

land, ME), and the region containing DNA strands sized 0.5 to 25 kb was sliced into 10 fractions. Gel slices were melted with Gelase (Epicentre) to release the DNA. DNA was collected by ethanol precipitation and then amplified by PCR. PCR was performed in saturating conditions (35 thermocycles), or during exponential amplification (28 cycles). The PCR products were analyzed by gel electrophoresis and visualized by ethidium bromide (EtBr) staining after saturation PCR. The PCR products derived from the exponential range of amplification that did not display visible bands on EtBr-stained agarose gels were visualized by transferring them to Hybond-N membranes and hybridizing them to digoxigenin-labeled oligonucleotide probes derived from a region within the expected amplification products. Hybridization signals were detected by chemiluminescence according to the manufacturer's instructions (Boehringer-Mannheim). To ensure specific reaction in the cell hybrids, we optimized the PCR conditions for every primer pair to yield a single fragment with human (K562) DNA and no signal with mouse (MEL) cell DNA. Primers were selected for efficient amplification of total human chromosomal DNA and lack of amplification of mouse DNA. Amplification of nascent strands from both cell lines with mouse β -globin primers yielded similar products, indicating that nascent strand size and integrity were similar between preparations. The above modifications proved essential for analyzing initiation in the β -globin locus as numerous attempts to use previously published PCR-based methods to detect initiation in the β -globin locus were unsuccessful (20).

19. Primer pairs used were as follows: position 33.3, upper primer, AACAAAGCAAAACCAAC, and lower primer, GTATGTAGGCACCCGATGAT; position 45.8, upper primer, AAGGGCTAGCTTGGACTCA, and lower primer, GCAATATCTAAGGGTAAAG; position 50.6, upper primer, TGAGAAATAATGTGAAAGC, and lower primer, TGTATGTAAGGAGGATGAGC; position 59.8, upper primer, CCTGAGGAGAAGTCTGCGGT, and lower primer, CAGTGCAGCTCAGTCTAGTGT; position 61.9, upper primer, GGAAGGGGAGAGTAACAGGGT, and lower primer, AAGGGGCTAGCTTGGACTCA; position 65.6, upper primer, TATCTTCATTTCCCTTCC, and lower primer, GAGTCTCATGCTGTACACCTG; position 71.8, upper primer, TTGACAAACCTGAGGGAAC, and lower primer, TGATGGCTAGTGATGATGAG.
20. M. I. Aladjem *et al.*, data not shown.
21. B. Trask *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **58**, 767 (1993).
22. B. J. Brewer, *Curr. Opin. Genet. Dev.* **4**, 196 (1994).
23. R. Kelly, M. DeRose, B. Draper, G. M. Wahl, *Mol. Cell. Biol.* **15**, 4136 (1995); S. M. Carroll *et al.*, *ibid.* **13**, 2971 (1993). CAD, carbamoyl phosphate synthetase-aspartate transcarbamylase-dihydroorotase.
24. D. M. Gilbert *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **58**, 475 (1993).
25. D. L. Dobbs, W. L. Shaiu, R. M. Benbow, *Nucleic Acids Res.* **22**, 2479 (1994).
26. J. L. Hamlin and P. A. Dijkwel, *Curr. Opin. Genet. Dev.* **5**, 153 (1995).
27. E. Epner, W. C. Forrester, M. Groudine, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8081 (1988).
28. V. Dhar, A. I. Skoultschi, C. L. Schildkraut, *Mol. Cell. Biol.* **9**, 3524 (1989).
29. R. Li and M. R. Botchan, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 7051 (1994).
30. M. DePamphilis, *Trends Cell Biol.* **3**, 161 (1993).
31. Z. S. Guo and M. L. DePamphilis, *Mol. Cell. Biol.* **12**, 2514 (1992); G. L. Bream, P. Vaillancourt, M. R. Botchan, *J. Virol.* **66**, 7319 (1992).
32. M. DePamphilis, *J. Biol. Chem.* **268**, 1 (1993).
33. P. Fraser, S. Pruzina, M. Antoniou, F. Grosfeld, *Genes Dev.* **7**, 106 (1993); N. Dillon and F. Grosfeld, *Trends Genet.* **9**, 134 (1993).
34. M. S. Caddle and M. P. Calos, *Nucleic Acids Res.* **20**, 5971 (1992).
35. G. H. Nonet and G. M. Wahl, *Somatic Cell. Mol. Genet.* **19**, 171 (1993).
36. Probes 1 through 15 are as follows: probe 1, 500-base pair (bp) Eco RI fragment from map unit 4 to 5; probe 2, 1400-bp Xba I fragment from map unit 8.8 to 10.1; probe 3, 450-bp Hind III-Xba I fragment from map unit 10.1 to 10.5; probe 4, 1300-bp Bam

HI-Hind III fragment from map unit 12.4 to 13.7; probe 5, 700-bp Bam HI fragment from map unit 19.3 to 20; probe 6, 1200-bp Eco RI fragment from map unit 42 to 44; probe 7, 2300-bp Eco RI fragment from map unit 54 to 56; probe 8, 1800-bp Eco RI fragment from map unit 56 to 57.8; probe 9, 800-bp Bam HI fragment from map unit 59.7 to 60.5; probe 10, 650-bp Hpa I fragment from map unit 60.5 to 61.3; probe 11, 550-bp Hpa I-Sna BI fragment from map unit 61.2 to 61.8; probe 12, 750-bp Sna BI-Bam HI fragment from map unit 61.8 to 62.6; probe 13, 900-bp Bam HI-Eco RI fragment from map unit 62.2 to 63.5; probe 14, 1800-bp Eco RI-Bgl II fragment from map unit 65.5 to 67.3; probe 15, 900-bp Hind II-Eco RI fragment from map unit 80. It should be noted that most of these probes are different from the probes used by Kitsberg *et al.* (2). All cloned fragments used in this work were tested for the absence of hybridization to repetitive sequences on Southern blots of total genomic DNA.

37. A. D. Bergemann and E. M. Johnson, *Mol. Cell. Biol.* **12**, 1257 (1992).
38. Cos B included the ϵ and 5' ϵ (5' ϵ) region, cos HG15 consisted of β and 3' β sequences (IR), and the HPFH probe was derived from a series of pooled plasmids from the HPFH region. The HPFH probe

was labeled with biotin and detected with avidin-Texas Red after amplification. The cosB and HG15 probes were labeled with digoxigenin (DIG) or biotin and detected with fluorescein isothiocyanate (FITC)-conjugated antibody to DIG or by avidin-Texas Red. Nuclei were counter-stained with 4',6'-diamidino-2-phenylindole, scored under a double band pass filter, and photographed.

39. G. M. Wahl, M. Stern, G. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).
40. We are very grateful to J. A. Huberman and A. Sanchez for communicating the details of the nascent strand assay before publication; F. Grosfeld for cosmid probes and D. Mager for HPFH plasmids; R. E. Kelly, S. O'Gorman, J. L. Kolman, J. Roberts, and W. Forrester for discussion and comments on the manuscript; and B. Trask and H. Yokoda for assistance with FISH. The technical assistance of C. Navarro and A. Telling is greatly appreciated. M.I.A. was supported by fellowships from the European Molecular Biology Organization and the Human Frontiers Science Project Organization. This work was supported by grants from NIH to G.M.W., M.G., and R.E.K.F. and by the G. Harold and Leila Y. Mathers Charitable foundation to GMW.

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Mutations in the Dystrophin-Associated Protein γ -Sarcoglycan in Chromosome 13 Muscular Dystrophy

Satoru Noguchi,* Elizabeth M. McNally, Kamel Ben Othmane, Yasuko Hagiwara, Yuji Mizuno, Mikiharu Yoshida, Hideko Yamamoto, Carsten G. Bönnemann, Emanuela Gussoni, Peter H. Denton, Theodoros Kyriakides, Lefkos Middleton, Faycal Hentati, Mongi Ben Hamida, Ikuya Nonaka, Jeffery M. Vance, Louis M. Kunkel, Eijiro Ozawa

Severe childhood autosomal recessive muscular dystrophy (SCARMD) is a progressive muscle-wasting disorder common in North Africa that segregates with microsatellite markers at chromosome 13q12. Here, it is shown that a mutation in the gene encoding the 35-kilodalton dystrophin-associated glycoprotein, γ -sarcoglycan, is likely to be the primary genetic defect in this disorder. The human γ -sarcoglycan gene was mapped to chromosome 13q12, and deletions that alter its reading frame were identified in three families and one of four sporadic cases of SCARMD. These mutations not only affect γ -sarcoglycan but also disrupt the integrity of the entire sarcoglycan complex.

The muscular dystrophies are genetically heterogeneous (1). X-linked recessive muscular dystrophy, or Duchenne muscular dys-

trophy (DMD), is the most common form and arises from mutations in the dystrophin gene (2). Autosomal inheritance is present in a significant percentage of muscular dystrophy cases (1, 3). In North Africa, the incidence of SCARMD [OMIM 253700 (1)], also referred to as limb girdle muscular dystrophy (LGMD) 2C (4), accounts for 10 to 50% of the total muscular dystrophy cases (5, 6). The early age of onset and severity of the clinical course seen in North Africa are features also seen in DMD (6). Segregation of the SCARMD phenotype with chromosome 13 markers was first documented in a number of Tunisian kindreds (7). Recently, strong linkage disequilibrium with the marker D13S232 was documented for eight Tunisian families and one Egyptian family, sug-

S. Noguchi, Y. Hagiwara, Y. Mizuno, M. Yoshida, H. Yamamoto, I. Nonaka, E. Ozawa, National Institute of Neuroscience, National Center for Neurology and Psychiatry, 4-1-1 Ogawa-higashi cho, Kodaira, Tokyo, 187 Japan.
E. M. McNally, C. G. Bönnemann, E. Gussoni, L. M. Kunkel, Division of Genetics and the Howard Hughes Medical Institute, Children's Hospital, Boston, MA 02155, USA.
K. Ben Othmane, P. H. Denton, J. M. Vance, Division of Neurology, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA.
T. Kyriakides and L. Middleton, Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus.
F. Hentati and M. Ben Hamida, Institute of Neurology, Tunis, Tunisia.

*To whom correspondence should be addressed.

Fig 1. The deduced amino acid sequence of human γ -sarcoglycan (16). The amino acids listed below the human γ -sarcoglycan polypeptide are those residues of rabbit γ -sarcoglycan that differ from human γ -sarcoglycan. The rabbit cDNA was identified with a monoclonal antibody against the 35-kD DAG (15, 17). The putative transmembrane domain (amino acids 36 to 60) is boxed. Potential asparagine-linked glycosylation and phosphorylation sites are boldface and shaded, respectively. Five conserved cysteine residues are present in the distal carboxyl portion (underlined). The human and rabbit sequences have been deposited in GenBank under the accession numbers U34976 and U36822, respectively.

MVREQYT	AT	EGICIERPEN	QYVYKIGIYG	WRKRC	<u>LYLFV LLLLTILVNN</u>	50
AG	L	TH	C		L	
<u>LALTTWILKY</u>		MFSPAGMGH	LCVTKDGLRL	EGESEFLFPL	YAKEIHSRVD	100
		T	H			
SSLLQLSTON	VTVNARNSEG	EVTGRLKVGPF	KMVEVQNOQF	QINSNDGKPL	RE S	150
	D		Q	S		
FTVDEKEVVV	GTDKLRVTGP	EGALFEHSVE	TPLVRADPFQ	DLRLSPSTRS		200
E	R		T			
LSMDAPRGVH	IQAHAGKIEA	LSQMDILFHS	SDGMLVLDAE	TVCLPKLVQG		250
	E	EV	VL	T		
TWGPSGSSQS	LYEIQVCPDG	KLYLSVAGVS	TTCQEHSHIC	L*		292
OAA	G	AG				

gesting a widespread founder mutation in this geographic region (8). Linkage of SCARMD with chromosome 13 markers has been confirmed in Algerian and Moroccan as well as non-North African families (8, 9).

Dystrophin is an elongated, cytoskeletal protein whose amino terminus binds actin (10). The carboxyl terminus of dystrophin is anchored to the sarcolemma as a macromolecular complex with the dystrophin-associated glycoproteins (DAGs) (11, 12). The DAGs can be separated into at least two complexes (13). The first, dystroglycan, binds the extracellular matrix protein laminin, completing a bridge from actin to the extracellular matrix (12, 14). The second, sarcoglycan, comprises three distinct transmembrane proteins: α -sarcoglycan (adhalin, 50-kD DAG, or A2), β -sarcoglycan (A3b), and the 35-kD DAG (A4) (13). We show that alterations of the gene encoding the 35-kD DAG, here named γ -sarcoglycan, are likely to be the primary genetic

defect in North African and a subset of sporadic SCARMD cases.

Rabbit and human complementary DNAs (cDNAs) encoding γ -sarcoglycan were identified (15). The deduced amino acid sequence of rabbit γ -sarcoglycan includes an amino-terminal peptide, AGEQYLATTEGT (16), and a proteolytically derived peptide, DGLR-LEGES, prepared from purified rabbit skele-

tal-muscle γ -sarcoglycan (15), proving the identity of this cDNA as rabbit γ -sarcoglycan. The predicted proteins encoded by the human and rabbit γ -sarcoglycan cDNA sequences contain 291 amino acids and are highly conserved (89% identity and 93% similarity) (Fig. 1). The deduced molecular mass of rabbit γ -sarcoglycan is 31,840 daltons and that of human γ -sarcoglycan is 32,350 daltons. The isoelectric points of rabbit and human γ -sarcoglycan are 5.6 and 5.0, respectively, a difference consistent with the slightly more acidic nature of human γ -sarcoglycan on two-dimensional electrophoresis (17). The γ -sarcoglycan protein is considered a type II transmembrane protein with an extracellular carboxyl terminus (amino acids 61 to 291), because it has only a single transmembrane domain (amino acids 36 to 60), lacks an amino-terminal signal sequence, and has charged residues at amino acids 32 to 34. The cytoplasmic domain contains a potential phosphorylation site for casein kinase II, and the extracellular domain contains one conserved asparagine-linked glycosylation site. There were no significant homologies with other proteins in the electronic databases

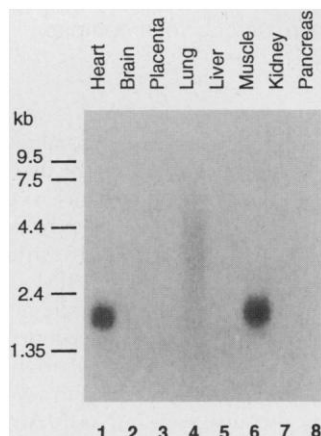


Fig 2. Expression of γ -sarcoglycan mRNA in human tissues. The γ -sarcoglycan cDNA was radio-labeled and hybridized to poly A⁺ mRNA from the tissues shown. The size of the γ -sarcoglycan mRNA is 1.7 kilobases (kb), consistent with the size of the cDNAs isolated from the λ gt10 cDNA libraries.

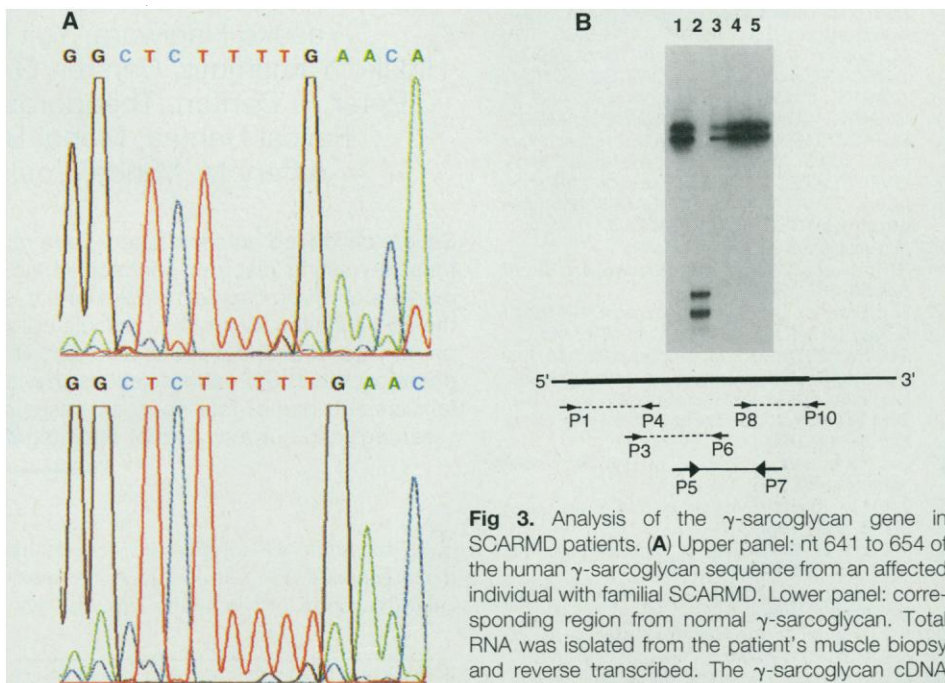


Fig 3. Analysis of the γ -sarcoglycan gene in SCARMD patients. (A) Upper panel: nt 641 to 654 of the human γ -sarcoglycan sequence from an affected individual with familial SCARMD. Lower panel: corresponding region from normal γ -sarcoglycan. Total RNA was isolated from the patient's muscle biopsy and reverse transcribed. The γ -sarcoglycan cDNA was amplified and sequenced. The homozygous loss of one of the thymine residues from nt 645 to 649 changes the γ -sarcoglycan reading frame, causing the loss of the carboxyl-terminal amino acids 175 to 291. Consistent with linkage disequilibrium in SCARMD families in northwest Africa (8), this same mutation was identified in an affected sibling and in two other Tunisian families (22). (B) SSCP analysis of human γ -sarcoglycan. Four dystrophic muscle biopsies were selected that had a deficiency of all or part of the sarcoglycan complex (23). Total RNA was isolated, reverse transcribed, and amplified with primers specific to γ -sarcoglycan. Lane 1, normal; lanes 2 to 5 represent the muscle biopsies from four SCARMD patients. Shown below is a schematic of the four different primer pairs spanning the coding region of the human γ -sarcoglycan gene that were used in the SSCP analysis. The primer pair P5-P7 was used to generate the conformers shown in the upper panel, amplifying nt 516 to 847 of γ -sarcoglycan. Patient 2 (lane 2) shows conformer changes reflecting a minimum deletion of 73 bp. The primer pair P3-P6 failed to amplify a product from the γ -sarcoglycan cDNA of patient 2, consistent with the deletion. Within the region spanning from nt 630 to 702, no normal allele was detected.

(18). The human γ -sarcoglycan mRNA is expressed exclusively in striated muscle (Fig. 2), both cardiac and skeletal. This expression pattern agrees well with the immunocytochemical survey of primate tissues that used a monoclonal antibody specific for γ -sarcoglycan (17).

Two overlapping genomic phages for the human γ -sarcoglycan gene were found to hybridize to metaphase human chromosomes at 13q12 (19), the same region implicated in SCARMD families by linkage analysis. The minimal candidate region for SCARMD was recently refined to a small region surrounding D13S232, and a physical map of this minimal candidate region has been constructed (20). Primers homologous to the 5' and to the 3' untranslated regions of the human γ -sarcoglycan sequence hybridized within this minimal candidate region, and analysis of cDNAs obtained from direct selection with a yeast artificial chromosome spanning this same region included a γ -sarcoglycan cDNA (20).

Linkage disequilibrium with the marker D13S232 suggests that both alleles of the responsible gene should carry an identical mutation in North African SCARMD families. This linkage disequilibrium and the relatively

large percentage of patients with this autosomal recessive disorder are consistent with the high degree of consanguinity present in Tunisia. To determine the homozygous mutation responsible for SCARMD, we prepared RNA from a skeletal-muscle biopsy taken from an affected Tunisian SCARMD patient (21) whose family is consanguineous and demonstrates linkage disequilibrium with D13S232 (7, 8). Direct sequencing of γ -sarcoglycan polymerase chain reaction (PCR) products demonstrated that one of the thymine residues from nucleotides (nt) 645 to 649 was homozygously deleted in the patient (Fig. 3A). The thymine deletion was confirmed in the DNA of this patient (22). This mutation was also found in an affected sibling in this family and in two additional Tunisian SCARMD families but was not seen in 10 unaffected chromosomes (22). The deletion of one thymine changed the reading frame at amino acid 175, creating a premature stop codon at amino acid 193. The aberrant γ -sarcoglycan would retain its transmembrane anchor and its asparagine-linked glycosylation site but lose the cluster of cysteine residues present in the distal portion of the protein. Immunostaining of the patient's muscle showed a deficiency of all three sarcoglycan subunits, reflecting an instability of the entire complex (Fig. 4).

To ascertain whether γ -sarcoglycan mutations contribute to sporadic cases of SCARMD, we analyzed γ -sarcoglycan cDNA from several dystrophic muscle biopsies. Muscle biopsies from four Japanese patients were selected for this study because they displayed dystrophic architecture on hematoxylin and eosin staining, deficiency of sarcoglycan, and preservation of normal dystrophin staining (23). Figure 3B shows a single-strand conformation polymorphism (SSCP) analysis of the region from nt 516 to 847 of the human γ -sarcoglycan sequence (24). The lower band in lane 2 was sequenced; it corresponded to a deletion of 73 base pairs (bp), producing a stop codon at amino acid 170 of the aberrant γ -sarcoglycan. In this case, as in the familial SCARMD, the carboxyl-terminal one-third of the γ -sarcoglycan protein was lost, including the distal cysteine residues. At the time of this biopsy, the patient was too young to fully determine the severity of her disorder (25). Immunostaining of this patient's muscle showed that all of the sarcoglycan components of the muscle had been lost (23, 26).

The tight association of the sarcoglycan subunits, as demonstrated by their ability to be biochemically cross-linked (13), suggests that sarcoglycan may function as a unit, although its role remains unknown. The observation that mutations in any one of the subunits result in the loss of the other subunits further supports this concept. Mutations that result in muscular dystrophy have been documented in the α -sarcogly-

can locus (adhalin) and the β -sarcoglycan locus (27, 28). Given that mutations in any one of the sarcoglycan subunit genes can result in muscle weakness, we propose the term "sarcoglycanopathy" as a collective name for these muscular dystrophies (29).

Mutations in dystrophin produce a broad range of phenotype that include severe and mild forms of muscular dystrophy. We expect to find a similar broad range in each of the sarcoglycanopathies. Missense mutations in α -sarcoglycan are usually associated with a milder course of muscle wasting that may begin in the second or third decade. An exception to this mild course is seen when the mutations produce truncating stop codons (27). In the case of β -sarcoglycan, mutations that produce stop codons on both alleles result in a more severe, earlier onset muscular dystrophy similar to classical SCARMD whereas missense mutations are associated with a milder course (28). Mutations in γ -sarcoglycan that produce a milder phenotype will likely be identified, and a genotype-phenotype correlation may emerge for predicting the clinical course, as has been successful for DMD and its milder allelic counterpart, Becker muscular dystrophy. The identification of the γ -sarcoglycan gene will aid in genetic counseling and diagnosis. Understanding the role of the sarcoglycan complex may ultimately affect therapy for these disorders.

REFERENCES AND NOTES

1. Online Mendelian Inheritance in Man (OMIM). The Human Genome Data Base Project, Johns Hopkins University, Baltimore, MD [cited 10 September 1995]. World Wide Web URL: <http://gdbwww.gdb.org/omim/docs/omimtop.html>.
2. A. P. Monaco *et al.*, *Nature* **323**, 646 (1986); A. H. Burghes *et al.*, *ibid.* **328**, 434 (1987); M. Koenig *et al.*, *Cell* **50**, 509 (1987).
3. M. Zatz, R. Passos-Bueno, D. Rapaport, *Am. J. Med. Genet.* **32**, 407 (1989).
4. K. M. D. Bushby and J. S. Beckmann, *Neuromuscular Dis.* **5**, 337 (1995).
5. K. Azibi *et al.*, *Cytogenet. Cell Genet.* **58**, 1907 (1991); T. I. Farag and A. S. Teebi, *Am. J. Med. Genet.* **37**, 290 (1990).
6. M. Ben Hamida, M. Fardeau, N. Attia, *Muscle Nerve* **6**, 469 (1983).
7. K. Ben Othmane *et al.*, *Nature Genet.* **2**, 316 (1992).
8. K. Ben Othmane *et al.*, *Am. J. Hum. Genet.* **57**, 732 (1995).
9. K. Azibi *et al.*, *Hum. Mol. Genet.* **2**, 1423 (1993); F. el Kerch *et al.*, *J. Med. Genet.* **31**, 342 (1994); M. R. Passos Bueno *et al.*, *Am. J. Hum. Genet.* **55**, A199 (1994).
10. E. P. Hoffman, R. J. Brown, L. M. Kunkel, *Cell* **51**, 919 (1987); B. A. Levine, A. J. Moir, V. B. Patchell, S. V. Perry, *FEBS Lett.* **263**, 159 (1990); L. Hemmings, P. A. Kuhlman, D. R. Critchley, *J. Cell Biol.* **116**, 1369 (1992).
11. M. Yoshida and E. Ozawa, *J. Biochem. (Tokyo)* **108**, 748 (1990); J. Ervasti and K. P. Campbell, *Cell* **66**, 1121 (1991).
12. A. Suzuki, M. Yoshida, H. Yamamoto, E. Ozawa, *FEBS Lett.* **308**, 154 (1992).
13. M. Yoshida *et al.*, *Eur. J. Biochem.* **222**, 1055 (1994).
14. O. Ibraghimov-Beskrovnya *et al.*, *Nature* **355**, 696 (1992).
15. We obtained the rabbit γ -sarcoglycan cDNA by screening a λ gt11 rabbit skeletal-muscle library

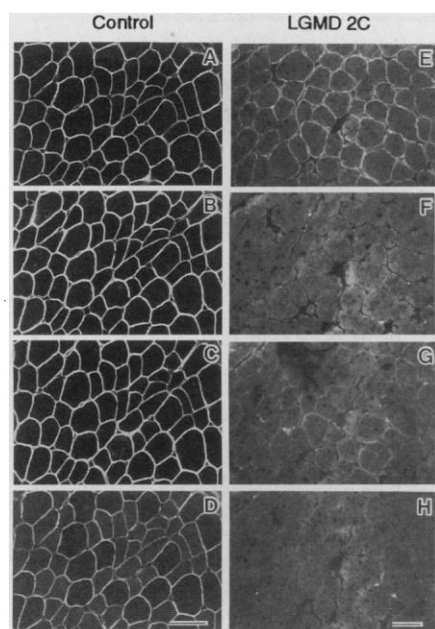


Fig 4. Immunocytochemical analysis of the muscle biopsy from the patient with chromosome-13-linked SCARMD (LGMD 2C), who carries a homozygous thymine deletion in the γ -sarcoglycan gene. Cryosections from unaffected muscle (A through D) and the patient's muscle (E through H) were immunostained for dystrophin (A and E), α -sarcoglycan (adhalin) (B and F), β -sarcoglycan (C and G), and γ -sarcoglycan (D and H) (27). In the patient sample, the staining for three subunits of sarcoglycan was lost or greatly reduced (F, G, and H), whereas dystrophin staining was preserved (E). Bar = 50 μ m.

- (Clontech, Palo Alto, CA) with the monoclonal antibody MA4-2 (17). One positive plaque was found in 5×10^5 screened. The insert was subcloned into Bluescript II SK (Stratagene, La Jolla, CA) and sequenced. Two human libraries, a λ gt10 fetal cardiac library (Clontech) and a λ gt10 adult cardiac library [E. M. McNally, M. Yoshida, Y. Mizuno, E. Ozawa, L. M. Kunkel, *Proc. Natl. Acad. Sci. U.S.A.* **91**; 9690 (1994)], were screened for the human γ -sarcoglycan sequence. Fifteen positive clones were characterized. All sequences were determined by Taq cycle sequencing. We confirmed the identity of the γ -sarcoglycan cDNA by isolating the DAG complex using two-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) and transfer to polyvinylidene difluoride membranes (13). The spot corresponding to γ -sarcoglycan was excised and applied to an automated gas-phase protein sequencer (Applied Biosystems, Foster City, CA). Internal amino acid sequences were determined by an analysis of the peptides released from the γ -sarcoglycan band separated on PAGE after digestion with *Acromobacter* protease I (Wako Pure Chemical Industries, Osaka). We also confirmed the identity of the γ -sarcoglycan cDNA by showing that an antibody to rabbit γ -sarcoglycan-glutathione-S-transferase fusion protein reacts with the 35-kD DAG (S. Noguchi and E. Ozawa, unpublished results).
16. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
 17. H. Yamamoto *et al.*, *J. Biochem. (Tokyo)* **115**, 162 (1994).
 18. Databases searched include GenBank, European Molecular Biology Laboratory database; National Biomedical Research Foundation-Protein Identification Research database; Swiss Protein Sequence database; and the database of Expressed Sequence Tags [cited September 1995].
 19. Two genomic phages encoding γ -sarcoglycan were isolated from an EMBL library and used as probes in fluorescence in situ hybridization against human metaphase chromosomes (E. M. McNally, E. Gussoni, L. M. Kunkel, unpublished results).
 20. K. Ben Othmane, P. H. Denton, J. M. Vance, unpublished results.
 21. We isolated RNA by homogenization of 5 to 10 mg of muscle in guanidinium isothiocyanate and subsequent cesium chloride centrifugation. Total RNA (1 to 2 μ g) was reverse transcribed with Superscript (GIBCO BRL). The cDNA was used as a template for PCR with the following primers: P1 (5'-CATTCTGTCTGTGGTAGAGCTCGG-3'), P4 (5'-TGGAGAAACACATCACTTTAAGAA-3'), P3 (5'-CTTCTTTACTCATCATCCTCGT-3'), P6 (5'-CCTTCAGGCCAGTTTACTCGAAG-3'), P5 (5'-AAATGGTAGAGTCCAGAGTCAACA-3'), P7 (5'-GTTTCAGCATCAAGCACAAGCATTC-3'), P8 (5'-GGAGTCTAGCATGGATGCCCAA-3'), and P10 (5'-GCGTTTACTTCCCATCCAGCTGC-3'). Conditions for PCR were 94°C for 2 min, 60°C for 1 min, 72°C for 45 s, and 94°C for 45 s for a total of 35 cycles. The PCR products were subjected to Taq cycle sequencing.
 22. Primers were designed to amplify the genomic span surrounding nt 645 to 649 of the cDNA sequence, and the PCR products were directly sequenced, showing the same mutation in an affected sibling in this family and in an affected member of two other Tunisian SCARMD families that show linkage disequilibrium with the marker D13S232 (7, 8).
 23. Y. Mizuno *et al.*, *Biochem. Biophys. Res. Commun.* **203**, 979 (1994).
 24. The PCR was performed in the presence of [α - 32 P]deoxycytidine 5'-triphosphate. The products were denatured in a formamide-NaOH mixture and separated by electrophoresis in an MDE Hydrolink (AT Biochem, FMC Bioproducts, Rockland, ME) gel. Conformers were excised, eluted, and directly sequenced. The primers P5 and P7 showed the deleted product. The primers P3 and P6 failed to amplify any product in patient 2.
 25. Patient 2 has no known family history of muscular dystrophy or consanguinity and underwent muscle biopsy at 1 year of age for evaluation of elevated serum creatine kinase [18,630 U/ml (normal, <200 U/ml)]. The patient was unavailable for further diagnostic testing. Because the DNA from this patient and her parents is unavailable, we can only confirm that the minimal deletion on both alleles involves nt 630 to 702. It is possible that one allele carries a larger deletion that causes a complete loss of the γ -sarcoglycan transcription unit.
 26. Y. Mizuno and E. Ozawa, unpublished results.
 27. S. L. Roberds *et al.*, *Cell* **78**, 625 (1994); F. Piccolo *et al.*, *Nature Genet.* **10**, 243 (1995); M. R. Passos-Bueno *et al.*, *Hum. Mol. Gen.* **4**, 1163 (1995); A. Ljunggren *et al.*, *Ann. Neurol.* **38**, 367 (1995).
 28. C. G. Bönnemann *et al.*, *Nature Genet.*, in press; L. E. Lim *et al.*, *ibid.*, in press.
 29. E. Ozawa *et al.*, *Hum. Mol. Genet.*, in press.
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