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Genetics

Human chromosomal localization of genes encoding the γ1 and γ2 subunits of the γ-aminobutyric acid receptor indicates that members of this gene family are often clustered in the genome

(ligand-gated ion channel/gene mapping/somatic cell hybrids/yeast artificial chromosomes/sequence-tagged-site)

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ABSTRACT **The γ-aminobutyric acid (GABA) receptors are the major inhibitory neurotransmitter receptors in the brain and the site of action of a number of important pharmacological agents including barbiturates, benzodiazepines, and ethanol. The γ1 and γ2 subunits have been shown to be important in mediating responses to benzodiazepines, and a splicing variant of the γ2 subunit, γ2L, has been shown to be necessary for ethanol actions on the receptor, raising the possibility that the γ2 gene may be involved in human genetic predisposition to the development of alcoholism. We have assigned the human genes encoding the γ1 and γ2 subunits of the $GABA_A$ receptor to chromosomes 4 and 5, respectively, by PCR amplification of human-specific products from human–hamster somatic cell hybrid DNAs. Using panels of chromosome-specific natural deletion hybrids, we have further localized the γ1 gene (*GABRG1*) to 4p14–q21.1 and the γ2 gene (*GABRG2*) to 5q31.1–q33.2. These data indicate that the γ1 gene may be clustered together with the previously mapped α2 and β1 genes on chromosome 4 and that the γ2 gene may be close to the previously localized α1 gene on chromosome 5. To further examine the latter possibility the α1 gene was mapped using the chromosome 5 deletion hybrids and shown to be within the same region as the γ2 gene, 5q31.1–q33.2. A PCR-based screening strategy was used to isolate a 450-kilobase human genomic yeast artificial chromosome clone containing both the α1 and γ2 genes. Pulsed-field gel restriction mapping of the yeast artificial chromosome indicates that the two genes are within 200 kilobases of each other. The data presented here provide further evidence for the nonrandom organization of the human genome by demonstrating that members of the $GABA_A$ receptor gene family often occur in small gene clusters widely distributed in the genome.**

The major inhibitory neurotransmitter in the vertebrate brain, γ-aminobutyric acid (GABA), exerts its effects primarily through $GABA_A$ receptors (1). Binding of GABA to these receptors causes an influx of chloride ions that leads to hyperpolarization (and thus inhibition) of postsynaptic neurons. This action can be allosterically facilitated by the binding of several classes of important pharmacological agents including the sedative hypnotics (benzodiazepines and barbiturates) and ethanol (1, 2).

$GABA_A$ receptors, together with glycine and acetylcholine receptors, are members of a superfamily of ligand-gated heterooligomeric ion channels (3). Molecular cloning of $GABA_A$ receptor subunit cDNAs has resulted in the identification of at least 15 distinct but related subunits of five different sequence classes: α, β, γ, δ, and ρ (for reviews see refs. 4 and 5). *In vivo*, single $GABA_A$ receptors appear to be composed of four or five subunits arranged to form a central channel that conducts chloride ions.

Because of the importance of GABA in neuronal function, mutations in the human genes encoding subunits of the $GABA_A$ receptor may result in significant deleterious effects on human brain function. Functional expression studies, using either cloned subunits or antisense oligonucleotide strategies, have provided some insight into the role various subunits play in $GABA_A$ receptor pharmacology (6–8). One of the more striking findings to emerge from such investigations is that an alternative splicing variant derived from the γ2 gene, γ2L, is required for ethanol action at the receptor (8). Interestingly, the other splicing product (γ2S), which differs from γ2L only by the absence of an 8-amino acid segment, does not appear to contribute to ethanol action. These data suggest that the human γ2 gene may be an important factor influencing genetic sensitivity to alcohol in humans and, as such, represents a candidate gene for involvement in genetic predisposition to develop alcoholism. To facilitate investigation of this possibility we have isolated a yeast artificial chromosome (YAC) clone containing the human γ2 gene and localized it in the genome. We have also mapped the human γ1 gene and provide evidence that genes encoding $GABA_A$ receptor subunits are often arranged in small clusters widely distributed in the genome. This additional knowledge of the genomic organization of the human $GABA_A$ receptor genes should lead to a more complete understanding of the molecular phylogeny of this important gene family.

METHODS AND MATERIALS

To increase the likelihood of generating a human-specific PCR (9) product, primers were designed from the 3′ untranslated region of the human γ2 (10), α1 (11), and γ1 (P.W., unpublished data) subunit cDNAs (12). The sequences of the γ2 primers were 5′-GATTCAGATACTTATCAACCAC-3′ and 5′-ATGGGTTTTACTGATATGGTTC-3′, and the predicted size of the amplified product was 104 base pairs (bp). The α1 primers were 5′-CAATAGATCTTTTACTCAC-3′ and 5′-CAGGGGTCTCTTGTCTTAA-3′, and the predicted size of the product was 159 bp. Primers for γ1 were 5′-

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Abbreviations: GABA, γ-aminobutyric acid; YAC, yeast artificial chromosome.

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TAATTTACTGTGTGTTCCCAGC-3′ and 5′-TGCTCACTTTCAAAACATCC-3′ and amplified a 226-bp product. Oligonucleotides were synthesized on an Applied Biosystems model 391A DNA synthesizer using the trityl-on mode. After cleavage and deprotection, primers were purified on oligonucleotide purification cartridges as directed by the supplier (Applied Biosystems).

Oligonucleotides used as hybridization probes for construction of the YAC restriction map were as follows: γ2, 5′-CCTTCTCTGGCCTCTTGCCTCTTGGTTGCA-3′ [from the 5′ untranslated region of the human γ2 cDNA (10)]; α1, 5′-GAGGGTCCAGGCCCAAAGATAGTCAGAGAGACC-3′ [from the N-terminal region of the mouse α1 cDNA (13)].

Human and hamster genomic DNA and the human–hamster somatic cell hybrid DNAs were obtained from Bios (New Haven, CT). PCR amplification was performed using 50 ng of template DNA in a 100-μl reaction mixture containing 1.5 mM $MgCl_2$, 200 μM each dNTP, 90 pmol of each primer, and 2.5 units of *Taq* polymerase in 50 mM KCl/10 mM Tris·HCl, pH 8.3. Samples were amplified in a Perkin–Elmer/Cetus thermal cycler under the following conditions: 20 sec at 72°C, then 35 cycles consisting of 20 sec at 94°C, 20 sec at 55°C (40°C for α1), and 30 sec at 72°C (the extension segment was lengthened by 5 sec for each cycle). This was followed by a final extension period of 5 min at 72°C. Products were analyzed by electrophoresis in either 2% agarose/2% NuSieve (FMC) agarose gels (γ2) or 1.5% agarose (α1 and γ1) gels stained with ethidium bromide. PCR amplification and analysis of YAC DNA was done as for genomic DNA except that each PCR segment was lengthened to 1.5 min.

PCR of natural deletion hybrid DNAs (refs. 14 and 15; J.J.W., unpublished work) was carried out in a total volume of 50 μl containing 500 ng of DNA, 67 mM Tris·HCl (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 1.25 mM each dNTP, 25 pmol of each primer, and 2 units of *Taq* polymerase. PCR conditions for the γ1 and γ2 genes included an initial denaturation period at 94°C for 10 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Amplification using α1 primers was as above, except that annealing was done at 45°C. Products were analyzed in 6% acrylamide gels stained with ethidium bromide.

Preparation of YAC DNA for PCR was carried out as follows: a small amount of material was removed from a yeast colony and placed into a 30°C solution containing 1 M sorbitol, 100 mM trisodium citrate, 50 mM EDTA, lyticase (5 units/μl), and 150 mM dithiothreitol. Spheroplast production was allowed to continue for at least 1 hr, and then cells were spun in a microcentrifuge for 1 min. Cells were suspended in 100 μl of water, then heated to 100°C for 15 min and spun in a microcentrifuge for 2 min.

The St. Louis human YAC library was screened by the method of Green and Olson (17) as modified (18). In brief, pools of DNA were created by combining YAC clones from 20 microtiter plates, and the pools were screened with PCR primers derived from the gene of interest. Once a positive pool was obtained, the single positive colony was identified by PCR analysis of successively smaller pools derived from the original positive pool.

DNA plugs from the positive YAC (called yGABRγ2) were prepared for pulsed-field studies as described by Gardiner *et al.* (19), using 1/8th-inch-diameter tubing and a final DNA concentration of 10 μg/ml. The size of the YAC was determined by separating the yeast chromosomes in a 1% agarose gel in the TAFE apparatus (20) for 18 hr at 250 V with a pulse time of 20 sec. YAC DNA was then digested with nine rare-cutting restriction enzymes (*Not* I, *Bss*HII, *Mlu* I, *Nru* I, *Eag* I, *Sst* II, *Sfi* I, *Sal* I, *Cla* I) and separated as above. The DNA was transferred to a nylon membrane (GeneScreen-*Plus*; NEN) and hybridized with ^{32}P-labeled oligonucleotide probes derived from the left and right arms of the pYAC4 vector (21) in order to generate a restriction map. Positions of the γ2 and α1 genes were determined using ^{32}P-labeled oligonucleotide probes specific for each of the two genes. For those enzymes with multiple sites (*Bss*HII, *Mlu* I, *Sal* I), partial digests were carried out and the fragments were separated on the ED gel apparatus (22) for 30 hr at 200 V, with a 25-sec pulse time. DNA was transferred to nylon filters and probed as described above.

RESULTS

Primers to the γ2 subunit were first shown to amplify a distinct product of 104 bp from human genomic DNA, whereas a smaller band was generated from hamster DNA (Fig. 1, lanes 1 and 2). To assign the γ2 subunit gene to a specific human chromosome, the primers were used to am-

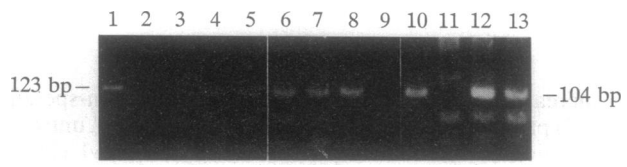

FIG. 1. Mapping of the GABA receptor γ2 subunit gene (*GABRG2*) to human chromosome 5 by use of somatic cell hybrid DNAs. Human, hamster, and human–hamster hybrid DNAs were PCR-amplified with primers derived from the 3′ untranslated region of the γ2 gene. PCR products were visualized by gel electrophoresis. Lane 1 contained a 123-bp "ladder" to provide molecular size markers. A 104-bp product was generated from human (lane 2) but not hamster DNA (lane 3). Lanes 4–13 show PCR products amplified from somatic cell hybrid DNAs. The human-specific 104-bp product was generated from all hybrids except those in lanes 9 and 11. All hybrids shown contain human chromosome 5 except those in lanes 9 and 11.

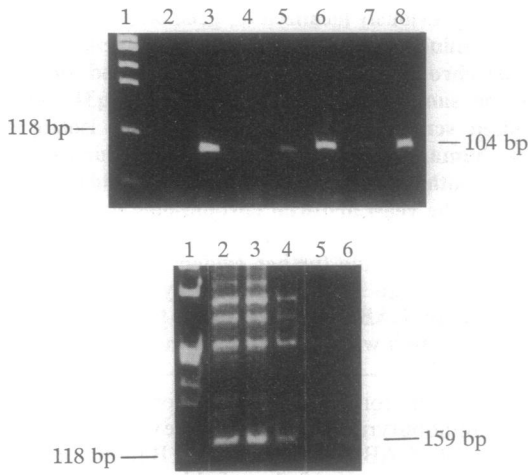

FIG. 2. (*Upper*) Mapping of the GABA receptor γ2 subunit gene to bands 5q31.1–q33.2 by use of natural deletion hybrids. Lane 1, *Hae* III-digested φX174 phage DNA (size markers); lane 2, hamster genomic DNA (negative control); lane 3, hybrid containing a whole human chromosome 5 (positive control); lanes 4–8, all other hybrids generated the 104-bp product, indicating that the chromosomal fragment common to all hybrids (5q31.1–q33.2) includes the γ2 gene. (*Lower*) Mapping of the GABA receptor α1 subunit gene to bands 5q31.1–33.2 by use of natural deletion hybrids. The expected 159-bp human-specific band was amplified from human genomic DNA but not from hamster DNA (data not shown). The human-specific band was generated from the same hybrids (lanes 2–6) as in *Upper* (lanes 3–8).

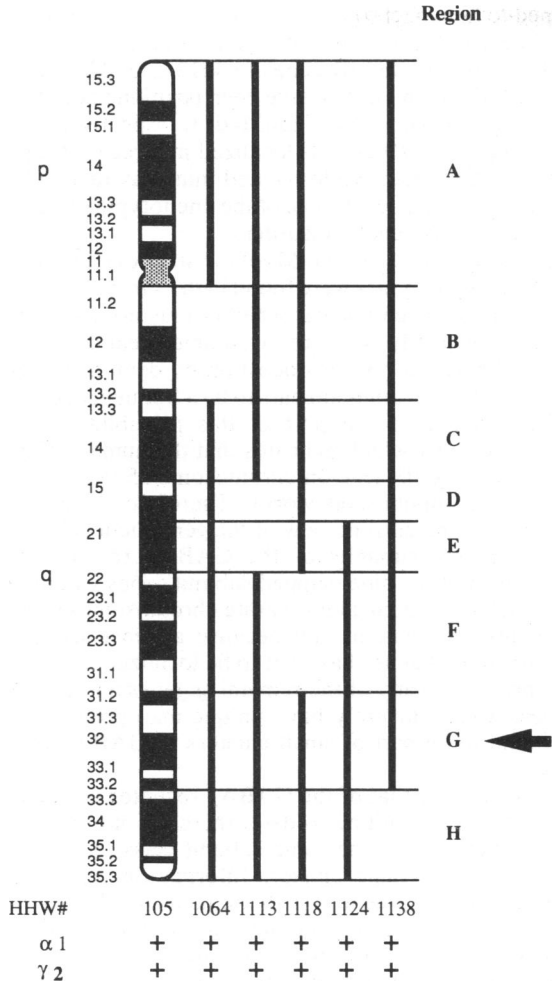

FIG. 3. Representation of natural deletion hybrid panel spanning chromosome 5. The region of chromosome 5 retained by each of the hybrids is indicated by a solid bar above the hybrid cell line designations. HHW105 retains an intact chromosome 5 as its only human DNA. The eight cytogenetic regions defined by this panel are indicated by A–H. The presence of the human-specific DNA fragment amplified by the γ2 and α1 subunit PCR primers in the different cell hybrids is indicated (+). All hybrids were positive for both the γ2 and the α1 gene. Based upon these results the GABA receptor γ2 and α1 genes can be assigned to region G (5q31.3–q33.2).

plify a mapping panel of 18 human–hamster somatic cell hybrid DNAs. The human-specific product was present in all hybrids except numbers 8 and 16 (lanes 9 and 11). The highest concordance frequency (94%) was for chromosome 5.

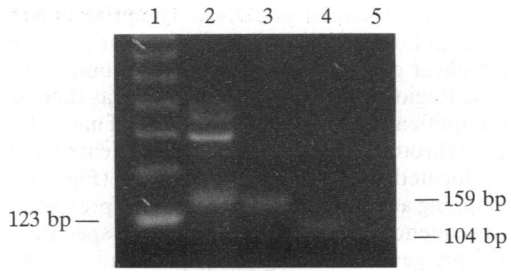

FIG. 4. PCR amplification of GABA receptor γ2 and α1 genes from a single 450-kb YAC clone. Lane 1, 123-bp marker; lane 2, the α1 159-bp human-specific product amplified from human genomic DNA; lane 3, the same 159-bp product obtained from the YAC; lanes 4 and 5, the γ2 104-bp human-specific product was amplified from human genomic and YAC DNA, respectively.

Regional localization of the gene was carried out by using the γ2 primers to amplify DNA from a panel of natural deletion hybrids containing only portions of chromosome 5. Analysis of the results indicated that the human γ2 gene, designated *GABRG2*, mapped to the long arm of chromosome 5, in bands q31.3–q33.2 (Fig. 2 *Upper*).

Because the gene encoding the α1 subunit of the $GABA_A$ receptor had been mapped to 5q34–q35 by radioactive *in situ* hybridization (23), the possibility existed that the two genes might colocalize if they were mapped by the same technique. To test this hypothesis, PCR primers to the 3′ untranslated region of the human α1 subunit cDNA (11) were synthesized and used to amplify DNA from the chromosome 5 natural deletion hybrid panel. Results obtained with the α1 primers were identical to those found with the γ2 primers (Fig. 2 *Lower*), indicating that both genes lie within the 5q31–5q33 region (Fig. 3).

To facilitate the analysis of the human γ2 gene and to explore the possibility that the γ2 and α1 genes may be adjacent in the genome, a human genomic YAC library was screened by a PCR-based method using primers specific for the two genes. Screening of the library identified a 450-kilobase (kb) YAC clone that was positive for both the γ2 and the α1 gene (Fig. 4). Pulsed-field restriction mapping of the YAC with subunit-specific oligonucleotides and probes derived from the left and right arms of the pYAC4 vector indicated that the γ2 probe could be localized, based on partial-digest analysis, to a 25-kb *Sal* I fragment (Fig. 5). The α1 probe was located on the right-end *Bss*HII fragment of ≈185 kb and on an internal *Cla* I fragment of ≈120 kb. Partial digests with *Cla* I narrowed the localization to the common 90-kb *Bss*HII–*Cla* I fragment. The γ2 and α1 fragments and the segment between them span a region of ≈200 kb.

For localization of the human γ1 gene, a set of PCR primers was designed from sequences derived from the 3′ untrans-

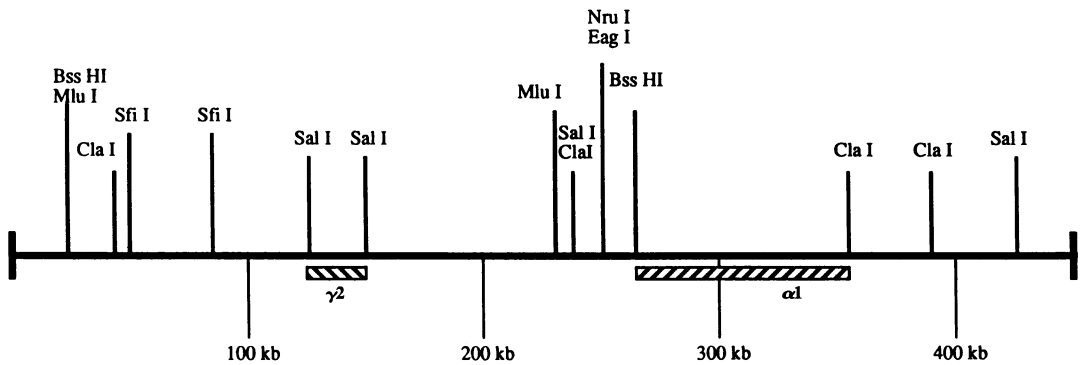

FIG. 5. Pulsed-field restriction map of a YAC containing the human GABA receptor γ2 and α1 genes. The γ2 and α1 probes (hatched bars) are indicated. No sites were observed for *Not* I and *Sst* II.

lated region of the human γ1 cDNA. The primers were used for PCR amplification of the panel of somatic cell hybrids, and the highest concordance (94.4%) was found with chromosome 4. Regional mapping information was then obtained by PCR amplification of DNA from a panel of natural deletion hybrids for chromosome 4. This analysis indicated that the γ1 gene was located between 4p14 and 4q21.1 (Fig. 6).

The mapping and PCR primer information presented above defines a sequence-tagged site (STS; ref. 24) specific for each of these three genes encoding the γ2, α1, and γ1 subunits of the $GABA_A$ receptor.

DISCUSSION

Because GABA is the most abundant inhibitory neurotransmitter in the brain and acts primarily through binding to $GABA_A$ receptors, it is not surprising that some of the genes encoding subunits of the receptor have been implicated as potential candidate genes for several human neurogenetic diseases—e.g., alcoholism (8), manic depression (23), and Angelman syndrome (25). Excluding the present data, 6 of the 15 known $GABA_A$ receptor subunit genes have been mapped to human chromosomes. Buckle *et al.* (23) localized the α1 gene to the long arm of chromosome 5, the α3 gene to Xq28, and the α2 and β1 genes to 4p12–p13. More recently the δ and β3 subunit genes have been positioned on the short arm of chromosome 1 and at 15q11–q13, respectively (25, 26). Interestingly, the β3 gene is localized in a region frequently deleted in Angelman syndrome and thus may be involved in the generation of at least some of the phenotypic features that characterize this genetic disorder.

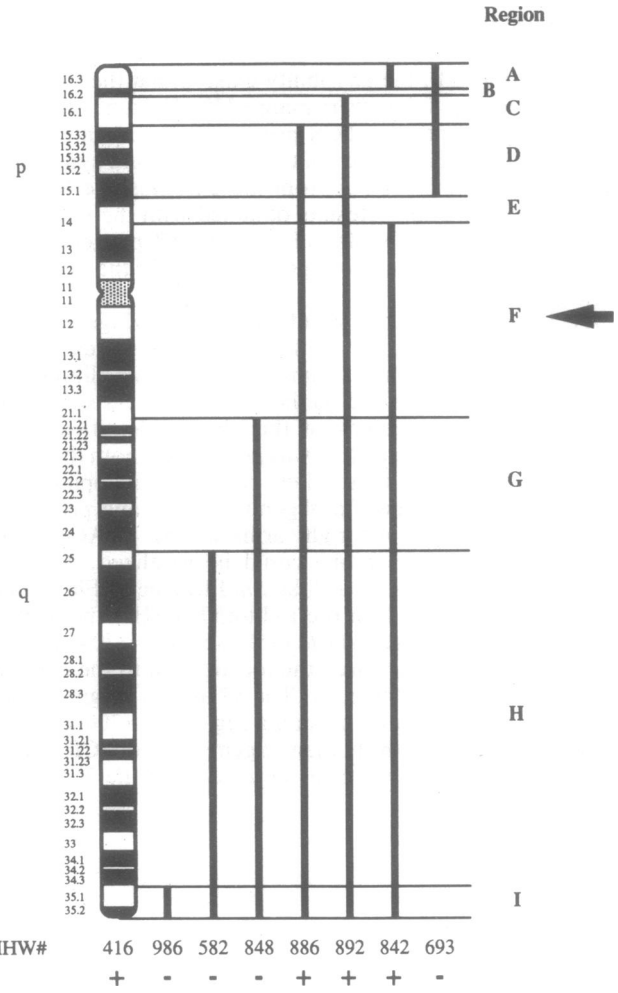

FIG. 6. Chromosome 4 regional mapping panel. An ideogram of chromosome 4 is shown at left. The region of chromosome 4 retained by each of the cell hybrids is indicated by a solid bar above the hybrid cell line designations. HHW416 retains an intact chromosome 4 as its only human DNA. The nine cytogenetic regions defined by this panel are indicated by A–I. The presence (+) or absence (−) of the human-specific DNA fragment amplified by the γ1 subunit PCR primers in the different cell hybrids is indicated. Based upon these results the GABA receptor γ1 locus can be assigned to region F (4p14–q21.1).

Because only two (α2 and β1) of the six previously mapped $GABA_A$ receptor genes were found in the same chromosomal location, it has been unclear whether clustering of genes of this receptor family is a rare or common feature. The data presented here address this question by demonstrating that clustering of these genes appears to be a frequent occurrence. Further evidence in support of this possibility has been obtained recently which indicates that the human α5 gene is located near the β3 gene on chromosome 15 (Marc Lalande and J.M.S., unpublished work). Therefore, including the present data, the current view of the genomic localization of genes encoding subunits of the $GABA_A$ receptor family places seven of the nine mapped subunit genes within one of three clusters occurring on separate chromosomes (Table 1). Given this information, and because at least six $GABA_A$ receptor subunit genes have yet to be localized, it would not be surprising if many of the remaining genes and the α3 and δ genes, which currently have unique map positions, were also found to be part of small clusters of $GABA_A$ receptor genes.

It is interesting that of the $GABA_A$ receptor gene clusters that have been identified to date, there are no examples of multiple members of the same subunit class (e.g., α, β, γ) occurring in the same cluster. Information regarding the genomic location of genes for the remaining subunits should allow determination of whether or not this pattern is a characteristic feature of this gene family.

While GABA is the major inhibitory neurotransmitter in the brain, glutamate is the major excitatory neurotransmitter (27). The human gene encoding the α1 subunit of the glutamate receptor has been localized to 5q33 (28). Our data place the α1 and γ2 genes of the $GABA_A$ receptor in the same region of chromosome 5. Given the functional importance and similarity of these two classes of amino acid receptors, it will be interesting to determine how close these glutamate and GABA receptor genes are in the genome and whether such close proximity has functional significance.

The restriction mapping data of the human YAC clone presented here does not allow determination of the precise distance separating the γ2 and α1 genes. However, there is precedent for members of the ligand-gated ion-channel receptor superfamily to be directly adjacent in the genome. For example, the genes encoding the δ and γ subunits of the chicken nicotinic acetylcholine receptor have been shown to be separated by only 750 bp (29). In addition, the α3, α5, and β4 genes of the rat acetylcholine receptor have been shown to be tightly clustered, with the α3 and α5 genes separated by

Table 1. Human chromosomal mapping information for genes encoding subunits of the $GABA_A$ receptor

Gene(s)	Chromosomal location
α1, γ2	5q31–5q33
α2, β1, γ1	4p12–4p13 (4p14–4q21.1)
β3, [α5]	15q11–15q13
α3	Xq28
δ	1p

Chromosomal location for the γ1 subunit gene is in parentheses. The α5 gene (bracketed) has been positioned in the same region as the β3 gene (Marc Lalande and J.M.S., unpublished work). Mapping references are as indicated in the text.

only a few kilobases (30). While the sizes and orientations of the $\gamma 2$ and $\alpha 1$ genes could not be determined from the available data, 200 kb represents the maximal distance separating the two genes and does not exclude the possibility that they are much closer together and perhaps even immediately adjacent. More detailed mapping of the location of the $\gamma 2$ and $\alpha 1$ subunit genes within the YAC should resolve this issue.

The close proximity of the $\gamma 2$ and $\alpha 1$ genes raises the question as to whether their expression may be coordinately regulated. Consistent with such a possibility, the two genes exhibit very similar spatial and temporal expression profiles and, when expressed together *in vitro*, appear to function efficiently (16, 31). For these reasons it has been suggested that these subunits may combine to form functional $GABA_A$ receptors *in vivo*. Thus, while sufficient information regarding other $GABA_A$ receptor genes is not yet available, the data for the $\alpha 1$ and $\gamma 2$ genes suggest that the colocalization of these genes may indeed be related to their coordinately regulated expression. If such a relationship is found to be a general characteristic of genes of the $GABA_A$ receptor family, then knowledge of the chromosomal location of other members of this family could potentially provide insight into the challenging question of which subunits combine to form functional $GABA_A$ receptors *in vivo*.

Finally, the isolation of a 450-kb human YAC clone containing both the $\gamma 2$ and $\alpha 1$ genes should provide a useful resource for the identification of polymorphic sequences that, in turn, could be utilized for linkage studies of the possible involvement of these genes in human neurogenetic disease. This may be particularly useful with respect to the $\gamma 2$ gene because of its demonstrated importance in mediating the effects of alcohol in the brain.

This paper is dedicated to the memory of Leland H. Schantz (14 October 1928 to 17 August 1991). We thank Jan Hopkins, Misi Robinson, and Donna Wilson-Shaw for excellent technical assistance. This research was supported by Public Health Service Grants NS27322 and MH44212 and the Veterans Administration Medical Research Service (J.M.S.), National Institutes of Health Grant NS25631 and the Hereditary Disease Foundation (J.J.W.), the Huntington Disease Society of America (M.R.A.), National Institutes of Health Grant AG00029 (D.P.), and National Institutes of Health Grant HG0001 (K.G.). A.S.W. was supported in part by a fellowship from the National Institute for Drug Abuse (DA05480).

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