年１回空腹時血糖、HbA1c を評価すべきである。

男性患者で、疲労感の増悪や性的活力の低下がある場合には性腺機能不全を検査するべきである。補充療法により効果がみられるかを判断するため、2-3年に一度は症状がなくても検査をおこなう。

研究中の治療法

広範囲の疾患に対する臨床研究の情報に調べる場合には ClinicalTrials.gov を検索すること。

注：本疾患に対する臨床治験はされていない可能性がある。

その他

遺伝クリニックでは、患者や家族は、本疾患の自然経過や治療、遺伝形式、他の家族への遺伝リスクだけでなく、利用可能な民間の情報資源に関する情報を、遺伝の専門家より得ることができる。GeneTests Clinic Directory 参照。
本疾患に対する疾患特異的または包括的支援組織に関する民間の情報資源を参照。これらの組織は患者と家族に情報、支援、他の患者への連絡情報などを提供するために設立されてきた。

遺伝カウンセリング

「遺伝カウンセリングは個人や家族に対して遺伝性疾患の本質，遺伝，健康上の影響などの情報を提供し，彼らが医療上あるいは個人的な決断を下すのを援助するプロセスである．以下の項目では遺伝的なリスク評価や家族の遺伝学的状況を明らかにするための家族歴の評価，遺伝子検査について論じる．この項は個々の当事者が直面しうる個人的あるいは文化的な問題に言及しようと意図するものではないし，遺伝専門家へのコンサルトの代用となるものでもない．」

遺伝形式

筋強直性ジストロフィー2型は優性遺伝形式で遺伝する。

患者家族のリスク

発端者の両親

- 現在の所、生物学的両親の遺伝子検査が行われたすべての発端者は両親のどちらかが CNBP 遺伝子の伸長を持つ。
- 新規突然変異を持つ発端者は報告されていない。
- CNBP 遺伝子の伸長は世代間および体細胞内で不均一性を示す。一般に、リピートの長さは次の世代に伝わる際に短縮し、個人の中で年齢を経るにつれて伸長する。母親由来、父親由来による伸長、短縮に関する一定の傾向はない。

発端者の同胞

- 発端者の同胞の危険性は両親の遺伝的状況による。
- 両親の片方が伸長した CNBP 遺伝子の伸長を持つ場合は、各同胞は 50%の可能性でその伸長を引き継ぐ

発端者の子

- CNBP 遺伝子の伸長を片方のアレルで持つ患者の子供は、それぞれ 50%の確率で遺伝子の伸長を引き継ぐ。
- CNBP 遺伝子の CCTG リピートは次の世代に遺伝する際、短縮し、個人の中で年齢を経るにつれて伸長する傾向にある。

他の血縁者

他の血縁者のリスクは、発端者の親の状況による。もし親も発症者であれば、その血縁者もリスクがある。

遺伝カウンセリングに関連した問題

測定されたリピートの大きさと疾患の重症度との間に相関関係はない、そのため発症年齢や臨床経過を分子遺伝学的検査から推測することはできない。

体細胞内不安定性　CNBP 遺伝子の CCTG リピート伸長は非常に不安定であり、年齢を経るに従い増加する傾向にある。異なる血液サンプルを使って複数回検査を行った場合、伸長の程度が異なることがある。さらに一回の末梢血サンプル中にも、サザンブロット解析で検出できる複数の伸長したアレルサイズが含まれている。一回のサンプルの中で主たるアレルの大きさからも、検出可能な異なる伸長の合計からも疾患の重症度、発症年齢、臨床症状を予測できない。

無症状のリスクのある成人の検査　無症状のリスクのある成人の検査は、分子遺伝学的検査の項で記述した方法により実施可能である。予測検査を行うことによって、被検者が CNBP 遺伝子の伸長を持っているかすなわち被検者が発病するリスクがあるかどうかを判定することができる。リピートの大きさから、発症年齢、重症度、臨床症状を予測することはできない。リスクのある家系の複数の人に検査の提案をする前に、症状のある人の遺伝子検査をして、その家系で本当に CNBP 遺伝子の伸長があるかを確認する必要がある。分子遺伝学的検査の限界と予測検査に関連するリスクを理解するため、それぞれの被検者は、予測検査の前に遺伝カウンセリングを受けるべきである。

無症状のリスクのある小人の検査　本疾患は成人発症であり、病気を阻止したり、転帰を改善させたりする有効な治療法がないことから、無症状のリスクのある小人の検査は勧められない。症状のある小人に対して遺伝子検査をすることは、診断を確立するという利点がある。the National Society of Genetic Counselors resolution on genetic testing of children や the American Society of Human Genetics and American College of Medical Genetics points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents (Genetic Testing; pdf).を参照のこと

家族計画

遺伝学的リスク評価や出生前検査の可否などについての議論は妊娠前に行うのが望ましい。同様に、リスクのある無症状の家族に対する遺伝学的検索も妊娠前に行うべきである。

DNA バンク

DNA バンクは主に白血球から調製した DNA を将来利用することを想定して保存しておくものである。検査技術や遺伝子、変異、あるいは疾患に対するわれわれの理解が将来さらに進歩すると考えられるので、DNA 保存が考慮される。

出生前診断

DM2の 50%のリスクのある妊娠について出生前診断が技術的に可能である。 DNA は胎生 15－18 週に採取した羊水中細胞や 10－12 週＊に採取した絨毛から調製する。成人発症型疾患の出生前診断の希望に対しては注意深い遺伝カウンセリングを必要とする。出生前診断を行う以前に、DM2の診断を確認するために、罹患している家族において CNBP 遺伝子伸長が同定されている必要がある。

DM2のような通常成人発症で知能障害をきたさない疾患に対する出生前診断の依頼はよくあることではない。発症前診断の施行に関して将来的なことを評価できる能力が、医療従事者と家族内で異なる。特に初期の診断というより中絶の目的で、検査を行うと考える場合、その差は顕著である。多くの医療施設では、両親が出生前診断を受けるかどうかを選択するとしているが、これらの問題に対する十分な議論を行うことが望まれる。

注：胎生週数は最終月経の開始日あるいは超音波検査による測定に基づいて計算される。

着床前診断(Preimplantation genetic diagnosis, PGD)は罹患している家族において病因となる遺伝子変異が同定されている場合に利用できることがある。（訳注：本邦では着床前診断に対応可能な施設はきわめて少なく、個別の倫理審査が必要など利用には障害が多い。）

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Chapter Number

Congenital Myasthenic Syndromes – Molecular Bases of Congenital Defects of Proteins at the Neuromuscular Junction –

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1. Introduction

Congenital myasthenic syndromes (CMS) are heterogeneous disorders caused by mutations in molecules expressed at the neuromuscular junction (NMJ) (Fig. 1). Each mutation affects the expression level or the functional properties or both of the mutant molecule. No fewer than 11 defective molecules at the NMJ have been identified to date. The mutant molecules include (i) acetylcholine receptor (AChR) subunits that forms nicotinic AChR and generate endplate potentials (Ohno *et al.*, 1995; Sine *et al.*, 1995), (ii) rapsyn that anchors and clusters AChRs at the endplate (Ohno *et al.*, 2002; Milone *et al.*, 2009), (iii) agrin that is released from nerve terminal and induces AChR clustering by stimulating the downstream LRP4/MuSK/Dok-7/rapsyn/AChR pathway (Huze *et al.*, 2009), (iv) muscle-specific receptor tyrosine kinase (MuSK) that transmits the AChR-clustering signal from agrin/LRP4 to Dok-7/rapsyn/AChR (Chevessier *et al.*, 2004; Chevessier *et al.*, 2008), (v) Dok-7 that interacts with MuSK and exerts the AChR-clustering activity (Beeson *et al.*, 2006; Hamuro *et al.*, 2008), (vi) plectin that is an intermediate filament-associate protein concentrated at sites of mechanical stress (Banwell *et al.*, 1999; Selcen *et al.*, 2011), (vii) glutamine-fructose-6-phosphate aminotransferase 1 encoded by *GFPT1*, the function of which at the NMJ has not been elucidated (Senderek *et al.*, 2011), (viii) skeletal muscle sodium channel type 1.4 ($Na_V1.4$) that spreads depolarization potential from endplate throughout muscle fibers (Tsujino *et al.*, 2003), (ix) collagen Q that anchors acetylcholinesterase (AChE) to the synaptic basal lamina (Ohno *et al.*, 1998; Ohno *et al.*, 1999; Kimbell *et al.*, 2004), (x) β2-laminin that forms a cruciform heterotrimeric lamins-221, -421, and -521 and links extracellular matrix molecules to the β-dystroglycan at the NMJ (Maselli *et al.*, 2009), (xi) choline acetyltransferase (ChAT) that resynthesizes acetylcholine from recycled choline at the nerve terminal (Ohno *et al.*, 2001). AChR (Lang & Vincent, 2009), MuSK (Hoch *et al.*, 2001; Cole *et al.*, 2008), and LRP4 (Higuchi *et al.*, 2011) are also targets of myasthenia gravis, in which autoantibody against each molecule impairs the neuromuscular transmission.
CMS are classified into three groups of postsynaptic, synaptic, and presynaptic depending on the localization of the defective molecules. Among the eleven molecules introduced above, AChR, rapsyn, MuSK, Dok-7, plectin, and $Na_V1.4$ are associated with the

postsynaptic membrane. Agrin, ColQ, and β2-laminin reside in the synaptic basal lamina. The only presynaptic disease protein identified to date is choline acetyltransferase (ChAT). A target molecule and its synaptic localization of glutamine-fructose-6-phosphate aminotransferase 1 (GFPT1) are still unresolved but the phenotypic consequence is the postsynaptic AChR deficiency. This chapter focuses on molecular bases of these three groups of CMS.

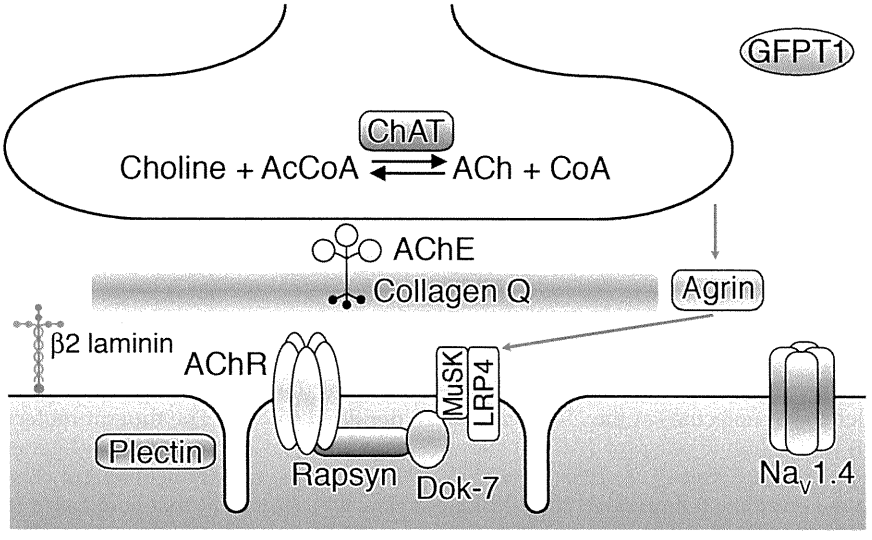

Fig. 1. Schematic of molecules expressed at the NMJ

2. Physiology of the NMJ

This section introduces molecular basis of development and maintenance of the NMJ, and physiological features of nicotinic muscle AChR.

2.1 NMJ synaptogenesis

At the NMJ, MuSK is an indirect receptor for agrin with (Valenzuela *et al.*, 1995; Dechiara *et al.*, 1996). Agrin released from the nerve terminal binds to LRP4 on the postsynaptic membrane (Kim *et al.*, 2008; Zhang *et al.*, 2008). Binding of LRP4 to agrin phosphorylates MuSK. Phosphorylated MuSK recruits the noncatalytic adaptor protein Dok-7 (Okada *et al.*, 2006). Once recruited, Dok-7 further facilitates phosphorylation of MuSK, and induces clustering of rapsyn and AChR by phosphorylating the β subunit of AChR. Rapsyn self-associates and makes a homomeric cluster at the endplate, which serves as a scaffold for AChR. Rapsyn and AChR bind each other with a stoichiometry of 1:1. Rapsyn also binds to β-dystroglycan and links the rapsyn scaffold to the subsynaptic cytoskeleton (Froehner *et al.*, 1990; Cartaud *et al.*, 1998; Ramarao & Cohen, 1998; Ramarao *et al.*, 2001). Except for LRP4, each of the above molecules is a CMS target.

2.2 Physiology of the nicotinic muscle AChR

Nicotinic AChRs are pentameric ligand-gated ion channels. The family of pentameric ligand-gated ion channels includes cationic AChRs, cationic serotonergic receptors ($5HT_3$), anionic glycine receptors, and anionic $GABA_A$ and $GABA_C$ receptors (Keramidas *et al.*, 2004). Heteromeric neuronal nicotinic AChRs are comprised of various combinations of α (α2-α7) and β subunits (β2-β4), whereas homomeric AChRs are formed only by a single α subunit (e.g., α7-α9) (Mihailescu & Drucker-Colin, 2000). On the other hand, nicotinic muscle AChRs have only two forms: fetal AChR that carries the α, β, δ, and γ subunits encoded by *CHRNA1, CHRNB1, CHRND, CHRNG*, respectively, in the stoichiometry $\alpha_2\beta\delta\gamma$; and adult-type AChR that carries the ε subunit instead of the γ subunit in the stoichiometry $\alpha_2\beta\delta\varepsilon$ (Mishina *et al.*, 1986). The ε subunit is encoded by *CHRNE*. Nicotinic muscle AChR harbors two binding sites for ACh at the interfaces between the α–δ and α–γ/α–ε subunits (Lee *et al.*, 2009; Mukhtasimova *et al.*, 2009). Binding of a single ACh molecule opens the channel pore but for a short time. Binding of two ACh molecules stabilizes the open state of AChR, and AChR stays open for longer time. Only cations pass through the channel pore of nicotinic AChRs. Unlike sodium, potassium, or calcium channels, AChRs, in general, have no selectivity for cations, but α7 AChRs have 10-20 times higher permeability for Ca^{2+} than for Na^+.

3. Postsynaptic CMS

Postsynaptic CMS is classified into four phenotypes: (i) endplate AChR deficiency due to defects in AChR, rapsyn, agrin, MuSK, Dok-7, plectin, glutamine-fructose-6-phosphate aminotransferase 1, (ii) slow-channel congenital myasthenic syndrome, (iii) fast-channel congenital myasthenic syndrome, and (iv) sodium channel myasthenia.

3.1 Endplate AChR deficiency

Endplate AChR deficiency is caused by defects in AChR, rapsyn, agrin, MuSK, Dok-7, plectin, and GFPT1.

3.1.1 Endplate AChR deficiency due to defects in AChR subunits

Endplate AChRs deficiency can arise from mutations in *CHRNA1, CHRNB1, CHRND*, and *CHRNE*, but not *CHRNG*.

Two different groups of mutations of the AChR subunit genes cause endplate AChR deficiency. The first group includes null mutations in *CHRNE* encoding the ε subunit. The null mutations are caused by frameshifting DNA rearrangements, *de novo* creation of a stop codon, and frameshifting splice-site mutations, or mutations involving residues essential for subunit assembly. Large-scale in-frame DNA rearrangements also abolish expression of the AChR ε subunit (Abicht *et al.*, 2002). Mutations in the promoter region (Ohno *et al.*, 1999) and most missense mutations (Ohno *et al.*, 1997) do not completely abolish expression of the ε subunit but the molecular consequences are indistinguishable from those of null mutations. Lack of the ε subunit can be compensated for by the presence of the fetal γ subunit that is normally expressed in embryos (Engel *et al.*, 1996). The patients can survive with γ-AChR even in the absence of ε-AChR. If a null mutation resides in the other AChR subunit genes,

the affected individual will have no substituting subunit and cannot survive. Indeed, two homozygous missense low expressor or null mutations in *CHRNA1* and *CHRND* caused lethal fetal akinesia (Michalk *et al.*, 2008).

The second group of mutations affecting the AChR subunit genes includes missense mutations of *CHRNA1*, *CHRNB1*, and *CHRND*. These mutations compromise expression of the mutant subunit and/or the assembly of AChRs, but do not completely abolish AChRs expression. The main difference between mutations in *CHRNE* and those in *CHRNA1*, *CHRNB1*, and *CHRND* is tolerance to low or no expression of the ε subunit whereas similar mutations in other subunits generally have devastating consequences and cause high fatality. Some missense mutations in *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE* also affect the AChR channel kinetics and vice versa. The kinetic effects will predominate if the second mutation is a low expressor, or if the kinetic mutation has slow-channel features with dominant gain-of function effects.

In endplate AChR deficiency, the postsynaptic membrane displays a reduced binding for peroxidase- or ^{125}I-labeled α-bungarotoxin and the synaptic response to ACh, reflected by the amplitude of the miniature endplate potential, endplate potential, and endplate current, is reduced. In some but not all cases the postsynaptic region is simplified. In most cases, the muscle fibers display an increased number of small synaptic contacts over an extended length of the muscle fiber. In some patients quantal release is higher than normal. In patients with null mutations in *CHRNE*, single channel recordings of AChRs at patient endplates reveal prolonged opening bursts that open to an amplitude of 60 pS, indicating expression of the fetal γ-AChR in contrast to the adult ε-AChR that has shorter opening bursts and opens to an amplitude of 80 pS. In contrast, in most patients with low-expressor mutations in the *CHRNA1*, *CHRNB1*, or *CHRND*, single channel recordings demonstrate no or minor kinetic abnormalities.

As in autoimmune myasthenia gravis, endplate AChR deficiency is generally well controlled by regular doses of anticholinesterases. Anticholinesterase medications inhibit the catalytic activity of AChE; this prolongs the dwell time of ACh in the synaptic space and allows each ACh molecule to bind repeatedly to AChR.

3.1.2 Endplate AChR deficiency due to defects in rapsyn

Congenital defects of rapsyn also cause endplate AChR deficiency. Rapsyn makes a homomeric cluster and binds to AChR as well as to β-dystroglycan, and forms AChR clusters at the endplate (Froehner *et al.*, 1990; Cartaud *et al.*, 1998; Ramarao & Cohen, 1998; Ramarao *et al.*, 2001). The structural domains of rapsyn include an N-terminal myristoylation signal required for membrane association (Ramarao & Cohen, 1998), seven tetratrico peptide repeats at codons 6 to 279 that subserve rapsyn self-association (Ramarao & Cohen, 1998; Ramarao *et al.*, 2001), a coiled-coil domain at codons 298 to 331 that binds to the long cytoplasmic loop of each AChR subunit (Bartoli *et al.*, 2001), a Cys-rich RING-H2 domain at codons 363-402 that binds to the cytoplasmic domain of β-dystroglycan (Bartoli *et al.*, 2001) and mediates the MuSK induced phosphorylation of AChR (Lee *et al.*, 2008), and a serine phosphorylation site at codon 406. Transcription of rapsyn in muscle is under the control of helix-loop-helix myogenic determination factors that bind to the *cis*-acting E-box sequence in the *RAPSN* promoter (Ohno *et al.*, 2003).

Loss-of-function mutations in *RAPSN* have been reported in the coding region (Ohno *et al.*, 2002; Burke *et al.*, 2003; Dunne & Maselli, 2003; Maselli *et al.*, 2003; Muller *et al.*, 2003; Banwell *et al.*, 2004; Yasaki *et al.*, 2004; Cossins *et al.*, 2006; Muller *et al.*, 2006) as we as in the

promoter region (Ohno *et al.*, 2003). N88K in *RAPSN* is one of the most frequently observed mutations in CMS (Muller *et al.*, 2003). We reported lack of a founder haplotype for N88K (Ohno & Engel, 2004), but analysis of markers closer to *RAPSN* later revealed possible presence of a shared haplotype (Richard *et al.*, 2003) suggesting that N88K is an ancient founder mutation but subsequent multiple recombination events and divergence of microsatellite markers have narrowed the shared haplotype region. Functional analysis L14P, N88K, and 553ins5 disclosed that these mutations have no effect on self-association of rapsyn but impair colocalization of rapsyn with AChR (Ohno *et al.*, 2002). Analysis of A25V, N88K, R91L, L361R, and K373del later revealed diverse molecular defects affecting colocalization of rapsyn with AChR, formation of agrin-induced AChR clusters, self-association of rapsyn, and expression of rapsyn (Cossins *et al.*, 2006). Although there are no genotype-phenotype correlations in mutations at the coding region, arthrogryposis at birth and other congenital malformations occurs in nearly a third of the patients. In addition, the -38A>G mutation affecting an E-box in the promoter region observed in Near-Eastern Jewish patients exhibits unique facial malformations associated with prognathism and malocclusion (Ohno *et al.*, 2003).

Most patients respond well to anticholinesterase medications. Some patients further improve with addition of 3,4-diaminopyridine, ephedrine, and albuterol (Banwell *et al.*, 2004). The drug 3,4-diaminopyridine blocks the presynaptic potassium channel, which slows the repolarization of the presynaptic membrane (Wirtz *et al.*, 2010) enhancing the influx of Ca^{2+} through the presynaptic voltage-gated P/Q-type and N-type channels. This, in turn, facilitates the exocytosis of synaptic vesicles and the quantal content of the endplate potential.

3.1.3 Endplate AChR deficiency due to a defect in agrin

Neural agrin released from the nerve terminal is a key mediator of synaptogenesis at the NMJ. A reported homozygous G1709R agrin mutation, however, did not cause AChR deficiency but mutations in agrin are potential causes of AChR deficiency by interfering with the activation of MuSK and by impeding synaptic maturation.

The patient harboring the G1709R mutation was a 42-year-old woman with right lid ptosis since birth, no oculoparesis, and mild weakness of facial, hip-girdle and anterior tibial muscles, and refractoriness to pyridostigmine or 3,4-diaminopyridine (Huze *et al.*, 2009). The mutation is in the laminin G-like 2 domain, upstream of the neuron-specific y and z exons that are required for MuSK activation and AChR clustering. AChR and agrin expression at the endplate were normal. Structural studies showed endplates with misshaped synaptic gutters partially filled by nerve endings and formation of new endplate regions. The postsynaptic regions were preserved. Expression studies in myotubes using a mini-agrin construct revealed the mutation did not affect MuSK activation or agrin binding to α-dystroglycan. Forced expression of the mutant mini-agrin gene in mouse soleus muscle induced changes similar to those at patient endplates. Thus, the observed mutation perturbs the maintenance of the endplate without altering the canonical function of agrin to induce development of the postsynaptic compartment.

3.1.4 Endplate AChR deficiency due to defects in MuSK

MuSK and LRP4 form a heteromeric receptor for agrin. Five *MUSK* mutations have been

reported in three papers. The first report describes heteroallelic frameshift (220insC) and missense (V790M) mutations in a patient with respiratory distress in early life, mild ptosis, decreased upward gaze, and fatigable weakness of the cervical and proximal more than distal muscles. The symptoms were worsened by pregnancy. Treatment with pyridostigmine and 3,4-diaminopyridine was ineffective (Chevessier *et al.*, 2004). The frameshift mutation prevents MuSK expression and the missense mutation decreases MuSK expression and impairs its interaction with Dok-7. Forced expression of the mutant protein in mouse muscle decreased AChR expression at the endplate and caused aberrant axonal outgrowth (Chevessier *et al.*, 2004). Interestingly, mice homozygous for MuSK V789M (which corresponds to the human MuSK V790M) are normal but mice hemizygous for V789M are severely affected suggesting that MuSK V790M in humans is a haploinsufficient only when accompanied by a null mutation (Chevessier *et al.*, 2008).
A second report describes heteroallelic M605I and A727V mutations in MuSK in a patient with severe myasthenic symptoms since early life that improved after puberty but worsened after menstrual periods. The MEPP and MEPC amplitudes in anconeus muscle were reduced to about 30% of normal and the EPP quantal content was half-normal. Synaptic contacts were small and electron microscopy showed simplified postsynaptic regions with too few secondary synaptic clefts. The patient failed to respond to pyridostigmine, ephedrine or 3,4-diaminopyuridine but responded partially to albuterol (Maselli *et al.*, 2010).
A third report describes a homozygous P31L mutation in the extracellular domain of MuSK in 5 patients in a consanguineous Sudanese kinship. The findings included ptosis from an early age, partial ophthalmoparesis, and weakness of torso and limb girdle muscles. Pyridostigmine therapy gave only slight benefit (Mihaylova *et al.*, 2009).

3.1.5 Endplate AChR deficiency due to defects in Dok-7

Phosphorylated MuSK recruits a noncatalytic adaptor protein, Dok-7. Recruited Dok-7 further facilitates phosphorylation of MuSK (Okada *et al.*, 2006). Dok-7 is highly expressed at the postsynaptic region of skeletal muscle and in heart. It harbors an N terminal pleckstrin homology domain (PH) important for membrane association, a phosphotyrosine-binding (PTB) domain, and C-terminal sites for phosphorylation. The PH and PTB domains are required for association with and phosphorylation of MuSK. Phosphorylation of two C terminal residues is a requisite for Dok-7 activation by Crk and Crk-L (Hallock *et al.*, 2010).
Numerous mutations have been identified in *DOK7* (Beeson *et al.*, 2006; Muller *et al.*, 2007; Anderson *et al.*, 2008; Selcen *et al.*, 2008; Vogt *et al.*, 2009; Ben Ammar *et al.*, 2010). Nearly all patients carry a common 1124_1127dupTGCC mutation in exon 7. This and other mutations upstream of the C-terminal phosphorylation sites abrogate the ability of Dok-7 to associate with Crk1/Crk1L and hence its activation (Hallock *et al.*, 2010; Wu *et al.*, 2010). Mutations disrupting or eliminating the PH and PTB domains of Dok-7 prevent dimerization and association of Dok-7 with MuSK (Bergamin *et al.*, 2010).

3.1.6 Endplate AChR deficiency due to defects in plectin

Plectin, encoded by *PLEC*, is a highly conserved and ubiquitously expressed intermediate filament-linking protein concentrated at sites of mechanical stress, such as the postsynaptic membrane of the endplate, the sarcolemma, Z-disks in skeletal muscle, hemidesmosomes in skin, and intercalated disks in cardiac muscle. Pathogenic mutations in *PLEC* result in

epidermolysis bullosa simplex, a progressive myopathy (Smith *et al.*, 1996), and, in some patients, myasthenic syndrome (Banwell *et al.*, 1999; Selcen *et al.*, 2011). We reported two cases of CMS associated with plectin deficiency (Banwell *et al.*, 1999; Selcen *et al.*, 2011). The dystrophic changes in muscle are attributed to dislocation of the fiber organelles no longer anchored by the cytoskeletal intermediate filaments and to sarcolemmal defects allowing Ca^{2+} ingress into the muscle fibers. The myasthenic syndrome is attributed to destruction of the junctional folds lacking adequate cytoskeletal support.

3.1.7 Endplate AChR deficiency due to defects in glutamine-fructose-6-phosphate aminotransferase 1 (GFPT1)

Glutamine-fructose-6-phosphate transaminase 1, encoded by *GFPT1*, catalyzes transfer of an amino group from glutamine onto fructose-6-phosphate, yielding glucosamine-6-phosphate and glutamate. GFPT1 is a rate-limiting enzyme that controls the flux of glucose into the hexosamine biosynthesis pathway. GFPT1 thus initiates formation of UDP-N-acetylglucosamine (UDP-GlcNAc), which is a source of multiple glycosylation processes including addition of N-acetylglucosamine to serine or threonine residues (O-linked GlcNAc) (Wells *et al.*, 2001). The disease gene was discovered by linkage analysis and homozygosity mapping of 13 kinships with a limb-girdle CMS often associated with tubular aggregates in skeletal muscle (Senderek *et al.*, 2011). Immunoblots of muscle of affected patients revealed decreased expression of O-linked GlcNAc, but the responsible molecule(s) causing CMS remain elusive.

3.2 Slow-channel congenital myasthenic syndrome (SCCMS)

The second class of postsynaptic CMS due to mutations in the AChR subunit genes is SCCMS. SCCMS is an autosomal dominant disorder, in which a gain-of-function mutation on a single allele compromises the neuromuscular signal transduction (Ohno *et al.*, 1995). The mutation causes prolonged AChR channel openings and increases the synaptic response to ACh (Fig. 2). There is a single reported case of autosomal recessive SCCMS, in which an εL78P mutation minimally prolongs channel opening events but the mutant channel arising from a single allele is not sufficient to cause disease (Croxen *et al.*, 2002). In general, dominantly inherited disorders, including SCCMS, tend to present after adolescence and have a relatively mild course. Some patients with SCCMS, however, present early in life and become severely disabled even in the first decade.

In SCCMS, neuromuscular transmission is compromised by three distinct mechanisms. First, staircase summation of endplate potentials causes depolarization block of the postsynaptic membrane by rendering the voltage-gated skeletal muscle sodium channel go into an inactivated state and thereby inhibit action potential generation (Maselli & Soliven, 1991). Second, some mutant AChRs are prone to become desensitized (Milone *et al.*, 1997), which reduces the number of AChRs that respond to the released ACh quanta. Third, prolonged opening of AChR causes excessive influx of extracellular calcium, which results in focal degeneration of the junctional folds as well as apoptosis of some of the junctional nuclei (Groshong *et al.*, 2007). In normal adult human ε-AChR, 7% of the synaptic current is carried by Ca^{2+}, which is higher than that carried by the human fetal γ-AChR or by muscle AChRs of other species (Fucile *et al.*, 2006). This predisposes endplate to Ca^{2+} overloading when the channel opening events are prolonged. In addition, at least two SCCMS mutations, εT264P

(Ohno *et al.*, 1995) and αV259F (Fidzianska *et al.*, 2005), increase the Ca^{2+} permeability 1.5- and 2-fold, respectively (Di Castro *et al.*, 2007).

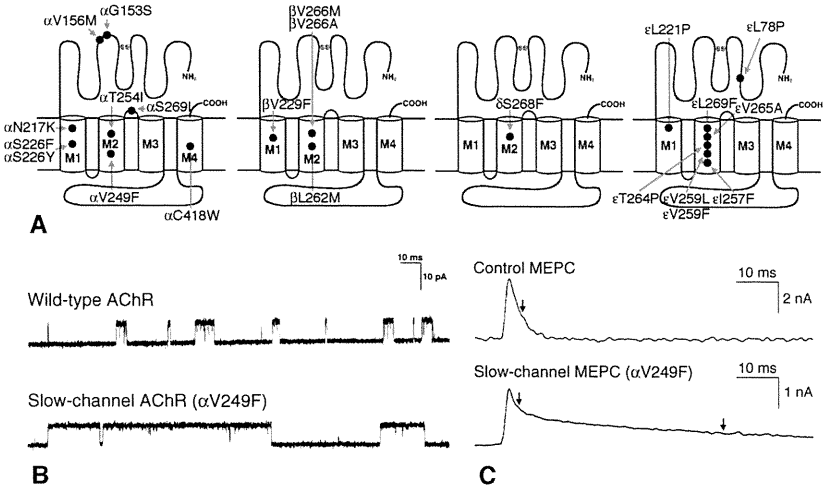

Fig. 2. Slow channel CMS. (A) Schematic diagram of AChR subunits with SCCMS mutations. (B) Single channel currents from wild-type and slow channel (αV249F) AChRs expressed on HEK293 cells. (C) Miniature endplate current (MEPC) recorded from endplates of a control and a patient harboring αV249F. The patient's MEPC decays biexponentially (arrows) due to expression of both wild-type and mutant AChRs.

Slow channel mutations can be divided into two groups. The first group includes mutations at the extracellular domain like αG153S (Sine *et al.*, 1995), as well as at the N-terminal part of the first transmembrane domain like αN217K (Wang *et al.*, 1997) and εL221F (Hatton *et al.*, 2003). These mutations increase the affinity for ACh binding, probably by retarding the dissociation of ACh from the binding site, which gives rise to repeated channel openings after a single event of ACh biding. The second group includes mutations at the second transmembrane domain (M2) that lines the ion channel pore. These mutations mostly introduce a bulky amino acid into the channel lining face, but εT264P (Ohno *et al.*, 1995) introduces a kink into the channel pore, whereas βV266A (Shen *et al.*, 2003) and εV265A (Ohno *et al.*, 1998) introduce a smaller amino acid into the pore. Mutations in M2 retard the channel closing rate α and variably enhance the channel opening rate β. Some mutations in M2 also increase affinity for ACh, which include αV249F (Milone *et al.*, 1997), εL269F (Engel *et al.*, 1996), and εT264P (Ohno *et al.*, 1995).

SCCMS can be treated with conventional doses of long-lived open channel blockers of AChR, such as the antiarrhythmic agent quinidine (Fukudome *et al.*, 1998; Harper & Engel, 1998) and the antidepressant fluoxetine (Harper *et al.*, 2003). Quinidine reduces the prolonged burst duration of SCCMS to the normal level at 5 μM (Fukudome *et al.*, 1998). As the concentration of quinidine in the treatment of cardiac arrhythmia is 6-15 μM, 5 μM is readily attainable in clinical practice and indeed demonstrates significant effects (Harper & Engel, 1998). Similarly, fluoxetine reduces the prolonged burst duration to the normal level at 10 μM, which is clinically attainable without adverse effects at 80 to 120 mg/day of fluoxetine (Harper *et al.*, 2003).