1~3)、乳児期発症1例(症例4)、幼児期発症1例(症例5)で、4例が生後1年以内に発症した。妊娠経過中の異常は全例指摘されなかった。1例(症例 1)で生直後から自発呼吸が確立せず人工呼吸管理を行った。初発症状は4例(症例1~4)に運動発達遅滞を伴う筋緊張低下または筋力低下を認めたが、経過中に無投薬で徐々に改善し3例が1歳7か月までに、最も遅い例(症例 1)でも 4 歳時に独歩を獲得した。1例(症例 5)は 3 歳時に長距離歩行後の動揺性歩行で発症した。家族歴は1例のみに認め父方祖母が重症筋無力症であった(症例5)。

研究時には全身の筋力低下を2例、頚部・四肢のみの筋力低下を3例に認めた。筋力変動は全例に認め、夕方や運動負荷後に筋力低下を呈し同日中に改善する日内変動 1 例、1日ごとに程度の異なる間欠的変動3例、筋力低下が数日単位で持続する長期変動3例であった(症例重複あり)。日内変動のみを呈した例はなかった。具体的には間欠的変動では歩行距離と呼吸器離脱可能な時間(症例 1)、階段昇降の段数(症例2)、運動不耐の程度(症例5)が1日ごとに変動した。長期変動では疲労による歩行距離の短縮(症例 1)運動負荷による疲労(症例 3)が数日続く例と、感冒罹患や喘息発作から1週間後に筋力低下を認めるがさらに1週間かけて徐々に回復する(症例4)例を認めた。

2. 検査所見

遠位の運動神経では、正中神経1例、尺骨神経3例(うち1例は手掌を最大収縮で30秒間握らせた後)、後脛骨神経2例で減衰を認めた。尺骨神経と後脛骨神経では repetitive CMAP をそれぞれ1例に認めた。近位の運動神経である副神経では評価した全例が減衰した。症例1, 2の波形を図1に示す。テンシロンテストは投与後のCMAP減衰率の改善を認めた2例と、運動負荷への所要時間の短縮を認めた1 例を陽性と判定した。また投与後に呼吸不全を呈した1例を悪化と判定した。眼瞼下垂の改善を認めた例はなかった。抗 AChR 抗体、抗 MuSK 抗体は検索した全例で陰性であった。筋生検は施行した4例全てで非特異的所見のみであった。遺伝子変異は *COLQ* 変異 3 例、*CHRNE* 変異1例、*DOK7* 変異1例であった。

3. 治療

COLQ 変異例(症例 2~4)は塩酸エフェドリンが全例で有効であり、易疲労性、運動不耐の改善を認めた。症例2では6分間歩行距離が130mから280mに延長した。*DOK7* 変異例(症例1)ではピリドスチグミン臭化物開始後の筋力改善は明らかでなかったが、3,4-Diaminopyridine(3,4-DAP)開始後に階段昇降の時間が短縮しジャンプが可能となった。さらに塩酸エフェドリンを追加したところ両上肢の挙上時間が10秒から20秒へ延長し、臥位から立位への体位変換に要する時間が10秒から3~4秒に短縮した。易疲労性を訴えることもなくなった。症例3は気管切開を行なっており、特に冬になると呼吸器感染症で頻回の入院を要していたが、塩酸エフェドリン開始後は入院を要する機会がなくなった。症例4ではピリドスチグミン臭化物、3,4DAP、易疲労感が若干改善したが、階段昇降の所要時間は変動が大きく明らかな運動不耐の改善は認めなかった。そこで塩酸エフェドリンの投与を併用したところ筋力低下の数日単位の大きな変動の頻度の減少を認めた。

D. 考察

CMS は先天性ミオパチー、抗体陰性重症筋無力症、肢帯型筋無力症、中枢性低緊張、代謝性疾患、先天性筋ジストロフィー、ミトコンドリア病[2)5)13)14)]と診断されている例が多く、これらの疾患との鑑別が重要である。遺伝子変異ごとに呈する症状が若干異なることが報告されており、今回の検討例にある遺伝子変異では *DOK7* 変異例での肢帯型の筋力低下[5)14)]、*COLQ* 変異例での早期からの筋力低下、呼吸不全、進行性の体幹の筋力低下と側彎、拘束性呼吸不全[15)]、*CHRNE* 変異例では新生児期発症、眼球運動制限、球麻痺、軽度の近位筋力低下、遠位筋萎縮、呼吸器感染の反復[16)17)]である。今回の検討例での *COLQ* 変異例と報告での臨床像は一致していたが、*DOK7* 変異例では呼吸不全、運動発達遅滞、眼線下垂を伴う重症例であり、*CHRNE* 変異例では球麻痺、眼瞼運動制限を伴わずより軽症であった。また *DOK7* 変異例では長期間での筋力変動を呈することがある[14)18)]が今回の検討では *COLQ* 変異例、*CHRNE* 変異例も間欠的あるいは長期での筋力変動を呈し、日内変動のみを呈した例はなかった。また間欠的変動、長期変動ともに眼瞼下垂の程度には変化がなく、易疲労性、

運動不耐、呼吸筋疲労が変動の中心であった。休息によって回復しない筋力変動を呈する理由は不明であるが、遺伝子変異に関係なくCMSに特徴的な症状である可能性がある。

代表的な神経筋接合部評価手法にRNSとテンシロンテストがある。RNSでは遠位の運動神経である正中神経、尺骨神経、後脛骨神経でCMAPの減衰を認めない例があり、近位の運動神経である副神経では評価した全例が減衰を認めた。Benらは筋力低下を呈する遠位筋に複数回行ったRNSのうち12例中9例が1回は減衰を呈さなかったと報告しており、[14] Violeta Mihaylovaらは近位筋、遠位筋ともに減衰を呈さなかった*COLQ*変異例2例を報告している[19]。我々の症例でも症例1, 2は遠位筋の筋力低下を認めるにもかかわらず、これらの筋を支配する運動神経はRNSでの減衰を示さなかった。筋力低下と易疲労性はそれぞれ独立した症状であり、筋力低下を認める筋でのRNSが減衰しなくてもCMSを否定することにはならないと考えられた。テンシロンテストの評価は眼瞼下垂を呈さない例が半数を占めていたため、負荷前後のRNS減衰率や階段昇降の所要時間を比較することで効果を判定したが、眼瞼下垂を呈する例でもテンシロンテストでの眼瞼下垂の改善は認めなかった。眼瞼下垂のみをテンシロンテストの評価対象としていると異常なしと判断する可能性が高い。眼瞼下垂の改善が見られない理由は不明であるが、CMSに特徴的な症状と考えられた。

塩酸エフェドリンを投与した全例で何らかの臨床的有用性を認めた。一例で塩酸エフェドリンの副作用と思われる体重減少を認めたが、中止に至る例はなく、塩酸エフェドリンは先天性筋無力症候群の病因に関わらず有効性が期待できる薬剤であることが考えられた。ただし投与量をどのように決めてよいのか根拠に乏しく、また他剤を併用すべきなのか今後の検討が必要である。

E. 結論

CMSではこれまで報告されている全身の筋力低下、易疲労性、生後1年以内の発症[1-3]以外に、間欠的または長期的な筋力変動、近位筋支配の運動神経でのRNS減衰、テンシロンテストで眼瞼下垂の変化を認めないことが特徴であった。これらは原因遺伝子にかかわらず共通する症状であった。塩酸エフェドリンは病因に関わらず投与を試みる価値のある薬剤と考えられた。

CMSは丁寧な診察と電気生理検査により疑うことが可能であり遺伝子解析によって確定診断が可能な、薬物治療が行える疾患である。

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F. 研究発表

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G. 知的財産権の出願・登録状況（予定を含む）

１．特許取得
　　なし

２．実用新案登録
　　なし

３．その他
　　なし

研究成果の刊行に関する一覧表

III. 研究成果の刊行に関する一覧表

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著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
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8. Molecular defects of acetylcholine receptor subunits in congenital myasthenic syndromes

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Abstract. Congenital myasthenic syndromes (CMS) are caused by mutations in molecules expressed at the neuromuscular junction. Among eight defective molecules identified to date in CMS, mutations in the muscle nicotinic acetylcholine receptor (AChR) subunits are the first to be characterized and are prevalent. Mutations in the AChR subunit genes cause three phenotypes: (i) endplate AChR deficiency in which the number of AChR at the endplate is critically reduced; (ii) slow channel syndrome in which mutations either at the acetylcholine binding sites or at the ion channel pore increases synaptic response to acetylcholine and prolongs AChR channel opening events; and (iii) fast channel syndrome in which mutations either at the acetylcholine binding sites or at the long cytoplasmic loop between the third and fourth transmembrane domains compromise synaptic response to acetylcholine and shortens AChR channel openings. In addition, mutations in AChR subunit genes also cause fetal akinesia deformation sequence, and a single nucleotide polymorphism in the promoter region of the AChR α1 subunit is associated with early onset myasthenia gravis.

Introduction

Congenital myasthenic syndromes

Congenital myasthenic syndromes (CMS) are heterogeneous disorders caused by congenital defects of molecules expressed at the neuromuscular junction (Fig. 1). Each mutation affects the expression level of the mutant molecule and/or compromises the functional properties of the mutant molecule. The mutant molecules identified to date

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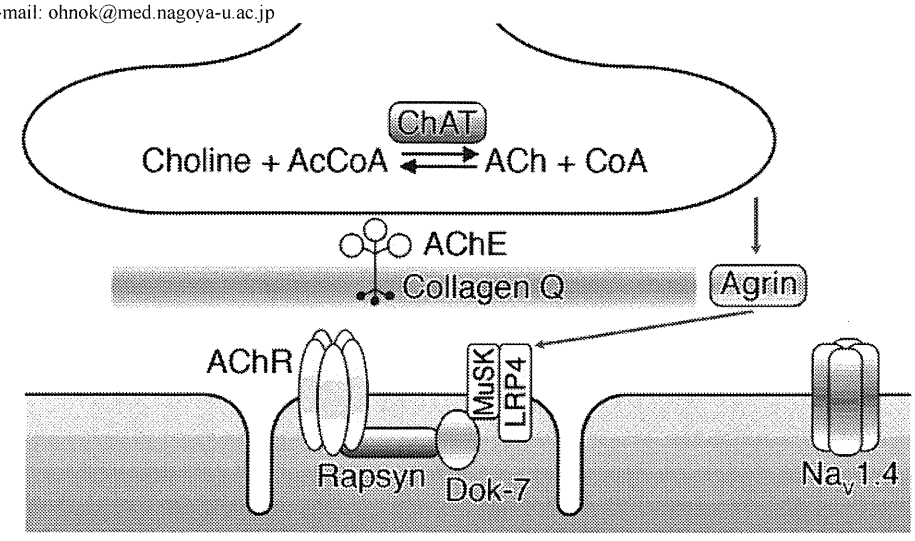

Figure 1. Schematic of molecules expressed at the neuromuscular junction. Mutated molecules in CMS include acetylcholine receptor (AChR), rapsyn, agrin, MuSK, Dok-7, skeletal muscle sodium channel type 1.4 ($Na_V1.4$), collagen Q, and choline acetyltransferase (ChAT).

include (i) acetylcholine receptor (AChR) subunits [1,2], (ii) rapsyn that anchors and clusters AChRs at the neuromuscular junction [3,4], (iii) agrin that is released from the nerve terminal and induces AChR clustering by stimulating the downstream LRP4/MuSK/Dok-7/rapsyn/AChR pathway [5], (iv) MuSK that transmits the AChR-clustering signal from agrin/LRP4 to Dok-7/rapsyn/AChR [6,7], (v) Dok-7 that transmits the AChR-clustering signal from agrin/LRP4/MuSK to rapsyn/AChR [8,9], (vi) skeletal muscle sodium channel type 1.4 ($Na_V1.4$) that spreads depolarization potential from endplate throughout muscle fibers [10], (vii) collagen Q that anchors acetylcholinesterase (AChE) to the synaptic basal lamina [11-13], (viii) choline acetyltransferase (ChAT) that resynthesizes acetylcholine from recycled choline at the nerve terminal [14]. AChR [15], MuSK [16,17], and LRP4 [18] are also targets of myasthenia gravis, in which autoantibody against these molecules compromises the neuromuscular transmission. This review focuses on molecular defects of AChR in CMS.

Muscle nicotinic acetylcholine receptor

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 [19]. Heteromeric neuronal nicotinic AChRs are comprised of various combinations of α (α2-α7) and β subunits (β2-β4), whereas homomeric AChRs are formed by just one subunit type (e.g., α7-α9) [20]. On the other hand, muscle nicotinic AChRs have only two forms: fetal AChR that carries the α1, β1, δ, and γ subunits encoded by *CHRNA1*, *CHRNB1*, *CHRND*, *CHRNG*, respectively, in the stoichiometry $\alpha 1_2\beta 1\delta\gamma$; and adult-type AChR that carries the ε subunit instead of the γ subunit in the stoichiometry

$\alpha 1_2\beta 1\delta\varepsilon$ [21]. The ε subunit is encoded by *CHRNE*. The muscle nicotinic AChR harbors two binding sites for ACh at the interfaces between α-δ and α-γ/ε subunits [22,23]. 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. Cations but no anions pass through the channel pore of nicotinic AChRs. Unlike sodium, potassium, or calcium channels, AChRs, in general, have no selectivity for cations, but α7AChRs have 10-20 times higher permeability for Ca^{2+} than for Na^{+}.

Endplate AChR deficiency

Congenital deficiency of endplate AChRs is caused by mutations in genes encoding the AChR subunits. The mutated genes include *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE*, but not *CHRNG*. Endplate AChR deficiency is also caused by mutations in molecules that transmit signals for AChR clustering. These include *AGRN* encoding agrin [5], *MUSK* encoding MuSK [6,7], *DOK7* encoding Dok-7 [8,9], and *RAPSN* encoding rapsyn [3,4]. Mutations in the signaling molecules, however, are not within the scope of this review and are not addressed.

Two different groups of mutations of the AChR subunit genes cause endplate AChR deficiency. The first group includes null mutations in *CHRNE* encoding the AChR ε subunit. The null mutations are caused by frameshifting DNA rearrangements, *de novo* creation of a stop codon, and frameshifting splicing mutations. Large-scale in-frame DNA rearrangements also abolish expression of the AChR ε subunit. Mutations in the promoter region [24] and missense mutations [25] do not completely nullify the expression of ε, but the molecular pathological consequences are indistinguishable from those of null mutations. Lack of the ε subunit can be compensated for by the presence of the γ subunit that is normally expressed in embryos [26]. The patients can survive with γ-AChR even when ε-AChR is lacking. If a null mutation resides on the other AChR subunit genes, the affected individual should have no substituting subunit and cannot survive. Indeed, two such homozygous missense mutations are reported in *CHRNA1* and *CHRND* in lethal fetal akinesia disorders [27]. In general, mutations causing monogenic diseases should be very rare, because a single nucleotide substitution among the 3.0×10^9 nucleotides in a single allele should exhibit a certain phenotype that is recognized as a disease. Most single nucleotide substitutions are likely to be silent or to partially confer a variable phenotype observed in normal individuals. Most of the other nucleotide changes cause early embryonic lethality and we cannot observe such mutations in patients.

The second group of mutations affecting the AChR subunit genes includes missense mutations of *CHRNA1*, *CHRNB1*, and *CHRND* encoding the AChR α1, β1, and δ subunits, respectively. These mutations compromise the expression level of the mutant subunit and/or the assembly of AChRs, but do not completely abolish the expression of AChRs. Differences between mutations in *CHRNE* and those in *CHRNA1*, *CHRNB1*, and *CHRND* are the tolerance to low expression of the affected subunit. The expression level of the ε subunit may goes to zero, whereas a patient needs a certain amount of AChRs to be expressed at the endplate to survive when a mutation is in a gene for either the α1, β1, or δ subunit. Patients with low-expressor mutations in *CHRNA1*, *CHRNB1*, and *CHRND* tend to have a devastating course with high fatality. Some missense mutations in *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE* also affect the AChR channel kinetics. If a pathological effect due to aberration of the

channel kinetics is more than the degree of aberration of AChR expression, such a mutation is classified as slow channel or fast channel mutation.

In biopsied skeletal muscle, we observed several lines of evidence indicating a decreased number of AChRs at the endplate. Ultrastructural studies demonstrate simplified junctional folds at the endplate and reduced staining for AChRs. Miniature endplate currents (MEPC) are small in amplitude. As the number of ACh in a synaptic vesicle (quanta) is rather increased, the low MEPC amplitude directly indicates a reduced number of AChRs at the endplate. Endplate potentials (EPP) are also small in amplitude. Again, as the number of ACh released by a single nerve stimulus (quantal content) is rather elevated, the low EP amplitude indicates a reduced number of AChRs at the endplate. In patients with null mutations in *CHRNE*, single channel recordings of AChRs at the patient's endplates demonstrate low conductance and prolonged opening bursts, indicating expression of the fetal γ-AChR instead of the adult-type ε-AChR. The conductance of the adult-type ε-AChR is 80 pS, whereas that of the fetal γ-AChR is 60 pS. In patients with low-expressor mutations in either *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 dosages of anticholinesterases. Anticholinesterases inhibit the catalytic activity of AChE, which in turn increases the dwell time of ACh at the synaptic space and enables reassociation of ACh and AChRs. Anticholinesterases are effective in a wide range of diseases where the number of AChRs is reduced independent of its cause. Inadvertent or unexpected overdose of anticholineseterase, however, simulates endplate AChE deficiency [11,13,28]. In endplate AChE deficiency, the neuromuscular signal transduction is compromised by an excessive amount and prologed dwell time of ACh in the synaptic space, which in turn induces three pathomechanisms: (i) staircase summation of endplate potentials, (ii) excessive desensitization of AChRs, and (iii) endplate myopathy caused by excessive influx of extracellular calcium. The three molecular mechanisms are identical to those observed in the slow channel congenital myasthenic syndrome, as described in the following section.

Slow channel congenital myasthenic syndrome

The second class of CMS due to mutations in the AChR subunit genes is the slow channel congenital myasthenic syndrome (SCCMS) (Table 1). SCCMS is an autosomal dominant disorder, in which a gain-of-function mutation on a single allele compromises the neuromuscular signal transduction [1]. 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 the εL78P mutation minimally prolongs channel opening events and a mutant channel arising from a single allele is not sufficient to cause the disease phenotype [29]. In general, dominantly inherited disorders tend to develop after adolescence, because an individual carrying a mutant allele should get married and transmit the mutant allele to the next generation. In concordance with this notion, SCCMS tend to develop later in life and progresses slowly. Some patients with SCCMS, however, present early in life and become severely disabled even in the first decade.

Table 1. AChR mutations causing slow and fast channel syndromes.

Gene	Mutation	Domain	Reference
Slow channel syndrome			
CHRNA1	α1G153S	ECD	[2,30]
(AChR α1 subunit)	α1V156M	ECD	[30]
	α1N217K	M1	[31-33]
	α1S226Y	M1	[34]
	α1S226F	M1	[34,35]
	α1V249F	M2	[36]
	α1T254I	M2	[30]
	α1S269I	M2-M3 linker	[30]
	α1C418W	M4	[37]
CHRNB1	β1V229F	M1	[33,38]
(AChR β1 subunit)	β1L262M	M2	[39]
	β1V266M	M2	[31,33]
	β1V266A	M2	[40]
CHRND	δS268F	M2	[33,41]
(AChR δ subunit)			
CHRNE	εL78P	ECD	[29,42]
(AChR ε subunit)	εL221F	M1	[29,43]
	εI257F	M2	[44]
	εV259F	M2	[45]
	εV259L	M2	[46]
	εT264P	M2	[1]
	εV265A	M2	[47]
	εL269F	M2	[31,48]
Fast channel syndrome			
CHRNA1	α1V132L	ECD	[49]
(AChR α1 subunit)	α1F256L	M2	[50]
	α1V285I	M3	[51]
CHRND	δL42P	ECD	[52]
(AChR δ subunit)	δE59K	ECD	[53]
CHRNE	εP121L	ECD	[54]
(AChR ε subunit)	εP121T	ECD	[55]
	εD175N	ECD	[56]
	εN182Y	ECD	[56]
	ε1254ins18	LCP	[57]
	εA411P	LCP	[58]
	εN436del	LCP	[59,60]

ECD, extracellular domain; M1-M4, transmembrane domains 1 to 4; LCP, long cytoplasmic loop.

In SCCMS, neuromuscular transmission defects are caused by three distinct mechanisms. First, staircase summation of endplate potentials causes depolarization of the membrane potential. Prolonged depolarization makes the voltage-gated skeletal sodium channel less responsive to endplate potential generated by opening of AChR ion channels. Second, mutant AChRs somehow tend to be desensitized [36], which reduces the number of AChRs that respond to the released ACh quanta. Third,

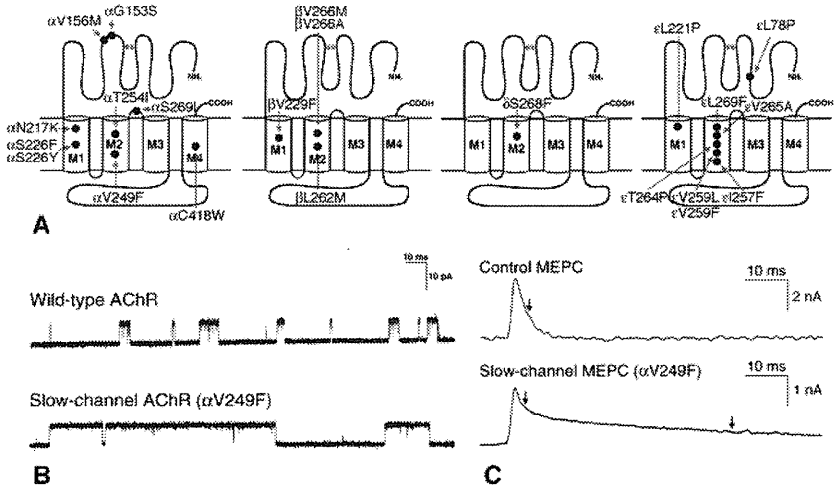

Figure 2. Slow channel syndrome. **(A)** Schematic diagram of AChR subunits with SCCMS mutations. **(B)** Single channel currents from wild-type and slow channel (α1V249F) AChRs expressed on HEK293 cells. **(C)** Miniature endplate current (MEPC) recorded from endplates of a control and a patient harboring α1V249F. The patient's MEPC decays biexponentially (arrows) due to expression of both wild-type and mutant AChRs.

prolonged opening of AChR causes excessive influx of extracellular calcium, which triggers the apoptosis pathway and gives rise to endplate myopathy [61]. In normal adult human ε-AChR, 7% of the synaptic current is carried by Ca^{2+}, which is higher than that in fetal human γ-AChR or muscle AChRs from other species [62]. This predisposes to endplate Ca^{2+} overloading when the channel opening events are prolonged. In addition, at least two SCCMS mutations, εT264P [1] and εV259F [45], increase the Ca^{2+} permeability 1.5- and 2-fold, respectively [63].

Slow channel mutations can be divided into two groups (Table 1). The first group includes mutations at the extracellular domain like α1G153S [2], as well as at the N-terminal part of the first transmembrane domain like α1N217K [32] and εL221F [43]. 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 [1] introduces a kink into the channel pore, whereas β1V266A [40] and εV265A [47] rather 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 α1V249F [36], εL269F [31], and εT264P [1].

SCCMS can be effectively treated with conventional dosages of long-lived open channel blockers of AChR, such as the antiarrhythmic agent quinidine [64,65] and the antidepressant fluoxetine [66]. Quinidine reduces the prolonged burst duration of SCCMS to the normal level at 5 μM [64]. 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

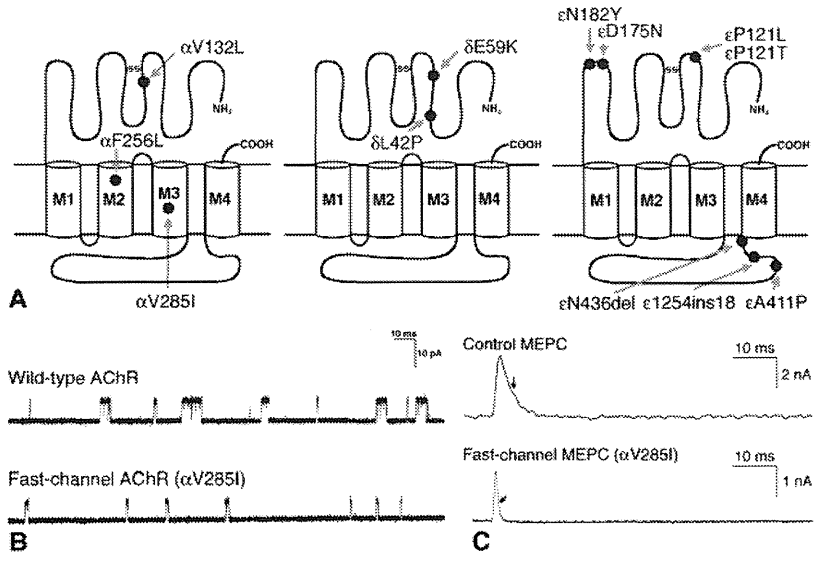

Figure 3. Fast channel syndrome. **(A)** Schematic diagram of AChR subunits with FCCMS mutations. **(B)** Single channel currents from wild-type and fast channel (α1V285I) AChRs expressed on HEK293 cells. **(C)** Miniature endplate current (MEPC) recorded from endplates of a control and a patient harboring α1V285I. The patient's MEPC decays faster than that of the normal control.

demonstrates significant effects [65]. 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 [66].

Fast channel congenital myasthenic syndrome

The third class of CMS due to mutations in AChR subunit genes is the fast channel congenital myasthenic syndrome (FCCMS) (Table 1). FCCMS is kinetically opposite to SCCMS (Fig. 3). In FCCMS, the AChR becomes resistant to be transferred to an open state and prematurely comes back to a closed state, which results in insufficient depolarization of the endplate potential. The resulting pathophysiology is thus similar to endplate AChR deficiency, but the cause of inefficient endplate depolarization is due to qualitative defects of AChRs but not to quantitative defects as in AChR deficiency.

FCCMS is an autosomal recessive disorder. One allele carries a missense mutation that confers a fast closure of AChRs, and the other allele usually harbors a low-expressor or null mutation. As in heterozygous healthy parents of endplate AChR deficiency, we humans may completely lack 50% of each AChR subunit without any clinical symptoms. In FCCMS, a low-expressor or null mutation on one allele unmasks kinetic abnormalities of a FCCMS mutation on the second allele. Detailed kinetic analyses of FCCMS mutations have unmasked yet uncharacterized molecular architectures of the AChR subunits. Three such examples are presented here.

ε1254ins18 causes a duplication of STRDQE codons at positions 413 to 418 close to the C-terminal end of the long cytoplasmic loop (LCP) linking the third (M3) and fourth (M4) transmembrane domains. ε1254ins18-AChR expressed on HEK293 cells opens in

three different modes. The opening probabilities of normal AChRs are clustered into a single large peak, whereas the ε1254ins18-AChR shows three different peaks [57]. In all the three modes, the AChR is activated slowly and inactivated rapidly, which gives rise to an inefficient synaptic response to ACh. Another FCCMS mutation, εA411P, in the LCP also destabilizes the channel opening kinetics. The channel opening probabilities of εA411P-AChRs are widely distributed and do not form any discernible peaks [58]. Our analysis first disclosed that the function of LCP is to stabilize the open conformation of the AChR.

εN436del is a deletion of Asn at the C-terminal end of the LCP. The deletion shortens the LCP and shifts a negatively charged Asp residue at codon 435 against M4. εN436del-AChR decreases the duration of channel opening bursts 2.7-fold compared to the wild type due to a 2.3-fold decrease in gating efficiency and a 2.5-fold decrease in agonist affinity of the diliganded closed state. A series of artificial mutations established that the effects of εN436del are not due to juxtaposition of a negative charge against M4 but to the shortening of the LCP. Deletion of the C-terminal residue of the LCP of the β1 and δ subunits also results in fast-channel kinetics, but that in the α1 subunit dictates slow-channel kinetics. Thus, the LCPs of four AChR subunits contribute in an asymmetric manner to optimize the activation of AChRs through allosteric links to the channel and to the agonist binding sites [59].

The mutation α1V285I introduces a bulky amino acid into the M3 transmembrane domain and causes FCCMS (Fig. 3). Kinetic studies demonstrate that the mutation slows the channel opening rate β and speeds the channel closing rate α, which gives rise to a 15.1-fold reduction in the channel gating equilibrium constant θ (= β/α). On the other hand, the mutation minimally affects affinity for ACh. The probability of channel openings decreased when we introduced Leu, a bulky amino acid, at position V285, but rather increased when we introduced smaller amino acids such as Thr and Ala. We observed similar effects when we introduced similar substitutions into the β1, δ, and ε subunits. Thus, introduction of bulky amino acids narrows the channel pore, while introduction of smaller amino acids widens the channel pore. Our analysis first disclosed that the M3 domain backs up the channel-lining pore that is composed by the M2 transmembrane domains and has sterochemical effects on channel gating kinetics [51].

FCCMS can be effectively treated with anticholinesterases and 3,4-diaminopyridine. The mechanism of action of anticholinesterases is described in the section devoted to endplate AChR deficiency. The drug 3,4-diaminopyridine blocks the presynaptic potassium channel, which slows the repolarization of the action potential delivered to the nerve terminal [67]. The enhanced nerve action potential stimulates the presynaptic voltage-gated P/Q-type and N-type Ca^{2+} channels and increases Ca^{2+} influx to the nerve terminal, which then enhances synaptotagmin and the SNARE complex to facilitate the fusion of ACh vesicles to the presynaptic membrane. This increases the amount of ACh released by a single nerve stimulus and enhances AChR channel openings.

Other phenotypes associated with AChR mutations and a single nucleotide polymorphism

Mutations or a single nucleotide polymorphism (SNP) in muscle nicotinic AChR subunits also give rise to phenotypes other than CMS.

The first phenotype is fetal akinesia deformation sequence (FADS). Mutations in the AChR subunit genes cause neuromuscular transmission defects in embryos and restrict

intrauterine movements. As human embryos use the fetal γ-AChR by 33 weeks of gestation [68], mutations in *CHRNG* [69,70], as well as in *CHRNA1* and *CHRND* [27], cause FADS.

The second phenotype is early onset myasthenia gravis [71]. Promiscuous expression of a set of self-antigens occurs in medullary thymic epithelial cells to impose T-cell tolerance and to provide protection against autoimmune disorders. The AChR αisubunit is one of those self-antigens. A SNP in the promoter region of *CHRNA1* compromises expression of the α1 subunit in thymic epithelial cells, which increases the chance of developing myasthenia gravis 2.01- to 2.35-fold in individuals carrying the SNP.

Conclusions

We addressed three types of CMS that are caused by mutations in the AChR subunit genes.

Congenital deficiency of endplate AChRs is caused by mutations in *CHRNA1*, *CHRNB1*, *CHRND*, and *CHRNE* encoding the AChR α1, β1, and δ and εsubunits, respectively. The mutations are classified into two groups. The first group includes mutations in *CHRNE* that nullify or significantly reduce the expression of the ε subunit. Patients survive with embryonic γ-AChR even when the adult-type ε-AChR is lacking. Null mutations in the other AChR subunit genes are likely to be fatal, which supports a general notion that we have no chance to identify mutations that result in lethal phenotypes. The second group of mutations includes missense mutations of *CHRNA1*, *CHRNB1*, and *CHRND*. These mutations compromise the expression level of the mutant subunit and/or the assembly of AChRs, but do not completely abolish the expression of AChRs. Differences between mutations in *CHRNE* and those in *CHRNA1*, *CHRNB1*, and *CHRND* are the tolerance to low expression of the affected subunit. As in autoimmune myasthenia gravis, endplate AChR deficiency is well controlled by anticholinesterases.

The slow channel congenital myasthenic syndrome (SCCMS) is an autosomal dominant disorder, in which a gain-of-function mutation causes prolonged AChR channel openings and increases the synaptic response to ACh. In SCCMS, neuromuscular transmission defects are caused by (i) staircase summation of endplate potentials, (ii) excessive desensitization of AChRs, and (iii) endplate myopathy caused by excessive influx of extracellular calcium. SCCMS mutations cause neuromuscular transmission defects either by increasing the affinity of AChR for ACh binding or by retarding the channel closing rate α and variably enhancing the channel opening rate β. SCCMS can be effectively treated with conventional dosages of long-lived open channel blockers of AChR, such as the antiarrhythmic agent quinidine and the antidepressant fluoxetine.

The fast channel congenital myasthenic syndrome (FCCMS) is caused by loss-of-function missense mutations in the AChR subunit genes. The mutations render the AChR resistant to be transferred to an open state and prematurely coming back to a closed state. Detailed kinetic analyses of FCCMS mutations have unmasked yet uncharacterized molecular architectures of the AChR subunits especially in the third transmembrane domain and in the long cytoplasmic loop. FCCMS can be effectively treated with anticholinesterases and 3,4-diaminopyridine.

Two more clinical phenotypes are associated with variations of the AChR subunit genes. Mutations in *CHRNG* encoding the AChR γ subunit cause another phenotype FADS by restricting intrauterine movement of an embryo. A SNP in the promoter region