MTM1 mutation associated with X-linked myotubular myopathy in Labrador Retrievers

Alan H. Beggs^{a,1,2}, Johann Böhm^{b,1}, Elizabeth Snead^c, Marek Kozłowski^a, Marie Maurer^d, Katie Minor^e, Martin K. Childers^f, Susan M. Taylor^c, Christophe Hitte^g, James R. Mickelson^e, Ling T. Guo^h, Andrew P. Mizisin^h, Anna Buj-Belloⁱ, Laurent Tiret^d, Jocelyn Laporte^b, and G. Diane Shelton^{h,2}

^aDivision of Genetics and Program in Genomics, The Manton Center for Orphan Disease Research at Children's Hospital Boston and Harvard Medical School, Boston, MA 02115; ^bDepartment of Neurobiology and Genetics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut National de la Santé et de la Recherche Médicale U964, Centre National de la Recherche Scientifique Unité Mixte de Recherche 7104, Université de Strasbourg, Collège de France, Chaire de Génétique Humaine, 67400 Illkirch, France; ^cWestern College of Veterinary Medicine, University of Saskatchewan, SK, Canada S7N 5B4; ^dUnité Mixte de Recherche 955, Génétique Fonctionnelle et Médicale, Institut National de la Recherche Agronomique, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, 94700 Maisons-Alfort, France; ^eSchool of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108; ^fWake Forest University Health Sciences, Winston-Salem, NC 27157; ^gInstitut de Génétique et Développement de Rennes, Centre National de la Recherche Scientifique Unité Mixte de Recherche 6061, Université de Rennes 1, F-35000 Rennes, France; ^hDepartment of Pathology, University of California at San Diego, La Jolla, CA 92093; and ⁱGénéthon, 91000 Evry, France

Edited* by Louis M. Kunkel, Children's Hospital Boston, Boston, MA, and approved July 12, 2010 (received for review March 19, 2010)

Mutations in the *MTM1* gene encoding myotubularin cause X-linked myotubular myopathy (XLMTM), a well-defined subtype of human centronuclear myopathy. Seven male Labrador Retrievers, age 14–26 wk, were clinically evaluated for generalized weakness and muscle atrophy. Muscle biopsies showed variability in fiber size, centrally placed nuclei resembling fetal myotubes, and subsarcolemmal ringed and central dense areas highlighted with mitochondrial specific reactions. Ultrastructural studies confirmed the centrally located nuclei, abnormal perinuclear structure, and mitochondrial accumulations. Wild-type triads were infrequent, with most exhibiting an abnormal orientation of T tubules. *MTM1* gene sequencing revealed a unique exon 7 variant in all seven affected males, causing a nonconservative missense change, p.N155K, which haplotype data suggest derives from a recent founder in the local population. Analysis of a worldwide panel of 237 unaffected Labrador Retrievers and 59 additional control dogs from 25 other breeds failed to identify this variant, supporting it as the pathogenic mutation. Myotubularin protein levels and localization were abnormal in muscles from affected dogs, and expression of GFP-MTM1 p.N155K in COS-1 cells showed that the mutant protein was sequestered in proteasomes, where it was presumably misfolded and prematurely degraded. These data demonstrate that XLMTM in Labrador Retrievers is a faithful genetic model of the human condition.

congenital myopathy | myotubularin | necklace fibers | canine myopathy | animal model

X-linked myotubular myopathy (XLMTM) is a well-defined subgroup of the centronuclear myopathies (CNMs) characterized by early onset and the presence of uniformly small muscle fibers with centrally placed nuclei resembling fetal myotubes (1, 2). Although centrally located nuclei can be found in many myopathies, clinical, genetic, and pathological factors can help distinguish these myopathies from XLMTM. Onset of clinical signs is typically at or near birth, and affected males have profound hypotonia and weakness accompanied by respiratory difficulties that usually require ventilatory support. The defective gene, *MTM1*, was identified in 1996 by positional cloning (3). Myotubularin, the protein encoded by the *MTM1* gene, is a ubiquitously expressed phosphoinositide phosphatase implicated in intracellular vesicle trafficking and autophagy (4, 5). In skeletal muscle, myotubularin localizes to the triadic regions, where it likely plays a role in lipid biogenesis or metabolism (6).

Animal models have played an important role in understanding the pathogenesis of how loss of *MTM1* function leads to clinically evident myotubular myopathy. A classical knockout (KO) for the murine *Mtm1* gene showed that myotubularin-deficient mice developed a progressive CNM during postnatal life that severely reduced life expectancy (7). Studies in this model, as well as in

a related muscle-specific KO line, have demonstrated that myotubularin plays a role in muscle maintenance rather than maturation, and have confirmed that the primary tissue involved in XLMTM is muscle. Recently, a zebrafish model was created using morpholino antisense technology (8). Impaired motor function and centrally placed nuclei were similar to those seen in *Mtm1* KO mice and XLMTM humans. Skeletal muscles of *mtm1* morphants exhibited elevated levels of phosphoinositide-3-phosphate, a key substrate of myotubularin. Both morphants and human XLMTM patient muscle biopsy specimens exhibited abnormalities in triad organization, suggesting that myotubularin plays an important role in the development or maintenance of tubuloreticular network structure and function. Recently, similar morphological abnormalities in T tubules have been identified in the *Mtm1* KO mouse model, accompanied by reductions in transcript and protein levels for several key excitation-contraction coupling proteins, including type 1 ryanodine receptors, as well as depressed sarcoplasmic reticulum (SR) Ca²⁺ release, demonstrating that a defect in excitation-contraction coupling might be a primary cause of weakness and hypotonia in XLMTM (9).

Along with their use in studies of the pathogenesis and mechanisms of disease, animal models are critical in preclinical trials of promising new drugs and therapies (10). Results of preclinical therapeutic trials in rodent models cannot always be directly extrapolated to the human condition, however. Thus, the identification and use of larger animal genetic homologs of human neuromuscular diseases is of increasing importance. Established models of X-linked dystrophin-deficient muscular dystrophy in Golden Retrievers (11, 12) and Beagles (13) have recently been used in therapeutic trials evaluating mesoangioblast stem cells (14) and systemic morpholino exon-skipping (15). Similarly, a colony of Labrador Retrievers with CNM due to a mutation in the *PTPLA* gene has been established in France (16–18); these dogs are being considered for upcoming preclinical trials. In a recent case report, we described a novel canine inherited myopathy presenting with progressive weakness and muscle atrophy beginning in the first few months of life (19). Here we describe this disorder in two kindreds of Labrador Retrievers and demonstrate

Author contributions: A.H.B., E.S., S.M.T., L.T., J.L., and G.D.S. designed research; J.B., E.S., M.K., M.M., K.M., M.K.C., S.M.T., L.T.G., and A.P.M. performed research; J.R.M. and A.B.-B. contributed new reagents/analytic tools; A.H.B., J.B., C.H., L.T., J.L., and G.D.S. analyzed data; and A.H.B., E.S., S.M.T., L.T., and G.D.S. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

¹A.H.B. and J.B. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: beggs@enders.tch.harvard.edu or gshelton@ucsd.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1003677107/-DCSupplemental.

that these dogs represent a new genetic homolog of XLMTM with a missense variant in the *MTM1* gene.

Results

Clinical Features of X-Linked Myopathy in Labrador Retrievers from Saskatchewan, Canada. Recently we reported an apparently novel inherited myopathy affecting a 5-mo-old male Labrador Retriever who presented to the Western College of Veterinary Medicine (19). This puppy suffered from a 4-wk progressive course of weakness and muscle atrophy, leading to euthanasia at the end of the fourth week (Fig. 1A). Four male littermates developed similar clinical signs between 12 and 16 wk of age and were euthanized. Over the course of the intervening 3 y, five male Labrador Retrievers from two additional litters were referred to Western College of Veterinary Medicine with clinically similar presentations, and another affected male was evaluated at Wake Forest Medical Center.

Affected puppies were considered by their owners to be normal until they were 10–19 wk of age. However, three pups from kindred II were reported to be of small stature with muscle atrophy and pelvic limb weakness at the time of vaccination at 7 wk of age. Affected dogs exhibited pelvic limb weakness that progressed to an inability to rise or walk unassisted. Dogs that could stand exhibited kyphosis with a ventroflexed neck and walked with a short, choppy stride, collapsing after a few steps (Fig. 14). All dogs had severe generalized muscle atrophy but no apparent muscle pain. Proprioception, as assessed by knuckling and hopping, was normal in all dogs that were able to move their limbs. Patellar reflexes were absent in all dogs, while withdrawal reflexes and cranial tibial reflexes were maintained in moderately affected dogs but absent in severely affected dogs. Dysphagia, a hoarse bark, and weakness of the muscles of mastication, leading to a dropped jaw, were noted terminally in all dogs. Dogs were non-ambulatory by 3–4 wk after the initial veterinary presentation. All dogs were euthanized at 15–26 wk of age.

Affected dogs came from three different litters that were initially thought to be unrelated; however, further investigation revealed that a female sibling of the dam of proband I.a was the maternal grand-dam of the independently ascertained proband I.e. Insufficient pedigree details are available for the third litter (kindred II in Fig. 1B) to prove or disprove a relationship. Inspection of the pedigrees clearly suggests that the myopathy in these dogs is inherited as an X-linked trait (Fig. 1B). All of the affected dogs evaluated were male, and each had multiple affected male siblings and only clinically normal female siblings. The presence in kindred I of affected male cousins whose dams were unaffected female siblings also clearly supports an X-linked pattern of inheritance.

Muscle Biopsies Show “Necklace Fibers” and Exhibit T Tubule Disorganization. Cryostat sections of muscle biopsy specimens from all seven studied affected male Labrador Retrievers showed similar morphologic abnormalities that were not evident in unaffected female carrier littermates or normal control dogs (Fig. 2). Excessive variability in myofiber size was present (Fig. 2*A* and *C*), with a type 1 fiber predominance evident on ATPase staining. Many small fibers contained centrally placed nuclei resembling fetal myotubes; triceps biopsies from four affected males contained 5%,

Fig. 1. (A) A 4-mo-old male Labrador Retriever (dog 1.a) affected with XLMTM illustrating the generalized muscle atrophy and characteristic kyphosis and ventroflexion of the neck indicative of generalized muscle weakness. (B) Pedigrees and genetic status of affected Labrador Retrievers from Saskatchewan, Canada. In the five litters depicted, only males were affected, supporting an X-linked recessive mode of inheritance. Three apparently unrelated probands (arrows) were eventually linked to two identifiable kindreds (I and II). All affected males (■) are related through carrier females (circles with red dots). Males marked with an asterisk were all genetically confirmed to be hemizygous for the *MTM1* c.465C>A mutation, and females with red dots are confirmed heterozygous carriers. "CNM clear" indicates dogs whose DNA tested negative for the *PTLPA* SINE insertion mutation that causes canine CNM. Numbers within or below male or female symbols indicate the number of additional pups of a particular gender and affection status as inferred from their owners' testimony. Not shown are two unaffected male and two unaffected female littermates of proband 1.e and unknown additional unaffected littermates of dams 1.b and 1.c.

Fig. 2. Skeletal muscle histology in an affected male, i.e. (A, C, and E), and unaffected carrier female, i.e. (B, D, and F), whose muscle exhibited no detectable abnormal findings. H&E-stained fresh frozen muscle biopsy sections from the triceps (A and B) reveal extensive fiber size variation with numerous small, centrally nucleated myofibers resembling fetal myotubes characteristic of XLMTM, in the affected male (A). The vastus lateralis muscles (C–F) consistently exhibited similar but less extreme pathology. Numerous “necklace” fibers were highlighted with the oxidative stain NADH-TR (E and F). (Scale bar: 100 μ m.)

6%, 15%, and 37% myofibers with central nuclei, respectively, compared with 4% in the vastus lateralis from the same four animals. A female carrier was indistinguishable from unaffected animals, with <1% centrally nucleated myofibers in both muscles. Subsarcolemmal ringed and central dense areas, so-called “necklace fibers” (20), were revealed with the oxidative reactions NADH-TR (Fig. 2E), succinic dehydrogenase, and cytochrome C oxidase. Necklace fibers were not observed in similarly stained sections from unaffected littermates or pups with CNM caused by *PTPLA* mutation (18). Immunofluorescence staining of myofibers from affected dogs using antibodies against markers for T tubules (DHPR α 1) and SR (RyR1) revealed an abnormal localization of both structures, with T tubules and surrounding SR concentrated in irregular densities within numerous myofibers (Fig. 3A), but in none of an unaffected littermate’s myofibers. Immunostaining of affected dog muscles also revealed an abnormally condensed pattern of myotubularin staining in some myofibers that colocalized

with the T tubule marker DHPR α 1 (Fig. 3B). The frequency and quality of these fibers was reminiscent of the necklace fibers detected with oxidative stains, suggesting abnormal retention of myotubularin in these fibers. Immunostaining for dystrophin, spectrin, alpha sarcoglycan, dysferlin, caveolin 3, and utrophin was normal in muscles from affected dogs.

Central Nuclei, Skeletal Muscle Degeneration, and Loss of Triad Structures in Affected Dog Muscle. Recent studies have revealed structural and functional defects of the triads and excitation-contraction coupling in humans with XLMTM, as well as murine and zebrafish models of myotubularin deficiency (8, 9). Ultrastructural studies of affected dogs confirmed the centrally located nuclei with surrounding mitochondria (Fig. 4A), myofibrillar disarray (Fig. 4B, D, and E) with and without mitochondrial accumulations, and perinuclear (Fig. 4C) and centrally located (Fig. 4D and E) whirled membranous structures similar to that seen in other species. Cristae-like structures (Fig. 4E) were evident within some membranous whorls, suggesting a mitochondrial origin of these abnormal structures. Triads were infrequent and disorganized in affected dogs (Fig. 4F) compared with a control littermate (Fig. 4G). The average number of morphologically normal triads per field was 2 ± 2 (range, 0–6) in affected pups, compared with 18 ± 9 (range, 6–32) in unaffected controls ($P = 0.0004$).

Identification of a Novel *MTM1* Gene Mutation in Affected Dogs. Genetic analyses of the first affected dog was negative for both the SINE exonic insertion mutation in *PTPLA* known to cause autosomal recessive CNM and the X-linked *DMD* gene mutation responsible for Golden Retriever muscular dystrophy (19). Because the histopathological findings were similar in the affected dog and human XLMTM, we sequenced all 15 exons of the canine *MTM1* gene. A C-to-A transversion in exon 7, causing the change c.465C>A, was present in two affected male dogs and in one ob-

Fig. 3. (A) Indirect immunofluorescence analysis of biopsies from the vastus lateralis muscle of two male Labrador Retrievers affected with X-linked myotubular myopathy (dogs II.d and I.e in Fig. 1B) and an unaffected carrier female (I.f in Fig. 1B) whose muscle histology and immunohistochemistry were indistinguishable from normal. Biopsy specimens from affected males revealed abnormal distribution of the SR and T tubule markers, ryanodine receptors (RyR1) and dihydropyridine receptors (DHPR α 1), in numerous myofibers that exhibited subsarcolemmal and internal localizations; this pattern was not seen in myofibers from the unaffected littermate (I.f). (B) Indirect immunofluorescence analysis of the triceps muscle from dog I.e (Fig. 1B) showing apparent colocalization of a triad marker (DHPR α 1 antibody) and myotubularin (R2867 C-terminal antibody) in abnormal distributions consistent with occurrence of necklace fibers. Staining of muscle from carrier I.f (bottom row) is indistinguishable from that in control biopsies. All panels are the same magnification (scale bar: 50 μ m) except the magnified images of I.e in row 5 (scale bar: 10 μ m).

Fig. 4. Ultrastructural studies of a Labrador Retriever (I.e in Fig. 1B) with XLMTM (A–F) and an unaffected female carrier littermate (I.f in Fig. 1B) (G) showing centrally located nuclei (A), myofibrillar disarray (B–E) with or without mitochondrial accumulation, and whirled membranous structures (C–E). Triads were infrequent and in disarray in the affected dog (F, arrow and inset showing higher-power view) compared with the unaffected littermate (G, arrow and inset showing higher-power view). (Scale bars: 3.6 μ m for A, 2.2 μ m for B and G, 3.0 μ m for C, and 1.8 μ m for E and insets.)

ligate carrier at the heterozygous state, compared with several unaffected Labrador Retrievers as well as the canine (Boxer breed) reference sequence XM_850116.1 (Fig. 5B). This mutation results in a nonconservative change from asparagine to lysine, p.N155K, at a residue located in the linker region between the GRAM-PH and phosphatase domains (Fig. 5A and C). Subsequently, seven affected males, representing each of the affected families, were found to be hemizygous for this change, whereas three obligate female carriers were all heterozygous (Fig. 1B). To determine whether the association of *MTM1* c.465C>A with canine congenital myopathy was absolute, we used both direct genomic PCR and DNA sequencing, along with a rapid 96-well plate-based TaqMan assay, to screen for this change in a panel of control canine DNA samples. The change was not seen in any of 237 unrelated and unaffected Labrador Retrievers from throughout North America, Europe, and Australia, nor was it detected in any of 59 additional control dogs from 25 other breeds.

Abnormal Expression of p.N155K Myotubularin in Vivo and in Vitro. Western blot analysis of myotubularin in skeletal and cardiac muscles of affected dogs revealed a profound loss of immunoreactive protein using two antibodies directed against the N- or C-termini of myotubularin (Fig. 6A and B). Muscle from WT and *Mtm1* KO mice was used as a control to demonstrate antibody specificity. These studies showed greatly reduced myotubularin protein in extracts from either the quadriceps muscle or heart of affected Labrador Retrievers relative to levels in a control littermate (Fig. 6).

To demonstrate whether the altered localization and reduced quantities of myotubularin in affected dogs was a direct consequence of the p.N155K mutation, COS-1 cells were transfected with either the WT or a p.N155K canine GFP-MTM1 expression

construct. Expression of the WT (control) construct led to a previously reported (21) characteristic fluorescence pattern representing a dense cytoplasmic filamentous network containing myotubularin (Fig. 6C). Remarkably, a high proportion of cells expressing the mutated myotubularin exhibited an unusual punctate pattern of fluorescence (Fig. 6F). Quantitation of cells containing these aggregates revealed significant differences ($P < 0.001$) in the proportions of cells expressing these phenotypes (Fig. 6I). Coimmunostaining with antibodies for several subcellular markers showed colocalization with the proteasome marker SUG1 (Fig. 6C–H), and additional studies revealed that these structures also labeled positively for polyubiquitinated proteins, but were negative for markers of microtubules (beta-tubulin), Golgi apparatus (golgin-97), lysosomes (Lamp-1), early endosomes (EEA1), and endoplasmic reticulum (calnexin) (Figs. S1 and S2). A similar punctate staining pattern of aggregated proteins has been observed after expression of a catalytically inactive mutant of myotubularin-related protein 3 (22), suggesting that this may be a common consequence of destabilizing mutations of this family of proteins.

Transcript Analysis of *MTM1* in Affected Skeletal Muscles. To determine whether the reduction in detectable myotubularin protein in skeletal muscles is more likely related to the effects of c.465C>A on mRNA or on protein stability, we performed rtPCR and DNA sequence analysis on *MTM1* transcripts in control and affected canine skeletal muscles. mRNA from muscles of three affected males and one carrier female yielded robust PCR products of similar size and quantity compared with normal control RT-PCR products. DNA sequence analysis demonstrated that affected animals expressed only the mutated mRNAs, whereas muscle from the carrier female contained both

Fig. 5. (A) Schematic diagram of mammalian myotubularin indicating the phosphoinositide-binding “GRAM-PH” domain, the catalytically active “phosphatase” domain, the “coiled-coil” domain likely responsible for binding other myotubularin-related proteins, and the C-terminal “PDZ-binding” domain. Exon 7 (indicated) encodes residues in a short linker between the GRAM-PH and phosphatase domains. (B) DNA sequence of a portion of *MTM1* exon 7 from an unaffected control Labrador Retriever (Control), affected males (II.d and I.e), and a carrier female (I.b). An *MTM1* c.465C>A transversion (in red box) was found to be hemizygous in all affected males and heterozygous in the obligate female carriers. (C) The affected amino acid, p.N155, is located directly at the interface between the beta pleated sheet-containing GRAM-PH (Upper Left) and the alpha-helical phosphatase (Lower Right) domains. (D) Amino acid alignment of *MTM1* orthologs demonstrate that this region is highly conserved among mammals and p.N155 (in red box) is conserved in all vertebrates examined as far as amphibians (*Xenopus*). (E) Amino acid lineup of human myotubularin (*MTM1*) with the seven catalytically active human *MTMRs* demonstrates that p.N155 (red box) is not conserved in myotubularin-related proteins.

There is no record of a missense mutation of p.N155 in a human patient; the nearest missense mutation is p.E157K, which was found in a family with unusually mild XLMTM (25). Although myotubularin protein was not examined in this case report, the extremely mild nature of the patients' conditions strongly suggests that their muscle contained residual and partially active myotubularin (26, 27). In contrast, the dramatic reduction in myotubularin protein on Western blot analysis of affected dogs' skeletal muscles, along with the mislocalization and aggregation of the mutated protein in proteasomes of transfected cells and in what might be necklace myofibers in affected dogs, suggests that the p.N155K mutation, which is in a linker region between the PH-GRAM and phosphatase domains, destabilizes the protein, leading to aggregation and premature degradation. This is consistent with the observation that most human missense *MTM1* mutations tested by Western blot analysis result in significant reduction in myotubularin levels (28). The recessive nature of the mutation and lack of overt pathology in carrier females suggests that the pathogenic mechanism of this mutation involves a loss of function, which is also consistent with the observation of greatly decreased overall protein levels. Vertebrate myotubularins are all collinear and highly conserved, and p.N155 is invariant in all myotubularins examined from human to *Xenopus* (Fig. 5D); however, it is not conserved in the other myotubularin-related proteins (Fig. 5E), suggesting a critical and specific role for this residue in maintaining the correct spatial relationships between the PH-GRAM and phosphatase domains of myotubularin.

The genetic haplotype data demonstrating a recent local origin for the *MTM1* p.N155K mutation suggest that Labrador Retrievers and other canine breeds from other parts of the world are unlikely to be at significant risk for this particular mutation. However, the recurring nature of XLMTM mutations in human populations also suggests that canine XLMTM likely has occurred independently before and is likely to recur in the future. As in humans, veterinarians and veterinary pathologists should consider an *MTM1* mutation as a possible cause of any unexplained progressive myopathy in dogs, especially when necklace fibers or excessive central nucleation are prominent features of the myopathology. Canine XLMTM is an excellent model for the human condition, representing the only known large animal model

available for preclinical trials of potential therapies. As such, these animals represent an invaluable resource that might be instrumental in developing effective treatments for this severe human congenital myopathy.

Materials and Methods

Animals. Over a 3-y period after the report on the original litter (19), five additional male Labrador Retrievers of age 14–26 wk, from two apparently unrelated litters were evaluated for generalized weakness and muscle atrophy at the Veterinary Medical Teaching Hospital, University of Saskatchewan. A fourth litter, whelped at Wake Forest University Medical Center, was also evaluated and included one additional affected male. All dogs tested negative for the known CNM mutation in the *PTPLA* gene (18). Complete blood counts and routine serum biochemistry profiles were unremarkable. Serum CK activities were normal to only mildly elevated. Electromyography was performed under general inhalational anesthesia in three of the dogs, showing abnormal spontaneous activity in several muscle groups. Muscle biopsy specimens were collected from multiple sites, including the lateral head of the triceps and vastus lateralis muscles in all five dogs. All animal studies were conducted during the course of routine clinical care under the auspices of the University of Saskatchewan's Animal Care Committee.

Experimental Details. Experimental details of histopathology, histochemistry, and immunofluorescence staining; immunoblotting; electron microscopy and quantification of triad structures; DNA extraction and *MTM1* gene sequencing; rapid TaqMan assay for identification of an *MTM1* mutation; SNP selection and genotyping of dogs; haplotype analysis; and transfection and subcellular localization of cDNA constructs are available in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank the various families of the dogs whose love and concern for their charges made this study possible. Special thanks to Alison Frase, whose untiring personal commitment and support were key ingredients driving this project. This work was supported in part by the Joshua Frase Foundation, the Anderson Family Foundation, National Institutes of Health (Grants P50 NS040828 and R01 AR044345), the Muscular Dystrophy Association, the CNM Project, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, University of Strasbourg, Collège de France, Association Française contre les Myopathies, Fondation Recherche Médicale, Agence Nationale de la Recherche, the Myotubular Trust, and Génopole d'Evry.

- North KN (2004) Congenital myopathies. *Myology*, eds Engel AG, Franzini-Armstrong C (McGraw-Hill, New York), 3rd Ed, Vol Vol 2, pp 1473–1533.
- Pierson CR, Tomczak K, Agrawal P, Moghadassadeh B, Beggs AH (2005) X-linked myotubular and centronuclear myopathies. *J Neuropathol Exp Neurol* 64:555–564.
- Laporte J, et al. (1996) A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* 13:175–182.
- Robinson FL, Dixon JE (2006) Myotubularin phosphatases: Policing 3-phosphoinositides. *Trends Cell Biol* 16:403–412.
- Nicot AS, Laporte J (2008) Endosomal phosphoinositides and human diseases. *Traffic* 9:1240–1249.
- Buj-Bello A, et al. (2008) AAV-mediated intramuscular delivery of myotubularin corrects the myotubular myopathy phenotype in targeted murine muscle and suggests a function in plasma membrane homeostasis. *Hum Mol Genet* 17:2132–2143.
- Buj-Bello A, et al. (2002) The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. *Proc Natl Acad Sci USA* 99:15060–15065.
- Dowling JJ, et al. (2009) Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. *PLoS Genet* 5:e1000372.
- Al-Qusairi L, et al. (2009) T-tubule disorganization and defective excitation-contraction coupling in muscle fibers lacking myotubularin lipid phosphatase. *Proc Natl Acad Sci USA* 106:18763–18768.
- Paoloni M, Khanna C (2008) Translation of new cancer treatments from pet dogs to humans. *Nat Rev Cancer* 8:147–156.
- Cooper BJ, et al. (1988) The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs. *Nature* 334:154–156.
- Sharp NJ, et al. (1992) An error in dystrophin mRNA processing in golden retriever muscular dystrophy, an animal homologue of Duchenne muscular dystrophy. *Genomics* 13:115–121.
- Shimatsu Y, et al. (2003) Canine X-linked muscular dystrophy in Japan (CXMDJ). *Exp Anim* 52:93–97.
- Sampaioles M, et al. (2006) Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* 444:574–579.
- Yokota T, et al. (2009) Efficacy of systemic morpholino exon-skipping in Duchenne dystrophy dogs. *Ann Neurol* 65:667–676.
- Kramer JW, Hegreberg GA, Bryan GM, Meyers K, Ott RL (1976) A muscle disorder of Labrador retrievers characterized by deficiency of type II muscle fibers. *J Am Vet Med Assoc* 169:817–820.
- McKerrell RE, Braund KG (1986) Hereditary myopathy in Labrador retrievers: A morphologic study. *Vet Pathol* 23:411–417.
- Pelé M, Tirté L, Kessler JL, Blot S, Panthier JJ (2005) SINE exonic insertion in the *PTPLA* gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. *Hum Mol Genet* 14:1417–1427.
- Cosford KL, Taylor SM, Thompson L, Shelton GD (2008) A possible new inherited myopathy in a young Labrador retriever. *Can Vet J* 49:393–397.
- Bevilacqua JA, et al. (2009) "Necklace" fibers, a new histological marker of late-onset *MTM1*-related centronuclear myopathy. *Acta Neuropathol* 117:283–291.
- Laporte J, et al. (2002) The PtdIns3P phosphatase myotubularin is a cytoplasmic protein that also localizes to Rac1-inducible plasma membrane ruffles. *J Cell Sci* 115:3105–3117.
- Walker DM, et al. (2001) Characterization of MTMR3, an inositol lipid 3-phosphatase with novel substrate specificity. *Curr Biol* 11:1600–1605.
- Herman GE, Finegold M, Zhao W, de Gouyon B, Metznerberg A (1999) Medical complications in long-term survivors with X-linked myotubular myopathy. *J Pediatr* 134:206–214.
- Shelton GD, Cardinet GH, 3rd, Bandman E (1988) Expression of fiber type specific proteins during ontogeny of canine temporalis muscle. *Muscle Nerve* 11:124–132.
- Yu S, et al. (2003) X-linked myotubular myopathy in a family with three adult survivors. *Clin Genet* 64:148–152.
- Biancalana V, et al. (2003) Characterisation of mutations in 77 patients with X-linked myotubular myopathy, including a family with a very mild phenotype. *Hum Genet* 112:135–142.
- Pierson CR, Agrawal PB, Blasko J, Beggs AH (2007) Myofiber size correlates with *MTM1* mutation type and outcome in X-linked myotubular myopathy. *Neuromuscul Disord* 17:562–568.
- Laporte J, Kress W, Mandel JL (2001) Diagnosis of X-linked myotubular myopathy by detection of myotubularin. *Ann Neurol* 50:42–46.