Candidate Gene Study of Eight $GABA_A$ Receptor Subunits in Panic Disorder

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Objective: *γ-Aminobutyric acid type A ($GABA_A$) receptor subunit genes are candidate genes for panic disorder. Benzodiazepine agonists acting at this receptor can suppress panic attacks, and both inverse agonists and antagonists can precipitate them. The human $GABA_A$ receptor subtypes are composed of various combinations of 13 subunits, each encoded by a unique gene. The authors tested eight of these subunits in a candidate gene linkage study of panic disorder.* *Method:* *In 21 U.S. and five Icelandic multiplex pedigrees of panic disorder, 104 individuals had DSM-III-R panic disorder (the narrowly defined affected phenotype) and 134 had either this diagnosis or subsyndromal panic disorder characterized by panic attacks that failed to meet either the criterion of attack frequency or the number of criterion symptoms necessary for a definite diagnosis (the broadly defined affected phenotype). The authors conducted lod score linkage analyses with both phenotypes using both a dominant and a recessive model of inheritance for the following loci: GABRA1–GABRA5 (α1–α5), GABRB1 (β1), GABRB3 (β3), and GABRG2 (γ2).* *Results:* *The results failed to support the hypothesis that any of these genes cause panic disorder in a majority of the pedigrees.* *Conclusions:* *Within the limitations of the candidate gene linkage method, panic disorder does not appear to be caused by mutation in any of the eight $GABA_A$ receptor genes tested.*

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The γ-aminobutyric acid type A ($GABA_A$) receptor appears to play a key role in modulating anxiety and could be involved in either the etiology or the pathogenesis of anxiety disorders (1). A benzodiazepine binding site is located on this receptor, and ligands that bind to this site can either increase or decrease anxiety (2). Benzodiazepine agonists are anxiolytic, but inverse agonists, such as several of the β-carbolines, are anxiogenic and can precipitate panic attacks. The β-carboline 3-carboxylic acid ethyl ester causes behavioral and physiological responses in primates that parallel those of anxiety in humans (2), and β-carboline monomethylamide has caused severe anxiety in human volunteers (3). The benzodiazepine antagonist flumazenil displaces both agonists and inverse agonists from the benzodiazepine binding site and neutralizes their actions. These observations demonstrate that the $GABA_A$-benzodiazepine receptor can modulate anxiety in humans.

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In mammals, 13 $GABA_A$ receptor polypeptides have been identified to date: six α, three β, three γ, and one δ; receptors composed of different subunits have different pharmacological properties (4). Each subunit is encoded by a distinct gene, and these are differentially expressed in the brain (5, 6). This degree of heterogeneity in the structure and expression of the receptor speaks for a highly complex system.

In a system this complex, altered function of one or more genes could conceivably cause limited symptoms and otherwise be compatible with normal life. Mutations are known to alter the binding characteristics of the $GABA_A$ receptor. For example, a single amino acid substitution in the α3 subunit increases by a factor of 10 the affinity of certain compounds for the benzodiazepine site (7). A splice variant of the γ2 subunit confers ethanol responsiveness on the receptor complex (8). A naturally occurring point mutation in the α6 subunit gene confers abnormal sensitivity to certain benzodiazepines on a rat strain selected for sensitivity to alcohol (9). These examples illustrate how this receptor provides multiple opportunities for mutations that could predispose to anxiety. The familial periodic paralyses, diseases that resemble panic disorder by their paroxysmal nature, are due to mutations in voltage-gated ion

channels (10). This raises the possibility that mutations in ligand-gated ion channels, such as the $GABA_A$ receptor, could cause panic attacks.

Limited clinical observations suggest that individuals with panic disorder may indeed have altered $GABA_A$ receptor function (11, 12). They are more sensitive than healthy individuals to the panicogenic effects of flumazenil, a benzodiazepine site antagonist, which suggests that they could have greater receptor affinity for that drug (11, 12). On the other hand, they are less sensitive to other benzodiazepines, as judged by their effect on saccadic eye movement velocity, which would suggest less receptor affinity for benzodiazepines (11), although this could be explained by previous exposure to sedative-hypnotic drugs (13).

The possibility that genetic variation in one or more subunits of the $GABA_A$ receptor commonly predisposes to panic disorder can be tested by linkage analysis. Polymorphisms have been characterized for eight of the 13 known receptor genes. If any of these genes are involved in the etiology of panic disorder, then the associated polymorphism should cosegregate with the disorder in multiplex pedigrees. We have tested this hypothesis in 26 panic disorder pedigrees.

METHOD

Clinical Methods

This is a study of 21 multiplex panic disorder pedigrees from the Midwestern United States and five multiplex panic disorder pedigrees from Iceland. The U.S. pedigrees were recruited from the psychiatry clinic at the University of Iowa, referred by community practitioners, and recruited by advertisement. Selection criteria required the proband to have a DSM-III-R diagnosis of panic disorder and three relatives with definite or subsyndromal panic disorder. The latter term refers to panic attacks that fail to meet diagnostic criteria for panic disorder by virtue of having either fewer attacks or fewer criterion symptoms than required. The inclusion criteria also required panic disorder to be transmitted unilineally.

Psychiatrists or senior psychiatry residents interviewed the probands and family members with the Structured Clinical Interview for DSM-III-R (SCID) (14). The first eight families were originally assessed with the Schedule for Affective Disorders and Schizophrenia—Lifetime Version (15), but they were reinterviewed later with the SCID for consistency. Interviewers wrote a narrative summary of the case history and obtained records of psychiatric treatment. All of the family members who were genotyped, and thus contributed to the lod scores, gave interviews. Written informed consent was obtained from all participants.

The diagnosticians (R.R.C. and R.N.Jr.) made best-estimate diagnoses based on the clinical material using DSM-III-R criteria and resolved differences by consensus agreement. In addition, they made diagnoses of subsyndromal panic disorder in individuals who had panic attacks that failed to meet DSM-III-R criteria because either the frequency of attacks or the number of criterion symptoms was too limited. In patients with comorbid disorders, a study diagnosis of definite or subsyndromal panic disorder required a history of panic attacks occurring independently of other axis I disorders; otherwise the case was classified as "diagnosis unknown" in the linkage analyses.

Five Icelandic pedigrees from an earlier study were added to the U.S. pedigrees to increase the sample size (16). They had been ascertained from the practice of one of the authors (T.Z.) using the same ascertainment criteria described for the U.S. pedigrees. A fifth-year medical student interviewed the families with an Icelandic language version of Anxiety Disorders Interview Schedule—Revised (17) after being trained to use the instrument by one of us (T.Z.). Diagnoses were made at a consensus conference of the three authors in the same manner as that described for the U.S. pedigrees (16). The study was approved for the use of human subjects by the institutional review board of the National University of Iceland; in accordance with their practice, verbal informed consent based on a narrative summary of the study was obtained from all participants.

The interviewers and diagnosticians of both pedigree sets were aware that all of the probands had panic disorder. Although diagnoses were completed before the marker typings were begun, the marker typings were performed and interpreted without knowledge of diagnostic status.

Laboratory Methods

All of the $GABA_A$ receptor loci tested have simple sequence repeat polymorphisms that can be genotyped by the polymerase chain reaction amplification of the polymorphic sequence, size fractionation of the products by gel electrophoresis, and autoradiography. The polymerase chain reaction amplification was performed in a 10-μl reaction containing 10 ng of genomic DNA; 50 mM of KCl; 10 mM of Tris-Cl (pH 8.0); 1.5 mM of $MgCl_2$; 200 μm each of dCTP, dGTP, dTTP; 62.6 μM of dATP; 3 μCi of α-[^{35}S]-dATP; 0.5 μM of each primer; and 0.5 units of Taq DNA polymerase. The conditions were 35 cycles of denaturation (94°C for 30 seconds), annealing (50–58°C for 30 seconds), and extension (72°C for 30 seconds). The products were size-fractionated in 6% (weight/volume) polyacrylamide denaturing gels run at 60 watts for 2–3 hours. Gels were dried and exposed to X-ray film at room temperature overnight. Two raters interpreted the autoradiographs without knowledge of the diagnoses, and differences were resolved either by discussion or by repeating the genotyping.

Data Analysis

Genotypes were entered into the LABMAN/LINKMAN database (18) for analysis. Lod scores were computed with the MLINK program of the LINKAGE v.5.1 package (19).

The linkage analyses employed two definitions of the affected phenotype and two genetic models. The narrowly defined phenotype included only cases of definite panic disorder, with or without agoraphobia; cases of subsyndromal panic disorder were classified as unknown phenotypes, and all others were considered unaffected. In contrast, the broadly defined phenotype included both definite and subsyndromal panic disorder, with or without agoraphobia, and all other cases were considered unaffected.

The linkage analyses modeled two phenotypes (narrowly and broadly defined) and two modes of inheritance (autosomal dominant and recessive) with age and sex-dependent penetrance. Since the α3 subunit locus (GABRA3) is located on the X chromosome, this analysis required an X-linked dominant model (X-linked recessive inheritance is incompatible with the observed transmission of panic disorder). The autosomal dominant model assumed a disease allele frequency of 0.015 and that heterozygotes and homozygotes for that allele had a penetrance of 0 at age 10, increasing linearly to a maximum of 0.80 by age 40 in females; the model also assumed that the penetrance in males was half the value for females at each respective age. Age- and sex-dependent penetrance was modeled with six age-related liability classes for each sex. The autosomal recessive model assumed a disease allele frequency of 0.20 with the homozygote penetrance being the same as the heterozygote penetrance in the dominant model. The X-linked model assumed a disease allele frequency of 0.015 with hemizygote and heterozygote penetrances identical to those in the autosomal models. The results of all of the linkage analyses were tested for locus heterogeneity with the program HOMOG (20) as implemented by LINKMAN (18). Markers for which lod scores greater than 0.00 were obtained at any recombination fraction were tested by using affected-pedigree-member analysis (21), a nonparametric method that computes the probability of the affected members of each family being identical by state at the marker locus.

TABLE 1. Lod Scores of 21 U.S. and Five Icelandic Multiplex Panic Disorder Pedigrees at Eight $GABA_A$ Receptor Subunit Gene Loci[a]

	Lod Score at Each Recombination Fraction						
Locus	0.00	0.05	0.10	0.20	0.30	0.40	Alpha[b]
GABRA1 (α1)							
Autosomal dominant genetic model	–19.78	–7.48	–4.01	–1.11	–0.17	0.04	0.40
Autosomal recessive genetic model	–10.87	–4.22	–1.68	0.20	0.45	0.21	0.40
GABRA2 (α2)							
Autosomal dominant genetic model	–15.01	–7.29	–4.87	–2.24	–0.91	–0.29	0.40
Autosomal recessive genetic model	–12.04	–7.13	–4.59	–1.92	–0.69	–0.17	0.40
GABRA3 (α3)							
X-linked dominant model	–3.59	–1.08	–0.23	0.41	0.41	0.11	0.95
GABRA4 (α4)							
Autosomal dominant genetic model	–15.31	–6.43	–3.86	–1.52	–0.53	–0.11	0.55
Autosomal recessive genetic model	–12.09	–7.82	–5.34	–2.42	–0.93	–0.23	0.40
GABRA5 (α5)							
Autosomal dominant genetic model	–16.39	–7.80	–4.97	–2.20	–0.92	–0.32	0.40
Autosomal recessive genetic model	–11.08	–6.45	–4.02	–1.53	–0.52	–0.15	0.45
GABRB1 (β1)							
Autosomal dominant genetic model	–16.06	–8.44	–5.51	–2.40	–0.91	–0.23	0.45
Autosomal recessive genetic model	–12.12	–7.32	–4.77	–2.06	–0.80	–0.22	0.40
GABRB3 (β3)							
Autosomal dominant genetic model	–22.26	–10.12	–6.00	–2.09	–0.51	0.01	0.40
Autosomal recessive genetic model	–12.97	–7.82	–4.97	–1.94	–0.65	–0.16	0.45
GABRG2 (γ2)							
Autosomal dominant genetic model	–9.43	–4.21	–2.42	–0.77	–0.15	0.03	0.60
Autosomal recessive genetic model	–1.14	0.05	0.55	0.75	0.52	0.20	—

[a]Lod scores obtained under a narrow diagnostic model including only definite panic disorder, with or without agoraphobia.
[b]The proportion of pedigrees in this study that could be linked to each locus but remain compatible with exclusion (i.e., lod < –2.00 at θ=0.00) of linkage in the whole data set. The recombination fraction of 0.00 was actually run at 0.0001 to avoid negative infinity errors.

The Loci Studied

Polymorphisms have been reported for eight of the $GABA_A$ receptor subunit genes, and these were tested for linkage in the pedigrees (22–26; unpublished results of M.E.S. Bailey et al.). The eight subunit genes were α2 (GABRA2), α4 (GABRA4), and β1 (GABRB1) on chromosome 4p14-q12 (27–29); α1 (GABRA1) and γ2 (GABRG2) on chromosome 5q32-q35 (28–30); α5 (GABRA5) and β3 (GABRB3) on chromosome 15q11-q13 (31); and α3 (GABRA3) on chromosome Xq28 (28). Recent studies (unpublished results of M.E.S. Bailey et al.) have shown that the polymorphism previously reported to be specific for the α6 subunit gene (32) is, in fact, within the GABRG2 gene. The allele frequencies for GABRA2, GABRA4, and GABRG2 were based on 80 parents of 40 Centre d'Etude du Polymorphisme Humain families (unpublished results of M.E.S. Bailey), and those for the other five loci were taken from published studies (22–26).

RESULTS

We included 370 individuals from the 21 U.S. pedigrees in the linkage analyses. Of these, 252 (107 men and 145 women) contributed diagnostic and genotypic data to the analyses; the remainder connected the assessed relatives within the pedigrees. The mean age of the 21 probands (10 men and 11 women), was 41 years (SD=8.6). The mean age of the family members who were assessed was 46.2 years (SD=15.6); thus, the majority were well into or through the age of risk for panic disorder.

Eighty pedigree members had panic disorder; this represents 32% of those who were interviewed. An additional 22 (9%) had subclinical panic disorder. Thus, 31.8% of the sample was affected with the narrowly defined phenotype and 40% with the broadly defined phenotype.

The Icelandic pedigrees included 125 individuals in the linkage analyses. Their mean age was 39.7 years (SD=16.0). Ninety-six of them were genotyped; 24 of these had DSM-III-R panic disorder with (N=11) or without (N=13) agoraphobia. An additional eight subjects had subsyndromal panic disorder. Thus, 32 individuals were affected with the broadly defined phenotype and 24 of these were affected with the narrowly defined phenotype.

The results of the linkage analyses based on the narrow model are presented in table 1. Since this was a candidate gene study, the relevant lod scores are those at the 0.00 recombination fraction because they test the hypothesis that a mutation in a candidate gene invariably cosegregates with the disease in the families. None of the $GABA_A$ receptor subunit genes was linked to panic disorder.

Most of the genes tested occur in clusters on chromosomes 4, 5, and 15. GABRA2 (α2), GABRA4 (α4), and GABRB1 (β1) are located in the chromosome 4p14-q12 region, and the GABRG1 (γ1) locus is in the 4p14-q21 region (27–29). The lod scores at the first three loci were strongly negative under both the dominant and recessive models. GABRA5 (α5) and GABRB3 (β3) are located on chromosome 15q11-q13 (with the GABRG3 [γ3] locus) (31), and the lod scores at GABRA5 and GABRB3 were negative under both models of inheritance. GABRA3 (α3) is located in the Xq28 region (28).

The frequent occurrence of father-to-son transmission of panic disorder excludes the possibility that this

gene could account for the disease in all of the families, but not the hypothesis that it could be responsible for panic disorder in a subgroup of them. This latter possibility cannot be dismissed because the magnitude of the negative lod score was small and excluded only the hypothesis that all of the pedigrees were segregating a mutation in the α3 subunit gene.

The last cluster to be considered comprised GABRA1 (α1) and GABRG2 (γ2) on chromosome 5q32-q35 along with GABRB2 (β2) (28–30). Neither of the first two loci showed evidence of linkage, although the γ2 subunit gene was essentially uninformative under the recessive model.

Although the results exclude the hypothesis that mutations at any one of the loci tested cause panic disorder in all of the pedigrees, could any of these genes account for the disease in a subset of the families? Lod scores were positive at recombination fractions of 0.20–0.30 at the GABRA1/GABRG2 loci under the recessive model and at the GABRA3 locus under the X-linked dominant model, which would be consistent with linkage heterogeneity. Lod scores at all of the loci were tested for heterogeneity with the program HOMOG (20), and no statistically significant evidence of locus heterogeneity was found. Nonparametric analyses were run at these three loci using the affected-pedigree-member method (21) to exclude the possibility that the lod score method could have missed a linkage due to misspecification of the genetic model. The probability values at the three loci using the 1/p marker allele weighting function were as follows: GABRA1, p=0.90 (Z= 0.13); GABRG2, p=0.47 (Z=0.72); and GABRA3, p=0.21 (Z=1.27).

The results of similar analyses using the broadly defined phenotype (i.e., either definite or subsyndromal panic disorder were considered affected) paralleled those obtained using the narrowly defined phenotype, and in most cases the lod scores were even more negative.

We found no evidence of heterogeneity, but how well did we exclude the possibility that a subgroup of the pedigrees might be linked to one or more of the loci we tested? For example, a lod score of –19.78 at the GABRA1 locus tells us that a mutation in this gene is highly unlikely to account for panic disorder in all 26 pedigrees, but it does not exclude the possibility that a mutation could cause the disease in a few of the families. We examined this question by correcting the lod scores for heterogeneity as described by Ott (33) to determine what proportion of the families (alpha) could be linked and still give a lod score less than –2.00 (the conventional level at which a locus is considered to be excluded). These alpha values are presented in table 1. Conversely, if panic disorder was linked to one of the loci tested, what magnitude of lod score might have been expected? Simulations using the program SIMLINK (34) indicated that if more than half of the pedigrees were linked, the probability of obtaining a lod score greater than 2.00 at the 0.00 recombination fraction was 0.75 for the dominant model and 0.68 for the recessive one.

DISCUSSION

We found no evidence of linkage between panic disorder and eight of the 13 known human $GABA_A$ receptor subunit genes: GABRA1–GABRA5 (α1–α5), GABRB1 (β1), GABRB3 (β3), and GABRG2 (γ2). The results need to be interpreted with caution in view of some of the limitations of linkage studies using candidate genes. In addition, the study did not test all of the subunit genes that have been isolated. GABRA6 (α6), GABRB2 (β2), GABRG1 (γ1), GABRG3 (γ3), and GABRD1 (δ) still need to be examined when polymorphisms at these loci are found. Furthermore, genes for additional $GABA_A$ receptor subunits may yet be discovered.

Regulation of gene expression is a second mechanism by which receptor subunit genes could be involved in psychopathology, and such alterations would not necessarily be detected through linkage to the $GABA_A$ receptor subunit genes themselves. The $GABA_A$ receptor is highly polymorphic with respect to subunit composition, and the expression of different subunits in the brain shows considerable anatomical specificity (5, 6). If the expression of a particular receptor subtype were disturbed in a critical region, this could be reflected in the baseline level of activity in the GABA system and conceivably predispose an individual to panic attacks.

The lod score method of linkage analysis assumes that the mode of inheritance is known. With behavioral disorders it must be estimated from the genetic epidemiology of the disease, and its misspecification weakens the power to detect linkage. Segregation analyses indicate that panic disorder is compatible with either autosomal dominant or autosomal recessive inheritance and that the penetrance of the gene is dependent on age and sex (35, 36). Consequently, we used both models to test for linkage. Since neither model detected linkage, it is unlikely that we missed a disease gene due to wrong assumptions about the inheritance of panic disorder.

Genetic heterogeneity can also cause disease genes to be missed by linkage studies. Our results indicate that none of the genes that we tested is likely to cause panic disorder in a majority of the pedigrees, but they do not exclude the possibility that a $GABA_A$ receptor gene mutation underlies panic disorder in rare families.

Finally, additive inheritance, like genetic heterogeneity, can be difficult to detect with linkage. As a rule, genes for common traits are unlikely to be Mendelian and are more likely to act additively rather than independently (37). Thus, two or more mutations working in concert may be required to develop panic disorder.

In conclusion, mutations in any of the eight $GABA_A$ receptor subunit genes studied do not appear to account for most cases of familial panic disorder. The remaining five subunit genes still need to be studied when polymorphisms at these loci are identified. Conceivably, $GABA_A$ receptor mutations could be responsible for rare cases of panic disorder, and to exclude this possibility these genes would need to be scanned for mutations in affected individuals.

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