Original Paper

European Journal of Human Genetics

Eur J Hum Genet 1996;4:199–204

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Assignment of Genes Encoding $GABA_A$ Receptor Subunits α_1, α_6, β_2, and γ_2 to a YAC Contig of 5q33

Key Words
$GABA_A$ receptor subunit genes
Gene cluster
Chromosome 5
YAC contig

Abstract
A gene cluster consisting of the four γ-aminobutyric $acid_A$ ($GABA_A$) receptor subunit genes GABRA1, GABRA6, GABRB2, and GABRG2 was assigned to a yeast artificial chromosome (YAC) contig of 5q33. Two of the 26 YACs of the contig are positive for all four subunit genes. The order of the GABR subunit genes with respect to known anonymous gene loci is cen – D5S380 – D5S403 – D5S529 – GABRB2 – GABRA1/A6 – GABRG2 – D5S422 – tel. This novel YAC contig lies between known YAC contigs of 5q34/q35 and 5q31-q33.

Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian brain. GABA mediates its function via specific receptors, mainly of the type $GABA_A$. $GABA_A$ receptors are ligand-gated ion channels and permeable to Cl^-. Biochemical analyses suggest that the receptors are pentameres of various subunit isoforms [for reviews see ref. 1, 2]. Presently, 17 different $GABA_A$ receptor subunit isoforms are known. Based on their degree of homology, the subunits are assigned to families and classes (α, β, γ, δ, and ρ). Homology within families is 60–80% at the amino acid level and 20–40% between classes. Family α comprises six members (α_{1-6}), families β and γ four each (β_{1-4}, and γ_{1-4}), δ one, and ρ two (ρ_{1-2}) members. The GABR receptor subunits are encoded by the genes GABRA1–6, GABRB1–4, GABRG1–4, GABRD and GABRR1–2 and can be alternatively spliced [3–7].

Most of the GABR subunit genes have been mapped to specific human chromosomes. The genes coding for the two subunits ρ_{1-2}, GABRR1 and GABRR2, are on chromosome 6q14-q21 [8], GABRD is located in the short arm of chromosome 1 [9] and GABRA3 has been mapped to Xq28 [10, 11]. Several additional subunit genes appear to be parts of gene clusters. One cluster consisting of GABRA5, GABRB3, and GABRG3 has been assigned to 15q11-q13 [12–14]. Evidence of another cluster comprising GABRA2, GABRB1, GABRA4, and GABRG1 comes from both in situ hybridization and deletion mapping experiments [11, 15–18]. These experiments have located the four genes to 4p14-q21.1. Four additional subunit

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1018–4813/96/0044–0199$10 00/0

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Received:
January 30, 1996
Revision received:
March 27, 1996
Accepted: April 9, 1996

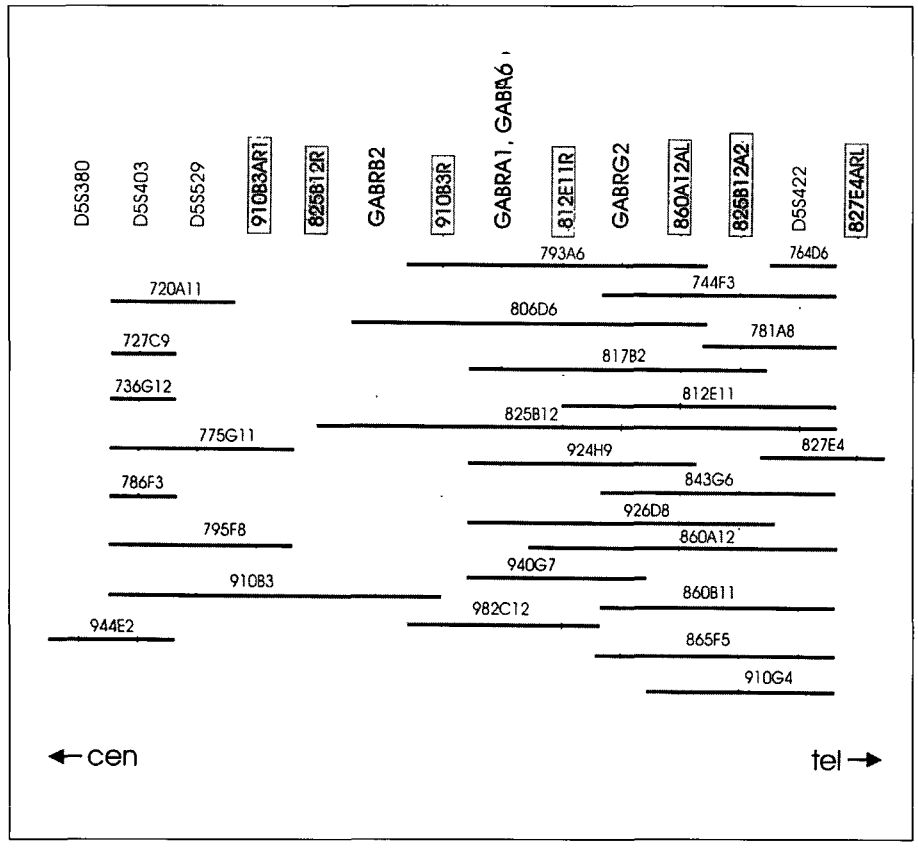

Fig. 1. YAC contig of 5q33. The newly isolated markers are shaded. GABR genes are typed in bold. The present YAC contig is located in the region of the doubly-linked contig WC-1432 of the Whitehead Institute for Biomedical Research.

genes, GABRA1, GABRA6, GABRB2, and GABRG2, have been mapped to the distal long arm of chromosome 5 (5q31.1-q35) and GABRA1 and GABRG2 have been assigned to a yeast artificial chromosome (YAC) of about 450 kb [11, 17, 19–22]. These findings suggest the existence of yet another cluster of GABR subunit genes.

Here we demonstrate by YAC mapping and fluorescence in situ hybridization (FISH) that subunits GABRA1, GABRA6, GABRB2, and GABRG2 are indeed clustered in the long arm of chromosome 5 (5q33).

Materials and Methods

The CEPH Mega YAC library pools were screened by PCR following the instructions of CEPH (Centre d'Etude du Polymorphisme Humain). Pulsed-field gel electrophoresis (PFGE) was carried out using a CHEF system (Bio Rad). Electrophoresis was performed at 14°C for 25 h (0.5 × TBE, 6 V/cm, 120°, ramp 50–100 s). Sizes of YACs were determined by comparison with the known size of chromosomes of *Saccharomyces cerevisiae* strain YPH49.

YAC end fragments and inter-Alu-sequences were isolated as previously decribed [23]. PCR products were blunt-end-cloned into the *Eco*RV site of pBluescript II SK + and sequenced. Sequences were used to generate new primer pairs for chromosome 5 STSs. The STSs were tested for chromosome 5 specificity using human/hamster somatic cell hybrid GM10114 that contains chromosome 5 as the only human chromosome.

Published primer sequences were used for the identification of $GABA_A$ receptor subunit genes (GABRA1 [19], GABRA6 [22], GABRB2 [21], GABRG2 [20]). Anonymous D5S markers were obtained from the Genome Data Base (D5S380, GDB ID: G00-186-594; D5S403, GDB ID: G00-188-048; D5S422, GDB ID: G00-188-376; D5S529, GDB ID: G00-195-023).

FISH was done according to standard procedures. YAC DNA was either used directly after digoxigenin-11-dUTP or biotin labeling or after amplification using various Alu primers [24, 25].

Results

YACs from the distal long arm of chromosome 5 were screened to localize $GABA_A$ receptor subunit genes GABRA1, GABRA6, GABRB2 and GABRG2 that had previously been assigned to 5q31.1-q35. The genes were

Table 1. Newly developed markers in 5q33

Marker name	D number	Accession number	Primer sequence	Product size, bp	Annealing temp., °C
812E11R	D5S2513	X94235	CAACACATCACAAATAGAAT TAATTGATTTGTCAGAGTTG	135	52
825B12R	D5S2514	X94236	GAAAACATAACATCATCGCC AATTGGAGAAATAATGAAACATG	154	60
910B3R	D5S2515	X94237	TGCCCATCCTTACAGAATCA GCTTCTTCCCTTTCTTATTTCA	151	62
825B12AR	D5S2516	X94238	AGGGAATAGAATGACACTCTGT TATACATGTGATTGGCCTGA	149	59
827E4ARL	D5S2517	X94239	TTTTCCGATTCTGGTTACTG AAAGAAAATATTCAATGCCTGT	126	60
860A12AL	D5S2518	X94240	CTGATGACAATATACCTGGGTG TTCGAGGGAATAATTGAGGA	128	62
910B3AR	D5S2519	X94241	CTGGGCAAATGACAAGTAGG GCTATCAAAAACAGGTGGCA	105	62

excluded from known YAC contigsof 5q34-q35 [23, 26] and of 5q31-q33 [27] (not shown). YACs located between these contigs [28] were positive for $GABA_A$ receptor subunit genes. Primers for GABRB2 amplified DNA of YAC 910B3 that contains locus D5S403. Seven additional D5S403 positive YACs (720A11, 727C9, 736G12, 775G11, 786F3, 795F8, 944E2) were negative for all GABA receptor subunit genes. Six additional YACs (744F3, 812E11, 843G6, 860A12, 860B11, 865F5) all of which include D5S422 types positive for GABRG2. Four D5S422-positive YACs (764D6, 781A8, 827E4, 910G4) did not contain any GABR subunit gene. Since GABRA1 and GABRA6 could not be localized on any known YAC from the region, we screened the CEPH library. A total of 8 YACs was isolated. One, 982C12, was positive for both GABRA1 and GABRA6, 5 YACs (793A6, 817B12, 924H9, 926D8, 940G7) included GABRG2 in addition to GABRA1 and GABRA6 and all four $GABR_A$ subunit genes (GABRA1, GABRA6, GABRB2, and GABRG2) were detected in two YACs (806D6 and 825B12). In order to refine the resulting YAC contig, several known markers (D5S380, D5S403, D5S422, D5S529) were assigned map positions. In addition, three YAC insert endpieces (812E11R, 825B12R, 910B3R) and four inter-Alu sequences (825B12AR, 827E4ARL, 860A12AL, 910B3AR) were located on the contig (fig. 1). Sequences of newly developed STSs are given in table 1.

The sizes of the 26 YACs of the contig were determined by PFGE and are listed in table 2. All YACs were

Table 2. YAC sizes and FISH results

YAC	Size, kb	Chimerism
720A11	400; 1,300	+
727C9	700; 945	+
736G12	360	+
744F3	850	+
764D6	615; >1,500	+
775G11	>1,500	+
781A8	500	−
786F3	400	+
793A6	850	−
795F8	945; 1,125; 1,500	−
806D6	960	−
812E11	1,050	−
817B2	870	−
825B12	1,500	−
827E4	1,300	−
843G6	1,050	−
860A12	1,050	−
860B11	1,050	−
865F5	1,300	n.d.
910B3	1,500	−
910G4	>1,500	+
924H9	820	−
926D8	1,300; 1,600	+
940G7	800	n.d.
944E2	630	−
982C12	730	+

Different sizes of the same YACs are probably due to in vivo truncation of an ancestral YAC.

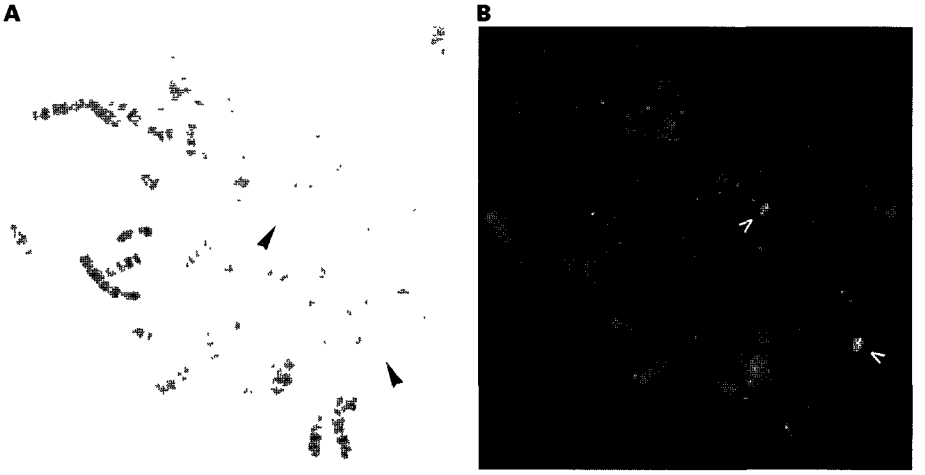

Fig. 2. A GTG-banded metaphase. **B** Same metaphase exhibiting the FISH signal of YAC 806D6 in 5q33. Relevant regions are indicated by arrow heads.

tested for chimerism by FISH. Fourteen YACs appeared to be nonchimeric, and 10 YACs were chimeric (table 2). No conclusive results were obtained for YACs 944E2 and 865F5. The YACs containing the GABR subunit genes were assigned to 5q33 (fig. 2).

Discussion

We have demonstrated that genes coding for $GABA_A$ receptor subunit isoforms α_1, α_6, β_2, and γ_2 are clustered within 5q33. All four subunits are contained within a YAC (806D6) of less than 1 Mb. The chromosomal order of the subunit genes is cen – GABRB2 – GABRA1/GABRA6 – GABRG2 – tel. The two GABRA subunit genes cannot be separated on this YAC contig indicating close proximity of both genes. Clearly, a high-resolution map is required for their eventual separation. This order of GABR subunit genes is in contrast to that of Warrington and Bengtsson [29]. Their method combining radiation hybrid mapping, interphase FISH and PFGE indicated the order cen – GABRG2 – GABRA1 – tel.

Another cluster of GABR genes, GABRB3, GABRA5, and GABRG3, was demonstrated on a YAC contig of 900 kb of proximal 15q [30]. The order of the GABR gene cluster in 5q11-q13 is comparable to that of the cluster described here. In both cases, genes coding for subunits β and γ flank the α-subunit gene(s) and the GABRB genes are most centromeric. A third cluster of GABR genes may be located on chromosome 4 and four subunit genes, GABRA2, GABRA4, GABRB1, and GABRG1, have been assigned to 4p14-q21.1 [6, 11, 15, 17, 18]. It needs to be determined, however, whether these genes are indeed clustered or whether they are dispersed over a relatively large region of chromosome 4.

The origin of the GABR gene clusters is presently unknown. One cluster may have arisen by duplication and subsequent mutation events on one chromosome and then was transposed to other regions of the genome. Accordingly, the original cluster consisted of one α-, one β-and one γ-subunit as on chromosome 15. This unit was dispersed by transposition and underwent further mutations including a duplication of the α-subunit gene.

It is not known whether clustering of GABR genes has any functional implications. One could speculate that clustering facilitates coordination of gene expression. Support for this notion comes from head-to-head arrangement of GABRB3 and GABRA5 on chromosome 15 within less than 100kb [14] that would allow for simple coordination of expression of these two genes. In contrast, GABRA6 of the cluster on chromosome 5 is expressed almost exclusively in the cerebellum [31, 32] while the remaining subunit genes are more widely expressed [33]. This finding argues against simultaneous expression of genes within a GABR cluster.

A potential function of GABA and its receptors in the origin of human disease has not been proven. A gene locus possibly involved in manic depression has been assigned to distal 5q [34] and one may speculate that GABR genes are involved in this disorder. However, there is presently no direct supporting evidence of this hypothesis. Another study speculates that $GABA_A$ receptors might be involved in the pathogenesis of spinal myoclonus [35]. Again, there is no convincing data in favour of this assumption. Final-

ly, GABRB3 has been implicated in normal facial development. The cleft palate of mice carrying a deletion in the *cp1* (cleft palate) locus is rescued by the introduction of transgenic *Gabrb3* [36].

Acknowledgements

We gratefully acknowledge Dr. D. LePaslier (Centre d'Etude du Polymorphisme Humain, Paris) for the isolation of YACs. We thank Oliver Buckolt for his skillful generation of the artwork and Karen Davis for help in FISH analysis. This work was supported by the European Community [EC GENE-CT93-0050 (DG 12 SSMA)]. APM is a Wellcome Trust Principal Research Fellow.

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