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Genetic mapping near the *myd* locus on mouse Chromosome 8

K.A. Mills,[1] K.D. Mathews,[1] T. Scherpbier-Heddema,[2] R.L. Schelper,[3] R. Schmalzel,[1] H.L. Bailey,[1] J.H. Nadeau,[4] K.H. Buetow,[2] J.C. Murray[1]

[1]Departments of Pediatrics, University of Iowa, Iowa City, Iowa 52242, USA
[2]Division of Population Sciences, Fox Chase Cancer Center, Philadelphia, Pennsylvania, 19111, USA
[3]Department of Pathology, University of Iowa, Iowa City, Iowa 52242, USA
[4]Department of Human Genetics, Montreal General Hospital, 1650 Cedar Avenue, Montreal, PQ, Canada H3G 1A4

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Abstract. Myodystrophy (*myd*), an autosomal recessive mutation of the mouse characterized by progressive weakness and dystrophic muscle histology, maps to the central portion of Chromosome (Chr) 8 (Lane et al. J. Hered 67, 135, 1976). This portion of Chr 8 contains the genes for a mitochondrial uncoupling protein (*Ucp*) and kallikrein (*Kal3*), which map to distal 4q in the human, providing evidence for a segment of homology. Characteristics of the *myd* phenotype coupled with this homology suggest that *myd* may be a mouse homolog of facioscapulohumeral muscular dystrophy (FSHD), which maps to human 4q35. We have confirmed and expanded the region of mouse 8–human 4 homology by generating a map of Chr 8 in an interspecific backcross of C57BL/6J and a partially inbred strain derived from *M. spretus*. The map is comprised of the genes for *Ucp*, coagulation factor XI (*Cf11*), and chloride channel 5 (*Clc5*), all of which have homologs on distal human 4q, 15 microsatellite loci, and the membrane cofactor protein pseudogene (*Mcp-ps*). To place *myd* in the genetic map, 75 affected progeny from an intersubspecific backcross of animals heterozygous for *myd* with *Mus musculus castaneus* were genotyped with Chr 8 microsatellite loci. The mutation maps between *D8Mit30* and *D8Mit75*, an interval that is flanked by genes with human homologs at distal 4q. These results are consistent with the possibility that *myd* is the mouse homolog of FSHD.

Introduction

Myodystrophy (*myd*) is a spontaneous autosomal recessive mouse mutant that was originally described and mapped to Chr 8 by linkage with oligosyndactilism (*Os*) and the extension coat color locus sombre allele (E^{so}; Lane et al. 1976). Affected mice have retarded growth with smaller overall body and organ size (as measured by weight). By 2 weeks of age they display abnormal adduction of the hind limbs when suspended by the tail. Life span is reduced, and reproductive performance is poor. Muscle histology reveals myopathic features indicative of a dystrophic process. A detailed phenotypic characterization of the *myd* mutant is reported elsewhere (Mathews et al. submitted).

The most recent composite linkage map of mouse Chr 8 (Ceci 1993) positions *myd* centrally, at 39 of 84 centimorgans (cM). This region of the chromosome also includes two genes with homologs at human 4q31 and 4q35. A mitochondrial uncoupling protein (*Ucp*/UCP) was localized to mouse Chr 8 by linkage (Jacobson et al. 1985), and plasma kallikrein (*Kal3*/KLK3) by in situ hybridization (Beaubien et al. 1991). However, regions of synteny or homology with mouse Chr 8 are more strongly established for human Chrs 1, 8, 16, and 19, while the known syntenic conservation for human Chr 4 is with mouse Chrs 3 and 5 (Copeland et al. 1993).

In the work reported here, we first characterize the homology between mouse Chr 8 and human Chr 4. A 19-locus map of Chr 8 for an interspecific backcross of C57BL/6J and *Mus spretus* was generated. It contains three genes with homologs on distal human Chr 4: *Ucp*, coagulation factor XI (*Cf11*), and chloride channel 5 (*Clc5*). Our second goal was to place *myd* in the genetic map. We mapped the mutation in an intersubspecific backcross using microsatellite markers, and show that it is flanked by the genes with homologs on distal human Chr 4. This refined genetic localization is a first step in the positional cloning of the *myd* gene.

Materials and methods

Animals. A panel consisting of DNA from 173 animals of a (C57BL/6J × *M. spretus*) F_1 × C57BL/6J backcross (Nadeau et al. 1991) was used to construct a reference map of Chr 8 (henceforth referred to as the interspecific backcross panel and map).

An intersubspecific cross was made to map the *myd* mutation. Four male B6C3Fe-*a/a-myd*/+ mice and five female CAST/Ei mice purchased from The Jackson Laboratory were bred, and F_1's heterozygous for *myd* (identified by their production of affected offspring) were backcrossed. Disease status (affected versus unaffected) was ascertained by inspection of hindlimb posture when animals were suspended by the tail at 2–3 weeks of age. Hind limb muscle was paraffin-fixed and stained with hematoxolin and eosin, then examined for dystrophic changes to confirm clinical status. All critical recombinants had muscle histology typical of *myd* homozygotes. DNA from the affected animals was prepared from harvested organs by standard procedures.

Polymorphisms. Human probes were used (at reduced hybridization stringency) to identify mouse restriction fragment length polymorphisms (RFLPs) for the human 4q genes Factor XI (F11; 4q35), glycophorin A (GYPA; 4q31), and beta fibrinogen (FGB; 4q28). A mouse *Ucp* probe (Kozak et al. 1988) was also used for genotyping by RFLP. Southern blotting was performed by standard procedures (Murray et al. 1987).

Single-strand conformation polymorphisms (SSCPs) were developed for two loci. *Mcp-ps* primers from the 3′ untranslated sequence (Paul et al. 1990: F, 5′ TGG AAT CCT CTT CTG GCC AAA 3′; R, 5′ TGA ATA CCC ACT TCT TTA TAC T 3′) give a product of 170 bp that was run on 5% acrylamide (49:1 acrylamide:bis), 10% glycerol, 0.5 × TBE gels at room temperature, 20 W with a fan. *Clc5* primers from the 5′ untranslated sequence (Borsani et al. submitted: F, 5′ CTG CTC CTG TAG TCC TTG G 3′; R, 5′ GCT TTC AGG AGA GGT TAC GT 3′) give a product of 153 bp that was run on 0.4-mm-thick 33 × 42 cm MDE (AT Biochem, Malvern, Pa.) plus 5% glycerol gels, in 0.6 × TBE buffer at room temperature, 20 W with a fan.

Mouse Chr 8 microsatellite polymorphisms (Dietrich et al. 1994) were typed by PCR as previously described (Mills et al. 1992) with primers purchased from Research Genetics (Huntsville, Ala.).

Correspondence to: K.A. Mills

Analysis. For the interspecific backcross panel, genotypes were scored as either "spretus" or "musculus" according to the allele inherited from the hybrid parent. Genotypes were entered into the program Map Manager (Manley 1993) and loci arranged in the order giving the fewest number of recombinants.

The *myd* mutation was localized in the affected animals by examination of recombinants.

Results

Interspecific backcross map with two newly localized genes. A *Sac*I RFLP for the *Cf11* gene produces a 9.8-kb band in *M. spretus* and two bands of 7.6 kb and 4.2 kb in C57BL/6J. This locus has the fewest animals typed and is the site of three double recombinants. For *Ucp,* two RFLPs were identified, and either was used for mapping. A *Sac*I digest produces fragments of 7.8, 4.0, and 2.5 kb in *M. spretus,* and 7.4, 3.4, and 3.2 kb in C57BL/6J; a *Hin*dIII digest produces fragments of 4.7 and 2.1 kb in *M. spretus,* and 5.3, 2.6, and 2.2 kb in C57BL/6J. More animals are genotyped for the PCR-based markers than for *Cf11* and *Ucp* (74 and 115 typings), since blots were made for only a subset of the interspecific backcross panel, and limited DNA availability made error checking by repeat digest impossible.

Figure 1 presents a linkage map of mouse Chr 8 based on 15 microsatellites, 3 genes, and a pseudogene. Both newly mapped genes—*Cf11* and *Clc5*—fall in the central portion of the chromosome, just proximal to *Ucp.* No difference in the order of markers was observed relative to the MIT map (July 1994 release; Dietrich et al. 1994), and the length of the segment is also comparable (56.6 cM versus 61.3 cM in the MIT map).

*Hin*dIII RFLPs were identified for both GYPA and FGB. These genes were unlinked to selected microsatellite markers at each end of the chromosome in pairwise comparisons (data not shown) and do not map to mouse Chr 8. Pairwise comparisons for FGB with the *IL2* locus (Todd et al. 1991) on mouse Chr 3 show linkage with a recombination fraction of 0.29 ± 0.054. A similar analysis with GYPA did not show linkage to Chr 3.

Haplotype analysis of recombinants from myd panel. Seventy-five affected mice have been genotyped with 12 microsatellite markers on Chr 8. Results are shown in Fig. 2 and allow *myd* to be localized to the interval between *D8Mit30* and *D8Mit75.* The microsatellites *D8Mit9, 28, 72, 73,* and *74* lie between these two markers and show no recombination with *myd.* In the MIT map (July 1994 release; Dietrich et al. 1994), the first four of these loci are unordered and at the same location as *D8Mit30,* while *D8Mit74* is 1.1 cM distal to this large unordered cluster. With this cross we have been able to localize *D8Mit30* as the most proximal marker of the group, but have been unable to separate out *D8Mit74* as lying more distally.

In addition to the 12 markers shown in Fig. 2, another five unordered microsatellites (*D8Mit26, 27, 29, 31,* and *71*) are in the cluster including *D8Mit30.* We have genotyped a subset of the affected mice, including the critical recombinant animals, with these markers, and all show complete linkage to the mutation. In our affected mice, the length of the interval between *D8Mit30* and *D8Mit75,* calculated as the recombination fraction, is 2.7 ± 1.9 cM. For all loci lying between these two markers and showing no recombination, the upper confidence limit for a 95% probability of having zero recombinants in 75 meioses is 0.040.

Six microsatellite markers were typed in both the *myd* mice and the interspecific backcross panel to facilitate comparison of the two crosses (bold typeface in both Figs. 1 and 2). Identical sets

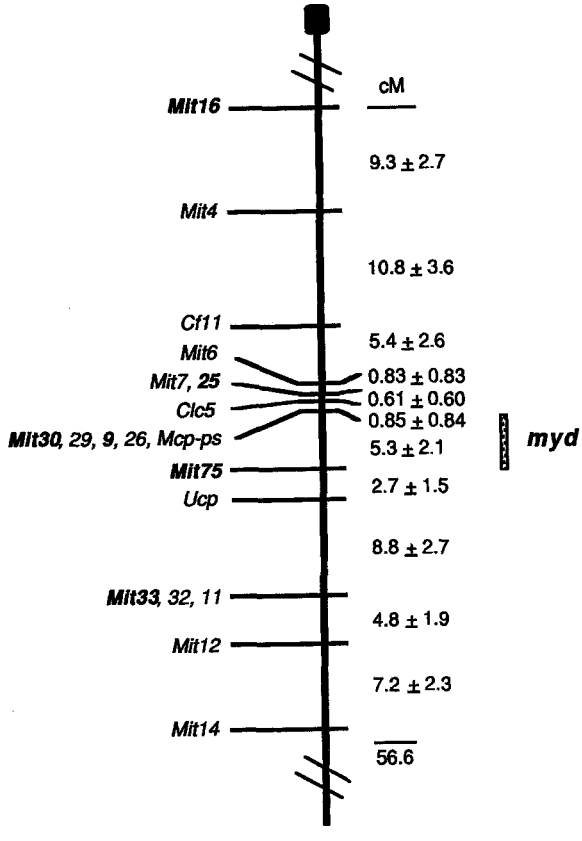

Fig. 1. Linkage map spanning 56.6 cM of mouse Chr 8. Distances between pairs of loci are shown in centiMorgans with standard errors, as calculated with Map Manager. At points with more than one locus that cannot be ordered, the loci are listed adjacent to one other. **Boldface** type indicates loci that are also analyzed in the *myd* cross (Fig. 2). The **shaded bar** to the right of the map indicates the location of the *myd* mutation by haplotype analysis as determined from data in Fig. 2.

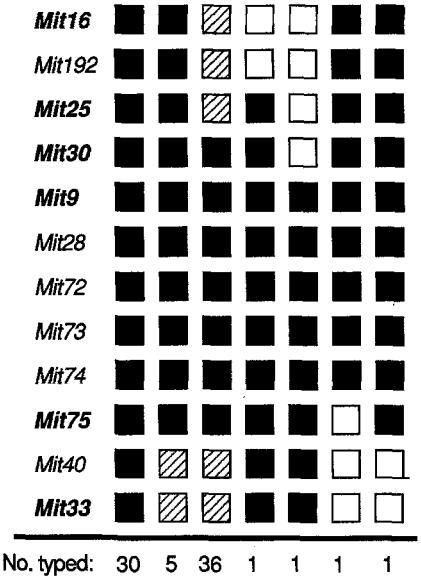

Fig. 2. Haplotype analysis of *myd* mice for Chr 8 microsatellite loci. Alleles inherited from the heterozygous parent are scored as either CAST/Ei-derived (**white squares**) or B6C3Fe-*a/a-myd*/+-derived (**black squares**); hatched squares are untyped. The "cast" phenotype at a locus is observed only outside the region containing the mutation, since for the mice to be affected, both alleles must be from the parental chromosome carrying *myd* and hence "black." Recombination events indicate the flanking loci for *myd* are *D8Mit30* proximally and *D8Mit75* distally. **Boldface** type indicates loci also analyzed in the interspecific backcross panel for the map in Fig. 1. Markers are arranged from most proximal to most distal.

of markers could not be used because not all markers are polymorphic in all three (sub)species. For example, the C57BL/6J and *M. m. castaneus* alleles are identical for *Clc5, Mcp-ps,* and *Ucp.*

Discussion

We have generated a genetic map of approximately 80% of mouse Chr 8 that is based mainly on markers typed by PCR and includes three genes with homologs on distal human Chr 4. The map is generally consistent in locus order and length with other published maps of Chr 8, and five of the loci (*D8Mits4, 6, 9, 11, 12*) are integrating markers used in generating the composite map of Chr 8 (Copeland et al. 1993).

One goal of this work was to characterize further the homology between mouse Chr 8 and human Chr 4q. Previously, one gene from 4q31 (*Ucp*) was genetically mapped to mouse 8, and a second gene (*Kal3*) was mapped to human 4q35 and mouse 8B by FISH. We have confirmed with linkage the localization of *Ucp* and added two genes from distal human 4q—*Cf11* and *Clc5*—to the same region of the chromosome. F11 maps to proximal human 4q35, and CLCN5, a recently identified gene (G. Borsani, pers. comm.), maps to human 4q32 by linkage, and with a somatic cell panel (Mills et al., manuscript in preparation). Thus, four genes are now known to map to both the midregion of mouse Chr 8 and the most distal band of human Chr 4. Two other genes on human 4q (GYPA, FGB) do not seem to have homologs on mouse Chr 8. A 14-cM span in the middle of mouse Chr 8 contains genes found on human Chrs 8p, 2q, 19p, 16q, and 1p (from proximal to distal) in addition to 4q, suggesting that multiple complex rearrangements of the ancestral mammalian karyotype in this region have occurred before emergence of the human and mouse genomes. Hence, the location of human homologs for genes in the region is difficult to predict.

The second goal of this work was to place *myd* in the genetic map. Haplotype analysis of affected mice localizes the mutation to the interval between *D8Mit30* and *D8Mit75.* The position of *Ucp* on our reference map is distal to *D8Mit75.* Taken together, these data suggest that *myd* is proximal to *Ucp,* in contrast with the current composite map (Ceci 1993), which has *myd* 4 cM distal to *Ucp.* The more proximal location is probably the correct one, since it was shown by mapping the mutation and the gene in the same cross. In our map, the interval containing *myd* is flanked proximally by *Cf11* and *Clc5,* whose human homologs are at 4q35 and 4q32 respectively, and distally by *Ucp,* whose homolog is at 4q31. The genes *Clc5* and *Ucp* cannot be considered candidate genes for the mutation, since they lie outside the interval containing it.

The interval containing *myd* is 3.3 cM in length on the MIT map (July 1994), and 5.3 ± 2.1 cM on our interspecific backcross map. A size difference is not unexpected, given that the two maps are constructed with two different crosses. The interval in our affected animals of 2.7 ± 1.9 cM is comparable to that in the MIT map, which is constructed with the same cross.

We have confirmed and further delineated the homology between the most distal band of human 4q3 and the midregion of mouse Chr 8, and have placed *myd* into an interval flanked by homologs to human 4q3 genes. This work supports the possibility that the *myd* mutant is a mouse homolog to the human disease facioscapulohumeral muscular dystrophy located at 4q35.

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