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Attention deficit hyperactivity disorder (ADHD) is a common early-onset childhood disorder with a strong genetic component. Results from previous studies have suggested that there may be a parent-of-origin effect for ADHD candidate genes. In particular, a recent investigation identified a pattern of paternal over-transmission of risk alleles for nine ADHD candidate genes. We examined this phenomenon in a sample of 291 trios for five genes previously associated with ADHD (HTR1B, SNAP-25, DRD5, DAT1, and BDNF). Using a dense map of markers and two analytic methods in this relatively large family-based sample, we do not find any evidence for significant paternal over-transmission of risk alleles in these candidate loci. Thus, we conclude that a substantial parent-of-origin effect is unlikely for these leading ADHD candidate genes.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a common childhood disorder with prevalence of 8–12% worldwide [Faraone et al., 2003]. Previous epidemiological studies provide compelling evidence that genes play a strong role in mediating susceptibility to ADHD and candidate gene studies have already implicated several genes in the etiology of ADHD [Faraone et al., 2005]. Interestingly, several family-based candidate gene studies have reported paternal over-transmission of alleles to ADHD offspring [Brophy et al., 2002; Hawi et al., 2002, 2005; Kustanovich et al., 2003; Quist et al., 2003; Mill et al., 2004; Kent et al., 2005; Smoller et al., 2006]. For example, three studies have reported paternal over-transmission of the G861 allele of HTR1B [Hawi et al., 2002; Quist et al., 2003; Smoller et al., 2006]. Additionally, prior studies have suggested paternal over-transmission of alleles of the SNAP-25 locus in ADHD [Brophy et al., 2002; Kustanovich et al., 2003; Mill et al., 2004] and for the Val66Met polymorphism of BDNF in which the common valine allele showed significant paternal over-transmission [Kent et al., 2005]. Most recently, Hawi et al. [2005] investigated 17 candidate genes and observed parent-of-origin effect in 9 of them. When they summed the transmission of risk alleles by parent-of-origin across these genes, they observed a significant excess of paternal transmissions.

If a parent-of-origin effect does exist for ADHD, the mechanism or biological basis is unclear. Whereas parent-of-origin effects have been demonstrated for several rare disorders [Mann and Bartolomei, 1999], such effects have not been well established for more common, complex disorders. Establishing whether such a phenomenon applies to a disorder as complex as ADHD would therefore be of substantial interest.

Herein, we describe our examination of parent-of-origin effects for five loci that have been associated with ADHD in several previous studies (HTR1B, SNAP-25, DRD5, DAT1, and BDNF), including three (HTR1B, SNAP-25, and BDNF) that have previously been reported to show paternal over-transmission of risks alleles in ADHD families [Brophy et al., 2002; Hawi et al., 2002; Kustanovich et al., 2003; Quist et al., 2003; Mill et al., 2004; Kent et al., 2005; Smoller et al., 2006]. We report that, using a dense map of single nucleotide polymorphisms (SNPs) across each gene in a sample of 291 trios, we find no significant evidence of paternal over-transmission.

MATERIALS AND METHODS

Subjects

A total of 229 families (comprising 291 parent-affected offspring trios) with ADHD were recruited from several ongoing family studies of ADHD conducted at the Massachusetts General Hospital as previously described [Smoller et al., 2006]. The largest number (90 families) was ascertained from longitudinal case-control family studies of boys and girls with ADHD [Biederman et al., 1992, 1999]. In these studies, probands were recruited from either consecutive referral to the MGH Pediatric Psychopharmacology program or by pediatric referral from a large Health Maintenance Organization (HMO) in the Boston area. Because ascertainment
ocurred prior to the publication of DSM-IV, affection status for probands and their relatives was based on DSM-III-R criteria. During the screening phase, mothers were administered a telephone questionnaire containing criteria for DSM-III-R ADHD and exclusionary criteria for their referred child. Potential probands were excluded if they were adopted, if their nuclear family was not available for study or if they had major sensorimotor handicaps, psychosis, autism, or a Full Scale IQ less than 70. Individuals who screened positive for DSM-III-R ADHD were invited to enroll in the study along with their first-degree relatives. Proband with subthreshold ADHD diagnoses were excluded. Those classified as having ADHD at both the screen and the interview (described below in diagnostic assessment) were included as index cases. Families were also ascertained from a family study of adults with ADHD, an affected sibling pair linkage study of ADHD, family studies of bipolar disorder, and family studies of ADHD and substance abuse. The screening methods for these studies were similar to the methods for the family studies of ADHD boys and girls with the following exceptions: (1) ADHD cases were obtained from the psychiatry clinics at MGH (and for the linkage study, the child psychiatry clinic at Children’s Hospital in Boston) as well as referrals from individual child psychiatrists in the community and adolescent psychiatrists; (2) ascertainment was based on DSM-IV diagnoses; (3) the pediatric bipolar studies ascertained cases for bipolar disorder and did not screen out cases with psychosis. Affection status for these studies was based on DSM-IV criteria for ADHD. Individuals whose age were 18 years or older provided written informed consent for themselves. Mothers provided written informed consent for minor children, and children provided written assent.

### Diagnostic Assessment

For children, lifetime history of psychopathology was assessed using the semi-structured K-SADS-E diagnostic interview [Orvaschel and Puig-Antich, 1987] administered to the mother, and, for children 12 and older, by direct assessment of the child. Final diagnostic assignment was made after blind review of all available information by a Diagnostic Committee composed of three board-certified child and adolescent psychiatrists and licensed clinical psychologists. The interviewers were instructed to take extensive notes about the symptoms for each disorder. These data were reviewed by the diagnostic committee and confirmed only if a consensus was achieved that criteria were met to a degree that would be considered “clinically meaningful” (i.e., that the structured interview indicated that the diagnosis should be a clinical concern due to the nature of the symptoms, the associated impairment, and the coherence of the clinical picture). To combine discrepant parent and offspring reports, the most severe diagnosis from either source was used as the consensus diagnosis, unless the diagnosticians suspected the source was not supplying reliable information. In addition to the ADHD diagnosis, we examined DSM-IV diagnostic subtypes of ADHD and co-morbidities for all subjects.

### Selection of Markers

Affected offspring and their parents were genotyped at SNPs spanning each candidate gene (HTR1B, SNAP-25, DRD5, DAT1, and BDNF). The selected region for each gene encompasses genomic areas of high linkage disequilibrium (LD) in and around each gene. The average inter-marker distance ranged from 2 to 6 kb for each gene, except for BDNF. For BDNF, the region spans a genomic area of ~210 kb which includes BDNF iso-form a (the shortest transcribed form of the gene) and its 3’ downstream area. Although SNPs covering all iso-forms of BDNF were not typed, the functional Val66Met polymorphism [Kent et al., 2005] and surrounding areas of high LD were included. SNPs were selected from the National Center for Biotechnology Information (NCBI) public database (http://www.ncbi.nlm.nih.gov/SNP), and Celera databases (http://www.celera.com). We selected “double-hit” SNPs (i.e., SNPs found either by independent submitters to dbSNP or in both the dbSNP and Celera databases) spanning each locus as others and we have demonstrated that these SNPs validate at a high rate. SNPs were selected for analysis if they met the following quality control metrics: (a) genotyping call rate >90%; (b) genotypes in Hardy-Weinberg equilibrium; and (c) less than 1% Mendelian errors.

### Genotyping

The genotyping for SNPs was performed using a single base extension reaction with allele discrimination by MassArray mass spectrometry system (Bruker-Sequenom) as previously described [Sklar et al., 2002].

### Analytic Strategy

We used two approaches to examine parent-of-origin effects in this sample. First, we applied the strategy reported by Hawi et al. [2005] by performing a TDT analysis on the full sample and selecting markers within each gene that demonstrated the highest T/NT ratio (i.e., “risk” alleles) and for which the TDT-P-value was <0.1. This resulted in one putative risk allele per gene by design. Then, according to the method of Hawi et al. [2005], we performed an omnibus test combining transmissions by parent-of-origin across all five loci and compared the T/NT ratios of paternal versus maternal alleles using a standard Pearson’s chi square test. TDT analysis was performed using TDTPHASE [Dudbridge, 2003]. Second, we examined whether any of the full set of 141 SNP markers that we genotyped across the five candidate genes show evidence of parent-of-origin effects. This analysis also included all variants of these five loci that have been reported to show significant parental over-transmission in ADHD in prior published studies (Brophy et al., 2002; Hawi et al., 2002, 2005; Kustanovich et al., 2003; Quist et al., 2003; Mill et al., 2004; Kent et al., 2005; Smoller et al., 2006). These analyses were performed using TDTPHASE [Dudbridge, 2003] and repeated using the PLINK package (http://pngu.mgh.harvard.edu/~purcell/plink/index.shtml) for confirmation. The P-values were essentially the same using both statistical packages and thus we report the results of the TDTPHASE analyses.

### RESULTS

Table I shows the results of the “risk allele” analysis based on the method of Hawi et al. [2005]. As shown, the omnibus test of paternal versus maternal transmission across the five genes showed no evidence of a parent-of-origin effect (P = 0.956).

When we examined the full array of SNPs (N = 141), only one (rs4813925 of SNAP-25) showed a nominally significant parent-of-origin effect (uncorrected P = 0.025) (see Fig. 1) (see supplementary Tables 1 and 2 for full TDT results and transmissions from each parent); however, in this case the evidence favored maternal rather than paternal over-transmission. In addition, this result does not survive correction for the number of markers (n = 63) tested in this gene. For the HTR1B G861 allele, which has been the most frequently reported to show paternal over-transmission [Hawi et al., 2002; Quist et al., 2003; Smoller et al., 2006], we observed nominally significant association for paternally transmitted alleles (P = 0.013) as previously reported [Smoller et al., 2006], but there was no significant difference when paternal versus
Maternal transmissions were compared (uncorrected \( P = 0.079 \)). Other markers, which were previously reported to show paternal over-transmission to ADHD offspring (Val66-Met polymorphism of BDNF [Kent et al., 2005]; MnII–DdeI haplotype of SNAP-25, MnlI polymorphism of SNAP-25 [Kustanovich et al., 2003]; DdeI polymorphism of SNAP-25 [Brophy et al., 2002]; -2015 A/T & 80609 G/A of SNAP-25 [Mill et al., 2004]) did not yield any statistically significant results. Thus, this more extensive examination is consistent with the first analysis in failing to detect any parent-of-origin effect for these five ADHD candidate loci.

We also examined how well our genotyped SNPs captured genetic variation at each locus by evaluating their performance as tag SNPs for each gene region. We downloaded genotype data for each locus from the HapMap website (www.hapmap.org; Release #19) and applied the Tagger algorithm [de Bakker et al., 2005] as implemented in the “Haploview” program [Barrett et al., 2005] to calculate the mean \( r^2 \) value between our SNPs and HapMap SNPs that we did not genotype. This analysis revealed that our SNP set captures most of the genetic variation (mean \( r^2 > 0.8 \)) for four loci; HTR1B, SNAP-25, DRD5, and BDNF. For DAT1, the mean \( r^2 \) was 0.54. However, it should be noted that these estimates of captured variation are conservative because we also genotyped genetic markers that were not found in the HapMap dataset. For instance, 20 of our 35 markers in and around DAT1 were not available in the HapMap SNP data (release #19). For the other genes which showed better performance, there were fewer markers that were not in the HapMap data: HTR1B (5 out of 21), SNAP-25 (14 out of 63), DRD5 (8 out of 13), BDNF (4 out of 10).

**DISCUSSION**

In light of a recent report that there may be systematic over-transmission of paternal alleles at candidate genes associated with ADHD [Hawi et al., 2005], we examined parent-of-origin effects using a dense set of SNPs across five leading candidate genes (HTR1B, SNAP-25, DRD5, DAT1, BDNF). We were unable to find any evidence suggesting systematic paternal over-transmission of risk alleles in ADHD for these loci. This conclusion was supported by two types of analyses: first,
applying the method of Hawi et al. [2005] to identify the strongest “risk allele” within each locus and second, by testing all 141 SNPs genotyped across these genes. Several features of our analyses suggest that our results are unlikely to represent Type II error. First, our family sample is actually somewhat larger than that in which Hawi et al. [2005] observed systematic paternal over-transmission (291 trios vs. 179 trios). Power estimates indicate that we had >80% power at alpha = 0.05 to detect association of paternal alleles under a multiplicative genotype relative risk (GRR) model for risk alleles with frequency of 0.1–0.8 and GRR > 2. Second, we saw no evidence of parent-of-origin effects in either analytic approach, including an examination of a dense set of markers encompassing each gene. Third, the full set of genetic markers that we genotyped appear to capture most of the genetic variation in the five candidate loci.

Of the individual markers tested, only the BDNF Val66Met polymorphism has previously been reported to have a statistically significant excess of paternal versus maternal transmission in ADHD [Kent et al., 2005]. We did not observe this effect in our analysis; however, since we examined only the iso-form a of the BDNF gene, we cannot rule out the possibility of parent-of-origin effects for other markers in the gene. Data from three independent samples [Hawi et al., 2002; Quist et al., 2003; Smoller et al., 2006] has provided some support for paternal over-transmission of the G861 allele of the HTR1B, although chi-square comparison of paternal versus maternal transmissions does not support a parent-of-origin effect in previous studies or the current analysis.

Our results appear to conflict with those of Hawi et al. [2005] who reported paternal over-transmission when they summed transmissions by parent-of-origin across nine candidate loci. When we applied this method, we did not observe a significant overall difference based on the parent-of-origin. The genes that we examined are overlapping but not identical with those tested by Hawi et al. [2005]. We examined an additional gene, BDNF, for which paternal over-transmission in ADHD has been reported [Kent et al., 2005] but which was not included in the report of Hawi et al. [2005]. In addition, we did not examine five genes that were included in their report (DRD4, TH, DDC, SERT, and TPH2). Although the lack of any signal for our five genes (P > 0.956) makes it unlikely that adding the additional five genes would produce an overall pattern of paternal transmission, we cannot rule out that our results differed from those of Hawi et al. [2005] due to examining this smaller set of genes.

Our failure to replicate parent-of-origin effects of Hawi et al. [2005] could also be related to differences in sample characteristics. For example, subtle differences in the percentage of each ADHD diagnostic subtype (combined, inattentive, hyperactive-impulsive) or in co-morbidity status might have also contributed to our discrepant result compared to the Hawi et al. [2005] study.

Parent-of-origin effects have been determined to be the cause of several rare disorders such as Prader-Willi syndrome (PWS) and Angelman syndrome (AS) [Mann and Bartolomei, 1999]. PWS and AS result from molecular defects in 15q11-13 that cause loss of expression of paternally transcribed (PWS) and maternally transcribed genes (AS), respectively. Whether it plays a substantial role in the etiology of more complex disorders has not been established. Linkage studies of several psychiatric disorders including autism [Lamb et al., 2005], bipolar disorder [McInnis et al., 2003], and alcoholism [McInnis et al., 2003; Liu et al., 2005] have provided some evidence for such effects, however, these remain to be confirmed.

In sum, using two analytic methods in a relatively large family-based sample, we do not observe evidence for systematic paternal transmission of risk alleles of five candidate loci for ADHD. We conclude that a robust parent-of-origin effect in ADHD is unlikely for these genes.

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evidence for linkage at 8q24, 18q22, 4q32, 2p12, and 13q12. Mol Psychiatry 8(3):288–298.


