SHORT COMMUNICATION

Further Evidence for Clustering of Human GABA_A Receptor Subunit Genes: Localization of the α_6 -Subunit Gene (GABRA6) to Distal Chromosome 5q by Linkage Analysis

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GABA_A receptors are hetero-oligomeric ion-channel complexes that are composed of combinations of α , β , γ , and δ subunits and play a major role in inhibitory neurotransmission in the mammalian brain. We report here a microsatellite polymorphism within the human α_6 -subunit gene (GABRA6). Mapping of this marker in a human-hamster hybrid cell-line panel and typing of the repeat in the Centre d'Étude du Polymorphisme Humain (CEPH) reference families enabled the localization of this gene to chromosome 5q and established its linkage to the GABA_A receptor α_1 -subunit gene (GABRA1) with a maximum lod score ($Z_{\max}$) of 39.87 at a θ of 0.069 (males) and 0.100 (females). These results reveal the clustering of GABRA6, GABRA1, and the GABA_A receptor γ_2 -subunit gene (GABRG2) on distal chromosome 5q. © 1994 Academic Press, Inc.

Neuronal inhibition in the mammalian brain is largely mediated by the binding of γ -aminobutyric acid (GABA) to GABA type A (GABA_A) receptors, each of which possesses an intrinsic chloride-selective channel. These receptors are of importance because they are the site of action of several clinically useful classes of compounds such as the benzodiazepines and barbiturates. Subtypes of the GABA_A receptor, which are hetero-oligomers, arise by the association of different combinations of

polypeptides. To date, 13 distinct subunits, each of which is encoded by a separate gene, have been identified in mammals (2, 17). Several of these genes have been localized to human chromosomal segments and this has revealed clustering of loci. For example, genes for the α_1 (GABRA1) and γ_2 (GABRG2) subunits occur together on chromosome 5q31-q33 (1, 4, 15), with at least parts of both genes residing on a 450-kb human genomic yeast artificial chromosome clone (16). Genes for the α_2 (GABRA2), β_1 (GABRB1), and γ_1 (GABRG1) subunits are located on chromosome 4 (1, 16). Furthermore, genes for the β_3 (GABRB3), α_5 (GABRA5), and γ_3 (GABRG3) subunits map to chromosome 15q11-q13 (10, 13), with GABRA5 and GABRB3 being separated by less than 100 kb and oriented in a "head to head" configuration (13).

GABA_A receptor genes have been implicated in the pathogenesis of several neurological and psychiatric disorders, and interest in the α_6 -subunit gene (GABRA6), which is expressed almost exclusively in the cerebellum (5, 8), has been stimulated by a recent study implicating it in the diazepam (benzodiazepine)-induced impairment of postural reflexes in a rat model of alcohol intolerance (6).

In an effort to isolate parts of the GABRA6 gene, 800,000 clones from a human genomic library constructed in λ EMBL3A were screened with a 1.2-kb *SacI*-*PstI* restriction fragment from a mouse GABA_A receptor α_6 -subunit cDNA (the *SacI* site used is in the polylinker of the vector; Ref. 5). The probe encodes the signal peptide, all of the amino-terminal extracellular domain, the first three membrane-spanning domains, and a small part of the large intracellular loop between the third and fourth membrane-spanning segments. Hybridization was performed using standard techniques (12), and filters were washed at a final stringency of 0.5 $\times$ SSC at 65°C prior to autoradiography; 12 positively hybridizing plaques were detected. A *Sau3AI* digest of one clone (λ hGR1 α_6) was blotted and probed with the same

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1.2-kb mouse GABA_A receptor α_6 -subunit cDNA fragment, and a positively hybridizing 400-bp fragment was subcloned into pVZ1 (a kind gift of Dr. S. Henikoff, Seattle, WA) and sequenced. This was found to contain exon 8 (Ref. 3) of a GABA_A receptor subunit gene encoding the majority of the second membrane-spanning domain, all of the third membrane-spanning domain, and part of the large intracellular loop. The latter region is that which is most variable in different GABA_A receptor polypeptides. Comparison of the sequence (86 amino acids in length) encoded by exon 8 of the human gene with the corresponding region of the mouse GABA_A receptor α_6 subunit revealed 86% identity (data not shown). Much lower similarity was seen when the human sequence was compared with those of the other known GABA_A receptor polypeptides. We therefore conclude that the DNA we have cloned is a fragment of the human GABA_A receptor α_6 -subunit gene (GABRA6).

Clone λ hGR1 α 6 was also digested with a series of restriction enzymes that have hexanucleotide recognition sequences, blotted, and probed with oligonucleotide (TG)₉, 5'-TGTGTGTGTGTGTGTGTG-3'. Hybridization was for 2 h in 5× SSC, 1× Denhardt's solution, 7% (w/v) SDS, 20 mM sodium phosphate, pH 7.2, 1 mM EDTA, and 100 μ g/ml yeast tRNA at 45°C, and filters were washed twice in 2× SSC, 0.1% (w/v) SDS, for 15 min each wash, at 50°C prior to autoradiography. A positively hybridizing 1.2-kb *Bgl*III fragment was subse-

quently subcloned into *Bam*HI-restricted pVZ1. Two 25-base oligonucleotide primers flanking a (dC-dA)₁₆ repeat were synthesized (GABRA6F, 5'-TAGACACTC-ATCTTTCCTGACTGAC-3', and GABRA6R, 5'-GAT-GATTGGTAGATGGATAGATGCC-3'), based on partial sequence from this subclone, and used in the polymerase chain reaction (PCR; Ref. 11) to characterize the repeat. Genomic DNA was either isolated from human nucleated blood cells (9) or obtained from CEPH. PCR amplifications were carried out under standard conditions using an annealing temperature of 63°C and *Taq* DNA polymerase purchased from Boehringer Mannheim. PCR products were analyzed in agarose gels and in denaturing 6% (w/v) polyacrylamide DNA sequencing gels. Allele sizes were determined by comparison with an α -³⁵S-labeled ladder of known sequence that was run in parallel; the top band of each allele was scored. Typings were also performed on some CEPH families for the GABRA1 (dC-dA)_n polymorphism (as described in Ref. 4) to eliminate a few mistypings.

A human-hamster hybrid cell-line panel described previously (14) was used to localize the GABRA6-specific marker to chromosome 5 (data not shown). PCR amplification of the (dC-dA) repeat associated with the GABRA6 gene in unrelated members of the CEPH reference panel of families, and in a small number of unrelated individuals from a group of German families being used in a different study, revealed that it was polymorphic in length. From these typings, allele sizes and their

TABLE 1

(A) Sizes and frequencies of the alleles of the GABRA6 (dC-dA) _n polymorphism in 108 healthy unrelated individuals							
Allele No.	Allele size (bp)	No. of chromosomes			Allele frequency		
1	208	7			0.032		
2	206	3			0.014		
3	204	148			0.685		
4	202	26			0.121		
5	200	32			0.148		
Total		216			1.000		

(B) Pairwise lod scores for GABRA6 and GABRA1 and markers on 5q31-q35 obtained by typing CEPH families							
Marker	Locus	GABRA6			GABRA1		
		θ		Z_{max}	θ		Z_{max}
		Male	Female		Male	Female	
GABRA1		0.069	0.100	39.87			
p93-121	D5S198	0.025	0.001	29.65	0.061	0.049	48.03
CRI-L45	D5S61	0.027	0.050	25.07	0.034	0.065	47.18
Mfd6	D5S119	0.090	0.060	22.76	0.167	0.096	23.18
CRI-C61	D5S55	0.040	0.001	20.41	0.117	0.112	22.39
pHVON 9-2	SPARC/OS-1	0.104	0.086	15.59	0.181	0.159	12.55
CRI-P152	D5S66	0.033	0.085	13.45	0.074	0.136	11.63
p92.27	GLR/D5S193	0.074	0.001	12.47	0.147	0.036	13.48
CRI-P148	D5S72	0.114	0.217	11.97	0.140	0.156	19.48
CRI-V1022	D5S54	0.084	0.060	9.86	0.057	0.269	16.68
1cYN5.62	D5S168	0.139	0.001	9.37	0.134	0.059	22.72
CRI-CVFM55	CSF1R	0.093	0.184	7.70	0.122	0.232	11.04

frequencies were calculated (Table 1A). The observed frequency of heterozygotes and the calculated PIC value were 44% and 0.49, respectively. There were no significant differences in allele frequencies between the unrelated CEPH members and the German group. Inheritance was shown to be codominant and Mendelian in all the CEPH families evaluated. The sizes of the alleles seen in CEPH parents 133101 and 133102 were deduced to be 204 and 204 and 204 and 204, respectively.

Linkage data obtained by typing the CEPH families were analyzed using version 5.2 of the LINKAGE package of programs (7). Family typings were added to the CEPH database (release 6), and two-point analyses were performed using the CLODScore option of the LINKAGE package to assign the GABRA6 gene to a linkage group. An anchor map was constructed with the CLINK program using several markers that showed good evidence for linkage to GABRA6, and GABRA6 was placed on this map using the CMAP program. All analyses were performed under a model that assumes a varying sex ratio of recombination frequencies. Twenty-eight of 40 reference families from the CEPH database were found to be informative for linkage analysis. Lod scores and recombination fractions for several markers that are closely linked to GABRA6 are given in Table 1B. In addition, lod scores were determined for GABRA1 at the same loci.

For GABRA6, several highly significant lod scores, the highest being with GABRA1 ($Z_{\max} = 39.87$) at recombination fractions of 0.069 for males and 0.100 for females, were observed. An anchor map giving the highest log-likelihood and the most resistance to local marker inversions was constructed from a subset of the linked markers (Fig. 1A). All other marker orders were at least 565-fold less likely than that shown, and no linked marker that could be placed with the required degree of certainty (1000-fold) on the anchor map distal to GABRA1 was found. This anchor map, which was generated using the latest CEPH database release, and which incorporated revised GABRA1 data for four families (due to previous mistyping), is slightly different to that

published earlier (4) and shows a considerable improvement in the log-likelihood and an increased resistance to marker inversion. GABRA6 could not be oriented unambiguously relative to p93-121 (D5S198) on the anchor map, but could be placed with odds of better than 1:10⁹ in the interval bounded by CRI-L45 (D5S61) and GABRA1 (Fig. 1B).

Previous studies (15, 16) have demonstrated the colocalization of the GABA_A receptor γ_2 -subunit gene, GABRG2, with GABRA1. Pulsed-field restriction mapping revealed that the maximal distance separating GABRA1 and GABRG2 is 200 kb, and it could not be ruled out that these two genes lie adjacent to one another. Our linkage data indicate that GABRA6 is located close to GABRA1 and GABRG2. Unfortunately, no typings for the latter gene appear in release 6 of the CEPH database, precluding the incorporation of GABRG2 into the linkage map that we have generated. The best estimate of the recombination fraction between GABRA1 and GABRA6 presented here suggests either that the distance between these two genes is greater than that between GABRA1 and GABRG2 or that a recombination hot spot lies between them.

In conclusion, we have mapped the human GABA_A receptor α_6 -subunit gene (GABRA6) to a subtelomeric region of chromosome 5q using a polymorphic (dC-dA)_n motif by demonstrating its tight linkage to several closely spaced markers. In addition, this study provides further evidence that GABA_A receptor genes occur in clusters; in this case, three genes (for one γ and two α subunits) are linked together. This clustering has implications both for the future mapping of genes predisposing to, or otherwise involved in, hereditary neurological and psychiatric disorders and for the analysis of GABA_A receptor gene expression.

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FIG. 1. Genetic linkage mapping of the GABRA6 gene. (A) Anchor map of markers on chromosome 5q32-q35 and (B) location scores for the placement of GABRA6 on the map. The numbers in B represent improvements in the map (2 ln likelihood) for the given order after insertion of GABRA6 in the indicated interval.

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