X-Linked Dominant Scapuloperoneal Myopathy Is Due to a Mutation in the Gene Encoding Four-and-a-Half-LIM Protein 1

Catarina M. Quinzii,[1] Tuan H. Vu,[1,2] K. Christopher Min,[1] Kurenai Tanji,[3] Sandra Barral,[1] Raji P. Grewal,[4] Andrea Kattah,[1] Pilir Camaño,[1,5] David Otaegui,[1,5] Teruhito Kunimatsu,[1] David M. Blake,[1] Kirk C. Wilhelmsen,[1,6] Lewis P. Rowland,[1] Arthur P. Hays,[2] Eduardo Bonilla,[1,3] and Michio Hirano[1,*]

Scapuloperoneal (SP) syndrome encompasses heterogeneous neuromuscular disorders characterized by weakness in the shoulder-girdle and peroneal muscles. In a large Italian-American pedigree with dominant SP myopathy (SPM) previously linked to chromosome 12q, we have mapped the disease to Xq26, and, in all of the affected individuals, we identified a missense change (c.365G→C) in the *FHL1* gene encoding four-and-a-half-LIM protein 1 (FHL1). The mutation substitutes a serine for a conserved trypophan at amino acid 122 in the second LIM domain of the protein. Western blot analyses of muscle extracts revealed FHL1 loss that paralleled disease severity. FHL1 and an isoform, FHL1C, are highly expressed in skeletal muscle and may contribute to stability of sarcomeres and sarcolemma, myofibrillary assembly, and transcriptional regulation. This is the first report, to our knowledge, of X-linked dominant SP myopathy and the first human mutation in FHL1.

Scapuloperoneal syndrome (SPS) was initially described more than 120 years ago by Jules Broussard as "une forme héréditaire d'atrophie musculaire progressive" beginning in the lower legs and affecting the shoulder region earlier and more severely than distal arm;[1] however, the etiology of this condition remains unclear. Descriptions of neurogenic SP (MIM 181400) with distal limb sensory loss (Davidenkow syndrome) or without sensory involvement (Kaeser syndrome) as well as SP myopathy (SPM [MIM 181430]) suggest that at least three pathogenically distinct forms exist.[2–5] Molecular genetic studies have confirmed the existence of more than one cause of SP syndrome. In 1996, we linked an apparently autosomal-dominant SPM in a large Italian-American family (family C) to chromosome 12q[6] whereas an autosomal-dominant neurogenic SP in New England kindred of French-Canadian origin (MIM 181405) was mapped to a second distinct locus on chromosome 12q24.[7] In the original family reported by Kaeser, a pathogenic missense mutation (R350P) of the desmin gene (*Des* [MIM 125660]) was identified,[4,8] and in two of 17 patients with scapuloperoneal myopathy, a missense mutation (R1845W) in the *MYH7* gene encoding myosin heavy chain 7 (MIM 160760) was observed.[9]

In family C, 14 of 44 members were definitely affected by SPM and two other deceased individuals were probably affected based on clinical history.[6] The diagnosis of SPS was based on clinical features including footdrop as an "invariable early sign," proximal arm weakness always preceding hand weakness, and scapular winging on examination of all patients. Elevated serum creatine kinase (CK) levels in all patients, normal nerve conduction studies with electromyographic myogenic changes, and muscle biopsies in four patients revealing typical myopathic changes indicated that myopathy was the cause of weakness. Detailed analysis of two muscle biopsy samples revealed desmin-positive cytoplasmic bodies indicative of a myofibrillary myopathy.

The prior linkage study mapped the disease to chromosome 12q between markers 12S88 (94.49 cM) and 12S306 (105.18) based on a maximum 2-point LOD score of 2.95 (marker D12S82 at recombinant fraction [θ] = 0) and peak multipoint LOD score of 3.0. However, 10 individuals who were "not affected" shared the chromosome 12q haplotype with the affected individuals suggesting incomplete penetrance, double recombination in these nonaffected individuals, or false-positive linkage. Therefore, we performed a new genome-wide scan with microsatellite markers to identify the chromosomal locus of the disease. Sex chromosome markers were included because X-linked dominant inheritance could not be excluded by male-to-male transmission.

In this study, we re-evaluated 27 adult members of family C (Figure 1). Fourteen individuals (8 women and 6 men) were considered affected because they had weakness of shoulder-girdle and peroneal muscles (MRC grade ≤ 4/5), scapular winging, and functional impairment. Affected individuals appear in five generations. Clinical features, electrophysiology, morphology, and immunohistochemistry of the family have been described.[6] We included DNA samples from the 12 affected individuals analyzed previously[6] plus two additional definitely affected individuals previously considered not affected. Two affected individuals (III-32 and IV-6) had died since the prior report. Tissue samples from the family were collected under Columbia University Institutional Review Board protocols.

[1]Department of Neurology, Columbia University Medical Center, New York, NY 10032, USA; [2]Wellspan Neurology, York, PA 17402, USA; [3]Department of Pathology, Columbia University Medical Center, New York, NY 10032, USA; [4]New Jersey Neuroscience Institute, Edison, NJ 08818, USA; [5]Unidad Experimental, Hospital Donostia, San Sebastian 20014, Spain; [6]Department of Genetics, University of North Carolina, Chapel Hill, NC 27599, USA
*Correspondence: mh29@columbia.edu
DOI 10.1016/j.ajhg.2007.09.013.

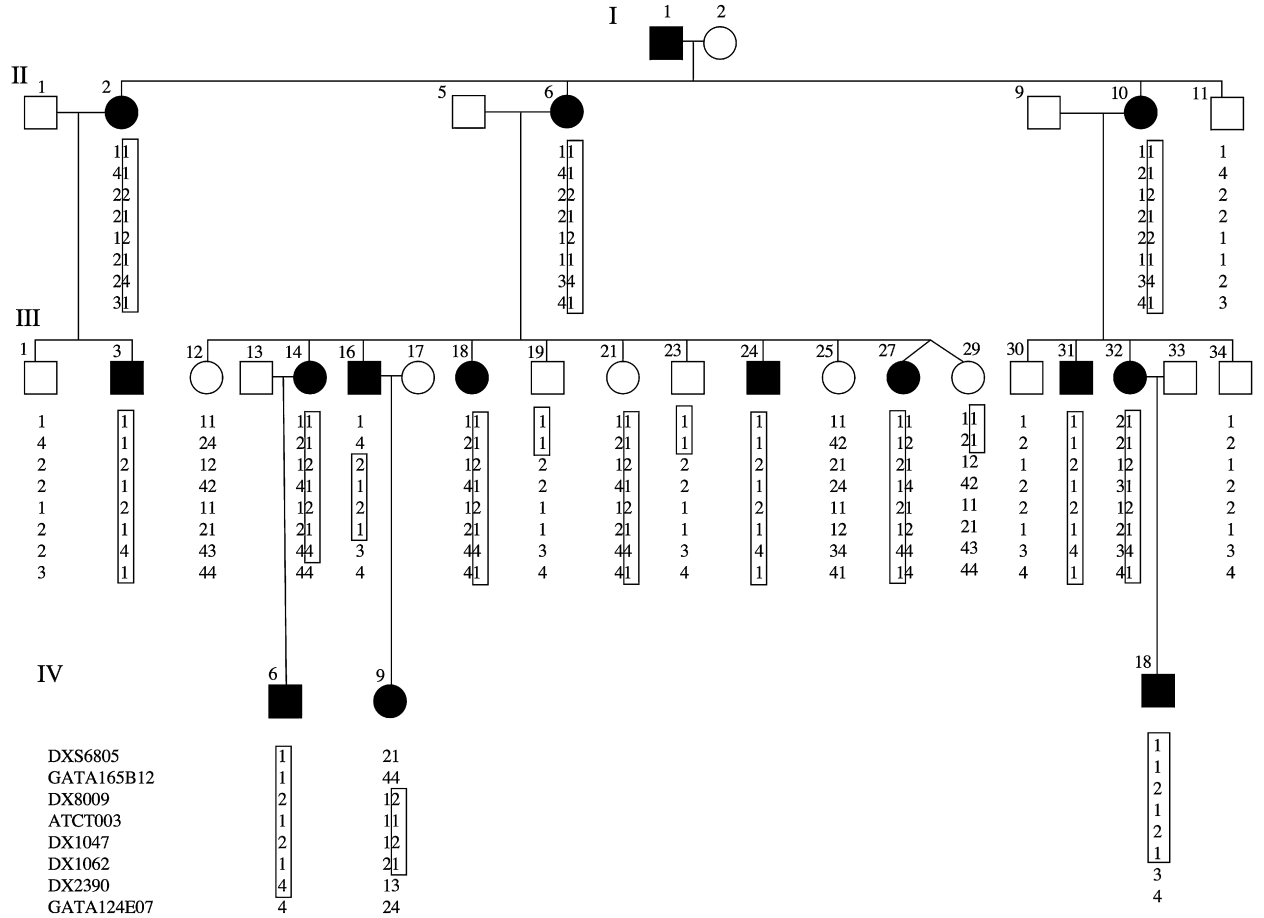

Figure 1. Pedigree of SPM Family C

Dark symbols indicate affected individuals. Genotypes are listed below each tested individual (two clinically unaffected are not shown as they requested). Haplotypes shared among the affected individuals are boxed. Individuals are numbered according to a prior publication.[6]

We performed molecular genetic linkage studies with leukocyte DNA from 27 family members (14 affected and 13 unaffected individuals). Three unaffected female individuals allowed us to analyze their DNA but refused publication of their genetic information; therefore, their haplotypes are not included in the pedigree (Figure 1). 411 fluorescently labeled microsatellite markers were initially tested (Prevention Genetics, Marshfield, WI). To confirm the results and narrow down the candidate region, we tested additional fluorescently labeled microsatellite markers in the ABI Prism Linkage Mapping Set-MD10 (Applied Biosystems, Foster City, CA). We performed two-point LOD score analysis with the MLINK option of FASTLINK 5.23 (X-linked dominant inheritance under the 90% females and 100% male penetrances models; we have used a disease allele frequency of 0.00 corresponding to 1 in 1000).

We screened three candidate genes for mutations: *MBNL3* (MIM 300413), *ZNF75* (MIM 314997), and *FHL1* (MIM 300163). To sequence *FHL1*, we designed 11 primer pairs flanking the exons, the promoter region, and the 5′ and 3′ untranslated region. Sequencing was performed with BigDye 3.1 with an ABI 3100 Genetic Analyzer (Applied Biosystems). Screening of 181 healthy individuals for the G-to-C transition at nucleotide 365 of *FHL1* was performed by restriction fragment length polymorphism analysis with BsmBI restriction endonuclease digestion of PCR-amplified DNA fragments.

Western blot studies were performed with rabbit polyclonal antibody anti-FHL1 (AVIVA Systems Biology) at a dilution of 1:1000 and with mouse monoclonal anti-vinculin antibody (Sigma) at a dilution of 1:5000. After washing, the membranes were incubated with the corresponding anti-rabbit or anti-mouse IgG secondary antibodies (Dako) at a dilution of 1:1000 for 45 min at room temperature. The membranes were developed with the chemiluminescence ECL system (Pharmacia) followed by exposure of the membranes to autoradiographic films. Quantitation of immunoreactive bands was performed by densitometric analysis with the NIH Scion Image software package (version 1.61).

To generate a model of W122S mutant FHL1, we used the coordinates of PDB entry 1X63 and replaced tryptophan 122 with a serine residue via a common rotamer with the program COOT. The molecular surface was generated with

Table 1. Two-Point LOD Score of Chromosome X Microsatellite Markers

Marker	Genetic Position (cM)	θ Values						
		0	0.01	0.05	0.1	0.2	0.3	0.4
GATA165B12	77.15	−6.05	−3.81	−1.45	−0.43	0.39	0.58	0.42
DXS8009	82.07	0.54	0.55	0.58	0.59	0.54	0.41	0.23
ATCT003	82.07	**3.66**	3.65	3.57	3.38	2.82	2.06	1.10
DXS1047	82.07	2.86	2.87	2.85	2.74	2.33	1.72	0.94
DXS1062	82.24	1.52	1.50	1.40	1.28	1.01	0.71	0.38
DXS2390	95.00	−4.90	0.43	1.67	2.02	2.00	1.58	0.89

the program GRASP, and figures were generated with the program PYMOL.

Only three microsatellite markers produced LOD scores ≥ 1.0: TPO (chromosome 2p25), D12S1294 (12q15), and ATCT003 (Xq26) (see Table S1 available online). We disproved the prior linkage to chromosome 12q because one affected individual (III-27), who was "not affected" in the prior study, did not share the haplotype with her affected relatives. We also excluded linkage to the desmin gene locus at chromosome 2q35.

We tested additional microsatellite markers at chromosome Xq26. Recombinations detected with markers GATA16B12P and DXS2390 restricted the SPM locus to a 19 megabase interval with a maximum two-point LOD score of 3.66 at $\theta = 0$ with marker ATCT003 (Table 1). Although the critical region was large, we identified three candidate genes, which encode proteins highly expressed in muscle and are involved in muscle sarcomeric organization or muscle differentiation: *MBNL3*, *ZNF75*, and *FHL1*. Direct DNA sequencing of *MBNL3* and *ZNF75* did not reveal any potential mutations; however, in *FHL1*, we identified a G-to-C transition at nucleotide 365 in exon 3 (c.365G→C) that changes a tryptophan in position 122 to serine (W122S). The skeletal muscle LIM protein 1, FHL1 or SLIM1, is a member of the four-and-a-half-LIM (FHL) domain protein family.[10–12] LIM is an acronym of the three transcription factors Lin-11, Isl-1, and Mec-3 in which the cysteine-rich double zinc finger motif $(CX_2CX_{17\text{-}19}HS_2C)X_2(CX_2CX_{16\text{-}20}CX_2(H/D/C)$ was originally identified.[13] The W122S mutation alters a conserved residue in the second LIM domain of the protein (Figure 2).

The mutation was hemizygous in all the affected males and heterozygous in all affected females. Affected men had earlier age at onset (26.1 ± 4.7 years, mean ± standard deviation; n = 8) than women (34.4 ± 11.9 years; n = 8) (p = 0.04, t test). In addition, men were more severely affected than women: 7 of 8 men became wheelchair bound earlier (age 35.9 ± 4.0 years) and only one was walking with a cane at age 63, whereas only 4 of 8 women became wheelchair bound later (age 54.8 ± 19) and four were ambulating at ages 33–74. Two unaffected females (III-21 and an individual who requested to be anonymous) carry the mutation, but because both are <58 years old, the oldest age at onset among definitely affected individuals, they are presumed to be presymptomatic. We did not detect

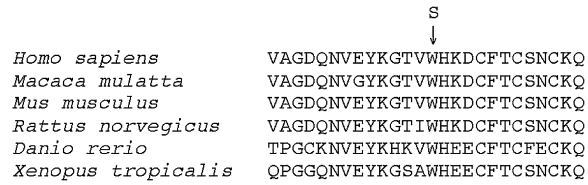

Figure 2. Evolutionary Conservation of Tryptophan-122 in FHL1
Tryptophan at amino acid 122 (arrow) in human FHL1 is highly conserved across species.

the mutation in 181 unrelated and unaffected Italian-American individuals, 54 females and 127 males (235 X chromosomes); 40 individuals were of Italian origin. We also excluded *FHL1* mutations in 10 unrelated patients with myofibrillar myopathy and 5 unrelated probands with the clinical diagnosis of SP syndrome.

We performed western blot analyses to quantitate levels of FHL1 and vinculin (a sarcolemmal protein) with quadriceps muscle from two affected men (III-24 and IV-18), two affected women (III-14 and III-32), and two controls (Figure 3). Levels of vinculin were comparable in all muscle samples, but amounts of FHL1 normalized to vinculin were inversely related to the duration of symptoms at the time of biopsy. Individual IV-18 had weakness for 12 years and his FHL1/vinculin ratio, 0.74, was comparable to both controls (0.75 and 1.0). In contrast, in relatives affected for longer periods, the ratios were reduced to 0.3 in III-14 (weak for 26 years), 0.4 in III-32 (weak for 39 years), and 0.0001 in IV-18 (weak for 25 years). It is noteworthy that the severely affected man had significantly lower FHL1 than his female relatives, who had less weakness with similar durations of symptoms.

To confirm that loss of protein was disease specific, western blot analyses of FHL1 and vinculin were performed with extracts of muscles from two patients with cytoplasmic body myopathy, two patients with myofibrillar myopathy not caused by mutations in FHL1, and two controls. The ratios of FHL1/vinculin were comparable in all muscle samples (controls, 1 and 1.15; cytoplasmic body myopathies, 1.17 and 0.9; and myofibrillar myopathies, 0.7 and 0.73).

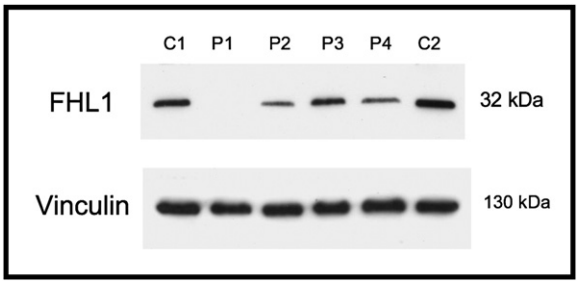

Figure 3. FHL1 Protein Expression in SPM Patients
Western blot analysis of FHL1 and vinculin in skeletal muscle of four patients (P1, III-24; P2, III-14; P3, III-32; and P4, IV-18) with the W122S FHL1 mutation and two controls.

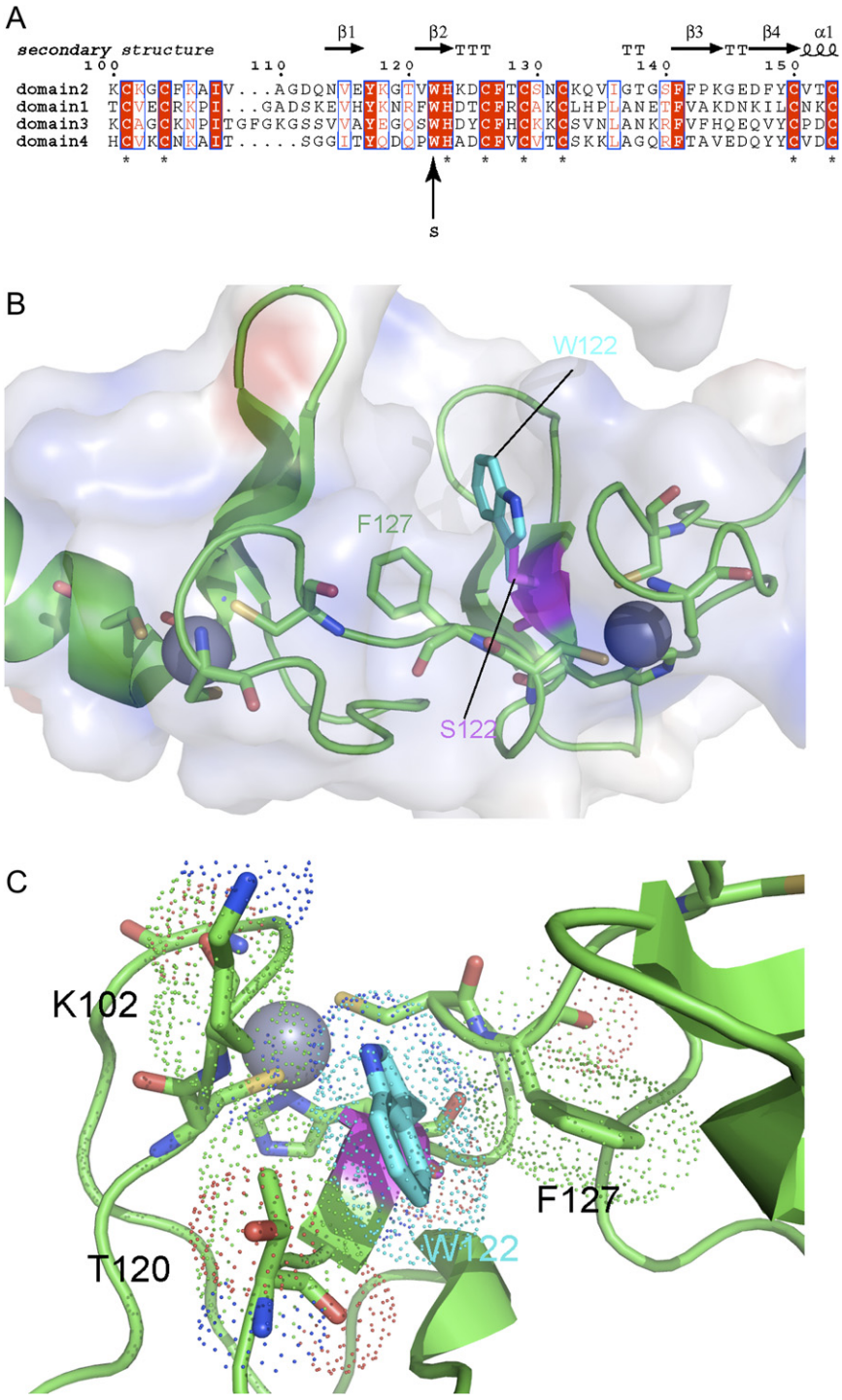

Figure 4. Structural Analysis of the FHL1 W122S Mutation

(A) Sequence alignment of the 4 LIM domains in FHL1 demonstrates 13 conserved amino acids (highlighted in red) including 8 residues (indicated by asterisks) that coordinate the two zinc ions. The secondary structure indicated on the first line is derived from an NMR model containing the second LIM domain (PDB, 1X63). Amino acid numbers refer to the second LIM domain. An arrow indicates the position of the disease-associated W122S mutation.

(B) The structure of LIM domain 2 of FHL1 is shown as a cartoon diagram. Wild-type residue W122 (in cyan) is replaced by serine (in magenta) in affected individuals. A molecular surface calculated for the mutant protein with GRASP[26] and colored to demonstrate the predicted electrostatic potential (blue is positive charge and red is negative). Substitution of the normal Trp residue by a smaller Ser side chain is likely to change the surface of the protein. Side chains of the residues that coordinate the zinc ions (shown as spheres) are shown in stick representation, as well as the nearby conserved F127.

(C) Apparent van der Waals contacts between wild-type W122 with neighboring F127, T120, and K107 are shown as overlapping clouds of small spheres in an image generated with PYMOL (DeLano Scientific LLC).

Trytophan at amino acid 122 of FHL1 is conserved in all four LIM domains of FHL1 and in the consensus LIM motif (Figure 4A). To assess the potential structural effects of the mutation on FHL1, we used a model derived from an NMR structural determination of a segment containing residues 91–159 of FHL1 (PDB entry 1x63) (Figures 4B and 4C). In this structural model, there are apparent van der Waals contacts between wild-type W122 with neighboring F127, T120, and K107. The interactions with T120 and K102 would be lost when W122 is replaced by a smaller serine, and therefore, the mutation may affect folding and stability of this LIM domain, conformation of the adjacent second zinc finger binding domain, or both. Alternatively, because W122 is adjacent to the predicted protein-binding surface of the FHL1, interactions with protein partners could be affected by the mutation (Figures 4B and 4C). Based on our western blot analysis indicating loss of FHL1 in parallel with disease severity, we hypothesize that the mutation may cause instability or selective destruction of misfolded protein.[14]

The LIM motif mediates protein-protein interactions with transcription factors, cell-signaling molecules, and cytoskeleton-associated proteins.[15] The FHL family includes FHL1, FHL2, FHL3, FHL4, and ACT in humans. Whereas expression of FHL4 and ACT is restricted to testis, FHL1, FHL2, and FHL3 are mainly expressed in muscle. FHL2 and FHL3 also contribute to the mechanical stabilization of muscle cells.[16]

FHL1 is the least characterized of the FHL proteins; it contains an N-terminal single zinc finger followed by four LIM domains, which are separated by eight intervening amino acids. FHL1 is highly expressed in adult human skeletal muscle, moderately in heart, and weakly in placenta, ovary, prostate, testis, small intestine, colon, and spleen.[10–12] In cultured C2C12 skeletal muscle cell lines, levels of FHL1 and FHL3 transcripts appear to be coordinated with expression of myogenic factors including MyoD, MRF4, and myogenin, suggesting that FHL1 and FHL3 participate in myogenesis.[17]

FHL1 may contribute to muscle cytoarchitecture by interacting with myosin-binding protein C (MyBP-C) and has been localized to the I-band and M-line of sarcomeres as well as to sarcolemma, where it colocalizes with MyBP-C.[18] RNAi knockdown of FHL1 in C2C12 cells impaired myosin thick filament formation and reduced MyBP-C incorporation into sarcomeres, indicating that FHL1 is required for sarcomere assembly.[18] In addition, FHL1 may have a role in α5β1-integrin-dependent myocyte elongation, adhesion, spreading, and migration.[19,20]

Two additional transcripts of the *FHL1* gene have been described: FHL1B (SLIMMER) and FHL1C (KyoT2).[21–23] FHL1B is expressed mainly in brain whereas FHL1C is primarily in skeletal muscle. All three transcripts share exons 1–3, which encode the N-terminal single zinc finger and two LIM domains. Isoforms FHL1B and 1C lack exon 4 (encoding two LIM domains of FHL1) and contain a C-terminal putative binding domain for J-recombination signal protein (RBP-J),[21–23] a transcription factor required for development and negatively regulated by KyoT2 in mice;[24,25] therefore, human FHL1C may modulate muscle transcription by repressing RBP-J transcription.

Based on the putative functions of FHL1 and FLH1C proteins in skeletal muscle, we hypothesize that the W122S mutation causes focal myofibrillary disarray though possible disruption of stability of sarcomeres and sarcolemma, myofibrillary assembly, transcriptional regulation, or a combination of these functions. The identification of *FHL1*, *MHC7*, and *Des* mutations in patients with scapuloperoneal myopathy suggests that myofibrillary disarray may be a common feature of SP syndrome.[8,9] Further studies are needed to elucidate the role of FHL1 in skeletal muscle and the pathomechanism of FHL1 in this novel X-linked dominant myopathy.

Supplemental Data

One supplemental table can be found with this article online at http://www.ajhg.org/cgi/content/full/82/1/208/DC1/.

Acknowledgments

We are grateful to all of the patients and relatives for their collaboration. We appreciate Dr. Osvaldo Graña (Structural Computational Biology Group, Spanish National Cancer Research Centre, Madrid, Spain) for assistance. This work was partially supported by the CFSR Foundation. The authors are supported by grants from Telethon Italy, the Muscular Dystrophy Association, National Institutes of Health (NS11766), and the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF).

Received: May 21, 2007
Revised: August 30, 2007
Accepted: September 11, 2007
Published online: January 10, 2008

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for neurogenic SP and scapuloperoneal myopathy)
Protein Data Bank (PDB), http://www.pdb.org/pdb/home/home.do

References

1. Broussard, J. (1886). Étude clinique sur une forme hérèditaire d'atrophie musculaire progressive débutant par les membres inférieurs (type fémoral avec griffe desl orteils). PhD thesis (Paris: Steinheil).
2. Davidenkow, S. (1939). Scapuloperoneal amyotrophy. Arch. Neurol. Psychiatry *41*, 694–701.
3. Hausmanowa-Petrusewicz, I., and Zielinska, S. (1962). Zur nosologiscen stellung des scapulo-peronealen syndroms. Dtsch. Z. Nervenheilkd. *183*, 377–382.
4. Kaeser, H.E. (1965). Scapuloperoneal muscular atrophy. Brain *88*, 407–418.
5. Steidl, L., and Urbanek, K. (1973). The dystrophic type of scapuloperoneal syndrome. Cesk. Neurol. *36*, 147–150.
6. Wilhelmsen, K.C., Blake, D.M., Lynch, T., Mabutas, J., De Vera, M., Neystat, M., Bernstein, M., Hirano, M., Gilliam, T.C., Murphy, P.L., et al. (1996). Chromosome 12-linked autosomal dominant scapuloperoneal muscular dystrophy. Ann. Neurol. *39*, 507–520.
7. Isozumi, K., DeLong, R., Kaplan, J., Deng, H.X., Iqbal, Z., Hung, W.Y., Wilhelmsen, K.C., Hentati, A., Pericak-Vance, M.A., and Siddique, T. (1996). Linkage of scapuloperoneal spinal muscular atrophy to chromosome 12q24.1-q24.31. Hum. Mol. Genet. *5*, 1377–1382.
8. Walter, M.C., Reilich, P., Huebner, A., Fischer, D., Schroder, R., Vorgerd, M., Kress, W., Born, C., Schoser, B.G., Krause, K.H., et al. (2007). Scapuloperoneal syndrome type Kaeser and a wide phenotypic spectrum of adult-onset, dominant myopathies are associated with the desmin mutation R350P. Brain *130*, 1485–1496.
9. Pegoraro, E., Gavassini, B.F., Borsato, C., Melacini, P., Vianello, A., Stramare, R., Cenacchi, G., and Angelini, C. (2007). *MYH7* gene mutation in myosin storage myopathy and scapuloperoneal myopathy. Neuromuscul. Disord. *17*, 321–329.
10. Greene, W.K., Baker, E., Rabbitts, T.H., and Kees, U.R. (1999). Genomic structure, tissue expression and chromosomal location of the LIM-only gene, *SLIM1*. Gene *232*, 203–207.
11. Lee, S.M., Tsui, S.K., Chan, K.K., Garcia-Barcelo, M., Waye, M.M., Fung, K.P., Liew, C.C., and Lee, C.Y. (1998). Chromosomal mapping, tissue distribution and cDNA sequence of four-and-a-half LIM domain protein 1 (FHL1). Gene *216*, 163–170.

12. Morgan, M.J., and Madgwick, A.J. (1996). Slim defines a novel family of LIM-proteins expressed in skeletal muscle. Biochem. Biophys. Res. Commun. *225*, 632–638.
13. Freyd, G., Kim, S.K., and Horvitz, H.R. (1990). Novel cysteine-rich motif and homeodomain in the product of the *Caenorhabditis elegans* cell lineage gene *lin-11*. Nature *344*, 876–879.
14. Goldberg, A.L. (2003). Protein degradation and protection against misfolded or damaged proteins. Nature *426*, 895–899.
15. Kadrmas, J.L., and Beckerle, M.C. (2004). The LIM domain: from the cytoskeleton to the nucleus. Nat. Rev. Mol. Cell Biol. *5*, 920–931.
16. Johannessen, M., Moller, S., Hansen, T., Moens, U., and Van Ghelue, M. (2006). The multifunctional roles of the four-and-a-half-LIM only protein FHL2. Cell. Mol. Life Sci. *63*, 268–284.
17. Morgan, M.J., and Madgwick, A.J. (1999). The LIM proteins FHL1 and FHL3 are expressed differently in skeletal muscle. Biochem. Biophys. Res. Commun. *255*, 245–250.
18. McGrath, M.J., Cottle, D.L., Nguyen, M.A., Dyson, J.M., Coghill, I.D., Robinson, P.A., Holdsworth, M., Cowling, B.S., Hardeman, E.C., Mitchell, C.A., and Brown, S. (2006). Four and a half LIM protein 1 binds myosin-binding protein C and regulates myosin filament formation and sarcomere assembly. J. Biol. Chem. *281*, 7666–7683.
19. McGrath, M.J., Mitchell, C.A., Coghill, I.D., Robinson, P.A., and Brown, S. (2003). Skeletal muscle LIM protein 1 (SLIM1/FHL1) induces alpha 5 beta 1-integrin-dependent myocyte elongation. Am. J. Physiol. Cell Physiol. *285*, C1513–C1526.
20. Robinson, P.A., Brown, S., McGrath, M.J., Coghill, I.D., Gurung, R., and Mitchell, C.A. (2003). Skeletal muscle LIM protein 1 regulates integrin-mediated myoblast adhesion, spreading, and migration. Am. J. Physiol. Cell Physiol. *284*, C681–C695.
21. Lee, S.M., Li, H.Y., Ng, E.K., Or, S.M., Chan, K.K., Kotaka, M., Chim, S.S., Tsui, S.K., Waye, M.M., Fung, K.P., and Lee, C.Y. (1999). Characterization of a brain-specific nuclear LIM domain protein (FHL1B) which is an alternatively spliced variant of FHL1. Gene *237*, 253–263.
22. Ng, E.K., Lee, S.M., Li, H.Y., Ngai, S.M., Tsui, S.K., Waye, M.M., Lee, C.Y., and Fung, K.P. (2001). Characterization of tissue-specific LIM domain protein (FHL1C) which is an alternatively spliced isoform of a human LIM-only protein (FHL1). J. Cell. Biochem. *82*, 1–10.
23. Brown, S., McGrath, M.J., Ooms, L.M., Gurung, R., Maimone, M.M., and Mitchell, C.A. (1999). Characterization of two isoforms of the skeletal muscle LIM protein 1, SLIM1. Localization of SLIM1 at focal adhesions and the isoform slimmer in the nucleus of myoblasts and cytoplasm of myotubes suggests distinct roles in the cytoskeleton and in nuclear-cytoplasmic communication. J. Biol. Chem. *274*, 27083–27091.
24. Oka, C., Nakano, T., Wakeham, A., de la Pompa, J.L., Mori, C., Sakai, T., Okazaki, S., Kawaichi, M., Shiota, K., Mak, T.W., and Honjo, T. (1995). Disruption of the mouse *RBP-J* κ gene results in early embryonic death. Development *121*, 3291–3301.
25. Taniguchi, Y., Furukawa, T., Tun, T., Han, H., and Honjo, T. (1998). LIM protein KyoT2 negatively regulates transcription by association with the RBP-J DNA-binding protein. Mol. Cell. Biol. *18*, 644–654.
26. Nicholls, A., Sharp, K.A., and Honig, B. (1991). Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. Proteins *11*, 281–296.