BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **238,** 683–685 (1997)
ARTICLE NO. RC977360

Polymorphisms and Intron Sequences Flanking the Alternatively Spliced 8-Amino-Acid Exon of γ2 Subunit Gene for GABAA Receptors

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Received August 15, 1997

Gamma-amminobutyric acid (GABA) is a major inhibitory neurotransmitter. Two alternatively spliced forms of the γ2 subunit of GABAA receptor (γ2L and γ2S), which differ by an exon of eight amino acids, show different sensitivities to modulatory effects of ethanol on receptor activities. A 2.7 kb DNA fragment and an 1.7 kb DNA fragment covering respectively the introns upstream and downstream from the 8-amino-acid exon were obtained through PCR-amplification of human genomic DNA using primers derived from cDNA sequences. Total sequencing of these fragments showed a composite 4.2 kb segment containing the 8-amino-acid exon and consensus sequences for RNA splice junctions. Restriction fragment length polymorphisms (RFLP) based on NciI restriction digestion were found among Chinese in Taiwan. This RFLP provides a useful DNA marker for allelic association or linkage analyses of the role of GABAA receptors in predisposition to alcoholism or other neuropsychiatric disorders.

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the brain. The GABAA receptor is a ligand-gated chloride ion channel modulated by barbiturates, benzodiazepines, and ethanol (1). Many behavioral actions of ethanol may be explained by enhancement of GABAA receptor-mediated ion flux and chronic effects of alcohol on the receptors (1). An alternative form of the γ2 subunit, γ2L, which contains an additionally spliced 8-amino-acid exon with a phosphorylation site, may mediate the action of ethanol (2-6). A point mutation in a cerebellum-specific GABAA receptor subunit (α6), may underlie the motor-impairing effect of ethanol (7). These results implicate possible roles of GABAA receptor genes in susceptibility to alcoholism. Here we report the cloning and sequencing of the two introns flanking the 8-amino-acid exon and the presence of NciI restriction fragment length polymorphisms (RFLP) in the downstream intron.

MATERIALS AND METHODS

Subcloning and sequencing of PCR fragments. PCR fragments were cloned into the pCR2.1 vector (TA cloning kit, Invitrogen) according to manufacturer's instructions. Plasmid DNA containing the inserts were then purified and used for automatic DNA sequencing.

Determination of NciI RFLP. The condition for PCR are as follows: The primer G-CVijI sequence is 5′-AAT TTA CCA ACT GGT CTA GCC GG, and the primer RI-GR sequence is 5′-GAA TGT CAA CAA TGT TTA CCT ACA TGT G (see Fig. 1). The reaction mixture contained 15 pmoles of each of the primers, dNTP (0.25 mM of each of the four nucleotides), $MgCl_2$ (2.0 mM), Tris-HCl (pH 8.3, 10 mM), KCl (50 mM) and Taq polymerase (1 unit, Boerhinger-Maniheim). The thermal cycles were 20 s at 94 °C, 30 s at 62 °C and 35 s at 70 °C for 40 cycles. The PCR fragments (in 6 μl of reaction mixture) were then digested with 5 units of restriction endonuclease NciI (New England Biolab) in a final volume of 20 μl at 37 °C overnight. The digestion products were then run on 6% polyacrylamide gels and stained with ethidium bromide for viewing under UV light.

RESULT AND DISCUSSION

Based on human cDNA sequences (2, 8), we first designed a pair of primers to amplify sequences between the 8-amino-acid exon and the 3′ untranslated region. The primers sequences were: G-F (5′-CCT CTT CGG ATG TTT TCC TTC AAG-3′) from the 8-amino-acid exon (24 bp) and G-R (5′-GAT TCA GAT ACT TAT CAA CCA C-3′) from the 3′ untranslated region. The PCR reaction mixture contained 200-500 ng of human genomic DNA, 100 pmoles of

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1     AAAGATAAAA AGAAGAAAAA CCCTgtatgt atcattttcc attggcacca
      LE3GF -->
51    ttgaaatttt tatgatttcg gtttagtttg ttttcattag cctatctgca
101   ggctaaggct cagcagtttg ggctccaaaa tgaaaacagc atgtatgatt
151   ttagccaggc cataacaatt catttacagt cattagttac ttgaaaagac
201   tcaagtctgt ttctattttc tgtgtcaaag ttcttatgca aatataatta
251   cctgctctct ttattttgtg gagactaaag ccatttttga gaaatgtgac
301   cttcttcttg ttgctattat tccaggtttc actgattttt tgaaatggag
351   tgtcactctg ttgctcaggc tggagtgcag tggcgggatc tcggcttact
401   gcaacctctg cctcctaggt tcaagcaatt ctcctgcctc acccccctga
451   gtagctggga ctataggcac acgccaccat gcctggttaa tttttgtatt
501   tttagtacag acggggtttc accatattgg tcaggctggt ctcaaactcc
551   tgacctcagg tgatccacct gtctcggcct cccaaagtgc tgggattaaa
601   tgcgtgagcc actgcgcctg gccatgataa tattattaaa tcactgatat
651   tttaaattaa aacttccatc tcaggcattc cactaggaag ctataaggtc
701   cttgaagttt caaggctgac tacatttttg caaatgattt agtgtgtgta
751   tggaggtgga atgtgggtat tgtcaccaaa taatttctat tgatttcatt
801   ctcgaaatgt atctttttgt ttttaaaaca aatgatttac tattatatgg
851   gcaagttagt tagcctcctt gtgtgtttta tatatatata tgtagtgtat
901   tatttaaaaa cacttgtctt atggggctcc tatgaaaaat aaatgtggca
951   atgaagggca aaacaaaaaa tcccaaaata ttacatagta ctctacacat
1001  gaatgtactt aacattagta gttggtgatg atagttgatt ttgatgattt
1051  atacagtttc agcctggat tgacttcagt gccacaattt gaaaacaggg
1101  aggatgactt ctacttgcaa ccaaacttta aatagtgagg atgaagcata
1151  aacacaaatg cctaaagcaa ctgtttttta ttgtgagtgt tgaggagagc
1201  acatagttac ccatttacac acctgaacaa agtggctggc tcatagaaat
1251  ccccatgaaa gttaaggtcc attcccttcc aagatatggg caaagagaat
1301  cacctgacaa tcctggatca tggccttctg ctttgagagc cgtaataacg
1351  ctctacctat ccaacccaga gactttggag gttgaattgt aaggaaggaa
1401  tttagctgat tttaggttgt attctgatgc agctgcagaa acccaagtga
1451  aatactacaa gagtaatgtg tttatttgaa tgtaagactt tggaacatgg
1501  aaagaaagaa acatcattat ttttgattta aaaaatgctt tctttaattg
1551  ctcaaatatt tacaatgctt tatgcagtcc ttcccttccc tgtttgtttt
1601  gcctctctct gaatttccat tttctctatc cttgttttat tttccttgtt
1651  gtgttgtttt tgtttgctcc ctcttgttca atttccattt cactctccct
1701  caagaaggtg gttttattga gagttggcat attacctgcc agctataaca
1751  aggacatgag gactggcttt caaacaagtt ttgatgagtc ctttggaaaa
1801  agccccttgg ccctctctgt caggtacgcc attgcagagt agattttgtt
1851  gcaaagatag aggcagattc ccttattcag ggtcatggaa atggcagaag
1901  aagaaaaggc agaggagaac aggataaaaa tttgagaaaa tgaaattacc
1951  tgagaatttc atgcacttct ttttaggcaa ttagatgatt catcagaact
2001  agcaagaaaa taaactagga atgagaagct gaaagtttat tttcctgttt
2051  aatatgcctt ttggataatt gtgtcaagac tcacctatta agttatccgt
2101  gatgataatt agaaaataga atcatagtat ttttaaacag gggaaccagg
2151  caatagaaaa cttccttgca attgaacaac tctggcttct tcagtcaagt
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FIG. 1. NciI RFLP and intron sequences flanking the 8-amino-acid exon of $\gamma 2$ subunit gene for human GABAa receptors. PCR primer sequences are underlined, with the arrows (→) and (←) indicating the sense strands, and the antisense strands respectively. The recognition sequence for restriction endonuclease NciI and the polymorphic base (**g** or **a** at nucleotide position 3145) are indicated by boldface types. Nucleotides in exons and introns are shown in capitalized letters and lower case letters, respectively.

each primer (1 μM), $MgCl_2$ (4 mM), dNTPs (0.25 mM for each nucleotide), 4 units of Taq polymerase (Gibco) in manufacturer-supplied buffer in a total volume of 100 ul. Thermal cycles were repeated for 35 times at 95 °C for 25 s, 53 °C for 50 s and 65 °C for 120 s. Agarose gel electrophoresis revealed an 1.7 kb PCR product. The PCR fragment was subjected to automatic DNA sequencing using a Applied Biosystem Prism 380 machine. Sequences (Fig. 1, nucleotides 3803-4186) matching the 3′ region of published cDNA sequence of human GABAa $\gamma 2$ subunit (8) were found in this 1.7 kb fragment. For intron sequences upstream of the 8-amino-acid exon, a primer, LE3GF (5′-AAA GAT AAA AAG AAG AAA AAC CCT), from the cDNA sequence 5′ to the splice site, and a primer, LE2GR (5′-CAG GGA TTA TAG CTT TTG GGC) downstream from the 8-amino-acid exon in the 1.7 kb fragment (Fig. 1) were used to amplify genomic DNA by PCR and generated a 2.7 kb product. Total sequencing of this fragment confirmed the presence of 0.25 kb of sequences from the 1.7 kb fragment including the 8-amino-acid exon (Fig. 1). Combination of sequences from the 2.7 kb and the 1.7 kb fragments gives a 4.2 kb fragment (Fig. 1). An RFLP revealed by NciI was found (see Material and Methods) among Chinese in Taiwan. This NciI RFLP is about 0.7 kb downstream from the 8-amino-acid exon and therefore is more tightly linked than previously reported dinucleotide repeat polymorphisms (9). The newly found RFLP marker and the intron sequences can therefore lead to further molecular genetic analysis of the role of GABAergic systems in the behavioral and cellular actions of alcohol (10).

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2201  aattgtagat taatttatta aaaatttcac tagtaattac aaaattacac
2251  ttaactttaa gcaaatactt tatcccaagc tcagaactct ccttctgtgt
2301  ttataatttc agttcatttc acttactgtg ttttcaaaat gtatttttaa
2351  tttatcttgt ctctctcttt ttttttcatt ttttttctcc tttttattaa
2401  aaacaaatgc aattctcttt tctgtctaca aacccaaagC TTCTTCGGAT
                                                G-F -->
2451  GTTTTCCTTC AAGgtataat gtttttggaa tggaaattca ctgcatgcaa
2501  ctgctaaatt taactattaa tgcttacatg gtgttttatt ttgttttatg
2551  agtagacatt taagcattct actagagata atatgttgga gaaagttcta
2601  cagacttcta gagttgattc agcaactata cagtaattca cataggtttt
2651  aatcttacac caagcccaaa agctataatc cctgtgagat gtcataggta
                          <-- LE2GR
2701  aagaaggctg ctttatccat caaagccaaa atgagcttcc tcttttcaga
2751  tgaaaatggg attgagtctt actgattacg actaccaagt ttgtactctt
2801  ttaaaactaa aatttaactc tgtaaatctt aattcaaaat gtattggttc
2851  attactgttt gtaaacttaa tgatttctgt ttttctgaac actacctgta
2901  agttctgtaa gaacaatatt ctttttgaaa ggctttgagt taaaatatcc
2951  attccttatt tccagagtga tccctgagcc ttttggctct actataatgt
3001  gttgcaatga gatttctatt caaatataat agctgattct gagacgttag
3051  aatttctgtt tagattatgc taaaatgaaa tcatccaaat taacattaaa
                                                      a
3101  tatatcattt agttgcatag aaatttacca actggtctag ccgggaattt
                          G-CVijI -->          NciI^
3151  gacaagtagt aaccttcttt tctcaatttc cattcacatg taggtaaaca
3201  ttgttgacat tcttaagctt atagatggtg aatattagaa aaagaatcat
        <-- RI-GR
3251  agtcctttaa aacataagcc ataccattgt ggtatacaat caggaaaatt
3301  ctgtatttat atatatttta acattttcta ttaacattta atacatttgc
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3351  tcagagtaat gggtttctat ttgcctataa ctaagcatga cacaatattt
3401  gatttcagaa ttgatgcatt tttatttctc tgttccactc acatgcagca
3451  ccatcaaatg ccttcatgta accaatgtca catctaatac ttactagaat
3501  aagattccat gttaatttga tttaaatatt taactttgag ttcttttggt
3551  gttcaatctc cttgctatag ctattagctt gatgatatta ttttcagctg
3601  cactgcttaa gctcaaaatt tgaaatctgc aaatgtgcta tctttctaag
3651  ttcaatttta ccattgtaga tcatgatgtc atagcaattt cctgagtacc
3701  cattttcaga ttcatcatca cattggtgac attgtggaaa aacagcctag
3751  gatctctcga gaacaactaa ctgatccctc tccttcccta ccctcgtccc
3801  agGCCCCTAC CATTGATATC CGCCCAAGAT CAGCAACATT CAAATGAATA
3851  ATGCTACACA CCTTCAAGAG AGAGATGAAG AGTACGGCTA TGAGTGTCTG
3901  GACGGCAAGG ACTGTGCCAG TTTTTTCTGC TGTTTTGAAG ATTGTCGAAC
3951  AGGAGCTTGG AGACATGGGA GGATACATAT CCGCATTGCC AAAATGGACT
4001  CCTATGCTCG GATCTTCTTC CCCACTGCCT TCTGCCTGTT TAATCTGGTC
4051  TATTGGGTCT CCTACCTCTA CCTGTGAGGA GGTATGGGTT TTACTGATAT
4101  GGTTCTTATT CACTGAGTCT CATGGAGAGA TGTCTGTTCT AAGTCCACTT
4151  AAATAATCCT CTATGTGGTT GATAAGTATC TGAATC
                                    <-- G-R
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FIG. 1—*Continued*

ACKNOWLEDGMENTS

We thank Mr. See-Voon Seow and technical assistance from the core facility at IBMS, Academia Sinica for automated sequencing. This research project was supported by grants from National Council to ATAC (NSC-84-2331-B001-046) and YPPH (NSC85-2331-B007-005-M31).

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