If only one mutation is identified in a simplex case (i.e., a single occurrence in a family), it is difficult to distinguish between the following:

- A *de novo* dominant mutation
- Autosomal recessive inheritance with a known *RYR1* mutation on one allele and a second as-yet unidentified mutation on the second allele.

To resolve this issue, the following can be considered:

- Testing both parents for the mutation, when possible, can confirm or exclude a *de novo* mutation.
- If autosomal recessive inheritance is suspected, the entire coding sequence of the gene should be sequenced in an effort to identify the mutation on the second allele.

Note: The pathogenicity of a mutation may be established by functional studies or testing in an animal model if one exists.

Testing Strategy

To confirm the diagnosis of CCD in a proband

- If clinical evaluation reveals characteristic findings (see Clinical Diagnosis), muscle biopsy to establish the diagnosis based on histologic findings
- Molecular genetic testing of *RYR1* to confirm the diagnosis

Carrier testing for relatives at risk of being heterozygous for autosomal recessive CCD requires prior identification of the disease-causing mutations in the family.

Note: (1) In the majority of cases CCD is inherited in an autosomal dominant manner; therefore, carrier testing is relevant in only that minority of CCD in which inheritance is autosomal recessive. (2) Carriers are heterozygous for one of the mutations causing autosomal recessive CCD and are not at risk of developing CCD.

Prenatal diagnosis and preimplantation genetic diagnosis (PGD) for pregnancies at increased risk for autosomal dominant CCD require prior identification of the disease-causing mutation in the family.

Note: It is the policy of *GeneReviews* to include clinical uses of testing available from laboratories listed in the GeneTests Laboratory Directory; inclusion does not necessarily reflect the endorsement of such uses by the author(s), editor(s), or reviewer(s).

Genetically Related (Allelic) Disorders

Malignant hyperthermia susceptibility (MHS) is a pharmacogenetic disorder of skeletal muscle calcium regulation resulting in uncontrolled skeletal muscle hypermetabolism.

Manifestations of malignant hyperthermia (MH) are triggered by certain volatile anesthetics (i.e., halothane, isoflurane, sevoflurane, desflurane, enflurane) either alone or in conjunction with depolarizing muscle relaxants (succinylcholine). The triggering substances release calcium stores from the sarcoplasmic reticulum, causing contracture of skeletal muscles, glycogenolysis, and increased cellular metabolism, resulting in production of heat and excess lactate. Affected individuals experience acidosis, hypercapnia, tachycardia, hypoxemia, rhabdomyolysis with subsequent increase in serum creatine kinase (CK), hyperkalemia with a risk of cardiac arrhythmia or even arrest, and myoglobinuria with a risk of renal failure. In nearly all cases, the first manifestations of MH, tachycardia, and tachypnea occur in the operating room, but MH may also occur in the early postoperative period. Death results unless the individual is promptly treated.

A clinical grading scale helps determine if a malignant hyperthermia (MH) episode has occurred. Contracture testing, the standard diagnostic test for MH since the mid-1970s, relies on the in vitro measurement of contracture response of biopsied muscle to graded concentrations of caffeine and the anesthetic halothane. Alternatively, calcium-induced calcium release (CICR) test can be performed, but has only been done in Japan. (For further information see Malignant Hyperthermia Susceptibility).

RYR1 is one of three known MHS-related genes. Domains 1 and 2 of *RYR1* are located in the soluble cytoplasmic regions of the protein and are hot spots for MH; however, mutations in these two domains have also been associated with CCD (see Molecular Genetic Testing).

The precise association of MHS and *RYR1* mutations is not clear and thus all individuals with a *RYR1* mutation are considered at risk for malignant hyperthermia and advised of appropriate precautions.

In several reports cores have been present in muscle biopsy of persons proven to have MH, thus raising controversy as to whether these individuals have CCD with MHS or MHS with cores. For example, Ibarra et al [2006] reported that 50% of persons with MHS with *RYR1* mutations had cores on muscle pathology, although most cores appear not to be as well-demarcated as those found in CCD. Further analysis is needed.

Multiminicore disease (MmD). The diagnosis of MmD is based on the presence of multiple "minicores" visible on muscle biopsy oxidative stains. Minicores are small zones of sarcomeric disorganization and/or diminished oxidative activity typically extending only a few sarcomeres in the fiber longitudinal axis that correlate with lack of mitochondria in muscle fibers. Because minicores are not specific to MmD, the diagnosis of MmD is based on the presence of minicores in a large proportion of muscle fibers associated with static or slowly progressive weakness and absence of findings diagnostic of other disorders.

Four clinical categories of MmD have been identified: classic form (75% of individuals), moderate form with hand involvement (<10%), antenatal form with arthrogryposis multiplex congenita (<10%), and ophthalmoplegic form (<10%). Onset of the classic form is usually congenital or occurs in early childhood with neonatal hypotonia, delayed motor development,

axial muscle weakness, scoliosis, and significant respiratory involvement (often with secondary cardiac impairment). Spinal rigidity of varying severity is present.

Mutations in two genes account for about half the cases of MmD. Although further genetic heterogeneity is suggested, no other candidate region or gene has been identified to date.

- *SEPN1* mutations inherited in an autosomal recessive manner account for about 30% of all cases of MmD and about 40% of cases of classic MmD.
- *RYR1* mutations inherited in an autosomal recessive manner account for some forms of MmD, and in particular, those with ophthalmoplegia. Ophthalmoplegia is an exclusion criterion for *SEPN1* mutations.

Congenital neuromuscular disorder with uniform fiber type 1 (CNMDU1). CNMDU1 is pathologically defined by the almost exclusive presence of type 1 fibers in muscle sections (i.e., type 1 fibers comprise more than 99% of the fibers) and the absence of specific structural abnormalities such as cores and nemaline bodies.

CNMDU1 histologic findings are thought to be an earlier manifestation of CCD, as an individual with pathologically confirmed CCD had a muscle biopsy consistent with CNMDU1 earlier in childhood [Sewry et al 2002]. Furthermore, Quinlivan et al [2003] reported *RYR1* mutations in a family with CCD in which the youngest member showed uniform fiber typing, suggesting that adults have CCD while children had CNMDU1. These data imply that CNMDU1 is an earlier manifestation of the CCD spectrum; however, this may not be the case. Recently, mutations in the C-terminal region of *RYR1* were identified in 40% of individuals with CNMDU1 [Sato et al 2008]. In this report, electron microscopic analysis of a muscle biopsy from a person with CNMDU1 showed virtually normal histology, devoid of signs of early core formation, also suggesting that CNMDU1 may be a distinct entity and more possibly allelic to CCD. Moreover, there has been no report of overlap between the two disorders with respect to histologic findings (i.e., uniform type 1 fiber with cores in only a few fibers), casting doubt on the hypothesis that these two diseases belong to a single spectrum.

Centronuclear myopathy is a genetically heterogeneous disorder characterized clinically as congenital myopathy and the presence of centrally placed nuclei in a significant proportion of myofibers. So far, causative mutations have been identified in myotubularin (*MTM1*), dynamin 2 (*DNM2*), amphiphysin 2 (*BIN1*), and myotubularin-related protein 14 (*MTMR14*). Jungbluth et al [2007] reported a 16-year-old who was diagnosed at age one year with centronuclear myopathy with multiple central nuclei in up to 50% of fibers and central accumulation of oxidative enzyme stains. However, muscle biopsy eight years later revealed some core-like areas, raising the suspicion of CCD. Molecular genetic testing revealed a *de novo* missense mutation in exon 90 of *RYR1*. These findings suggest that the presence of an increased number of fibers with centrally placed nuclei may be a part of the CCD spectrum.

Clinical Description

Natural History

The expressivity of central core disease (CCD) is variable, ranging clinically from mild (i.e., almost asymptomatic) to severe (i.e., ventilator-dependent) and histologically varying in the extent and localization of cores in the muscle fibers.

Most individuals have mild disease characterized by mild, symmetric weakness that preferentially affects the proximal muscles. The facial and neck muscles may be mildly involved in some cases. The extraocular muscles are often spared in the classic, autosomal dominant form, but are typically involved in the autosomal recessive form. Motor development is usually delayed, but in general, most affected individuals acquire independent ambulation. Hypotonia in infancy and respiratory insufficiency can also occur in those with mild disease. Life span is usually normal.

Muscle cramps have been documented in some individuals with CCD, and this may be associated with MH susceptibility.

Severe disease is characterized by infantile onset associated with profound hypotonia and respiratory dysfunction requiring continuous assisted ventilation. In severely affected individuals, death may result from respiratory infection or respiratory insufficiency.

Fetal akinesia has been associated with both autosomal dominant and autosomal recessive forms of *RYR1*-related CCD [Romero et al 2003]. The clinical phenotype consisted of severe hypotonia, arthrogryposis multiplex congenita, amyotrophy, and respiratory failure, requiring mechanical ventilation. The outcome, however, was variable (ranging from early death to survival beyond age five years).

Typically CCD is not progressive, although slow progression has been reported . Scoliosis can be progressive, resulting in respiratory insufficiency.

Intellectual ability is intact.

Other. Serum creatine kinase concentration may be normal or mildly elevated.

Electromyography may confirm the presence of myopathy and reveal brief, short action potentials and early recruitment.

Muscle imaging has demonstrated that certain muscles are selectively involved in *RYR1*-related myopathies, including quadriceps, sartorius, adductor magnus, soleus, gastrocnemius, and peroneal group; certain muscles are relatively spared, including rectus femoris, gracilis, adductor longus, and tibialis anterior [Jungbluth et al 2004]. These findings were supported by Fischer et al [2006] who described distinct MRI findings in persons with CCD who have an *RYR1* mutation, including predominant involvement of the gluteus maximus, adductor magnus, sartorius, vastus intermediolateralis, soleus, and lateral gastrocnemius muscles, as compared to those who do not have an *RYR1* mutation.

Genotype-Phenotype Correlations

Although most *RYR1* mutations that result in CCD are inherited in an autosomal dominant manner, reports of autosomal recessive inheritance are increasing. At the moment, it is not possible to predict the mode of inheritance based on the mutation alone.

Some studies have shown that autosomal recessive CCD, often associated with *RYR1* mutations outside the C-terminal region, can be severe [Romero et al 2003, Zhou et al 2006b]. Thus, it may be possible to consider most autosomal dominant forms of CCD as milder in phenotype than autosomal recessive forms of CCD.

In a study of 25 individuals with genetically-confirmed CCD, Wu et al [2006] determined that:

- The 16 individuals with C-terminal *RYR1* mutations had certain clinical features including hypotonia during infancy, delayed motor development, and limb muscle weakness and certain pathologic findings on muscle biopsy that delineate C terminal mutations from other groups including (1) type 2 fiber deficiency and interstitial fibrosis, (2) characteristic cores with clearly demarcated borders that are observed in almost all type 1 muscle fibers, (3) higher than average frequency of "rimming" on the borders of these cores.
- Most individuals with CCD with at least one *RYR1* mutation outside the C-terminal region had only mild musculoskeletal abnormalities such as joint contractures and scoliosis. Inheritance was autosomal dominant, consistent with previous reports of mild CCD phenotype.

Malignant hyperthermia susceptibility (MHS)-related *RYR1* mutations are predominantly located in the hydrophilic N-terminal and central portions of the ryanodine receptor 1 (RyR1) protein, whereas CCD-related *RYR1* substitutions mainly occur in the hydrophobic pore-forming region of the channel [Monnier et al 2000, Monnier et al 2001, Davis et al 2003, Zorzato et al 2003]. Previous reports have asserted that persons without muscle disease who are susceptible to malignant hyperthermia (MH) have mutations in the C-terminal region of ryanodine receptor 1; however, limited histopathologic evaluation of these individuals has revealed the presence of cores that are not characteristic of the cores of CCD [Ibarra et al 2006]; thus, they are most appropriately labeled as having "MH with cores."

Individuals with CCD who have mutations in the N-terminal domain may have a higher probability of malignant hyperthermia susceptibility than those with mutations in the C-terminal domain [Wu et al 2006].

Penetrance

In general, the penetrance of CCD is variable. Mutations in the C-terminal region of ryanodine receptor 1, including p.Ile4898Thr [Lynch et al 1999] and p.Tyr4796Cys [Monnier et al 2000] in the luminal domain were associated with more severe phenotype, and, hence, full penetrance, and autosomal dominant inheritance.

Anticipation

Anticipation is not observed.

Nomenclature

CCD has also been referred to as Shy-Magee syndrome, after the individuals who initially reported it.

Some cases called core-rod disease are not associated with a *RYR1* mutation; thus, "core-rod disease" is not a true synonym for CCD.

Prevalence

The precise incidence and prevalence of CCD, considered to be the most frequently occurring congenital myopathy, are unknown.

Differential Diagnosis

For current information on availability of genetic testing for disorders included in this section, see GeneTests Laboratory Directory. —ED.

The clinical findings of central core disease (CCD) are variable and not disease specific; they can be seen in other congenital myopathies. Thus, from a clinical standpoint CCD cannot be readily distinguished from other congenital myopathies, such as multiminicore disease, CNMDU1 (see Allelic Disorders), the intermediate form of nemaline myopathy, fingerprint body myopathy, congenital fiber-type disproportion, hyaline body myopathy, reducing body myopathy, and cylindrical spirals myopathy.

The phenotype of CCD is relatively heterogeneous with a variable age of onset. Thus, CCD must be considered in persons of all ages with scoliosis or severe spinal deformity, unexplained muscle weakness, and multiple joint problems [Mertz et al 2005, Sestero & Perra 2005].

The 'central core' histologic changes are nonspecific and may occur in other myopathies. Cores that have been noted in CCD have also been reported with mutations in the following genes:

- *SEPN1*. Mutations in this gene are also associated with minicores [Ferreiro et al 2002b], but no individual with a *SEPN1* mutation and the typical long, well-delimited central cores characteristic of CCD has been reported.
- *MYH7*, in hypertrophic cardiomyopathy
- *ACTA1* and *TNNT1* in nemaline myopathy [Ilkovski et al 2001]. *ACTA1* mutations were found in a congenital myopathy with few cores on muscle biopsy [Kaindl et al 2004]; like other disorders with cores, however, these disorders are better considered as myopathies with cores, not CCD.
- *CFL2*, encoding cofilin-2, has recently been associated with nemaline myopathy with minicores [Agrawal et al 2007].

- Structures similar to cores have been observed in the myofibers of individuals with neurogenic atrophy but are more appropriately called "target fibers" in this setting because of the darker band around the pale central area, giving it a target-like appearance. In addition, core-like lesions devoid of this band can also be seen in these conditions.

Management

Evaluations Following Initial Diagnosis

To establish the extent of disease in an individual diagnosed with central core disease (CCD), the following evaluations are recommended:

- Neurologic examination with attention to features of congenital myopathy (hypotonia, failure to thrive, joint contractures, scoliosis), weakness of the limbs, and muscle cramps
- Physical and occupational therapy assessments
- Evaluation for feeding difficulties, including assessment for sucking and ability to swallow
- Pulmonary function testing in most patients, especially those with scoliosis, hypotonia, signs of respiratory distress, and/or history of recurrent chest infections. History should be taken for symptoms of nocturnal hypoxia including early morning headaches, daytime drowsiness, loss of appetite, and deteriorating school performance.

Treatment of Manifestations

Since prognosis is mainly influenced by respiratory status and scoliosis, treatment geared towards these manifestations is essential.

Treatment depends on the severity of symptoms, but mainly consists of supportive measures and rehabilitation that address the following problems:

- Hypotonia and weakness. Patients may benefit from physical therapy. Interventions may include stretching programs and mild to moderate low-impact exercise; activities should be balanced in such a way that exhaustion is avoided.
- Scoliosis and joint contractures. Some patients may only require physical therapy, while others may need orthopedic surgery (e.g., scoliosis surgery, corrective surgery for congenital hip dislocation and foot deformities).
- Respiratory. Patients with more severe symptoms may require respiratory support. Breathing exercises and chest physiotherapy for handling secretions may also be beneficial.
- Feeding difficulties. Individuals may require diet supplementation and feeding by means of nasogastric/orogastric routes or gastrostomy.

Prevention of Secondary Complications

Secondary complications can include respiratory compromise from scoliosis; hence, orthopedic intervention may reduce the risk of this problem.

Immunization against influenza is encouraged.

Prompt treatment of respiratory infection is important.

Joint contractures may be prevented by encouraging mobility and by active participation in physical therapy.

Surveillance

The following are appropriate:

- Routine assessment of the spine for scoliosis and joints for contractures
- Routine assessment of respiratory parameters such as respiratory rate, peak expiratory flow rate (PEFR), forced vital capacity (FVC), and forced expiratory volume in one second (FEV1)
- Sleep studies especially when patients show signs of nocturnal hypoxia
- Regular assessment of motor abilities in order to determine need for physical therapy, occupational therapy, and assistive devices for ambulation, such as a wheelchair

Agents/Circumstances to Avoid

Although it is unknown how CCD is associated with malignant hyperthermia susceptibility or which mutations in *RYR1* are absolutely related to MH susceptibility, it is prudent for individuals with CCD to avoid inhalational anesthetics and succinylcholine. See Malignant Hyperthermia Susceptibility for more details.

Individuals suspected of having MH susceptibility are advised to avoid extremes of heat, but this does not mean restriction of athletic activity.

Testing of Relatives at Risk

Because CCD is associated with an increased risk for MH susceptibility, it is appropriate to test at-risk relatives of a proband (whether symptomatic or not) for the *RYR1* mutation identified in the proband in order to caution those with the mutation about potential risks of inhalational anesthetics and succinylcholine. See Malignant Hyperthermia Susceptibility for more details.

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Therapies Under Investigation

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Search ClinicalTrials.gov for access to information on clinical studies for a wide range of diseases and conditions. Note: There may not be clinical trials for this disorder.

Other

Genetics clinics, staffed by genetics professionals, provide information for individuals and families regarding the natural history, treatment, mode of inheritance, and genetic risks to other family members as well as information about available consumer-oriented resources. See the GeneTests Clinic Directory.

See Consumer Resources for disease-specific and/or umbrella support organizations for this disorder. These organizations have been established for individuals and families to provide information, support, and contact with other affected individuals.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. To find a genetics or prenatal diagnosis clinic, see the GeneTests Clinic Directory.

Mode of Inheritance

Central core disease (CCD) is usually inherited in an autosomal dominant manner, but it can be inherited in an autosomal recessive manner [Ferreiro et al 2002a, Jungbluth et al 2002, Romero et al 2003, Wu et al 2006, Zhou et al 2006b, Kossugue et al 2007].

Risk to Family Members — Autosomal Dominant Inheritance

Parents of a proband

- Most individuals diagnosed with autosomal dominant CCD have an affected parent or an asymptomatic parent who has a disease-causing mutation.
- A proband with autosomal dominant CCD may have the disorder as the result of a new gene mutation. The proportion of cases caused by *de novo* mutations is unknown.
- If the disease-causing mutation found in the proband cannot be detected in the DNA of either parent, two possible explanations are germline mosaicism in a parent or a *de novo* mutation in the proband. Although no instances of germline mosaicism have been reported, it remains a possibility.
- Recommendations for the evaluation of parents of a proband with an apparent *de novo* mutation include muscle biopsy and molecular genetic testing. Evaluation of parents may

determine that one is affected but has escaped previous diagnosis because of failure by health care professionals to recognize the syndrome and/or a milder phenotypic presentation. Therefore, an apparently negative family history cannot be confirmed until appropriate evaluations have been performed.

Note: (1) Although most individuals diagnosed with CCD have an affected parent, the family history may appear to be negative because of failure to recognize the disorder in family members, early death of the parent before the onset of symptoms, late onset of the disease in the affected parent, or reduced penetrance. (2) If the parent is the individual in whom the mutation first occurred s/he may have somatic mosaicism for the mutation and may be mildly/minimally affected.

Sibs of a proband

- The risk to the sibs of the proband depends on the genetic status of the proband's parents.
- If a parent of the proband is affected, the risk to the sibs is 50%.
- When the parents are clinically unaffected, the risk to the sibs of a proband appears to be low.
- If the disease-causing mutation found in the proband cannot be detected in the DNA of either parent, the risk to sibs is low, but greater than that of the general population because of the possibility of germline mosaicism.

Offspring of a proband. Each child of an individual with autosomal dominant CCD has a 50% chance of inheriting the mutation.

Other family members of a proband. The risk to other family members depends on the status of the proband's parents. If a parent is affected, his or her family members may be at risk.

Risk to Family Members — Autosomal Recessive Inheritance

Parents of a proband

- The parents of an affected child are obligate heterozygotes and therefore carry one mutant allele.
- Heterozygotes (carriers) are often asymptomatic.

Sibs of a proband

- At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier.
- Once an at-risk sib is known to be unaffected, the risk of his/her being a carrier is 2/3.
- Heterozygotes (carriers) are asymptomatic.

Offspring of a proband. The offspring of an individual with autosomal recessive CCD are obligate heterozygotes (carriers) for a disease-causing mutation.

Other family members of a proband. Each sib of the proband's parents is at a 50% risk of being a carrier.

Carrier Detection

Carrier testing for family members at risk of being heterozygous for autosomal recessive CCD is possible if the disease-causing mutations have been identified in the family.

Related Genetic Counseling Issues

See Testing of Relatives at Risk for information on testing of relatives for malignant hyperthermia susceptibility.

Simplex cases. Kossugue et al [2007] reported several simplex cases with CCD in whom at least one mutation was identified. The cause of CCD in these individuals may be (1) a *de novo* dominant mutation or (2) autosomal recessive inheritance of a known *RYR1* mutation and a second as-yet unidentified mutation.

Considerations in families with an apparent *de novo* mutation. When neither parent of a proband with an autosomal dominant condition has the disease-causing mutation or clinical evidence of the disorder, it is likely that the proband has a *de novo* mutation. However, possible non-medical explanations including alternate paternity or maternity (e.g., with assisted reproduction) or undisclosed adoption could also be explored.

Family planning.

- The optimal time for determination of genetic risk and discussion of the availability of prenatal testing is before pregnancy.
- It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are affected or at risk.

DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, mutations, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals. See **Testing** for a list of laboratories offering DNA banking.

Prenatal Testing

Although prenatal diagnosis has not been reported, prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15 to 18 weeks' gestation or chorionic villus sampling (CVS) at about ten to 12 weeks' gestation. The disease-causing allele(s) of an affected family member must be identified or linkage established in the family before prenatal testing can be performed.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutation(s) has/have been identified. For laboratories offering PGD, see **Testing**.

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Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A. Central Core Disease: Genes and Databases

Gene Symbol	Chromosomal Locus	Protein Name	Locus Specific	HGMD
RYR1	19q13.1	Ryanodine receptor 1	RYR1 @ LOVD	RYR1

Data are compiled from the following standard references: gene symbol from HGNC; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from UniProt. For a description of databases (Locus Specific, HGMD) linked to, click here.

Table B. OMIM Entries for Central Core Disease (View All in OMIM)

117000 CENTRAL CORE DISEASE OF MUSCLE
180901 RYANODINE RECEPTOR 1; RYR1

Molecular Genetic Pathogenesis

The skeletal muscle isoform of ryanodine receptor 1 (RyR1) mediates Ca^{2+} release during excitation-contraction (EC) coupling; hence, mutations in the *RYR1* gene are expected to cause disturbance in this process. However, the precise pathophysiology of central core disease (CCD) is not fully understood and remains controversial. Two fundamentally distinct cellular mechanisms (leaky channels and EC uncoupling) are proposed to explain how altered release channel function caused by different mutations in *RYR1* could result in muscle weakness in CCD [Dirksen & Avila 2002]. Although it is commonly believed that cores are not specific to CCD, it has recently been demonstrated that calcium-handling proteins are abnormally distributed in *RYR1*-associated core myopathies: RyR1 protein was depleted from the cores, while calsequestrin, SERCA1/2, triadin, and DHPR had accumulated within or around the lesions [Herasse et al 2007]. These findings suggest that EC uncoupling may indeed lead to muscle weakness.

Certain *RYR1* mutations are associated with both CCD and MH susceptibility. In a previous report, the effects of mutations that involve CCD plus MH susceptibility and MH susceptibility only on Ca^{2+} handling and EC coupling have been characterized; it has been suggested that sarcoplasmic reticulum (SR) Ca^{2+} depletion and increased basal Ca^{2+} levels are preferentially associated with *RYR1* mutations that result in combined MH susceptibility and CCD [Dirksen & Avila 2004]. Furthermore, the authors also found that MH susceptibility-only mutations modestly increase basal release-channel activity in a manner insufficient to alter net SR Ca^{2+} content ("compensated leak"), whereas the combined MH susceptibility and CCD phenotype

arises from mutations that enhance basal activity to a level sufficient to promote SR Ca^{2+} depletion, elevate $[Ca^{2+}]i$, and reduce maximal VGCR ("decompensated leak").

Zhou et al [2006a] presented evidence that in individuals with autosomal recessive core myopathies, *RYR1* frequently undergoes polymorphic, tissue-specific, and developmentally regulated allele silencing apparently mediated by hypermethylation. The resulting monoallelic expression of *RYR1* can unveil recessive mutations in the remaining *RYR1* allele in persons with core myopathies. Zhou et al [2006a] also suggested that imprinting is a likely mechanism for this phenomenon, which can play a role in human phenotypic heterogeneity and in irregularities of inheritance patterns.

Normal allelic variants. *RYR1* consists of 106 exons (two of which are alternatively spliced) encompassing a total of 160 kb and producing one of the largest proteins in humans with 5038 amino acids [Phillips et al 1996]. Several normal allelic variants have been noted in *RYR1*, including: p.Ala1832Gly, p.Val2550Leu [Monnier et al 2000]; p.Val4849Ile [Monnier et al 2001]; p.Gly2060Cys, and p.Met485Val [Zhou et al 2006b]. See Table 2.

Table 2. Selected *RYR1* Normal Allelic Variants

DNA Nucleotide Change	Protein Amino Acid Change	Reference Sequences
--	p.Met485Val	NM_000540.2 NP_000531.2
--	p.Ala1832Gly	
--	p.Val2550Leu	
--	p.Val4849Ile	
--	p.Gly2060Cys	

See Quick Reference for an explanation of nomenclature. *GeneReviews* follows the standard naming conventions of the Human Genome Variation Society (www.hgvs.org).

Pathologic allelic variants. More than 80 reported *RYR1* mutations have been associated with the autosomal dominant or autosomal recessive forms of CCD, including 67 missense mutations and five deletions, clustered in three regions of the gene. More than half of the *RYR1* mutations are private.

The most common mutations are shown in Table 3 (pdf).

Table 4 (pdf) shows the most common *RYR1* pathogenic amino acid variants associated with autosomal dominant central core disease.

Normal gene product. *RYR1* encodes the ryanodine receptor 1 protein, a skeletal muscle calcium-release channel located in the sarcoplasmic reticulum (SR). The functional channel is a homotetramer of 560-kd subunits; it releases calcium stored in the SR in response to membrane depolarization transduced by the dihydropyridine receptor (DHPR). The cytoplasmic domain of ryanodine receptor 1, also called the foot structure, comprises the first 4000 amino acids that

bridge the gap between the SR and the transverse tubular system. The last 1000 amino acids from the transmembrane domain contain the pore of the channel [Tilgen et al 2001, Lehmann-Horn et al 2003].

Ryanodine receptors belong to the superfamily of intracellular Ca^{2+} release channels present on endoplasmic reticulum/sarcoplasmic reticulum (SR) membranes, having three different isoforms. Functional units are homotetramers of approximately 5,000 amino acids per subunit coded by 150-kb genes. *RYR1*, forming the SR calcium release channel, has a large hydrophilic NH_2-terminal domain and a hydrophobic COOH-terminal domain containing several transmembrane domains as well as the channel pore. The 563-kd protein is predominantly expressed not only in skeletal muscle but also in human B-lymphocytes and immature murine dendritic cells.

Abnormal gene product. Alterations in the ryanodine receptor 1 protein lead to an abnormal, sustained increase in myoplasmic calcium concentration in skeletal muscle because of a "leaky channel" or uncoupling with its voltage sensor, which is encoded by the voltage-gated calcium channel gene *DHPR* [Nelson 2001, Wehner et al 2003].

In vitro studies suggest that a high basal activity of the mutant Ca^{2+} channel could explain the muscle weakness and muscle atrophy observed in persons with CCD in one family [Lynch et al 1999]. In vitro expression of ryanodine receptor 1 with a single mutation (p.Ile4898Thr) in the C-terminal transmembrane/luminal domain in HEK293 cells resulted in loss of channel activation and reduction in ryanodine binding, possibly by disrupting the ligand binding site located in the C terminus of the protein. Further analysis, however, showed that this mutation leads to a significant increase in the sensitivity of the channel to the activating effects of calcium.

The association of C-terminal mutations with clinically evident muscle weakness may be explained by the leaky-channel model and the excitation-contraction (EC) uncoupling model.

- Some non-C-terminal mutations in ryanodine receptor 1 promote the leak of Ca^{2+} ions from the SR that may or may not be compensated by the activity of the sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA), resulting in elevation of resting cytosolic Ca^{2+} and depletion of SR Ca^{2+} stores.

C-terminal mutations, especially those in the pore region of ryanodine receptor 1, may directly affect the channel gating properties, resulting in an abolition of orthograde activation by the voltage-gated L-type Ca^{2+} channel or, in other words, EC uncoupling. However, no compensatory mechanism increases Ca^{2+} release as the SERCA pumps do in the leaky model. Nevertheless, the effect of mutations in the C-terminal region remains controversial and at best unlikely because a number of mutations in this area were also shown to be "leaky." Interestingly, several mutations in *RYR1* exon 102 were shown to lead to varying degrees of EC uncoupling, indicating that this region is a primary locus of EC uncoupling in CCD [Avila et al 2003b].

Resources

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See Consumer Resources for disease-specific and/or umbrella support organizations for this disorder. These organizations have been established for individuals and families to provide information, support, and contact with other affected individuals. GeneTests provides information about selected organizations and resources for the benefit of the reader; GeneTests is not responsible for information provided by other organizations.—ED.

References

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Literature Cited

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The cathepsin L gene is a direct target of FOXO1 in skeletal muscle

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FOXO1 (forkhead box O1), a forkhead-type transcription factor whose gene expression is up-regulated in the skeletal muscle during starvation, appears to be a key molecule of energy metabolism and skeletal muscle atrophy. Cathepsin L, a lysosomal proteinase whose expression is also up-regulated in the skeletal muscle during starvation, is induced in transgenic mice overexpressing FOXO1 relative to wild-type littermates. In the present study, we conducted *in vivo* and *in vitro* experiments focusing on FOXO1 regulation of *Ctsl* (cathepsin L gene; *CTSL1* in humans) expression in the skeletal muscle. During fasting and refeeding of C57BL/6 mice, *Ctsl* was regulated in parallel with FOXO1 in the skeletal muscle. Fasting-induced *Ctsl* expression was attenuated in transgenic mice overexpressing a dominant-negative form of FOXO1 or in skeletal-muscle-specific *Foxo1*-knockout mice relative to respective wild-type controls. Using C2C12 mouse myoblasts overexpressing a constitutively active form of FOXO1, we showed that FOXO1 induces *Ctsl* expression. Moreover, we found FOXO1-binding sites in both the mouse *Ctsl* and human *CTSL1* promoters. The luciferase reporter analysis revealed that the mouse *Ctsl* and human *CTSL1* promoters are activated by FOXO1, which is abolished by mutations in the consensus FOXO1-binding sites. Gel mobility-shift and chromatin immunoprecipiation assays showed that FOXO1 is recruited and binds to the *Ctsl* promoter. The present study provides *in vivo* and *in vitro* evidence that *Ctsl* is a direct target of FOXO1 in the skeletal muscle, thereby suggesting a role for the FOXO1/cathepsin L pathway in fasting-induced skeletal muscle metabolic change and atrophy.

Key words: atrophy, cathepsin L, forkhead box O1 (FOXO1), forkhead transcription factor, muscle metabolism, starvation.

INTRODUCTION

The skeletal muscle is the largest organ in the human body, with important roles in exercise, glucose uptake and energy expenditure. Skeletal muscle metabolism is changed by the supply of nutrients and circulating hormones [1,2]. Starvation and disease states (such as diabetes and cancer cachexia) lead to a rapid reduction in skeletal muscle mass (atrophy) [2]. What is the physiological role of muscle atrophy? As the brain mainly uses glucose as an energy source, during starvation it needs to be supplied with glucose. Thus, for short periods of fasting, skeletal muscle increases utilization of lipids instead of glucose. On the other hand, for longer periods of fasting or starvation resulting in muscle atrophy, skeletal muscle protein is degraded and mobilized as a source of amino acids for gluconeogenesis that occurs mainly in the liver [3].

The FOXO (forkhead box O) members FOXO1, FOXO3a and FOXO4 belong to a subfamily of the forkhead transcription factors [4,5]. The FOXO family regulates a variety of biological processes such as metabolism, cell proliferation, apoptosis, stress response and longevity [6–9]. FOXO1 activates gluconeogenic enzyme genes in the liver, such as those for PEPCK (phosphoenolpyruvate carboxykinase) and G6Pase (glucose-6-phosphatase). A dominant-negative form of FOXO1 (DN-FOXO1), which contains the DNA-binding domain, but lacks the transcriptional activation domain, suppressed the fasting-induced increase of *Pepck* and *G6Pase* expression in liver cells [10]. We showed previously that energy-deprived conditions in mice, such as fasting and diabetes, up-regulated expression of *Foxo1* in skeletal muscle of mice [11]. Several FOXO1 target genes have been identified in skeletal muscle. For instance, FOXO1 up-regulates *PDK4* (pyruvate dehydrogenase kinase 4), a kinase that suppresses glycolysis [12], and *LPL* (lipoprotein lipase), an enzyme that increases lipid incorporation [11], and down-regulates *SREBP1c* (sterol-regulatory-element-binding protein 1c), a master regulator of lipogenesis [13]. The FOXO1 target genes may be involved in the utilization of lipids instead of glucose in the skeletal muscle. On the other hand, forced expression of FOXO1 or FOXO3a up-regulates the expression of a variety of atrophy-related genes including the *MuRF1* and *atrogin/MAFbx* ubiquitin ligases [14,15], as well as *Bnip3* and *LC3*, important molecules for autophagy [16,17], thus inducing skeletal muscle atrophy *in vitro* and *in vivo*. We have created transgenic mice that overexpress FOXO1 in skeletal muscle (FOXO1 mice) and found

Abbreviations used: ChIP, chromatin immunoprecipitation; DBE, DAF16 (decay-accelerating factor 16)-binding element; DMEM, Dulbecco's modified Eagle's medium; DN-FOXO1, dominant-negative forkhead box O1; ER, oestrogen receptor; FBS, fetal bovine serum; FOXO, forkhead box O; GADD45α, growth-arrest and DNA-damage-inducible protein 45α; G6Pase, glucose-6-phosphatase; HEK, human embryonic kidney; PEPCK, phosphoenolpyruvate carboxykinase; PLSD, protected least-significant difference; TAM, tamoxifen.

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