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# The gene encoding the glutamate receptor subunit GluR5 is located on human chromosome 21q21.1-22.1 in the vicinity of the gene for familial amyotrophic lateral sclerosis

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**ABSTRACT** **Genomic clones of the human non-*N*-methyl-D-aspartate (non-NMDA) glutamate receptor subunit GluR5 were isolated by high-stringency screening of a cosmid library using the rat cDNA as a probe. The chromosomal localization of the human GluR5 gene has been established. Southern hybridization of DNA isolated from mapping panels of Chinese hamster–human hybrid cell lines and high-resolution *in situ* suppression hybridization localize the GluR5 gene to chromosome 21q21.1-22.1. This coincides with the localization of a mutant gene causing familial amyotrophic lateral sclerosis (ALS), as Siddique *et al.* established by linkage analyses [Siddique, T., Figlewicz, D. A., Pericak-Vance, M. A., Haines, J. L., Rouleau, G., Jeffers, A. J., Sapp, P., Hung, W. Y., Bebout, J., McKenna-Yasek, D., Deng, G., Horvitz, H. R., Gusella, J. F., Brown, R. H. & Roses, A. D. (1991) *N. Engl. J. Med.* 324, 1381–1384]. Convergent evidence from other investigators suggests that chronic pathologic activation of motor neurons via non-NMDA glutamate receptors might induce excitotoxic injury of motor neurons, culminating in ALS. Together with the demonstration that GluR5 transcripts are expressed in the ventral horn of the spinal cord, the region in which susceptible motor neurons reside, the chromosomal localization suggests that a mutated GluR5 gene may be responsible for the familial form of ALS.**

Synapses using glutamate and related amino acids are the principal excitatory synapses of the mammalian nervous system (1). Apart from their central role in a diversity of physiologic processes, these synapses have been implicated in a number of pathologic processes. Excessive activation of excitatory synapses has been proposed to cause neuronal death in neurodegenerative disorders (2–4) through a process termed excitotoxicity (5). The cornerstone of this proposal is that sustained exposure to high concentrations of glutamate can kill neurons (6, 7).

Amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease refers to a form of neurodegenerative disease characterized by the insidious onset and gradual progression of death of neurons in the ventral horn of the spinal cord, motor nuclei of the brainstem, and Betz cells of motor cortex (8, 9). Beginning with onset in adulthood, affected individuals exhibit progressive weakness and wasting of skeletal muscles, a condition ordinarily culminating in death within 5 years. Epidemiologic studies of a form of ALS in Guam implicated ingestion of excessive amounts of a glutamate analog, β-*N*-methylaminoalanine (BMAA) (10), in the pathogenesis, thereby suggesting an excitotoxic mechanism for ALS. Although the majority of ALS occurs sporadically, roughly 5–10% of cases are familial (11). Multipoint linkage analyses of an autosomal dominant form of ALS localized the mutant gene to the long arm of chromosome 21 (12).

Mutations of glutamate receptor (GluR) genes could alter synaptic function and thereby lead to a neurodegenerative disease. The molecular cloning of a family of non-*N*-methyl-D-aspartate (non-NMDA) glutamate-gated receptor subunits from rat brain (13–22) provided the tools to investigate this possibility. Correlation of the chromosomal locale of a human GluR gene with the location of the ALS locus would further support the hypothesis that a mutated GluR gene could cause this disease. We previously reported the chromosomal locations of the human GluR subunit receptor genes GluR1–GluR4, none of which resides on chromosome 21 (23). We report here the physical mapping of the human GluR5 gene to chromosome 21q21.1-22.1, a location concordant with the ALS locus.

## MATERIALS AND METHODS

**Cosmids.** Genomic cosmid clones were isolated from a human placental library constructed in the vector pWE-15 (24). Cosmid clones containing genomic sequences homologous to the rat GluR5 cDNA sequence were isolated as described (23).

**Cell Lines and Southern Hybridization.** DNA from somatic cell hybrids for chromosome mapping were obtained from the Coriell Cell Repositories (mapping panel 1). Ten micrograms of somatic cell hybrid DNA was digested with 25 units of *Bam*HI for 12 hr and separated on a 0.8% agarose gel. The DNA was transferred to nitrocellulose filters by Southern blotting and hybridized with a 1.3-kilobase *Pst* I fragment, containing the 3′ region of the rat GluR5 cDNA, clone RB20 (15). Hybridization was carried out using a final concentration of 2.6× Denhardt's solution, 43% formamide, 4.25× SSC (1× SSC = 0.15 M sodium chloride/15 mM sodium citrate), 1.0 mg of denatured salmon sperm DNA per ml, and 10% dextran sulfate, at 42°C, for 12–18 hr. Filters were washed with 0.5× SSC/0.1% $NaDodSO_4$ at 60°C. Blots were exposed to Kodak XAR film at −70°C for 36 hr.

**Subcloning of Cosmid GluR5 Fragments and DNA Sequence Analysis.** Restriction fragments from the cosmid clone were purified and subcloned into the pGEM-3Z (Pharmacia) vector. DNA sequences were determined using double-stranded sequencing. DNA sequencing was carried out using the modified T7 DNA polymerase (United States Biochemical) and primers specific for the T7 or SP6 promoter sequences in the vector. DNA sequences were analyzed using programs

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Abbreviations: ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; BMAA, β-*N*-methylaminoalanine; BOAA, β-*N*-oxalylamino-L-alanine; GluR, glutamate receptor; KA, kainic acid; NMDA, *N*-methyl-D-aspartate.

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Table 1. Nucleotide homology between the 210-base-pair human GluR5 *Eco*RI fragment and rat non-NMDA GluR subunit cDNAs

| Rat cDNA | % homology to human GluR5 |
|---|---|
| GluR1 | 50 |
| GluR2 | 52 |
| GluR3 | 54 |
| GluR4 | 55 |
| GluR5 | 91 |
| GluR6 | 80 |
| GluR7 | 76 |
| KA1 | 59 |
| KA2 | 61 |

In each case, the maximum homology was present in similar regions of the GluR cDNAs. KA, kainic acid.

from the University of Wisconsin Genetics Computer Group version 6.0 (25).

**Fluorescent *in Situ* Suppression Hybridization to Chromosomes.** Fluorescent *in situ* suppression hybridization was carried out as described (26). Metaphase chromosomes were prepared from human peripheral blood lymphocytes as described (27). Purified GluR5 cosmid DNA was labeled with biotinylated-dCTP and dUTP, repetitive sequences were blocked by prehybridization with CoT-1 DNA (Bio-Rad), and hybridization was carried out as described (26, 27). Chromosomes were counterstained with propidium iodide at a concentration of 100 μg/ml. Using confocal microscopy (Bio-Rad MRC-500 equipped with an argon gas laser), propidium iodide staining allows a G-banding-like pattern to be observed. The location of the GluR5 gene was determined by (*i*) observing the position of the hybridization signal relative to the G-like banding pattern (as an approximate position of the signal with respect to conventional chromosome banding designations) and by (*ii*) measuring the distance of the hybridization signal from the 21p telomere (FLpter) using the method of Lichter *et al.* (26).

## RESULTS

Two cosmid clones (designated C3 and B5) containing human sequences homologous to rat GluR5 cDNA were isolated by high-stringency screening of a genomic cosmid library with a random primed rat GluR5 cDNA probe. These cosmids were restriction mapped and shown to overlap by ≈8 kilobases (not shown). An *Eco*RI fragment of 478 base pairs, common to both cosmids, which hybridizes with a 1.4-kilobase 3′ *Pst* I fragment of the rat GluR5 cDNA (clone RB20) (15), was subcloned and partially sequenced. This fragment contains 210 base pairs of coding sequence, with an overall nucleotide homology to the rat GluR5 sequence of 91%. In-frame translation of this fragment generated a single amino acid change between rat and human, from serine to glutamate at position 794 of the rat subunit (15). Table 1 shows the homology of the coding sequence found in the *Eco*RI fragment to other rat GluR genes, indicating that the cosmids contain sequences corresponding to the 3′ open reading frame region of the human GluR5 cDNA.

To determine the chromosome on which the GluR5 gene resides, Southern hybridization was performed using the 3′ *Pst* I fragment of the rat GluR5 cDNA as a probe on DNA isolated from a chromosome mapping panel. The result of this hybridization (Table 2) discloses zero discordance with chromosome 21; a single discordant cell line (9925) was obtained with chromosome 5. To confirm this localization and further sublocalize the position of this gene on chromosome 21, fluorescent *in situ* suppression hybridization was conducted on karyotypically normal metaphase chromosomes using cosmid B5 as the probe (Fig. 1). Clear signals are present on both sister chromatids for each homologous chromosome 21. No evidence of signals on larger chromosomes was obtained, thereby providing additional evidence against a localization to chromosome 5. Use of computer-enhanced propidium iodide banding disclosed that the signal most likely resides on band 21q21.1-22.1. Fractional length measurements by the method of Lichter *et al.* (26) are consistent with the positioning of the GluR5 gene on the long arm of the chromosome.

Table 2. Concordance of human chromosome 21 with the rat GluR5 cDNA in a panel of somatic cell hybrids

| | Hybrid Cell Line | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHR | 9925 | 9926 | 9927 | 9928 | 9929 | 9930 | 9931 | 9932 | 9933 | 9934 | 9935 | 9936 | 9937 | 9938 | 9940 | 10324 | 10567 | 10611 | %Disc. |
| 1 | + | + | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | 55 |
| 2 | + | + | + | + | - | + | - | - | - | + | - | - | - | - | - | - | - | - | 44 |
| 3 | - | + | + | + | + | + | - | - | + | - | + | - | + | - | + | - | - | - | 50 |
| 4 | + | + | + | - | + | - | - | + | + | - | + | + | + | + | - | - | - | - | 55 |
| 5 | + | - | - | + | - | + | + | + | + | + | + | - | - | + | - | - | - | - | 6 |
| 6 | + | + | + | + | + | - | - | + | + | + | + | + | + | + | - | - | - | - | 44 |
| 7 | + | + | + | - | - | + | + | - | + | - | - | + | + | + | + | - | - | - | 55 |
| 8 | + | + | + | + | + | - | - | + | + | + | - | + | + | - | + | - | - | - | 61 |
| 9 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | 50 |
| 10 | - | + | + | - | - | - | + | - | - | - | - | + | - | - | - | - | - | - | 55 |
| 11 | - | - | - | - | + | - | - | + | - | + | - | + | - | + | - | - | - | - | 39 |
| 12 | + | - | - | - | + | + | + | + | + | + | + | - | + | + | - | - | - | - | 22 |
| 13 | - | + | + | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | 39 |
| 14 | + | + | + | + | + | + | + | - | + | - | + | + | + | + | - | - | - | - | 44 |
| 15 | + | + | + | + | - | + | - | - | + | + | - | - | + | - | + | - | - | - | 50 |
| 16 | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | 55 |
| 17 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | 39 |
| 18 | + | + | + | - | - | + | - | - | + | + | + | - | + | - | - | - | - | - | 44 |
| 19 | + | + | + | + | - | - | - | - | + | - | - | + | - | - | - | - | - | - | 55 |
| 20 | + | + | + | - | + | + | + | - | + | + | + | + | - | + | - | - | - | - | 39 |
| 21 | - | - | - | + | - | + | + | + | + | + | + | - | - | + | - | - | - | - | 0 |
| 22 | + | - | - | + | - | + | - | - | + | - | + | + | - | + | - | - | - | - | 28 |
| X | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | 50 |
| Y | - | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | - | - | 28 |
| Probe | - | - | - | + | - | + | + | + | + | + | + | - | - | + | - | - | - | - | |

Results of GluR5 Southern blot analysis of polychromosomal mapping panel 1. A 1.4-kilobase *Pst* I fragment obtained from the rat GluR5 cDNA was labeled by random priming and used to probe the mapping panel. A positive was scored if hybridization in a somatic cell hybrid DNA matched the pattern of hybridization to control human genomic DNA. From this blot, the DNA was digested with *Bam*HI. At least four specific hybridizing bands were present on control human DNA, indicating that this cDNA fragment is likely interrupted by multiple introns. CHR, chromosome; % Disc., % discordance.

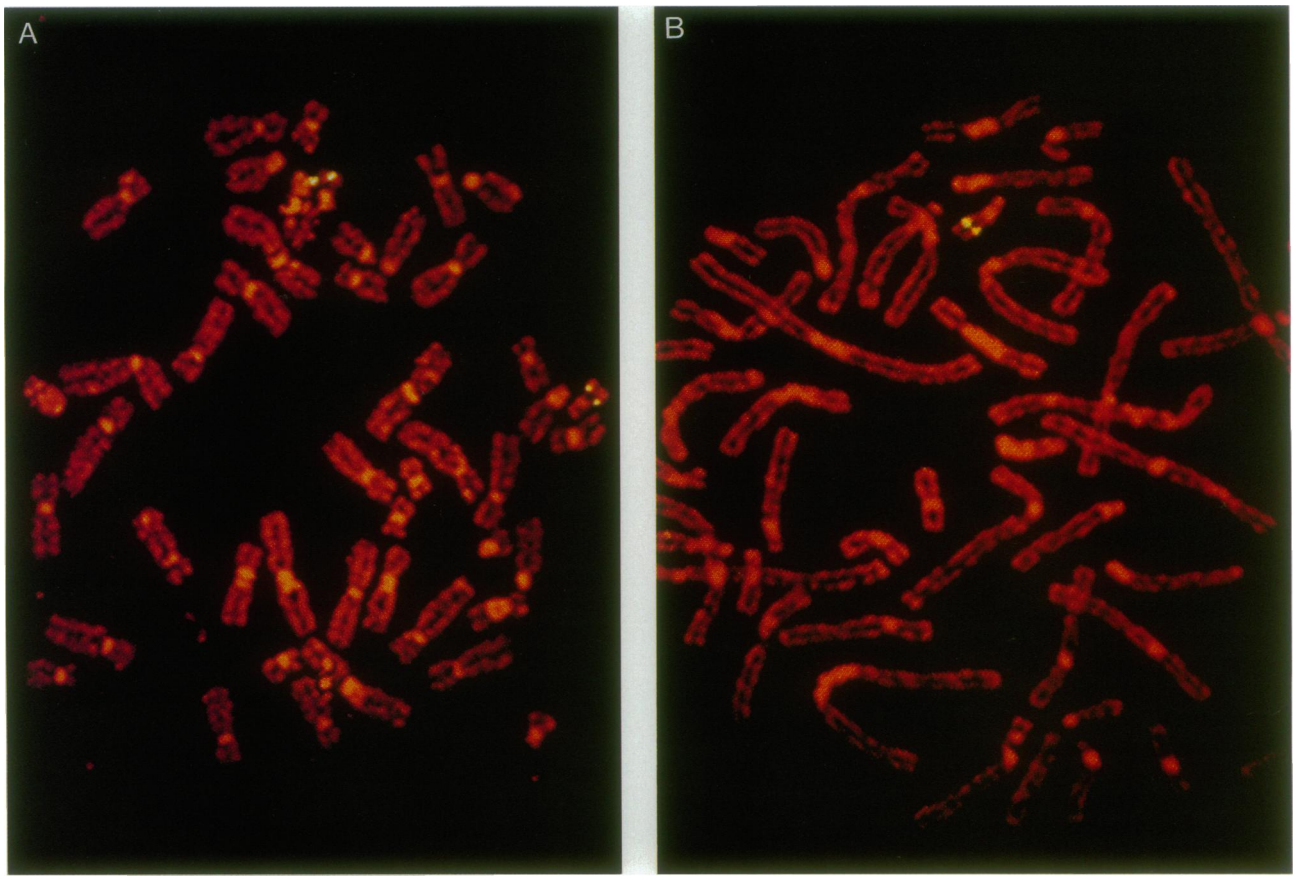


FIG. 1. *In situ* hybridization of cosmid clones containing human GluR5 gene sequences to a metaphase spread of human chromosomes. (*A*) The two pairs of bright yellow spots are located on equivalent positions of sister chromatids of chromosomes identified as 21 based upon Southern hybridization of GluR5 cDNA with mapping panel and upon G-type banding pattern. The single pair of bright yellow spots (*B*) provides enhanced resolution of the location on the long arm of chromosome 21.

This region of chromosome 21 is known to contain the mutation that confers a form of familial ALS (Fig. 2) (12).

## DISCUSSION

Three principal findings emerge from this work. (*i*) Hybridization data together with partial sequence analysis indicate that the genomic cosmid clones isolated represent at least part of the human GluR5 gene (15). (*ii*) Southern hybridization with DNA isolated from a chromosome mapping panel pinpointed the location of the human GluR5 gene to chromosome 21. (*iii*) Fluorescent *in situ* suppression hybridization provided regional localization of the gene to 21q21.1-22.1. The present findings, together with evidence of selective localization of GluR5 mRNA in spinal cord to ventral horn (28), the site at which susceptible motor neurons reside, indicate that GluR5 is a candidate gene for the familial form of the neurodegenerative disease ALS.

Increasing evidence implicates the excitatory neurotransmitter glutamate in the pathogenesis of ALS. The idea that excitatory amino acids might contribute to neuronal injury in neurodegenerative diseases emerged from the discovery of two distinct neurodegenerative disorders, sulfite oxidase deficiency and a form of olivopontocerebellar atrophy, in association with enzyme deficiencies resulting in elevated levels of excitatory amino acids (2, 3). Glutamate was linked to motor neuron disease in particular when ingestion of excessive amounts of the glutamate analog BOAA was implicated in the degeneration of upper and lower motor neurons in a disorder termed lathyrism (29). The link of ALS to glutamate was strengthened when ingestion of excess amounts of a related compound, BMAA, was implicated in the Guam form of ALS, Parkinson dementia (10). The causal role of BMAA was strengthened when feeding excess amounts of BMAA to non-human primates damaged motor neurons (10). Direct study of patients with the sporadic form of ALS disclosed increased levels of glutamate and aspartate in the brain, spinal cord, and cerebrospinal fluid (30, 31), increases that may be due to a selective reduction in the $V_{max}$ of a high-affinity glutamate transporter (32).

Among the diversity of receptor subtypes through which synaptically released glutamate might produce the insidious and progressive neuronal injury in ALS, the non-NMDA or KA/$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) subtypes have emerged as the leading candidates. The excitotoxic effects of BMAA and BOAA, the GluR agonists implicated in ALS-like syndromes, appear to be mediated by non-NMDA receptors, since these toxins kill neurons selectively susceptible to injury by non-NMDA receptor agonists (33). Moreover, exposure of spinal cord explant cultures to glutamate uptake blockers selectively kills motor neurons, an action prevented by coincubation with non-NMDA, but not NMDA, receptor antagonists (34).

In view of the pivotal role of receptors in regulation of synaptic efficacy, it seems plausible that a mutation of a gene encoding a KA/AMPA receptor could result in excitotoxic injury of motor neurons and culminate in ALS. Among the multitude of genetic isoforms encoding non-NMDA receptor subunits, GluR5 in particular is implicated because its physical position on chromosome 21 colocalizes to the position determined by linkage analysis to contain the gene responsible for familial ALS (Fig. 2). Importantly, GluR5 mRNA

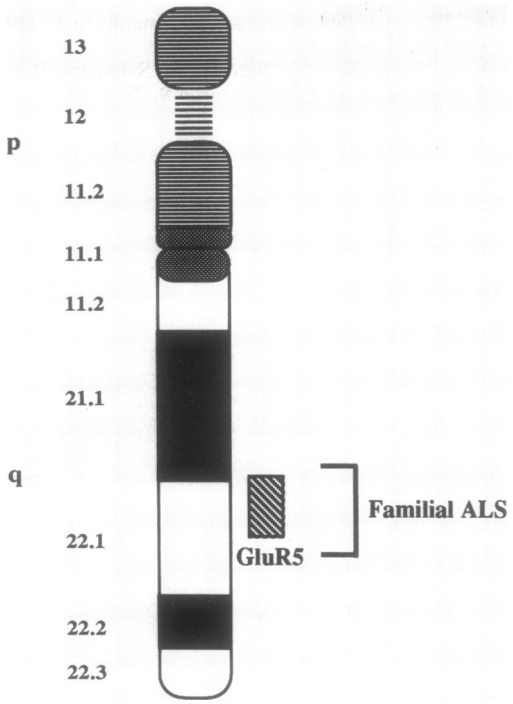


FIG. 2. Ideograms of banded chromosome 21. The hatched area to the right of the chromosome on the left corresponds to regional localization of GluR5 based upon *in situ* hybridization in Fig. 1. The bracket to the right of the chromosome on the right corresponds to genetic localization familial ALS (12).

expression in the spinal cord has been selectively localized to the ventral horn (28), the site at which the population of spinal cord motor neurons undergoing premature death in ALS resides. The hypothetical involvement of GluR5 must account for the time course of the disease—namely, delay in onset of the disease until middle age and indolent course thereafter—as well as the selective involvement of motor neurons in spinal cord, brainstem, and precentral gyrus. The excitotoxic effects of non-NMDA receptor agonists are calcium dependent (35, 36). Importantly, mutation of a single amino acid appears to control ion permeation properties in GluR1–GluR4 and GluR5–GluR7 families of non-NMDA receptors (37, 38), including the GluR5 subtype in particular (38). It therefore seems plausible that a point mutation might trigger subtle elevations of intracellular calcium following synaptic activation, thereby producing an indolent pace of excitotoxicity over a lifetime resulting in appearance of clinical symptoms in middle age. The predilection of the excitotoxic process for motor neurons might be due to a high synaptic density of GluRs and/or less effective calcium buffering systems in these cells compared to other neuronal populations expressing GluR5 [e.g., pyriform cortex (15)]. In summary, the increasingly convergent evidence implicating the interaction of glutamate with non-NMDA receptors in excitotoxic injury of motor neurons together with concordant localizations established by genetic mapping of familial ALS and physical mapping of GluR5 support the candidacy of GluR5 as the mutant gene accounting for familial ALS.

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