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N-methyl-D-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: Polymorphisms and mRNA levels

Livia Martucci, Albert H.C. Wong, Vincenzo De Luca, Olga Likhodi, Gregory W.H. Wong, Nicole King, James L. Kennedy *

Neurogenetics Section, CAMH, Clarke Division, University of Toronto, R-31, 250 College Street, Toronto (ON), Canada M5T 1R8

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Abstract

The NR2B protein is a critical structural and functional subunit of the NMDA glutamate receptor. The glutamate neurotransmitter system has been implicated in psychosis and schizophrenia, and so we looked for genetic association and measured gene expression in human DNA and brain samples, respectively, of the GRIN2B gene that codes for the NR2B protein. We tested three genetic polymorphisms: G-200T (5′UTR), A5806C and T5988C (both 3′UTR) in 180 matched schizophrenia case–control pairs, 86 schizophrenia nuclear family trios, and 318 bipolar disorder trios (of which 158 probands had psychotic symptoms). We measured brain GRIN2B mRNA levels in schizophrenia, bipolar disorder and unaffected controls ($n=35$ each). We detected genetic association between the G-200T marker and schizophrenia ($p=0.002$), between T5988C and bipolar disorder ($p=0.02$), and between A5806C and bipolar disorder with psychotic symptoms ($p=0.0038$). The T–C–C haplotype was transmitted more frequently with bipolar disorder, but less often with schizophrenia, while the G–C–T haplotype was transmitted more often in schizophrenia. Significant differences were found in overall haplotype frequencies between schizophrenia cases and controls ($p=0.005$). GRIN2B expression levels in schizophrenia, bipolar disorder and controls were not significantly different. The genetic findings suggest a role for GRIN2B in schizophrenia and bipolar disorder.

Keywords: Schizophrenia; Bipolar disorder; Polymorphisms; Family-based association test; Gene expression; NR2B subunit gene (GRIN2B)

1. Introduction

Schizophrenia and bipolar disorder are distinct diagnoses, but there is evidence of an overlap in genetic susceptibility loci (Badner and Gershon, 2002; Lewis et al., 2003; Segurado et al., 2003). The glutamate neurotransmitter system has also been

* Corresponding author. Tel.: +1 416 979 4987; fax: +1 416 979 4666.
E-mail address: james_kennedy@camh.net (J.L. Kennedy).

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implicated in susceptibility or pathophysiology for both disorders. In schizophrenia, the *N*-methyl-D-aspartate receptor (NMDAR) may modulate psychotic symptoms and disease progression (Olney and Farber, 1995). Investigations into mRNA expression levels of individual NMDA receptor subunits (including NR2B) in schizophrenia have produced conflicting results (Akbarian et al., 1996; Grimwood et al., 1999), suggesting that further studies are needed. In bipolar disorder, lithium and valproate exert neuroprotective effects through reducing NMDAR-induced excitotoxicity (Hokin et al., 1996; Nonaka et al., 1998; Dixon et al., 1999; Loscher, 1999).

The NMDA receptor is a heteromeric ligand-gated ion channel that mediates synaptic functions such as long-term potentiation (LTP) and long-term depression (LTD) (Lijam et al., 1997; Ishii et al., 1993; Laurie et al., 1997; Cull-Candy et al., 2001). The NR2B subunit is a critical structural and functional component of the NMDA receptor (Krupp et al., 1996; Laube et al., 1997; Vicini et al., 1998; Tang et al., 1999; Tovar et al., 2000; Moon et al., 1994; Smart, 1997; Aoki et al., 1998; Jan de Beur et al., 2000; Loftis and Janowsky, 2003). The gene encoding the NR2B subunit (GRIN2B: glutamate receptor, ionotropic, *N*-methyl-D-aspartate 2B) is located on chromosome 12p12 (Mandich et al., 1994; Schito et al., 1997). In both rats and humans, the NR2B subunit is primarily expressed in forebrain structures such as the cortex, hippocampus, striatum, thalamus, and olfactory bulb (Laurie et al., 1997). Altered expression of GRIN1 and GRIN2B has been reported in both SCZ and bipolar disorder (Grimwood et al., 1999; Law and Deakin, 2001; Gao et al., 2000; Benes et al., 2001; Scarr et al., 2003).

Previous genetic association studies of GRIN2B with schizophrenia or antipsychotic drug response have been inconsistent (Nishiguchi et al., 2000; Hong et al., 2001; Ohtsuki et al., 2001; Chiu et al., 2003). Miyatake et al (2002) examined the T-200G single-nucleotide polymorphism (SNP) in the 5′UTR of GRIN2B. This substitution shortens the dinucleotide repeat to (GT)6(CT)(GT)6, and alters a putative Sp1 binding site. Luciferase reporter assays with transfected cell lines demonstrated that the G-variant is associated with lower gene activity (Miyatake et al., 2002). Therefore, Sp1 may be an important transcription factor in regulating NR2B gene expression in both adult and developing neurons (Henson et al., 1992). A Japanese case–control study reported a significantly higher frequency of the −200G allele in schizophrenia ($n=200$). Although protein variants were not detected, these findings support the possibility that the GRIN2B gene, or a nearby site in linkage disequilibrium with it, may confer susceptibility to schizophrenia. To our knowledge, there have not been previous genetic association studies with the GRIN2B gene and bipolar disorder, although a genetic association between the GRIN1 gene and bipolar disorder has been reported (Mundo et al., 2003).

Based on the data above, we hypothesized that there would be significant genetic association between GRIN2B and both schizophrenia and bipolar disorder, and that GRIN2B mRNA levels in the cortex would be altered. To test this hypothesis, we examined three GRIN2B SNPs in both case–control and family-based samples in both disorders. We also performed a separate analysis of all patients with psychotic symptoms, regardless of diagnosis. We quantified GRIN2B mRNA levels in postmortem cortex from people with schizophrenia, bipolar disorder, or unaffected controls.

2. Materials and methods

2.1. Subject recruitment and demographics

Subjects for this study were recruited with fully informed written consent, and in accordance with University of Toronto and Canadian Institutes of Health Research (CIHR) guidelines for the ethical treatment of human subjects. Approval for the recruitment protocol was given by our hospital research ethics board. A total of 86 nuclear families consisting of probands with schizophrenia and at least one first-degree relative were collected from hospitals in Toronto, Ontario, in addition to 192 schizophrenia case–control pairs. All probands had an independent clinical DSM-IIIR/DSM-IV diagnosis of schizophrenia from their referring psychiatrist. (American Psychiatric Association, 1994), confirmed with a SCID (Structured Clinical Interview for DSM diagnosis). Final diagnosis was decided by a consensus between two psychiatrists (AHCW, JLK), based on all