Congenital Myasthenic Syndromes


Angela Abicht, MD
Department of Neurology
Friedrich-Baur Institute
Munich, Germany
angela.abicht@med.uni-muenchen.de

Juliane Müller, S, PhD
Institute of Genetic Medicine
Newcastle University
Newcastle upon Tyne, United Kingdom
juliane.mueller@newcastle.ac.uk

Hanns Lochmüller, MD
Institute of Genetic Medicine
Newcastle University
Newcastle upon Tyne, United Kingdom
hanns.lochmueller@ncl.ac.uk

Initial Posting: May 9, 2003; Last Revision: June 28, 2012.

Summary

Disease characteristics. Congenital myasthenic syndromes (designated as CMS throughout this entry) are characterized by fatigable weakness of skeletal muscle (e.g., ocular, bulbar, limb muscles) with onset at or shortly after birth or in early childhood; rarely, symptoms may not manifest until later in childhood. Cardiac and smooth muscle are not involved. Severity and course of disease are highly variable, ranging from minor symptoms to progressive disabling weakness. In some subtypes of CMS, myasthenic symptoms may be mild, but sudden severe exacerbations of weakness or even sudden episodes of respiratory insufficiency may be precipitated by fever, infections, or excitement. Major findings of the neonatal onset subtype include: feeding difficulties; poor suck and cry; choking spells; eyelid ptosis; facial, bulbar, and generalized weakness. In addition arthrogryposis multiplex congenital may be present; respiratory insufficiency with sudden apnea and cyanosis may occur. Later childhood onset subtypes show abnormal muscle fatigability with difficulty in activities such as running or climbing stairs; motor milestones may be delayed; fluctuating eyelid ptosis and fixed or fluctuating extraocular muscle weakness are common presentations.

Diagnosis/testing. The diagnosis of CMS is based on clinical findings, a decremental EMG response of the compound muscle action potential (CMAP) on low-frequency (2-3 Hz) stimulation, absence of anti-acetylcholine receptor (AChR) and anti-MuSK antibodies in the serum, and lack of improvement of clinical symptoms with immunosuppressive therapy. Mutations in one of multiple genes encoding proteins expressed at the neuromuscular junction are currently known to be associated with subtypes of CMS, including the genes encoding different subunits of the acetylcholine receptor:

- **CHRNE** (εAChR subunit)
- **CHRNA1** (αAChR subunit)
- **CHRNB1** (βAChR subunit)
- **CHRND** (δAChR-subunit)
- **AGRN** encoding agrin
- **CHAT** encoding choline O-acetyltransferase
- **COLQ** encoding acetylcholinesterase collagenic tail peptide
- **DOK7** encoding protein Dok-7
- **GFPT1** encoding glucosamine--fructose-6-phosphate aminotransferase 1
- **MUSK** encoding muscle, skeletal receptor tyrosine protein kinase
• *RAPSN* encoding rapsyn (43-kd receptor-associated protein of the synapse)

• *SCN4A* encoding the sodium channel protein type 4 subunit alpha

Clinical molecular genetic testing is available for all of these genes.

**Management.** Treatment of manifestations: Most individuals with CMS benefit from acetylcholine esterase (AChE) inhibitors and/or the potassium channel blocker 3,4-diaminopyridine (3,4-DAP); however, caution must be used in giving 3,4-DAP to young children and individuals with fast-channel CMS (FCCMS). Individuals with *COLQ* and *DOK7* mutations usually do not respond to long-term treatment with AChE inhibitors. Some individuals with slow-channel CMS (SCCMS) are treated with quinidine, which has some major side effects and may be detrimental in individuals with acetylcholine receptor (AChR) deficiency. Fluoxetine is reported to be beneficial for SCCMS. Ephedrine and albuterol have been beneficial in a few individuals, especially as a therapeutic option for those with *DOK7* or *COLQ* mutations.

**Prevention of primary manifestations:** Prophylactic anticholinesterase therapy to prevent sudden respiratory insufficiency or apneic attacks provoked by fever or infections in those with mutations in *CHAT* or *RAPSN*. Parents of infants are advised to use apnea monitors and be trained in CPR.

**Agents/circumstances to avoid:** Drugs known to affect neuromuscular transmission and exacerbate symptoms of myasthenia gravis (e.g., ciprofloxacin, chloroquine, procaine, lithium, phenytoin, beta-blockers, procainamide, quinidine).

**Evaluation of relatives at risk:** If the disease-causing mutations in the family are known, molecular genetic testing can be used to clarify the genetic status of at-risk asymptomatic family members, especially newborns or young children, who could benefit from early treatment to prevent sudden respiratory failure.

**Genetic counseling.** Congenital myasthenic syndromes are inherited in an autosomal recessive, or, less frequently, autosomal dominant manner.

In autosomal recessive CMS (AR-CMS), the parents of an affected child are obligate heterozygotes and therefore carry one mutant allele. Heterozygotes (carriers) are asymptomatic. 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.

In autosomal dominant CMS (AD-CMS), some individuals have an affected parent while others have a *de novo* mutation. The proportion of cases caused by *de novo* mutations is unknown. Each child of an individual with AD-CMS has a 50% chance of inheriting the mutation.

Prenatal testing for pregnancies at increased risk is possible in a clinical laboratory for most subtypes of CMS if the disease-causing mutations in a family are known; prenatal testing for the remaining subtypes may be available through laboratories offering custom prenatal testing.

**Diagnosis**

**Clinical Diagnosis**

The clinical diagnosis of congenital myasthenic syndromes (CMS) is based on the following:

• A history of fatigable weakness involving ocular, bulbar, and limb muscles

• Onset at or shortly after birth or in early childhood. Post-childhood onset has been observed, but is rare [Burke et al 2003, Beeson et al 2005, Müller et al 2007a].

• A decremental EMG response of the compound muscle action potential (CMAP) on low-frequency (2-3 Hz) stimulation (see Testing, *Electrophysiologic testing*)

• Absence of anti-acetylcholine receptor (anti-AChR) and anti-MuSK antibodies in the serum

  Note: (1) Absence of anti-AChR antibodies in the serum can help distinguish CMS from myasthenia gravis (MG), but does not exclude seronegative types of MG or MG with anti-MuSK antibodies [Hoch et al 2001]. (2) One case of autoimmune MG developing in an individual with CMS has been reported [Croxen et al 2002b].

• Lack of improvement of clinical symptoms with immunosuppressive therapy

• Absence of major pathology in a skeletal muscle biopsy specimen despite considerable muscle weakness

• A family history consistent with either autosomal recessive or autosomal dominant inheritance

**Testing**

**Laboratory testing.** Serum creatine kinase (CK) concentration may be normal or slightly elevated (usually not more than tenfold the normal).

**Electrophysiologic testing**

• Generally, individuals should be tested for a decremental EMG response of the CMAP on low-frequency (2- to 3-Hz) stimulation.

• In some cases, 2- to 3-Hz stimulation elicits no decremental response from rested non-weak muscle, but elicits a
significant decremental response after five to ten minutes of stimulation at 10 Hz.

- If the amplitude of the CMAP is normal in two distal and two proximal muscles, facial muscles should be tested.
- Alternatively or in addition, a single-fiber EMG is a good determinant of a neuromuscular transmission defect.
- A single nerve stimulus may elicit a repetitive CMAP (the so-called “double response to single nerve stimulus”) in individuals with endplate acetylcholinesterase deficiency or slow-channel CMS (SCCMS; caused by autosomal dominant gain-of-function mutations of the genes encoding the AChR subunits that prolong the time that the AChR channel is open) or in those taking high doses of AChE inhibitors.

**Response to acetylcholinesterase inhibitors** is assessed using intravenous injection of edrophonium (Tensilon®), a fast-acting acetylcholinesterase inhibitor. An initial dose of 2.0 mg is injected over 15 seconds, followed by additional doses of 3.0 mg and 5.0 mg at intervals of 60 seconds, if necessary. Maximum improvement occurs within 30 seconds of the injection and persists for minutes. An objective endpoint (e.g., improvement in ptosis, extraocular muscle weakness, tongue weakness, decremental EMG response) needs to be established prior to the injection and then carefully followed. In practice, a controlled/supervised trial of oral medication often replaces the edrophonium test.

**Morphologic studies.** Conventional skeletal muscle biopsy and routine histochemical studies in individuals with CMS generally show no major abnormalities except for type I fiber predominance and occasionally minor myopathic changes. Note: Tubular aggregates have been described in GFPT1-associated limb-girdle CMS [Senderek et al 2011].

More detailed studies can be performed on an intercostal or motor point skeletal muscle biopsy including estimation of acetylcholine receptors (AChRs) per endplate, light and electron microscopic analysis of endplate morphology, and *in vitro* electrophysiologic studies of endplate function [Engel & Franzini-Armstrong 1994]. Such studies – usually carried out within a research setting – allow a more precise classification by pointing to a defect in an endplate-associated gene or protein.

**Molecular Genetic Testing**

**Genes.** Mutations in one of several genes encoding different proteins expressed at the neuromuscular junction are currently known to be associated with CMS [Beeson et al 2005, Engel & Sine 2005, Beeson et al 2006, Müller et al 2007b, Palace et al 2007, Engel et al 2010]. These include AGRN, CHAT, the genes encoding different subunits of the acetylcholine receptor (CHRNA1, CHRNBI, CHRND, CHRNE), COLQ, DOK7, GFPT1, MUSK, RAPSN, and SCNA4 (see also Table 1 and Table A).

**Evidence for locus heterogeneity.** To date, no other loci are known to be associated with CMS; however, families with CMS not linked to any of the known candidate genes have been identified. Further genetic studies may reveal new loci or candidate genes underlying CMS [Engel & Sine 2005, Müller et al 2007b].

Table 1. Summary of Molecular Genetic Testing Used in Congenital Myasthenic Syndromes

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Proportion of CMS Attributed to Mutations in this Gene</th>
<th>Test Method</th>
<th>Mutations Detected</th>
<th>Test Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGRN</td>
<td>Rare ²</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>None reported ⁵</td>
<td></td>
</tr>
<tr>
<td>CHAT</td>
<td>4%-5%</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targeted mutation analysis</td>
<td>c.914T&gt;C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>None reported ⁵</td>
<td></td>
</tr>
<tr>
<td>CHRNA1</td>
<td>&lt;1%</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>None reported ⁵</td>
<td></td>
</tr>
<tr>
<td>CHRNBI</td>
<td>&lt;1%</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>Exonic or whole-gene deletions ⁶</td>
<td></td>
</tr>
<tr>
<td>CHRND</td>
<td>&lt;1%</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>None reported ⁷</td>
<td></td>
</tr>
<tr>
<td>CHRNE</td>
<td>50%</td>
<td>Sequence analysis</td>
<td>Sequence variants ³</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targeted mutation analysis</td>
<td>c.1327delG ⁸</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis ⁴</td>
<td>Exonic or whole-gene deletions ¹⁰</td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Frequency</td>
<td>Test Availability</td>
<td>Analysis Methods</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-------------------</td>
<td>------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>COLQ</td>
<td>10%-15%</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td>None reported</td>
</tr>
<tr>
<td>DOK7</td>
<td>10%-15%</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targeted mutation analysis</td>
<td>c.1124_1127dupTGCC</td>
<td>Exonic or whole-gene deletions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFPT1</td>
<td>2%</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUSK</td>
<td>Rare</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td>None reported</td>
</tr>
<tr>
<td>RAPSN</td>
<td>15%-20%</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Targeted mutation analysis</td>
<td>c.264C&gt;A</td>
<td>Exonic or whole-gene deletions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCN4A</td>
<td>Rare</td>
<td>Sequence analysis</td>
<td>Sequence variants</td>
<td>Clinical Testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deletion / duplication analysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test Availability refers to availability in the GeneTests™ Laboratory Directory. GeneReviews designates a molecular genetic test as clinically available only if the test is listed in the GeneTests™ Laboratory Directory by either a US CLIA-licensed laboratory or a non-US clinical laboratory. GeneTests does not verify laboratory-submitted information or warrant any aspect of a laboratory’s licensure or performance. Clinicians must communicate directly with the laboratories to verify information.

1. Estimated percentage based on individuals with CMS investigated at the authors’ laboratory and on published data [Beeson et al 2005, Engel & Sine 2005, Chaouch et al 2012b]

2. Mutations in AGRN have been reported in one family [Huze et al 2009].

3. Examples of mutations detected by sequence analysis may include small intragenic deletions/insertions and missense, nonsense, and splice site mutations.

4. Testing that identifies deletions/duplications not readily detectable by sequence analysis of the coding and flanking intronic regions of genomic DNA; included in the variety of methods that may be used are: quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), and chromosomal microarray (CMA) that includes this gene/chromosome segment. See CMA.

5. Utility of deletion/duplication analysis for this gene is unknown.

6. Quiram et al [1999]

7. Müller et al [2006]

8. The CHRNE founder mutation c.1327delG in exon 12 is present in up to 50% of individuals of European Roma and/or southeastern European origin with CMS [Abicht et al 1999, Karcagi et al 2001].

9. The CHRNE founder mutation c.1353dup is present in up to 20% of persons from the Maghreb (especially Algeria and Tunisia) [Beeson et al 2005].

10. Abicht et al [2002]

11. Most affected individuals have the European founder mutation c.1124_1127dupTGCC in DOK7 on at least one allele.

12. Selcen et al [2008]

13. Mutations in MUSK have been reported in eight individuals from three families [Chevessier et al 2004, Mihaylova et al 2009, Maselli et al 2010].

14. RAPSN mutations are dispersed throughout the translated region. Mutations have been identified in the E-box element of the promoter (See Molecular Genetics).

15. Most affected individuals of European origin, especially those with respiratory failure, have the c.264C>A mutation on at least one allele; about 50% are homozygous for c.264C>A. When only one c.264C>A mutation is identified in an individual, it is appropriate to sequence the entire gene including the promoter to detect a second heteroallelic mutation.

17. Mutations in SCN4A have been reported in one individual [Tsujino et al 2003].

**Interpretation of test results.** For issues to consider in interpretation of sequence analysis results, click here. Information on specific allelic variants may be available in Molecular Genetics (see Table A. Genes and Databases and/or Pathologic allelic variants).

**Testing Strategy**

**To confirm/establish the diagnosis in a proband.** Targeted mutation analysis may be carried out depending on the ethnic origin of the patient:

- **German or central / western European origin.** RAPSN mutation c.264C>A and DOK7 mutation c.1124_1127dupTGCC
- **Southeastern European or Roma origin.** CHRNA mutation c.1327delG
- **From the Maghreb** (especially Algeria and Tunisia). CHRNE mutation c.1353dup

In case of heterozygosity for one of the mutations mentioned above, the entire gene including the promoter should be sequenced to detect a second heteroallelic mutation.

Sequential molecular genetic testing should be based on the proportion of CMS attributed to a mutation in each of the genes included in Table 1.

Some clinical clues may help the clinician pinpoint the gene most likely to be involved:

- **Apneas.** Perform molecular genetic testing of RAPSN, CHAT, and COLQ.
- **No response to treatment with AChE inhibitors.** Consider testing for mutation in COLQ or RAPSN.
- **Limb-girdle phenotype.** Consider testing for mutation in DOK7, COLQ, and GFPT1.
- **Double response to a single nerve stimulus.** Consider:
  - A mutation in COLQ;
  - OR
  - A slow-channel CMS with a mutation in one of the genes encoding the AChR subunits (CHRHA, CHRNB1, CHRND, CHRNE).
- **Contractures.** Consider testing for mutation in RAPSN.
- **Autosomal dominant family history.** Consider slow-channel CMS caused by mutations of the genes encoding AChR subunits: CHRNA1, CHRN, CHRND, CHRNE. (All other CMS subtypes are inherited in an autosomal recessive manner.)

**Carrier testing for at-risk relatives** when the mode of inheritance is autosomal recessive requires prior identification of the disease-causing mutations in the family.

Note: Carriers are heterozygotes for an autosomal recessive disorder and are not at risk of developing the disorder.

**Prenatal diagnosis and preimplantation genetic diagnosis (PGD)** for at-risk pregnancies require prior identification of the disease-causing mutations in the family.

Note: It is the policy of GeneReviews to include in GeneReviews™ chapters any 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**

No other phenotypes are known to be associated with mutations in AGRN, CHAT, CHRNA1, CHRN, CHRND, CHRNE, COLQ, DOK7, GFPT1, MUSK, and RAPSN.

**SCN4A.** Gain-of-function mutations in this gene (encoding the sodium channel protein type 4 subunit alpha) have been identified in several disorders of muscle membrane excitability: potassium-aggravated myotonia, paramyotonia congenita, and hyperkalemic periodic paralysis [Jurkat-Rott et al 2010].

**Fetal akinesia deformation sequence (FADS), prenatal CMS.** Fetal hypomobility can result in the intrauterine phenotype of FADS, characterized by severe joint contractures, subcutaneous edema, growth retardation, lung hypoplasia, and other malformations. FADs have multiple etiologies and can be lethal or non-lethal. If multiple ptterygia are present the phenotype is described as Escobar syndrome. In recent years, biallelic null mutations in genes associated with CMS (RAPSN, DOK7, CHRNA1, CHRN, CHRND) as well as in the fetally expressed gamma subunit of AChR (CHRNG) have been causative of FADS [Ravenscroft et al 2011].

**Clinical Description**
Natural History

In the congenital myasthenic syndromes (CMS), the first myasthenic symptoms occur in general early in life, usually in the first two years. Rarely, onset is in the second to third decade of life [Milone et al 1999, Croxen et al 2002a, Burke et al 2003, Müller et al 2007a, Ben Ammar et al 2010, Guergueltcheva et al 2011].

Severity and course of disease are highly variable, ranging from minor symptoms (e.g., mild exercise intolerance) to progressive disabling weakness. Minor myasthenic symptoms may be exacerbated by sudden onset of severe weakness or respiratory insufficiency precipitated by fever, infections, or excitement especially in individuals with CMS with episodic apnea (CMS-EA) or endplate rapsyn deficiency [Ohno et al 2001, Byring et al 2002, Ohno et al 2002].

Some myasthenic symptoms are present at birth. Respiratory insufficiency with sudden apnea and cyanosis are common findings in neonates.

Neonates with CMS can have multiple joint contractures (often described as arthrogryposis multiplex congenita [AMC]) resulting from a lack of fetal movement in utero. AMC seems to be particularly common in infants with truncating RAPSN mutations [Brownlow et al 2001, Burke et al 2003, Beeson et al 2005]. (See also Genetically Related Disorders, Fetal akinesia deformation sequence).

Other major findings in the neonatal period may include feeding difficulties, poor suck and cry, choking spells, eyelid ptosis, and facial, bulbar, and generalized weakness. Stridor in infancy may be an important clue to CMS, particularly in those with DOK7 mutations [Kinali et al 2008].

Individuals with onset later in childhood show abnormal muscle fatigability, with difficulty in running or climbing stairs. Motor milestones may be delayed. Affected individuals present with fluctuating eyelid ptosis and fixed or fluctuating extraocular muscle weakness. Ptosis may involve one or both eyelids. In addition, facial and bulbar weakness with nasal speech and difficulties in coughing and swallowing may be present.

Spinal deformity or muscle atrophy may occur.

Some individuals display a characteristic ‘limb-girdle’ pattern of weakness with ptosis and a waddling gait, with or without ptosis and ophthalmoparesis (‘limb girdle myasthenia’ [LGM]).

In some individuals, a high-arched palate and distinctive facial features have been reported.

CMS is limited to weakness of the skeletal muscles. Cardiac and smooth muscle are not involved. Cognitive skills, coordination, sensation, and tendon reflexes are normal.

Major CMS subtypes are recognized based on molecular genetic studies in research laboratories (see Table 2).

<table>
<thead>
<tr>
<th>Gene in which Mutation is Causative</th>
<th>CMS Subtype</th>
<th>Clinical Findings</th>
<th>Response to AChE Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAT</td>
<td>CMS with episodic apnea (CMS-EA)</td>
<td>Hypotonia, respiratory failure at birth Episodic apnea Improvement with age</td>
<td>Improvement</td>
</tr>
<tr>
<td>AChR subunit genes: CHRN1, CHRN2, CHRD</td>
<td>Acetylcholine receptor (AChR) deficiency</td>
<td>Early onset Varies from mild to severe Ptosis, EOP; bulbar, arm, leg weakness</td>
<td>Improvement</td>
</tr>
<tr>
<td></td>
<td>Slow-channel CMS (SCCMS)</td>
<td>Selective severe neck, wrist, finger extensor weakness Onset from childhood to adult Varies from mild to severe Progressive ventilatory insufficiency; may require assisted ventilation</td>
<td>Often deterioration</td>
</tr>
<tr>
<td></td>
<td>Fast-channel CMS (FCCMS)</td>
<td>Varies from mild to severe</td>
<td>Improvement</td>
</tr>
<tr>
<td>COLQ</td>
<td>Endplate acetylcholinesterase (EP AChE) deficiency</td>
<td>Often severe In some with C-terminal missense mutations: later presentation, milder clinical course Ophthalmoparesis General muscle weakness / severe involvement of axial muscles Slow pupillary light response</td>
<td>Deterioration or no response</td>
</tr>
<tr>
<td>DOK7</td>
<td>DOK7-associated limb-girdle-myasthenia</td>
<td>Limb-girdle pattern of weakness with predominantly proximal weakness, waddling gait, and ptosis but no ophthalmoparesis</td>
<td>Deterioration or no response</td>
</tr>
</tbody>
</table>
**RAPSN**

<table>
<thead>
<tr>
<th>Endplate (EP) rapsyn deficiency</th>
<th>Varies from mild to severe Rapsyn-LO (late onset) Limb weakness in adolescence or adulthood resembling seronegative myastenia gravis Other 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement</td>
<td></td>
</tr>
</tbody>
</table>

**GFPT1**

<table>
<thead>
<tr>
<th>GFPT1-associated limb-girdle-myasthenia (CMS-TA)</th>
<th>“Limb-girdle” pattern of weakness with predominantly proximal weakness but usually no ptosis or ophthalmoparesis; sometimes tubular aggregates (TA) in muscle biopsy 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement</td>
<td></td>
</tr>
</tbody>
</table>

Includes only those genes in which more than a few individuals/families have been reported. Does not include AGRN, MUSK, and SCN4A, as only single individuals or a few families have been described with mutations in these genes. Does not include either PLEC (as mutations are primarily associated with epidermolysis bullosa simplex [Selcen et al 2011]) or LAMB2 (as mutations are primarily associated with kidney problems (nephrosis) [Maselli et al 2009]).

1. Because of the many private mutations and the limited number of genotype-phenotype correlations, the clinical spectrum may be broader or different from the findings listed.
2. See Testing, **Response to acetylcholinesterase inhibitors**
3. EOP = external ophthalmoplegia
5. Senderek et al [2011], Guergueltcheva et al [2011]

**Other subtypes of CMS** have been reported in the literature in a few kinships without identification of the underlying genetic defect [Walls et al 1993, Banwell et al 1999, Rodolico et al 2002, Beeson et al 2005, Milone et al 2006]. Some have been thoroughly characterized by morphologic and in vitro electrophysiologic studies pointing toward a presumed lesion, but others are not yet completely classified.

### Genotype-Phenotype Correlations

Mutations of the genes encoding the AChR subunits (CHRNA1, CHRNBI, CHRNND, CHRNHE) can be inherited in an autosomal dominant or autosomal recessive manner.

- **Gain-of-function mutations** of CHRNA1, CHRNBI, CHRNND, CHRNHE that alter the kinetic properties of the AChR result in the autosomal dominant slow channel CMS (SCCMS).
- **Loss-of-function mutations** of the AChR subunit genes (CHRNA1, CHRNBI, CHRNND, CHRNHE) are associated with autosomal recessive CMS [Hantai et al 2004, Ohno & Engel 2004a, Engel & Sine 2005].


Genotype-phenotype correlations are difficult to establish for rare CMS subtypes with identified mutations in only a few patients worldwide (AGRN, MUSK, SCN4A).

### Penetrance

In general, reported CMS mutations have complete penetrance.

One case of reduced penetrance has been reported for slow-channel CMS (SCCMS) resulting from mutations in CHRNHE [Croxen et al 2002a].

### Nomenclature

An outdated and misleading term is familial infantile myasthenia (FIM) [Deymeer et al 1999].

### Prevalence

The prevalence of CMS is estimated at one tenth that of myasthenia gravis (which has a prevalence of 25:1,000,000-125:1,000,000); however, it may be higher. At least 1000 independent kinships with identified mutations have been documented worldwide [Chaouch et al 2012b].

Founder mutations have been identified in several populations:

- The population of the southeastern European Roma may be at higher risk for CMS because of an increased carrier rate (>4%) for the mutation c.1327delG in CHRNHE, the gene encoding the eAChR subunit [Morar et al 2004].
Individuals from the Maghreb (especially Algeria and Tunisia) may be at higher risk for CMS because of another CHRNE founder mutation, c.1353dup [Beeson et al 2005].

Another recurrent mutation in the central European population is DOK7 c.1124_1127dup [Srour et al 2010].

RAPSN mutations are likely one of the most common causes of CMS in individuals of central or western European origin [Müller et al 2003, Richard et al 2003].

Differential Diagnosis

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

Myasthenia gravis. The clinical picture of congenital myasthenic syndromes (CMS) is similar to that of myasthenia gravis (MG), in which individuals have a history of fatigable weakness involving ocular, bulbar, and limb muscles; however, the myasthenic symptoms of CMS usually start at or shortly after birth rather than in adulthood, as is usual for MG. Because seronegative autoimmune MG has been reported on occasion in children younger than age two years, MG may be difficult to differentiate from CMS, especially in later childhood or adulthood. Furthermore, immunosuppressive therapy does not improve clinical symptoms in CMS, whereas it does in MG.

Transient neonatal myasthenia gravis. Autoimmune MG can be passed across the placenta from mother to fetus and so can affect offspring at birth.

Childhood. Other disorders partially resembling CMS to consider include:

- Spinal muscular atrophy
- Spinal muscular atrophy with respiratory distress 1 (SMARD1)
- Congenital muscular dystrophies
- Congenital myopathies including X-linked myotubular myopathy, nemaline myopathy, and multiminicore myopathy
- Infantile myotonic dystrophy type 1
- Mitochondrial myopathies
- Brain stem anomalies
- Mobius syndrome
- Infantile botulism

Adulthood. Other disorders partially resembling CMS to consider include:

- Motor neuron disease including spinal and bulbar muscular atrophy (SBMA, Kennedy disease)
- Limb girdle muscular dystrophy
- Facioscapulohumeral dystrophy
- Mitochondrial myopathy and chronic progressive external ophthalmoplegia (CPEO) (see Mitochondrial Diseases Overview)
- Autosomal dominant progressive external ophthalmoplegia (ADPEO caused by mutations in ANT1, TWINKLE, or POLG)
- Chronic fatigue syndrome

A few individuals with myasthenic findings and major findings as a part of other systemic disorders have been reported.

- Biallelic mutation of LAMB2 causes Pierson syndrome (OMIM 609049), an autosomal recessive disorder characterized by severe congenital nephrotic syndrome and extremely small nonreactive pupils (microcoria) resulting from aplasia or atrophy of the dilator pupillae muscle. One individual with biallelic LAMB2 mutations and Pierson syndrome with end stage renal disease (microcystic nephrosis) requiring kidney transplantation had the onset of myasthenic findings in early infancy [Maselli et al 2009].

- Biallelic mutation of PLEC1 causes epidermolysis bullosa simplex with muscular dystrophy of later onset (EBS-MD) (OMIM 226670). In three individuals from three families, biallelic PLEC mutations resulted in EBS-MD with variable skin findings and late-onset myasthenic symptoms associated with a neuromuscular transmission defect [Forrest et al 2010, Selcen et al 2011]. In addition, mutations in PLEC affecting the 1f isoform of plectin, which has a suggested specific role in skeletal muscle, cause limb-girdle muscular dystrophy type 2Q in Turkish families, characterized by early childhood onset of proximal muscle weakness and atrophy without skin involvement [Gundesli et al 2010].

Note to clinicians: For a patient-specific ‘simultaneous consult’ related to this disorder, go to SimulConsult®, an interactive diagnostic decision support software tool that provides differential diagnoses based on patient findings (registration or institutional access required).

- AChR deficiency
• FCCMS
• SCCMS
• CMS with episodic apnea
• Endplate rapsyn deficiency
• AChE deficiency

Management

Evaluations Following Initial Diagnosis

To establish the extent of disease in an individual diagnosed with congenital myasthenic syndromes (CMS), evaluation of respiratory function in children is recommended.

Treatment of Manifestations

AChE inhibitors (pyridostigmine). Although the majority of individuals with CMS benefit from acetylcholine esterase (AChE) inhibitors (pyridostigmine), some myasthenic symptoms may remain refractory to treatment even in individuals who are otherwise responsive. Certain CMS subtypes (see Table 2) such as endplate acetylcholinesterase deficiency (EP AChE deficiency), slow-channel CMS (SCCMS), or DOK7-related CMS are refractory to or deteriorate with AChE inhibitors [Engel 2007, Palace et al 2007, Kinali et al 2008, Schara & Lochmüller 2008].

3,4-diaminopyridine (3,4 DAP). Alternatively or in addition to AChE inhibitors, the potassium channel blocker 3,4-DAP may be used [Palace et al 1991, Anlar et al 1996, Banwell et al 2004, Beeson et al 2005, Engel 2007]. This drug increases the release of ACh and prolongs the presynaptic action potential. Of note, two children with fast-channel CMS (FCCMS; caused by autosomal recessive loss-of-function mutations of the genes encoding the AChR subunits that shorten the time that the AChR channel is open) died when started on 3,4-DAP [Beeson et al 2005]. Although a relation to 3,4-DAP has not been proven, clinicians must be cautious when using 3,4-DAP in young children and in individuals with FCCMS.

Ephedrine treatment shows positive effects in different subtypes of CMS, and may be an alternative treatment option for CMS subtypes that are refractory to AChE inhibitors, such as COLQ and DOK7-associated CMS [Bestue-Cardiel et al 2005, Schara et al 2009, Lashley et al 2010]. It is well tolerated by most patients and improvement in strength can be profound.

Albuterol, an alternative to ephedrine, may have a role in the treatment of COLQ and DOK7-associated CMS [Liewluck et al 2011].

Quinidine, fluoxetine. Some individuals with genetically defined slow-channel CMS (SCCMS; caused by autosomal dominant gain-of-function mutations of the genes encoding the AChR subunits that prolong the time that the AChR channel is open) have been successfully treated with quinidine, a long-lived open-channel blocker of AChR [Harper & Engel 1998]. Quinidine in turn may be detrimental in individuals with AChR deficiency.

Recently, the therapeutic benefit of fluoxetine in SCCMS has been shown [Harper et al 2003, Colomer et al 2006]; however, it may induce suicidal ideation; thus, caution is strongly suggested in its use in childhood [Engel 2007].

Overall fluoxetine appears to be the accepted first-line treatment in SCCMS, whereas quinidine is the treatment of choice in children and teenagers because of the risk of psychiatric side effects associated with fluoxetine [Chaouch et al 2012a, Chaouch et al 2012b].

Prevention of Primary Manifestations

Sudden respiratory insufficiency or apneic attacks provoked by fever or infections are common in individuals with underlying mutations in CHAT or RAPSN, even if the myasthenic symptoms are mild between crises. These individuals should receive prophylactic anticholinesterase therapy. Note: Less frequently, acute respiratory events may also occur in other CMS subtypes. Parents of infants are advised to use apnea monitors and be trained in CPR.

Prevention of Secondary Complications

Side effects of drugs used to treat myasthenic symptoms should be carefully monitored. If necessary, individual doses should be adjusted or treatment should be stopped. For example, quinidine has some major side effects including torsades de pointes (a potentially life-threatening arrhythmia), hypotension, cinchonism (or quininism), and hypersensitivity reactions. In individuals with CMS, adverse effects, such as exacerbation of weakness and development of respiratory failure, may occur.

Surveillance

Routine surveillance of muscle strength and respiratory function is recommended. In some patients, especially those with underlying COLQ and DOK7 mutations, slowly progressive respiratory impairment is seen with increasing age. Symptoms of nighttime hypoventilation should be considered.
Agents/Circumstances to Avoid

A number of drugs are known to affect neuromuscular transmission and therefore exacerbate symptoms of myasthenia gravis (e.g., ciprofloxacin, chloroquine, procaine, lithium, phenytoin, beta-blockers, procainamide, and quinidine). These drugs are not absolutely contraindicated and may be used with caution in CMS. A more complete list can be obtained online.

Evaluation of Relatives at Risk

If the disease-causing mutations in the family are known, molecular genetic testing can be used to clarify the genetic status of at-risk asymptomatic family members, especially newborns or young children, who could benefit from early treatment to prevent sudden respiratory failure.

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

Pregnancy Management

Limited data on pregnancy management in CMS are available [Terblanche et al 2008, Chaouch et al 2012b].

Therapies Under Investigation

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.

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

Apart from the autosomal dominant slow-channel CMS (SCCMS), all other CMS are inherited in an autosomal recessive manner.

Mutations in AGRN, CHAT, COLQ, DOK7, GFPT1, MUSK, RAPSN, and SCN4A are always associated with autosomal recessive CMS.

Mutations of the genes encoding the AChR subunits (CHRNA1, CHRNBI, CHRND, and CHRNE) can be inherited in an autosomal dominant or autosomal recessive manner.

- Gain-of-function mutations of CHRNA1, CHRNBI, CHRND, and CHRNE that alter the kinetic properties of the AChR result in autosomal dominant slow-channel CMS (SCCMS).
- Loss-of-function mutations of the AChR subunit genes (CHRNA1, CHRNBI, CHRND, and CHRNE) are associated with autosomal recessive CMS [Hantai et al 2004, Ohno & Engel 2004a, Engel & Sine 2005].

Risk to Family Members — Autosomal Recessive Inheritance

Parents of a proband

- The parents of a child with an autosomal recessive congenital myasthenic syndrome (AR-CMS) are obligate heterozygotes and therefore carry one mutant allele.
- Heterozygotes (carriers) are clinically 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 chance of his/her being a carrier is 2/3.
- Heterozygotes (carriers) are clinically asymptomatic.

Offspring of a proband

- The offspring of an individual with an AR-CMS are obligate heterozygotes (carriers) for a disease-causing mutation.
The risk that the offspring will inherit a second disease-causing CMS-causing allele depends on the carrier status of the proband's reproductive partner.

The carrier frequency of CMS-related mutations in the general population is low.

In populations with a high carrier rate and/or a high rate of consanguineous marriages, the risk to the offspring of an affected individual of being affected is increased.

**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 at-risk family members is possible once the mutations have been identified in the proband.

**Risk to Family Members — Autosomal Dominant Inheritance**

**Parents of a proband**

- Some individuals diagnosed with an autosomal dominant congenital myasthenic syndrome (AD-CMS) have an affected parent.
- A proband with an AD-CMS may have the disorder as the result of a *de novo* gene mutation. The proportion of cases caused by *de novo* mutations is unknown.

**Sibs of a proband**

- The risk to the sibs of the proband depends on the status of the parents.
- If a parent is affected and/or has a disease-causing mutation, the risk is 50%.
- When the parents are clinically unaffected and neither has the disease-causing mutation found in the proband, the risk to the sibs of a proband is low but still higher than in the general population because of the possibility of germline mosaicism in a parent.

**Offspring of a proband.** Each child of an individual with an AD-CMS 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 and/or has a CMS-causing mutation, his or her family members are at risk.

**Related Genetic Counseling Issues**

**Family planning**

- The optimal time for determination of genetic risk, clarification of carrier status, 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, or are carriers or at risk of being carriers.

**Considerations in families with an apparent *de novo* mutation.** When neither parent of a proband with an autosomal dominant condition has 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.

**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**

Prenatal diagnosis for pregnancies at increased risk for CMS caused by most mutations in most known genes 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 before prenatal testing can be performed.

If no laboratories offering molecular genetic testing for prenatal diagnosis of CMS caused by mutation in a specific gene are listed in the GeneTests Laboratory Directory, such testing may be available to families with identified disease-causing mutations through laboratories offering custom prenatal testing. See [Testing](#).

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 has been identified. For laboratories offering PGD, see [Testing](#).

Note: It is the policy of *GeneReviews* to include in *GeneReviews™* chapters any clinical uses of testing available from laboratories listed in the GeneTests™ Laboratory Directory; inclusion does not necessarily reflect the endorsement of such
Resources

GeneReviews staff has selected the following disease-specific and/or umbrella support organizations and/or registries for the benefit of individuals with this disorder and their families. GeneReviews is not responsible for the information provided by other organizations. For information on selection criteria, click here.

- **Myasthenia Gravis Foundation of America, Inc. (MGFA)**
  355 Lexington Avenue
  15th Floor
  New York NY 10017
  **Phone:** 800-541-5454 (toll-free); 212-297-2156
  **Fax:** 212-370-9047
  **Email:** mgfa@myasthenia.org
  www.myasthenia.org

- **National Institute of Neurological Disorders and Stroke (NINDS)**
  PO Box 5801
  Bethesda MD 20824
  **Phone:** 800-352-9424 (toll-free); 301-496-5751; 301-468-5981 (TTY)
  Congenital Myasthenia Information Page

- **Muscular Dystrophy Association - USA (MDA)**
  3300 East Sunrise Drive
  Tucson AZ 85718
  **Phone:** 800-572-1717
  **Email:** mda@mdausa.org
  www.mda.org

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. Congenital Myasthenic Syndromes: Genes and Databases

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Chromosomal Locus</th>
<th>Protein Name</th>
<th>Locus Specific</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN4A</td>
<td>17q23.3</td>
<td>Sodium channel protein type 4 subunit alpha</td>
<td>Sodium channel, voltage-gated, type IV, alpha subunit (SCN4A) @ LOVD</td>
<td>SCN4A</td>
</tr>
<tr>
<td>CHRNA1</td>
<td>2q31.1</td>
<td>Acetylcholine receptor subunit alpha</td>
<td>CHRNA1 homepage - Leiden Muscular Dystrophy pages</td>
<td>CHRNA1</td>
</tr>
<tr>
<td>CHRNBI</td>
<td>17p13.1</td>
<td>Acetylcholine receptor subunit beta</td>
<td>CHRNBI homepage - Leiden Muscular Dystrophy pages</td>
<td>CHRNBI</td>
</tr>
<tr>
<td>CHRNND</td>
<td>2q37.1</td>
<td>Acetylcholine receptor subunit delta</td>
<td>CHRNND homepage - Leiden Muscular Dystrophy pages</td>
<td>CHRNND</td>
</tr>
<tr>
<td>CHRNAE</td>
<td>17p13.2</td>
<td>Acetylcholine receptor subunit epsilon</td>
<td>CHRNAE homepage - Leiden Muscular Dystrophy pages</td>
<td>CHRNAE</td>
</tr>
<tr>
<td>RAPSN</td>
<td>11p11.2</td>
<td>43 kDa receptor-associated protein of the synapse</td>
<td>RAPSN homepage - Leiden Muscular Dystrophy pages</td>
<td>RAPSN</td>
</tr>
<tr>
<td>COLQ</td>
<td>3p25.1</td>
<td>Acetylcholinesterase collagentic tail peptide</td>
<td>ESTHER www server: ESTerases and alpha/beta Hydrolase Enzymes and Relatives COLQ homepage - Leiden Muscular Dystrophy pages</td>
<td>COLQ</td>
</tr>
<tr>
<td>CHAT</td>
<td>10q11.23</td>
<td>Choline O-acetyltransferase</td>
<td>CHAT @ LOVD</td>
<td>CHAT</td>
</tr>
<tr>
<td>MUSK</td>
<td>9q31.3</td>
<td>Muscle, skeletal receptor tyrosine protein kinase</td>
<td>MUSK homepage - Leiden Muscular Dystrophy pages</td>
<td>MUSK</td>
</tr>
<tr>
<td>DOK7</td>
<td>4p16.3</td>
<td>Protein Dok-7</td>
<td>DOK7 homepage - Leiden Muscular Dystrophy pages</td>
<td>DOK7</td>
</tr>
</tbody>
</table>
Molecular Genetic Pathogenesis

The understanding of the molecular basis of the different subtypes of CMS has been evolving since 1995. After the identification of mutations in the subunits of the nicotinic acetylcholine receptor (AChR), other genes encoding postsynaptic, presynaptic, or synaptic proteins were identified as candidate genes for CMS [Engel et al 2003, Hantai et al 2004, Ohno & Engel 2004a, Beeson et al 2005, Engel & Sine 2005, Müller et al 2007b]. Currently, several proteins expressed at the neuromuscular junction and involved in neuromuscular transmission have been found to be involved in CMS. CMS subtypes have been classified according to the site of the underlying defect into presynaptic, synaptic, and postsynaptic CMS. This classification is still tentative because it is likely that additional subtypes of CMS will be discovered.

The neuromuscular junction (NMJ). Neuromuscular transmission depends on the calcium-dependent release of acetylcholine (ACh) from the presynaptic nerve terminal and its interaction with AChRs on the postsynaptic membrane. ACh is first synthesized in the motor nerve terminal by the action of the enzyme choline acetyltransferase (ChAT), and is transported into the synaptic vesicles via a specific uptake mechanism. Following depolarization of the motor nerve terminal by the axonal action potential, calcium influx via voltage-gated calcium channels triggers events that lead to vesicle fusion and release of acetylcholine. Binding of ACh to AChR leads to the opening of the AChR ion channel resulting in depolarization of the postsynaptic membrane. If this depolarization exceeds that required to open the voltage-gated sodium channels on the postsynaptic side, an action potential is generated and propagated throughout the muscle fiber, leading to contraction of the muscle. ACh is hydrolyzed by the enzyme acetylcholinesterase (AChE), which is localized at the basal lamina of the NMJ, and the membrane potential of the presynaptic membrane is restored when voltage-gated potassium channels open.

Mutations in acetylcholine receptor subunit genes. The majority of postsynaptic CMS subtypes identified to date are caused by mutations in AChR subunit genes that either increase or decrease the response to acetylcholine. After binding acetylcholine, the AChR responds by an extensive change in conformation that affects all subunits and leads to the opening of an ion-conducting channel. The adult muscle AChR is composed of five homologous subunits: two α subunits, and one each of β, δ, and ε. Each subunit has a large N-terminal extracellular domain, four transmembrane segments (M1–M4) with the M2 domain lining the cation-selective pore. Each AChR has two acetylcholine binding pockets, one at the α/ε interface and one at the α/δ interface.

CHAT

Normal allelic variants. A presynaptic subtype of CMS has been linked to mutations in CHAT, the gene encoding choline O-acetyltransferase (ChAT) [Ohno et al 2001]. CHAT comprises 18 exons.

Pathologic allelic variants. Loss-of-function mutations (missense, frameshift, and stop mutations) causing autosomal recessive CMS have been identified in more than 40 kinships with CMS clinically characterized as CMS-EA [Chaouch et al 2012a].
Table 3. Selected CHAT Pathologic Allelic Variants

<table>
<thead>
<tr>
<th>DNA Nucleotide Change</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.914T&gt;C</td>
<td>p.Ile305Thr</td>
<td>NM_020549.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP_065574.3</td>
</tr>
</tbody>
</table>


**Normal gene product.** CHAT catalyzes the reversible synthesis of acetylcholine from acetyl-coenzyme A and choline.

**Abnormal gene product.** Biochemical studies have established reduced catalytic efficiency and/or reduced expression of mutated gene products.

**COLQ**

**Normal allelic variants.** A synaptic subtype of CMS has been linked to mutations in COLQ, the gene encoding the collagenic tail ColQ. COLQ comprises 17 exons (and two alternate exons: 1A and 11A).

**Pathologic allelic variants.** All individuals with endplate (EP) AChE deficiency described to date have pathogenic mutations in COLQ. To date, mutations (missense, frameshift, stop, and splice site mutations) causing autosomal recessive CMS have been identified in more than 100 CMS kinships [Chaouch et al 2012a].

**Normal gene product.** The endplate species of AChE is a heteromeric asymmetric enzyme composed of one, two, or three homotetramers of globular catalytic subunits (AChE7) attached to a triple-stranded collagenic tail (ColQ) anchoring the enzyme to the synaptic basal lamina. ColQ has an N-terminal proline-rich region attachment domain (PRAD), a collagenic central domain, and a C-terminal region enriched in charged residues and cysteines. Each ColQ strand can bind an AChE7 tetramer to its PRAD, giving rise to A4, A8, and A12 species of asymmetric AChE. Two groups of charged residues in the collagen domain (heparan sulfate proteoglycan binding domains [HSPBD]) plus other residues in the C-terminal region assure that the asymmetric enzyme is inserted into the synaptic basal lamina. The C-terminal region is also required for initiating the triple helical assembly of ColQ that proceeds from a C- to an N-terminal direction in a zipper-like manner.

**Abnormal gene product.** The COLQ-associated synaptic subtype of CMS is caused by the absence of acetylcholinesterase (AChE) from the synaptic cleft as a consequence of mutations in the triple-stranded collagenic tail (ColQ) anchoring the enzyme to the synaptic basal lamina [Donger et al 1998, Ohno et al 1998, Ohno et al 2000].

Biochemical studies have established three major functional consequences resulting from mutations:

- Mutations in the PRAD-domain prevent attachment of AChE7 to ColQ;
- Mutations in the collagen-domain produce a short, single-stranded ColQ that binds a single AChE7 tetramer and is insertion incompetent;
- Mutations in the C-terminus hinder the triple helical assembly of the collagen domain, or produce an asymmetric species of AChE that is insertion incompetent, or both.

Neuromuscular transmission in absence of AChE is compromised by a desensitization and depolarization block of AChR at physiologic rates of stimulation, an endplate myopathy caused by cholinergic overactivity with a likely compensatory smallness of the nerve terminals and their encasement by Schwann cells. Endplate potentials and currents are prolonged in the absence of AChE, eliciting repetitive compound muscle action potentials.

**AGRN**

**Normal allelic variants.** The complete cDNA of AGRN comprises 36 exons. Agrin mRNA undergoes cell-specific alternative splicing at several sites. An amino acid insert of the isoform secreted by motor neurons is required for MuSK activation and for formation of the neuromuscular junction.

**Pathologic allelic variants.** A single homozygous mutation (c.5125G>C) was identified in two affected siblings from a consanguineous family by Huze et al 2009.

Table 4. Selected AGRN Pathologic Allelic Variants

<table>
<thead>
<tr>
<th>DNA Nucleotide Change</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.5125G&gt;C</td>
<td>p.Gly1709Arg</td>
<td>NM_198576.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP_940978.2</td>
</tr>
</tbody>
</table>


1. Variant designation that does not conform to current naming conventions

**Normal gene product.** The protein (NP_940978.2) has 2045 amino acids.
Agrin is a heparan sulfate proteoglycan that has been shown to bind to laminins via its amino-terminal domain 14 and to interact via its carboxy-terminal part with LRP4 and a-dystroglycan. Agrin is a neuronal aggregating factor that induces the aggregation of acetylcholine receptors and other postsynaptic proteins on muscle fibers and is crucial for the formation of the neuromuscular junction. Agrin activates the MuSK by binding and activating the postsynaptic LRP4-MuSK-DOK7 complex to recruit downstream signaling components which triggers the local aggregation and synthesis of postsynaptic acetylcholine receptors (AChRs) and other postsynaptic proteins, such as the cytoskeletal protein rapsyn.

Abnormal gene product. The homozygous mutation c.5125G>C causes a p.Gly1709Arg substitution. The resulting mutant agrin is expressed and localized correctly in patient muscle, but the overall organization of the neuromuscular junction is disturbed, affecting both the pre- and postsynaptic regions. Experimental findings in rat muscle injected with mutant agrin indicate that the mutation does not interfere with the ability of agrin to induce postsynaptic structures but that it dramatically disturbs the maintenance of the neuromuscular junction.

AChR Subunit Genes: CHRNA1, CHRNB1, CHRND, CHRNE

CMS with kinetic abnormalities of the acetylcholine receptor (slow-channel syndromes and fast-channel syndromes) is caused by mutations in the acetylcholine receptor subunit genes CHRNA1, CHRNB1, CHRND, and CHRNE. Two major kinetic abnormalities of AChR, resulting in slow-channel syndromes and fast-channel syndromes, have emerged. The two kinetic syndromes are physiologic and morphologic opposites and call for different therapeutic modalities.

Normal allelic variants. The CHRNA1 reference sequence NM_000079.3 comprises ten exons. The CHRNB1 reference sequence NM_000747.2 comprises 11 exons. The CHRND reference sequence NM_000751.1 comprises 12 exons. The CHRNE reference sequence NM_000080.3 comprises 12 exons.

Pathologic allelic variants

- **Slow-channel syndrome.** Slow-channel syndromes are caused by dominant gain-of-function mutations. To date, several autosomal dominant missense mutations have been described [Ohno & Engel 2004a, Engel & Sine 2005, Navedo et al 2006, Shen et al 2006]. Mutations have been identified in different AChR subunits and in different functional domains of the subunits. Several mutations are located in the transmembrane domains (M2 domains of α, β, δ, and ε subunits, and in the M1 domain of the α, β, and ε subunit) or in the extracellular domain of the α and ε subunit (see OMIM 601462).

- **Fast-channel syndrome.** The fast-channel CMSs are caused by recessive loss-of-function mutations. A number of fast-channel mutations have been identified [Ohno & Engel 2004a, Engel & Sine 2005, Palace et al 2012]. The mutations are located in different functional domains of the AChR α, β, and δ subunit. Usually, the mutated allele causing the kinetic abnormality is accompanied by a null mutation in the second allele. In all cases, the kinetic mutation dominates the clinical phenotype.

Normal gene product. The five homologous subunits of the adult AChR (two α subunits, and one each of β, δ, and ε) each have a large N terminal extracellular domain and four transmembrane segments (M1-M4); the M2 domain lines the cation-selective pore.

The proteins encoded by these genes:

- **CHRNA1** (NP_000070.1) comprises 457 amino acids.
- **CHRNB1** (NP_000738.2) comprises 501 amino acids.
- **CHRND** (NP_000742.1) comprises 517 amino acids.
- **CHRNE** (NP_000071.1) comprises 493 amino acids.

Abnormal gene product

- **Slow-channel syndrome.** Patch-clamp studies of mutant AChR channels reveal prolonged activation episodes of the AChR in the presence of ACh. This results in prolonged endplate currents and potentials, exceeding the refractory period of the muscle fiber action potential. Therefore, a single nerve stimulus elicits one or more repetitive CMAPs as described in Harper & Engel [1998]. During physiologic activity, the prolonged endplate potentials may undergo staircase summation, producing a depolarization block. Moreover, these factors cause cationic overloading of the junctional sarcoplasm resulting in myopathic changes with loss of AChR from degenerating junctional folds and altered endplate geometry with widening of the synaptic space and subsynaptic alterations.

- **Fast-channel syndrome.** In this subtype of CMS with kinetic abnormalities of the AChR, the channel-opening events are abnormally brief and there are usually fewer activation episodes. Fast-channel mutations affect one or more of the following functions of AChR: affinity for ACh, efficiency of gating, and stabilization of channel kinetics. Endplate studies reveal normal or reduced AChR numbers. The structural integrity of the postsynaptic membrane is preserved. The common electrophysiologic features are rapidly decaying endplate currents, abnormally brief channel activation periods, and a reduced quantal response owing to the reduced probability of channel opening.

Acetylcholine receptor deficiency with or without minor kinetic abnormality: caused by mutations in the acetylcholine receptor subunit genes CHRNA1; CHRNB1; CHRND; CHRNE

Pathologic allelic variants. The AChR subunits in individuals with CMS have numerous homozygous or, more frequently, heteroallelic recessive mutations that result in a reduced number of functional AChRs at the postsynaptic membrane. These low-expressor or null mutations have been reported in all subunits of the adult AChR. However, they are
concentrated in the ε subunit and especially in its long cytoplasmic M3/M4 linker.

To date, more than 50 ε subunit mutations have been reported [Ohno & Engel 2004a, Engel & Sine 2005].

- Most such mutations are nonsense, splice site, or frameshift mutations resulting in a premature termination of the translational chain.
- Missense mutations alter residues essential for assembly (e.g., glycosylation sites, the cystine loop) or in the signal peptide, also resulting in reduced gene expression. Some missense mutations affecting AChR gene expression also have accompanying kinetic effects.
- Point mutations of a regulatory element (N-box) in the AChRe promoter region have been shown to result in reduced gene expression [Nichols et al 1999, Ohno et al 1999, Abicht et al 2002].
- A chromosomal microdeletion of 1290 bp encompassing parts of CHRNE has been shown to result in CMS [Abicht et al 2002].

One particular point mutation of the AChRe subunit (c.1327delG) resulting in endplate AChR deficiency has been shown to be common (~50%) in affected individuals of Romany and/or southeastern European ethnic origin [Abicht et al 1999, Karcagi et al 2001, Morar et al 2004].

Another mutation of the AChRe subunit (c.1353dup) may be frequent in the Maghreb (especially Algeria and Tunisia) because of an ancient founder effect [Besson et al 2005].

**Normal gene product.** The five homologous subunits of the adult AChR (two α-subunits, and one each of β, δ, and ε) each have a large N-terminal extracellular domain and four transmembrane segments (M1-M4); the M2 domain lines the cation-selective pore.

**Abnormal gene product.** Morphologic studies of endplates show an increased number of endplate regions distributed over an increased span of the muscle fiber. The integrity of the junctional folds is preserved, but AChR expression on the folds is patchy and faint. The fetal type γ subunit may partially compensate for absence of the ε subunit, thereby producing a less severe phenotype.

### Table 5. Selected Pathologic Allelic Variants of CHRNE

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>DNA Nucleotide Change (Alias ¹)</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHRNE</td>
<td>c.1327delG (ε1267delG)</td>
<td>p.Glu443LysfsX64</td>
<td>NM_000080.3</td>
</tr>
<tr>
<td></td>
<td>c.1353dup (ε1293insG)</td>
<td>p.Asn452GlufsX4</td>
<td>NP_000071.1</td>
</tr>
<tr>
<td></td>
<td>(del1290)²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


1. Variant designation that does not conform to current naming conventions
2. 1290-bp deletion from the promoter to exon 2 [Abicht et al 2002]

### DOK7

**Normal allelic variants.** DOK7, encoding the postsynaptic protein Dok-7, comprises seven exons.

**Pathologic allelic variants.** DOK7 mutations recently were identified in CMS patients with congenital myasthenic syndromes affecting primarily proximal limb muscles ('limb girdle myasthenia' [LGM]) [Besson et al 2006, Müller et al 2007a, Palace et al 2007]. Since then, a large number of autosomal recessive mutations have been reported in more than 150 kinships [Chaouch et al 2012a]. Most affected individuals have the C-terminal frameshift mutation c.1124_1127dupTGCC on at least one allele.

### Table 6. Selected DOK7 Pathologic Allelic Variants

<table>
<thead>
<tr>
<th>DNA Nucleotide Change</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1124_1127dupTGCC</td>
<td>p.Ala378SerfsX30</td>
<td>NM_001173660.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NP_0075931.3</td>
</tr>
</tbody>
</table>


**Normal gene product.** Dok ("downstream of kinase") -7 is a 43-kd MuSK-interacting postsynaptic protein [Okada et al 2006] that is essential for synaptogenesis. Previously known Dok-family proteins (Dok-1 to Dok-6) play a role in signaling downstream of receptor and non-receptor phosphotyrosine kinases. Like other Dok proteins, Dok-7 has a pleckstrin-
homology (PH) domain and a phosphotyrosine-binding (PTB) domain in the N-terminal moiety and multiple tyrosine residues in the C-terminal region.

Okada et al [2006] demonstrated that Dok-7 overexpression can induce the aneural activation of MuSK and subsequent clustering of AChR in cultured myotubes. Unique among adaptor proteins recruited to receptor tyrosine kinases, Dok7 is not only a substrate of MuSK, but also an activator of MuSK's kinase activity.

The crystal structure of the Dok7 PHPTB domains in complex with a phosphopeptide representing the Dok7-binding site on MuSK revealed a dimeric arrangement of Dok7 PH-PTB that facilitates transautophosphorylation of the kinase activation loop [Bergamin et al 2010].

Abnormal gene product. Analysis of AChR clusters induced by Dok-7 harboring the mutation c.1124_1127dupTGCC showed a significant reduction in the number of branched-type AChR plaques compared to wild-type Dok-7 in fully differentiated transfected C2C12 myotubes [Beeson et al 2006]. By contrast, the ability of the c.1124_1127dupTGCC mutant to bind and induce MuSK phosphorylation was not impaired in non-differentiated myoblasts and in heterologous cells, suggesting that the C-terminal domain could play a key role in the maturation of the synaptic structure. Other mutations of the C-terminal domain of Dok-7 may have similar effects in myotubes.

**RAPSN**

**Normal allelic variants.** RAPSN, encoding the postsynaptic protein rapsyn (43-kd receptor-associated protein of the synapse), comprises eight exons.

**Pathologic allelic variants.** Nearly 200 mutations in RAPSN have been identified to date in the coding region (missense, frameshift, stop, and splice site mutations) and the promoter region [Ohno & Engel 2004a, Engel & Sine 2005, Müller et al 2007b, Milone et al 2009, Chaouch et al 2012a].

- The missense mutation c.264C>A (p.Asn88Lys) has been identified in many individuals who have mutations in the coding region in at least one allele. There is evidence for an ancient Indo-European founder [Richard et al 2003, Müller et al 2004a]; however, not all affected individuals with c.264C>A have the same haplotype [Richard et al 2003, Ohno & Engel 2004b].
- Other mutations in the translated region are dispersed over different domains of the protein.
- One affected individual was found to be a compound heterozygote with the c.264C>A mutation and a large (~4.5-kb) deletion disrupting the other allele [Müller et al 2004b].
- Heteroallelic multiexonic deletions may be common and present in up to 15% of cases [Gaudon et al 2010].
- Mutations in E-box regions of the promoter have been identified (Table 7).

<table>
<thead>
<tr>
<th>DNA Nucleotide Change (Alias)</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.-209A&gt;G (-38A&gt;G)</td>
<td>NA</td>
<td>NM_005055.4</td>
</tr>
<tr>
<td>c.-198C&gt;G (-27C&gt;G)</td>
<td>NA</td>
<td>NP_005046.2</td>
</tr>
<tr>
<td>c.264C&gt;A</td>
<td>p.Asn88Lys</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Selected RAPSN Pathologic Allelic Variants


NA = not applicable

1. Variant designation that does not conform to current naming conventions
2. Promoter mutations. Number of nucleotides 5' of the ATG translation initiation codon
3. An E-box mutation, c.-209A>G observed in the homozygous state, arose from a common founder in Near-Eastern Jews with marked jaw and other facial malformations [Ohno et al 2003].
4. Alias designations are number of nucleotides 5' of the transcription start site.
5. One E-box mutation, c.-198C>G, was compound heterozygous with the p.Asn88Lys mutation [Ohno et al 2003].

**Normal gene product.** Rapsyn, a 43-kd postsynaptic protein, plays an essential role in the clustering of AChR at the endplate. It self-associates, aggregates AChRs, and links them to the subsynaptic cytoskeleton.

**Abnormal gene product.** EP studies in each case have shown decreased staining for rapsyn and AChR, as well as impaired postsynaptic development. Expression studies of mutant constructs indicate that many mutations diminished co-clustering of AChR with rapsyn. However, different pathogenic mechanisms for some missense mutations that inhibited self-association of rapsyn or reduced the stability of the rapsyn protein have been uncovered [Cossins et al 2006].
**MUSK**

**Normal allelic variants.** MUSK comprises 14 exons.

**Pathologic allelic variants.** Chevessier et al [2004] identified two heteroallelic mutations (frameshift and missense) in an individual with CMS. Muscle biopsy showed dramatic pre- and postsynaptic structural abnormalities of the neuromuscular junction and severe decrease in acetylcholine receptor (AChR) epsilon-subunit and MuSK expression [Chevessier et al 2004]. To date, MUSK mutations have been reported in eight individuals with CMS from three families [Chevessier et al 2004, Mihaylova et al 2009, Maselli et al 2010].

**Normal gene product.** MUSK encodes the postsynaptic muscle-specific receptor tyrosine kinase (MuSK; muscle, skeletal receptor tyrosine-protein kinase). MuSK plays an essential role in the agrin-MuSK-rapsyn pathway in organizing the postsynaptic scaffold and in inducing the high concentration of AChR and tyrosine kinases of the ErB family in the postsynaptic membrane.

**Abnormal gene product.** Expression studies of mutant constructs have indicated that the frameshift mutation prevents MuSK expression; that the missense mutation diminishes the expression and stability of MuSK but not its kinase activity; and that overexpression of the missense mutant in mouse muscle results in decreased EP AChR and aberrant axonal outgrowth [Chevessier et al 2004].

**GFPT1**

**Normal allelic variants.** GFPT1 comprises 19 exons, plus one additional alternative exon 8A, which is incorporated only into a longer isoform GFPT1L, resulting in the insertion of 18 additional amino acids. GFPT1L is mainly expressed in skeletal muscle and heart and is the predominant GFPT1 species in these tissues [Niimi et al 2001].

**Pathologic allelic variants.** GFPT1 mutations have been identified in 24 individuals from 14 families with a subtype of CMS named congenital myasthenic syndrome with tubular aggregates (CMS-TA) [Senderek et al 2011, Guergueltcheva et al 2011]. Nineteen different GFPT1 mutations consisting of 13 missense mutations, four frameshift mutations, one nonsense mutation, and one variant in the 3'-UTR were identified. No individual with CMS with two null mutations in the constitutive exons resulting in a complete loss of GFPT1 expression has been identified.

**Normal gene product.** GFPT1 encodes an 80-kd enzyme that is part of the hexosamine biosynthesis pathway. GFPT1 catalyzes the transfer of an amino group from glutamine onto fructose-6-phosphate, yielding glucosamine-6-phosphate (GlcN-6-P) and glutamate. This transamidase reaction has been identified as the first and rate-limiting step of the hexosamine biosynthesis pathway. Products of this biochemical pathway are the sugar building blocks for the glycosylation of proteins and lipids in all cells.

**Abnormal gene product.** Mutations in GFPT1 lead to reduced protein levels in muscle; some missense mutations also slightly reduce the enzymatic activity [Senderek et al 2011]. The precise mechanism by which GFPT1 deficiency induces a dysfunction of the neuromuscular junction is not yet understood.

**SCN4A**

**Normal allelic variants.** SCN4A (NM_000334.4) comprises 24 exons.

**Pathologic allelic variants.** Only one individual with CMS and two heteroallelic SCN4A mutations (c.4325T>A [p.Val1442Glu] and c.737C>T [p.Ser246Leu]) has been identified to date [Tsujino et al 2003]. Several other gain-of-function mutations of SCN4A have been identified in a variety of disorders of muscle membrane excitability: potassium-aggravated myotonia, paramyotonia congenita, and hyperkalemic periodic paralysis (for review, see Cannon [2000]).

Table 8. SCN4A Pathologic Allelic Variants Discussed in This GeneReview

<table>
<thead>
<tr>
<th>DNA Nucleotide Change</th>
<th>Protein Amino Acid Change</th>
<th>Reference Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.737C&gt;T</td>
<td>p.Ser246Leu</td>
<td>NM_000334.4</td>
</tr>
<tr>
<td>c.4325T&gt;A</td>
<td>p.Val1442Glu</td>
<td>NP_000325.4</td>
</tr>
</tbody>
</table>

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

**Normal gene product.** The reference sequence NP_000325.4 has 1836 amino acids. SCN4A encodes the sodium channel protein type 4 subunit alpha (Na+, 1.4), which mediates the voltage-dependent sodium ion permeability of the postsynaptic membrane to generate and propagate an action potential.

**Abnormal gene product.** Expression studies on the observed mutations in HEK cells revealed that the Na channel with the p.Val1442Glu substitution showed marked enhancement of fast inactivation close to the resting potential and enhanced use-dependent inactivation on high frequency stimulation; that with the p.Ser246Leu substitution showed only minor kinetic abnormalities, suggesting that it is a benign variant. Na+, 1.4 expression at the endplates and over the sarcolemma was normal by immunocytochemical criteria [Tsujino et al 2003].

**References**

Medical Genetic Searches: A specialized PubMed search designed for clinicians that is located on the PubMed Clinical
Literature Cited


63. Ohno K, Engel AG. Lack of founder haplotype for the rapsyn mutation; N88K is an ancient founder mutation or arises from multiple founders. J Med Genet. 2004;41:108. [PMC free article: PMC1757267] [PubMed: 14729848]


Congenital Myasthenic Syndromes - GeneReviews™ - NCBI Bookshelf

4/29/13


87
80
79
78
77
76
75
74
73
72
71
70
69
68
67
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
51
50
49
48
47
46
45
44
43
42
41
40
39
38
37
36
35
34
33
32
31
30

Suggested Reading


Chapter Notes

Revision History

- 28 June 2012 (cd) Revision: targeted mutation analysis of CHRNA1 no longer listed in the GeneTests™ Laboratory Directory as being available clinically
- 22 March 2012 (me) Comprehensive update posted live
- 26 September 2006 (aa) Revision: clinical testing available for: mutation scanning of RAPSN, CHAT, COLQ, CHRNA1, CHRNA1, and CHRNA1; targeted mutation analysis of RAPSN mutation p.N88K, CHAT mutation p.1305T, and CHRNA1 mutation p.G1538; prenatal diagnosis for CHAT, CHRNA1, CHRNA1, CHRNA1, CHRNA1, CHRNA1, COLQ, and RAPSN
- 20 September 2005 (aa) Revision: sequence analysis for RAPSN clinically available
- 8 August 2005 (me) Comprehensive update posted to live Web site
- 9 May 2003 (me) Review posted to live Web site
- 30 January 2003 (aa) Original submission

Copyright © 1993-2013, University of Washington, Seattle. All rights reserved.