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A syntrophin gene maps to mouse Chromosome 8 and is not the myodystrophy gene

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A complex of proteins and glycoproteins spans the muscle cell membrane and anchors the intracellular cytoskeletal proteins dystrophin and actin to the extracellular matrix. This dystrophin-associated complex is critical in maintaining the integrity of the muscle cell; disruption of its components causes various forms of muscular dystrophy (reviewed in Campbell 1995). Absence of dystrophin (Xp21.2) leads to Duchenne muscular dystrophy, and truncated or dysfunctional dystrophin causes Becker muscular dystrophy. Adhalin (17q) is absent in severe childhood autosomal recessive muscular dystrophy (SCARMD) and other muscular dystrophies with variable manifestations (Piccolo et al. 1995; Hayashi et al. 1995). Merosin (6q22-23) deficiency has been demonstrated in some cases of congenital muscular dystrophy. Other components of this complex are obvious candidates for dystrophies where the mutation is as yet unknown.

Syntrophin is a 59-kDa protein triplet that associates with the carboxy-terminus of dystrophin and the related protein utrophin. Syntrophin cDNA clones have been isolated from *Torpedo* and mouse (Adams et al. 1993), rabbit (Yang et al. 1994), and human (Ahn et al. 1994). Syntrophins can be characterized as acidic or basic (Yamamoto et al. 1993), and this distinction underlies the nomenclature. The acidic mouse isoform, α-syntrophin (*Snta1*), has a human homolog on Chr 20q11 (Campbell 1995). The basic isoform, β-syntrophin (*Sntb2*), is homologous to a human expressed sequence tag that maps to 16q23 (EST25263; unpublished, Genbank accession no. T29784). A third basic human isoform maps to 8q23-24 and has no known mouse homolog.

Sntb2 was likely to map to mouse Chr 8 on the basis of the location of its human homolog at 16q23 and the existence of a known region of synteny (Ceci, 1994). A mouse neuromuscular mutant, myodystrophy (*myd*), also maps to mouse Chr 8, near the region of synteny with 16q23. Myodystrophy is a spontaneously arising autosomal recessive mutation that is characterized by dystrophic muscle pathology and elevated serum CK. Affected animals have abnormal hindlimb posture when they are held suspended. The disorder is progressive and results in premature death (Lane et al. 1976; Mathews et al. 1995). *Sntb2* was an attractive candidate gene for *myd* because of the homologous chromosomal location and the association with dystrophin/utrophin. There is no disease process currently known to result from mutation of this gene in either human or mouse. We investigated *Sntb2* in *myd* mice through both genetic mapping and protein analysis.

As shown in Fig. 1, *Sntb2* maps to mouse Chr 8 distal to *D8Mit211* and proximal to *D8Mit116*. This places the gene within an interval of 4.82 ± 1.66 cM. The distance between these two flanking markers in the MIT map, a cross between different subspecies, is 5.5 cM (Release 9, 3/1/95; Dietrich et al. 1994). There were no recombinants with *D8Mit12*, which lies between these two markers. The 99% upper confidence limit for zero recombinants between *Sntb2* and *D8Mit12* in 166 meioses is a distance of 2.8 cM. This map location places *Sntb2* within the segment of mouse Chr 8 known to be syntenic with human Chr 16q23.

Myodystrophy was previously mapped to mouse Chr 8 in a cross between B6C3Fe-*a/a-myd*/+ and *M. m. castaneus* (Mills et al. 1995), distal to a cluster of five markers (*D8Mits 26, 29, 71, 73, 179*) and proximal to *D8Mit74*. In the current MIT map of Chr 8, *D8Mit74* is about 14 cM proximal to *D8Mit211*. In this report we have shown *D8Mit211* to be proximal to *Sntb2* by 3.49 ± 1.40 cM. Hence, *Sntb2* is clearly distal to the *myd* locus. The distances and possibly the order must be considered with caution since the mutation and *Sntb2* were mapped in different crosses; however, we have now typed 15 markers in both the cross used to map the *myd* mutation and the cross used to map *Sntb2*, and have found no evidence to suggest a chromosomal rearrangement in this region.

The exclusion of *Sntb2* as the *myd* gene based on genetic map location is supported by the Western blot analysis, shown in Fig. 2. This study shows similar expression of *Sntb2* in affected and control animals. Control, heterozygous (*myd*/+) and homozygous (*myd/myd*) mice all exhibited a syntrophin triplet of similar molecular weights in comparable amounts.

Our results are consistent with the available information about

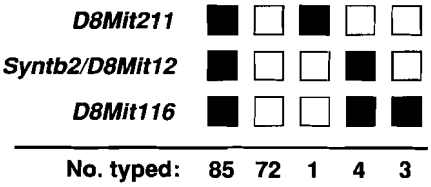

Fig. 1. Shown above is typing of selected markers localizing *Sntb2*. *Sntb2* was genetically mapped with DNA samples from a (C57BL/6J × *M. spretus*) F_1 × C57BL/6J backcross obtained from The Jackson Laboratory (Nadeau et al. 1991). Black squares indicate transmission from the heterozygous parent of the *M. spretus* allele; white squares, of the C57BL/6J allele. These DNAs were used by us previously to construct a framework map of mouse Chr 8 (Mills et al. 1995). Primers were selected from the 3′ untranslated sequence of mouse *Sntb2* (Adams et al. 1993; GenBank accession no. U00678); forward: 5′ TGT CAC AAG AAG TAT TTC CAC C 3′; reverse: 5′ ATT TCT GAC CAA GGC TGT GTG 3′. These primers amplify a 185-bp product from total mouse DNA and DNA purified from a mouse Chr 8-specific cell line (MC2/3; Stallings and Deaven 1994; T_a 58°C; methods as in Mills et al. 1992). Primer pairs were then tested for polymorphism among various parental strains of mice. An SSCP was detected for C57BL/6J and *M. spretus* on an MDE (AT Biochem, Malvern, Pa.) +5% glycerol, 0.4 mm gel run at 20 watts, with a fan, and the backcross panel was typed with *Sntb2*. The panel was then typed with *D8Mit116* and *D8Mit211* (primers purchased from Research Genetics, Huntsville, Ala.), dinucleotide repeat polymorphisms known to be within the interval (Dietrich et al. 1994) containing *Sntb2*. Map information was analyzed with Map Manager Version 2.6 (Manley 1993).

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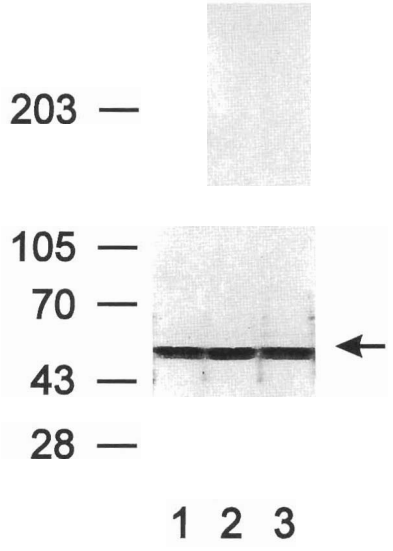

Fig. 2. Western blot analysis of skeletal muscle crude surface membranes prepared from age-matched normal control (+/+; lane 1), heterozygous (*myd/+*; lane 2), and homozygous (*myd/myd;* lane 3) mice as previously described (Ohlendieck and Campbell 1991). Animals heterozygous and homozygous for *myd* are from a breeding colony of B6C3Fe-*a/a-myd/+* crossed to *M.m. castaneus,* as described (Mills et al. 1995). 200 mg of crude surface membranes were separated on a 3–12% SDS-PAGE in the presence of 1% 2-mercaptoethanol and transferred to nitrocellulose. The nitrocellulose transfer was immunostained with a polyclonal antibody specific for the rabbit syntrophin triplet, which was affinity purified from sheep antiserum against the purified dystrophin glycoprotein complex (Ohlendieck and Campbell 1991). A 59-kDa triplet of equal amounts is detected in all three preparations of mouse skeletal muscle. Molecular weight standards ($\times 10^3$) are indicated at left.

Sntb2 expression in mice. Adams et al. (1993) showed by Northern blot analysis that both mouse isoforms of syntrophin, *Snta1* and *Sntb2,* are expressed in multiple tissues; however, *Snta1* is expressed most abundantly in muscle, whereas *Sntb2* is expressed at a low level in muscle. These authors suggested on the basis of this tissue distribution that mutation of *Snta1* may cause muscle disease, whereas mutation of *Sntb2* may result in a generalized phenotype.

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