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GAD1 Single Nucleotide Polymorphism Is in Linkage Disequilibrium with a Child Bipolar I Disorder Phenotype

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ABSTRACT

Background: **Pediatric bipolar I disorder (BP-I) and childhood schizophrenia (SZ) share certain symptoms (e.g., psychosis, aggression/irritability [A/I]), and the psychotic and A/I features are treated with neuroleptics in both disorders. Thus, it is of interest to examine the association of GAD1 to child BP-I because of its recently reported association to childhood SZ.**

Methods: **Child BP-I probands were obtained by consecutive new case ascertainment, and the phenotype was defined as current DSM-IV BP-I (manic or mixed phase) with at least one of the cardinal symptoms of mania (i.e., elation and/or grandiosity) and a Children's Global Assessment Scale score ≤60 (clinical impairment). These child BP-I probands are part of a large, ongoing, longitudinal study in which the phenotype has been validated by unique symptoms, longitudinal stability, and 7–8 times greater family loading than adult BP-I probands. Genotyping was performed using a TaqMan® Validated SNP Genotyping Assay, and FBAT was used for analysis.**

Results: **There were 48 families. The rs2241165 A allele was preferentially transmitted (FBAT $\chi^2 = 5.2$, df = 1, p = 0.022). No interaction between this GAD1 SNP and the Val66 BDNF allele was found.**

Conclusions: **These data are consistent with some shared genetic vulnerability between child BP-I and SZ, which may be related to similar treatments.**

INTRODUCTION

Pediatric bipolar I disorder (BP-I) and childhood schizophrenia (SZ) share certain symptoms (e.g., psychosis, aggression/irritability [A/I]) (Kumra et al. 1996; Geller et al. 2002a; Kowatch et al. 2005; Tillman and Geller, in press; Tillman et al., 2008), and the psychotic and A/I features are treated with neuroleptics in both disorders (Kumra et al. 1996; DelBello et al. 2002, 2006; Shaw et al. 2006). Thus, it is of interest to examine the relationship of GAD1 single nucleotide polymorphism (SNP) in pediatric BP-I, because four correlated SNPs ($r^2 > 0.92$) were found to be associated with childhood SZ (Addington et al. 2005). In addition,

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the GAD1 gene is of interest for child BP-I because of decreased expression of GAD67 (which is encoded by the GAD1 gene) in postmortem brains of BP adults (Guidotti et al. 2000; Knable et al. 2001; Heckers et al. 2002; Woo et al. 2004; Fatemi et al. 2005; Torrey et al. 2005). There are also two linkage studies (Cichon et al. 2001; Ewald et al. 2003) and one association study (Lundorf et al. 2005) that provide some suggestion of a relationship between GAD1 and BP in adults. Recently, Benes et al. (2007) reported down regulation of GAD67 in the hippocampus of BP subjects, further supporting study of this gene in a child BP population.

The GAD1 gene on chromosome 2q31 encodes for the GAD67 protein that is responsible for decarboxylation of glutamate and is expressed primarily in the brain (Bu and Tobin 1994). Glutamic acid decarboxylase is an enzyme that catalyzes the decarboxylation of glutamate to GABA and is present in GABA neurons in the brain.

METHODS AND MATERIALS

Child BP-I probands are part of the large, ongoing, NIMH-funded, longitudinal "Phenomenology and Course of Pediatric Bipolar Disorders" study, in which the phenotype has been validated by unique symptoms, longitudinal stability, and 7-8 times greater familiality than adult BP-I probands (Geller et al. 2002a, 2002b, 2002c, 2004a, 2006; Geller and Tillman 2005). The phenotype was defined as current DSM-IV BP-I (manic or mixed phase) with at least one of the cardinal symptoms of mania (i.e., elation and/or grandiosity) and a Children's Global Assessment Scale (CGAS) (Shaffer et al. 1983) score $\leq$60, to establish clinical impairment in the moderate and severe ranges (Bird et al. 1992; Geller et al. 2002a). Cardinal symptoms are those that occur only in a single disorder. By way of analogy, DSM-IV requires sad mood or anhedonia for major depressive disorder. Using cardinal symptoms avoided diagnosing mania only by symptoms that overlapped with those for attention-deficit/hyperactivity disorder (ADHD) (e.g., irritability, hyperactivity, distractibility).

Probands were obtained by consecutive new case ascertainment from designated pediatric and child psychiatry facilities (Geller et al. 2002a). To enhance generalization, probands were obtained from pediatric and child psychiatry sites by consecutive new case ascertainment, between 1995 and 1998 (Geller et al. 2002a). In this schema, every new case at the designated facilities was screened for exclusions (e.g., major medical illness), and those still eligible were interviewed by telephone, e.g., a child with a sore throat was given the same screening as a child with hyperactivity. Cases that were still not excluded after the initial telephone contact were given the complete research assessment (Geller et al. 2002a).

Subjects were assessed by experienced, blinded research nurses with instruments including the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al. 2001). The WASH-U-KSADS was administered separately to parents about their children and to children about themselves (Tillman et al. 2004). The child BP-I phenotype was defined as current DSM-IV BP-I (manic or mixed phase) with at least one of the two cardinal symptoms of mania (i.e., elation and/or grandiosity) (Geller et al. 2002a).

Informed consent was obtained from the parents and assent from the child. The study and consent forms were approved by the Washington University in St. Louis Human Studies Committee. Consents for genetic studies at Washington University in St. Louis are for all future genetic studies.

All laboratory procedures were performed blind to all clinical information, including family relationship. DNA extraction was performed by using the PureGene DNA extraction kit (Gentra Systems,Minneapolis, MN). For normalization of DNA concentration across the 384 well plate, DNA quantitation was conducted with the Quant-iT™ PicoGreen® dsDNA Assay Kit by Molecular Probes, Inc. (Eugene, OR). rs2241165 was selected because the A allele tagged the overtransmitted haplotype consisting of four associated SNPs in complete LD ($r^2 > 0.92$ between rs2241165 and the other associated SNPs) in the childhood SZ study (Addington et al. 2005). SNP genotyping of

rs2241165 was performed by using a TaqMan® Validated SNP Genotyping Assay (Applied Biosystems, Foster City, CA). Data acquisition was performed on the Fluoroskan Ascent (Thermo Electron Corporation, Waltham, MA) by using dual measurement of fluorescence intensity for VIC and FAM labeled probes. Thirty-one samples were rerun in a separate assay on a separate day and all duplicate calls were concordant.

Family Based Association Test (FBAT) software (http://www.biostat.harvard.edu/~fbat/fbat.htm) was used to analyze GAD1 genotype data. FBAT tests for preferential transmission of an allele to affected offspring. The particular SNP tested was rs2241165, which is the SNP that was associated with childhood SZ (Addington et al. 2005). Previously, the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism was found to be in linkage disequilibrium in our child BP-I sample (Geller et al., 2004b). Logistic regression models were used to test for an interaction between the BDNF and GAD1 alleles.

RESULTS

There were 55 probands with GAD1 rs2241165 SNP genotype data. This included 2 sibling pairs, so genotype data was available for 53 independent families. One family was not included in the analysis, because of a parent-child incompatibility. In this family, both parents were genotyped as having two A alleles, but the child was heterozygous for rs2241165. Four other families were not included because of a missing genotype in one family member. Therefore, FBAT analysis was conducted on 48 families (including 2 families with 2 probands). The overall call rate (i.e., the number of genotype calls divided by all samples attempted) was 96.9%.

The 50 PEA-BP-I probands in these families were aged 11.2 (SD = 2.9), age of onset of baseline mania episode was 7.9 (SD = 3.9), duration of baseline mania episode was 3.2 (SD = 2.6) years, and baseline CGAS was 43.4 (SD = 8.0). Female subjects comprised 40.0% of the sample, 54.0% were prepubertal, 88.0% were Caucasian, 2.0% were black, 2.0% were Hispanic, and 8.0% were of some other race.

Frequency of parental A alleles was 70.3%. This is consistent with the frequency of rs2241165 A alleles in the general Caucasian population (70.0%) (<http://www.ncbi.nlm.nih.gov/SNP/snp_ss.cgi?subsnp_id=44199983>). Proband and parental genotypes were in Hardy-Weinberg equilibrium (HWE) (proband: $\chi^2 = 0.3$, df = 1, p = 0.569; parental: $\chi^2 = 0.1$, df = 1, p = 0.822).

The FBAT analyses showed preferential transmission of the rs2241165 A allele ($\chi^2 = 5.2$, df = 1, p = 0.022). As illustrated in Fig. 1, the A allele was transmitted 29 times and not transmitted 14 times from heterozygous parents, giving an odds ratio of 2.07. The empirical level of significance for this test using the Monte Carlo method in FBAT was p = 0.025. Additional FBAT analyses by proband sex, pubertal status, and age of BP-I onset were not more significant than the FBAT for the overall group.

No significant interaction was found between the GAD1 and BDNF alleles in this sample.

DISCUSSION

These results provide preliminary evidence of association between the GAD1 SNP

		Non-Transmitted: G	Non-Transmitted: A
Transmitted	G	8	14
Transmitted	A	29	49

FIG. 1. GAD1 rs2241165 allele transmission in 48 families (2 families included 2 probands). Of the 96 parents in the 48 families, 41 were heterozygous for the GAD1 rs2241165 SNP (i.e., had one A and one G allele). Two of these parents were part of families with two probands. Shaded cells indicate transmission of alleles from heterozygous parents. Preferential transmission of rs2241165 A alleles to probands with a prepubertal and early adolescent bipolar I disorder phenotype was supported ($\chi 2 = 5.2$, df = 1, p = 0.022).

rs2241165 and child onset BP-I. Because preferential transmission of the same GAD1 SNP rs2241165 A allele (and 3 highly correlated SNPs) was also reported in a sample of child-onset schizophrenia, this finding is not specific to child BP (Addington et al. 2005), but is consistent with the symptomatic (e.g., psychosis, A/I) and treatment (e.g., neuroleptic) overlap between these disorders. If replicated, these data may suggest overlap in genetic vulnerability between child BP-I and child onset SZ that may be related to the use of neuroleptics for both disorders (Kumra et al. 1996; DelBello et al. 2002, 2006; Shaw et al. 2006). These findings are consistent with other examples of vulnerability genes for both SZ and BP, e.g., the DAOA gene has been implicated in both SZ and BP (Detera-Wadleigh and McMahon 2006).

Limitations include small sample size, and thus relatively low power. The selection of a single SNP is logical because it was the best tagging SNP being followed up from the previous study of childhood schizophrenia. However, this SNP would not be expected to be functional, and further resequencing of the coding region and conserved noncoding variation in future studies would be necessary to identify functional variation. Future studies should consider genotyping other polymorphisms within this region, including those in Straub et al. (2007).

DISCLOSURES

Dr. Cook has consulted for Lilly. Dr. Geller, Ms. Tillman, Ms. Bolhofner, and Ms. Hennessy have no conflicts of interest to disclose.

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REFERENCES

Addington AM, Gornick M, Duckworth J, Sporn A, Gogtay N, Bobb A, Greenstein D, Lenane M, Gochman P, Baker N, Balkissoon R, Vakkalanka RK, Weinberger DR, Rapoport JL, Straub RE: GAD1 (2q31.1), which encodes glutamic acid decarboxylase (GAD67), is associated with childhood-onset schizophrenia and cortical gray matter volume loss. Mol Psychiatry 10:581–588, 2005.

Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M: Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. Proc Natl Acad Sci U S A 104:10164–10169, 2007.

Bird HR, Gould MS, Staghezza B: Aggregating data from multiple informants in child psychiatry epidemiological research. J Am Acad Child Adolesc Psychiatry 31:78–85, 1992.

Bu DF, Tobin AJ: The exon-intron organization of the genes (GAD1 and GAD2) encoding two human glutamate decarboxylases (GAD67 and GAD65) suggests that they derive from a common ancestral GAD. Genomics 21:222–228, 1994.

Cichon S, Schumacher J, Muller DJ, Hurter M, Windemuth C, Strauch K, Hemmer S, Schulze TG, Schmidt-Wolf G, Albus M, Borrmann-Hassenbach M, Franzek E, Lanczik M, Fritze J, Kreiner R, Reuner U, Weigelt B, Minges J, Lichtermann D, Lerer B, Kanyas K, Baur MP, Wienker TF, Maier W, Rietschel M, Propping P, Nothen MM: A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q. Hum Mol Genet 10:2933–2944, 2001.

DelBello MP, Schwiers ML, Rosenberg HL, Strakowski SM: A double-blind, randomized, placebo-controlled study of quetiapine as adjunctive treatment for adolescent mania. J Am Acad Child Adolesc Psychiatry 41:1216–1223, 2002.

DelBello MP, Kowatch RA, Adler CM, Stanford KE, Welge JA, Barzman DH, Nelson E, Strakowski SM: A double-blind randomized pilot study comparing quetiapine and divalproex for adolescent mania. J Am Acad Child Adolesc Psychiatry 45:305–313, 2006.

Detera-Wadleigh SD, McMahon FJ: G72/G30 in schizophrenia and bipolar disorder: Review and meta-analysis. Biol Psychiatry 60:106–114, 2006.

Ewald H, Kruse TA, Mors O: Genome wide scan using homozygosity mapping and linkage analyses of a single pedigree with affective disorder suggests oligogenic inheritance. Am J Med Genet B Neuropsychiatr Genet 120:63–71, 2003.

Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, Eagan E: GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. Schizophr Res 72:109–122, 2005.

Geller B, Zimerman B, Williams M, Bolhofner K, Craney JL, DelBello MP, Soutullo C: Reliability of the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) mania and rapid cycling sections. J Am Acad Child Adolesc Psychiatry 40:450–455, 2001.

Geller B, Zimerman B, Williams M, Bolhofner K, Craney JL, Frazier J, Beringer L: DSM-IV mania symptoms in a prepubertal and early adolescent bipolar disorder phenotype compared to attention-deficit hyperactive

and normal controls. J Child Adolesc Psychopharmacol 12:11–25, 2002a.

Geller B, Craney JL, Bolhofner K, Nickelsburg MJ, Williams M, Zimerman B: Two-year prospective follow-up of children with a prepubertal and early adolescent bipolar disorder phenotype. Am J Psychiatry 159:927–933, 2002b.

Geller B, Zimerman B, Williams M, Delbello MP, Frazier J, Beringer L: Phenomenology of prepubertal and early adolescent bipolar disorder: Examples of elated mood, grandiose behaviors, decreased need for sleep, racing thoughts and hypersexuality. J Child Adolesc Psychopharmacol 12:3–9, 2002c.

Geller B, Tillman R, Craney JL, Bolhofner K: Four-year prospective outcome and natural history of mania in children with a prepubertal and early adolescent bipolar disorder phenotype. Arch Gen Psychiatry 61: 459–467, 2004a.

Geller B, Badner JA, Tillman R, Christian SL, Bolhofner K, Cook EH Jr: Linkage disequilibrium of the brain-derived neurotrophic factor Val66Met polymorphism in children with a prepubertal and early adolescent bipolar disorder phenotype. Am J Psychiatry 161: 1698–1700, 2004b.

Geller B, Tillman R: Prepubertal and early adolescent bipolar I disorder: Review of diagnostic validation by Robins and Guze criteria. J Clin Psychiatry 66 Suppl 7:21–28, 2005.

Geller B, Tillman R, Bolhofner K, Zimerman B, Strauss NA, Kaufmann P: Controlled, blindly rated, direct-interview family study of a prepubertal and early-adolescent bipolar I disorder phenotype: morbid risk, age at onset, and comorbidity. Arch Gen Psychiatry 63: 1130–1138, 2006.

Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E: Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: A postmortem brain study. Arch Gen Psychiatry 57: 1061–1069, 2000.

Heckers S, Stone D, Walsh J, Shick J, Koul P, Benes FM: Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. Arch Gen Psychiatry 59:521–529, 2002.

Knable MB, Torrey EF, Webster MJ, Bartko JJ: Multivariate analysis of prefrontal cortical data from the Stanley Foundation Neuropathology Consortium. Brain Res Bull 55:651–659, 2001.

Kowatch RA, Youngstrom EA, Danielyan A, Findling RL: Review and meta-analysis of the phenomenology and clinical characteristics of mania in children and adolescents. Bipolar Disord 7:483–496, 2005.

Kumra S, Frazier JA, Jacobsen LK, McKenna K, Gordon CT, Lenane MC, Hamburger SD, Smith AK, Albus KE, Alaghband-Rad J, Rapoport JL: Childhood-onset schizophrenia. A double-blind clozapine-haloperidol comparison. Arch Gen Psychiatry. 53:1090–1097, 1996.

Lundorf MD, Buttenschon HN, Foldager L, Blackwood DH, Muir WJ, Murray V, Pelosi AJ, Kruse TA, Ewald H, Mors O: Mutational screening and association study of glutamate decarboxylase 1 as a candidate susceptibility gene for bipolar affective disorder and schizophrenia. Am J Med Genet B Neuropsychiatr Genet 135:94–101, 2005.

Shaffer D, Gould MS, Brasic J, Ambrosini P, Fisher P, Bird H, Aluwahlia S: A Children's Global Assessment Scale (C-GAS). Arch Gen Psychiatry 40:1228–1231, 1983.

Shaw P, Sporn A, Gogtay N, Overman GP, Greenstein D, Gochman P, Tossell JW, Lenane M, Rapoport JL: Childhood-onset schizophrenia: A double-blind, randomized clozapine-olanzapine comparison. Arch Gen Psychiatry 63:721–730, 2006.

Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, Vakkalanka RK, Kolachana BS, Kleinman JE, Weinberger DR: Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry advance online publication 1 May 2007; doi: 10.1038/sj.mp.4001988.

Tillman R, Geller B, Craney JL, Bolhofner K, Williams M, Zimerman B: Relationship of parent and child informants to prevalence of mania symptoms in children with a prepubertal and early adolescent bipolar disorder phenotype. Am J Psychiatry 161:1278–1284, 2004.

Tillman R, Geller B: Diagnosis, prognosis and personalized medicine. In: Treatment of Child and Adolescent Bipolar Disorder. Edited by Geller B, DelBello MP. New York, Guilford Press, in press.

Tillman R, Geller B, Klages T, Corrigan M, Bolhofner K, Zimerman B: Psychotic phenomena in 257 young children and adolescents with bipolar I disorder: Delusions and hallucinations (benign and pathological). Bipolar Disord, 10:45–55, 2008.

Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB: Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. Biol Psychiatry 57:252–260, 2005.

Woo TU, Walsh JP, Benes FM: Density of glutamic acid decarboxylase 67 messenger RNA-containing neurons that express the N-methyl-D-aspartate receptor subunit NR2A in the anterior cingulate cortex in schizophrenia and bipolar disorder. Arch Gen Psychiatry 61:649–657, 2004.

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