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The mouse homolog of FRG1, a candidate gene for FSHD, maps proximal to the *myodystrophy* mutation on Chromosome 8

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Abstract. The human autosomal dominant neuromuscular disorder facioscapulohumeral muscular dystrophy (FSHD) is associated with deletions within a complex tandem DNA repeat (D4Z4) on Chromosome (Chr) 4q35. The molecular mechanism underlying this association of FSHD with DNA rearrangements is unknown, and, thus far, no gene has been identified within the repeat. We isolated a gene mapping 100 kb proximal to D4Z4 (FSHD Region Gene 1:FRG1), but were unable to detect any alterations in total or allele-specific mRNA levels of FRG1 in FSHD patients. Human Chr 4q35 exhibits synteny homology with the region of mouse Chr 8 containing the gene for the myodystrophy mutation (*myd*), a possible mouse homolog of FSHD. We report the cloning of the mouse gene (*Frg1*) and show that it maps to mouse Chr 8. Using a cross segregating the *myd* mutation and the European Collaborative Interspecific Backcross, we showed that *Frg1* maps proximal to the *myd* locus and to the *Clc3* and *Ant1* genes.

Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder characterized by progressive weakness and atrophy of the face, shoulder girdle, and upper arm muscles (Lunt and Harper 1991). The locus for FSHD has been mapped to human Chr 4q35 and is associated with deletions within a 3.3-kb tandem repeat, D4Z4 (van Deutekom et al. 1993). Despite extensive analysis of this D4Z4 locus, no gene has been found to reside there (Hewitt et al. 1994; Altherr et al. 1995; Lyle et al. 1995). However, considering the association of sequences related to D4Z4 with heterochromatic regions of the genome, a position effect variegation model has been proposed as the underlying genetic mechanism for FSHD (Hewitt et al. 1994; Winokur et al. 1994; Lyle et al. 1995).

The search for genes in the FSHD candidate region has been hindered by the presence of high and low copy number repeats (Altherr et al. 1995; van Deutekom et al. 1995). Despite this, we recently reported the isolation and characterization of the first FSHD candidate gene (FRG1;FSHD Region Gene 1) located approximately 100 kb centromeric of the repeat units (van Deutekom et al. 1996). Although allele-specific RT-PCR experiments suggested that transcript levels of FRG1 were not affected by FSHD-associated deletions, we could not exclude the possibility of a subtle change in transcription levels (van Deutekom et al. 1996).

The spontaneously arising mouse mutation, myodystrophy (*myd*), maps to mouse Chr 8 (Lane et al. 1976; Mathews et al. 1995b), which shows homology of synteny with distal human Chr 4q. *myd* is flanked distally by mitochondrial uncoupling protein (*Ucp*) with a homolog on human 4q31 and proximally by chloride channel 3 (*Clc3*) and coagulation factor XI (*Cfl1*), genes with homologs on human 4q32 and 4q35 respectively (Mathews et al. 1995b; Mills et al. 1995, 1996b). The *myd* mutation may be homologous to FSHD. The isolation of FRG1 thus provided a promising candidate gene for involvement in *myd*. The aim of this study was to isolate the mouse homolog of FRG1 and map its position relative to the *myd* locus.

Materials and methods

PCR analysis of somatic cell hybrid panel. The CV panel of mouse × Chinese hamster somatic cell hybrids (Williamson et al. 1995) was used for chromosomal assignment of mouse *Frg1*. DNA from this panel was obtained from the MRC HGMP Resource Center. PCR primers used: GEGF: 5′ AGAAATCTTAAAAAGGCTCGG 3′; GEG2R: 5′ AAGCTACACAAAATGTCCACG 3′. These primers amplified a 1500-bp product in mouse CBA/H DNA; no product was amplified in hamster V79 TOR DNA. Fifty nanogram of template DNA was used in a final reaction volume of 25 μl containing 10 mM Tris-HCl pH 8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 150 ng of each primer, and 0.5 U Taq polymerase (Bioline, London). The PCR was carried out in a Hybaid Omnigene Thermocycler for 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s extension at 72°C for 30 s.

Screening cDNA libraries. The 1.5-kb GEG2 PCR product, precompeted with mouse Cot1 DNA (Gibco BRL, Paisley) for 1.5 h at 65°C, was used as a probe to screen a 10-dpc mouse embryo cDNA library cloned into λSH/*lox* vector (Novagen). Final washes were performed at 65°C in 0.5–1 × SSC, 0.1% SDS for 30 min. Four positive plaques were picked and suspended in SM buffer 100 mM NACl, 10 mM Mg504, 35 mM Tris[pH 7.5] and rescreened twice before further analysis.

Genetic mapping of Frg1. DNA samples, prepared by the European Collaborative Interspecific Backcross (EUCIB) between laboratory strain C57BL/6 and *Mus spretus* (Breen et al. 1994), were obtained from the MRC HGMP Resource Centre. Using the PCR conditions described for the STS GEG2, a product was amplified from the parental strains. Although the two species did give slightly different-sized products, the difference between the two strains was too small to resolve accurately on agarose gels; therefore, an *Hae*III polymorphism was used. PCR product was digested with 1U of the restriction enzyme *Hae*III in a total volume of 30 μl for at least 1 h to identify the polymorphism. Several constant bands were produced, with strain-specific fragments of 750 bp and 1 kb in C57BL/6 and *M. spretus* respectively. Both fragments could be clearly identified in heterozygotes. Samples were scored on 3% NuSieve (Flowgen) gel stained with ethidium bromide. A polymorphism was produced for *Ant1* based on the genomic structure of the human ANT1 gene (Li et al. 1989) and the mouse mRNA sequence deposited in GenBank (Accession no. U27315). Mouse-specific PCR primers (AntF: 5′ GCTGCCAGACCCCAAGAATG 3′; AntR: 5′GTCCCCGTGTACATAATATC 3′) amplify a product of approximately 1 kb from mouse genomic DNA with PCR conditions as

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  1 aagcgaagaccgagagttctcggggactcgctcagctccggccgttagagccacttcctcctcctcccagtcctacggaagctctgccagcggctgccacttcagcgagccgggccATGG  120
                                                                                                                      M  A

121 CCGAATATTCCTATGTAAAGTCCACCAAACTCGTGCTCAAGGGCACTAAAGCCAAGAGTAAAAAGAAAAAGAGCAAAGATAAGAAGAGAAAACGGGAAGAAGACGAGGAGACCCAACTTG  240
     E  Y  S  Y  V  K  S  T  K  L  V  L  K  G  T  K  A  K  S  K  K  K  K  S  K  D  K  K  R  K  R  E  E  D  E  E  T  Q  L  D

241 ATATTGTGGGAATCTGGTGGACTGTATCCAACTTTGGGGAGATTTCAGGAACTATTGCCATTGAAATGGATAAAGGAGCCTATATCCATGCACTGGACAATGGACTGTTTACACTGGGAG  360
     I  V  G  I  W  W  T  V  S  N  F  G  E  I  S  G  T  I  A  I  E  M  D  K  G  A  Y  I  H  A  L  D  N  G  L  F  T  L  G  A

361 CTCCACACAGAGAAGTTGATGAAGGCCCCAGTCCTCCAGAGCAGTTTACTGCTGTCAAGTTGTCTGACTCCAGAATTGCCCTGAAGTCTGGCTATGGAAAATATCTTGGTATAAATTCAG  480
     P  H  R  E  V  D  E  G  P  S  P  P  E  Q  F  T  A  V  K  L  S  D  S  R  I  A  L  K  S  G  Y  G  K  Y  L  G  I  N  S  D

481 ATGGACTTGTTGTTGGGCGTTCGGATGCAATTGGGCCCAGAGAACAATGGGAACAAGTGTTTCAAGATGGGAAGATGGCTTTACTGGCCTCAAATAGCTGCTTTATTAGATGTAATGAAG  600
     G  L  V  V  G  R  S  D  A  I  G  P  R  E  Q  W  E  Q  V  F  Q  D  G  K  M  A  L  L  A  S  N  S  C  F  I  R  C  N  E  A

601 CAGGCGATATAGAAGCAAAGAATAAAACGGCAGGAGAAGAAGAAATGATAAAGATCAGATCCTGTGCGGAAAGAGAAACCAAGAAAAAGGATGACATCCCAGAAGAAGACAAGGGAAGTG  720
     G  D  I  E  A  K  N  K  T  A  G  E  E  E  M  I  K  I  R  S  C  A  E  R  E  T  K  K  K  D  D  I  P  E  E  D  K  G  S  V

                                                                                            f ------------------>
721 TGAAGCAGTGTGAAATCAACTATGTAAAGAAATTTCAGAGCTTCCAGGACCACAAACTCAAAATAAGCAAAGAAGATAGTAAAATTCTTAAAAAGGCTCGGAAAGATGGATTTTTACATG  840
     K  Q  C  E  I  N  Y  V  K  K  F  Q  S  F  Q  D  H  K  L  K  I  S  K  E  D  S  K  I  L  K  K  A  R  K  D  G  F  L  H  E

                                                 <-------------- r
841 AGACACTTCTGGACAGGAGAGCCAAATTGAAAGCTGACCGATACTGCAAGtgacgtggacatttgtgtagctttgggtttttttctgtggtttttttgttgttgttgtttctttctttct  960
     T  L  L  D  R  R  A  K  L  K  A  D  R  Y  C  K  *

961 ttgtcccctctctctctctctctctttctctgaataaaaatatttgatataagtaaaaaa  1022
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B

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mouse   1 MAEYSYVKSTKLVLKGTKAKSKKKKSKDKKRKREEDEETQLDIVGIWWTVSNFGEISGTIAIEMDKGAYIHALDNGLFTLGAPHREVDEGPSPPEQFTAV
human   1 ------------------T-------------------------------T----------------T---------------K----------------

mouse 101 KLSDSRIALKSGYGKYLGINSDGLVVGRSDAIGPREQWEQVFQDGKMALLASNSCFIRCNEAGDIEAKNKTAGEEEMIKIRSCAERETKKKDDIPEEDKG
human 101 ---------------------------------------P---N------------------------S-------------------------------

mouse 201 SVKQCEINYVKKFQSFQDHKLKISKEDSKILKKARKDGFLHETLLDRRAKLKADRYCK 258
human 201 N--------------------------------------------------------- 258
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Fig. 1. (**A**) Nucleotide and deduced amino acid sequence of *Frg1* cDNA (GenBank Accession number U62105). The positions corresponding to the PCR primers GEGF and GEGR are shown by arrows labeled f and r, respectively. The polyadenylation signal is underlined. (**B**) A comparison of the amino acid sequences of the human and mouse *Frg1* genes. Amino acid identity is indicated by –.

above, with an annealing temperature of 58°C. Again, an *Hae*III polymorphism was identified with bands of 250 bp and 400 bp being diagnostic for C57BL/6 and *M. spretus,* respectively. *Clc3* was mapped with PCR primers and conditions described in Mills et al. (1995) except that an AlwNI polymorphism was used to score alleles, rather than SSCP. For D8Mit markers, polymorphisms were typed on 4% NuSieve gels; genotypes were scored according to the allele inherited from the hybrid parent. The genotypes were entered into the program Map Manager (Manly 1993) and loci arranged in the order giving the fewest number of recombinants.

The localization of *Frg1* with respect to the *myd* locus was determined in a panel of animals developed to map the mutation (Mills et al. 1995). B6C3Fe-*a/a-myd*/+ animals were crossed with CAST/Ei mice. Heterozygotes (identified by production of affected offspring) were backcrossed to the parental strain carrying the mutation to generate affected animals (this cross is referred to below as the B6C3-CAST cross). Unaffected siblings have also been genotyped to order markers in the region. From inspection of the EUCIB data, a subset of affected animals and unaffected sibs were identified that would localise *Frg1* in the cross, and these were genotyped. Approximately 100 additional chromosomes from affected and unaffected mice were also examined to confirm the predicted order. PCR amplification of *Frg1* was carried out as described above, and the products were separated on a 1.2% agarose, 0.5× TBE gel (approximate band sizes: *cast*–1.4 kb; B6C3–1.5 kb). In this cross, no digestion was required to score the alleles.

Northern blot hybridization. Total RNA was isolated from mouse (normal and *myd* homozygote) tissues according to RNAzol protocol and using RNA Instapure (Eurogenetic) as described previously (van Deutekom et al. 1996). Northern blots were prepared with 20 μg total RNA. Hybridizations were performed in Church buffer for 16 h at 65°C with a-^{32}P-labeled probes. The *Frg1* probes were produced by amplification of a 535-bp product from cDNA clone MC10 with the FRG1 primers 3f and 8r (van Deutekom et al. 1996). As a control for RNA loading, a 1200-bp mouse rRNA probe was used. Blots were washed at 65°C and to a stringency of 2 × SSC, 0.1% SDS for *Frg1* and 0.1 × SSC, 0.1% SDS for the rRNA probe.

Subcloning and sequencing. Phage DNA from the cDNA clones was isolated by the method of Chisholm (1989). Inserts were released by digestion with *Eco*RI and *Hin*dIII and cloned into pBluescript SKII+ (Stratagene). Double-stranded plasmid DNA was sequenced with a Sequenase (USB) kit with modifications as described by Rouer (1994). Sequence analysis was carried out with the GCG package of programs (Genetics Computer Group, 1994), and database searches used the BLAST program (Altschul et al. 1990).

Results

Isolation of the mouse homolog of FRG1 (Frg1). Searching the GenBank database with the human FRG1 cDNA sequence revealed a 3′ EST clone (GenBank accession no. X71639) with 88% nucleotide identity to human FRG1. Mouse-specific PCR primers were designed to span intron 8, which in the human gene is 1 kb (van Deutekom et al. 1996). These primers amplified an approximately 1.5-kb product in mouse genomic DNA. The PCR product was then used to screen a 10-dpc mouse embryonic cDNA library and 4 clones identified. One clone (MC10) was then sequenced completely on both strands. The sequence showed there to be errors in the published EST, including the region used to design the forward PCR primer. Although the original primer pair amplified the correct product, to improve the PCR reliability a second set of primers corresponding to the correct sequence (GEG2F/GEG2R) was used in subsequent PCR analysis (Materials and methods). The two arrows in Figure 1A indicate the positions of the redesigned PCR primers used to amplify the GEG2 PCR product.

The nucleotide and predicted amino acid sequence of the mouse cDNA (GenBank Accession number U62105) is shown in Fig. 1A. The cDNA is 1022bp and contains an open reading frame encoding 258 amino acids with 97% amino acid identity to the human FRG1 protein (Fig. 1B). This suggests that the cDNA clone

Table 1. PCR analysis of *Frg1* with a mouse/hamster somatic cell hybrid panel. The table shows the mouse chromosome content of each hybrid cell line. '+' and '−' indicate presence or absence of the chromosome, respectively. (+) indicates that the chromosome is thought to be present in only a portion of the cells. (±) indicates that inconsistent results have been reported in previous PCR experiments. Cell lines are indicated as to whether they were positive or negative for the GEG2 PCR product. Discordance for each chromosome is given underneath.

	Mouse chromosome																					
Hybrid name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	GEG2
MH1	+	−	−	−	−	−	−	(+)	−	−	−	−	−	+	−	+	−	+	−	+	+	−
MH2	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	+	+	−	−	+	−	−
MH3	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	+	−	−	−	+	−	−
MH4	−	−	+	−	−	+	−	−	−	−	−	−	+	+	−	+	+	−	+	+	+	−
MH6	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	−	−	+	+	−
MH7	−	−	+	−	−	+	−	−	−	−	−	−	+	+	−	+	+	−	+	+	+	−
MH8	+	+	−	+	−	+	−	−	−	−	−	−	+	+	+	−	+	−	−	+	+	−
MH9	+	+	−	+	−	+	−	−	−	−	−	−	−	+	+	−	+	−	−	+	+	−
MH12	−	+	−	+	−	+	−	−	−	−	−	−	+	+	−	+	+	−	−	+	+	−
MH13	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	+	+	−
MH14	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	+	−
MH15	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	−
MH18	+	+	−	+	−	+	+	+	−	+	−	+	+	+	+	−	−	−	+	+	+	+
MH19	+	+	−	+	−	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	+
MH20	−	+	−	+	−	+	+	+	−	+	−	+	+	+	+	−	−	−	−	+	−	+
MH21	−	−	+	−	−	+	+	+	−	−	−	−	−	+	−	±	−	+	−	+	+	+
MH22	−	−	−	−	−	+	+	+	−	−	−	−	+	+	−	+	+	+	−	+	+	+
MH23	−	−	+	−	−	+	+	−	−	−	−	−	+	+	−	+	+	+	−	+	+	−
MH24	−	−	−	−	−	+	+	−	−	−	−	−	+	+	−	+	+	+	−	+	+	−
MH25	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	+	−	+	−	−	+	−
MH26	±	−	+	+	+	+	+	−	−	+	−	+	+	−	+	−	−	+	+	+	−	−
MH27	+	(+)	+	+	+	+	+	−	+	−	−	+	+	−	+	−	+	+	+	+	−	−
Discordance	11/22	6/22	9/22	7/22	7/22	11/22	6/22	1/22	6/22	3/22	5/22	4/22	13/22	14/22	6/22	15/22	12/22	9/22	7/22	16/22	14/22	

MC10 is the mouse homolog of FRG1, and we have designated this gene *Frg1*. Within the coding region, the human and mouse cDNAs have 90% nucleotide identity, but there is no significant homology in either the 5′ or 3′ untranslated regions. The Frg1 protein contains a high percentage of charged amino acids (38%). Searching of protein databases revealed no significant homology to any known proteins or protein domains.

Mapping of Frg1 to mouse Chr 8. To determine the chromosomal location of *Frg1*, we analyzed the mouse–hamster somatic cell hybrid panel of Williamson and associates (1995) using the GEG2 PCR. The data are consistent with *Frg1* mapping to Chr 8 (Table 1). Importantly, this result showed this STS to be locus specific; co-amplification of closely related loci on other chromosomes had complicated analysis of the human gene (van Deutekom et al. 1996).

Fine genetic mapping. The *Frg1* polymorphism was used to type 68 random backcross progeny from the European Collaborative Interspecific Backcross (EUCIB) (Breen et al. 1994). Linkage was found between *Frg1* and mouse Chr 8 markers, confirming the somatic cell hybrid panel localization. To refine the location of *Frg1* and to determine its relationship to *myd*, we then also typed these mice using D8Mit markers known to be closely linked to *myd* (Mills et al. 1995). In addition, the location of *Frg1* was determined with respect to *Clc3* and *Ant1*, mouse genes with human homologs on 4q32 and 4q35 respectively (Mills et al. 1996a, 1996b).

Figure 2A shows a genetic map of the region of mouse Chr 8 containing *Frg1* determined in the EUCIB. Haplotype data are shown in Fig. 2B. This map places *Frg1* outside the *myd* critical region as it is proximal to *D8Mit9*, which is completely linked to *myd* (Mathews et al. 1995a). Furthermore, both *Ant1* and *Clc3* map between *Frg1* and *D8Mit9*. The order of markers is in agreement with the MIT map (Dietrich et al. 1996) and previously published maps of this region (Mathews et al. 1995a; Mills et al. 1995, 1996a, 1996b); the only major difference being that in the EUCIB cross three recombination events were identified placing *D8Mit261* proximal to *D8Mit9*, while these markers are unordered in the MIT map.

Mapping of Frg1 in a cross segregating the myd mutation. To confirm that the *Frg1* gene maps outside the region containing the *myd* locus, we then used the B6C3-CAST cross described in Materials and methods. Figure 2C shows the localization of *Frg1* by haplotype analysis in a subset of animals from this cross that are recombinant in the interval of interest. None of the genotyping of the 100 other chromosomes contradicted the location of *Frg1* based on the recombinants. Additional animals have been typed to confirm marker order (data not shown). The major difference between the two maps is that *D8Mit224* is proximal to *Frg1* in the B6C3-CAST cross, whereas no recombinants between these markers were identified in our study. The location of *Frg1* relative to *D8Mit192* and *D8Mit9* in both maps is in agreement. Four affected animals which showed recombination events proximal to the mutation and distal to *Frg1* were identified.

Northern blot analysis. Although the genetic mapping has placed the *Frg1* gene proximal to the *myd* mutation, we were aware that a possible disease mechanism for FSHD is a long-range position effect. As a similar mechanism could also be occurring in the mouse, we investigated the expression of the *Frg1* gene in normal and *myd* mice by Northern blot analysis (Fig. 3). A single transcript of approximately 1.2 kb was detected in all tissues analyzed, with highest levels of expression in testis. No significant differences in *Frg1* mRNA levels were seen in homozygous *myd* mice compared with controls.

Discussion

On the basis of the high level of sequence conservation and mapping of the mouse gene to the region with homology of synteny on Chr 8, we have isolated a cDNA for the mouse homolog of the human FRG1 gene. FRG1 is highly conserved between human and mouse with only eight amino acid differences, the majority of which are conservative substitutions.

The Northern blots showed no evidence of alternative splicing of *Frg1*, which differs from the findings of López-Alanón and del Mazo (1995), who used EST X71639 as a probe and identified

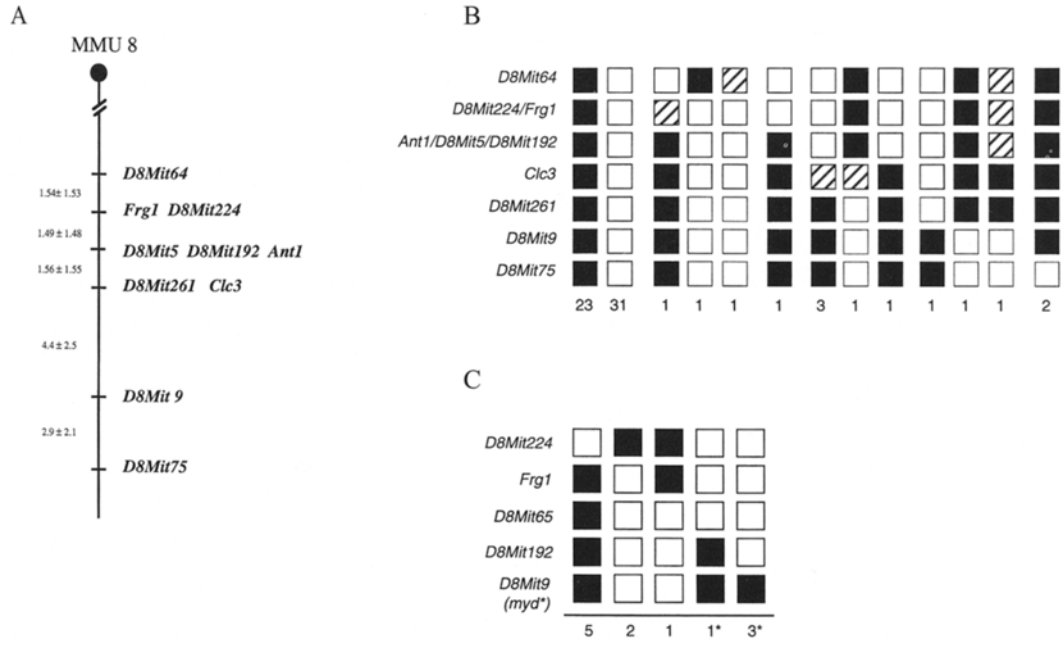

Fig. 2. Genetic mapping of *Frg1*.**(A)** Partial Chr 8 linkage map (95% confidence limits) based on typing 68 random mice from the EUCIB. Genetic distances (in centimorgans) and standard errors are listed to the left. **(B)** Haplotype of animals in EUCIB. Black and white squares represent loci typed as homozygous or heterozygous, respectively. Hatched boxes indicate unknown genotype. The number of animals with each chromosome haplotype is listed at the bottom of each column. **(C)** Haplotype analysis of *Frg1* in the B6C3-CAST cross. Black squares indicate transmission of the B6C3 allele, white squares of the CAST allele. All *D8Mit* dinucleotide markers were typed in this cross previously. Haplotypes marked with an asterisk are from affected animals. No recombinants have been observed between *D8Mit9* and *myd* in 275 chromosomes (Mills et al. 1996c).

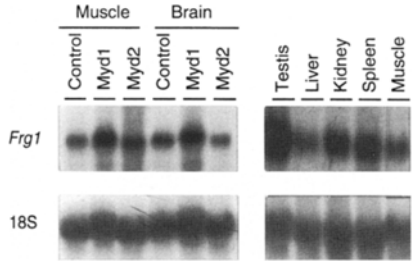

Fig. 3. Northern blot containing 20 μg of total RNA from tissues isolated from one normal and two *myd* mice. The filter was hybridized with either a probe for the mouse *Frg1* cDNA clone MC10 or an 18S rRNA probe. With *Frg1*, a 1.2-kb transcript was detected in all tissues, with highest levels in testes. There are no significant differences in the levels of *Frg1* mRNA between normal and *myd* mice.

transcripts of 1.2, 1.9, and 5 kb, although the 1.9 kb transcript was detected only in fetal tissue. The probe used in our study did not contain the entire cDNA, which may account for this discrepancy. However, there is no evidence that the human FRG1 gene undergoes alternative splicing (van Deutekom et al. 1996). Further studies on the expression profile of *Frg1* will be needed to investigate these differences. The mouse EST used to design *Frg1* primers was isolated from a fetal ovary cDNA library in an approach designed to identify genes involved in gametogenesis (López-Alanón and del Mazo 1995). Consistent with this, by Northern blot analysis much higher expression levels of *Frg1* were seen in testis, and it seems likely that a function of this gene may be related to gametogenesis.

There are at least 14 genes from human Chr 4q28–q35 with identified homologs in a span of approximately 20 cM on mouse Chr 8 (Mathews and Mills 1996). We have extended this region with homology of synteny on mouse Chr 8 to include *Frg1*. It is not known whether there is linkage conservation between mouse and human because many of these genes have been mapped in different crosses. However, the current synteny homology maps of human Chr 4q35 and mouse Chr 8 suggest a general relationship between the most distal genes on human 4q and the most proximal genes in the mouse (Mathews and Mills 1996). Our data show linkage conservation between *Frg1, Ant1,* and *Clc3,* which all map proximal to the *myd* locus.

Phenotypically the *myd* mouse is a good candidate as a homolog of FSHD (Mathews et al. 1995a). However, if the relative gene order is conserved between human and mouse, the human homolog of *myd* should map to a more proximal region of Chr 4q than FRG1, and the two mutations may not be homologous. At present, however, the mouse map is still at a relatively low resolution, and small inversions and rearrangements could have resulted in different locations of the FSHD/*myd* gene. Ultimately, the relationship between FSHD and *myd* can be determined only by cloning of the genes responsible for each disorder.

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