The GABA<sub>A</sub>-Receptor γ2 (GABRG2) Gene in obsessive-compulsive disorder

Margaret A. Richter,¹,² Gwyneth Zai,² Joanna C. McBride,¹ Emanuela Mundo,³ Richard P. Swinson,⁴ James L. Kennedy²

Abstract

Objective: The γ-aminobutyric acid type A (GABA<sub>A</sub>) system may be implicated in obsessive-compulsive disorder, based on its major role in modulation of anxiety and its function as the principal inhibitory neurotransmitter system in the cortex. In addition, glutamatergic/GABAergic mechanisms appear to play a role in the pathophysiology of obsessive-compulsive disorder, making the GABA<sub>A</sub>-receptor-γ<sub>2</sub> (GABRG2) gene a good candidate for susceptibility in this disorder. Method: 118 probands meeting DSM-IV criteria for primary obsessive-compulsive disorder and their available parents were recruited for participation in this study and informed consent was obtained. An NciI restriction site polymorphism in the second intron was genotyped and data was analyzed using the Transmission Disequilibrium Test. Results: In total, 61 of the participating families were informative (i.e., with at least one heterozygous parent). No biases were observed in the transmission of either of the two alleles ($c^2 = 0.016, 1$ d.f., $p = 0.898$) to the affected probands in the total sample. Conclusion/Discussion: While these results do not provide support for a major role for the GABA<sub>A</sub>-receptor-γ<sub>2</sub> in obsessive-compulsive disorder, further investigations of this gene in larger samples are warranted.

Descriptors: Obsessive-compulsive disorder; Genetics; Linkage disequilibrium; Receptors, GABA; Allelic imbalance

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Correspondence
Margaret A. Richter
Staff Psychiatrist, Department of Psychiatry, Sunnybrook Health Sciences Centre
FG42, 2075 Bayview Avenue
Toronto, ON M4N 3M5, Canada
Phone: 416-480-6100 ext. 6832; Fax: 416-979-6853
E-mail: peggy.richter@sunnybrook.ca

Gwyneth Zai
Neurogenetics Section, Centre for Addiction and Mental Health – Clarke Division
R-76, 250 College Street
Toronto ON M5T 1R8, Canada
Phone: 416-535-8501 ext. 4883; Fax: 416-979-4666
Email: gwyneth_zai@camh.net

Introduction

Obsessive-compulsive disorder (OCD) is characterized by recurrent unwanted thoughts (obsessions), usually accompanied by repetitive behaviours (compulsions) intended to alleviate anxiety. Insight is generally preserved into the senseless nature of the symptoms. OCD is a relatively common disorder, with a lifetime prevalence of 2-3%. Psychosocial morbidity of this chronic condition is extremely high, and can render the individual completely debilitated in some cases. There is solid evidence for the involvement of genetic factors in the etiology of OCD. Family studies have typically reported increased prevalence of OCD and related disorders amongst first-degree relatives of OCD probands, thus providing support for a genetic diathesis. Furthermore, segregation analyses have been consistent with a single gene of major effect. The etiology of OCD is unclear. While it is widely accepted that serotonergic mechanisms are important in the neurobiology of OCD, other neurotransmitter systems may also be involved. Benzodiazepines (BDZ) have long been observed to be specifically helpful in augmentation of serotonin-reuptake inhibitor treatment of OCD, as well as non-specifically useful in modulating the intense anxiety, which is a core feature of this disorder. BDZs pharmacologically act at the \( \gamma \)-aminobutyric acid (GABA)-benzodiazepine receptor complex, via positive allosteric modulation of the GABA\(_A\) receptor-binding site. The GABA\(_A\) receptor is an oligomeric glycoprotein, which forms a pentameric chloride channel assembled from different genetic variants of 3 subunits of the following: alpha (\( \alpha \)), beta (\( \beta \)), delta (\( \delta \)), and gamma (\( \gamma \)), with \( \alpha _2 \beta _2 \gamma \) being the most common pentamer in the brain; BDZs bind at the junction of the \( \alpha \) and \( \gamma \) subunits. The amino acid neurotransmitter, GABA, is the principal inhibitory neurotransmitter in the brain, and may also be functionally important in cortical disinhibition. It may therefore be relevant to explore the potential role of GABAergic mechanisms in OCD, in particular the GABA\(_A\) receptor, in OCD. The relevance of GABAergic mechanisms in OCD has also been supported by observations that the anticonvulsants, gabapentin and topiramate, may be effective in OCD. The cortical silent period (CSP) assay has reported a decrease in inhibition studies with short interval cortical inhibition (SICI) and topiramate, may be effective in OCD. Furthermore, cortical inhibition studies with short interval cortical inhibition (SICI) and the cortical silent period (CSP) assay have reported a decrease in inhibition studies with short interval cortical inhibition (SICI) and topiramate, may be effective in OCD. The reaction mixture was first denatured at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds. A final extension step was added at 72°C for 4 minutes. Ten microlitres of PCR product was digested with 5 Units of NciI (NEB), 1 mM PCR buffer (NEB4), 2x BSA (NEB), and ddH\(_2\)O to a volume of 15\( \mu \)l. The NciI digested PCR fragments were detected by 3.0% agarose gel electrophoresis at 100V for 1.5 hours. The fragment size of the uncut product, which has the A allele (allele 1), is 287bp and the cut product sizes (G allele, allele 2) are 263bp and 24bp after digested with NciI.

Method

1. Sample

A total of 118 adults with OCD were recruited from consecutive referrals to the Anxiety Disorders Clinic at the Centre for Addiction and Mental Health. All subjects were diagnosed with primary OCD by an experienced psychiatrist. OCD and other Axis I disorders were confirmed using the Structured Clinical Interview for DSM-IV (SCID-IV). All subjects had one or more first-degree biological relatives (parent or sibling) willing to participate in the study. Probands with a history of neurologic or metabolic diseases, bipolar or psychotic disorder, or current substance dependence were excluded. In total, 198 first-degree relatives participated; all participants provided written informed consent. For demographic details, see Table 1. This study was approved by the Centre for Addiction and Mental Health Research Ethics Board.

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<th>Table 1 - Demographic data</th>
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1. Genetic typing

Genomic DNA was extracted from whole blood using a non-enzymatic procedure. Polymerase chain reactions were used to amplify the segment of genomic DNA containing a polymorphic restriction site in the GABRG2 gene. The PCR reaction was performed in a 25\( \mu \)l volume containing: 150ng genomic DNA, 1´ GeneAmp PCR Buffer II (Applied Biosystems, Foster City), 2.5mM MgCl\(_2\), 160\( \mu \)M each of dATP, dTTP, dCTP, dGTP, 0.8\( \mu \)M of each primer [F: 5’ – AGA AAT TTA CCA ACT GGT CTA GCC GG – 3’, R: 5’ – AAA TCA TAA ATT GTG TCA TGC TTA GT – 3’], and 0.04 Unit of Taq polymerase (Applied Biosystems, Foster City). The reaction mixture was first denatured at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 68°C for 30 seconds, and 72°C for 30 seconds. A final extension step was added at 72°C for 4 minutes. Ten microlitres of PCR product was digested with 5 Units of NciI (NEB), 1 mM PCR buffer (NEB4), 2x BSA (NEB), and ddH\(_2\)O to a volume of 15\( \mu \)l. The NciI digested PCR fragments were detected by 3.0% agarose gel electrophoresis at 100V for 1.5 hours. The fragment size of the uncut product, which has the A allele (allele 1), is 287bp and the cut product sizes (G allele, allele 2) are 263bp and 24bp after digested with NciI.

3. Statistical analysis

We tested for the presence of transmission disequilibrium between the GABRG2 gene NciI RFLP polymorphism and OCD using the Transmission Disequilibrium Test (TDT). McNemar chi-square tests (\( \chi^2 \)) were performed on the sample of informative trios (n = 61 in total), i.e. those with one or more heterozygous relative. Power for TDT was determined with the Genetic Power Calculator. In this study, the statistical analyses were significant based on p < 0.05.

Results

The genotype frequencies were in Hardy-Weinberg equilibrium using PedStats. The frequency of allele 1 (A allele) in our total was 0.544 and allele 2 (G allele) was 0.456. The genotype distribution was: 0.270 for A/A, 0.547 for A/G, and 0.182 for G/G. We did not detect biased transmission of alleles from parents to their affected offspring in our informative sample (\( \chi^2 = 0.016, 1 \) df, \( p = 0.898 \)) (Table 2).

Discussion

Our results from this study do not provide support for the hypothesis of linkage disequilibrium between the GABA\(_A\) receptor-\( \gamma \)2 gene and OCD.
The role of the neurotransmitter GABA in OCD is unclear. Evidence implicating the GABA<sub>A</sub>-BDZ receptor in this disorder is mainly derived from suggestions that BDZ may play a useful role in the management of this condition. However, this literature has been generally based on studies of clonazepam, a 7-nitro-benzodiazepine derivative that in addition to binding with the GABA<sub>A</sub>-BDZ receptor, it uniquely impacts on serotonin synthesis and upregulates cortical serotonin binding sites. Thus it is not clear that the possible therapeutic effects of clonazepam are in fact mediated by alteration in GABA neurotransmission, but may rather relate to its serotonergic effect. Alternately, BDZ may also modulate anxiety via binding with the peripheral BDZ receptor. Additionally, observations of the potential anxiolytic effects of the anticonvulsants gabapentin and topiramate also suggest a role for GABA neurotransmission in OCD. Topiramate has been shown to have anti-obsessional benefits in two OCD studies, and the GABA analogue, gabapentin, has been reported helpful in OCD in a few case reports. However, the mechanism of action of gabapentin is unclear, and potentially may not directly involve GABAergic mechanisms.

This study is clearly limited by the small size of the sample available. Although a total of 118 families were tested, only 61 were actually informative. Nonetheless, this sample had a power of 93% to detect a relative risk as low as 1.5 due to the high variant frequency of 0.456. However, only one polymorphism was tested. The Nci1 RFLP polymorphism is itself silent, but is located 0.7kB downstream from an alternately spliced 8 nucleotide long exon implicated in alcohol effects. Thus this negative finding for this one polymorphism does not rule out the possibility of linkage disequilibrium elsewhere in this gene.

To our knowledge, this is the first published study of the GABRG2 gene in obsessive-compulsive disorder. While this analysis does not clearly support a major role for this gene, further investigations utilizing larger samples are warranted.

Acknowledgement
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Disclosures

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* Modest
** Significant
*** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.
For more information, see instructions for authors.

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