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G. 知的所有権の取得状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

先天性筋無力症候群の診断・病態・治療法開発研究

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研究要旨　先天性筋無力症候群5例の臨床的および電気生理学的特徴、ならびに治療法について検討した。4例が乳児期に運動発達遅滞で発症し初発時は筋力低下、筋緊張低下が主症状であった。眼瞼下垂、眼球運動制限は新生児期発症例にのみ認めた。筋力変動は幼児期以降に明らかになった。筋力変動は間欠的または数日間持続する筋力低下を全例に認め、日内変動を呈したのは1例のみであった。副神経の反復神経刺激では評価した全例が減衰を示したが、正中神経、尺骨神経、後脛骨神経では筋力低下を呈していても減衰を認めない例があった。テンシロンテストでは眼瞼下垂が改善せず反復神経刺激での減衰率改善が判定に有用であった。診断まで平均6年を要しており診断に難渋した例が多かった。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。

A. 研究目的

先天性筋無力症候群(congenital myasthenic syndrome：以下CMS)は、神経筋接合部の先天的分子欠損により全身の筋力低下、易疲労性を呈し[1)2)]、生後1年以内に発症することが多い[3)]稀な疾患である。多くは近位筋優位の筋力低下に加えて外眼筋障害、眼瞼下垂を呈し自己免疫性の重症筋無力症や先天性ミオパチーと似た臨床像を呈する[4)5)]が、軽度の筋力低下のみを呈し眼瞼下垂を欠く例5)や成人発症例[2)3)6)]があり、診断に長期間を要する例が少なくない[2)7)]。欧米を中心にすでに200家系以上のCMSが報告されている[8)]が本邦での報告は我々の検索する限り4例[9)~12)]であり、未診断例が多い可能性がある。我々は日本人小児のCMS5例の臨床情報をもとに、他疾患との鑑別となる症状と電気生理学的特徴について検討した。

B. 研究方法

発症時期、臨床症状、自己抗体陰性、反復神経刺激(repetitive nerve stimulation：以下RNS)減衰からCMSを疑い、遺伝子解析にて病的な変異が判明した5例を対象とし、診療録に基づいて臨床的特徴について検討した。自己抗体は抗アセチルコリン受容体(acetylcholine receptor：以下AChR)抗体、抗muscle specific kinase(以下MuSK)抗体を検索した。反復神経刺激(repetitive nerve stimulation；RNS)は正中神経2例、尺骨神経3例、後脛骨神経3例、副神経3例を1,3,5,10Hzの刺激頻度で評価した(重複含む)。第1反応と最小振幅の波形を比較してその差が第1反応の10%以上あれば減衰現象陽性とした。テンシロンテストは投与後のcompound muscle action potential(以下CMAP)減衰率の改善または運動負荷の所要時間の短縮を陽性とし、症状の悪化を悪化とした。

（倫理面への配慮）

本研究は診療録などを用いて情報収集した後方視的研究であり、倫理委員会の申請は行っていない。

C. 研究結果

1.臨床的特徴

症例は男児4例、女児1例で研究時の年齢は6~19歳であった。新生児期発症3例(症例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。

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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

書籍

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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研究成果の刊行物・別刷

RNA Pathologies in Neurological Disorders

Kinji Ohno and Akio Masuda

Abstract RNA is not a simple intermediate linking DNA and protein. RNA is widely transcribed from a variety of genomic regions, and extensive studies on the functional roles and regulations of noncoding RNAs including antisense RNAs and small RNAs are in progress. In addition, the human genome project revealed that we humans carry as few as ~22,000 genes. Humans exploit tissue-specific and developmental stage-specific alternative splicing to generate a large variety of molecules in specific cells at specific developmental stages. Neurological disorders are also subject to aberrations of the splicing mechanisms. This review focuses mostly on splicing abnormalities due to pathological alterations of splicing *cis*-elements and *trans*-factors. Pathomechanisms associated with disrupted splicing *cis*-elements can be applied to any human diseases, and we did not restrict the descriptions to neurological diseases. On the other hand, we limited the descriptions of dysregulated splicing *trans*-factors to neurological disorders. Neurological diseases covered in this review include congenital myasthenic syndromes, spinal muscular atrophy, myotonic dystrophy, Alzheimer's disease, frontotemporal dementia with Parkinsonism linked to chromosome 17, facioscapulohumeral muscular dystrophy, fragile X-associated tremor/ataxia syndrome, Prader–Willi syndrome, Rett syndrome, spinocerebellar atrophy type 8, and paraneoplastic neurological disorders.

Keywords The RNA world · Pre-mRNA splicing · Splicing *cis*-elements · Splicing *trans*-factors · Branch point sequence (BPS) · Exonic splicing enhancer (ESE) · Exonic splicing silencer (ESS) · Intronic splicing enhancer (ISE) · Intronic splicing silencer (ISS) · Nonsense-mediated mRNA decay (NMD) · Nonsense-associated skipping of a remote exon (NASRE) · Congenital myasthenic syndromes · Spinal muscular atrophy (SMA) · Myotonic dystrophy (DM1, DM2) · Alzheimer's disease· Frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) ·

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Facioscapulohumeral muscular dystrophy (FSHD) · Fragile X-associated tremor/ataxia syndrome (FXTAS) · Prader–Willi syndrome, Rett syndrome · Spinocerebellar atrophy type 8 (SCA8) · Paraneoplastic neurological disorders (PND)

Contents

1 Introduction

The central dogma first enunciated by Francis Crick depicts RNA as an intermediate that links DNA and protein (Crick, 1970). The beginning of life, however, was the RNA world where there were no DNA or proteins (Gilbert, 1986). In the RNA world, RNA was the only carrier of genetic information that DNA currently serves as, and the only functional molecule that proteins currently serve as. Although the RNA transmits no genetic information to progeny and constitutes a limited number of functional molecules in our human body, the RNA world is still in effect in our body. Humans transcribe more than half of our entire genome including noncoding regions. The transcripts work as *antisense RNAs, microRNAs, and snoRNAs*. Researchers are now working to disclose the functional significance of these noncoding RNAs.

The human genome project and the subsequent annotation efforts revealed that we humans carry as few as 22,000 genes. Tissue-specific and developmental stage-specific splicing enables to us to generate more than 100,000 molecules from a limited number of genes (Black, 2003; Licatalosi and Darnell, 2006). Small RNA molecules and RNA splicing mechanisms potentially become targets of neurological diseases (Ranum and Cooper, 2006). This review focuses mostly on splicing aberrations associated with neurological disorders.

2 Physiology of Splicing Mechanisms

In higher eukaryotes, pre-mRNA splicing is mediated by degenerative splicing *cis*-elements comprised of the branch point sequence (BPS), the polypyrimidine tract (PPT), the 5′ and 3′ splice sites, and exonic/intronic splicing enhancers/silencers (Fig. 1). Stepwise assembly of the spliceosome starts from recruitment of *U1 snRNP* to the 5′ splice site, *SF1* to the BPS, *U2AF65* to the PPT, and *U2AF35* to the 3′ end of an intron to form a spliceosome complex E (Sperling et al., 2008). SF1, a 75 kDa protein, is a mammalian homologue of yeast BBP (branch point-binding protein). U2AF65 and U2AF35 bring *U2 snRNP* to the BPS in place of SF1 (Wu et al., 1999; Zorio and Blumenthal, 1999). The BPS establishes base pairing interactions with a stretch of "GUAGUA" of U2 snRNA (Arning et al., 1996; Abovich and Rosbash, 1997), which then bulges out the branch site nucleotide, usually an adenosine to form a spliceosome complex A (Query et al., 1994). Thereafter, pre-mRNAs are spliced in two sequential transesterification reactions mediated by the spliceosome. In the first step, the 2′-OH moiety of the branch site nucleotide carries out a nucleophilic attack against a phosphate at the 5′ splice site, generating a free upstream exon, as well as a lariat carrying the intron and the downstream exon. In the second step, the 3′-OH moiety of the upstream exon attacks the 3′ splice site of the

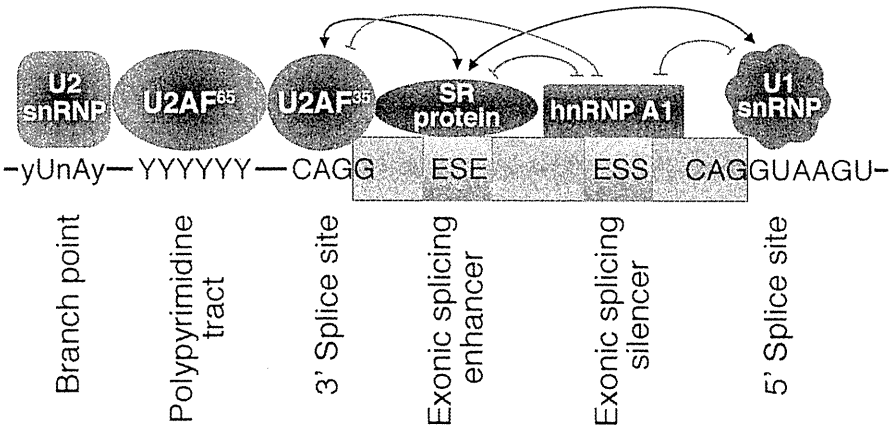

Fig. 1 Representative splicing *cis*-elements and *trans*-factors. Tissue-specific and developmental stage-specific expressions of splicing *trans*-factors including SR proteins and hnRNP A1 enable precise regulations of alternative splicing. ISE and ISS have similar activities as ESE and ESS, but are omitted from the figure

lariat leading to intron excision and ligation of the upstream and downstream exons (Query et al., 1996).

In addition to the "classical" spliceosomal mechanisms, splicing is modulated by exonic/intronic splicing enhancers/silencers (ESE, ISE, ESS, ISS). The *trans*-factors for the splicing enhancers/silencers carry repeats of arginine and serine are accordingly called SR proteins. Tissue-specific and developmental stage-specific expressions of the splicing *trans*-factors enable precise spatial and temporal regulations of the gene expressions. In addition, the splicing *trans*-factors also work on constitutively spliced exons to compensate for highly degenerative "classical" splicing *cis*-elements.

3 Disorders Associated with Disruption of Splicing *Cis*-Elements

3.1 Aberrations of the 5′ Splice Sites

Mutations disrupting the 5′ splice sites have been most frequently reported. U1 snRNA recognizes three nucleotides at the end of an exon and six nucleotides at the beginning of an intron (Fig. 2). The completely matched nucleotides to U1 snRNA are CAG|GTAAGT, where the vertical line represents the exon/intron boundary. The completely matched sequence is observed at 1597 sites out of the entire 189,249 5′ splice sites in the human genome (Sahashi et al., 2007), which is the tenth most common sequence. The completely matched 5′ splice site is rather avoided because, in the second stage of splicing, U1 snRNA is substituted for U5 snRNA. If U1 snRNA is tightly bound to the 5′ splice site, it hinders binding of U5 snRNA.

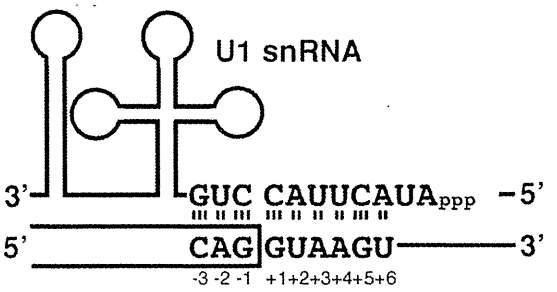

Fig. 2 U1 snRNA recognizes three nucleotides at the 3′ end of an exon and six nucleotides at the 5′end of an intron

Degeneracy of the 5′ splice site and its vulnerability to disease-causing mutations have been extensively studied. Three algorithms have been proposed. First, Shapiro and Senapathy collated nucleotide frequencies at each position of the 5′ splice site. They assumed that nucleotide frequencies at each position of the 5′ splice site represent the splicing signal intensity. They thus constructed a linear regression model so that the most preferred 5′ splice site becomes 1.0 and the most unfavorable 5′ splice site becomes 0.0 (Shapiro and Senapathy, 1987). Second, Rogan and Schneider

invented the information contents, R*i*. For example, at a specific position, if a single nucleotide is exclusively used, the information content at this position becomes $-\log_2(1/4) = 2$ bits. Similarly, if two nucleotides are equally used, the information content becomes $-\log_2(2/4) = 1$ bit. In R*i*, the similarity to the consensus sequence is represented by the sum of information bits (Rogan and Schneider, 1995; O'Neill et al., 1998). Third, we found that a new parameter, the SD-Score, which represents a common logarithm of the frequency of a specific 5′ splice site in the human genome, efficiently predicts the splicing signal intensity (Sahashi et al., 2007).

Our algorithm predicts the splicing consequences of mutations with the sensitivity of 97.1% and the specificity of 94.7%. Simulation of all the possible mutations in the human genome using the SD-score algorithm predicts high frequencies of splicing mutations from exon –3 to intron +6 (Table 1). Especially at exon position –3, about one third of mutations are predicted to cause aberrant splicing. Using our algorithm, we predicted and proved that *DYSF* G1842D in Miyoshi myopathy, *ABCD1* R545W in adrenoleucodystrophy, *GLA* Q333X in Fabry disease, and *DMD* Q119X and Q1144X in Duchenne muscular dystrophy are not missense or nonsense mutations but are splicing mutations. Algorithms by us and by others all point to the notion that aberrant splicing caused by mutations at the 5′ splice sites is likely to be underestimated.

Table 1 Predicted ratios of exonic and intronic splicing mutations

Position	–3	–2	–1	+1	+2	+3	+4	+5	+6
Complementary nucleotide	C (%)	A (%)	G (%)	G	T	A (%)	A (%)	G (%)	T (%)
A	1.8	–	93.7	–	–	–	–	93.9	56.9
C	–	89.6	99.7	–	–	99.9	94.4	98.6	75.4
G	35.0	90.5	–	–	–	48.7	96.2	–	56.7
T	76.7	86.2	97.1	–	–	99.9	94.3	97.0	–
All mutations	37.8	88.8	96.8	–	–	82.8	95.0	96.5	63.0

3.2 Human Branch Point Consensus Sequence

In an effort to seek an algorithm to predict the position of the branch point sequence (BPS) in humans, we sequenced 367 clones of lariat RT-PCR products arising from 52 introns of 20 human housekeeping genes and identified that the human consensus BPS is simply yUnAy, where "y" represents U or C (Gao et al., 2008) (Fig. 3). The consensus BPS was more degenerative than we had expected and we failed to construct a dependable algorithm that predicts the position of the BPS. Sixteen disease-causing mutations and a polymorphism, however, have been reported to date that disrupt a BPS and cause aberrant splicing (Gao et al., 2008). Among these, eight mutates U at position –2, whereas nine affects A at position 0, which also supports the notion that U at –2 and A at 0 are essential nucleotides.

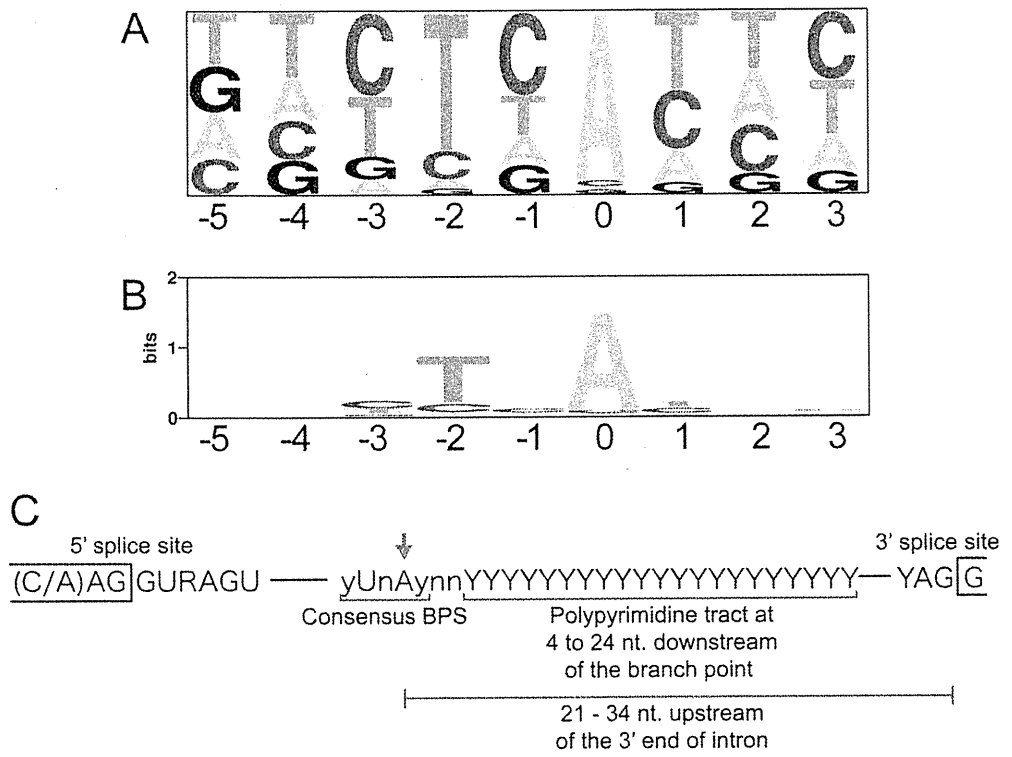

Fig. 3 Human consensus BPS. (**a**) Pictogram and (**b**) WebLogo presentations of BPS. Position 0 represents the branch point. (**c**) Representative sequences and positions of splicing *cis*-elements

3.3 Ectopic AG Dinucleotide Abrogates the AG-Scanning Mechanism

The 3′ end of an intron and the 5′ end of an exon carry a consensus sequence of CAG|G, where the vertical line represents the intron/exon boundary. The AG dinucleotide is scanned from the branch point and the first AG is recognized as the 3′ end of the intron (Chen et al., 2000). In a patient with congenital myasthenic syndrome, we identified duplication of a 16-nt segment comprised of 8 intronic and 8 exonic nucleotides at the intron 10/exon 10 boundary of *CHRNE* encoding the acetylcholine receptor epsilon subunit (Ohno et al., 2005). We found that the upstream AG of the duplicated segment is exclusively used for splicing and that one or two mutations in the upstream BPS had no effect whereas complete deletion of the upstream BPS partially activated the downstream AG. Similar exclusive activation of the upstream AG is reported in *HEXB* (Dlott et al., 1990) and *SLC4A1* (Bianchi et al., 1997). Creation of a cryptic AG dinucleotide close to the 3′ end of an intron should be carefully scrutinized in mutation analysis.

3.4 Mutations That Disrupt ESE *and* ESS

Gorlov and colleagues predicted that more than 16–20% of missense mutations are splicing mutations that disrupt an ESE (Gorlov et al., 2003). According to our own

experience, their estimates are likely to be too high. Most ESE/ESS-disrupting mutations, however, are likely to be underestimated, because the positions and sequences of ESE/ESS are highly degenerative.

Four Web services provide valuable information to locate ESE and ESS. First, the ESE Finder (http://rulai.cshl.org/ESE/) calculates the similarity of a given nucleotide sequence to the consensus sequences of four splicing *trans*-factors, SF2/ASF, SC35, SRp40, and SRp55 (Cartegni et al., 2003; Smith et al., 2006). Second, the RESCUE-ESE Web server (http://genes.mit.edu/burgelab/rescue-ese/) shows the similarity of a given sequence to ESE elements of unidentified splicing *trans*-factors (Fairbrother et al., 2002). The same group also provides the FAS-ESS Web service to screen for ESS elements (http://genes.mit.edu/fas-ess/) (Wang et al., 2004). Third, the PESX Web server (http://cubweb.biology.columbia.edu/pesx/) indicates an RNA octamer with putative exonic splicing enhancing or silencing activities (Zhang and Chasin, 2004; Zhang et al., 2005). Fourth, the ESRsearch Web server (http://ast.bioinfo.tau.ac.il/) shows 285 candidate ESE/ESS sequences (Goren et al., 2006), as well as ESE/ESS elements indicated by the RESCUE-ESE, FAS-ESS, and PESX services.

In patients with congenital myasthenic syndromes, we identified that *CHRNE* E154X and EF157V (Ohno et al., 2003), as well as *COLQ* E415G (Kimbell et al., 2004), disrupt an ESE and cause aberrant splicing. The ESE/ESS servers above indicate disruption of candidate splicing *cis*-elements for all three mutations, but we frequently obtain false positives and we cannot simply rely on the servers. Analysis of patient mRNA or analysis using a minigene is generally expected.

3.5 Mutations That Disrupt ISE *and* ISS

Identification of mutations disrupting intronic splicing *cis*-elements is more challenging than that of exonic mutations, because introns are longer than exons and splicing mutations can be anywhere in the introns, and because we do not have a dependable algorithm to predict ISE/ISS. The ESRsearch Web server described above is able to indicate consensus sequences recognized by a variety of splicing *trans*-factors including intronic ones.

In a patient with congenital myasthenic syndrome, we identified that *CHRNA1* IVS3-8G>A attenuates binding of *hnRNP H* ~100-fold and causes exclusive inclusion of the downstream exon P3A (Masuda et al., 2008) (Fig. 4). We also identified that polypyrimidine tract binding protein (PTB) silences recognition of exon P3A and tannic acid facilitates the expression of PTB by activating its promoter region (Gao et al., 2009).

3.6 Spinal Muscular Atrophy (SMA)

SMA is an autosomal recessive disorder characterized by degeneration of the anterior horn cells of the spinal cord, which causes muscular weakness and atrophy. SMA is caused by loss-of-function mutations including deletion of the *SMN1* gene