kit (Promega, Madison, WI) were incubated with 50 μl of the stock Sepharose G at 4°C for 2 h in the presence of 2 μg of either E-20 or C-15 (Santa Cruz Biotechnology, Santa Cruz, CA). Antibody-beads complex was collected by centrifugation and eluted with PBS containing 1% SDS at 65°C for 10 min. Presence of recombinant ADAR2a and FLAG-ADAR2a proteins in the eluate were verified with western blotting.

Because E-20 more effectively bound to recombinant ADAR2a and Flag-ADAR2a proteins than C-15 (Fig. 1a), we used only E-20 for the immunoprecipitation of nuclear eluate. One ml of the nuclear eluate obtained from 0.75 g of human cerebellum was incubated at 4°C for 2 h in the presence of 2 μg of E-20 or control goat anti-human IgG (H + L) (Jackson ImmunoResearch, West Grove, PA) after pre-clearing once with 50 μl of the untreated Sepharose G suspension in PBS, and then for another h with additional 50 μl of stock Sepharose G beads. The nuclear eluate-antibody-bead complex was collected by centrifugation and eluted with 50 μl of PBS containing 1% SDS at 65°C for 10 min and then the eluate was treated with proteinase-K at 37 °C for 60 min. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA).

2.3. RNAi of ADAR1 and ADAR2

HeLa cells were cultured in MEMα (Wako, Osaka, Japan), and then in Opti-MEM I Reduced-Serum Medium (GIBCO, Langley, OK) without fetal bovine serum (FBS) or antibiotics, containing 30 nM of one of the small interference RNA (siRNA) listed in Supple. Table S1 (Qiagen HP GenomeWide siRNAs; Qiagen, Valencia, CA) and Lipofectamine RNAiMAX (1:600; Invitrogen) (Forward Transfection). The

following siRNAs were used: siR1a and siR1b were used to target human ADAR1, and siR2a and siR2b to target human ADAR2. Cells cultured in Opti-MEM containing 30 nM ALLStars Negative Control siRNA (siR n/c; Qiagen) were used as the negative control. After 5 h of incubation, the medium was switched back to the original MEMα. Total RNA was extracted 96 h after the administration of siRNAs using an RNA spin Mini RNA Isolation kit (Qiagen), and reverse transcription (RT)-polymerase chain reactions (PCRs) were carried out (Supple. Table S1, S2).

2.4. Analyses for extents of A-to-I editing sites

Editing efficiencies at the A-to-I editing sites in GluR2, kv1.1 and CYFIP2 mRNAs were calculated by quantitative analyses of the digests of PCR products with restriction enzymes (Kawahara et al., 2003; Kawahara et al., 2004; Bhalla et al., 2004). The PCR products for GluR2 and kv1.1 were cleaved by *Bbv*I (New England Biolabs, Ipswich, MA) and *Mfe*I (New England Biolabs), respectively, and those for CYFIP2 were cleaved by *Mse*I (New England Biolabs). Relative amounts of the resulting digests of PCR products were analyzed with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). The extent of GluR2 Q/R site-editing was calculated as the ratio of the molarity of the 129-bp band (derived from edited GluR2 mRNA) to the sum of the 129- and 91-bp bands (derived from unedited GluR2 mRNA). Similarly, the extent of kv1.1 I/V site-edtiting was calculated as the ratio of the molarity of the 117-bp band (derived from edited kv1.1 mRNA) to the sum of the 117- and 66-bp bands (derived from unedited kv1.1 mRNA). The extent of CYFIP2 K/E site-editing was calculated as the ratio of the molarity of the 216-bp band (derived from edited CYFIP2 mRNA) to the sum of those of both the 216- and 60-bp bands (derived from unedited CYFIP2 mRNA).

Editing efficiencies at the A-to-I(G) editing sites in FLNA and BLCAP mRNAs were evaluated by sequencing the PCR products with a 3100 Genetic Analyzer sequencer (Applied Biosystems, Faster City, CA). Expression level of CYFIP2 mRNA was measured using a LightCycler System (Roche Diagnostics, Mannheim, Germany). The internal standards, primers, and probes for quantitative PCR for ADAR1, ADAR2 and CYFIP2 were designed as previously described (Kawahara et al., 2003) (Supple. Table S1).

2.5. ADAR2 mRNA expression in human tissues

Quantitative PCR was performed using a LightCycler System (Roche Diagnostics, Indianapolis, IN), as described previously (Kawahara et al., 2003). Each cDNA human tissue sample was amplified in a reaction mixture (20 μl total volume) composed of 10 μl of 2× LightCycler 480 Probes Master Roche (Roche Diagnostics), 0.5 μM each primer, 0.1 μM probes (Universal ProbeLibrary Set, Human, #42 for ADAR2 and #64 for β-actin, Roche Diagnostics) (Supple. Table S1).

2.6. Statistics

Differences between groups were evaluated using Student's *t*-tests. Differences were considered statistically significant at $P<0.05$.

3. Results and discussion

mRNAs co-precipitated with ADAR2 in human brain

To identify mRNAs with ADAR2-mediated editing sites, we analyzed the

ADAR2-immunoprecipitates of human cerebellum. First we confirmed that recombinant ADAR2 and Flag-ADAR2 proteins could be successfully immunoprecipitated with the E-20, and to a lesser extent with the C-15 antibodies against human ADAR2 peptides (Fig. 1a). As a second control of the method, immunoprecipitation using the E-20 antibodies was performed on nuclear extract of human cerebellum, and GluR2 and kv1.1 mRNAs, which were already known to have ADAR2-mediated editing sites, were selectively amplified from the RNA fraction of the complexes with RT-PCR (Fig. 1b; upper, middle). Thus, the natural substrates for ADAR2 should also be immunoprecipitable from human brain.

We found that CYFIP2 and FLNA mRNAs, but not BLCAP or IGFBP7 mRNAs, were specifically recovered from the ADAR2-precipitate (Fig. 1c, d), suggesting that CYFIP2 and FLNA mRNAs should have ADAR2-mediated editing sites. Then, we analyzed the A-to-I editing sites in kv1.1, CYFIP2, FLNA and GluR2 mRNAs extracted from cerebellar tissues and their ADAR2-immunoprecipitates. All CYFIP2 mRNAs recovered from the immunoprecipitates had U(G) instead of A at the predicted A-to-I editing position (K/E site) in genomic DNA (Levanon, E.Y. et al., 2005), whereas 84% of the mRNA in the human cerebellar tissue was edited at this site (Fig. 2c, d). On the other hand, the previously reported A-to-I editing position (Q/R site) of FLNA mRNA (Levanon, E.Y. et al., 2005) was edited to only 51% in the immunoprecipitate, and not to a detectable level in human cerebellum (Fig. 2e, f). In addition, the I/V site of kv1.1 mRNA was edited to a greater extent (36%) in the immunoprecipitate than in the cerebellar tissue (20%) (Fig. 2a, b), although the Q/R site of GluR2 mRNA was fully edited in both the immunoprecipitate (data not shown) and the human cerebellum (Kawahara et al., 2003). These results suggest that ADAR2 seems to bind

predominantly to these mRNAs with ADAR2-mediated editing positions, more efficiently when these sites were edited than when unedited (Fig. 2 a, c, e vs. b, d, f).

Editors at A-to-I positions in ADAR2-associated mRNAs

To determine whether ADAR2 specifically edits the putative editing sites of CYFIP2 and FLNA mRNAs but not BLCAP or IGFBP7 mRNAs, we investigated changes in the extent of RNA editing at these sites after knockdown of ADAR1 and ADAR2 using siRNAs (Supple. Table S1) in HeLa cells. However, because the Q/R site in FLNA mRNA was not edited at all in HeLa cells (data not shown), and because the base sequence around the K/R site of IGFBP7-derived cDNA was GC-rich and was not suitable for sequencing analysis, we could determine the effects of siRNAs only at the K/E site of CYFIP2 and at the Y/C site of BLCAP mRNAs. Applications of siR1a and siR1b, to target ADAR1, and siR2a and siR2b, to target ADAR2, specifically and significantly decreased the copy numbers of ADAR1 and ADAR2 mRNAs, respectively (Fig. 3a, b). In addition, extent of GluR2 Q/R site-editing was markedly decreased to nearly 0% after ADAR2 knockdown, but not significantly decreased after ADAR1 knockdown (data not shown), indicating that the knockdown of ADAR2 by this method sufficiently suppressed ADAR2 activity but not ADAR1 activity. Although the effects of ADAR1 knockdown could not be tested on natural substrates, previous studies have demonstrated that ADAR1 activity was significantly decreased with a similar method (Wong & Lazinski, 2002). The editing efficiencies at the CYFIP2 K/E site in HeLa cells were significantly reduced after applications of siR2a and siR2b (both 0.0±0.0%), and also, but to a lesser extent, after siR1a and siR1b, as compared to those after the application of siR n/c or normal culture medium alone (Fig. 3c). The results suggested

that ADAR2 played a major role in RNA editing at this site, and ADAR1 may also have taken a part (Fig. 3b, c). On the other hand, sequence analysis of BLCAP mRNA-derived cDNA indicated that editing extent of the Y/C site of BLCAP mRNA was significantly lower after the application of ADAR1 siRNAs than after ADAR2 siRNAs or contol siRNA (siR n/c and normal culture medium alone) (Fig. 3d). These results suggest that the Y/C site of BLCAP mRNA was edited predominantly with ADAR1 (Fig. 3d). Together with the results of immunoprecipitation (Fig. 1b-d), it seems likely that only mRNAs that have ADAR2-mediated editing positions form a complex with ADAR2.

The majority of A-to-I editing sites were localized in the non-coding regions particularly in the repetitive sequences within *Alu* and long interspersed element (LINE) sequences (Levanon, K. et al., 2005) but the editors responsible for these sites have been only sporadically demonstrated (Kawahara et al., 2007). BLCAP mRNA has a long dsRNA hairpin structure (Levanon, E.Y. et al., 2005) similar to *Alu* sequence, which suggests ADAR1 as a possible editor of A-to-I editing sites frequently seen in *Alu* sequence.

ADAR2 specifically catalyzes GluR2 Q/R site-editing in mammalian and human brains, and the reduction of its editing activity may play a causative role in death of motor neurons in sporadic ALS patients (Kwak & Kawahara, 2005). Therefore knowledge about the regulatory mechanism of ADAR2 activity may promote our understanding of ALS pathogenesis. However, GluR2 Q/R site-editing is always complete in neurons, and is somewhat reduced only when the expression of ADAR2 mRNA is reduced to a certain threshold level in human white matter (Kawahara et al., 2003), hence editing extents at this site may remain 100% when the reduction of

ADAR2 activity was mild. On the other hand, CYFIP2 mRNA is ubiquitously expressed with the K/E site edited to variable extents among human tissues, especially abundant in the nervous system and lymphocytes (Mayne et al., 2004; Affymetrix HG-U133A: 215785_s_at). In this study, editing efficiencies at the CYFIP2 K/E site were 80.3 ± 6.0% in the cerebral cortex, 85.0 ± 8.4% in the cerebellum, 28.8% in the spinal cord, 14.5% in the spleen, 5.1% in the heart, and less than 1.5% in the other tissues. In addition, we found that the expression level of ADAR2 mRNA relative to that of β-actin mRNA was higher in the cerebral cortex (2.49×10^{-2}) and the cerebellum (2.30×10^{-2}) with high CYFIP2 K/E site-editing efficiency than in tissues with low CYFIP2 K/E site-editing efficiency (from 6.12×10^{-3} in the lung to 1.66×10^{-3} in the tonsil) (Fig. 4). However, CYFIP2 K/E site-editing was inactive in the leukocytes and the lung in which ADAR2 mRNA expression was higher than in the spinal cord and the spleen where CYFIP2 K/E site-editing was active (Fig. 4).

Consistent with the results of the knockdown experiment, there was a rough correlation between the CYFIP2 K/E site-editing and ADAR2 mRNA expression. However, this correlation was less clear in some tissues such as leukocyte, suggesting the participation of certain unidentified tissue-specific ADAR2-regulatory factor(s) other than the expression level of ADAR2 mRNA. Indeed, ADAR2 mRNA-self-editing produced an mRNA isoform with premature stop codon in rat and mouse (Rueter et al., 1999, Feng et al., 2006), and protein-coding ADAR2 mRNA variants accounted for a small proportion among more than 48 splicing variants in human cerebellum (Kawahara et al., 2005). These results indicate that the total amount of ADAR2 mRNA may not directly correlate with that of active ADAR2 proteins. Furthermore expression level of ADAR1 may influence the ADAR2 activity by forming inactive heterodimers with

ADAR2 (Cenci et al., 2008).

Extent of editing at the CYFIP2 mRNA K/E site, a novel ADAR2-mediated A-to-I editing site, might become a potential marker for ADAR2 activity and hence a potential tool for ALS research.

Acknowledgement

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4. Figure legends

Fig 1. Immunoprecipitated ADAR2 protein complex specifically contains substrates with selective editing sites.

(a) Western blot analysis of eluates immunoprecipitated with anti-human ADAR2 polyclonal antibodies, E-20 (lanes 2, 6) or C-15 (lanes 3, 7), containing recombinant Flag-ADAR2a (lanes 2-4) or ADAR2a proteins (lanes 6-8) synthesized *in vitro*. Goat anti-human IgG (H+L) was used as a precipitating control (lanes 4, 8). Untreated recombinat Flag-ADAR2a (lane 1) and ADAR2a proteins (lane 5) are also shown. Flag-ADAR2a (arrowhead) and ADAR2a proteins (arrow) were immunoprecipitated more effectively by E-20 than by C-15 or control goat anti-human IgG. Total RNA was extracted from the eluate of immunoprecipitate (IP) with E-20 and that of control IgG.

(b-d) RT-PCR conducted on these eluates demonstrated mRNAs of GluR2 (b) and kv1.1 (b) (known to have ADAR2-mediated editing sites) as well as those of CYFIP2 (cytoplasmic fragile X mental retardation protein interacting protein 2) (c) and FLNA (filamin A) (c) in the IP with E-20 (lane 1) but not that with control goat anti-human IgG (lane 2). The mRNA of β-actin demonstrated in total RNA extracted from the human cortex (b, lane 3) was not detected in either of the eluates (b). Moreover, both the mRNAs of BLCAP (bladder cancer-associated protein) and IGFBP7 (insulin-like growth factor binding protein 7) demonstrated in total RNA extracted from the human cerebellum (d, lane 4) were not detected in either of the eluates (d).

Fig 2. Extent of editing in situ and in ADAR2-immunoprecipitates

The editing efficiency at the I/V site of kv1.1 mRNA was calculated as the ratio of the

molarity of the 117-bp band (derived from edited kv1.1 mRNA) to the sum of those of both 117- and 66-bp bands (derived from unedited kv1.1 mRNA) after digestion of the PCR products with *MfeI*. Similarly, the editing efficiency at the K/E site of CYFIP2 mRNA was calculated as the ratio of the molarity of the 216-bp band (derived from edited CYFIP2 mRNA) to the sum of those of both the 216- and 60-bp bands (derived from unedited CYFIP2 mRNA) after digestion of the PCR products with *MseI*.

(a, c, e) In the cerebellar tissue, extent of RNA editing was 20% at the kv1.1 I/V site (a), 84% at the CYFIP2 K/E site (c), and 0% at the FLNA Q/R site (G in e; arrowhead).

(b, d, f) In the ADAR2-immunoprecipitates, on the other hand, extent of RNA editing was 36% at the kv1.1 I/V site (b), 100% at the CYFIP2 K/E site (d) and 51% at the FLNA Q/R site (G in f; arrowhead). LM; lower marker, HM; higher marker. Each value is the mean of two samples.

Fig 3. Knockdown of ADAR1 and ADAR2

(a) The amount of ADAR1 mRNA relative to that of β-actin mRNA in HeLa cells was significantly decreased following knockdown treatment with siR1a and siR1b (1, 2) but not with siR2a or siR2b (3, 4).

(b) The amount of ADAR2 mRNA relative to that of β-actin mRNA in HeLa cells was significantly decreased following knockdown treatment with siR2a and siR2b (3, 4), compared to with siR1a or siR1b (1, 2).

(c) The editing efficiencies at the K/E site of CYFIP2 mRNA in HeLa cells were markedly decreased after ADAR2 knockdown (siR2a and siR2b; both 0.0±0.0%), and to a lesser extent after ADAR1 knockdown (siR1a; 1.1±0.2%, siR1b; 0.4±0.5%), than

control siRNA (siR n/c; 2.1±0.2%, normal medium alone; 1.9±0.5%). Each bar represents the value after administration of siRNA (mean±SD; n=3). Bar 1, siR1a; Bar 2, siR1b; Bar 3, siR2a; Bar 4, siR2b; Bar 5, siR negative control (n/c); Bar 6, no siR. (Student's *t*-test, $*P < 0.05$ and $**P<0.01$ vs siR n/c and $\dagger\dagger P<0.01$ between bars as shown).

(d) The sequence chromatography of the peaks at the Y/C site of BLCAP mRNA after the application of siR n/c (negative control; left), siR1a (ADAR1 knockdown; middle) or siR2a (ADAR2 knockdown; right). These demonstrate the lower peak of G at the Y/C site of BLCAP mRNA (arrows) after ADAR1 knockdown (siR1a; 2%, siR1b; 0%) than after ADAR2 knockdown (siR2a; 18%, siR2b; 16%) or control siRNA (siR n/c; 17%, normal medium alone; 13%). Each value is calculated as the mean of two samples.

Fig4. CYFIP2 mRNA-editing extent and ADAR2 mRNA expression level in human tissues

Tissues with high expression levels of ADAR2 mRNA in the β-actin mRNA base (black columns) tend to show higher extent of RNA editing at the CYFIP2 K/E site (gray columns) than those with low ADAR2 mRNA expression level, whereas some regions with high ADAR2 mRNA expression level (eg. leukocytes) showed very low extents of CYFIP2 mRNA-editing. (Value represents the mean±SD for multiple samples of cerebral cortex (n=4), cerebellum (n=5) and leukocytes (n=7).)

Fig.1

Nishimoto et al., Determination of editors at the novel A-to-I editing positions, Fig.1

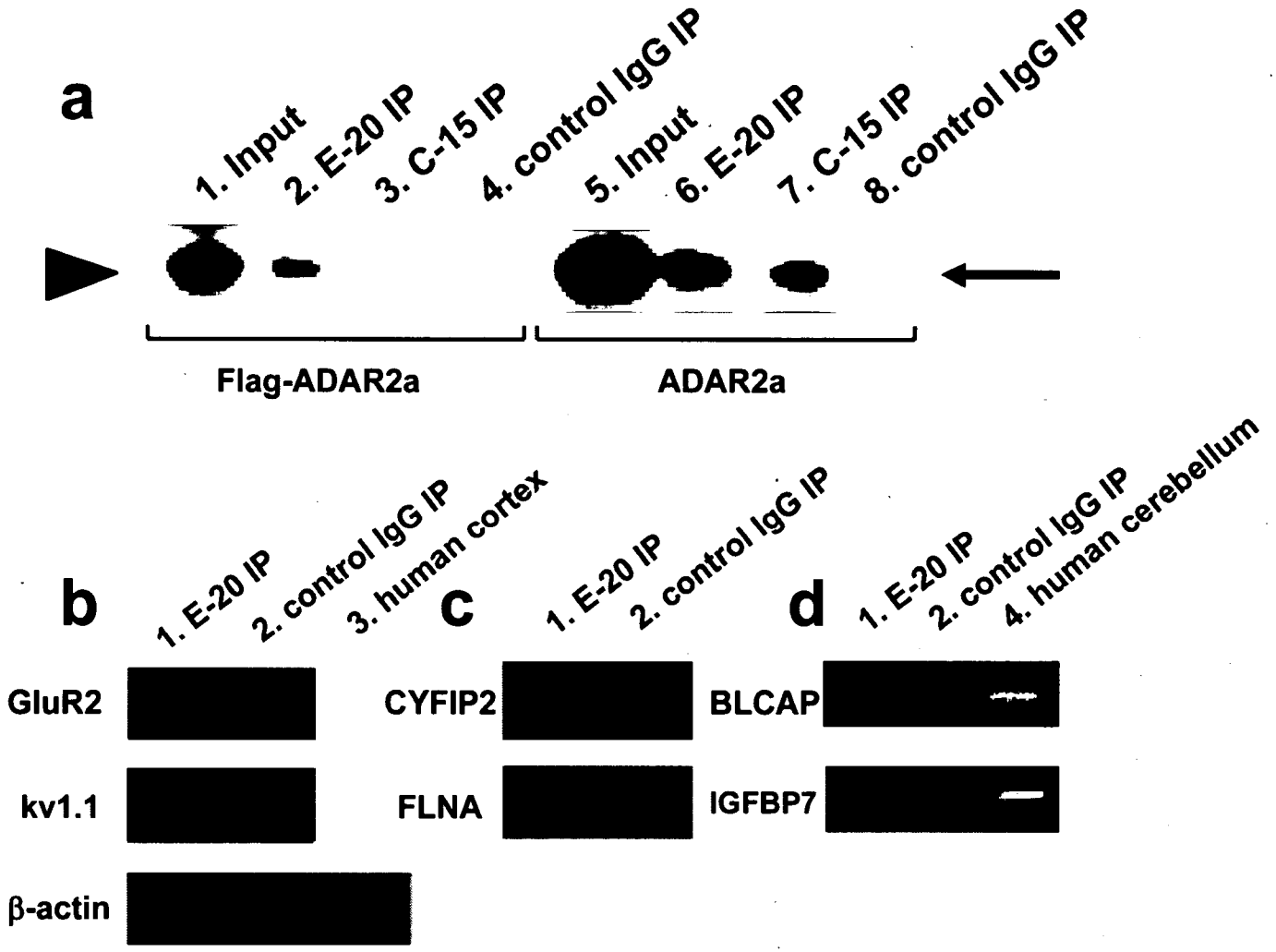


figure 2

Fig.2

Nishimoto et al., Determination of editors at the novel A-to-I editing positions, Fig.2

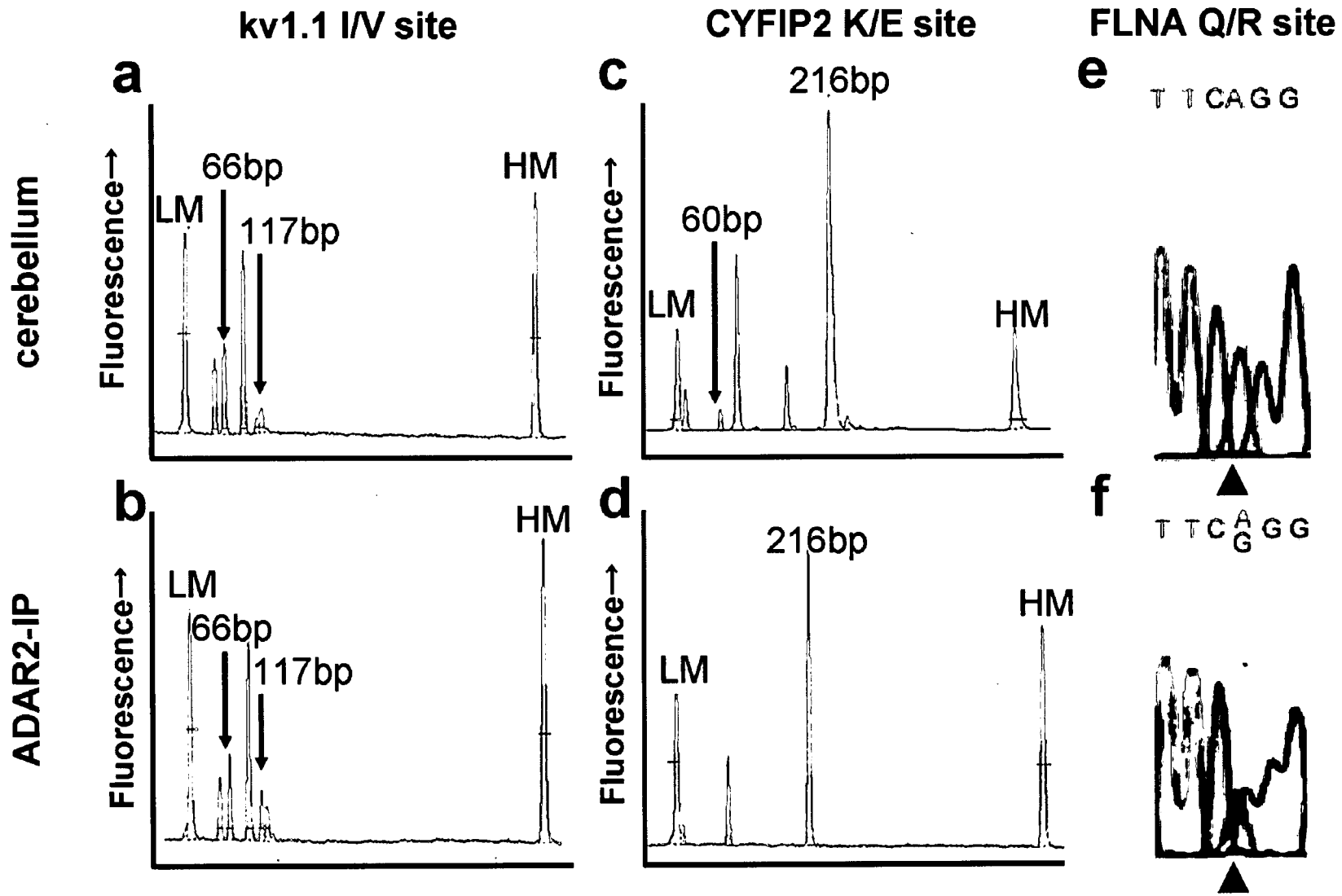


Fig.3

Nishimoto et al., Determination of editors at the novel A-to-I editing positions, Fig.3

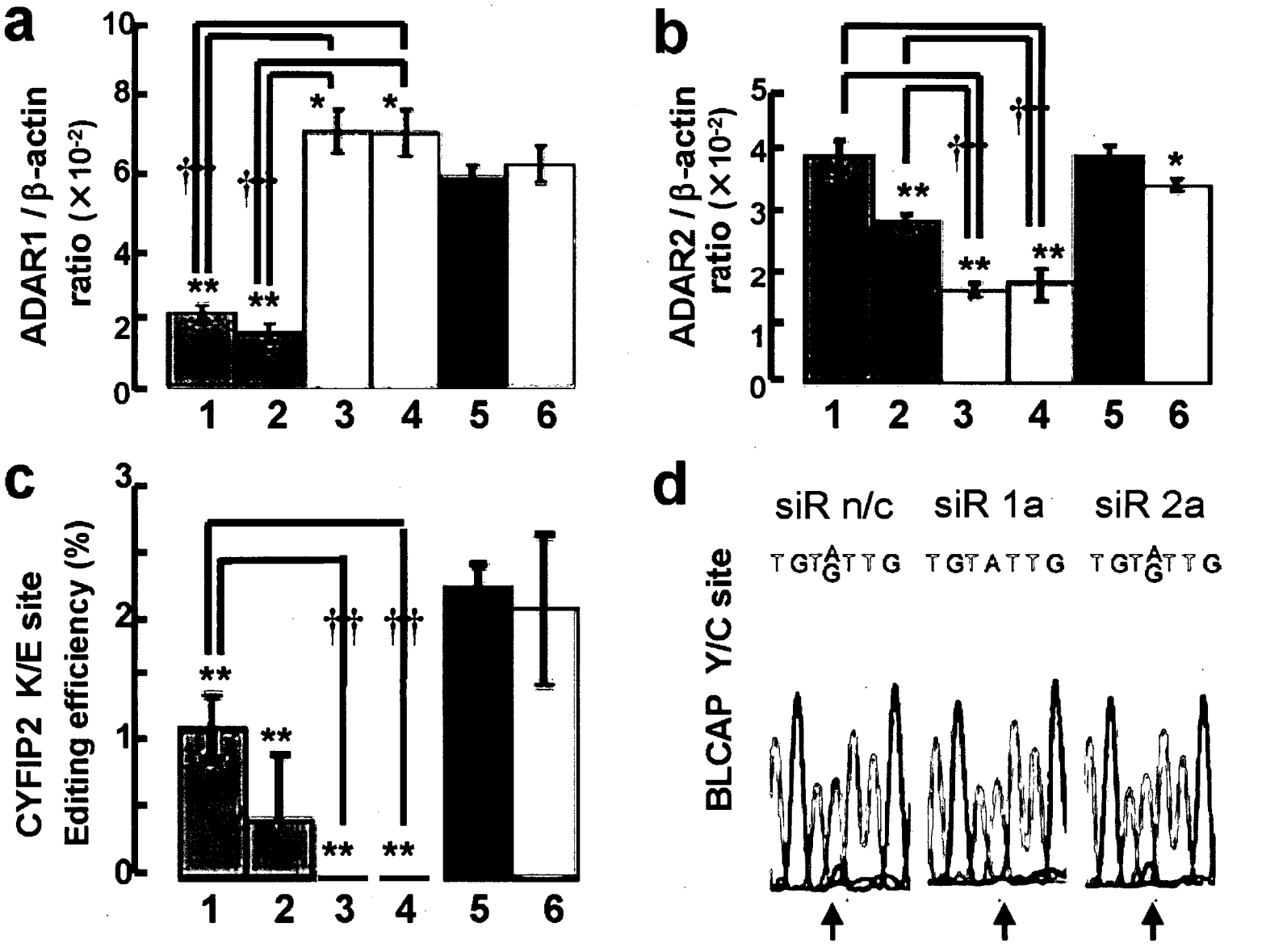
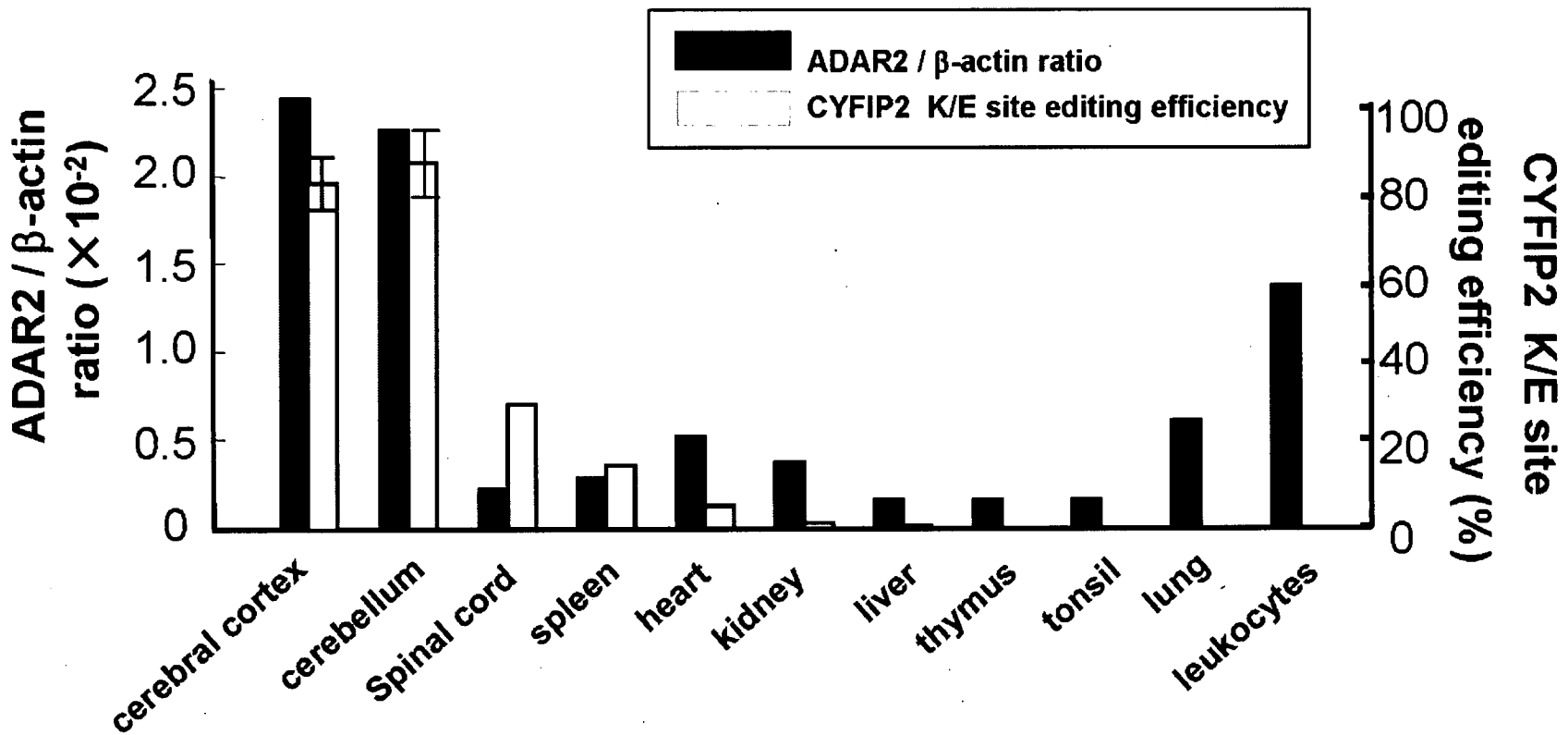


Fig.4

Nishimoto et al., Determination of editors at the novel A-to-I editing positions, Fig.4



孤発性ALSの病因

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孤発性ALS

皆様ご存じのように筋萎縮性側索硬化症（amyotrophic lateral sclerosis：以下、ALS）は原因不明で治療方法に乏しい難病ですが、その治療に一日でも早く近づけるように私たちのグループは日々努力をしております。現時点での成果を2006年7月28日に行われたALS班会議ワークショップ「ALSの克服に向けて」において「孤発性ALSの病因」として疾患概念として発表した内容からご紹介致します。

ALSは1870年代にJean-Martin Charcotという当時一流の神経学者により病気が世間に紹介されてから約130年となりますが、今なお原因不明で治療方法の乏しい難病で多くは中年以降に、手足に力が入らないといった四肢の症状、飲み込みにくい、喋りづらいという球症状で気づかれます。手足の運動をつかさどる脊髄の運動ニューロンだけがある時期から何故突然死ぬのか、という原因は依然として分かっていません。ALSの発症率は人口10万人にあたり年間0.5～3人程度、有病率は2～8人程度で90%以上が遺伝性を持たない孤発性と言われるものです。患者さんは手足が動かせず、自力での呼吸が難しくなっても意識、知能は正常で、日常生活動作に介助が必須となるため、患者本人のみならず介助者の物心面での負担は大きく、治療法の早期開発が切望される疾患のひとつです。

私たちのグループは近年、孤発性ALSの原因としてこの病気の脊髄運動ニューロンだけに起こっている変化を分子レベルで明らかにしました。専門的な話になるのですが、ニューロンは電気的な活動によりニューロンからニューロンへと情報を伝達します。ニューロンの電気的活動を調節しているものが神経細胞の表面にあるチャネルです。その1つにグルタミン酸受容体であるAMPA（α-3-hydroxy-5-methyl-4-isoxazole propionic acid）受容体というチャネルがあり、細胞内外のナトリウムイオンの濃度を調整することで細胞膜の興奮性の調節をしています。ナトリウムイオン以外にイオンチャネルを通じて細胞内外を行き来しているイオンにはカルシウムイオン（以下Ca^{2+}）があります。通常、Ca^{2+}は細胞内に比べて細胞外にはるかに多く存在します。Ca^{2+}は適切な量以上細胞内に入るとその細胞は死んでしまいます。AMPA受容体は通常の哺乳類のニューロンではCa^{2+}を通しませんが、孤発性ALSの脊髄ではCa^{2+}を通してしまう分子変化が起こっていました。この発見は、ALSへの治療の道を切り拓く可能性を大いに期待させるものと考えています。

ALSにおける興奮性神経細胞死とAMPA受容体

孤発性ALSの原因の仮説としては、グルタミン酸受容体サブタイプであるAMPA受容体を介した興奮性神経細胞死仮説が最有力で、支持する知見が積み重ねられてきました。脳から手足を動かすという指令を伝える錐体路はグルタミン酸を神経伝達物質として用いていて、脊髄運動ニューロンもこの興奮性入力を豊富に受けています。グルタミン酸を受け取るのはグルタミン酸受容体であり、運動ニューロンはグルタミン酸受容体を沢山持っており、運動ニューロンの興奮を伝達します。ところが、グルタミン酸による指令過剰により興奮が多くなりすぎると細胞内環境の変化を補償する機能の限界を越えてしまい、元に戻す機構が破綻し、細胞死へと進む、というのが興奮性神経細胞死のメカニズムです。興奮性神経細胞死は主に脳梗塞のときの虚血や低血糖、外傷、てんかん重積などの急性の神経細胞死に働くと考えられていました。一方で近年、変性疾患と言われる年単位でゆっくり進む、原因未解明の病気の神経細胞死にも関わっていることが注目されるようになっています。動物実験レベルでも急性には神経細胞死を引き起こさない濃度でも受容体が長期

間持続的に興奮することで遅発性のゆっくりとした神経細胞死が起こることが次々と明らかにされ、慢性に進行する神経変性疾患の中でも特にALSでグルタミン酸受容体を介した経路が関与している可能性が注目されるようになりました。

グルタミン酸受容体

グルタミン酸受容体の仲間にはいろいろあり、大きくはその特性から種々のイオンの透過に関わるイオンチャネル型と細胞内シグナル伝達に関わる代謝調節型に分けられます。そしてイオンチャネル型は更にNMDA受容体、カイニン酸受容体、AMPA受容体に分けられます。

NMDA受容体が急性の神経細胞死に関与するのに対して、特に速いシナプス伝達に関わるAMPA受容体は、ニューロンの遅発性の細胞死に関わっており、運動ニューロンは、特に後者が関与する興奮性細胞死に弱いことが知られています。その分子メカニズムとして細胞死に先立つ過剰なCa^{2+}流入による細胞内Ca^{2+}濃度の持続的上昇が培養ニューロンで明らかにされ、それに引き続いて細胞の生命維持に必要な機構の障害（例えばミトコンドリア障害）が段階的に起こることにより細胞死を引き起こすことが様々な実験系により明らかにされました。

AMPA受容体について

それでは、AMPA受容体がどのような構造をしているか説明します。AMPA受容体は、4種のサブユニット（GluR1-GluR4）の単独または様々な組み合わせからなる四量体（4つのサブユニット構成の分子）です（図1a）。各サブユニットは共通構造を持っており、相互に約70%のアミノ酸配列の相同性といって同じような配列を持ち、細胞外のN端（ニトロ茎［N_2］側の末端）、4つの膜ドメイン（M1～M4）とそれらをつなぐリンカー、細胞内のC端（カルボキシ基側の末端）からなります。

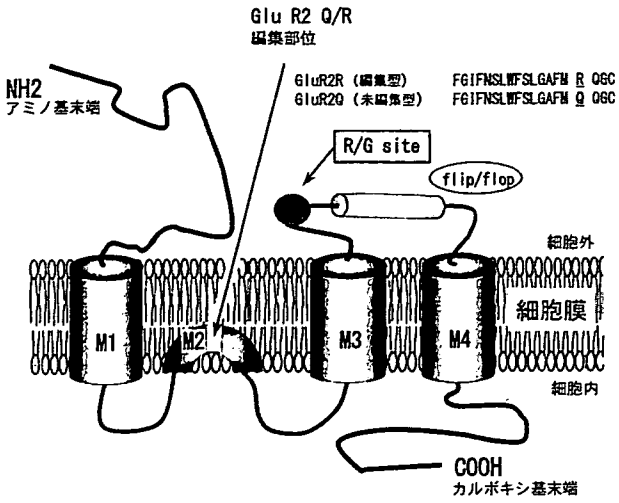
AMPA受容体のCa^{2+}透過性を決める因子には、いろいろなものがあります。分かっていることとしてAMPA受容体の4つのサブユニットにGluR2サブユニットが含まれるかどうか、GluR2サブユニットがRNA編集（特にQ/R部位）を受けているかどうか、などが挙げられます。

まず、サブユニットの中で、チャネルがCa^{2+}を通すがどうかを決めているのはGluR2です。AMPA受容体を構成する4つのサブユニットのうちGluR2を含む受容体は、Ca^{2+}透過性が低いのですが、Glu R1, 3, 4のサブユニットだけで構成された受容体は、高いCa^{2+}透過性を示します（図1b）。つまりAMPA受容体がCa^{2+}を通すかどうかを決めているのは、GluR2があるかどうかによって決定されています。実際、ラット小脳プルキニエ細胞や海馬錐体細胞などでは、他のサブユニットに比べGluR2が多く発現しており、その結果、AMPA型受容体はCa^{2+}を通しにくく、海馬のバスケット細胞、新皮質の非錐体細胞、小脳のBergmannグリア細胞のようにGluR2サブユニットがほとんど発現していないような細胞では、Ca^{2+}が通りやすいことが分かっています。

第二にAMPA受容体の各サブユニットの第2膜ドメイン（M2）にはQ/R部位と呼ばれる部位があり、同部位はGluR2以外ではグルタミン（アミノ酸は略語で示されますが、グルタミンはQというアルファベットで表します）であるのに対して、GluR2だけはアルギニン（R）です（図1a）。しかしGluR2の遺伝子を調べてみるとこの部位はQをコードしています。すなわち遺伝子に書かれた情報が書き換えられたタンパクが発現しているのです。

その理由はDNAからRNAへ転写される際にmRNAの1個のアデノシン（A）がイノシン（I）へ置換されることにより、そのmRNAがコードしているアミノ酸が置換（CAG→CIG）されるためです。この機構は、RNA編集と呼ばれ、adenosine deaminase acting on RNA type 2（以下ADAR2）と呼ばれる編集酵素により、アデノシン（A）はイノシン（I）へと置換され、リボソームでIはグアノシン（G）と同等であると見なされるため、CIGはCGGと見なされRとして翻訳されます。

通常、タンパク質が出来るときは、DNAからmRNAに転写され、そのmRNAの配列をリボソームが読み、3塩基ずつに対応したアミノ酸が作られ、いくつか集まりタンパク質になります。つまり、DNAの配列が決まっていれば出来上がるアミノ酸は決まっていて、DNAの配列が分かっていれば出来上がるアミノ酸が予測出来ます。このように遺伝情報は正確に転写されなければ生死に関わるような重大な障害を来たす可能性がありますが、遺伝子からmRNAが形成される過程で、遺伝情報が書き換え



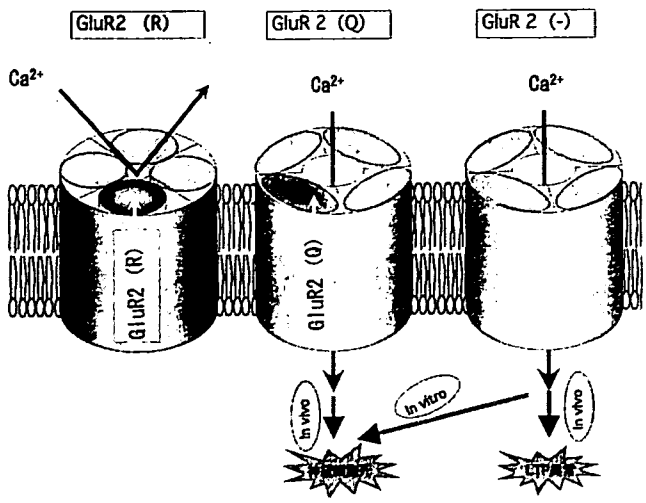


図1a　AMPA受容体の構造

AMPA受容体は4種類のサブユニットGluR1～GluR4による4量体からなります．M1，M3，M4は膜貫通部分で各サブユニットにはそれぞれ選択的スプライシングにより異なるアミノ酸配列をもったflip型とflop型という2つの変異種があり，受容体はこれらのサブユニットの組み合わせからなる4量体であると考えられています．GluR2～4にはR/G部位があり，RNA編集によりアルギニン（R）からグリシン（G）へ置換します．この部位は受容体脱感作を修飾すると考えられています．更にGluR2の多様性が生じるメカニズムとしてQ/R編集部位があり，翻訳過程でCAGまたはCAA（グルタミン（Q））がCGGまたはCGA（アルギニン（R））になります．そのため，サブユニットの組み合わせからは機能的にも多様性がもたらされると推測されています．

図1b　Ca^{2+}に対する透過性とサブユニットGluR2の関係

GluR2によりCa^{2+}透過性が変化する．GluR2（R）がサブユニットに入っているとCa^{2+}を通さないのですが，入っていないAMPA受容体はCa^{2+}を通します．未編集型GluR2（Q）が入っていてもやはりCa^{2+}透過性になります．

られることがあります。これをRNA editing（RNA編集）と呼びます。RNA編集は、GluR2 Q/R部位以外にもカイニン酸受容体サブユニットであるGluR5、GluR6のQ/R部位やGluR2, GluR3, GluR4サブユニットのR/G（グリシン/アルギニン）部位（図1a）など様々なRNAのそれも複数の部位で生じており、その効率は様々です。中でも、GluR2のQ/R部位は、胎生期から成熟期に至るまでほぼ100%編集されているという点で他の編集部位と異なり、これほど高い編集率を示す部位は、今のところ他には見付かっていません。Q/R部位がCa^{2+}を通すかどうかに重要なのは、この部位がチャネル孔に面しており、陽電化のRがCa^{2+}を弾くのに対して電気的に中性のQではこの作用が弱いためであると考えられています。

AMPA受容体サブユニット発現とALSの運動ニューロン死

いよいよ本題に入りますが、これらの結果を踏まえ、神経細胞死に関連する分子変化であるGluR2の減少（Ca^{2+}を通すAMPA受容体の割合の増加）ないしGluR2 Q/R部位の編集率低下（Ca^{2+}を通すAMPA受容体の実質的増加）が生じているのかどうかをALSの運動ニューロンで検討することにしました。

郭らはlaser microdissectorを用いて薄くスライスした凍結剖検組織からレーザービームによりニューロン1つのみを切り出しました。この1つのニューロンからRNAを取り出し、RT-PCR法によりGluR2 mRNA由来のcDNAを増幅し、AMPA受容体各サブユニットmRNAを定量する方法及びGluR2 Q/R部位RNA編集率を定量する方法を確立しました。

そして、孤発性ALS脊髄運動ニューロンの単一神経細胞レベルで、GluR2 mRNA発現量に有意な減少が無いこと、及びALS脊髄前角組織レベルで、部位選択的・疾患特異的にGluR2 Q/R部位の編集率が低下していることを発見しました。図2に示すように、正常対照群の運動ニューロンでは、全例GluR2 Q/R部位は100%RNA編集されていましたが、ALS群では0～100%とばらつき、平均値は38～75%と低下していました。ALS群における小脳プルキンエ細胞の編集率は、正常対照群と同様にほぼ100%に保たれていました。また、他の神経変性疾患の同細胞を検索しましたが、編集率は正常対照と同様のレベ