Unequal Expression of Allelic Kainate Receptor GluR7 mRNAs in Human Brains

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We describe here the first example of an exonic polymorphism that affects the primary structure of a human ionotropic glutamate receptor. The human kainate receptor GluR7 gene contains a thymine (T)/guanine (G) nucleotide variation that determines a serine or alanine at position 310 in the extracellular region of GluR7 receptor subunits. Our finding contrasts with a previous report that suggested that GluR7 transcripts were RNA-edited at this site. Whole-cell patch-clamp recordings did not detect differences in receptor activation and desensitization between the human GluR7 receptor isoforms expressed in HEK-293 cells. Analysis of 41 tissue samples obtained from 30 human brains revealed expression level differences between GluR7 alleles expressed in the same brain. The expression level of the allelic GluR7 mRNAs differed in 27 samples from 1.2- to 12.7-fold. Unequal expression level of allelic mRNAs is characteristic for genes that are affected by genomic imprinting or that contain mutations. Genomic imprinting in most cases is conserved between human and mice. However, we did not detect unequal expression of allelic GluR7 mRNAs in mice. Our results are important for future studies that explore a potential role or roles for GluR7 receptors in the brain and for neurological disorders.

Key words: kainate receptor; GluR7; polymorphism; allele expression; genomic imprinting; RNA editing

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Materials and Methods

Source of DNA and RNA samples. The rat genomic DNA (#6750-1), rat total RNA (#64060-1), human genomic DNA (#6550-1), and human total RNA (#64020-1) that were used in the experiments described in Figure 1 were obtained from Clontech (Palo Alto, CA). Genomic DNA and brain total RNA from neuropsychiatric disorder cases were isolated from brain tissue samples kindly provided by the Stanley Foundation at the National Alliance for the Mentally Ill Research Institute (NAMI, Bethesda, MD). The human genomic control DNAs used for the estimation of GluR7 allele frequency and genotype distribution in the group of Caucasian people (Table 1) were obtained from the Alzheimer Disease Research Center at the University of California, San Diego.
Table 1. Frequency of the GluR7 T and G allele and genotype distribution of the GluR7 T/G polymorphism in 35 Caucasians

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Genomic PCR fragments that cover the T/G site of the human GluR7 gene were obtained from 35 genomic DNA samples (Caucasian) and genotyped by using the cycled primer extension assay (see Materials and Methods). n = 35.

The university of California, San Diego. E. Masliah from the Department of Neurosciences, University California, San Diego, provided all other human brain tissue samples. Primers used to analyze nucleotide variation in rat, human, and mouse GluR7 genes and mRNAs. Rat GluR7 cDNA synthesis was initiated with the gene-specific primer R7.3170 (GATGAAGCCCAAGCAGCTGCTG) and H7.110 (TTCCCATTGAGCCTCCTTGAT). Genomic DNA products covering either the T or G allele at the G/A site were amplified by using the following primer pairs, respectively: primer R7.890 (GAATTACATCCATCTTAC(T)(C)ACC) and primer R7.1095 (TCTTCTTGAGGCTGATGATGTC). Rat RT-PCR fragments containing the GluR7 T/G and G/A site were amplified with the primer pair R7.1109 (GCAGCCCTGCTCTACGATGCGGTC) and primer R7.1309 (CATCACTCCATCCAGCAGGCCTGACT) or primer R7.1095 (TCTTCTTGAGGCTGATGATGTC) and primer R7.1110 (GCAAAGCGGCTCTCTCGTGATC). The RT-PCR products containing the GluR7 T/G and G/A site were amplified by using the primer pair H7.890 (GAATTACATCCATCTTAC(T)(C)ACC) and H7.1309 (TCTTCTTGAGGCTGATGATGTC), and the dideoxy terminator ddTTP. Human or mouse GluR7 cDNA was synthesized on total brain RNA by using the GluR7 gene-specific primer R7.3170 (GATGAAGCCCAAGCAGCTGCTG) or R7.PE.G/A (GAAGCGCCAGGGCTGA). The T/G polymorphism identified in the human GluR7 gene was analyzed for the presence of nucleotide variation at the T/G or G/A site via a homogenizer and disposable generator probes obtained from Omni (Warrenton, VA). cDNA was generated by the Thermoscript RT-PCR system (Ambion, Austin, TX). The RT-PCR products containing the GluR7 T/G and G/A site were amplified with the primer pair H7.890 (GAATTACATCCATCTTAC(T)(C)ACC) and H7.1309 (TCTTCTTGAGGCTGATGATGTC), and the dideoxy terminator ddTTP. The primer M7.342 (GACCATCACGCGGCTGATGTA) or R7.PE.G/A (GAAGCGCCAGGGCTGA) and the dideoxy terminator ddTTP. Human or mouse GluR7 cDNA and mRNA was studied by using a cycled primer extension assay with the dideoxy terminator ddTTP as the dideoxy terminator (Fig. 1A). RT-PCR reactions were performed on human fetal whole brain total RNA by that represented pooled RNAs derived from 13 brains. Primer extension analysis of the obtained RT-PCR products revealed a nucleotide variation at the T/G site. Thus, we observed both a 21 bp (T-containing) and 27 bp (non-T-containing) extension product (Fig. 1A, lane 1). RT-PCR reactions were performed on human fetal whole brain total RNA that represented pooled RNAs derived from 13 brains. Primer extension analysis of the obtained RT-PCR products revealed a nucleotide variation at the T/G site. Thus, we observed both a 21 bp (T-containing) and 27 bp (non-T-containing) extension product (Fig. 1A, lane 1). The same result was obtained in two independent assays and is consistent with the result of Nutt et al. (1994). Phosphorimaging analysis determined that a thymine was present at the T/G site in ~92% of the analyzed fetal RT-PCR products. However, we could not detect a nucleotide variation at the G/A site in primer extension assays analyzing RT-PCR fragments containing the G/A site (data not shown).

In the rat GluR7 cDNA a guanine nucleotide was found at both corresponding sites (Bettler et al., 1992; Lomeli et al., 1992). Because RNA editing at the well characterized Q/R site in GluR2, GluR5, and GluR6 subunits proceeds to a similar extent in rat and humans (Seeburg, 1996), we next determined whether residues at the T/G and G/A sites in the rat GluR7 cDNA also showed variability. We performed primer extension assays with ddTTP as a dideoxy terminator on RT-PCR products (see Materials and Methods; Schiffer and Heinemann, 1999). The cDNA products were obtained by amplifying a region that included the putative thymine/guanine (T/G) site at position 928 and the guanine/adenine (G/A) site at position 1055. The GluR7 cDNAs used as

RESULTS

A genetic polymorphism underlies the observed variation of the human GluR7 cDNA sequence

Two nucleotide variations found in cDNAs coding for the human GluR7 receptor (also known as EAAs) were postulated to result from RNA editing (Nutt et al., 1994). In the human GluR7 cDNA, guanine nucleotides were found at cDNA position 928, and adenosine nucleotides were found at position 1055; these nucleotides did not match the reported human genomic sequences of thymine and guanine, respectively (Nutt et al., 1994). To characterize the expressed editing events in the human GluR7 receptor, we analyzed the RT-PCR products containing the G/A site (data not shown). The same result was obtained in two independent assays and is consistent with the result of Nutt et al. (1994). Phosphorimaging analysis determined that a thymine was present at the T/G site in ~92% of the analyzed fetal RT-PCR products. However, we could not detect a nucleotide variation at the G/A site in primer extension assays analyzing RT-PCR fragments containing the G/A site (data not shown).

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template for the amplifications were derived from embryonic (E18) and adult (ad) whole rat brain total RNA. Primer extension analyses with dideoxy terminators were performed to detect nucleotide variations at the T/G site of the human GluR7 mRNA or gene. A \textsuperscript{32}P-labeled primer was annealed to GluR7-specific RT-PCR (derived from cDNA) or genomic PCR products containing the T/G site and was extended in the presence of ddTTP. Lane 1, T/G site in cDNA (total RNA derived from 13 fetal brains, fe). Lane 2, T/G site in gene (DNA derived from one adult brain, ad). The detection of two extended primer products, a 21- and 27-mer in both the cDNA and genomic DNA, indicates that the human GluR7 gene \textit{GRIK3} is polymorphic at the T/G site. B, Primer extension analyses with dideoxy terminators were performed to detect potential editing events at the G/A and T/G site in rat brain GluR7 transcripts. \textsuperscript{32}P-labeled primers were mixed with GluR7-specific RT-PCR products derived from brain cDNA and were extended in the presence of the dideoxy terminator ddATP. Lanes 1, 3, Rat GluR7 T/G site in embryonic (E18) and adult (ad) brain, respectively. Lanes 2, 4, Rat GluR7 G/A site in embryonic (E18) and adult (ad) brain, respectively. If the nucleotide variations reported for these sites existed in rat, we would have detected two extended primer bands in each lane: a 29-mer and a 23-mer primer band for the T/G site and a 23- and a 20-mer band for the G/A site. The detection of only one 29-mer band (T/G site, lanes 1, 3) and only one 23-mer band (G/A site, lanes 2, 4) indicated that the GluR7 mRNA sequence in the rat brain is not variable (edited) at these sites. C, Illustration of the genomic polymorphism identified in the \textit{GRIK3} gene (nucleotide position 928 in \textit{EA5} cDNA; GenBank accession number U16127). Nucleotide and amino acid sequences of the region surrounding the T/G site in the GluR7 cDNA/\textit{GRIK3} gene are shown. The variable nucleotide position found in the GluR7 gene and cDNA and the predicted alternating amino acids in the GluR7 receptor protein at amino acid position 310 are indicated in \textbf{bold letters}. Amino acid position number 1 corresponds to methionine in the GluR7 receptor precursor containing the signal peptide. D, Schematic illustration of the cycled primer extension assay with the dideoxy terminator ddATP used to detect the T/G nucleotide variation at the T/G site of the human GluR7 receptor gene and mRNA. A radiolabeled primer (H7.PE.T/G), annealed to a genomic primer extension assay we found that thymine was incorporated in \textasciitilde 50\% of the extension products and that a nucleotide other than thymine was present in the rest of the extension products (Fig. 1A, lane 2). This observation is consistent with the interpretation that the individual was heterozygotic at this site in the \textit{GRIK3} alleles. Subcloned RT-PCR (cDNA) and genomic PCR products were sequenced to verify the results of the primer extension assays. As expected, guanine and thymine residues were found at the T/G site in both the GluR7 cDNA and the gene. Five genomic clones (derived from the single individual) were analyzed; two clones contained a thymine and one guanine at the T/G site. In 10 cDNA clones (derived from 13 pooled embryonic brains), one contained a guanine and nine contained a thymine at the T/G site.

Prime extension assays with ddGTP as the dideoxy terminator were performed on genomic PCR fragments derived from an additional three individuals that were heterozygotic for the T/G polymorphism. In all three samples we identified guanine in \textasciitilde 50\% of the extension products (data not shown). Additionally, we analyzed PCR fragments containing the G/A site derived from genomic DNA from 15 individuals. However, we could not detect a nucleotide variation in the GluR7 gene at the G/A site in three independent primer extension assays (data not shown).

These results demonstrate that the T/G nucleotide variation in the GluR7 mRNA is caused by a bi-allelic polymorphism in the human GluR7 gene \textit{GRIK3} and that the T/G site contains no nucleotide types other than guanine or thymine. This exonic nucleotide variation predicts either a serine (S) or alanine (A) at position 310 (S/A site) in the N-terminal extracellular domain of the GluR7 receptor subunit (illustrated in Fig. 1C).

We were interested in determining the allele frequency and genotype distribution of the GluR7 T/G polymorphism in the
human population. To address these questions, we analyzed the genomes of 35 healthy control Caucasians and determined the nucleotide identity at the T/G site of each GluR7 allele. The results of these experiments are summarized in Table 1. The genotypes for each individual were obtained by performing primer extension assays with dideoxy terminators on genomic PCR fragments derived from these individuals (data not shown).

The frequency of the T and G allele among the group of 35 individuals (21 female and 14 male) was estimated as 0.70 and 0.30, respectively (Table 1). Of the individuals, 42.8% were heterozygous for the T/G polymorphism in the GluR7 gene, 48.6% were homozygous for the T allele, and 8.6% were homozygous for the G allele (Table 1). The observed genotype distributions correlated well with the Hardy–Weinberg equilibrium, as predicted from the observed allele frequencies. These results suggest that the T/G polymorphism in the GluR7 gene is common in the human population from which our genomic DNA was obtained (estimated heterozygosity, 0.428). Although we have not found evidence for a genetic polymorphism at the G/A site, we cannot exclude the existence of a rare genetic polymorphism at this site of the GRIK3 gene. The polymorphism at the T/G site of the GluR7 gene is the first example of a genetic polymorphism that affects the primary structure of a human ionotropic glutamate receptor subunit.

**Electrophysiological and pharmacological properties of the serine and alanine isoforms of the human GluR7 receptor**

We previously demonstrated that the rat GluR7 receptor subunit forms functional homomeric receptor channels with low sensitivity to glutamate (Schiffer et al., 1997). Because the T/G polymorphism occurs within the coding sequence of GluR7, we assayed for differences in the functional behavior of the human GluR7 receptor isoforms. Additionally, we were interested in comparing the functional properties of human GluR7 receptors with those of rat GluR7 receptors. The detected nucleotide variation at the T/G site lies in a codon that codes for the amino acid serine when thymine is present or that codes for alanine when guanine is present (illustrated in Fig. 1C). The affected amino acid at position 310 is localized in the N-terminal extracellular domain of the GluR7 receptor protein (Nutt et al., 1994). This domain of glutamate receptors has been shown to affect receptor desensitization (Krupp et al., 1998) and to participate in ligand binding (Stern-Bach et al., 1994; Armstrong et al., 1998). The thymine residue at the T/G site of the human GluR7(S310) cDNA, encoding the serine variant, was replaced by PCR-based site-directed mutagenesis with guanine to obtain the human GluR7(A310) cDNA, coding for the alanine isoform. HEK-293 cells were transiently transfected with the human GluR7(A310) or GluR7(S310) cDNAs, and receptor currents evoked by fast application of glutamate (30 mM) were recorded in whole-cell patch-clamp configuration (Fig. 2A).

Homomeric human GluR7(A310) or GluR7(S310) receptors had similar functional properties (Fig. 2A). The mean peak amplitudes of glutamate-evoked currents for the human GluR7(A310) and GluR7(S310) receptors were 1.9 ± 0.3 nA, with a 10–90% rise time of 1.4 ± 0.2 msec, and 1.3 ± 0.2 nA, with a 10–90% rise time of 1.3 ± 0.1 msec, respectively. These data were not significantly different from each other or from that of the previously characterized rat GluR7a(S310) receptor (1.1 ± 0.2 nA mean peak amplitude and 1.4 ± 0.1 msec rise time) (Schiffer et al., 1997). Glutamate-evoked currents from the human GluR7(A310), GluR7(S310), and rat GluR7a(S310) receptor desensitized with similar time courses (τdes of 7.1 ± 0.7, 6.3 ± 0.4, and 8.4 ± 0.5 msec, respectively). Dose–response analyses of peak currents evoked by l-glutamate gave EC50 values for human GluR7(A310) and GluR7(S310) receptors of 4.1 and 3.8 mM, respectively (Fig. 2B). Interestingly, the estimated EC50 values for these GluR7 receptor isoforms were lower than those of rat GluR7a(S310) receptors (EC50 = 7.5 mM in parallel experiments), although the amino acid sequences of the mature rat and human GluR7(S310) receptor proteins differ by only eight amino acids. Consistent with this observation, Nutt et al. (1994) reported l-glutamate binding affinities that differed by approximately threefold between human and rat GluR7 receptors (Nutt et al., 1994).
human alanine and serine variants of the GluR7 receptor have distinct functional behavior when expressed in the brain, perhaps in assemblies with additional subunits.

**GluR7 alleles are expressed unequally in human brains**

The human GluR7 gene has been mapped to chromosome one at region 1p34–33 (Puranam et al., 1993). Recent studies identified two tumor suppressor genes at this region, which are genomic imprinted. These genes code for the tumor suppressor protein p73 and NOEY2 and have been mapped to 1p36 and 1p31, respectively (Caron et al., 1995; Kaghad et al., 1997; Yu et al., 1999). Genomic imprinting is an epigenetic mechanism that silences the expression of an allele that is dependent on whether the inheritance of the allele is from the father (paternal) or the mother (maternal). We were interested in testing whether the human GluR7 gene (GRIK3) is affected by genomic imprinting, which would be manifested by unequal expression of GluR7 alleles in human brains. The T/G polymorphism identified in the human GluR7 gene served in these experiments as a genetic marker to identify the differential expression of GluR7 alleles.

First, we analyzed the GluR7 allele expression levels in total RNAs isolated from brain tissue samples derived from nine individuals heterozygous for the T/G polymorphism (three fetuses and six adults). The brain regions from which the samples were derived were not identified for these individuals. The total RNA isolated from each individual brain sample was reverse-transcribed with GluR7-specific primers, and the cDNAs were used as templates to obtain pools of RT-PCR products covering the T/G site of the GluR7 mRNA.

Each pool of RT-PCR products contained a mix of DNA fragments that had either a T or G at the T/G site, because only individuals heterozygous for the T/G polymorphism were analyzed. Individual RT-PCR product pools were used as template for the cycled primer extension assay with the dideoxy terminator ddTTP to determine the ratio between the T allele- and G allele-derived PCR fragments in these pools. We have demonstrated previously that our assay is a sensitive method to quantify nucleotide variations in genomic PCR or RT-PCR fragment fractions (Schiffer and Heinemann, 1999). It was assumed that the relative proportion of the T or G allele in the analyzed DNA fragment pools reflected the relative expression level in the brain tissue samples. In our study the term “unequally expressed” was used when the relative GluR7 allele expression levels differed by >1.2-fold from equal expression (based on the analysis of mouse GluR7 allele expression; see also Fig. 5 and Discussion).

Our assays revealed that GluR7 alleles were represented unequally in most of the RT-PCR product fractions. In six samples the estimated T/G allele ratio varied between 0.30 and 0.73, indicating a lower expression of the T allele as compared with the G allele (Fig. 3, lanes 1–4, 6, 8). In contrast, one sample showed a T/G ratio of 4.7, indicating a higher expression of the T allele as compared with the G allele (Fig. 3, lane 5). Two of the nine analyzed samples had a ratio of 0.89 and 0.87, reflecting a nearly equal GluR7 allele expression (Fig. 3, lanes 7, 9). In summary, seven of the nine analyzed samples showed from 1.4- to 4.7-fold reduction in the expression levels of one GluR7 allele as compared with the second allele. All assays shown were repeated at least three times and gave reproducible results. The estimated SD for each analyzed sample ranged between 0.9 and 5.2% (n = 3). Furthermore, repeating the analysis with independent cDNA synthesis and PCR reactions gave similar results (data not shown). Unequal expression as a result of polyploidy was excluded, because genomic PCR fragments derived from each sample had the expected T/G ratio of ~1 (data not shown).

To analyze the unequal expression of GluR7 mRNA further, we analyzed additional human brain total RNA samples. We obtained total RNA samples isolated from the temporal lobes of neuropsychiatric patients matched for age and postmortem interval. Analyzing genomic DNA with the cycled primer extension assay identified twelve individuals that were heterozygous for the T/G polymorphism in the GluR7 gene (data not shown). The corresponding 12 total RNA samples were analyzed as described before, and the relative GluR7 allele expression levels were determined (data not shown). Similar to our first observation, we detected unequal expression levels in most of the analyzed samples. Six samples showed a T/G ratio between 0.52 and 0.72, whereas four samples showed a T/G ratio of 1.27, 1.4, 3.0, and 5.8. Two samples showed a fairly equal expression of GluR7 alleles, with a T/G ratio of 1.01 and 0.78. These results indicate that the T and G allele of the GluR7 gene were expressed unequally in most of the human temporal lobes that we analyzed. The detection of unequal GluR7 allele expression in brain samples matched for brain region and age supported our initial observations and suggested that unequal GluR7 allele expression occurs in the majority of human brains.

**Regional diversity of unequal expression of allelic GluR7 mRNAs in individual brains**

The presence of mutations and genomic imprinting of the GluR7 gene are two possible mechanisms that account for our observation that unequal GluR7 allele expression occurred in human brain samples and that the difference between the GluR7 T allele and G allele expression levels varied over a wide range. To analyze this observation further, we determined the relative expression level of GluR7 alleles in various brain regions isolated from individual human brains. Genotyping of brain samples from adult individuals without disease history identified two brains (A and B) that were heterozygous for the T/G polymorphism in the GRIK3 gene. Total RNA from frontal cortex, occipital cortex, parietal cortex, mesen-

![Figure 3.](image-url)
unequal expression of GluR7 alleles in human brains. To screen mice strains for nucleotide variations in the GluR7 gene, we needed to identify a transcribed nucleotide variation between GluR7 genes in the mouse brain cDNA library and cloned the 3' cDNA sequence was only partially available, we first screened a mouse brain cDNA library and cloned the 3' cDNA sequence (1.8 kb) that we obtained allowed us to identify a transcribed nucleotide variation between GluR7 genes in the mouse brain cDNA library and cloned the 3' cDNA sequence (1.8 kb) that we obtained allowed us to identify a transcribed nucleotide variation between GluR7 genes from genetically divergent mice strains. Because the mouse GluR7 gene showed unequal allele expression in a mouse model. To generate such a mouse model, we needed to identify a transcribed nucleotide variation between GluR7 genes in those strains and use this variation to screen for allelic GluR7 mRNAs in human, but not in mouse, brains. An exonic genetic polymorphism accounts for the expression of GluR7 receptor variants. We identified a common T/G polymorphism in the human GRIK3 gene (heterozygosity, 0.428), which codes for the glutamate receptor subunit GluR7. The exonic nucleotide variation predicts either a serine or alanine at position 310 (S/A site) in the first half of the N-terminal extracellular domain of the receptor. The function of this domain is not well understood, but it may play a role in receptor assembly (Kuusinen et al., 1999; Leuschner and Hoch, 1999) or in determining functional properties. Our electrophysiological characterization of the alanine and serine isoforms of the human GluR7 receptors showed no difference in pharmacology, receptor desensitization and rise time (see Fig. 2), or in receptor assembly (data not shown). However, these results do not exclude the possibility that the T/G polymorphism in the GRIK3 gene affects GluR7-mediated neurotransmission or that functionally different GluR7 receptor isoforms might be related to neurological disease.

**DISCUSSION**

**An exonic genetic polymorphism accounts for the expression of GluR7 receptor variants.** We identified a common T/G polymorphism in the human GRIK3 gene (heterozygosity, 0.428), which codes for the glutamate receptor subunit GluR7. The exonic nucleotide variation predicts either a serine or alanine at position 310 (S/A site) in the first half of the N-terminal extracellular domain of the receptor. The function of this domain is not well understood, but it may play a role in receptor assembly (Kuusinen et al., 1999; Leuschner and Hoch, 1999) or in determining functional properties. Our electrophysiological characterization of the alanine and serine isoforms of the human GluR7 receptors showed no difference in pharmacology, receptor desensitization and rise time (see Fig. 2), or in receptor assembly (data not shown). However, these results do not exclude the possibility that the T/G polymorphism in the GRIK3 gene affects GluR7-mediated neurotransmission or that functionally different GluR7 receptor isoforms might be related to neurological disease.

**Polymeric GluR7 genes reveal differentially expressed allelic GluR7 mRNAs in human, but not in mouse, brains.**

Recent studies discovered that the tumor suppressor genes p73 and NOEY2 are modulated by genomic imprinting (Kaghad et al., 1997; Yu et al., 1999). The p73 and NOEY2 genes are mapped to the small arm of chromosome one at region p36.2–3 and p31, respectively. The vicinity of these genes to the GluR7 gene, which has been mapped to region 1p33–34 (Puranam et al., 1993), led us to investigate whether the GluR7 gene similarly is imprinted. To address this question, we performed gene-specific RT-PCRs on total RNA isolated from 41 tissue samples, which were derived from 30 individual human brains. All 30 brains were identified as male 129/SvEvTac mice were crossed with female CBA/CaJ mice and female 129/Sv/EvTac mice were crossed with male CBA/CaJ mice to obtain F1 generation mice that were heterozygous for the C/T polymorphism. The relative GluR7 allele expression levels were analyzed in F1 generation mice at postnatal ages P3, P13, and P21. Total RNA was isolated from P3 whole brains, P13 cerebella, and P21 cortex, cerebella, and hippocampi. GluR7-specific primers were used to synthesize cDNA and to amplify RT-PCR products covering the C/T site. The cycled primer extension assay with dideoxy nucleotide ddATP was used to quantify the relative expression level of GluR7 alleles and to analyze the mouse GluR7 gene (see Materials and Methods; Schiffer and Heinemann, 1999).
samples obtained from two individual human brains. 32P-labeled primers in the GluR7 gene are shown. The analysis combined 13 total RNA samples from individuals. Primer extension analysis with the dideoxy terminator ddTTP was performed to analyze the expression of GluR7 alleles in various brain regions of individual human brains (see Materials and Methods). Only individuals that were heterozygous for the T/G polymorphism in the GluR7 gene are shown. 32P-labeled primers were annealed to GluR7-specific RT-PCR products obtained from each total RNA sample and were extended in the presence of the dideoxy terminator ddATP. Primers were separated by gel electrophoresis and were visualized by autoradiography. The detected primer products, a 21-mer (T allele-specific) and a 22-mer (C allele-specific), were quantified by phosphorimaging, and the T/G ratio was determined. Each total RNA sample was analyzed three times to obtain the mean values and the SD shown in the bar graph. The bar graph shows the relative expression level of the GluR7 allele in regions from brain A (lanes 1–6) and brain B (lanes 1–7); also shown is the relative representation of T and G allele in the genomic PCR fraction obtained for brain B (lane 8).

![Figure 4](image_url)

**Figure 4.** Varying unequal expression of GluR7 allelic mRNAs in brain regions from individuals. Primer extension analysis with the dideoxy terminator ddTTP was performed to analyze the expression of GluR7 alleles in various brain regions of individual human brains (see Materials and Methods). Only individuals that were heterozygous for the T/G polymorphism in the GluR7 gene are shown. The analysis combined 13 total RNA samples obtained from two individual human brains. 32P-labeled primers were annealed to GluR7-specific RT-PCR products obtained from each total RNA sample and were extended in the presence of the dideoxy terminator ddATP. Primers were separated by gel electrophoresis and were visualized by autoradiography. The detected primer products, a 21-mer (T allele-specific) and a 22-mer (C allele-specific), were quantified by phosphorimaging, and the T/G ratio was determined. Each total RNA sample was analyzed three times to obtain the mean values and the SD shown in the bar graph. A. Autoradiogram of brain A; analysis of tissue derived from frontal cortex (FC), occipital cortex (OC), mesencephalon (MES), cerebellum (CER), basal ganglia (BG), and thalamus (TH). The total RNA samples are derived from one individual brain (lanes 1–6). Also shown is an analysis of T/G polymorphism in the GluR7 gene (lane 7). B. Bar graph showing the relative expression level of the GluR7 T allele in regions from brain A (lanes 1–6). Relative representation of T and G allele in genomic PCR fraction is shown in lane 7. C. Bar graph showing the relative expression level of GluR7 T and G allele in regions from brain B (lanes 1–7); also shown is the relative representation of T and G allele in the genomic PCR fraction obtained for brain B (lane 8).

Table 2. Summary of estimated GluR7 T/G ratios, indicating unequal GluR7 allele expression level in 27 of 41 human brain samples

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*Four of seven brain samples derived from human brain A.

![Figure 5](image_url)

**Figure 5.** No unequal expression of GluR7 allelic mRNAs in brains from 129/SvEvTac × CBA/CaJ F1 generation mice. Primer extension analysis with the dideoxy terminator ddATP was performed to analyze the expression of GluR7 alleles in various brain regions of 129/SvEvTac × CBA/CaJ F1 generation mice (see Materials and Methods). Only mice heterozygous for the C/T polymorphism in the GluR7 gene are shown. 32P-labeled primers were annealed to GluR7-specific RT-PCR products obtained from each total RNA sample and were extended in the presence of the dideoxy terminator ddATP. Primers were separated by gel electrophoresis and were visualized by autoradiography. The detected primer products, a 20-mer (T allele-specific) and a 22-mer (C allele-specific), were quantified by phosphorimaging, and the C/T ratio was determined. Each total RNA sample was analyzed three times to obtain the mean values and the SD shown in the bar graph. The bar graph shows the relative expression level of the GluR7 allele in brain regions from P21 mice (lanes 1–8). Also shown is the relative representation of C and T allele in the genomic PCR fraction (lanes 1, 2). F1 generation mice from crossing male CBA/CaJ mice with female 129/SvEvTac mice are labeled MC, in contrast to FC-labeled mice, which resulted from crossing female 129/SvEvTac mice with male 129/SvEvTac mice. CER, Cerebellum; COR, cortex; HIP, hippocampus.

The unequal representation of the T allele and G allele in the RT-PCR fragment fractions could have been the result of mutations or genomic imprinting in the human GluR7 gene. Genomic imprinting causes gene silencing that is dependent on the origin of inheritance. Imprinting is regulated during development and in a
cell- and tissue-specific way (Latham, 1999). In some cases, imprinting of human genes has been described as a sporadic occurrence of unequal allele expression, accompanied by variability in allele expression levels (Xu et al., 1993; Jinno et al., 1994; Bunzel et al., 1998). The genetic heterogeneity of the human genome has been suggested as a major cause for this observed polymorphic imprinting. For example, silencing of the serotonin receptor subunit gene HTR2A has been detected in only four of 18 tested individuals (Bunzel et al., 1998); the insulin growth factor 2 receptor gene Igf2r was found partially or completely silenced in only three of 14 fetuses (Xu et al., 1993). Expression of the homologous mouse genes that encode the IGFIIR-R and 5-HT2RA proteins was subject to allele-specific silencing in mouse populations (Barlow et al., 1993; Caron et al., 1998). Individual development-dependent relaxation of imprinting of the endogenous mouse gene K Igf1 also has been observed (Jiang et al., 1998). The results of these studies indicate that the imprinting efficiency is dependent on the genetic background.

It seemed possible that human GluR7 genes were imprinted polymorphically. We detected differences in GluR7 allele expression levels (more than twofold differences) only sporadically in nine brain samples. Smaller differences (twofold or less than twofold) were observed more frequently in 18 brain samples (Table 2). The observation of unequal GluR7 allele expression can be explained by the heterogeneity of the genome in the human population and/or cell type and tissue-specific imprinting.

Allele-specific methylation or chromatin conformation may be involved in the maintenance of parental origin-specific expression of imprinted genes (for review, see Kelsey and Reik, 1998). However, because GluR7 mRNA is expressed predominantly in the CNS, we were unable to determine the parental influence on unequal expression of human GluR7 alleles. Furthermore, the inconsistency in GluR7 allele expression in human brains and the lack of sufficient tissue prevented us from studying methylation or chromatin conformation of GluR7 alleles. Therefore, we could not obtain conclusive evidence from analysis of human genes that the GluR7 gene was imprinted. However, in a follow-up study, we obtained data that indicated linkage between the GluR7 gene and a neurological disorder and suggested that the GluR7 gene or a close locus is imprinted genomically (our unpublished data).

As an alternate way of testing whether GluR7 genes were imprinted, we turned to an analysis of mouse GluR7 genes. In most, but not all, studied cases, an imprinted human gene also is imprinted in mice (Falls et al., 1999). Furthermore, analyzing genomic imprinting of genes in mice models avoids imprinting differences caused by heterogeneity of the genetic background. We therefore generated a mouse model to study the GluR7 allele expression. We identified a transcribed cytosine (C)/thymine (T) nucleotide variation in the GluR7 gene between 129/SvEvTac and CBA/CaJ mice strains. Both strains were crossed to obtain F1 mice and therefore did not find support for genomic imprinting of the GluR7 gene in our mouse model (see Fig. 5). It should be noted, however, that the lack of evidence for genomic imprinting of the GluR7 gene in these mice does not eliminate the possibility that the human gene is imprinted. Human GABA<sub>A</sub> receptor subunit genes, specifically GABRB3, GABRA5, and GABRG3, were found to be imprinted in human tumor tissue (Kubota et al., 1994), but not in mice (Nicholls et al., 1993; Culiat et al., 1994). The imprinting status of these genes also was studied with somatic cell hybrids containing isolated human chromosomes. One study reported imprinting of these GABA<sub>A</sub> receptor genes (Meguro et al., 1997), in contrast to another study that did not detect evidence for genomic imprinting (Gabriel et al., 1998).

Our experiments with mice served a purpose peripheral to testing the imprinting hypothesis; that is, they allowed us to determine the variation observed between GluR7 allele expression levels in an isogenic genetic background. On the basis of this variation observed in our assays, we defined the term “unequal expression” to be a ratio of expression levels of GluR7 alleles that differed by >1.2-fold from the ideal expected ratio of 1.0.

Because of the lack of evidence for genomic imprinting of the GluR7 gene in CBA/CaJ mice, we also have to consider the possibility that the unequal GluR7 expression levels in human brains were the result of genetic alterations in the GluR7 gene itself. A correlation between gene alterations and differences in allele expression levels has been studied intensively in the disease neurofibromatosis type 1 (NF1; Hoffmeyer et al., 1994, 1995; Cowley et al., 1998). Unequal expression of NF1 alleles (from 2.0- to 20-fold) was detected frequently in neurofibromatosis samples and was correlated to the presence of gene mutations (Hoffmeyer et al., 1994, 1995; Cowley et al., 1998). Interestingly, unequal expression of NF1 alleles to a minor impact on GluR7 allele expression level.

In summary, our results suggest that the human GluR7 gene either is affected by genomic imprinting or carries sporadic mutations that cause unequal allele expression. It will be interesting to analyze further the structure and function of the human GluR7 gene to distinguish between these possibilities.

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