarcolemma. *J Biochem (Tokyo)* 1990; **108**: 748-752.
- 2 Ervasti JM, Ohlendieck K, Kahl SD, Gaver MG, Campbell KP: Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature* 1990; **345**: 315-319.
- 3 Ohlendieck K, Matsumura K, Ionasescu VV, Campbell KP: Duchenne muscular dystrophy: deficiency of dystrophin-associated proteins in the sarcolemma. *Neurology* 1993; **43**: 1267-1268.
- 4 Ervasti JM, Campbell KP: A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. *J Cell Biol* 1993; **122**: 809-823.
- 5 Roberds SL, Leturcq F, Allamand V *et al*: Missense mutations in the adhalin gene linked to autosomal recessive muscular dystrophy. *Cell* 1994; **78**: 625-633.

- 6 Bonnemann CG, Modi R, Noguchi S *et al*: β -sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. *Nat Genet* 1995; **11**: 266–273.
- 7 Lim LE, Duclos F, Broux O, Bourg N *et al*: β -sarcoglycan (43 DAG): Characterization and involvement in a recessive form of limb-girdle muscular dystrophy linked to chromosome 4q12. *Nat Genet* 1995; **11**: 257–265.
- 8 Noguchi S, McNally EM, Ben Othmane K *et al*: Mutations in the dystrophin-associated protein γ -sarcoglycan in chromosome 13 muscular dystrophy. *Science* 1995; **270**: 819–822.
- 9 McNally EM, Passos-Bueno MR, Bonnemann CG, Vainzof M, Moreira ES *et al*: Mild and severe muscular dystrophy caused by a single γ -sarcoglycan mutation. *Am J Hum Genet* 1996; **59**: 1040–1047.
- 10 Passos-Bueno MR, Moreira ES, Vainzof M, Marie SK, Zatz M: Linkage analysis in autosomal recessive limb-girdle muscular dystrophy (AR-LGMD) maps a sixth form to 5q33-34 (LGMD2F) and indicates that there is at least one more subtype of AR LGMD. *Hum Mol Genet* 1996; **5**: 815–820.
- 11 Nigro V, Moreira HS, Piluso G *et al*: The 5q autosomal recessive limb-girdle muscular dystrophy, LGMD2F, is caused by a mutation in the δ -sarcoglycan gene. *Nat Genet* 1996; **14**: 195–198.
- 12 Vainzof M, Passos-Bueno MR, Moreira ES *et al*: The sarcoglycan complex in the six autosomal recessive limb-girdle (AR-LGMD) muscular dystrophies. *Hum Mol Genet* 1996; **5**: 1963–1969.
- 13 Moreira ES, Vainzof M, Marie SK, Nigro V, Zatz M, Passos-Bueno MR: A first missense mutation in the δ -sarcoglycan gene associated with a severe phenotype and frequency of limb-girdle muscular dystrophy type 2F (LGMD2F) among Brazilian sarcoglycanopathies. (Submitted). *J Med Genet* 1998; **35**: (in press).
- 14 Ho-Kim MA, Bedard A, Vincent M, Rogers PA: Dystrophin: A sensitive and reliable immunochemical assay in tissue and cell culture homogenates. *Biochem Biophys Res Comm* 1991; **181**: 1164–1172.
- 15 Karpati G, Zubrzycka-Gaarn EE, Carpenter S, Bulman DE, Ray PN, Worton RG: Age-related conversion of dystrophin negative to positive fibre segments of skeletal but not cardiac muscle fibres in heterozygote mdx mice. *J Neuropathol Exp Neurol* 1990; **49**: 96–105.
- 16 Vainzof M, Nicholson LVB, Bulman DE *et al*: Sarcolemmal distribution of abnormal dystrophin in Xp21 carriers. *Neuromusc Disord* 1993; **3**: 135–140.
- 17 Vainzof M, Passos-Bueno MR, Pavanetto RCM, Zatz M: Is dystrophin always altered in Becker muscular dystrophy patients? *J Neurol Sci* 1995; **131**: 99–104.