Brief Report

Linkage Disequilibrium of the Brain-Derived Neurotrophic Factor *Val66Met* Polymorphism in Children With a Prepubertal and Early Adolescent Bipolar Disorder Phenotype

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Objective: Transmission of the brain-derived neurotrophic factor (BDNF) *Val66* allele in children with a prepubertal and early adolescent bipolar disorder phenotype was examined.

Method: The prepubertal and early adolescent bipolar disorder phenotype was defined as current DSM-IV bipolar I disorder (manic or mixed phase) with at least one cardinal mania criterion (i.e., euphoria and/or grandiosity) to ensure differentiation from attention deficit hyperactivity disorder. Probands (mean age=10.7 years, SD=2.7) were obtained by consecutive new case ascertainment from designated pediatric and psychiatric venues. Parents and probands were interviewed separately by research nurses who were blind to the probands' diagnoses. Genotyping was done with TaqMan Assay-on-Demand. Analysis was done with the Family Based Association Test program.

Results: There were 53 complete, independent trios. The BDNF *Val66* allele was preferentially transmitted (Family Based Association Test: χ^2=6.0, df=1, p=0.014).

Conclusions: This finding in child bipolar disorder is consistent with data for adults with bipolar disorder that show preferential transmission of the *Val66* allele.

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Brain-derived neurotrophic factor (BDNF) is heavily expressed in human brain and has increased expression beginning in young adulthood (1). Relevance to child psychiatry of differential BDNF expression by age is not yet known. Preclinically, BDNF has been implicated in numerous mood-relevant neurobiological actions (e.g., antidepressant drug response [2] and the neuroprotective action of lithium [3]), and thus it was hypothesized to be a good candidate for investigation in bipolar disorders. Moreover, the *Val66* allele is a functional variant in both human and preclinical studies (4).

Recently, two independent research groups, using family-based methods, reported that the *Val66* allele (at amino acid position 66 in exon 1 of the BDNF gene on chromosome 11p13) was preferentially transmitted to predominantly Caucasian adult probands with bipolar disorder (5, 6). One Japanese case-control study had negative findings (7).

Based on functionality of the *Val66Met* single nucleotide polymorphism (SNP) (4) and on family-based findings in adult bipolar disorder (5, 6), preferential transmission of the *Val66* allele in children with a prepubertal and early adolescent bipolar disorder phenotype was hypothesized.

Method

Probands were a subset of subjects in the NIMH-funded Phenomenology and Longitudinal Course of Pediatric Bipolar Disorders study (8, 9) and were obtained by consecutive new case ascertainment from designated pediatric and child psychiatry venues, using methods described in detail elsewhere (9). In brief, research nurses screened every new child at multiple pediatric and child psychiatric sites. All children who were not excluded because of a priori exclusion criteria (e.g., major medical illness) were interviewed by phone, and, if they still were not excluded, they were given the complete research assessments.

Comprehensive assessment by research nurses who were blind to the probands' diagnoses included the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (10) given separately to the probands' parents about their children and to the children about themselves (11). The prepubertal and early adolescent bipolar disorder phenotype was defined as current DSM-IV bipolar I disorder (manic or mixed phase) with at least one of the two cardinal criteria of mania (i.e., euphoria and/or grandiosity). Use of this cardinal symptom approach was analogous to the DSM-IV requirement of sad mood or anhedonia for a diagnosis of major depressive disorder. This definition of a prepubertal and early adolescent bipolar disorder phenotype ensured differentiation from attention deficit hyperactivity disorder. This differentiation was a major contentious issue in the field of child bipolar disorder, because of overlapping symptoms (e.g., hyperactivity, distractibility) between the two disorders (8, 9). Moreover, the cardinal symptom approach also facilitated differentiation of a prepubertal and early adolescent bipolar disorder phenotype from other child psychiatry disorders that have aggression/irritability as a symptom (e.g., references 12, 13). The Children's Global Assessment Scale (14) score needed to be ≤60, which corresponds to definite clinical impairment (15). The prepubertal and early adolescent bipolar disorder phenotype has 4-year longitudinal validation (8).

After complete description of the study was provided to parents and children, written informed consent was obtained from parents and written assent from children.

DNA extraction was performed by using a PureGene DNA extraction kit by Gentra Systems Inc. (Minneapolis), and DNA quantitation was conducted with the PicoGreen dsDNA kit by Molecular Probes, Inc. (Eugene, Ore.). SNP genotyping was performed by using TaqMan Assay-on-Demand (Applied Biosystems, Foster City, Calif.). Data acquisition was performed on the Analyst AD

(Molecular Devices, Sunnyvale, Calif.) by using the fluorescence intensity method.

Genotype data were analyzed with the Family Based Association Test program (http://www.biostat.harvard.edu/~fbat/fbat.htm) and with the ASPEX/sib_tdt program (16). A one-tailed test was used for analysis because only preferential transmission of the *Val66* allele would be considered significant, based on adult bipolar disorder studies (5, 6).

Results

The probands' mean age was 10.7 years (SD=2.7), the mean age at onset of baseline mania episodes was 7.6 years (SD=3.6), and the mean duration of baseline mania episodes was 3.2 years (SD=2.5). The mean Children's Global Assessment Scale score was 44.3 (SD=8.5). The proportion of female subjects was 35.8%, of Caucasians was 88.7%, and of prepubertal subjects was 60.4%.

There were 53 complete, independent biological trios (probands and both biological parents), among which there were 27 informative trios. The frequency of parental *Val66* alleles was 79.2%. Proband alleles were in Hardy-Weinberg equilibrium (χ^2=0.05, df=2, p=0.98). Analyses showed preferential transmission of the BDNF *Val66* allele (Family Based Association Test: χ^2=6.0, df=1, p=0.014; sib_tdt: p=0.014). The *Val66* allele was transmitted 21 times and not transmitted nine times (both parents in a trio may transmit the allele). Exploratory analyses by prepubertal status (16 informative trios) were significant (Family Based Association Test: χ^2=6.8, df=1, p=0.009; sib_tdt: p=0.011). Analyses by gender and by postpubertal status were not significant, but the number of informative trios for postpubertal status was small (N=11).

Discussion

These data suggest that the BDNF *Val66* allele confers susceptibility to a prepubertal and early adolescent bipolar disorder phenotype. To our knowledge, this is the first significant molecular genetic finding in child bipolar disorder. Whether this finding has implications for continuities between child and adult bipolar disorder will be an important question for future research.

Specificity of preferential *Val66* allele transmission for bipolar disorder is unclear, as this finding has also been reported in one family-based study of probands with childhood-onset obsessive-compulsive disorder (17).

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