The Most Common Mutation in FKRP Causing Limb Girdle Muscular Dystrophy Type 2I (LGMD2I) May Have Occurred Only Once and Is Present in Hutterites and Other Populations

Patrick Frosk,1 Cheryl R. Greenberg,1 Alysa A.P. Tennesse,1 Ryan Lamont,1 Edward Nylen,1 Cheryl Hirst,1 Danielle Frappier,4 Nicole M. Roslin,4 Michaela Zaik,6 Kate Bushby,5 Volker Straub,6 Mayana Zatz,7 Flavia de Paula,7 Kenneth Morgan,3,4 T. Mary Fujiwara,3,4 and Klaus Wrogemann1,2*

1Departments of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Canada; 2Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Canada; 3Departments of Human Genetics and Medicine, McGill University, Montreal, Canada; 4The Research Institute of the McGill University Health Centre, Montreal, Canada; 5Institute of Human Genetics, University of Newcastle upon Tyne, United Kingdom; 6Department of General Pediatrics and Neuropediatrics, University of Essen, Essen, Germany; 7Human Genome Research Center, Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil

Communicated by Jacques Beckmann

Limb girdle muscular dystrophy (LGMD) is common in the Hutterite population of North America. We previously identified a mutation in the TRIM32 gene in chromosome region 9q32, causing LGMD2H in approximately two-thirds of the 60 Hutterite LGMD patients studied to date. A genomewide scan was undertaken in five families who did not show linkage to the LGMD2H locus on chromosome 9. A second LGMD locus, LGMD2I, was identified in chromosome region 19q13.3, and the causative mutation was identified as c.826C>A (L276I), a missense mutation in the FKRP gene. A comparison of the clinical characteristics of the two LGMD patient groups in this population reveals some differences. LGMD2I patients generally have an earlier age at diagnosis, a more severe course, and higher serum creatine kinase (CK) levels. In addition, some of these patients show calf hypertrophy, cardiac symptoms, and severe reactions to general anesthesia. None of these features are present among LGMD2H patients. A single common haplotype surrounding the FKRP gene was identified in the Hutterite LGMD2I patients. An identical core haplotype was also identified in 19 other non-Hutterite LGMD2I patients from Europe, Canada, and Brazil. The occurrence of this mutation on a common core haplotype suggests that L276I is a founder mutation that is dispersed among populations of European origin. Hum Mutat 25:38–44, 2005. © 2004 Wiley-Liss, Inc.

KEY WORDS: FKRP; TRIM32; limb girdle muscular dystrophy; LGMD; Hutterites; linkage mapping; linkage disequilibrium; founder effect

DATABASES:
TRIM32 – OMIM: 602290, 254110 (LGMD2H); GDB: 9957765; GenBank: NM_012210.2
FKRP – OMIM: 606596, 607155 (LGMD2I); GDB: 4589036; GenBank: NM_024301.2
www.dmd.nl (Leiden Muscular Dystrophy Pages)

INTRODUCTION

The limb girdle muscular dystrophies (LGMDs) are a clinically and genetically heterogeneous group of disorders characterized by weakness and wasting in the pelvic and shoulder girdles [Bushby, 1999a, 1999b]. The LGMDs are rare worldwide, with a prevalence of about 40 per million [Emery, 1991]. LGMDs have been mapped to 15 different loci; five LGMDs have an autosomal dominant mode of inheritance and 10 have an autosomal recessive mode of inheritance [Kaplan and Fontaine, 2004; Zatz et al., 2003]. Mutations in genes have been identified in three of the dominantly inherited LGMDs and for all of the recessively inherited LGMDs (see the Leiden muscular dystrophy website; www.dmd.nl).
A COMMON LGMD2I FOUNDER MUTATION

www.dmd.nl. Among the latter are LGMD2H (MIM# 254110) and LGMD2I (MIM# 607155). LGMD2H is a relatively mild form that was first described in the Manitoba Hutterites in 1976 [Shokeir and Kobrinsky, 1976] and has become known as the “Hutterite type” muscular dystrophy. We previously identified a missense mutation, c.1459G>A (D487N), in TRIM32 as the putative causative mutation, and all known Hutterite patients in families that showed linkage to chromosome region 9q32 were homozygous for this mutation [Frosk et al., 2002]. TRIM32 is a member of the tripartite-motif family of proteins [Reymond et al., 2001] and may be an E3 ubiquitin ligase due to the presence of a RING-finger domain [Fremont, 2000; Horn et al., 2004]. To date, mutations in this gene have not been found in any patients outside the Hutterite population. LGMD2I was first described in a large consanguineous Tunisian family and mapped to chromosome region 19q13.3 [Dris et al., 2002]. Concurrent with our study, another group [Brockington et al., 2001a, b] found mutations within the fukutin-related protein gene (FKRP) in LGMD2I families, as well as in families with a severe form of congenital muscular dystrophy, MDC1C (MIM # 606612). FKRP is thought to be a glycosyltransferase that may function in the O-linked glycosylation of proteins such as α-dystroglycan [Esapa et al., 2002; Hewitt and Grewal, 2003]. Recent work has shown that LGMD2I is one of the most common forms of LGMD worldwide [Bushby and Beckmann, 2003].

The Hutterites, also called Hutterite Brethren, live on farming colonies located predominantly in the Prairie Provinces and Great Plains of North America, and constitute a religious and genetic isolate. They immigrated to North America from Europe in the 1870s and formed three subdivisions. There has been very little intermarriage between subdivisions. The ancestry of the overwhelming majority of the Hutterites can be traced back to 89 ancestors [Nimgaonkar et al., 2002]. The history and social structure of the Hutterite Brethren are described in Hostetler [1985].

Here we report that the second type of LGMD in the Hutterite population maps to chromosome 19q31–q33 and is due to homozygosity for the L276I mutation in FKRP. We also present a comparison of the clinical variability of LGMD2I and LGMD2H and evidence that the L276I mutation observed in Hutterites and other populations is inherited from a common ancestor.

MATERIALS AND METHODS

Patients, Families, and Controls

DNA samples from 38 Hutterites, including 12 affected with LGMD, from five nuclear families, were included in a genomewide scan. Subsequent to the genomewide scan, we collected DNA from five other small Hutterite families with seven individuals affected with LGMD. The LGMD2H families referred to in this study are those reported in Frosk et al. [2002], along with one newly ascertained patient referred by Dr. Keith Brownell, University of Calgary. The clinical criteria for LGMD were newly ascertained patient referred by Dr. Keith Brownell, University of Calgary. The clinical criteria for LGMD were essentially the same as previously used [Weiler et al., 1998a].

DNA from 19 non-Hutterite LGMD2I patients was obtained. A total of 12 are from the UK, five are from Brazil (four Caucasian, one African-Brazilian), one is from Germany, and one is German-Canadian. A total of 14 of these patients are homozygous for the FKRP L276I mutation, and the remaining five are heterozygous for this mutation. The second mutation in four of the five compound heterozygotes is E310X, W279X, V300M, or Y307N, while no other mutation was identified in the coding sequence of FKRP for the remaining heterozygote. A control group of 111 healthy individuals from Manitoba, not known to have LGMD, was used to determine allele frequencies. Controls were obtained from the Rh Laboratory, University of Manitoba, and are 90% Caucasian, with the remainder being Aboriginal, Asian, and African. This study was approved by the Health Research Ethics Board of the University of Manitoba.

DNA Analysis

Protocols for DNA analysis have been published previously, as follows: genotyping of microsatellite markers in candidate genes and for fine mapping [Weiler et al., 1998a], genomewide scan at the Montreal Genome Centre [Mira et al., 2003], and DNA sequencing [Frosk et al., 2002]. PCR cloning was done using a TOPO TA cloning kit (Invitrogen) as per the manufacturer’s instructions. A total of 18 additional polymorphisms were added to this study in used were: D19S903, D19S918, D19S908, D19S219, DM, D19S412, FKRP52 (hsSNP rs8179080), a C>G SNP in the FKRP promoter (rs3810288), FKRP c.135C>T (rs2287717), EMS2 (rs3138636), D19S540, D19S606, D19S590, D19S596, D19S879, D19S550, D19S867, and D19S904. FKRP52 [Loubiichi et al., 2003], a C/A/C repeat found within an intron of the PRK2 gene, was amplified by PCR with FKRP52_F (5’TCTCCAAAAACAAACACAC-3’) and FKRP52_R (5’CTAGTTGTCTGCGGTTTCTT-3’), EMS2, a CA repeat found within the 3’ untranslated region (UTR) of SLC1A5 [Jones et al., 1994], was amplified with EMS2_F (5’GGAGAGGATTGCTGTTT-3’) and EMS2_R (5’-CACCACCTGCTTATTTTG-3’). The fragment was then digested with NgOIV (New England Biolabs, www.neb.com), the C allele yields two fragments (499 and 203 bp) and the T allele remains uncut. Rs3810288 in the FKRP promoter was amplified in a 118-bp fragment using rs3810288_F (5’TCCACACCTGCTGCTCTTCC-3’) and rs3810288_R (5’AGCTGGAGGTCCTGCGAATC-3’). A mismatch (denoted by lowercase t) creates an Hpy188I site (New England Biolabs), the C allele yields two fragments when digested (96 and 22 bp) and the G allele remains uncut. Samples for both SNPs, c.135C>T and rs3810288, were analyzed by electrophoresis on polyacrylamide gels (8 and 15%, respectively).

Addition to genotyping, FKRP c.826G>A (L276I) (NM_024301.2) by BfaI digestion [Brockington et al., 2002], we developed a nested allele-specific PCR assay. A 2-kb PCR product was generated using primers FKRP-ex4(1F) (5’-CTCGCTTCTCCTTGTGCTTCC-3’) and FKRP-ex4(3R) (5’-CCAAACTGGCGCCCTG-3’). This fragment contains most of exon 4 of the FKRP gene and was used as a template in a secondary allele-specific amplification step using the reverse primers FKRP826C (5’-CTCTCCCCAGCTCCACGTG-3’) and FKRP826A (5’-CTCTCCCCAGCTCCACGTG-3’). Each primer has an intentional mismatch at the second-to-last base pair (lowercase t) to destabilize the resultant DNA duplex, and the last base pair corresponds to either the normal (C) or mutant (A) sequence. A common forward primer, FKRP-ex2(2F) (5’-CTGCTGGAGGAGCCCTG-3’) was used and amplification with the two reverse allele-specific primers was done in separate PCR reactions. Samples were analyzed by electrophoresis in 1.5% agarose.

Protein Analysis

Muscle proteins were extracted in treatment buffer containing 0.125 M Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 5% mercaptoethanol, and 0.001% bromophenol blue. Soluble proteins were separated by SDS-PAGE on 3 to 10% linear gradient gels and transferred to a nitrocellulose membrane. The membrane was blocked in 3% milk powder in phosphate buffered saline (PBS), treated with anti-glycosylated α-dystroglycan antibody (IIH6C4, Upstate 1:1,000, www.upstate.com) or anti-β-dystroglycan antibody (Novocastra 1:50, www.novocastra.co.uk), and washed and
incubated with horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibodies (Dianova, www.dianova.de). Immunoreactive bands were detected using a chemiluminescence detection system (ECL, Amersham Biosciences, www.amershambiosciences.com).

Skeletal muscle tissues were embedded in tragant and frozen in liquid nitrogen-cooled isopentane. Cryosections (7 μm) were immunostained with anti-glycosylated α-dystroglycan (IHH6C4; Upstate) in TBS/1% bovine serum albumin (BSA) for 90 min. After washing, sections were incubated with the appropriate Texas Red dye-conjugated secondary antibody for 30 min. Sections were observed under a Zeiss Axioplan fluorescence microscope (www.zeiss.com).

**RESULTS**

**Identification of the LGMD2I Locus in Hutterites**

After the LGMD2H locus had been mapped, five Hutterite families did not show linkage to chromosome region 9q31–q33, two of which had been reported previously [Weiler et al., 1998b]. After excluding the known LGMD loci LGMD1A, IB, ID, and 2A–2G, we performed a genomewide scan, using 389 microsatellite markers with an average spacing of 9.1 cM, on 38 individuals, including 11 affected with LGMD. Single-point parametric linkage analysis with a fully penetrant autosomal recessive disease model was performed using GENEHUNTER 2.1 [Kruglyak et al., 1996; Markianos et al., 2001]. A maximum logarithmic odds (lod) score of 1.50 at D19S587 was obtained. The second highest score was 0.87 at the adjacent marker, D19S178, and the third highest score was 0.67 at D2S407. These lod scores were all at zero recombination. Using multipoint linkage analysis, the maximum multipoint lod score was 3.18 at D19S178. The region with the next highest multipoint lod score was 1.06 at D2S407.

To define a candidate gene interval, families were genotyped for 18 additional markers in a 13-cM region between two genomescan markers, D19S178 and D19S246 (see Materials and Methods section). Three markers, FMS2, FKRP52, and D19S902, in a 1.3-cM interval, had single-point lod scores of 3.33 at zero recombination. During our fine-mapping studies, Brockington et al. [2001a] reported that a form of congenital muscular dystrophy was caused by mutations in FKRP, a promising candidate gene for LGMD2I. The entire coding region of FKRP was sequenced in one Hutterite LGMD2I patient who was found to be homozygous for a missense mutation, c.826C>A (L276I), that results in the substitution of isoleucine for leucine. This same mutation was reported by Brockington et al. [2001b], with the patients from 15 of their 17 LGMD2I families being homozygous (five families) or heterozygous (10 families). We found that every Hutterite LGMD patient who was not homozygous for the TRIM32 D487N mutation, was homozygous for the FKRP L276I mutation. L276I was not found in our control group of 111 individuals.

The majority of Hutterites who are homozygous for the L276I mutation are also homozygous for D19S412 (109 bp), FKRP52 (110 bp), rs3810288 (G allele), c.135C>T (T allele), FMS2 (142 bp), and D19S540 (184 bp) (Fig. 1). This indicates that a genomic segment of about 0.5 Mb is shared among Hutterites carrying L276I and is likely identical by descent from a common ancestor. In comparison to the allele frequencies of our 111 controls (Table 1), there is a strong association of L276I in the non-Hutterite patients with the 110-bp allele of FKRP52 (52 kb centromeric to L276I), the G allele of rs3810288 (10 kb centromeric to L276I), the T allele of c.135C>T (0.7 kb telomeric to L276I), and the 142-bp allele of FMS2 (19 kb telomeric to L276I). The association of L276I with the 109-bp allele of D19S412 (250 kb centromeric to L276I) and the 184 bp allele of D19S540 (250 kb telomeric to L276I) does not appear as strong (Table 1). The markers flanking this region (DM, 1 Mb centromeric and D19S606, 0.7 Mb telomeric) show no association with L276I in the patients that we studied (Table 1). Recombination appears to have occurred between L276I and FMS2 on one chromosome (Patient C11.975) and between rs3810288 and c.135C>T on another (Patient NCL-10; Fig. 1). This results in a very small common core haplotype consisting of the mutation itself and c.135C>T. The likelihood that this set of associations has occurred by chance is low and reflects strong linkage disequilibrium. Thus, L276I appears to have arisen only once and is identical by descent in most, and possibly all patients. The presence of a homoygous African-Brazilian individual showing these same associations (Patient C10.882; Fig. 1) raises the possibility that the mutation is not
specific to Caucasians. However, with our limited data this cannot be conclusively determined, particularly in light of the extreme amount of admixture amongst Brazilians [Parra et al., 2003].

Of note are the discrepancies present at the FKRP locus. A possible mutation in FKRP52 due to slipped strand mispairing during DNA replication is present on five of the nine Brazilian chromosomes (110 bp > 108 bp). This suggests a recent Brazilian mutational event in a common ancestor of three of these five Brazilian patients [de Paula et al., 2003]. In addition, within the Hutterite population there are also individuals with a 112-bp allele instead of a 110-bp allele on the same haplotype as the L276I mutation. Through cloning and sequencing, we have found a large amount of variation in FKRP52 (data not shown). This hypervariability readily explains the discrepancies seen in patients with Becker muscular dystrophy has been noted. Two children presented with reactions to inhalation anaesthetic. Unlike LGMD2I, to our knowledge, LGMD2H patients have shown none of the following characteristics: muscle hypertrophy, reaction to general anaesthetics, or development of cardiomyopathy. In addition, none of the LGMD patients of either type that we have studied have shown signs of facial weakness or any respiratory symptoms; however, subtle respiratory difficulties cannot be ruled out.

Among the Hutterite LGMD families that we studied, there was one individual who was homozygous for FKRP L276I and heterozygous for TRIM32 D487N. This individual has proximal muscle weakness and a highly elevated CK level of 9,190 U/L. There were also six individuals who were homozygous for TRIM32 D487N and heterozygous for FKRP L276I, representing two families (a father and daughter from one family and a set of four siblings from another family). The father is ambulatory but has proximal muscle weakness, a dystrophic muscle biopsy, and a CK level of 669 U/L. The daughter is asymptomatic at this time and her CK level is 267 U/L. In the remaining family, all four siblings from another family). The father is ambulatory but has proximal muscle weakness, a dystrophic muscle biopsy, and a CK level of 669 U/L. The daughter is asymptomatic at this time and her CK level is 267 U/L. In the remaining family, all four siblings have CK levels ≥ 10 × maximum normal (range 1,700–2,960 U/L), one has had a clearly dystrophic muscle biopsy, and two show proximal weakness but they are all ambulatory. Within both of these families, there are eight individuals who are heterozygous for both mutations. Seven of these individuals were available for study and were clinically normal (as examined by C.R.G.). CK values for these individuals ranged from 35–294 U/L.

### DISCUSSION

We have demonstrated unexpected locus heterogeneity for LGMD in the Hutterite population, and have identified patients who are homozygous for a missense mutation (D487N) in TRIM32 or homozygous for a missense mutation (L276I) in FKRP. This provides another example of genetic heterogeneity of an autosomal recessive disease in a genetically isolated population. Both locus and allelic heterogeneity were found for LGMD in the Amish, another Anabaptist isolate [Duclos et al., 1998]. At this time, there is no evidence for a third locus causing LGMD in this population.
Table 2. Clinical Differences Between LGMD2H and LGMD2I in the Hutterites

<table>
<thead>
<tr>
<th>Mutation Status</th>
<th>Current Status</th>
<th>Number of Individuals</th>
<th>Age of Onset (years)</th>
<th>Calf Hypertrophy</th>
<th>Highest Resting Mean Multiples of Normal CK (U/litre)</th>
<th>Mean Male Normal</th>
<th>Cardiac Status</th>
<th>Mean Age of Consent</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD2H homozygote</td>
<td>12 Asymptomatic (7-42), 13 Deceased at birth (0-27), 14 Deceased at age 2 (0-2)</td>
<td>41</td>
<td>9-24</td>
<td>Absent (41)</td>
<td>Not studied (28)</td>
<td>10 × 81−5,556</td>
<td>Normal (23), not studied (28)</td>
<td>49</td>
</tr>
<tr>
<td>LGMD2I homozygote</td>
<td>17 Ambulatory (5-50), 26 Asymptomatic (2-25), 28 Deceased/congestive heart failure (40)</td>
<td>19</td>
<td>9-60</td>
<td>Absent (19)</td>
<td>Not studied (5)</td>
<td>1 × 322−26,087</td>
<td>Normal (10), not studied (5)</td>
<td>25</td>
</tr>
<tr>
<td>LGMD2H carrier</td>
<td>38 Ambulatory (6-77), 41 Deceased/congestive heart failure (40), 46 Ambulatory (5-46), 21 Asymptomatic (6-77)</td>
<td>49</td>
<td>9-42</td>
<td>Absent (49)</td>
<td>Not studied (23)</td>
<td>1 × 81−5,556</td>
<td>Normal (19), not studied (8)</td>
<td>24</td>
</tr>
<tr>
<td>LGMD2I carrier</td>
<td>36 Ambulatory (6-70), 37 Deceased/congestive heart failure (40), 38 Ambulatory (6-70), 39 Asymptomatic (6-77)</td>
<td>23</td>
<td>9-77</td>
<td>Absent (23)</td>
<td>Not studied (8)</td>
<td>1 × 81−5,556</td>
<td>Normal (20), not studied (13)</td>
<td>15</td>
</tr>
</tbody>
</table>

*Six of these are also heterozygous for LGMD2I and not obviously different phenotype.  
One of these is also heterozygous for LGMD2I and not obviously different phenotype.

LGMD2H appears to be more frequent in the Schmiedeleut subdivision of the Hutterites, whereas LGMD2I appears to be more frequent in the Dariusleut. Currently, we do not have an accurate estimate of the relative frequencies of the two mutations in each of the subdivisions. Overall, we have ascertained 58 Canadian Hutterite LGMD patients and the Canadian Hutterite population is estimated to be 28,020 (Statistics Canada; www.statcan.ca). Thus, the estimated prevalence of LGMD in this population is at least 1 in 483. This is very much higher than the highest prevalence reported to date of 1 in 14,493 (69 per million) in the Guipúzcoa population in Spain [Urtasun et al.,1998].

There is considerable clinical heterogeneity for both LGMD2H and LGMD2I in spite of a uniform communal lifestyle and only one mutation for each of the LGMDs. A wide spectrum in clinical severity has been previously reported for LGMD2I and ascribed to the various mutations found in compound heterozygotes [Mercuri et al., 2003]. We have observed similarly large clinical variation, although all our patients are homozygous for the FKRP L276I mutation. This was also seen in a recent study by Walter et al. [2004] in which 13 out of 20 patients from nonconsanguineous matings were homozygous for this mutation and showed similar clinical variability. Our impression is that patients with LGMD2I present earlier, follow a more severe course with possible cardiomyopathy (Poppe et al., submitted manuscript), and have higher serum CK levels than LGMD2H patients, although we may be underascertaining more mildly affected LGMD2I patients. A comprehensive ascertainment of LGMD in the Hutterites is needed to provide accurate information on the prevalence, penetrance of the genotypes, and clinical variability of LGMD. In addition, there is no indication of any interaction between the two loci as individuals with mutations at both loci are indistinguishable from those with mutations at only one locus (Table 1). This is not surprising due to the apparently different mechanisms by which these genes appear to cause muscular dystrophy [Esapa et al., 2002; Frosk et al., 2002]. Presumably, the only situation that might result in a more severe phenotype would be a double mutation homozygote.

It appears that all Hutterite LGMDs are of either type 2H or type 2I. This will make it possible to provide accurate noninvasive diagnostic and carrier testing for LGMD in Hutterites. Specific tests for these two mutations are currently being established in the molecular diagnostic laboratory at the Health Sciences Centre, Winnipeg, Manitoba. Such a DNA-based approach is not yet practical for the non-Hutterite LGMD population because of the marked locus and allelic heterogeneity. Given the high incidence of LGMD2I carriers in the Hutterite population, and the risk of cardiomyopathy and anaesthetic reactions in this group, we would suggest that genetic testing of at-risk individuals even below the normal age of consent for such testing should be considered and discussed with the families.

The FKRP L276I mutation appears to be common worldwide, with respect to LGMD-causing mutations. Carrier frequency is estimated to be 1 out of 306 on the basis of controls typed for L276I [Brockington et al., 2001b; de Paula et al., 2003; Walter et al., 2004; this study]. In addition, patients homozygous for L276I from 28 nonconsanguineous families have been reported [Brockington et al., 2001b; Poppe et al., 2003; Walter et al., 2004]. Our analysis indicates that L276I may be a founder mutation, as all Hutterite and non-Hutterite disease chromosomes tested to date carry the low frequency T allele (14%) at an intragenic SNP (c.135C>T) and the G allele at rs3810288 in the putative
promoter of FKRP (with one exception). The L276I mutation with the C allele at rs3810288 (Patient NCL-10; Fig. 1) is consistent with being a recombinant chromosome but due to the lack of phase information for most of the genotypes this cannot be firmly established. The T allele of c.135C>T and the G allele of rs3810288 have been shown to be associated with a further 26 L276I chromosomes from German patients [Walter et al., 2004], strengthening the evidence for a founder mutation. Markers as far away from the L276I mutation as 0.25 Mb in each direction show readily detectable linkage disequilibrium; however, markers 0.75–1.0 Mb away show very little linkage disequilibrium in the samples used in this study. This is strong evidence that L276I has arisen only once. The small common core haplotype is an indication that the mutation may have occurred long ago. Further analysis of SNPs in and around FKRP and additional patients will be needed to confirm that most, if not all, copies of L276I in the contemporary population are identical by descent and to accurately estimate the age of the mutation.

The relative frequency of L276I in Caucasians, compared to other LGMD-causing mutations, and the high likelihood that it is a founder mutation, are readily explained by genetic drift. However, given the postulated function of FKRP, it is tempting to speculate that a selective advantage may also be contributing to the maintenance of this allele. FKRP is thought to be a glycosyltransferase, and its mutations affect the glycosylation of α-dystroglycan, an essential component of muscle cell membranes [Durbeej et al., 1998a; Esapa et al., 2002; Hewitt and Grewal, 2003]. An immunoblot of muscle from an L276I homozygote shows a decreased level of fully glycosylated α-dystroglycan (Fig. 2). Two other groups have recently reported variably decreased levels of α-dystroglycan in patients homozygous for L276I as well [Brown et al., 2004; Walter et al., 2004]. α-Dystroglycan is known to be expressed in numerous tissues and has been shown to be the receptor for the entry of two different types of pathogens [Cao et al., 1998; Durbeej et al., 1998b; Rambukkana et al., 1998]. It is possible that an FKRP L276I heterozygote with a mild defect in glycosylation may have a partial resistance to these or other pathogens. Over long periods this advantage would then increase the prevalence of the FKRP L276I allele in areas where the pathogen is endemic. On the basis of the data presented here, it appears that the frequent occurrence of L276I is not the result of multiple de novo mutations as was previously thought [Bushby and Beckmann, 2003]. Instead, our findings suggest this mutation has occurred once and became prevalent through either genetic drift, selective advantage, or some combination of both.

ACKNOWLEDGMENTS

We are indebted to the patients and their families for their participation in this study. We thank Drs. T. Bree, F. Booth, C. Bourque, K. Brownell, A. Hoke, W. Ilse, T. Ladd, N. Lowry, C. Toth, and S. Wetzman for patient samples, and Dr. T. Zelinski for control samples. We thank Dr. T. Hudson and A. Verner for facilitating the genomewide scan in the Montreal Genome Centre (McGill University and Genome Quebec Innovation Centre), J. Crumley for maintaining the genealogical database for many years and for the Xbase computer programs, J. Loredo-Osti for helpful discussions, and L. Cree and M. Buddles for mutation analysis in the Newcastle patients. V. Straub and M. Zaik were supported by the German Research Society (DFG Str 498/3–2, SPP 1086) and the Krupp-Stiftung. The Newcastle Muscle Centre receives financial support from the Muscle Dystrophy Campaign.

REFERENCES


FIGURE 2. Immunodetection of glycosylated α-dystroglycan. Immunofluorescence staining of skeletal muscle from a control individual (a) and from LGMD2I Patient G-01 (b) with anti-glycosylated α-dystroglycan antibody (IIH6C4, Upstate). c: Reduction of α-dystroglycan expression in skeletal muscle of a patient (lane 2) compared to control muscle (lane 1) by immunoblot. No reduction is apparent in β-dystroglycan. Equal loading determined by Ponceau S staining (not shown).


