A Linkage Study Between the $GABA_A$ β_2 and $GABA_A$ γ_2 Subunit Genes and Major Psychoses

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FOCUS POINTS

- The involvement of the gabaergic system in major psychoses has been suggested by a large body of evidence.
- Nonparametric linkage analysis is a useful tool to identify genes that play a role in major psychoses.
- The use of homogenous populations in the genetic studies of complex disorders allows to minimize genetic heterogeneity.

ABSTRACT

Background: *Alterations of the γ-aminobutyric acid (GABA) system have been implicated in the pathophysiology of major psychoses.*

Objective: *Restriction fragment length polymorphisms associated with the human γ-aminobutyric acid type A ($GABA_A$) β_2 and $GABA_A$ γ_2 subunit genes on chromosome 5q32-q35 were tested to determine whether they confer susceptibility to major psychoses.*

Methods: *Thirty-two schizophrenic families and 25 bipolar families were tested for linkage.*

Results: *Nonparametric linkage (NPL) analysis performed by GENEHUNTER showed no significant NPL scores for both genes in schizophrenia ($GABA_A$ β_2: NPL narrow=–0.450; NPL broad=–0.808; $GABA_A$ γ_2: NPL narrow=0.177; NPL broad=–0.051) or bipolar disorder ($GABA_A$ β_2: NPL narrow=0.834; NPL broad=0.783; $GABA_A$ γ2: NPL narrow=–0.159; NPL broad=0.070).*

Conclusion: *Linkage analysis does not support the hypothesis that variants within the $GABA_A$ β_2 and $GABA_A$ γ_2 genes are significantly linked to major psychoses in a Portuguese population.*

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INTRODUCTION

There is accumulated evidence that heredity is a major factor in the pathogenesis of major psychoses. This hypothesis is supported by findings from family, twin, and adoption studies. The mode of inheritance is still unknown and may depend on a few (oligogenic) or many (polygenic) genes[1,2] in combination with different environmental factors, although genes with a major effect may be present in some multiplex families.

The increasing availability of polymorphic DNA markers has greatly expanded their potential utility in the identification of genes for susceptibility to the disease by applying linkage or association strategies. However, the application of linkage analysis to complex diseases such as schizophrenia and bipolar disorder has many difficulties, including reduced penetrance, unknown mode of inheritance, pheno-

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copies, and genetic heterogeneity.[2,3] Nonparametric linkage (NPL) analysis was developed to avoid some of these problems.[4] It is considered the most important method for linkage analysis of complex disorders because NPL analysis is independent of the mode of inheritance. Although linkage studies can be performed using chromosomal regions, these studies are particularly important when applied to specific candidate genes of known biological function implicated in the disease, identified within the candidate chromosomal region by linkage studies.[5] Based on several linkage studies,[6-10] a number of genomic regions that include the 5q region (which might harbor genes predisposing to schizophrenia and bipolar disorder) have been proposed to be either linked or implicated in these disorders.

Alterations in γ-aminobutyric acid (GABA) neurotransmission have been indirectly implicated in the etiology of schizophrenia,[11-15] and bipolar I disorder.[16,17] Because GABA is the major inhibitory neurotransmitter in the central nervous system (CNS), primarily synthesized by interneurons in the cerebral cortex, hippocampus, and limbic structures,[18] changes in the GABAergic activity may cause perturbation in other neurotransmitter pathways, as, for example, dopamine, serotonin (5-HT) and noradrenaline, which also have been implicated in these disorders. About 20% to 50% of all neuronal synapses use GABA as a transmitter,[19] and most of the physiological actions of GABA are produced via GABA A ($GABA_A$) receptors. Based on their pharmacologic action, it was concluded that $GABA_A$ receptors are involved in controlling the excitability of the brain[20,21] and in the modulation of anxiety,[22] cognition, vigilance, memory, and learning.[23,24]

Molecular cloning studies have revealed the existence of 16 mammalian subunits (α_{1-6}, β_{1-4}, γ_{1-4}, ρ_{1-2}, ε_{1-2}) for ionotropic $GABA_A$ receptors.[25] Chromosomal mapping indicates that $GABA_A$ subunit genes are often clustered in the genome. For example, $GABA_A$ α_1, $GABA_A$ α_6, $GABA_A$ β_2, and $GABA_A$ γ_2 have been localized to chromosome 5q32-q35.[26-29] $GABA_A$ receptors are chloride ion channels that can be opened by GABA and can be modulated by a variety of pharmacologically and clinically important drugs, such as barbiturates, steroids, anesthetics, anticonvulsants, and benzodiazepines (BZs).[30,31]

In clinical practice, BZs are routinely prescribed in schizophrenia and in acute psychotic states, although mostly in combination with neuroleptics. The benefit of such therapy appears to be the reduction of the dose of neuroleptics and increased responsiveness to therapy, particularly in neuroleptic-resistant patients.[12] BZs produce at least part of their clinically relevant effects by interacting with distinct allosteric binding sites on $GABA_A$ receptors.[32] Alterations in the density of these receptors in the dorsolateral prefrontal cortex from schizophrenia subjects and alterations in the number of BZ binding sites on the $GABA_A$ receptors have been reported.[14] Also, BZs can ameliorate symptoms of mania.[33] The antimanic and antidepressant effects of lithium, carbamazepine, and valproic acid may be partly achieved by their actions on GABAergic neurotransmission.[34,35]

BZ binding to $GABA_A$ receptors is modulated by γ_2 receptor subunit, and this subunit, along with α and β subunits, is the principal contributors to most native $GABA_A$ receptors.[36] The subunits α are responsible for the selectivity of the receptor for BZs, whereas the β_2 subunit is essential for high affinity BZ binding. Based on the aforementioned evidence, $GABA_A$ receptor genes, such as $GABA_A$ β_2 and $GABA_A$ γ_2 subunit genes, are located in chromosomal regions implicated in schizophrenia and bipolar disorder by linkage analysis are excellent candidate genes for these disorders. Therefore, the present linkage study investigates the hypothesis that allelic variants of $GABA_A$ β_2 and $GABA_A$ γ_2 genes confer susceptibility to major psychoses in a Portuguese population.

METHODS

Subjects

The families used in this study were ascertained from Azores and mainland Portugal and contained at least two affected members. The overall sample consisted of 32 schizophrenia families (including 66 schizophrenia, 9 unipolar, and 123 unaffected family members) and 25 bipolar families (including 51 bipolar, 23 unipolar, 3 schizophrenia, and 92 unaffected family members). Local ethical committee approval and written consent from each subject were obtained. All probands and available relatives were personally interviewed by a clinician with a extensive training. All participants were administered the Diagnostic Interview for Genetics Studies,[37] Portuguese version[38] and rated with the Operational Criteria checklist. Best estimate diagnosis were made according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*.[39] Ascertainment and diagnostic methods for these families have been described elsewhere.[40,41]

$GABA_A$ β_2 and $GABA_A$ γ_2 Polymorphisms

Blood samples were collected by venous puncture in test tubes containing ethylenediaminetetraacetic acid as the anticoagulant, and genomic DNA was prepared using the standard method,[42] with slight modifications. polymerase chain reaction (PCR) restriction fragment length polymorphism analysis of BanI and NciI polymorphisms of the $GABA_A$ β_2 and $GABA_A$ γ_2 genes was carried out according to Loh and colleagues,[43] with slight modifications. For polymorphism BanI, amplification reactions were carried out in a volume of 25 µl, containing 100 ng genomic DNA as template, 200 µM dinucleotides, 1µM of each primer, 10 mM Tris-HCL (pH=8.3), 1.67 mM MgCl2, and 1 unit of Taq polymerase. After initial denaturation at 95°C for 5 minutes, 40 cycles of PCR reaction were performed under conditions of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds. Amplification products were digested with BanI restriction enzyme, separated by electrophoresis in a 3% agarose gel, and visualized with ethidium bromide staining under ultraviolet light.

The polymorphism NciI in the intronic region of the $GABA_A$ γ_2 was determined by PCR in a final volume of 25 µl, containing 100 ng genomic DNA as template, 200 µM dinucleotides, 1 µM of each primer, 10 mM Tris-HCL (pH=8.3), 2.5 mM $MgCl_2$, and 1 unit of Taq polymerase. PCR amplification was initiated at 95°C for 4 minutes and performed for 40 cycles each, consisting of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 40 seconds. PCR products were digested using enzyme NciI. The digested fragments were separated in 2.5% agarose gel and were visualized by ethidium-bromide staining.

Statistical Analysis

Single-point NPL analysis was performed using GENEHUNTER (Version 1.2) program,[4] which allows NPL analysis of moderately sized pedigrees. This is a model-free test of the exact probability of observed identical by descent marker allele sharing among affected individuals in each pedigree. Two diagnostic phenotypes were tested in linkage analysis. Concerning schizophrenia, the narrow phenotype included only schizophrenia and the broad phenotype included schizophrenia and unipolar disorder. Relatively to bipolar disorder, the narrow phenotype included bipolar disorder, and the broad phenotype included bipolar disorder, unipolar disorder and schizophrenia.

FINDINGS

Thirty two schizophrenic families and 25 bipolar families with at least two affected members were recruited in this study. Concerning schizophrenia, we performed linkage analysis with GENEHUNTER and the results are presented in Table 1 for polymorphism BanI of $GABA_A$ β_2 gene and polymorphism NciI situated in the intronic region of the $GABA_A$ γ_2 gene. For polymorphism BanI of the $GABA_A$ β_2 gene (narrow and broad disease phenotype), the NPL score was –0.450 (*P*=.703) and –0,808 (*P*=.823), respectively, and for polymorphism NciI $GABA_A$ γ_2 gene (narrow and broad disease phenotype) the NPL score was 0.177 (*P*=.412) and –0.051 (*P*=.517), respectively. Similarly, single-point NPL analysis performed with GENEHUNTER used the 25 bipolar families. The results for both genes are presented in Table 2. For polymorphism BanI of the $GABA_A$ β_2 gene the NPL score was no significant for both narrow (0.834, *P*=.169) and broad (0.783, *P*=.189) diagnoses. For polymorphism NciI of the $GABA_A$ γ_2 gene the NPL score for the narrow disease phenotype was –0.159 (*P*=.563) and for broad disease phenotype was 0.070 (*P*=.458).

DISCUSSION

We tested two genes from GABAergic system, and we did not find evidence for linkage between the polymorphisms examined in these genes and both schizophrenia and bipolar disorder, following the guidelines proposed by Lander and Krugylak.[44] To our knowledge, no association and linkage studies between the polymorphism BanI of $GABA_A$ β_2 gene or polymorphism NciI of $GABA_A$ γ_2 gene and schizophrenia have been reported. Although we did not find any evidence for linkage, we cannot exclude the involvement of GABA receptors in the etiology of schizophrenia. Indeed, it has been

TABLE 1. NPL ANALYSIS FOR $GABA_A$ β_2 AND $GABA_A$ γ_2 GENES AND SCHIZOPHRENIA FAMILIES

	$GABA_A$ β2 gene		$GABA_A$ γ2 gene	
Phenotype definition	NPL score	*P* value	NPL score	*P* value
Narrow	–0.450	.703	0.177	.412
Broad	–0.808	.823	–0.051	.517

NPL=nonparametric linkage; $GABA_A$=γ-aminobutyric acid type A.

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reported an increase in the density of $GABA_A$ receptors in the dorsolateral prefrontal cortex from schizophrenic subjects.[45] Conversely, a reduction or no change in the number of BZ binding sites on the $GABA_A$ receptor in schizophrenia has also been reported.[46,47] It was also shown that glutamic acid decarboxylase mRNA levels are reduced in the prefrontal cortex of schizophrenia patients without loss of neurons.[48,49]

Concerning bipolar disorder these findings extended the results of other negative molecular genetic studies of the GABAergic system, including $GABA_A$ α_1, $GABA_A$ α_3, $GABA_A$ α_5, $GABA_A$ $\beta_2 1$ and $GABA_A$ β_3 genes.[50-54] Conversely, Papadimitiou and colleagues[55] showed association between $GABA_A$ α_5 gene and bipolar disorder, but case control association studies can generate false positives as a result of population stratification.

Several methods have been proposed for linkage analysis of complex traits with unknown mode of inheritance, including maximum likelihood-based methods and nonparametric approaches, such as NPL statistics.[4] The maximum likelihood method uses all the data available and is the most powerful method available when the true model is used. NPL analysis is less powerful when detecting linkage than parametric analyses,[56] but does not require specification of a mode of inheritance. Both methods are still limited by heterogeneity and definitive conclusions cannot be drawn from this study due to the small sample size, the low marker heterozygosity and consequently limited statistical power. The use of the Portuguese population, which can be considered a highly homogenous population,[57] allows us to minimize the problem of genetic heterogeneity.

Our findings cannot completely exclude a role of the $GABA_A$ β_2 and $GABA_A$ γ_2 genes in major psychoses. GABAergic neurons are widely distributed in the CNS, and their action influences the effects of other neurotransmitters such as dopamine, 5-HT, and norepinephrine.[58] For example, it was recently shown that the second intracellular loop of the $GABA_A$ γ_2 (short) receptor subunit interacts directly with the dopamine D_5 carboxy-terminal domain.[59] Failure to detect linkage with $GABA_A$ β_2 and $GABA_A$ γ_2 genes cannot exclude the possibility that there might be mutations in these genes that play a role in schizophrenia and bipolar disorder, for instance, potentially via interaction with genetic variation of the dopamine D_5. In linkage analyses of genetically complex traits, the inherent methodological difficulties may make it difficult to detect the presence of a rare or minor gene effect.

TABLE 2. NPL ANALYSIS FOR $GABA_A$ β_2 AND $GABA_A$ γ_2 GENES AND BIPOLAR FAMILIES

	$GABA_A$ β_2 gene		$GABA_A$ γ_2 gene	
Phenotype definition	NPL score	*P* value	NPL score	*P* value
Narrow	0.834	.169	–0.159	.563
Broad	0.783	.189	0.070	.458

NPL=nonparametric linkage; $GABA_A$=γ-aminobutyric acid type A.

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CONCLUSION

Although our results do not provide evidence for a role of the $GABA_A$ β_2 and $GABA_A$ γ_2 genes in the susceptibility to major psychoses in the Portuguese population, further work on larger samples is warranted. In addition, the potential role of gene-gene interaction between several neurotransmitter systems may be of particular interest. CNS

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