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REVIEW

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Deficient RNA editing of GluR2 and neuronal death in amyotropic lateral sclerosis

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Abstract One plausible hypothesis for selective neuronal death in sporadic amyotropic lateral sclerosis (ALS) is excitotoxicity mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, which are a subtype of ionotropic glutamate receptors. The Ca^{2+} conductance of AMPA receptors differs markedly depending on whether the GluR2 (or GluR-B) subunit is a component of the receptor. The properties of GluR2 are generated posttranscriptionally by RNA editing at the Q/R site in the putative second membrane domain (M2), during which the glutamine (Q) codon is substituted by an arginine (R) codon. AMPA receptors containing the unedited form of GluR2Q have high Ca^{2+} permeability in contrast to the low Ca^{2+} conductance of those containing the edited form of GluR2R. The role of Ca^{2+}-permeable AMPA receptors, particularly GluR2 Q/R site RNA editing status, in neuronal death has been clearly demonstrated both in mice deficient in editing at the GluR2 Q/R site and in mice transgenic for an artificial Ca^{2+}-permeable GluR2 subunit. We analyzed the expression level of mRNA of each AMPA receptor subunit in individual motor neurons, as well as the editing efficiency of GluR2 mRNA at the Q/R site in the single neuron level in control subjects and ALS cases. There was no significant difference as to the expression profile of AMPA receptor subunits or the proportion of GluR2 mRNA to total GluRs mRNA between normal subjects and ALS cases. By contrast, the editing efficiency varied greatly, from 0% to 100%, among the motor neurons of each individual with ALS, and was not complete in 44 of them (56%), whereas it remained 100% in normal controls. In addition, GluR2 editing efficiency was more than 99% in the cerebellar Purkinje cells of ALS, spinocerebellar degeneration and normal control groups. Thus, GluR2 underediting occurs in a disease specific and region selective manner. GluR2 modification by RNA editing is a biologically crucial event for neuronal survival, and its deficiency is a direct cause of neuronal death. Therefore, marked reduction of RNA editing in ALS motor neurons may be a direct cause of the selective motor neuron death seen in ALS. It is likely that the molecular mechanism underlying the deficiency in RNA editing is a reduction in the activity of ADAR2, a double- strand RNA specific deaminase. The restoration of this enzyme activity in ALS motor neurons may open the novel strategy for specific ALS therapy.

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Abbreviations *ADAR*: Adenosine deaminases acting on RNA · *ALS*: Amyotropic lateral sclerosis · *AMPA*: α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate · *PCR*: Polymerase chain reaction

ALS: history

The most common motor neuron disease, amyotrophic lateral sclerosis (ALS), is characterized by a selective loss of upper and lower motor neurons that initiates in mid-life by a progressive paralysis with muscle wasting. ALS has a uniform worldwide prevalence (0.8–7.3 cases per 100,000 individuals), with risk of disease increasing in a age-dependent manner after the sixth decade of life, and only approximately 5–10% of all ALS cases are familial. Although three causal genes have been so far identified in individuals affected with familial ALS (SOD1, ALS2, senataxin) [1–4], the mechanism underlying motor neuron death or familial ALS pathology has not been elucidated. With regard to the non-hereditary form of ALS (sporadic ALS), which accounts for most cases of ALS, virtually no clues to the causal mechanism have been gleaned since the establishment of the disease entity by Jean-M. Charcot in 1874.

ALS etiology: the AMPA receptor-mediated neuronal death hypothesis

Several hypotheses have been proposed to explain the etiology of sporadic ALS, including genetic and/or non-genetic abnormalities of cytoskeletal and axonal transporting proteins [5, 6], vascular endothelial growth factor (VEGF) [7, 8], and viruses [9, 10], among others. Of these, one plausible hypothesis for selective neuronal death in sporadic ALS is excitotoxicity mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors, which are a subtype of ionotropic glutamate receptors. Because the pyramidal tract that projects to the motor neurons in the spinal cord uses glutamate as the excitatory neurotransmitter, motor neurons express abundant glutamate receptors and hence are vulnerable to exaggerated receptor activation by glutamate. The first act of the excitotoxicity story opened by selective loss of glutamate uptake due to loss of a glutamate transporter in the ALS motor cortex [11, 12], which was later proved to be not disease selective [13]. Including the neuronal death induced by glutamate transporter blockade, it has been repeatedly shown that motor neurons are differentially more vulnerable to AMPA receptor-mediated slow neuronal death than are other neuronal subsets in rat and mouse spinal cord cultures [14–16] and in the adult rat spinal cord [17, 18]. An increased influx of Ca^{2+} through activated AMPA receptor-coupled channels appears to play a key role in this type of neuronal death [19, 20].

Functional AMPA receptors are homo- or heterooligomeric assemblies that are composed of four subunits, GluR1, GluR2, GluR3, and GluR4, in various combinations. The Ca^{2+} conductance of AMPA receptors differs markedly depending on whether the GluR2 subunit is a component of the receptor. AMPA receptors that contain at least one GluR2 subunit have low Ca^{2+} conductance, whereas those lacking a GluR2 subunit are Ca^{2+} permeable (Fig. 1A) [21–24]. These properties of GluR2 are generated posttranscriptionally by RNA editing at the Q/R site in the putative second membrane domain (M2; Fig. 1B), during which the glutamine (Q) codon is substituted by an arginine (R) codon [22, 23, 25]. Analyses of adult rat, mouse, and human brain RNA have demonstrated that almost all GluR2 mRNA in vivo is edited

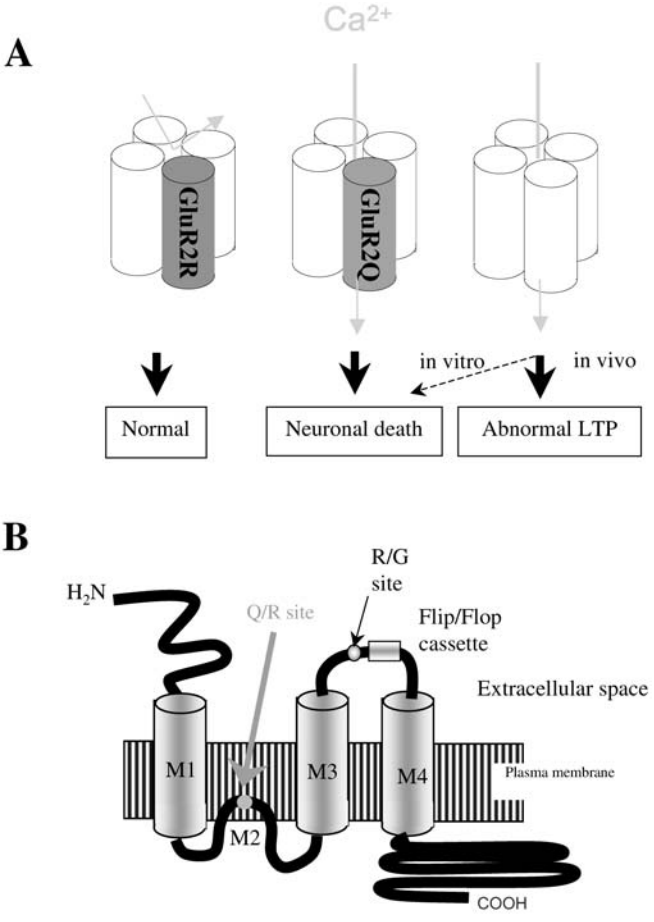

Fig. 1 Ca^{2+} permeability of AMPA receptors and the GluR2 subunit. **A** Functional AMPA receptors are homo- or heterooligomeric assemblies that comprise the four subunits GluR1, GluR2, GluR3, and GluR4 in various combinations. The Ca^{2+} conductance of AMPA receptors differs markedly depending on whether they contain the GluR2 subunit. AMPA receptors that contain at least one edited GluR2 subunit have low Ca^{2+} conductance (*left*), whereas those lacking a GluR2 subunit (*right*) and those containing unedited GluR2Q (*middle*) have high Ca^{2+} conductance. Neuronal death is induced when RNA editing at the GluR2 Q/R site is deficient. **B** Structure of GluR2: the Q/R site is located in the putative second membrane domain (*M2*)

(i.e., R is found at the Q/R site), whereas Q remains at this critical position in the GluR1, GluR3, and GluR4 subunits. AMPA receptors containing the unedited form of GluR2Q have high Ca^{2+} permeability in contrast to the low Ca^{2+} conductance of those containing the edited form of GluR2R (Fig. 1A) [26, 27].

The role of Ca^{2+}-permeable AMPA receptors in neuronal cell death has been demonstrated in animal models. Although low expression of GluR2 was found to influence AMPA receptor-mediated neuronal death in cultured hippocampal neurons from GluR2-null mice [28], low GluR2 expressed caused abnormal long-term potentiation (LTP) but not neuronal death in vivo [29]. By contrast, the effects of the GluR2 Q/R site RNA editing status on neuronal death have been clearly demonstrated both in mice deficient in editing at the GluR2 Q/R site [30] and in mice transgenic for an artificial Ca^{2+}-permeable GluR2 subunit [31]. Total Ca^{2+} influx depends on relative Ca^{2+} permeability, and on the number of open channels, as determined by the other factors including the proportion of flip/flop splice variants in AMPA receptor subunits and on the cellular density of AMPA receptors, although these factors rarely induce neuronal death.

Accordingly, two mechanisms have been proposed for the AMPA receptor-mediated neuronal death observed in ALS spinal motor neurons. The first is a selective reduction in GluR2 expression, which results in a decrease in the proportion of GluR2-containing, and therefore Ca^{2+}-impermeable, AMPA receptors owing to a low relative abundance of GluR2 among the four subunits. The second is a reduction in GluR2 RNA editing, which results in the number of GluR2Q-containing, Ca^{2+}-impermeable AMPA receptors increasing to a non-negligible amount.

AMPA receptor subunits in normal human CNS neurons

To our surprise, only inconsistent evidence had been available concerning the expression profile of AMPA receptor subunits in human brain. Therefore, we first quantified the expression level of each subunit in various neural subsets including spinal motor neurons. Notwithstanding studies reporting the expression of GluR2 [32, 33], some studies based on in situ hybridization or immunocytochemistry had claimed that GluR2 is not expressed in the spinal motor neurons of control human subjects [34, 35], and there was no evidence concerning whether or not GluR2 is present in ALS motor neurons. By means of a laser microdissector (Fig. 2) and real-time quantitative RT-PCR, we measured the relative abundance of GluR2 mRNA in situ in human spinal motor neurons and other neuronal tissues from control subjects. We found a stronger relative abundance of GluR2 mRNA, representing 77.8–95.5% of the total AMPA receptor subunits, as compared to other AMPA receptor subunit mRNAs throughout the neuronal subsets examined [36]. However, the motor neurons of control subjects expressed significantly lower levels of GluR2 (77.8±2.0%), as compared to other neuronal subsets (89.8±1.2% in Purkinje cells, 95.5±0.5% in cerebellar granule cells; Fig. 3) [36].

In rat spinal cords, the mRNA of all four AMPA receptor subunits has been detected in spinal motor neurons by in situ hybridization [37–40], and quantification of the relative abundance of GluR2 mRNA by single-cell RT-PCR has demonstrated a weaker predominance of GluR2 (from 2.1 to 63%) in cultured spinal motor neurons than what we found in human motor neurons [16, 41, 42]. The AMPA receptor subunit composition did not differ between motor neurons and dorsal horn neurons in immature rat dissociated spinal cord cultures [16], but significantly lower expression of GluR2 mRNA has been demonstrated in adult rat motor neurons than in dorsal horn neurons that are dissected with a laser microdissector (unpublished observation), which probably reflects age-dependent alterations of the AMPA receptor expression profile.

With regard to the other AMPA receptor subunits, the expression profiles differed among neuronal subsets, but were consistent between the same neuronal subsets analyzed from human and rat brains [36]. Low expression of GluR1 mRNA has been repeatedly shown in human and rat spinal motor neurons [36–38]. Rapid progress in the field of AMPA receptor trafficking has demonstrated the elaborate machinery that controls the activity-dependent trafficking of GluR1/GluR2 subunits differentially from the constitutive replacement of GluR2/GluR3 subunits [43, 44]. Thus, the different expression profiles of AMPA receptor subunits among neuronal subsets may reflect a subset-specific balance between activity-dependent and constitutive AMPA receptor regulatory mechanisms, rather than a species-specific difference.

Although the proportion of GluR2 varies relative to the other subunits, it is likely that GluR2 mRNA accounts for the major proportion of AMPA receptor subunits in neu-

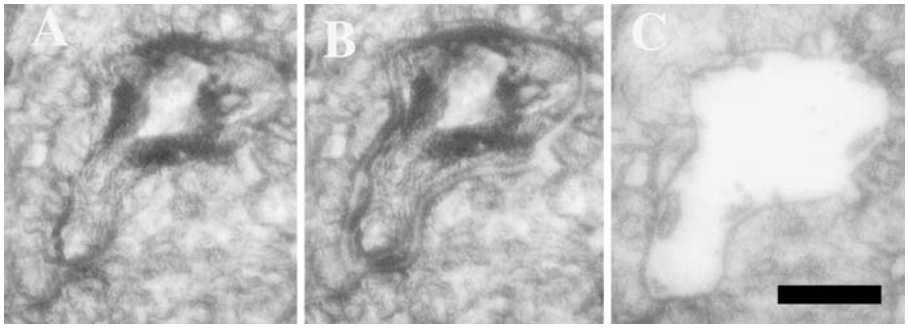

Fig. 2 Dissection of single motor neurons with a laser microdissector. **A** Before dissection; **B** demargination with a narrow laser beam; **C** after capturing the neuronal tissue. *Bar*= 40 μm

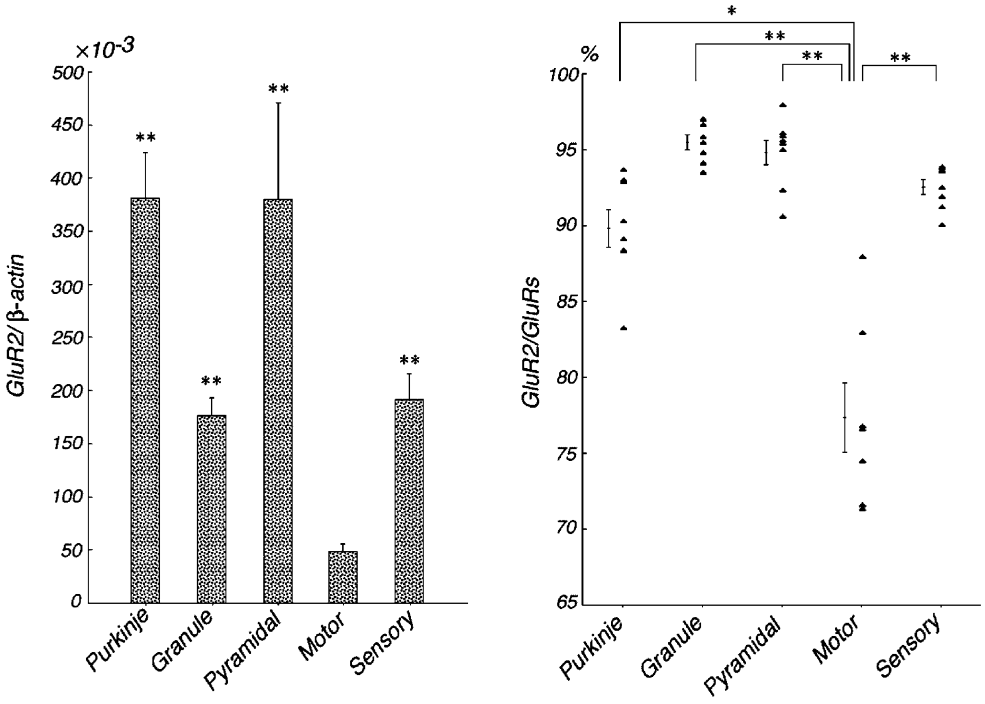

Fig. 3 Expression profile of AMPA subunit mRNA in single neuronal subsets of normal subjects. *Left*: The copy number of GluR2 mRNA has been normalized to the β-actin control (mean ±SEM; n=8). Samples significantly different from spinal motor neurons (*Motor*) are indicated (Mann-Whitney U test, $^{**}P<0.001$). *Right*: Each symbol represents the GluR2 mRNA copy number relative to the sum of the copy numbers of all GluR mRNAs (*GluRs*) in one subject. For each group, the mean ±SEM (n=8) is also displayed. The magnitude of GluR2 mRNA predominancy is significantly lower in spinal motor neurons (*Motor*) than in other neuronal subsets (Mann-Whitney U test, $^{*}P<0.01$, $^{**}P<0.001$). *Purkinje*, Purkinje cells; *Granule*, cerebellar granule cells; *Pyramidal*, cortical pyramidal neurons; *Sensory*, spinal dorsal horn neurons in substantia gelatinosa. (Modified from [36] with permission)

rons, including motor neurons of the spinal cords of humans as well as other mammals, and that the expression level of GluR2 mRNA is significantly lower in motor neurons than in other neuronal subsets in adult brains.

RNA editing

RNA editing has been defined as a posttranscriptional modification of the base sequence of mRNA and is recognized as a genetic mechanism for changing gene-specified codons and thus protein structure and function [45, 46]. In mammals, the main types of RNA editing are the base conversion of adenosine (A) to inosine (I) and that of cytidine (C) to uracil (U), which has been demonstrated to occur only in apolipoprotein B (ApoB) [47, 48] and neurofibromatosis type 1 (NF1) [49].

The conversion of A-to-I is most active in the central nervous system and has been found to occur predominantly in receptors and ion channels, including ionotropic glutamate receptor subunits [50–52], the serotonin -2C receptor $5HT_{2C}R$ [53], the voltage-dependent potassium channel Kv1.1 [54], and the RNA editing enzyme ADAR2 [55]. Among these, the biological effects of RNA editing have been most clearly demonstrated in ionotropic glutamate receptors, where editing positions have been identified in the subunits of AMPA receptors and kainate receptors, which, like AMPA receptors, are tetrameric assemblies comprising different combinations of the homologous subunits GluR5-GluR7, KA1, and KA2. Editing positions have been named after the amino acids that can occupy them, including the Q/R site in GluR2, GluR5, and GluR6 [50], the R/G site in GluR2, GluR3, and GluR4 [52], and the I/V and Y/C sites in GluR6 [51]. The change in amino acid residue caused by RNA editing, particularly at the Q/R site of GluR2, results in alterations in channel properties, including Ca^{2+} permeability [21, 22, 26, 27, 51, 56–59] and kinetic aspects of channel gating [52] in AMPA receptors.

AMPA receptors in ALS

As mentioned above, AMPA receptor-mediated neuronal death occurs after an increase in Ca^{2+} influx through AMPA receptor-coupled ion channels, and either a decrease in GluR2 expression or insufficient RNA editing at the GluR2 Q/R site is the causative molecular change. A decrease in GluR2 mRNA has been demonstrated after ischemia [60], but insufficient RNA editing had not been shown in any human neurological disease [61–63] or animal models under conditions inducing neuronal death after cerebral hypoxia [64] or kindling [65]. Owing to the lack of evidence on ALS motor neurons, we investi-

gated, both quantitatively and qualitatively, these molecular changes in AMPA receptors in motor neuron tissues of ALS patients, as compared to disease control and normal control subjects.

Quantitative analysis (RNA expression)

On the basis of the AMPA receptor mRNA expression profile in normal human neurons, we investigated whether the composition of AMPA receptor subunits is altered in ALS motor neurons. Quantitative analyses for AMPA receptor mRNA on both 100 motor neurons and single motor neurons showed that ALS motor neurons expressed almost the same amount of GluR2 mRNA relative to the β-actin baseline as control motor neurons (Fig. 4) [36]. Neither the ratio of β-actin mRNA-positive neurons to total dissected neurons (83.5% vs. 82.7%) nor the ratio of GluR2 mRNA-positive neurons to β-actin mRNA-positive neurons (57.9% vs. 53.7%) differed between the ALS and control groups, indicating that GluR2 mRNA expression is not altered in individual motor neurons of ALS [36]. Thus, selective reduction in GluR2 does not occur, and, therefore, an increase in the proportion of Ca^{2+}-permeable AMPA receptors owing to a reduced proportion of GluR2-containing AMPA receptors cannot be the mechanism underlying AMPA receptor-mediated neuronal death in ALS.

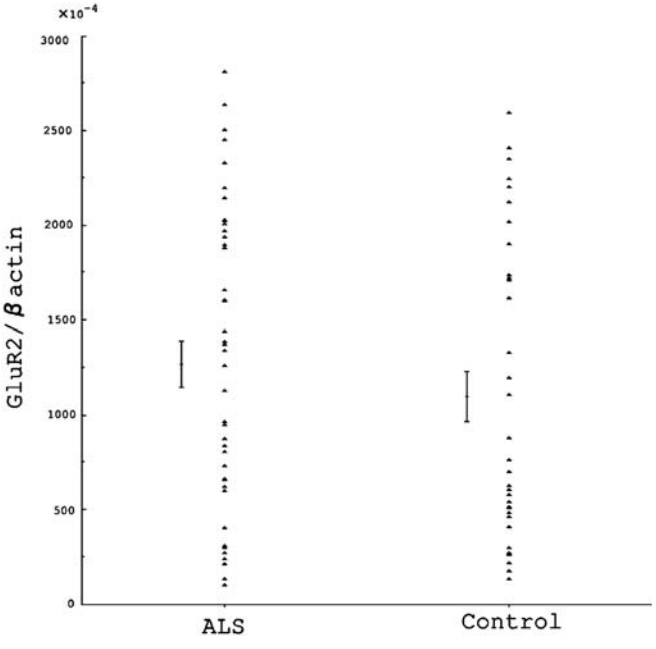

Fig. 4 Expression level of GluR2 mRNA in single motor neurons of ALS and control subjects. Each symbol represents the copy number of GluR2 mRNA normalized to the β-actin control in a single motor neuron. The expression levels in 44 motor neurons from three subjects with ALS and 36 motor neurons from three control subjects is shown. There is no significant difference between motor neurons from the ALS group and those from the control group (Mann-Whitney *U* test, $P>0.01$). For each group, the mean ±SEM is also displayed. (Adapted from [36] with permission.)

Qualitative analysis (RNA editing)

The alternative explanation for the AMPA receptor-mediated selective motor neuronal death in ALS is a deficiency in GluR2 Q/R site editing, which we have observed in the ventral gray matter of the ALS spinal cord [66]. In order to analyze neuron-selective tissue, we extracted RNA from single motor neurons isolated with a laser microdissector [36] from five individuals with ALS and five normal control subjects [67]. The editing efficiency was calculated by measuring differences in the digestion patterns of nested RT-PCR products of GluR2 mRNA obtained with the restriction enzyme *Bbv*I, whose cutting site depends on the occurrence of editing (Fig. 5A) [66–68]. The editing efficiency in GluR2 in cerebellar Purkinje cells was also quantified in individuals with ALS, dentatorubral-pallidoluysian atrophy (DRPLA), and multiple system atrophy (MSA) and compared with that in normal subjects.

The frequency of GluR2 mRNA positivity did not differ significantly between the ALS and the control groups (two-sample test for equality of proportions, $P>0.05$). The editing efficiency varied greatly, from 0% to 100%, among the motor neurons of each individual with ALS, and was not complete in 44 of them (56%); this was in marked contrast to the control motor neurons, of which all 76 examined showed 100% editing efficiency. The editing efficiency in Purkinje cells was virtually complete in the ALS, DRPLA, MSA, and normal groups (Fig. 5B) [67]. We confirmed that laser-captured neuronal tissue was not contaminated with tissues derived from glial cells or other cells, by PCR for microtubulus associate protein 2 (MAP2) and glial fibrillary acidic protein (GFAP) on neural tissue and PCR for GluR2 on tissues from neuropil.

Albeit at a tissue level, GluR2 Q/R site editing has been reported to be preserved in the severely pathological brain areas of other neurodegenerative diseases including the striatum of Huntington disease, the neocortex and hippocampus of Alzheimer and Pick diseases, and the cerebellum of diseases of spinocerebellar degeneration [61–63, 69]. In addition, GluR2 editing was found to be virtually complete in Purkinje cells (Fig. 5B) [67] and the motor cortex [66] of ALS patients, indicating that the defect in GluR2 editing at the Q/R site is disease specific to ALS and site-selective to spinal motor neurons.

In accordance with our results, mice transgenic for an artificial Ca^{2+}-permeable GluR2 develop motor neuron disease late in life [31], indicating that motor neurons are specifically vulnerable to a deficiency in RNA editing. The proportion of the artificial Ca^{2+}-permeable GluR2 out of total GluR2 mRNA was only about 25% in that study, which raises questions about the type of molecular mechanism that could result in a small proportion of unedited GluR2Q altering the channel conductance of AMPA receptors to an extent sufficient to induce neuronal death. It has been reported that a GluR2Q subunit is more readily incorporated into functional AMPA receptors than is an edited GluR2R at the stage of tetramer (dimer of dimers) formation [70], as well as during receptor trafficking from

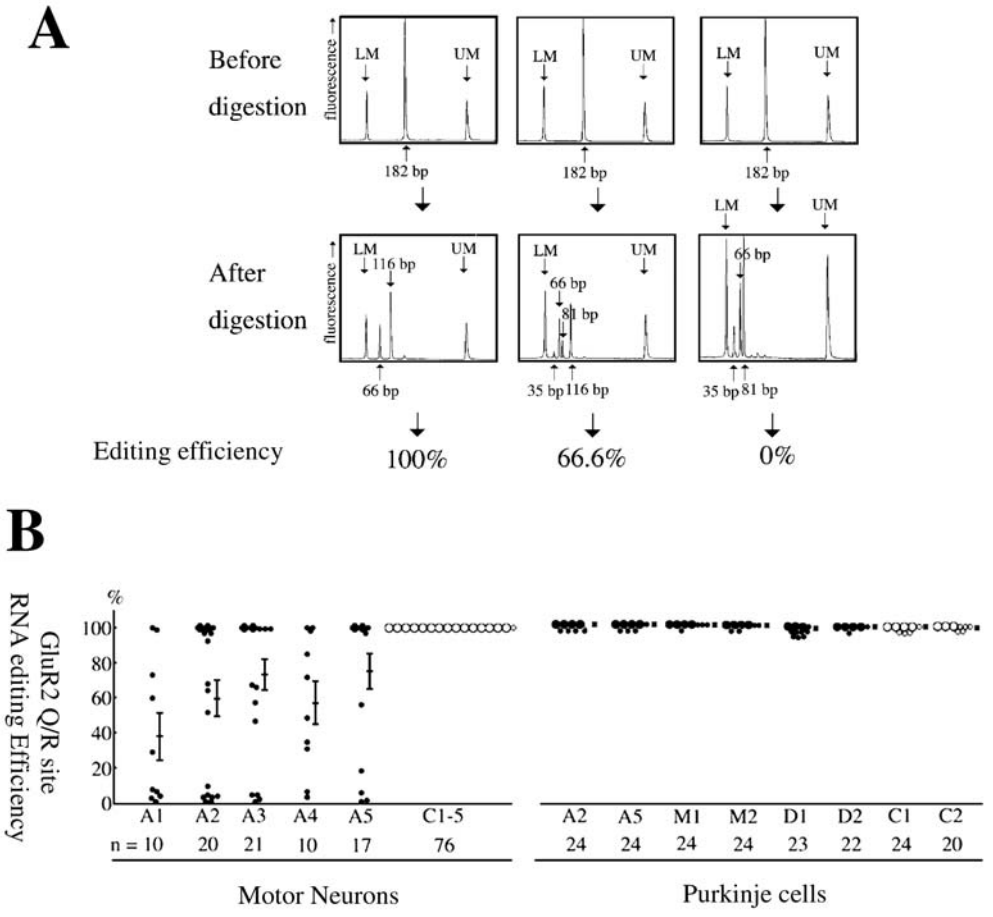

Fig. 5 **A** Analysis of RNA editing efficiency: When all of the RT-PCR products are derived from edited GluR2 mRNA, two bands (66 and 116 bp) are detected; by contrast, when all of the RT-PCR products are derived from unedited GluR2, three bands (35, 66, and 81 bp) are detected. When both edited and unedited GluR2 subunits coexist, four bands (35, 66, 81, and 116 bp) are detected. **B** Editing efficiency at the GluR2 Q/R site in single neurons of ALS, disease control and normal control subjects (modified from Fig. 1 in [67]). The editing efficiency varied greatly, from 0% to 100% (mean: 38.1–75.3%), among the motor neurons of each individual with ALS (A1–A5), and was not complete in 44 of them (56%); this was in marked contrast to the control motor neurons (C1–5), of which all 76 examined showed 100% editing efficiency. The editing efficiency in Purkinje cells was virtually complete (greater than 99.8%) in the ALS, disease control (dentatorubral-pallidoluysian atrophyDRPLA (D), multiple system atrophyMSA (M)) and the normal control groups (C)

the endoplasmic reticulum (ER) to the cell surface [71]. Therefore, if the RNA editing were deficient, even in a small proportion, then GluR2Q-containing receptors would be formed preferentially in the endoplasmic reticulumER, and receptors containing GluR2Q and GluR1 or GluR3 would be readily transported to the membrane, resulting in the preferential expression of Ca^{2+}-permeable functional AMPA receptors in the postsynaptic membrane. The effects of GluR2 RNA editing deficiency may be more conspicuous in spinal motor neurons, where the expression level of GluR2 is relatively low compared with that in other neuronal subsets [36]. According to this scheme, a small increase in GluR2Q with deficient GluR2 RNA editing will greatly increase the proportion of GluR2Q-containing, and therefore Ca^{2+}-permeable, functional AMPA receptors, thereby promoting excitotoxicity in ALS motor neurons [47, 48, 50–52].

Editing enzymes and RNA editing of GluR

Enzymes responsible for the A-to-I conversion have been termed "'adenosine deaminases acting on RNA"' (ADARs), and three structurally related ADARs (ADAR1 to ADAR3) have been identified in mammals [72–77]. ADAR1 and ADAR2 are expressed in most tissues [72, 74, 78, 79] and recognize the adenosine residue to be edited through the structure of the duplex that is formed between the editing site and its editing site complementary sequence (ECS), which is located in a downstream intron of the precursor (pre-) mRNA (Fig. 6) [80–82]. ADAR2 predominantly catalyzes RNA editing at the Q/R site of GluR2 both in vivo and in vitro, whereas both ADAR1 and ADAR2 catalyze the Q/R sites of GluR5 and GluR6, which are subunits of kainate receptors [83, 84]. ADAR3 is expressed

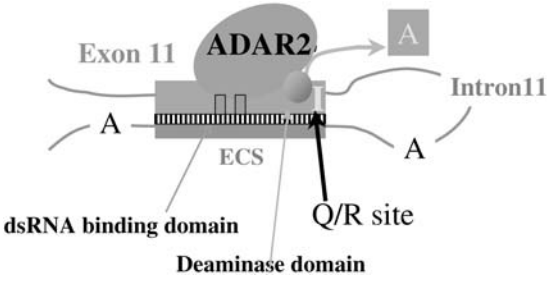

Fig. 6 ADAR2 and RNA editing. ADAR2 has two double-stranded RNA-binding domains (*square*) and one catalytic domain (*cube*), and it recognizes the adenosine residue (*A*) to be edited through the structure of the duplex that is formed between the editing site (e.g., the Q/R site in exon 11 of GluR2 pre-mRNA) and its editing site complementary sequence (*ECS*), which is located in a downstream sequence (e.g., in intron 11 of GluR2 pre-mRNA). Adenosine in the edited site is substituted to inosine (*I*), but adenosine that is not located in the editing site or the ECS remains unedited

exclusively in the brain, but is catalytically inactive on both extended double-stranded RNA and known pre-mRNA editing substrates [75, 77].

In mammalian and human brains, the editing efficiency at each editing position of GluRs is developmentally and regionally regulated [52, 56, 62, 85, 86], and the Q/R sites of GluR5 and GluR6 have been reported to be edited less in white matter than in gray matter [58, 68, 86–91]. By contrast, the GluR2 Q/R site is almost completely edited in various brain regions, including white matter in neonatal and adult rodent brains [56, 87, 92, 93]. GluR2 mRNA in human brains including white matter is, however, not always completely edited at the Q/R site [61, 62, 65, 66, 68, 91, 94, 95]. We have demonstrated an editing efficiency of virtually 100% in single neuron tissues from various neuronal subsets, but a significantly low editing efficiency in adult, but not in immature, human white matter [68, 91]. It seems likely that, in contrast to neurons, human glial cells physiologically express Ca^{2+}-permeable AMPA receptors: oligodendrocytes express those containing unedited GluR2 subunits, and astrocytes and Bergmann's glial cells express those lacking edited GluR2 subunits [23, 96, 97]. Therefore, the regional, and thus presumably cell-specific, regulation of GluR2 Q/R editing might occur in the human central nervous system.

ADAR2

It has been shown that the mRNA expression of ADAR2, the most efficient double-stranded RNA deaminase for editing GluRs, is regulated in a cell-specific manner throughout development and in rodent brain, is first detected by in situ hybridization in the thalamic nuclei formation at embryonic day 19 (E19), with more extensive and widespread distribution by the third postnatal week when the expression is highest in the thalamic nuclei and very low in white matter [98]. Editing efficiency at the editing sites of GluRs have been found to parallel the expression levels of ADAR2 mRNA in developmental rat [86, 87, 99–101] and human [91] brains, in a cultured human teratocarcinoma cell line [102], and in surgically excised hippocampus from patients affected with refractory epilepsy [103].

An association between the level of ADAR2 expression and editing efficiency at the GluR2 Q/R site has been also demonstrated by the observation that an RNA editing efficiency of less than 100% occurred only when the ratio of ADAR2 mRNA to total AMPA receptor subunit (GluR1-GluR4) mRNA was below 20×10^{-3} in human white matter (Fig. 7B) [68]. No such correlation was observed between the editing efficiency at GluR2 mRNA and the ratio of ADAR1 mRNA to total GluR mRNA, or between the editing efficiency at GluR5 or GluR6 mRNA and the ratio of either ADAR1 or ADAR2 mRNA to total GluR mRNA (Fig. 7A) [68]. Our results on human brain are in agreement with in vivo and in vitro evidence obtained in animal brains, indicating that ADAR2 predominantly catalyzes RNA editing at the Q/R site of GluR2 [55, 74, 78, 83, 104, 105], whereas the Q/R sites of GluR5 and GluR6 are edited not only by ADAR2 but also by ADAR1 [74, 75, 78, 81, 83, 84].

Taken together, the expression level of ADAR2 mRNA is one factor determining the editing efficiency of GluR2 at the Q/R site, although the nonlinear correlation suggests that another factor or factors may be also involved in the regulation of editing activity. Indeed, inhibitors of ADARs have been identified as sequence-nonspecific double-stranded RNA-binding proteins in *Xenopus* oocytes [106].

Significance of GluR2 underediting

GluR2 Q/R site editing in neurons occurs with virtually 100% efficiency throughout life from an embryonic stage, and the early demise of mice deficient in GluR2 RNA editing caused by neuronal death [30] can be rescued by restoring RNA editing [84], indicating that GluR2 modification by RNA editing is a biologically crucial event for neuronal survival and its deficiency is a direct cause of neuronal death. Thus, marked reduction of RNA editing in ALS motor neurons [67] may be a direct cause of the selective motor neuron death seen in ALS.

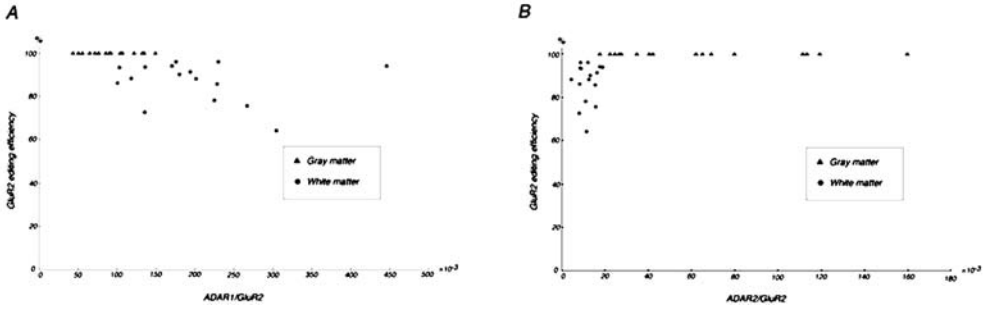

Fig. 7 Relationship between editing efficiency at the Q/R site of GluR2 and the abundance of ADAR1 mRNA (**A**) or ADAR2 mRNA (**B**) relative to GluR2 mRNA. **A** No relationship is found between GluR2 editing and the expression of ADAR1 mRNA relative to GluR2 mRNA. **B** In samples where the abundance of ADAR2 mRNA relative to GluR2 mRNA is less than 20×10^{-3}, GluR2 is not completely edited. By contrast, when the relative abundance is greater than 20×10^{-3}, GluR2 is completely edited. *Cbl*, Cerebellum; *BW*, cerebellar white matter; *Cx*, cerebral cortex; *CW*, cerebral white matter. (Adapted from Fig.7 in [68] with permission)

As mentioned above, AMPA receptors in glial cells seem to be more Ca^{2+}permeable than those in neurons, and a deficiency in RNA editing at the GluR2 Q/R site seems to cause significant cell death only when it occurs in neurons. Therefore, the recent finding of a reduction in GluR2 Q/R editing in malignant gliomas [94] may have different biological significance in comparison to the editing deficiency that occurs in neuronal tissue in ALS [67]. Indeed, the magnitude of the editing efficiency that was observed in malignant gliomas (69–88%) was within the range that we detected in normal white matter (64–99%) [68].

It is likely that the molecular mechanism underlying the deficiency in RNA editing is a reduction in ADAR2 deaminase activity. Our results suggest that the expression level of ADAR2 mRNA is a determining factor in this reduced activity; thus, restoring ADAR2 activity selectively in motor neurons, for example, by transfection of an ADAR2 cDNA construct using a motor neuron-specific vehicle for enhancing ADAR2 mRNA levels may be a specific therapeutic strategy for ALS. With this more focused research target, it is our sincere hope that a specific therapy for ALS will be developed in the near future.

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References

1. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62
2. Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, Devon RS, Miyamoto N, Showguchi-Miyata J, Okada Y, Singaraja R, Figlewicz DA, Kwiatkowski T, Hosler BA, Sagie T, Skaug J, Nasir J, Brown RH Jr, Scherer SW, Rouleau GA, Hayden MR, Ikeda JE (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29:166–173
3. Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, Hung WY, Ouahchi K, Yan J, Azim AC, Cole N, Gascon G, Yagmour A, Ben-Hamida M, Pericak-Vance M, Hentati F, Siddique T (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet 29:160–165
4. Chen YZ, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, Dierick I, Abel A, Kennerson ML, Rabin BA, Nicholson GA, Auer-Grumbach M, Wagner K, De Jonghe P, Griffin JW, Fischbeck KH, Timmerman V, Cornblath DR, Chance PF (2004) DNA/RNA Helicase Gene Mutations in a Form of Juvenile Amyotrophic Lateral Sclerosis (ALS4). Am J Hum Genet 74:1128–1135
5. Julien JP (2001) Amyotrophic lateral sclerosis. unfolding the toxicity of the misfolded. Cell 104:581–591
6. Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, Bowen S, Lalli G, Witherden AS, Hummerich H, Nicholson S, Morgan PJ, Oozageer R, Priestley JV, Averill S, King VR, Ball S, Peters J, Toda T, Yamamoto A, Hiraoka Y, Augustin M, Korthaus D, Wattler S, Wabnitz P, Dickneite C, Lampel S, Boehme F, Peraus G, Popp A, Rudelius M, Schlegel J, Fuchs H, Hrabe de Angelis M, Schiavo G, Shima DT, Russ AP, Stumm G, Martin JE, Fisher EM (2003) Mutations in dynein link motor neuron degeneration to defects in retrograde transport. Science 300:808–812
7. Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, Van Dorpe J, Hellings P, Gorselink M, Heymans S, Theilmeier G, Dewerchin M, Laudenbach V, Vermylen P, Raat H, Acker T, Vleminckx V, Van Den Bosch L, Cashman N, Fujisawa H, Drost MR, Sciot R, Bruyninckx F, Hicklin DJ, Ince C, Gressens P, Lupu F, Plate KH, Robberecht W, Herbert JM, Collen D, Carmeliet P (2001) Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. Nat Genet 28:131–138
8. Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, Carmeliet P, Mazarakis ND (2004) VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 429:413–417
9. MacGowan DJ, Scelsa SN, Waldron M (2001) An ALS-like syndrome with new HIV infection and complete response to antiretroviral therapy. Neurology 57:1094–1097
10. Moulignier A, Moulonguet A, Pialoux G, Rozenbaum W (2001) Reversible ALS-like disorder in HIV infection. Neurology 57:995–1001
11. Rothstein JD, Martin LJ, Kuncl RW (1992) Decreased glutamate transporter by the brain and spinal cord in amyotrophic lateral sclerosis. N Engl J Med 326:1464–1468
12. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl R, W. (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 38:73–84
13. Nagai M, Abe K, Okamoto K, Y. Itoyama (1998) Identification of alternative splicing forms of GLT-1 mRNA in the spinal cord of amyotrophic lateral sclerosis patients. Neurosci Lett 244: 165–168
14. Rothstein JD, Jin L, Dykes-Hoberg M, Kuncl RW (1993) Chronic inhibition of glutamate uptake produces a model of slow neurotoxicity. Proc Natl Acad Sci USA 90:6591–6595
15. Vandenberghe W, Ihle EC, Patneau DK, Robberecht W, Brorson JR (2000) AMPA receptor current density, not desensitization, predicts selective motoneuron vulnerability. J Neurosci 20:7158–7166
16. Vandenberghe W, Robberecht W, Brorson JR (2000) AMPA receptor calcium permeability, GluR2 expression, and selective motoneuron vulnerability. J Neurosci 20:123–132
17. Kwak S, Nakamura R (1995) Selective degeneration of inhibitory interneurons in the rat spinal cord induced by intrathecal infusion of acromelic acid. Brain Res 702:61–71
18. Kwak S, Nakamura R (1995) Acute and late neurotoxicity in the rat spinal cord in vivo induced by glutamate receptor agonists. J Neurol Sci 129 [Suppl]: 99–103
19. Carriedo SG, Yin HZ, Weiss JH (1996) Motor neurons are selectively vulnerable to AMPA/kainate receptor-mediated injury in vitro. J Neurosci 16:4069–4079
20. Lu YM, Yin HZ, Chiang J, Weiss JH (1996) Ca^{2+}-permeable AMPA/kainate and NMDA channels: high rate of Ca^{2+} influx underlies potent induction of injury. J Neurosci 16:5457–5465
21. Hollmann M, Hartley M, Heinemann S (1991) Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. Science 252:851–853
22. Verdoorn T, Burnashev N, Monye rH, Seeburg P, Sakmann B (1991) Structural determinants of ion flow through recombinant glutamate receptor channels. Science 252:1715–1718
23. Burnashev N, Monyer H, Seeburg P, Sakmann B (1992) Divalent ion permeability of AMPA receptor channels is dominated by the edited form of a single subunit. Neuron 8:189–198

24. Koh DS, Burnashev N, Jonas P (1995) Block of native Ca (2+)-permeable AMPA receptors in rat brain by intracellular polyamines generates double rectification. J Physiol (Lond) 486: 305–312
25. Hume RI, Dingledine R, Heinemann SF (1991) Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028–1031
26. Burnashev N, Zhou Z, Neher E, Sakmann B (1995) Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. J Physiol (Lond) 485:403–418
27. Swanson G, Kamboj S, Cull-Candy S (1997) Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. J Neurosci 17:58–69
28. Iihara K, Joo DT, Henderson J, Sattler R, Taverna FA, Lourensen S, Orser BA, Roder JC, Tymianski M (2001) The influence of glutamate receptor 2 expression on excitotoxicity in Glur2 null mutant mice. J Neurosci 21:2224–2239
29. Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna F, Velumian A, MacDonald J, Carlen P, Abramow-Newerly W, Roder J (1996) Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 17:945–956
30. Brusa R, Zimmermann F, Koh D, Feldmeyer D, Gass P, Seeburg P, Sprengel R (1995) Early-onset epilepsy and postnatal lethality associated with an editing-deficient GluR-B allele in mice. Science 270:1677–1680
31. Feldmeyer D, Kask K, Brusa R, Kornau HC, Kolhekar R, Rozov A, Burnashev N, Jensen V, Hvalby O, Sprengel R, Seeburg PH (1999) Neurological dysfunctions in mice expressing different levels of the Q/R site-unedited AMPAR subunit GluR-B. Nat Neurosci 2:57–64
32. Tomiyama M, Rodriguez-Puertas R, Cortes R, Christnacher A, Sommer B, Pazos A, Palacios JM, Mengod G (1996) Differential regional distribution of AMPA receptor subunit messenger RNAs in the human spinal cord as visualized by in situ hybridization. Neuroscience 75:901–915
33. Virgo L, Samarasinghe S, de Belleroche J (1996) Analysis of AMPA receptor subunit mRNA expression in control and ALS spinal cord. NeuroReport 7:2507–2511
34. Williams T, Day N, Ince P, Kamboj R, Shaw P (1997) Calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors: a molecular determinant of selective vulnerability in amyotrophic lateral sclerosis. Ann Neurol 42:200–207
35. Bar-Peled O, O'Brien RJ, Morrison JH, Rothstein JD (1999) Cultured motor neurons possess calcium-permeable AMPA/kainate receptors. NeuroReport 10:855–859
36. Kawahara Y, Kwak S, Sun H, Ito K, Hashida H, Aizawa H, Jeong S-Y, Kanazawa I (2003) Human spinal motoneurons express low relative abundance of GluR2 mRNA: an implication for excitotoxicity in ALS. J Neurochem 85:680–689
37. Furuyama T, Kiyama H, Sato K, Park HT, Takagi H, Tohyama J (1993) Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. Mol Brain Res 18:141–151
38. Tölle TR, Berthele A, Zieglgänsberger W, Seeburg PH, Wisden W (1993) The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. J Neurosci 13:5009–5028
39. Grossman SD, Wolfe BB, Yasuda RP, Wrathall JR (1999) Alterations in AMPA receptor subunit expression after experimental spinal cord contusion injury. J Neurosci 19:5711–5720
40. Laslo P, Lipski J, Nicholson LF, Miles GB, Funk GD (2001) GluR2 AMPA receptor subunit expression in motoneurons at low and high risk for degeneration in amyotrophic lateral sclerosis. Exp Neurol 169:461–471
41. Greig A, Donevan SD, Mujtaba TJ, Parks TN, Rao MS (2000) Characterization of the AMPA-activated receptors present on motoneurons. J Neurochem 74:179–191
42. Dai WM, Egebjerg J, Lambert JD (2001) Characteristics of AMPA receptor-mediated responses of cultured cortical and spinal cord neurones and their correlation to the expression of glutamate receptor subunits, GluR1–4. Br J Pharmacol 132:1859–1875
43. Shi S, Hayashi Y, Esteban JA, Malinow R (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. Cell 105:331–343
44. Passafaro M, Piech V, Sheng M (2001) Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nat Neurosci 4:917–926
45. Gerber AP, Keller W (2001) RNA editing by base deamination: more enzymes, more targets, new mysteries. Trends Biochem Sci 26:376–384
46. Keegan LP, Gallo A, O'Connell MA (2001) The many roles of an RNA editor. Nat Rev Genet 2:869–878
47. Chen SH, Habib G, Yang CY, Gu ZW, Lee BR, Weng SA, Silberman SR, Cai SJ, Deslypere JP, Rosseneu M et al (1987) Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. Science 238:363–366
48. Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J (1987) A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. Cell 50:831–840
49. Skuse GR, Cappione AJ, Sowden M, Metheny LJ, Smith HC (1996) The neurofibromatosis type I messenger RNA undergoes base-modification RNA editing. Nucleic Acids Res 24:478–485
50. Sommer B, Köhler M, Sprengel R, Seeberg P (1991) RNA editing in brain controls a determinant of ion flow in glutamate-gated channels. Cell 67:11–19
51. Köhler M, Burnashev N, Sakmann B, Seeburg P (1993) Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing. Neuron 10:491–500
52. Lomeli H, Mosbacher J, Melcher T, Hoger T, Geiger JR, Kuner T, Monyer H, Higuchi M, Bach A, Seeburg PH (1994) Control of kinetic properties of AMPA receptor channels by nuclear RNA editing. Science 266:1709–1713
53. Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB (1997) Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 387: 303–308
54. Hoopengardner B, Bhalla T, Staber C, Reenan R (2003) Nervous system targets of RNA editing identified by comparative genomics. Science 301:832–836
55. Rueter SM, Dawson TR, Emeson RB (1999) Regulation of alternative splicing by RNA editing. Nature 399:75–80
56. Burnashev N, Khodorova A, Jonas P, Helm P, Wisden W, Monyer H, Seeburg P, Sakmann B (1992) Calcium-permeable AMPA-kainate receptors in fusiform cerebellar glial cells. Science 256:1566–1570
57. Jonas P, Burnashev N (1995) Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. Neuron 15:987–990
58. Puchalski R, Louis J, Brose N, Traynelis S, Egebjerg J, Kukekov V, Wenthold R, Rogers S, Lin F, Moran Tea (1994) Selective RNA editing and subunit assembly of native glutamate receptors. Neuron 13:131–147
59. Swanson GT, Feldmeyer D, Kaneda M, Cull-Candy SG (1996) Effect of RNA editing and subunit co-assembly single-channel properties of recombinant kainate receptors. J Physiol (Lond) 492:129–142
60. Pellegrini-Giampietro DE, Gorter JA, Bennett MV, Zukin RS (1997) The GluR2 (GluR-B) hypothesis: Ca (2+)-permeable AMPA receptors in neurological disorders. Trends Neurosci 20:464–470
61. Akbarian S, Smith M, Jones E (1995) Editing for an AMPA receptor subunit RNA in prefrontal cortex and striatum in Alzheimer's disease, Huntington's disease and schizophrenia. Brain Res 699:297–304
62. Paschen W, Hedreen J, Ross C (1994) RNA editing of the glutamate receptor subunits GluR2 and GluR6 in human brain tissue. J Neurochem 63:1596–1602

63. Pellegrini-Giampietro DE, Bennett MV, Zukin RS (1994) AMPA/kainate receptor gene expression in normal and Alzheimer's disease hippocampus. Neuroscience 61:41–49
64. Rump A, Sommer C, Gass P, Bele S, Meissner D, Kiessling M (1996) Editing of GluR2 RNA in the gerbil hippocampus after global cerebral ischemia. J Cerebral Blood Flow Metabol 16:1362–1365
65. Kamphuis W, Lopes da Silva F (1995) Editing status at the Q/R site of glutamate receptor-A, -B, -5 and −6 subunit mRNA in the hippocampal kindling model of epilepsy. Mol Brain Res 29:35–42
66. Takuma H, Kwak S, Yoshizawa T, Kanazawa I (1999) Reduction of GluR2 RNA editing, a molecular change that increases calcium influx through AMPA receptors, selective in the spinal ventral gray of patients with amyotrophic lateral sclerosis. Ann Neurol 46:806–815
67. Kawahara Y, Ito K, Sun H, Aizawa H, Kanazawa I, Kwak S (2004) RNA editing and death of motor neurons. Nature 427:801
68. Kawahara Y, Ito K, Sun H, Kanazawa I, Kwak S (2003) Low editing efficiency of GluR2 mRNA is associated with a low relative abundance of ADAR2 mRNA in white matter of normal human brain. Eur J Neurosci 18:23–33
69. Suzuki T, Tsuzuki K, Kameyama K, Kwak S (2003) Recent advances in the study of AMPA receptors. Folia Pharmacol Jpn 122:515–526
70. Greger IH, Khatri L, Kong X, Ziff EB (2003) AMPA receptor tetramerization is mediated by Q/R editing. Neuron 40:763–774
71. Greger IH, Khatri L, Ziff EB (2002) RNA editing at Arg607 controls AMPA receptor exit from the endoplasmic reticulum. Neuron 34:759–772
72. Kim U, Wang Y, Sanford T, Zeng Y, Nishikura K (1994) Molecular cloning of cDNA for double-stranded RNA adenosine deaminase, a candidate enzyme for nuclear RNA editing. Proc Natl Acad Sci U S A 91:11457–11461
73. O'Connell MA, Krause S, Higuchi M, Hsuan JJ, Totty NF, Jenny A, Keller W (1995) Cloning of cDNAs encoding mammalian double-stranded RNA-specific adenosine deaminase. Mol Cell Biol 15:1389–1397
74. Melcher T, Maas S, Herb A, Sprengel R, Seeburg P, Higuchi M (1996) A mammalian RNA editing enzyme. Nature 379:460–464
75. Melcher T, Maas S, Herb A, Sprengel R, Higuchi M, Seeburg PH (1996) RED2, a brain-specific member of the RNA-specific adenosine deaminase family. J Biol Chem 271:31795–31798
76. O'Connell MA, Gerber A, Keller W (1997) Purification of human double-stranded RNA-specific editase 1 (hRED1) involved in editing of brain glutamate receptor B pre-mRNA. J Biol Chem 272:473–478
77. Chen CX, Cho DS, Wang Q, Lai F, Carter KC, Nishikura K (2000) A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. RNA 6:755–767
78. Lai F, Chen C, Carter K, Nishikura K (1997) Editing of glutamate receptor B subunit ion channel RNAs by four alternatively spliced DRADA2 double-stranded RNA adenosine deaminases. Mol Cell Biol 17:2413–2424
79. Gerber A, O'Connell M, Keller W (1997) Two forms of human double-stranded RNA-specific editase 1 (hRED1) generated by the insertion of an Alu cassette. RNA 3:453–463
80. Higuchi M, Single F, Kohler M, Sommer B, Sprengel R, Seeburg P (1993) RNA editing of AMPA receptor subunit GluR-B: a base-paired intron-exon structure determines position and efficiency. Cell 75:1361–1370
81. Herb A, Higuchi M, Sprengel R, Seeburg PH (1996) Q/R site editing in kainate receptor GluR5 and GluR6 pre-mRNAs requires distant intronic sequences. Proc Natl Acad Sci U S A 93:1875–1880
82. Aruscavage PJ, Bass BL (2000) A phylogenetic analysis reveals an unusual sequence conservation within introns involved in RNA editing. RNA 6:257–269
83. Wang Q, Khillan J, Gadue P, Nishikura K (2000) Requirement of the RNA editing deaminase ADAR1 gene for embryonic erythropoiesis. Science 290:1765–1768
84. Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N, Feldmeyer D, Sprengel R, Seeburg PH (2000) Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. Nature 406:78–81
85. Bernard A, Khrestchatisky M (1994) Assessing the extent of RNA editing in the TMII regions of GluR5 and GluR6 kainate receptors during rat brain development. J Neurochem 62:2057–2060
86. Paschen W, Djuricic B (1994) Extent of RNA editing of glutamate receptor subunit GluR5 in different brain regions of the rat. Cell Mol Neurobiol 14:259–270
87. Paschen W, Djuricic B (1995) Regional differences in the extent of RNA editing of the glutamate receptor subunits GluR2 and GluR6 in rat brain. J Neurosci Methods 56:21–29
88. Garcia-Barcina JM, Matute C (1996) Expression of kainate-selective glutamate receptor subunits in glial cells of the adult bovine white matter. Eur J Neurosci 8:2379–2387
89. de Zulueta MP de, Matute C (1999) Reduced editing of low-affinity kainate receptor subunits in optic nerve glial cells. Brain Res Mol Brain Res 73:104–109
90. Lowe D, Jahn K, Smith D (1997) Glutamate receptor editing in the mammalian hippocampus and avian neurons. Brain Res Mol Brain Res 48:37–44
91. Kawahara Y, Ito K, Sun H, Ito M, Kanazawa I, Kwak S (2004) Regulation of glutamate receptor RNA editing and ADAR mRNA expression in developing human normal and Down's syndrome brains. Brain Res Dev Brain Res 148:151–155
92. Carlson NG, Howard J, Gahring LC, Rogers SW (2000) RNA editing (Q/R site) and flop/flip splicing of AMPA receptor transcripts in young and old brains. Neurobiol Aging 21:599–606
93. Seeburg PH (2002) A-to-I editing: new and old sites, functions and speculations. Neuron 35:17–20
94. Maas S, Patt S, Schrey M, Rich A (2001) Underediting of glutamate receptor GluR-B mRNA in malignant gliomas. Proc Natl Acad Sci U S A 98:14687–14692
95. Kortenbruck G, Berger E, Speckmann EJ, Musshoff U (2001) RNA editing at the Q/R site for the glutamate receptor subunits GLUR2, GLUR5, and GLUR6 in hippocampus and temporal cortex from epileptic patients. Neurobiol Dis 8:459–468
96. Seifert G, Steinhauser C (1995) Glial cells in the mouse hippocampus express AMPA receptors with an intermediate Ca^{2+} permeability. Eur J Neurosci 7:1872–1881
97. Geiger JR, Melcher T, Koh DS, Sakmann B, Seeburg PH, Jonas P, Monyer H (1995) Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15:193–204
98. Paupard MC, O'Connell MA, Gerber AP, Zukin RS (2000) Patterns of developmental expression of the RNA editing enzyme rADAR2. Neuroscience 95:869–879
99. Schmitt J, Dux E. Gissel C, Paschen W (1996) Regional analysis of developmental changes in the extent of GluR6 mRNA editing in rat brain. Brain Res Dev Brain Res 91:153–157
100. Bernard A, Ferhat L, Dessi F, Charton G, Represa A, Ben-Ari Y, Khrestchatisky M (1999) Q/R editing of the rat GluR5 and GluR6 kainate receptors in vivo and in vitro: evidence for independent developmental, pathological and cellular regulation. Eur J Neurosci 11:604–616
101. Paschen W, Dux E, Djuricic B (1994) Developmental changes in the extent of RNA editing of glutamate receptor subunit GluR5 in rat brain. Neurosci Lett 174:109–112
102. Lai F, Chen CX, Lee VM, Nishikura K (1997) Dramatic increase of the RNA editing for glutamate receptor subunits during terminal differentiation of clonal human neurons. J Neurochem 69:43–52

103. Grigorenko EV, Bell WL, Glazier S, Pons T, Deadwyler S (1998) Editing status at the Q/R site of the GluR2 and GluR6 glutamate receptor subunits in the surgically excised hippocampus of patients with refractory epilepsy. NeuroReport 9: 2219–2224
104. Dabiri GA, Lai F, Drakas RA, Nishikura K (1996) Editing of the GLuR-B ion channel RNA in vitro by recombinant double-stranded RNA adenosine deaminase. EMBO J 15:34–45
105. Yang J, Sklar P, Axel R, Maniatis T (1997) Purification and characterization of a human RNA adenosine deaminase for glutamate receptor B pre-mRNA editing. Proc Natl Acad Sci USA 94:4354–4359
106. Saccomanno L, Bass BL (1994) The cytoplasm of Xenopus oocytes contains a factor that protects double-stranded RNA from adenosine-to-inosine modification. Mol Cell Biol 14: 5425–5432