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Genetic Mapping of the β1 GABA Receptor Gene to Human Chromosome 4, Using a Tetranucleotide Repeat Polymorphism

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Summary

As more coding loci for functional human genes are described, there is a growing need to identify DNA polymorphisms in specific genes. By examining DNA sequences within the introns of the β1 subunit of the gamma-aminobutyric acid receptor gene, *GABARB1,* we found a tetranucleotide repeat sequence (GATA). Amplification of this region by using PCR revealed seven alleles and a high degree of polymorphism (PIC = .75) in human populations. DNAs from the CEPH families were typed for the *GABARB1* intron polymorphism and were analyzed with respect to 20 linked markers on chromosome 4. The results permit placement of *GABARB1* on the linkage map of chromosome 4, between D4S104 and ALB. These results affirm that sequence analysis of noncoding segments included within or adjacent to functional genes has value as a strategy to detect highly informative polymorphisms.

Introduction

Gamma-aminobutyric acid (GABA) is formed from glutamic acid by the enzyme glutamic acid decarboxylase (GAD). The principal receptors ($GABA_A$) have been localized throughout the brain, and the principal function of GABA is as an inhibitory neurotransmitter (reviewed in Olsen and Venter 1986). $GABA_A$ receptors are allosterically modulated by a number of pharmacological agents, including benzodiazepines, barbiturates, and alcohol.

Purification of $GABA_A$ receptors suggested that the receptor-ion-channel complex comprised at least two polypeptides arranged in a tetrameric array (Stephenson 1988; Olsen and Tobin 1990). Cloning and sequence analysis of cDNAs encoding murine, bovine, and human $GABA_A$ receptor subunits have revealed a large number of distinct receptor subunit genes. To date, six α, three β, one δ, and two γ receptor subunits have been isolated (Schofield et al. 1987, 1989; Levitan et al. 1988; Pritchett et al. 1989; Ymer et al. 1989; Lüddens et al. 1990); these subunits share both 25%–35% amino acid sequence identity with each other and low but significant homologies with subunits of nicotinic acetylcholine and glycine receptors.

Recently, the gene encoding a human β1 subunit of the $GABA_A$ receptor has been isolated and sequenced (Kirkness et al. submitted). The β1 subunit is encoded by a relatively large (>65 kb) gene on nine exons. The eight intron-exon junctions of the β1 subunit gene are found at equivalent positions in a mouse δ-subunit gene (Sommer et al. 1990). We have used the intron sequences to identify microsatellite loci.

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Material and Methods

Genomic Cloning and Sequencing

A 1,761-bp *Bsm*1/*Bgl*II fragment of a cDNA that encoded the β1 subunit of the bovine $GABA_A$ receptor (a gift from Dr. E. A. Barnard) was used to initially screen an amplified human leukocyte genomic DNA library at high stringency. Six clones were isolated; an

TGATAGCTAG AAAGCTAGCA AGGTGGATGG ATGATGAT**GA TAGATAGATA GATAGATAGA**

TAGATAGATA GATAGATAGA TCGATCGATC GATCTATCTC CACATCAGGG AGGCACATCA

AGCCAGATGT TTAGGAACAC AGTGTTTAAT GAGC

Figure 1 Sequence of *GABARB1* microsatellite locus. The sequence surrounding the 4-bp repeat locus is shown. The primers used to amplify this region are underlined, and the repeat itself is shown in boldface type.

oligonucleotide derived from sequence at the 3′ end of a human β1 cDNA clone hybridized to one of these six clones (λβ1). A second cDNA clone hybridized with an oligonucleotide derived from sequence from the middle of the coding region of the bovine β1 subunit as well as from the 5′ end of the λβ1 insert. No additional clones isolated from this library hybridized with probes derived from the 5′ end of the bovine β1 subunit cDNA.

A second human genomic DNA library was therefore screened with an *Apa*I fragment from the 5′ end of the bovine β1 subunit cDNA. Twelve hybridizing clones were isolated. Characterization of these clones by oligonucleotide probes and sequence analysis revealed that the gene encoding the human β1 subunit of the $GABA_A$ receptor was contained in seven overlapping genomic clones and included nine exons (Kirkness et al., in press).

For DNA sequence analysis, genomic clones were digested with restriction endonucleases and subcloned into pVZ1 (Henikoff and Eghtedarzadeh 1987). Sets of ordered deletions were generated using a modification of the Erase-a-Base system (Promega). Vector inserts were sequenced from single-stranded templates by using a *Taq* polymerase sequencing system (Promega) designed for use on the Applied Biosystems 370A automated DNA sequencer. Nucleotide data were analyzed and assembled using the Intelligenetics Suite software package.

PCR Amplification

The primers employed were 5′ TGATAGCTAGA-AAGCTAGCAAG and 5′ GCTCATTAAACACTG-TGTTCCT. DNA samples (100 ng) were amplified in 10 μl 10 mM Tris pH 8.8, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 70 μM each dNTP, 0.1 μl

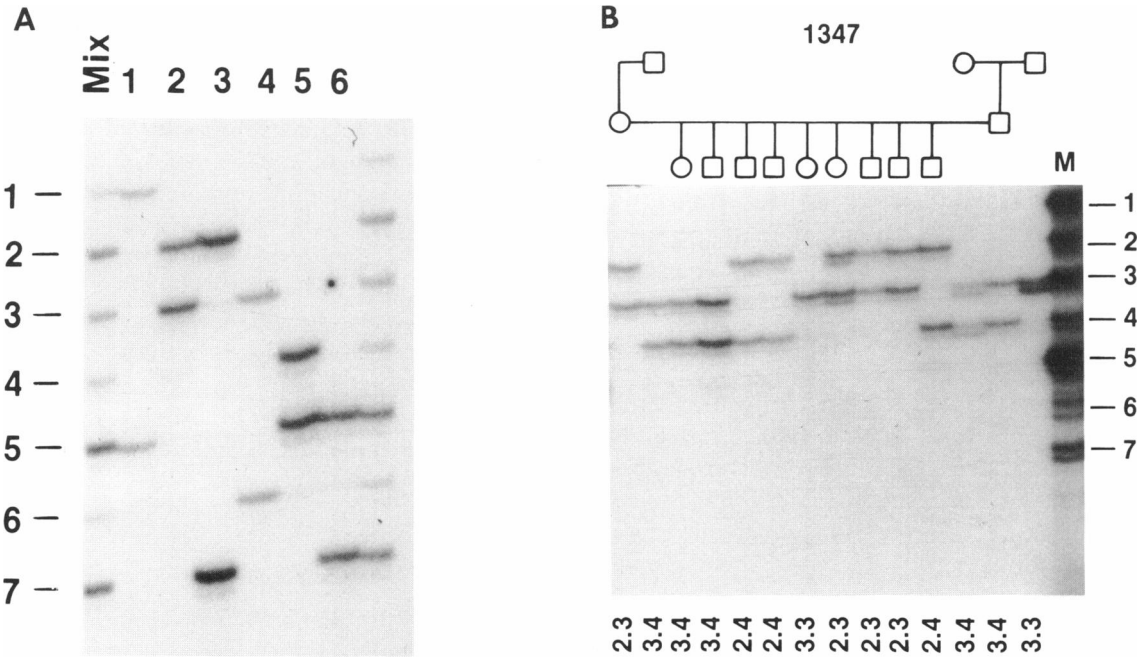

Figure 2 Segregation of *GABARB1* polymorphism. *A*, Six individuals with different genotypes analyzed as described in Material and Methods. Mix = mixture of all six samples. Alleles are numbered on the left. *B*, Transmission of polymorphism in CEPH pedigree 1347 (Dausett et al. 1990). Genotypes are shown below the pedigree. M = marker.

Table 1

Frequency of *GABARBI* Alleles

Allele[a]	No. of Repeats[b]	Frequency[c] (N^d)
1	12	.01 (2)
2	11	.12 (21)
3	10	.43 (77)
4	9	.22 (40)
5	8	.18 (32)
6	7	.02 (3)
7	6	.02 (3)

[a] Numbered from largest to smallest.
[b] Number of copies of tetranucleotide GATA.
[c] From unrelated individuals in CEPH pedigrees.
[d] Number of chromosomes containing indicated allele.

^{32}P-dCTP (3,000 Ci/mmol), 1 unit *Taq* polymerase (Cetus or Digene). The reactions contained 1 μl 1.0 O.D. (optical density units) each primer/ml solution, and 0.25 mM spermidine was included (Straub and Bale 1990). An aliquot of the PCR reaction (1.5 μl) was added to 9 μl 95% formamide loading dye and was heated at 90°C for 2 min, placed on ice, and loaded onto a 6% TBE/urea sequencing gel. The gel was run at 45 W for 3.5 h and was dried and exposed to film for 3–24 h. A marker lane was generated by mixing DNA from individuals with each of the known alleles.

Linkage Analysis

Data were entered into the programs provided by CEPH, and files with chromosome 4 markers were prepared using SETPED. Two-point LOD score analysis was performed using MAPMAKER (Lander et al. 1987), and the two-point values were employed for multipoint analysis. By the linkage map of Donis-Keller and Helms (1990), *GABARB1* was located on the map with the TRY function of MAPMAKER. All primary data have been contributed to CEPH and are freely available (Dausett et al. 1990).

Table 2

Markers Linked to *GABARBI*

Locus	Probe	Enzyme	Location	Maximum LOD Score[a]	Recombination Fraction[a] (Kosambi Morgans)
D4S1	3-6	*Bgl*I	4q11-q21	6.58	.22
D4S13	A46	*Msp*I	4pter-q26	8.41	.09
AREG	AR9	*Msp*IA	4q13-21	6.31	.13
		*Taq*I		3.10	.17
ALB	AlbB44	*Eco*RV	4q11-13	4.95	.08
	AlbF47	*Pst*I		4.44	.25
	F47-B44	Pst-RV		10.61	.23
ATP1BL1	B51-1-4	*Eco*RI		9.49	.10
		*Kpn*I		5.42	.00
D4S103	CRI-L1190	*Msp*I	4	9.25	.04
		*Taq*I		14.68	.07
D4S104	CRI-R171	*Bgl*II	4p	3.01	.33
D4S105	CRI-R234	*Bgl*II	4	5.38	.33
		*Hae*III		5.99	.29
D4S35	g920	haplo	4p11-q11	22.60	.03
		G9B	MspI	22.91	.03
		G9A	MspI	23.78	.00
D4S172	pYX1-11	*Bam*HI		14.67	.07
GC	Protein		4q12-13	11.01	.21
IL8		*Hin*dII	4q13-21	4.20	.19
INP10	gIFN31-7	*Eco*RI	4q21	7.57	.23
KIT	KIT	*Hae*III	4p11-q21	11.69	.02
	vkit	*Hin*dIII		11.02	.05
MT2P1	MTII	*Eco*RI	4p11-q21	6.20	.26
	pHM6	*Eco*RI		3.04	.37
PF4	PF4	*Eco*RI	4q12-21	3.52	.26
RAF1P1	RAF2p52	*Msp*I	4p16.1	3.00	.32

[a] Calculated under the assumption that there were no sex differences.

Results

In order to further characterize and identify polymorphisms in the *GABARB1* gene, genomic clones were isolated and sequenced (data not shown). The sequence of a portion of the intron between exons 8 and 9 revealed a repetitive sequence that contained 10 copies of the tetranucleotide GATA (fig. 1). Because many such simple-sequence repeat loci are polymorphic (Litt and Luty 1989; Weber and May 1989), we amplified this region by using PCR from several unrelated individuals and separated the products on a sequencing gel. Seven alleles were identified (fig. 2*A*), and the alleles segregated in Mendelian fashion (fig. 2*B*).

The *GABARB1* polymorphism was typed in all informative CEPH pedigrees (37 families), and allele frequencies in 84 unrelated individuals were calculated (table 1). Allele 3 was the most frequent, at .43, and the locus is heterozygous in 73% of individuals (data not shown).

GABARB1 has been physically assigned to chromosome 4p12–13 by in situ hybridization (Buckle et al. 1989; Kirkness et al., in press). In order to place the gene on the linkage map, two-point and multipoint linkage analyses were performed. Table 2 displays maximum LOD scores with respect to other chromosome 4 loci. As expected, *GABARB1* is tightly linked to several other markers in the centromeric region. Multipoint analysis with the closest markers places *GABARB1* between D4S104 and ALB. KIT, D4S35, and D4S103 also lie in this interval but could not be definitively ordered in relation to *GABARB1* (fig. 3).

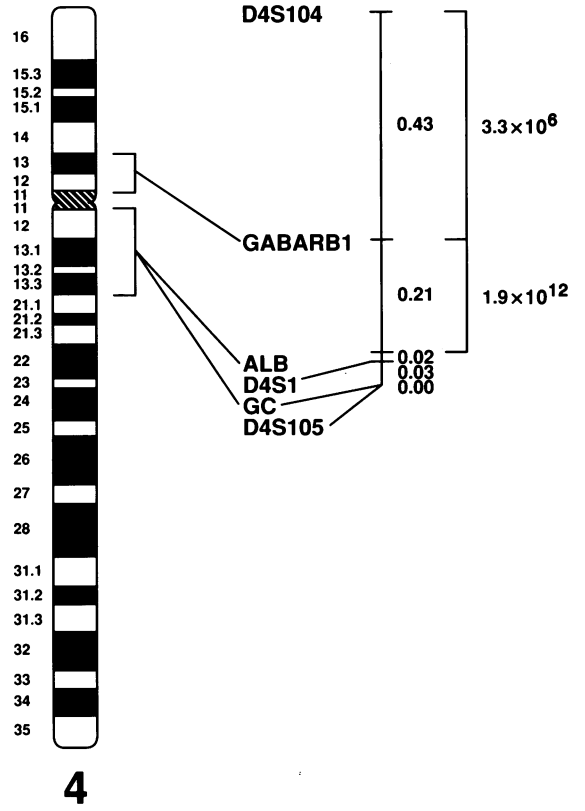

Figure 3 Genetic location of *GABARB1* gene. The location of the *GABAR1* gene is shown in relation to other mapped chromosome 4 loci. Odds against the placement of *GABARB1* in adjacent positions are shown.

Discussion

Microsatellite loci hold many advantages as genetic markers. They are highly polymorphic, abundant in the genome, and widely distributed—although the 4-bp repeat loci appear to be less common than the 2-bp repeats. Several polymorphic tri- and tetranucleotide repeat loci have been recently described within or near human genes (Boylan et al. 1990; Polymeropoulos et al. 1990*a*, 1990*b*, 1991 *a*, 1991*b*; van Amstel and Reitsma 1990; Zuliani and Hobbs 1990*a*, 1990*b*, 1990*c*). These sequences appear to be diverse, as TTTA, TTTC, TCTA, TGGA, GATT, and TTA repeats have been described. In our hands the tetranucleotide loci show only very faint background bands, because of slippage of the polymerase. This makes tetranucleotide repeats easier to score than CA repeats (fig. 2 and data not shown). This class of polymorphism could be very useful both in constructing high-resolution genetic maps and for forensic analysis.

We have shown that a tetranucleotide microsatellite sequence in an intron of the *GABARB1* gene is highly polymorphic, with an observed heterozygosity of over 70%. The alleles of this marker can be unambiguously scored, allowing an accurate determination of their frequency. Four of the alleles account for 95% of those found in the population. A highly informative marker in the *GABARB1* gene will allow this locus to be tested for linkage in neurological disorders. The $GABA_A$ of α2 subunit (*GABARA2*) gene also maps to the same region of chromosome 4p (Buckle et al. 1989), suggesting that there is a cluster of $GABA_A$ receptor genes in this region. The only neurological disorder mapped to 4p is Huntington disease (HD), but HD is located near the telomere (Gilliam et al. 1987).

The $GABA_A$ receptors are the site of action of a wide

range of pharmacological agents, including benzodiazepines and barbiturates. Benzodiazepines and barbiturates potentate the action of GABA by facilitating GABA binding to certain $GABA_A$ receptor subtypes. Evidence from a rat model supports a role of $GABA_A$ receptors in ethanol-induced motor impairment (Suzdak et al. 1986). Thus the $GABA_A$ genes represent plausible candidates for certain neurological diseases, particularly those that involve depression, anxiety, and/or alcoholism.

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