GENOMICS **45,** 1–10 (1997)
ARTICLE NO. GE974885

# A Gene in Human Chromosome Band Xq28 (GABRE) Defines a Putative New Subunit Class of the $GABA_A$ Neurotransmitter Receptor

Klaus Wilke,[1] Renate Gaul, Sabine M. Klauck, and Annemarie Poustka[2]

*Deutsches Krebsforschungszentrum, Abteilung Molekulare Genomanalyse, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*

Received March 10, 1997; accepted June 26, 1997

**We have isolated and sequenced a novel human gene (GABRE) of the $GABA_A$ neurotransmitter receptor family. A cDNA sequence of the gene coding for a 506 amino acid protein was identified, representing a member of a putative new class ($\epsilon$) of the $GABA_A$ receptor. The gene is transcribed at least at low level in several different tissues, with the highest levels being detected in adult heart and placenta. Alternative splicing of GABRE transcripts isolated from different tissues was observed at multiple positions of the gene, yielding an unusually complex variety of cDNA variants. The structure of the 5′ region of most cDNAs is compatible with expression of protein sequence $\epsilon$ only in adult brain, whereas in other tissues, the majority of transcripts code for truncated protein sequences. The GABRE gene extends over 14 kb and is clustered together with the $\alpha$3 and the putative $\beta$4 $GABA_A$ receptor subunit genes in an ≈0.8-Mb interval in chromosome band Xq28, located in the candidate regions of two different neurologic diseases. Based on features of conservation of protein sequences, gene structure, and genomic organization of $GABA_A$ receptor gene clusters, we propose that the $\epsilon$ and $\gamma$ subunit genes have a common ancestor and that $GABA_A$ receptor gene clusters in the human genome have diverged by multiple duplication events of an ancestral gene cluster containing one each $\alpha$, $\beta$, and $\gamma/\epsilon$ precursor gene.**



## INTRODUCTION

$GABA_A$ receptors are the main inhibitory neurotransmitter receptors in the vertebrate brain, occurring in virtually every neuron (for review on structure and function of $GABA_A$ receptors see Olsen and Tobin, 1990, and Seeburg *et al.*, 1990). They are heterooligomeric structures thought to be composed of five subunits, each containing four transmembrane domains, which form a chloride channel gated by the neurotransmitter GABA ($\gamma$-aminobutyric acid). Sixteen different $GABA_A$ receptor subunits have been identified in humans and can be divided into five classes: $\alpha$ (1–6), $\beta$ (1–4), $\gamma$ (1–3), $\delta$ (1), and $\rho$ (1–2). A $\gamma$4 subunit has been identified in chicken (Harvey *et al.*, 1993). In the human retina, the $\rho$1 and $\rho$2 subunits, which form receptors of the $GABA_C$ type, are expressed. In rat, an additional putative $\rho$3 subunit has been identified (Ogurusu and Shingai, 1996).

$GABA_A$ receptors are the targets of a variety of psychoactive drugs, including anxiolytics (e.g., benzodiazepines), sedatives (e.g., barbiturates, alcohol), antiepileptic agents, muscle relaxants, hypnotics, and certain steroids (Burt and Kamatchi, 1991). The pharmacological properties of $GABA_A$ receptor subtypes are decisively determined by their subunit composition. Each receptor subunit has a distinct pattern of expression in different parts of the brain. Involvement of $GABA_A$ receptor genes in genetic diseases has been demonstrated in rodents: In mice, deficiency of the $\beta$3 subunit of the $GABA_A$ receptor is the cause of a developmental defect (cleft palate; Culiat *et al.*, 1995), whereas a point mutation in the $\alpha$6 subunit of alcohol-intolerant rats results in impairment of postural reflexes by benzodiazepine agonists (Korpi *et al.*, 1993).

In the human chromosome band Xq28, genes GABRA3 and GABRB4 have been mapped, coding for the $\alpha$3 and putative $\beta$4 subunits of the $GABA_A$ receptor (Rogner *et al.*, 1994; Levin *et al.*, 1996). The genes are located within the candidate regions of two different neurologic disorders: early onset parkinsonism (Waisman syndrome; Laxova *et al.*, 1985; Gregg *et al.*, 1991) and X-linked mental retardation (MRX3; Gedeon *et al.*, 1991; Nordstrom *et al.*, 1992). In a search for candidate

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. Y09763–Y09765.

[1] Present address: Westfälische Wilhelms-Universität Münster, Institut für Humangenetik, Vesaliusweg 12-14, D-48149 Münster, Germany.

[2] To whom correspondence should be addressed. Telephone: x6221-424646. Fax: x6221-423454.



0888-7543/97 $25.00

genes for Waisman syndrome and MRX3, we have now identified a new gene (GABRE) of the $GABA_A$ receptor family, which resides together with GABRA3 and GABRB4 in an ≈0.8-Mb interval in Xq28, at a distance of ≈4.5 Mb from the telomere. Based on the degree of homology of deduced protein sequences and on unique features of alternative splicing, the gene defines a putative new subunit class of the $GABA_A$ receptor.

## MATERIALS AND METHODS

DNA was labeled with [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol) in a random-primed reaction (Feinberg and Vogelstein, 1983). Hybridizations were performed at 65°C as described by Poustka (1990). To block repeat sequences, the fragments were prehybridized with 50 $\mu$g of denatured human cot-1 DNA (BRL) at 65°C for 4 h (Sealey *et al.*, 1985). Hybridization probes and YACs are described by Rogner *et al.* (1995 and 1994, respectively). An interval E3 probe was generated by PCR amplification from cosmid 11C8, using primers 6 and 7. Cosmids were isolated from a cosmid library constructed from flow-sorted X chromosomes (cosmid ICRF B5117; Nizetic *et al.*, 1991) and from an Xq28-specific cosmid library constructed from cell hybrid Q1Z (Rogner *et al.*, 1994). A multiple human poly(A)$^+$ RNA Northern blot and a human placenta cDNA library, constructed by oligo(dT) and random priming, were purchased from Clontech. 5′ RACE was performed in two rounds using the Marathon cDNA amplification kit (Clontech), the Marathon adaptor, and oligonucleotide primers 7 (first round) and 5 (second round). Products were cloned into plasmid pCRII (Invitrogen). Poly(A)$^+$ RNA from different human tissues was purchased from Clontech. A total of 0.5 $\mu$g of each RNA was reverse transcribed using Superscript II reverse transcriptase (GIBCO BRL) and GABRE primer 9 under the conditions recommended by the manufacturer. PCR amplification of cDNA was performed at 65°C annealing temperature with 1.5 m*M* $MgCl_2$. Oligonucleotide sequences were: (1) CTTCCAGTCCTCCTAGGCATCTT; (2) GGCATCTTATTGATCCTCCAGTCG; (3) CTCAGGATAGTGTTCAGGATGCGAGA; (4) CCTCTCGCATCCTGAACACTATCCTGAGTA; (5) CGGAGATCTCAACAGTGACCACAGTGGGCT; (6) AGCCCACTGTGGTCACTGTTGAGATCTCCG; (7) CACCTTGCCATCCTTGTAGATGCGG; (8) CAACCAGATGGTCCGCATCTAC; (9) ACTGGAAGAGCTTCCAGGAGTT; (10) TATGTCCCTTCTTCCGTGACC; (11) CGGAGTTTAGGAGAAGCATGGGCTTTTGTC.

Human/hamster cell hybrids were kindly provided by Nigel Spurr. For PCR mapping on cell hybrids, YACs and cosmids, primer pairs 4/5, 8/9, and 10/11, were used. DNA was sequenced on an ABI 373A automated sequencer by the dideoxy termination method (Sanger *et al.*, 1977) using the *Taq* DyeDeoxy Terminator Cycle sequencing kit (Perkin–Elmer, Applied Biosystems). DNA and protein sequences were assembled and analyzed using XGAP (R. Staden) and HUSAR sequence analysis software. Sequence data from this article have been deposited with the EMBL database under Accession Nos. Y09763 and Y09764 (genomic sequence) and Y09765 (cDNA V1).

## RESULTS

### *Isolation of cDNAs*

To identify transcripts in a region 400 kb proximal to the GABRA3 gene in Xq28, the method of direct cDNA selection was employed (Korn *et al.*, 1992). Cosmids mapping to the region were used for enrichment of cDNA clones from human adult skeletal muscle, fetal brain, and fetal liver (average size 800 bp; Rogner *et al.*, 1995). Sequencing of cDNAs from the enriched sublibrary hybridizing with cosmid 11C8, which was used in the enrichment protocol, revealed 5 overlapping clones of a new gene of the $GABA_A$ receptor gene family that we named GABRE. Using these 5 cDNAs as probes, a total of 27 cDNAs were isolated from the enriched sublibrary and sequenced from both ends. Eight cDNAs were derived from adult skeletal muscle, 4 from fetal brain, and 15 from fetal liver.

Since Northern analysis revealed transcription of GABRE in placenta (see below), we used a placenta cDNA library for the isolation of longer conventional cDNA clones. By screening $10^6$ clones of the placenta cDNA library, 4 clones (CP1–CP4) corresponding to the GABRE gene were identified and sequenced by primer walking. The 5′ sequence of cDNAs from placenta was determined by 5′ RACE amplification of placenta cDNA, using nested gene-specific primers, followed by cloning and sequencing of 10 individual clones.

Eight additional cDNA sequences (expressed sequence tags, ESTs) corresponding to the 3′ end of GABRE were identified by a homology search in sequence databases (The IMAGE consortium; GenBank Accession Nos. H83086, R07883, R07942, R49718, R64082, R91732, T27015, T78142). R49718, T27015, and T78142 originated from infant brain and R07883 and R07942 from fetal liver or spleen, whereas the other ESTs are from nonspecified human tissues (Hillier *et al.*, 1995).

### *Genomic Sequence and Alignment of cDNA Variants*

Since a preliminary analysis of cDNA sequences indicated complex patterns of alternative splicing, it was essential for alignment of cDNA sequences to determine the genomic sequence of GABRE. The sequence was determined by sequencing PCR fragments obtained from cosmid Qc11C8 using PCR primers defined from cDNA sequences. GABRE has a length of 14 kb. The genomic organization is shown in Fig. 1A. Within interval I3, an *Alu* sequence that is 87% identical with the human *Alu*-Sx subfamily consensus sequence hsu14574 was identified (18). The simple repeat $(GTTTT)_8$ is located at a distance of 48 bp from the 5′ end of the *Alu* sequence, and a $(AGC)_5$ repeat resides in the 3′ untranslated region of interval E8 (Fig. 1A).

All identified cDNA sequences were aligned with the genomic sequence, as shown in Figs. 1B–1D. From the overlapping cDNA clones isolated by direct cDNA selection, the consensus cDNA sequences V1–V4 were assembled (Fig. 1B). cDNA variant V1 (containing the alternatively spliced interval E4) is the only variant containing the complete open reading frame with coding capacity for the 506-amino-acid sequence of the putative $GABA_A$ receptor subunit $\epsilon$ (Fig. 2).

In cDNA variants V2–V4, the deduced $\epsilon$ protein sequence is truncated at the N terminus. The first putative ATG translation initiation codon in these cDNA sequences is located in interval E3A and corresponds to

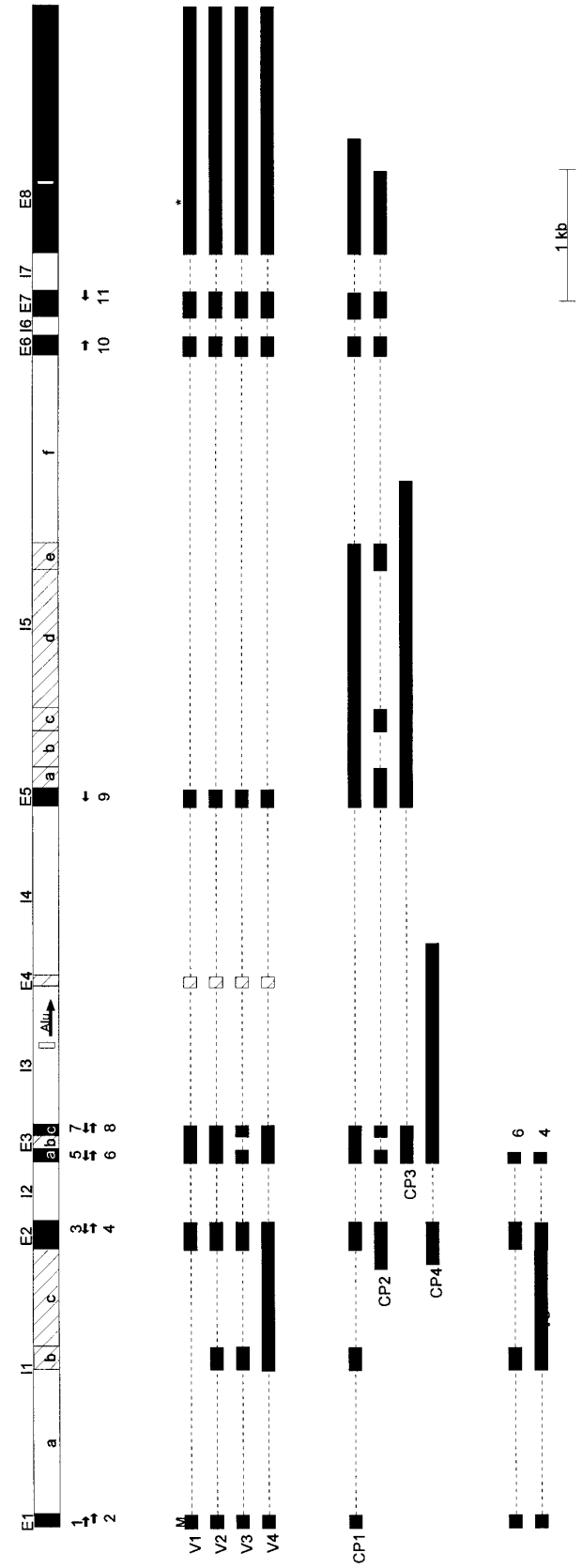


**FIG. 1.** Genomic organization of GABRE and structure of cDNAs. (**A**) Genomic organization of GABRE. The complete sequence of GABRE between the beginning of GABRE transcripts and the poly(A) tail was determined by primer walking, except for a 2.7-kb sequence gap within intron I1a. Intervals of the genomic sequence present in all isolated cDNA variants (compare B, C) are shown in black, whereas intervals present in only a subset of cDNA variants are hatched. Intervals E1–E8 are represented in cDNA variant V1 (B), coding for protein sequence $\epsilon$. The region of an *Alu* repeat in I3 is marked by an arrow. The simple repeat sequences $(GTTTT)_8$ and $(AGC)_5$ are marked by small boxes in intervals I3 and E8, respectively. Oligonucleotide primers used for analysis of cDNAs are indicated with arrows. (**B**) Structure of cDNAs isolated by direct cDNA selection. From the sequences of the overlapping cDNA fragments, the consensus sequences V1–V4 were assembled. The 3′ terminus of transcripts V1–V4 is represented by EST H83086 and contains the polyadenylation signal (AATAAA) at a distance of 15 nucleotides from a poly(A) tail. Intervals present in the cDNAs are black. Additional variation of cDNAs occurs when E3 is joined directly to E5. The alternatively spliced interval E4 is indicated by hatching. V1 (including E4) is the only cDNA variant containing an open reading frame and codes for $GABA_A$ subunit protein sequence $\epsilon$1. The positions of the ATG translation initiation codon (M) and the TAG translation stop codon (*) of the open reading frame are indicated. (**C**) Structure of four cDNAs (CP1–CP4) isolated from a conventional placenta cDNA library. Intervals present in the cDNAs are black. (**D**) Structure of cDNA fragments from placenta. 5′ ends were amplified by the 5′ RACE method and cloned into vector pCRII. Ten individual clones were sequenced. Two different cDNA variants were identified at the 5′ ends of cDNAs. Intervals present in the two cDNA variants are black. For each variant, the number of identified clones is indicated.

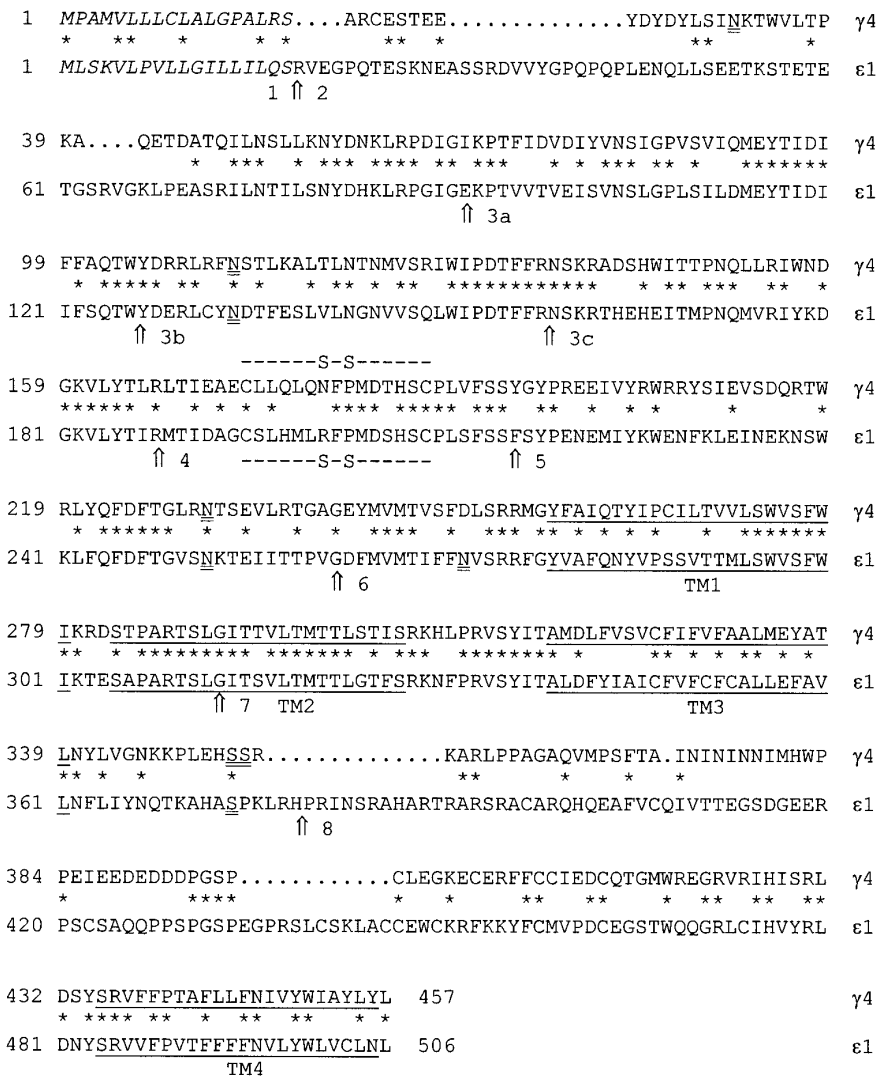


**FIG. 2.** Protein sequence $\epsilon$ (deduced from cDNA V1; see Fig. 1B) compared to the sequence of the $\gamma$4 subunit of the $GABA_A$ receptor from chicken. ⇑ indicates the beginnings of protein regions coded by the indicated intervals. The sequence of $\epsilon$ is 49% identical to the sequence of the the $\gamma$4 subunit of the $GABA_A$ receptor from chicken (Harvey *et al.*, 1993). Identical amino acids are marked by asterisks. In the protein sequences, the predicted positions of the signal peptide (italics), a disulfide bridge (-S-S-) in the predicted extracellular domain, transmembrane regions TM1–TM4, and N glycosylation (N) and protein kinase C phosphorylation (S) sites are indicated.

amino acid position 114 (Fig. 2). The truncated protein sequence does not contain a predicted signal sequence and has lost a conserved part of the predicted major extracellular domain of $\epsilon$ (Fig. 2). In variant V3, the alternative skipping of interval E3b leads to an additional internal deletion of 32 amino acids (positions 127–158), whereas alternative skipping of interval E4 leads to a C terminal truncation, beginning after position 188.

The structure of the 4 cDNAs isolated from placenta is depicted in Fig. 1C. Each cDNA has a different structure as a result of alternative splicing. Some of the splice events are the same as those in the cDNAs identified by direct cDNA selection (see Fig. 1B). Additional variation occurs by different patterns of splicing within interval I5 (CP1–CP3) and interval I3-E4-I4 (CP4). The consensus sequences of the 5′ ends of 10 placenta cDNA clones, identified by 5′ RACE analysis, is shown in Fig. 1D. In this analysis, two different splice variants were identified, involving the splice events previously observed at the 5′ ends of cDNA variants V2–V4 (compare Fig. 1B). In all 5′ splice variants from placenta the deduced $\epsilon$ protein sequence is truncated at the N terminus, beginning at position 114. In addition, all isolated cDNAs from placenta yield truncations of the deduced protein sequences, beginning after position 188.

The exon/intron boundaries of all cDNA variants identified in this study were compared with the splice consensus sequences derived from the analysis of 1800 human introns (Stephens and Schneider, 1992). All boundaries, including those identified in the placenta cDNAs, are in full agreement with the human consensus sequence (Table 1).

**TABLE 1**

**Exon/Intron Boundaries of cDNA Variants V1–V4 and Placenta cDNAs CP1–CP4 (Fig. 1)**

| Sequence name | Exon/ Intron /Exon |
|---|---|
| Splice consensus | aG/**GT**AAG.....tttttttttttttnC**AG**/g<br>ca Gga ccccccccccccc T a<br>tt ct<br>gc tc |
| V1 | AG/GTGAG..Ila-c....TCGTTTgaaTgTTCTAG/G |
| V2-4, CP1 | AG/GTGAG..Ila.....aCTTTTTaTggaaTTAG/A |
| V2, V3, CP1 | CG/GTGAG..I1c.....TCgTTTgaaTgTTCTAG/G |
| V1-4, CP1,2,4 | AG/GTGAG..I2.....TTCTgCTTCCCTTATAG/A |
| V3, CP2 | TG/GTACG..E3b.....CCggaCaCCTTTTTTAG/G |
| V1-4 | AG/GTATG..I3.....gTgTTgTaCaCaaTTAG/G |
| V1-4 | CT/GTGAG..I4.....CTCaCaTTCCTTTCCAG/t |
| V1-4, CP1-3 | AG/GTATG..I3,E4,I4...CTCaCaTTCCTTTCCAG/t |
| V1-4 | TG/GTGAC..I5a-f.....TTTTTTgTgTCTTTCAG/G |
| CP2 | TC/GTGAG..I5b.....ggTTTTCTgCaCCTCAG/c |
| CP2 | AG/GTAAG..I5d.....agTgTCTTaaaTTGCAG/t |
| CP1, CP2 | AG/GTGAG..I5f.....TTTTTTgTgTcTTTCAG/G |
| V1-4, CP1-2 | AG/GTAAG..I6.....CTCTTgTgCTCTTGCAG/G |
| V1-4, CP1-2 | AT/GTATG..I7.....TTCCTTgTCCaTTTTAG/c |

*Note.* The sequences were aligned with the splice consensus sequences derived from the analysis of 1800 human introns (Stephens and Schneider, 1992). In the splice consensus sequence, the most conserved nucleotides are printed in capital letters. In the exon/intron boundary sequences of GABRE transcripts, nucleotides that are identical to the consensus sequence are printed in capital letters. At the ends of all identified introns of GABRE transcripts, the dinucleotides GT or AG, respectively, which are present at the ends of nearly all human introns, are conserved.

To analyze the relatedness between GABRE and known chloride channel receptor genes on the level of gene structure, we compared the organization of coding regions between GABRE (coding for protein sequence $\epsilon$) and $GABA_A$ and glycine receptor subunit genes (Fig. 3). Figure 3 shows that the position of the first and last intron in known $GABA_A$ receptor subunit genes is characteristic for each subunit class. Also in GABRE, the first intron has a unique position, but approximately coincides—as in other $GABA_A$ receptor subunit genes—with the predicted signal sequence cleavage site in the protein sequence (compare Fig. 2). However, the position of the last intron in GABRE is conserved with the deduced position of an intron in $\gamma$2 subunit genes. The positions of the remaining introns are conserved between $GABA_A$ receptor subunit genes of all classes, including GABRE, and the glycine receptor gene. In contrast, one of these introns, located in the region homologous to interval E3a, is not present in GABRE. The alternative skipping of a subregion of interval 3, and of interval 4, are additional unique features of GABRE (Fig. 3).

### *Protein Sequence $\epsilon$*

The 506-amino-acid protein sequence $\epsilon$, coded by cDNA V1 (Fig. 1B), was compared to known sequences in the SwissPROT database. The highest homology (49% identical amino acids) is with the chicken $\gamma$4 subunit of the $GABA_A$ receptor (Harvey *et al.*, 1993). Homology to the rat $\gamma$3, human $\gamma$2, and rat $\gamma$1 subunits (Herb *et al.*, 1992; Pritchett *et al.*, 1989; Ymer *et al.*, 1990) is 46, 43, and 47%, respectively. In comparison, homology between members of the $\gamma$ class is much higher: As an example, the chicken $\gamma$4 sequence has 69, 67, and 67% identical amino acids with the rat $\gamma$3, human $\gamma$2, and rat $\gamma$1 subunit sequences, respectively. The highest scores in comparisons between $\epsilon$/chicken $\gamma$4, respectively, and members of other $GABA_A$ receptor subunit classes in humans are 39/45% ($\alpha$), 33/36% ($\beta$), 34/35% ($\delta$), and 32/34% ($\rho$). The highest scores with other ion channel proteins are 33/36% (glycine receptor) and 21/23% (achetylcholine receptor).

An alignment of the $\epsilon$ and chicken $\gamma$4 sequences is shown in Fig. 2. The alignment shows that homology extends over most of the protein sequence. Most notably, sequences of key structural elements are conserved: transmembrane domains TM1–TM4, the putative signal sequence, N-glycosylation and protein kinase C phosphorylation sites, and the putative Cys-Cys loop representing a "signature" of all ion-gated receptor proteins except for glutamate receptors (Montal, 1990; Dingledine *et al.*, 1990).

### *Tissue Specific Splicing and Expression*

Expression of GABRE in different tissues was analyzed by reverse transcription of poly(A)$^+$ RNA from different adult or fetal tissues followed by PCR amplification, using GABRE-specific primers in each step (Fig. 4). GABRE transcripts were detectable at least at a low level in all analyzed tissues, using PCR primers specific for the 5′ region of GABRE (Fig. 1A). A 209-bp band was the only band detected in adult brain, but was also weakly seen in adult skeletal muscle and adult heart. This band corresponds to cDNA V1, which codes

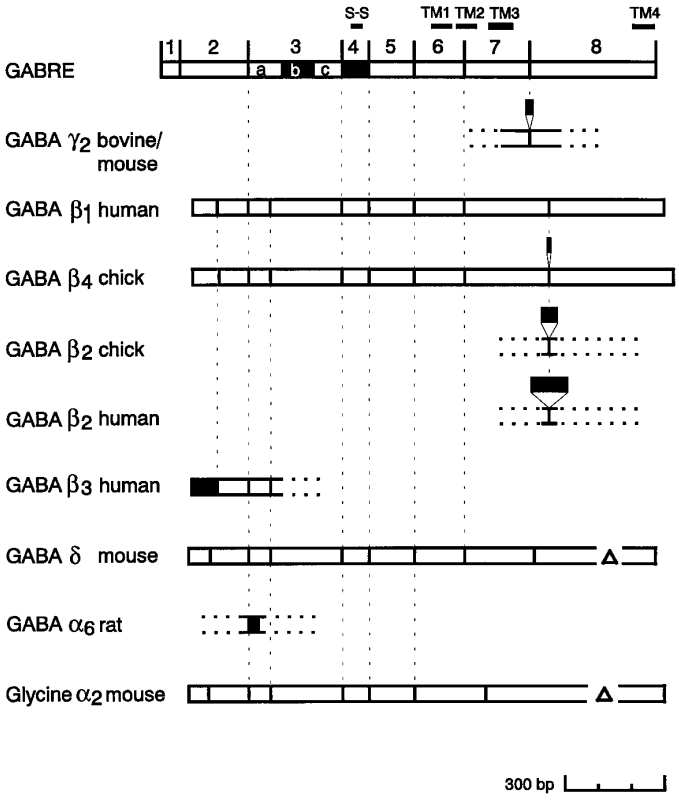


**FIG. 3.** Genomic organization of GABRE coding regions compared to coding regions of $GABA_A$ and glycine receptor subunit proteins. The positions of introns are indicated by vertical divisions. The names of GABRE intervals, coding for protein sequence $\epsilon$, are indicated. Alternatively spliced regions are black and are in some cases indicated as insertions. The coding sequences were aligned to yield maximum homology between the corresponding protein sequences (compare to Fig. 2). △ indicates regions not represented in the $\delta$ subunit gene of the $GABA_A$ receptor or in the $\alpha$2 subunit gene of the glycine receptor. The conserved positions of the predicted extracellular disulfide bond (S-S) and the predicted transmembrane domains TM1–TM4 are indicated. Information on the genomic organization of the chloride channel receptor genes is based on comparisons between cDNA and genomic sequences [$GABA_A$ receptor genes $\beta$1 human (Kirkness *et al.*, 1991), $\beta$4 chicken (Bateson *et al.*, 1991; Lasham *et al.*, 1991), $\beta$2 chicken (Harvey *et al.*, 1994), $\beta$2 human (McKinley *et al.*, 1995), $\beta$3 human (Kirkness and Fraser, 1993), $\delta$ mouse (Sommer *et al.*, 1990), $\alpha$6 rat (Korpi *et al.*, 1994), glycine receptor subunit $\alpha$2 mouse (Matzenbach *et al.*, 1994)]. The position of a putative intron in bovine and mouse $\gamma$2 subunit genes has been deduced from the sequences of alternative cDNA variants (Whiting *et al.*, 1990; Kofuji *et al.*, 1991).

for protein sequence $\epsilon$. In contrast, a 387-bp band corresponding to cDNAs V2/V3 was detected as the strongest band in all other tissues, including fetal brain. Neither of the cDNAs V2 or V3 has coding potential for protein sequence $\epsilon$.

The level of transcription of GABRE in different adult tissues was analyzed by Northern analysis. Figure 5 shows that transcripts were detected at this lower level of sensitivity in heart and placenta, but not in other tissues including brain. The size of the single band in heart (3.5 kb) is the same as expected for cDNA variant V2. Several bands in placenta in the range 5.7–14 kb may be the result of multiple alternatively spliced or unspliced transcripts, such as the transcripts shown in Fig. 1C.

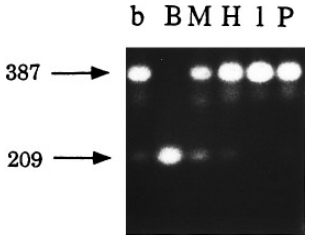


**FIG. 4.** Analysis of splicing in different tissues. GABRE transcripts in different human tissues were analyzed by reverse transcription of poly(A)$^+$ RNA using GABRE primer 9 followed by nested PCR amplification using GABRE primer pairs 1/3 and 2/3. Analyzed tissues: fetal brain (**b**), adult cerebral brain (**B**), adult skeletal muscle (**M**), adult heart (**H**), fetal liver (**l**), and placenta (**P**). The 387- and 209-bp products correspond to cDNA variants V2/V3 and V1, respectively. The identity of the PCR products was verified by sequence analysis after excision of bands from the agarose gel. No amplification of a 1124-bp fragment corresponding to variant V4 was obtained.

*Genomic Localization*

The localization of GABRE in Xq28 was verified by PCR mapping on human/hamster cell hybrid DNAs containing single human chromosomes or different portions of the X chromosome. By PCR mapping on YACs and cosmids, and hybridization of YAC and cosmid fingerprints, the location of GABRE within Xq28 was determined (Fig. 6). The gene was found to be clustered together with the $\alpha$3 and putative $\beta$4 subunit genes of the $GABA_A$ receptor in an ≈0.8-Mb interval. Two of the cosmids harboring GABRE (2C9, 8A1) in addition contain a gene of the melanoma antigen gene family (MAGE-4), which has previously been located on YAC I457 (Rogner *et al.*, 1995).

## DISCUSSION

The classification of subunit types of the $GABA_A$ receptor is based on the degree of homology of their pro-

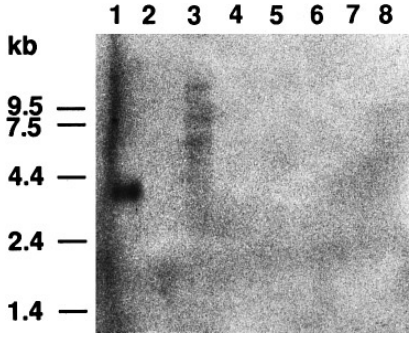


**FIG. 5.** Analysis of transcripts by Northern analysis. A Northern blot from different adult human tissues was hybridized with a GABRE probe generated by PCR amplification of a cDNA (variant V1) using primers 4 and 7. Lanes: **1,** heart; **2,** brain; **3,** placenta; **4,** lung; **5,** liver; **6,** skeletal muscle; **7,** kidney; **8,** pancreas.

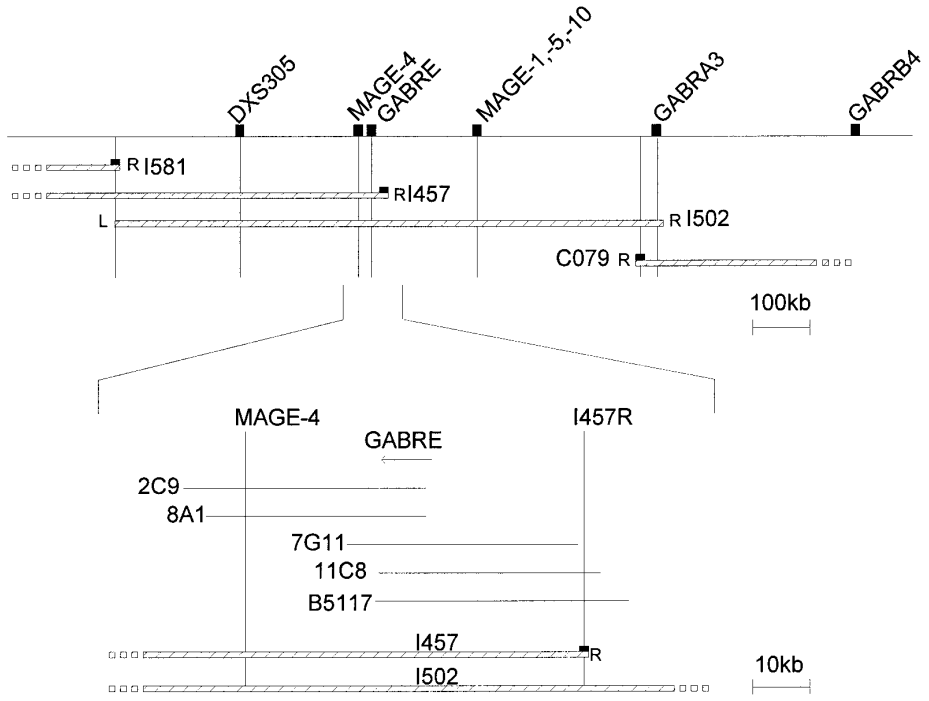


**FIG. 6.** Physical map of the GABRE region. (**Top**) YAC map. (**Bottom**) Cosmid map. The YAC map of Xq28, including the position of the gene for the $\alpha$3 subunit of the $GABA_A$ receptor (GABRA3), was described by Rogner *et al.* (1994). Localization of the putative $\beta$4 subunit gene of the $GABA_A$ receptor (GABRB4) on a different set of YACs was described by Levin *et al.* (1996). Mapping of genes of the melanoma antigen gene family (MAGE) was described by Rogner *et al.* (1995).

tein sequences. Proteins of one class (e.g., $\alpha$) are 60–80% identical to other members of the same class (Olsen and Tobin, 1990). Sequence homology and the conservation of predicted structural elements in protein identifies $\epsilon$ as a member of the family of $GABA_A$ receptor subunit protein sequences. The much lower conservation with glycine chloride channel and acetylcholine cation channel protein sequences excludes the possibility that $\epsilon$ is a member of any other known group of amino acid receptor ion channel proteins. Even though $\epsilon$ is most related to the $\gamma$ subunit class of the $GABA_A$ receptor, it shares only 43–49% identical amino acids with the members of this class. The $\epsilon$ and $\gamma$ subunit sequences are most divergent at the beginnings of the predicted major extracellular domains and in the predicted major intracellular domain (<25% sequence identity). Based on sequence homology, we have classified the new protein sequence as a member of the putative new subunit class $\epsilon$ of the $GABA_A$ receptor.

Comparison of the genomic organization of the coding sequences of GABRE and previously identified $GABA_A$ receptor subunit genes supports this classification: On the one hand, the positions of most introns of the GABRE gene are conserved with previously identified $GABA_A$ receptor genes, in agreement with the classification as a $GABA_A$ receptor subunit gene. The position of the last intron is conserved only with the deduced intron position of genes of the $\gamma$2 class, in agreement with the finding that $\epsilon$ and $\gamma$ protein sequences are most homologous. On the other hand, the unique position of the first intron and the absence of one of the internal introns of previously isolated $GABA_A$ receptor subunit genes support the interpretation that GABRE codes for a protein sequence of a putative new $GABA_A$ receptor subunit class.

During the final preparation of this paper, a publication appeared describing a new $GABA_A$ receptor subunit ($\epsilon$) conferring insensitivity to the potentiating effects of anaesthetic agents when expressed in combination with $\alpha$ and $\beta$ subunits (Davies *et al.*, 1997). Based on its biochemical and pharmacological properties, the $\epsilon$ subunit was described to represent a new subunit class of the $GABA_A$ receptor. The sequence of the $\epsilon$ subunit was deduced from a cDNA clone derived by PCR amplification from a hippocampal cDNA library and is nearly identical to the sequence of the $\epsilon$ subunit deduced by us from independent cDNA and genomic sources. The only difference between the two sequences is an exchange of serine 102 in the putative extracellular domain by alanine. The origin of the sequence difference, which might have an effect on the pharmacological properties of $GABA_A$ receptor complexes, is unknown. Most likely, both protein sequences correspond to allelic variants of the GABRE gene.

The findings of different levels of transcription in nonbrain tissues and tissue-specific alternative splicing are most intriguing features of GABRE. Our finding that GABRE is transcribed at least at low level in several brain and nonbrain tissues is based on Northern analysis (adult heart and placenta), RT-PCR (adult

heart and adult skeletal muscle, placenta, fetal liver, and fetal and adult brain), 5′ RACE (placenta), and the isolation of cDNA clones from different sources (conventional library from placenta, enriched libraries from human adult skeletal muscle, fetal brain, and fetal liver, and EST clones from infant brain and fetal liver or spleen). Expression of $GABA_A$ receptors in nonbrain tissues has to our knowledge previously only been described in pancreatic $\alpha 2$ cells, where it might be involved in the regulation of glucagone secretion by insulin (Rorsman *et al.*, 1989). In addition, receptors of the $GABA_C$ class are expressed in the retina (Enz *et al.*, 1996).

Alternative splicing has been observed in previously isolated $GABA_A$ receptor subunit genes, but was found to be restricted to a single position within the genes (see Fig. 3). It creates an alternative signal sequence in human $\beta 3$ (Kirkness and Fraser, 1993) or an insertion in the putative major intracellular domain in human $\beta 1$, human and chicken $\beta 2$, and chicken $\beta 4$ (Kirkness *et al.*, 1991; McKinley *et al.*, 1995; Harvey *et al.*, 1994). An insertion in chicken $\beta 2$ and bovine and mouse $\gamma 2$ yields a predicted protein kinase C phosphorylation site in the putative major intracellular domain (Harvey *et al.*, 1994; Moss *et al.*, 1992; Whiting *et al.*, 1990; Kofuji *et al.*, 1991). In contrast, alternative splicing of GABRE transcripts occurs at several possible positions and yields truncated protein sequences in most tissues. The possible function of alternative splicing of GABRE may therefore be to down-regulate the expression of GABRE in these tissues or to create truncated protein variants with unknown function. A possible mechanism of down-regulation of $GABA_A$ receptor subunit gene expression by alternative splicing has previously been observed in transcripts of the rat $\alpha 6$ subunit gene, where an alternative in-frame deletion of a 30-nucleotide sequence yields an inactive protein variant. However, no difference in the tissue-specific expression of the rat $\alpha 6$ splice variants was detected (Korpi *et al.*, 1994; compare Fig. 3). Generation of multiple protein isoforms by alternative splicing has been described in the case of neurexins, a class of cell surface proteins expressed in different parts of the brain. In neurexins, potentially more than 1000 different protein isoforms can be generated by alternative splicing in different gene regions. At least some of these isoforms are differentially expressed in parts of the brain (Ullrich *et al.*, 1995), therefore resembling the tissue specificity of GABRE splicing. Since in GABRE, adult brain was the only tissue in which the structure of the 5′ region of most cDNAs is compatible with expression of the complete protein sequence $\epsilon$, brain might be the major site of functional expression of the gene, analogous to the expression of previously isolated $GABA_A$ receptor subunit genes.

GABRE spans a region of 14 kb in chromosome band Xq28 and is therefore the shortest $GABA_A$ receptor gene with known gene structure. To our knowledge, it is the first described $GABA_A$ receptor subunit gene containing an *Alu* element. We have shown that GABRE is clustered together with the $\alpha 3$ and putative $\beta 4$ subunit genes within ≈0.8 Mb of Xq28. The gene cluster on the X chromosome resembles the clusters of $GABA_A$ receptor genes on human chromosomes 4, 5, and 15 (Kirkness *et al.*, 1991; Buckle *et al.*, 1989; Wagstaff *et al.*, 1991; Johnson *et al.*, 1992; Knoll *et al.*, 1993; Hicks *et al.*, 1994; Russek and Farb, 1994; McLean *et al.*, 1995). Each of these clusters contains one gene each of the $\alpha$, $\beta$, and $\gamma$ class. The clusters on chromosome 4 and 5 in addition contain a second $\alpha$ gene. The relative order of genes in Xq28 ($\epsilon$–$\alpha$–$\beta$) is analogous to the order of genes on chromosome 15 ($\gamma$–$\alpha$–$\beta$). The $\epsilon$ and $\gamma$ genes in both gene clusters have the same position and transcriptional orientation relative to their respective gene cluster, pointing away from the $\alpha$ and $\beta$ genes (Greger *et al.*, 1995). Our finding that the $\gamma$ subunit gene is apparently replaced by an $\epsilon$ subunit gene in the Xq28 gene cluster indicates that $\epsilon$ and $\gamma$ subunit genes are not only homologous on the levels of protein sequence and gene structure, but also on the level of genomic organization within the gene clusters.

It has been proposed that the $GABA_A$ receptor gene clusters in the human genome have been generated by gene duplication events from an ancestral gene cluster (Hicks *et al.*, 1994; McLean *et al.*, 1995). Our data suggest that the presumed ancestral gene cluster contained one each $\alpha$, $\beta$, and $\gamma/\epsilon$ ancestral gene. Although Xq28 is one of the most extensively investigated human chromosome bands in terms of gene isolation (Bione *et al.*, 1993; Sedlacek *et al.*, 1993; Chen *et al.*, 1996; Heiss *et al.*, 1996; Kioschis *et al.*, 1996; Rogner *et al.*, 1996), a second $\alpha$ subunit gene has not been detected in this chromosome band. We therefore propose that following the first duplication of the ancestral gene cluster, duplication of an $\alpha$ ancestral gene has taken place in only one of the two resulting "second generation" gene clusters and that the present-day $GABA_A$ receptor gene clusters have resulted from another duplication of each of the second generation gene clusters. In this model, divergence of the $\epsilon$ subunit gene from the $\gamma$ subunit genes would have taken place in one of the two "third generation" gene clusters containing only one $\alpha$ subunit gene each.

Since $GABA_A$ receptor genes have been shown to be involved in neurologic or developmental defects in rodents (Korpi *et al.*, 1993; Culiat *et al.*, 1995; Günther *et al.*, 1995), and because of their function in signal transmission in the brain, they are good candidates for related genetic defects in humans. Physical mapping of $GABA_A$ receptor subunit genes in Xq28 opens the possibility of comparing the positions of the genes with the candidate regions of genetic defects mapped in Xq28. The $GABA_A$ receptor genes in Xq28 are located within the candidate regions of early onset parkinsonism (Waisman syndrome) and X-linked mental retarda-

tion (MRX3). We are currently investigating the possible involvement of GABRE in both genetic defects.

## ACKNOWLEDGMENTS

We thank S. Wiemann and G. Haberhauer for excellent support concerning sequence analysis and computer applications, W. Gong for providing cosmids, Z. Sedlacek, S. Wiemann, and J. Coy for reading the manuscript, and H. Lüddens for helpful discussions. This work was supported by grants from Deutsche Forschungsgemeinschaft and Bundesministerium für Bildung und Forschung.

## REFERENCES

Bateson, A. N., Lasham, A., and Darlison, M. G. (1991). γ-aminobutyric acid A receptor heterogeneity is increased by alternative splicing of a novel β subunit gene transcript. *J. Neurochem.* **56:** 1437–1440.

Bione, S., Tamanini, F., Maestrini, E., Tribioli, C., Poustka, A., Torri, G., Rivella, S., and Toniolo, D. (1993). Transcriptional organization of a 450-kb region of the human X chromosome in Xq28. *Proc. Natl. Acad. Sci. USA* **90:** 10977–10981.

Buckle, V. J., Fujita, N., Ryder-Cook, A. S., Derry, J. M., Barnard, P. J., Lebo, R. V., Schofield, P. R., Seeburg, P. H., Bateson, A. N., Darlison, M. G., and Barnard, E. A. (1989). Chromosomal localization of $GABA_A$ receptor subunit genes: Relationship to human genetic diseases. *Neuron* **3:** 647–654.

Burt, D. R., and Kamatchi, G. L. (1991). $GABA_A$ receptor subtypes: From pharmacology to molecular biology. *FASEB J.* **5:** 2916–2923.

Chen, E. Y., Zollo, M., Mazzarella, R., Ciccodicola, A., Chen, C., Zuo, L., Heiner, C., Burough, F., Repetto, M., Schlessinger, D., and D'Urso, M. (1996). Long-range analysis in Xq28: Thirteen known and six candidate genes in 219.4 kb of high GC DNA between the RCP/GCP and G6PD loci. *Hum. Mol. Genet.* **5:** 659–668.

Culiat, C. T., Stubbs, L. J., Wyochik, R. P., Russell, L. B., Johnson, D. K., and Rinchik, E. M. (1995). Deficiency of the β3 subunit of the type A γ-aminobutyric acid receptor causes cleft palate in mice. *Nature Genet.* **11:** 344–346.

Davies, P. A., Hanna, M. C., Hales, T. G., and Kirkness, E. F. (1997). Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. *Nature* **385:** 820–823.

Dingledine, R., Myers, S. J., and Nicholas, R. A. (1990). Molecular biology of mammalian amino acid receptors. *FASEB J.* **4:** 2636–2645.

Enz, R., Brandstatter, J. H., Wassle, H., and Bormann, J. (1996). Immunocytochemical localization of the $GABA_C$ receptor ρ subunits in the mammalian retina. *J. Neurosci.* **16:** 4479–4490.

Feinberg, A. P., and Vogelstein, B. (1983). A technique for radiolabeling DNA endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.

Gedeon, A., Kerr, B., Mulley, J., and Turner, G. (1991). Localisation of the MRX3 gene for non-specific X linked mental retardation. *J. Med. Genet.* **28:** 372–377.

Greger, V., Knoll, J. H. M., Woolf, E., Glatt, K., Tyndale, R. F., DeLorey, T. M., Olsen, R. W., Tobin, A. J., Sikela, J. M., Nakatsu, Y., Brilliant, M. H., Whiting, P. J., and Lalande, M. (1995). The γ-aminobutyric acid receptor γ3 subunit gene (GABRG3) is tightly linked to the α5 subunit gene (GABRA5) on human chromosome 15q11–q13 and is transcribed in the same orientation. *Genomics* **26:** 258–264.

Gregg, R. G., Metzenberg, A. B., Hogan, K., Sekhon, K., and Laxova, R. (1991). Waisman syndrome, a human X-linked recessive basal ganglia disorder with mental retardation: Localization to Xq27.3–pter. *Genomics* **9:** 701–706.

Günther, U., Benson, J., Benke, D., Fritschy, J.-M., Reyes, G., Knoflach, F., Crestani, F., Aguzzi, A., Arigoni, M., Lang, Y., Bluethmann, H., Mohler, H., and Lüscher, B. (1995). Benzodiazepine-insensitive mice generated by targeted disruption of the $\gamma_2$ subunit gene of γ-aminobutyric acid type A receptors. *Proc. Natl. Acad. Sci. USA* **92:** 7749–7753.

Harvey, R. J., Kim, H.-C., and Darlison, M. G. (1993). Molecular cloning reveals the existence of a fourth γ subunit in the vertebrate brain $GABA_A$ receptor. *FEBS Lett.* **331:** 211–216.

Harvey, R. J., Chinchetru, M. A., and Darlison, M. G. (1994). Alternative splicing of a 51-nucleotide exon that encodes a putative protein kinase C phosphorylation site generates two forms of the chicken γ-aminobutyric $acid_A$ receptor β2 subunit. *J. Neurochem.* **62:** 10–16.

Heiss, N. S., Rogner, U. C., Kioschis, P., Korn, B., and Poustka, A. (1996). Transcriptional mapping in a 700-kb region around the DXS52 locus in Xq28: Isolation of six novel transcripts and a novel ATPase isoform (hPMCA5). *Genome Res.* **6:** 478–491.

Herb, A., Wisden, W., Lüddens, H., Puia, G., Vicini, S., and Seeburg, P. H. (1992). The third γ subunit of the γ-aminobutyric acid type A receptor family. *Proc. Natl. Acad. Sci. USA* **89:** 1433–1437.

Hicks, A. A., Bailey, M. E. S., Riley, B. P., Kamphuis, W., Siciliano, M. J., Johnson, K. J., and Darlison, M. G. (1994). Further evidence for clustering of human $GABA_A$ receptor subunit genes: Localization of the $\alpha_6$-subunit gene (GABRA6) to distal chromosome 5q by linkage analysis. *Genomics* **20:** 285–288.

Hillier, L., Clark, N., Dubuque, T., Elliston, K., Hawkins, M., Holman, M., Hultman, M., Kucaba, T., Le, M., Lennon, G., Marra, M., Parsons, J., Rifkin, L., Rohlfing, T., Soares, M., Tan, F., Trevaskis, E., Waterston, R., Williamson, A., Wohldmann, P., and Wilson, R. (1995). The WashU–Merck EST project. [Unpublished]

Johnson, K. J., Sander, T., Hicks, A. A., Marle, A. V., Janz, D., Mullan, M. J., Riley, B. P., and Darlison, M. G. (1992). Confirmation of the localization of the human $GABA_A$ receptor α1-subunit gene (GABRA1) to distal 5q by linkage analysis. *Genomics* **14:** 745–748.

Jurka, J., and Milosavljevic, A. (1991). Reconstruction and analysis of human Alu genes. *J. Mol. Evol.* **32:** 105–121.

Kioschis, P., Rogner, U. C., Pick, E., Klauck, S. M., Heiss, N. S., Siebenhaar, R., Korn, B., Coy, J. F., Laporte, J., Liechti-Gallati, S., and Poustka, A. (1996). A 900 kb cosmid contig and eleven new transcripts within the candidate region for myotubular myopathy (MTM1). *Genomics* **33:** 365–373.

Kirkness, E. F., Kusiak, J. W., Fleming J. T., Menninger, J., Gocaynde, J. D., Ward, D. C., and Venter, J. C. (1991). Isolation, characterization and localization of human genomic DNA encoding the β1 subunit of the $GABA_A$ receptor (GABRB1). *Genomics* **10:** 985–995.

Kirkness, E. F., and Fraser, C. M. (1993). A strong promoter element is located between alternative exons of a gene encoding the human γ-aminobutyric acid-type A receptor β3 subunit (GABRB3). *J. Biol. Chem.* **268:** 4420–4428.

Knoll, J. H., Sinnett, D., Wagstaff, J., Glatt, K., Wilcox, A. S., Whiting, P. M., Wingrove, P., Sikela, J. M., and Lalande, M. (1993). FISH ordering of reference markers and of the gene for the α5 subunit of the gamma-aminobutyric acid receptor (GABRA5) within the Angelman and Prader–Willi syndrome chromosomal regions. *Hum. Mol. Genet.* **2:** 183–189.

Kofuji, P., Wang, J. B., Moss, S. J., Huganir, R. L., and Burt, D. R. (1991). Generation of two forms of the γ2-subunit in mice by alternative splicing. *J. Neurochem.* **56:** 713–715.

Korn, B., Sedlacek, Z., Manca, A., Kioschis, P., Konecki, D., Lehrach, H., and Poustka, A. (1992). A strategy for the selection of transcribed sequences in Xq28 region. *Hum. Mol. Genet.* **4:** 235–242.

Korpi, E. R., Kuner, T., Kristo, P., Köhler, M., Herb, A., Lüddens, H., and Seeburg, P. H. (1994). Small N-terminal deletion by splicing in cerebellar α6 subunit abolishes $GABA_A$ receptor function. *J. Neurochem.* **63:** 1167–1170.

Korpi, E. R., Kleingoor, C., Kettenmann, H., and Seeburg, P. H.

(1993). Benzodiazepine-induced motor impairment linked to point mutation in cerebellar $GABA_A$ receptor. *Nature* **361:** 356–359.

Lasham, A., Vreugdenhil, E., Bateson, A. N., Barnard, E. A., and Darlison, M. G. (1991). Conserved organization of $\gamma$-aminobutyric acidA receptor genes: Cloning and analysis of the chicken $\beta$4-subunit gene. *J. Neurochem.* **57:** 352–355.

Laxova, R., Brown, E. S., Hogan, K., Hecox, K., and Opitz, J. M. (1985). An X-linked recessive basal ganglia disorder with mental retardation. *Am. J. Med. Genet.* **21:** 681–689.

Levin, M. L., Chatterjee, A., Pragliola, A., Worley, K. C., Wehnert, M., Zhuchenko, O., Smith, R. F., Lee, C. C., and Herman, G. E. (1996). A comparative transcription map of the murine bare patches (Bpa) and Striated (Str) critical regions and Xq28. *Genome Res.* **6:** 465–477.

Matzenbach, B., Maulet, Y., Sefton, L., Courtier, B., Avner, P., Guenet, J. L., and Betz, H. (1994). Structural analysis of mouse $\alpha$ subunit genes. Identification and chromosomal localization of a new variant. *J. Biol. Chem.* **269:** 2607–2612.

McKinley, D. D., Lennon, D. J., and Carter, D. B. (1995). Cloning, sequence analysis and expression of two forms of mRNA coding for the human $\beta$2 subunit of the $GABA_A$ receptor. *Brain Res.–Mol. Brain Res.* **28:** 175–179.

McLean, P. J., Farb, D. H., and Russek, S. J. (1995). Mapping of the $\alpha$4 subunit gene (GABRA4) to human chromosome 4 defines an $\alpha$2-$\alpha$4-$\beta$1-$\gamma$1 gene cluster: Further evidence that modern $GABA_A$ receptor gene clusters are derived from an ancestral gene cluster. *Genomics* **26:** 580–586.

Montal, M. (1990). Molecular anatomy and molecular design of channel proteins. *FASEB J.* **4:** 2623–2635.

Moss, S. J., Doherty, C. A., and Huganir, R. L. (1992) Identification of the cAMP-dependent protein kinase and protein kinase C phosphorylation sites within the major intracellular domains of the $\beta$1, $\gamma_2$S, and $\gamma_2$L subunits of the $\gamma$-aminobutyric acid type A receptor. *J. Biol. Chem.* **267:** 14470–14476.

Nizetic, D., Zehetner, G., Monaco, A. P., Gellen, L., Young, B. D., and Lehrach, H. (1991). Construction, arraying, and high density screening of large insert libraries of human chromosomes X and 21; Their potential use as reference libraries. *Proc. Natl. Acad. Sci. USA* **88:** 3233–3237.

Nordstrom, A.-M., Penttinen, M., and Koskull, H. (1992). Linkage to Xq28 in a family with nonspecific X-linked mental retardation. *Hum. Genet.* **90:** 263–266.

Ogurusu, T., and Shingai, R. (1996). Cloning of a putative gamma-aminobutyric acid (GABA) receptor subunit $\rho$3 cDNA. *Biochim. Biophys. Acta* **1305:** 15–18.

Olsen, R. W., and Tobin, A. J. (1990). Molecular Biology of $GABA_A$ receptors. *FASEB J.* **4:** 1469–1480.

Poustka, A. (1990). Physical mapping by PFGE. *Methods: Companion Methods Enzymol.* **1:** 204–211.

Pritchett, D. B., Sontheimer, H., Sivers, B. D., Ymer, S., Kettenmann, H., Schofield, P. R., and Seeburg, P. H. (1989). Importance of novel $GABA_A$ receptor for benzodiazepine pharmacology. *Nature* **338:** 582–585.

Rogner, U. C., Kioschis, P., Wilke, K., Gong, W., Pick, E., Dietrich, A., Zechner, U., Hameister, H., Pragliola, A., Herman, G. E., Yates, J. R. W., Lehrach, H., and Poustka, A. (1994). A YAC clone map spanning 7.5 megabases of human chromosome band Xq28. *Hum. Mol. Genet.* **3:** 2137–2146.

Rogner, U. C., Wilke, K., Steck, E., Korn, B., and Poustka, A. (1995). The melanoma antigen gene (MAGE) family is clustered in the chromosomal band Xq28. *Genomics* **29:** 725–731.

Rogner, U. C., Heiss, N. S., Kioschis, P., Wiemann, S., Korn, B., and Poustka, A. (1996). Transcriptional analysis of the candidate region for incontinential pigmenti (IP2) in Xq28. *Genome Res.* **6:** 922–934.

Rorsman, P., Berggren, P.-O., Bokvist, K., Ericson, H., Möhler, H., Östenson, C.-G., and Smith, P. A. (1989). Glucose-inhibition of glucagon secretion involves activation of $GABA_A$-receptor chloride channels. *Nature* **341:** 233–236.

Russek, S. J., and Farb, D. H. (1994). Mapping of the $\beta$2 subunit gene (GABRB2) to microdissected human chromosome 5q34–q35 defines a gene cluster for the most abundant $GABA_A$ receptor isoform. *Genomics* **23:** 528–533.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.

Sealey, P. G., Whittaker, P. A., and Southern, E. M. (1985). Removal of repeated sequences from hybridization probes. *Nucleic Acids Res.* **13:** 1905–1922.

Sedlacek, Z., Korn, B., Konecki, D. S., Siebenhaar, R., Coy, J. F., Kioschis, P., and Poustka, A. (1993). Construction of a transcript map of a 300 kb region around the human G6PD locus by direct cDNA selection. *Hum. Mol. Genet.* **2:** 1865–1869.

Seeburg, P. H., Wisden, W., Verdoorn, T. A., Pritchett, D. B., Werner, P., Herb, A., Lüddens, H., Sprengel, R., and Sakmann, B. (1990). The $GABA_A$ receptor family: Molecular and functional diversity. *Cold Spring Harbor Symp. Quant. Biol.* **55:** 29–40.

Sommer, B., Poustka, A., Spurr, N. K., and Seeburg, P. H. (1990). The murine $GABA_A$ receptor $\delta$-subunit gene: Structure and assignment to human chromosome 1. *DNA Cell. Biol.* **9:** 561–568.

Stephens, R. M., and Schneider, D. T. (1992). Features of spliceosome evolution and function inferred from an analysis of the information at human splice sites. *J. Mol. Biol.* **228:** 1124–1136.

Ullrich, B., Ushkaryov, Y. A., and Südhof, T. C. (1995). Cartography of neurexins: More than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. *Neuron* **14:** 497–507.

Wagstaff, J., Knoll, J. H., Fleming, J., Kirkness, E. F., Martin-Gallardo, A., Greenberg, F., Graham, J. M., Menninger, J., Ward, D., and Venter, J. C. (1991). Localization of the gene encoding the $GABA_A$ receptor $\beta$3 subunit to the Angelman/Prader–Willi region of human chromosome 15. *Am. J. Hum. Genet.* **49:** 330–337.

Whiting, P., McKernan, R. M., and Iversen, L. L. (1990). Another mechanism for creating diversity in $\gamma$-aminobutyric type A receptor: RNA splicing directs expression of two forms of the $\gamma$2 subunit, one of which contains a protein kinase C phosphorylation site. *Proc. Natl. Acad. Sci. USA* **87:** 9966–9970.

Ymer, S., Draguhn, A., Wisden, W., Werner, P., Keinaenen, K., Schofield, P. R., Sprengel, R., Pritchett, D. B., and Seeburg, P. H. (1990). Structural and functional characterization of the $\gamma$1 subunit of the $GABA_A$/benzodiazepine receptors. *EMBO J.* **9:** 3261–3267.