Association and linkage studies of candidate genes involved in GABAergic neurotransmission in lithium-responsive bipolar disorder

Anne Duffy, MD, MSc; Gustavo Turecki, MD, PhD; Paul Grof, MD; Patrizia Cavazzoni, MD; Eva Grof, MD; Ridha Joober, MD, PhD; Bernd Ahrens, MD; Anne Berghöfer, MD; Bruno Müller-Oerlinghausen, MD; Marta Dvořáková, PhD; Eva Libigerová, MD; Miloš Vojtěchovský, MD, PhD; Petr Zvolský, MD, DSc; Agneta Nilsson, MD; Rasmus W. Licht, MD; Nils A. Rasmussen, MD; Mogens Schou, MD; Per Vestergaard, MD; Anita Holzinger, MD; Claudia Schumann, MD; Kenneth Thau, MD; Carrie Robertson, BA; Guy A. Rouleau, MD, PhD; Martin Alda, MD

Duffy, Alda — Department of Psychiatry, Dalhousie University, Halifax, NS; Turecki, Joober, Rouleau — Centre for Research in Neuroscience, The Montreal General Hospital, McGill University, Montreal, Que.; Grof, Cavazzoni, Grof — Department of Psychiatry, University of Ottawa, Ottawa, Ont.; Ahrens, Berghöfer, Müller-Oerlinghausen — Department of Psychiatry, Free University, Berlin, Germany; Dvořáková, Libigerová, Vojtěchovský, Zvolský — Department of Psychiatry, Charles University, Prague and Hradec Králové, Czech Republic; Nilsson — Karolinska Hospital, Stockholm, Sweden; Licht, Rasmussen, Schou, Vestergaard — Psychiatric Hospital, University of Aarhus, Risskov, Denmark; Holzinger, Schumann, Thau — University Clinic of Vienna, Department of Psychiatry, Vienna, Austria; Robertson — Royal Ottawa Hospital, Ottawa, Ont.

Correspondence to: Dr. Martin Alda, Department of Psychiatry, Dalhousie University, Abbie J. Lane Bldg., 5909 Jubilee Rd, Halifax NS B3H 2E2; fax 902 473-4596; malda@is.dal.ca

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Objective: To test for genetic linkage and association with GABAergic candidate genes in lithium-responsive bipolar disorder. Design: Polymorphisms located in genes that code for GABRA3, GABRA5 and GABRB3 subunits of the GABA A receptor were investigated using association and linkage strategies. Participants: A total of 138 patients with bipolar I disorder with a clear response to lithium prophylaxis, selected from specialized lithium clinics in Canada and Europe that are part of the International Group for the Study of Lithium-Treated Patients, and 108 psychiatrically healthy controls. Families of 24 probands were suitable for linkage analysis. Outcome measures: The association between the candidate genes and patients with bipolar disorder versus that of controls and genetic linkage within families. Results: There was no significant association or linkage found between lithium-responsive bipolar disorder and the GABAergic candidate genes investigated. Conclusions: This study does not support a major role for the GABAergic candidate genes tested in lithium-responsive bipolar disorder.
Introduction

Bipolar disorder, as defined by the Diagnostic Statistical Manual of Mental Disorders, fourth edition (DSM-IV), represents heterogeneous disease processes that likely result from interplay between a number of different genes and the environment. The definition of a core phenotypic spectrum and the inclusion of genetically heterogeneous proband samples are factors that have impeded the progress of molecular genetic studies in identifying susceptibility genes.1

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain. It modulates the activity of many other neurotransmitter systems, including dopamine, serotonin and norepinephrine, all involved in the pathogenesis and pharmacologic mechanisms of antidepressant and mood stabilizing drugs.2 Low levels of GABA have been reported in the brain, plasma and cerebrospinal fluid of patients diagnosed with depression and in the plasma of those diagnosed with mania.3,4 Moreover, in depression, plasma GABA levels do not appear to normalize when patients are in remission or with treatment. Finally, different classes of antidepressants and mood stabilizers (including lithium) have been shown to cause an up-regulation of GABAB receptor density. Taken together, these findings are suggestive of a deficit GABA state in some individuals predisposed to major mood disorders.5

Because of the involvement of the GABAergic system in the etiology and treatment of mood disorders, genes involved in GABAergic neurotransmission are considered candidate genes. GABA_{A} receptors are ligand-gated ion channels composed of 5 classes of subunits — α1–6, β1–3, γ1–3, δ and ρ1–2 — which cluster on different chromosomes, namely, chromosomes 4, 5, 6, 15, and X. Historically, interest in genetic studies of bipolar disorder was stimulated by the hypothesis of a dominant X-linked pattern of inheritance, a theory based on a preponderance of affected females and a seeming lack of father-to-son transmission in a subset of families.6,7 Early genetic studies investigated linkage between bipolar disorder and phenotypic markers located on the X chromosome, including protan or deutan colour blindness and glucose-6-phosphate-dehydrogenase deficiency.8–17 Results of these studies were inconsistent, and it was argued that genetic heterogeneity may have significantly contributed to the conflicting findings.18,19 More recently, both negative (reviewed, for instance by Paterson et al.20) and moderately positive21,22 results have been reported.

Candidate gene studies, using highly polymorphic DNA markers (both linkage and association strategies) for GABA_{A} receptor subunit genes in patients with bipolar disorder, have yielded mostly negative or equivocal results.23–29 However, positive findings have been reported.30

This study improves upon the methodology of previous work by limiting inclusion to a homogeneous subgroup of patients with bipolar disorder. We identified a homoogenous subgroup of carefully diagnosed bipolar probands based on an excellent response to long-term lithium monotherapy.31,32 These patients presumably share genetic determinants, and mode-of-transmission studies suggest major gene effects, thereby increasing the suitability of this population for molecular genetic studies.33

In this patient group, we performed association and linkage studies of candidate genes involved in GABAergic neurotransmission, including the GABA_{A} receptor α3 subunit (GABRA3), located in Xq28, and the

Objectif : Vérifier le lien et l’association génétiques avec les gènes candidats GABAergiques chez les sujets atteints d’un trouble bipolaire qui réagit au lithium. Conception : On a étudié les polymorphismes localisés sur des gènes qui codent des sous-unités GABRA3, GABRA5 et GABRB3 du récepteur GABAA au moyen de stratégies d’association et d’établissement de liens. Participants : Au total, 138 patients atteints d’un trouble bipolaire réagissant clairement à une prophylaxie au lithium, sélectionnés dans des cliniques spécialisées en dehors de familles. Résultats : On n’a constaté aucune association ni aucun lien significatifs entre le trouble bipolaire réagissant au lithium et les gènes candidats GABAergiques étudiés. Conclusions : Cette étude n’appuie pas un rôle important pour les gènes candidats GABAergiques testés chez les sujets atteints d’un trouble bipolaire qui réagit au lithium.
α5 subunit (GABRA5) and β3 subunit (GABRB3) clustering in 15q11.2–q12. Linkage and association strategies are complementary in that linkage analyses can be helpful in identifying genes of major effect and association studies can identify susceptibility genes with modifying effects.

Methods

Subjects

The sample included 138 genetically unrelated patients and 108 healthy controls matched for ethnicity. All proband patients were selected from specialized affective disorders outpatient clinics at participating centres of the International Group for the Study of Lithium-Treated Patients (IGSLI) and gave informed consent to participate in the study. The study protocol was approved by ethics committees at the appropriate institutions.

All probands were interviewed using the Schedule for Affective Disorders and Schizophrenia–Lifetime (SADS-L) format and met Research Diagnostic Criteria (RDC) for bipolar I disorder. As well, all probands met predetermined criteria for an excellent response to lithium monotherapy (described in detail by Grof et al.). Briefly, all probands had to demonstrate a high recurrence risk before lithium was initiated and no recurrence requiring biological intervention (i.e., electroconvulsive therapy, antidepressants, antipsychotics) during the observation time and while they were receiving adequate lithium therapy (i.e., at least 3 years taking lithium with plasma levels of at least 0.7 mmol/L). The mean age of the probands was 50.0 (standard deviation [SD] 14.4) years, and the male to female ratio was 0.87. The mean number of illness episodes before lithium was initiated was 8.2 (SD 10.1), and probands had been fully stabilized on lithium monotherapy for a mean of 14.4 (SD 6.8) years. To ensure reliability of diagnoses and lithium response across centres, all case histories and records were reviewed by a senior clinical investigator (PG) who also personally reinterviewed all subjects in Canada, the Czech Republic and Germany.

The control group, consisting of 108 subjects of comparable age (mean 51.5 [SD 14.8] years) and sex ratio (0.86), were selected from a population of individuals who married into the families of probands and from hospital employees; they had no history of any major psychiatric disorder.

Twenty-four probands had families suitable for linkage analysis. From these families, 171 relatives were interviewed with the SADS-L, diagnosed according to RDC and genotyped. All interviews were conducted by 2 psychiatrists blind to family affiliation and group, and final diagnoses were made on a blind consensus basis between at least 2 other psychiatrists using all data available to them (i.e., interviews, hospital records, collateral information). Of the 171 relatives interviewed, 72 had a bipolar spectrum illness (bipolar I or II disorders, recurrent schizoaffective disorder or recurrent unipolar depressive disorder).

The genes for the GABA α5 and β3 subunits were tested in the association and linkage samples, but the gene for α3 was tested in a pilot association study only.

Laboratory analysis

The markers tested were GABRA3 (Xq28, Genome Database [GDB]:156286), GABRA5 (15q11.2–q12, GDB: 162554) and GABRB3 (15q11.2–q12, GDB:160763). All 3 markers were intragenic dinucleotide repeat polymorphisms.

Genomic DNA was extracted by a standard method from venous blood samples. Polymerase chain reaction (PCR) was carried out in a total volume of 12.5 µL containing 40 ng genomic DNA; 125 ng of each primer; 200 µmol/L each of dGTP, dCTP and dTTP; 25 µmol/L dATP; 1.5 µCi (1 Bq = 2.7 × 10^-11 Ci [approx.]) [35S] DATP; 0.5 U Taq DNA polymerase (Bio/Can Scientific, Toronto, Ont); and 2.0 µL of 10 × buffer (Bio/Can Scientific) with magnesium chloride included in the final concentration of 1.5 mmol/L. Samples were overlaid with mineral oil and processed throughout 35 cycles of denaturation at 94°C, annealing at 55°C, and elongation at 72°C, followed by a final elongation period at 72°C. PCR products were analyzed on 6% denaturing polyacrylamide gel (38:2, acrylamide:bisacrylamide). Samples were run for an average of 2 hours in a vertical electrophoresis gel apparatus (Life Technologies, Gaithersburg, Md.). Gels were dried and exposed to x-ray films for 48 to 72 hours at room temperature. All marker determinations were blind to subject identity and diagnostic status. Autoradiographs were read and interpreted independently by 2 different raters, and a consensus was reached on all typing results.

Statistical analyses

Association data were analyzed by χ² tests, with p val-
ues determined empirically because of low expected cell frequencies. The method described by Zaykin and Pudovkin\(^37\) was used both for comparison of allele frequencies and for testing for Hardy–Weinberg equilibrium in the genotype frequencies. The linkage data were analyzed by both nonparametric (GENEHUNTER\(^38\) and SimIBD\(^39\)) and parametric (FASTLINK\(^40\)) methods. For parametric analysis, recessive, dominant and intermediate models were tested, along with low or high penetrance, with or without phenocopies. The model parameters were set so that the population frequency of the phenotype was 0.01 for men and 0.017 for women, and the proportion of sporadic cases was between 0.45 and 0.5 to account for phenocopies.

## Results

### Association study

The allele frequencies from the association analyses are given in Tables 1–3. The genotype frequencies for each of the 3 markers conformed to Hardy–Weinberg equilibrium in the patient group and the control group. There was a nonsignificant trend toward a difference in the allele frequencies of the \(\text{GABRB3}\) gene between cases and controls \((p = 0.015)\). However, given the negative linkage findings and the fact that the significance level did not approach that required in association studies, we interpreted this as a chance finding. There were no statistically significant associations found for the other markers tested.

### Linkage study

The linkage results were also negative. LOD scores under all models tested were negative for all \(\theta \leq 0.1\). This was confirmed by the results of nonparametric linkage analyses: in SimIBD, we obtained \(Z_{\text{Obs}} = 56.22\) \((p = 0.17)\) for \(\text{GABRB3}\) and \(Z_{\text{Obs}} = 45.96\) \((p = 0.36)\) for \(\text{GABRA5}\), and in GENEHUNTER, we obtained NPL (nonparametric linkage) = 1.02 \((p = 0.14)\) for \(\text{GABRB3}\) and NPL = 0.58 \((p = 0.26)\) for \(\text{GABRA5}\).

## Discussion

Neither a series of association studies nor linkage analysis provided support for the hypothesis that GABA\(_\alpha\) receptor subunit genes confer susceptibility to lithium-responsive primary recurrent affective disorders. This finding is consistent with the bulk of previously reported negative or equivocal findings for these candidate genes in more heterogeneous patient populations.

Although the association studies reported here are somewhat limited by the numbers of cases, the sample size was estimated to have sufficient power to detect a difference as small as 25% in allelic frequencies between patients and controls. We cannot, however, exclude completely the possibility that our result was a false-negative one and that the GABA\(_\alpha\) receptor genes may contribute in a minor way to the likelihood of manifesting a recurrent mood disorder in certain patients. This is because association studies can usually detect linkage disequilibrium in only a small region. However, because the markers tested were intragenic, the probability of missing a locus elsewhere in the gene, but not in disequilibrium with either marker, is not very high. By focusing on a genetically based homogenous subgroup of carefully diagnosed patients, we attempted to minimize the confounding role of etiologic heterogene-

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<th>Table 1: Allele frequencies from the association analysis of the GABA receptor (\alpha)3 subunit gene (GABRA3)</th>
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<td>Patients with bipolar disorder</td>
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<td>Controls</td>
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<td>(\chi^2 = 4.03; p = 0.25).</td>
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<th>Table 2: Allele frequencies from the association analysis of the GABA receptor (\beta)3 subunit gene (GABRB3)</th>
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<td>Patients with bipolar disorder</td>
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<td>Controls</td>
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<td>(\chi^2 = 21.64; p = 0.015).</td>
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ity. In addition, the combination of linkage and association strategies further improved the chances of detecting true gene effects. Therefore, the convergent evidence presented here and in previous studies suggests that GABA<sub>a</sub> receptor genes do not play a major role in the etiology of recurrent mood disorders.

### Acknowledgement

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