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Family based association analysis of statistically derived quantitative traits for drug use in ADHD and the dopamine transporter gene

Jessica Lasky-Su [a], Joseph Biederman [b], Alysa E. Doyle [b], Timothy Wilens [b], Michael Monuteaux [b], Jordan W. Smoller [c], Stephen Faraone [a,*]

[a] *Genetics Research Program and Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, NY, USA*
[b] *Pediatric psychopharmacology Unit, Massachusetts General Hospital, Boston, MA, USA*
[c] *Psychiatric and Neurodevelopmental Genetics Unit, Department of Psychiatry, Massachusetts General Hospital, Charlestown, MA, USA*

Abstract

Objective: To determine whether SNPs within the dopamine transporter gene (*DAT*) are associated with quantitative phenotypes generated from drug frequency variables in an ADHD sample.
Method: 35 SNPs were genotyped in and around *DAT*. We developed a quantitative phenotype at each SNP by weighting the drug frequency variables. The weights were selected to maximize the heritability at each SNP. Once a quantitative phenotype was generated at each SNP, a screening procedure was used to select and test the SNPs with the greatest power to detect an association in *DAT*.
Results: No SNPs in *DAT* were associated with the quantitative phenotypes generated from the drug frequency variables after the multiple comparisons adjustment; however, some SNPs achieved nominal significance. A sliding window of analysis of 3 SNPs also resulted in only nominal associations.
Conclusions: SNPs in *DAT* do not appear to be associated with the phenotypes generated from drug frequency variables among individuals with ADHD.

Keywords: DAT; FBAT; Association; Phenotypes; Genetics

* Corresponding author. Department of Psychiatry, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA. Tel.: +1 315 464 3113.
E-mail address: faraones@upstate.edu (S. Faraone).

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1. Introduction

Genetic research has the potential to clarify the etiology of substance use disorders (SUDs) and foster the development of effective prevention and intervention efforts. Family, twin, and adoption studies indicate that genes play a significant etiologic role in the development of SUDs (Cloninger, 1987; Luthar & Rounsaville, 1993; Merikangas et al., 1998; Merikangas, Weissman, Prusoff, Pauls, & Leckman, 1985; Mirin, Weiss, & Michael, 1986; Pickens et al., 1991; Rounsaville, Anton et al., 1991; Tsuang et al., 1998) and of ADHD (Cantwell, 1975; Faraone & Biederman, 1994; Faraone et al., 1995; Levy, Hay, McStephen, Wood, & Waldman, 1997; Morrison & Stewart, 1974). These studies have led to the widely accepted conclusion that much of the familial transmission of SUDs is due to genes. However, the specific genes involved have been difficult to detect. Major obstacles to identifying genes for SUDs are the lingering uncertainties about how best to define SUDs, the possibility of genetic heterogeneity and the variable phenotypic expression of SUD genotypes. It is likely that multiple genes, each of small effect, combine to cause SUDs. If so, they may not be detectable without reducing measurement error and creating measures that more directly assess the genotype and its consequences. Furthermore, it seems unlikely that there will be a one-to-one correspondence between genetically influenced processes in the brain and the clinical phenomena that define diagnostic categories. The currently accepted psychiatric nosology, DSM-IV, provides four binary categories for measuring substance use phenotypes: alcohol and drug abuse and dependence with additional subgroups based on the type of drug used (American Psychiatric Association, 1994). There are drawbacks associated with this approach, namely, loss of information and efficiency and difficulties of interpretation stemming from the arbitrary distinction between “cases” and “non-cases.” By nature of adolescents having not passed entirely through the age of risk for SUDs, use and misuse of drugs may be more important to capture given the later potential of more substantial SUDs as adults. This nomenclature has been inadequate in providing genetic studies with precisely defined phenotypic measurements that allow for the successful detection of genes.

Molecular genetic studies may thus be more fruitful if they focus on alternative phenotypes explicitly developed to maximize the power to detect genes. By using a comprehensive set of assessment measures one can develop refined SUD phenotypic measurements that are maximally informative for genetic studies and, following the recommendation of Weinberg, Rahdert, Colliver, and Glantz (1998), make use of dimensional measurement approaches in the study of youth substance use. Candidate phenotypes for SUDs comprise variables that are, by definition, associated with substance use: number of substances used, frequency of substances use, number of DSM symptoms of abuse/dependence, age of first use, number of years from first use to abuse/dependence, impairment attributed to abuse/dependence and chronicity (defined as the duration of abuse/dependence divided by the subject’s age).

Research suggests a shared genetic vulnerability across various drug disorders (Kendler, Jacobson, Prescott, & Neale, 2003; Merikangas et al., 1998; Tsuang et al., 1998). For example, Tsuang et al. (1998) found that abusing one type of drug was associated with a large increase in the probability of abusing another type of drug. Evidence for a common vulnerability in this study spanned the following drugs: marijuana, sedatives, stimulants, heroin or opiates, and psychedelics. Such findings suggest that using information across many drugs may help identify a genetic association that is common to several drugs.

Due to the heterogeneity of SUDs (Cadoret, 1991; Glantz & Pickens, 1992; O’Brien & Jaffee, 1992), we chose to use data from families ascertained through referred youth with and without ADHD. There is evidence that ADHD drug abusers form a relatively homogeneous subgroup of SUDs (Kaminer, 1992; Lambert, 1988; Levin & Kleber, 1995; Schubiner et al., 1995; Tarter, McBride, Buonpane, & Schneider,

1977; Wilens, Biederman, Spencer, & Frances, 1994). Several studies have also shown that there is a familial association between ADHD and substance abuse (Faraone, Biederman, Keenan, & Tsuang, 1991; Morrison, 1980), suggesting that the two may share genetic or other familial etiologic factors. Independent reviews by Levin and Kleber (1995), Schubiner et al. (1995), and Wilens, Spencer, and Biederman (1996), Wilens, Biederman, Mick, Faraone, and Spencer (1997) found converging evidence indicating that the overlap between substance abuse (including alcohol and/or drug abuse or dependence) and ADHD is larger than expected by chance and is bi-directional, having been reported in samples of both substance abusers and ADHD individuals (Biederman et al., 1995; Carroll & Rounsaville, 1993; Levin & Kleber, 1995; Levin et al., 1996; Rounsaville, Kosten et al., 1991; Rounsaville, Weissman, Kleber, & Wilber, 1982; Tarter et al., 1977; Wilens et al., 1994).

A large body of literature suggests that an ADHD diagnosis is associated with an increase in substance use (Biederman et al., 1995; Biederman et al., 1997; Chilcoat & Breslau, 1999; Disney, Elkins, McGue, & Iacono, 1999; Lambert & Hartsough, 1998; Riggs, Mikulich, Whitmore, & Crowley, 1999; Wilens et al., 1997; Wilson & Levin, 2001). Other attributes of the relationship between ADHD and substance use disorders have also been studied. Wilens et al. (1997) found that an ADHD diagnosis is associated with a longer duration of substance use (Biederman, Wilens, Mick, Faraone, & Spencer, 1998) as well as with an earlier age of onset of substance use disorders, independent of psychiatric comorbidity. There is also evidence that an ADHD diagnosis is associated with an increase in the severity of non-tobacco substance use (Riggs et al., 1999).

Prior studies suggest that the dopamine transporter (*DAT*) represents a viable candidate gene for SUDs. Lerman et al. (1999) found associations of the 9 VNTR allele and several smoking phenotypes including lack of smoking, late initiation of smoking, and length of quitting attempts. Several positive associations were also shown with DAT and alcohol-related phenotypes (Bau et al., 2001; Gorwood et al., 2003; Kohnke et al., 2005; Limosin et al., 2004; Schmidt, Harms, Kuhn, Rommelspacher, & Sander, 1998; Wernicke et al., 2002). The dopamine transporter is also known to have an impact on the behavioral effects from cocaine use. Such influence makes this gene a good candidate gene for cocaine use.

Although there is initial evidence that *DAT* may be involved in the etiology of SUDs, more remains to be discovered about its potential role in the etiology of the disorder. In this paper we develop maximally heritable phenotypes that have high power to detect an association with SNPs in or near *DAT*. These phenotypes are developed using information on the frequency of drug use. By generating these phenotypes, we hope to further clarify the role that DAT may play in the etiology of SUDs.

2. Methods

2.1. Clinical study population

Two hundred twenty nine ADHD families were recruited through several ongoing research studies being conducted at Massachusetts General Hospital pediatric psychopharmacology clinic (MGHPPC): (1) 90 from the longitudinal case-control family studies of boys and girls; (2) 83 from an affected sibling pair linkage study of ADHD; (3) 37 from a family study of bipolar disorder; (4) 17 from a family study of ADHD adults and; (5) 2 from a study of ADHD and substance abuse. Because these studies were conducted by the same research group, the ascertainment criterion for ADHD did not differ among studies, e.g., children enrolled as bipolar probands for the family study of bipolar disorder would have

qualified for enrollment in the ADHD studies if they also met criteria for ADHD. For the longitudinal case-control family studies of boys and girls, probands were recruited from either MGHPPC or from HMOs in the Boston area. Ascertainment of the probands and their relatives was based on DSM-III-R criteria as subjects were recruited before the publication of DSM-IV. Individuals of 6 to 18 years of age were eligible to participate in this study. Potential subjects were excluded if they were adopted, had major sensorimotor handicaps, psychosis, autism, inadequate command of the English language, an IQ less than 80, or their nuclear family was not able to participate in the study. All of the ADHD probands met DSM-III-R diagnostic criteria for ADHD at the time of the clinical referral and had active ADHD symptoms at the time of recruitment. Recruitment, inclusion, and exclusion criteria for the other studies listed above were the same as the longitudinal study for ADHD boys and girls with the following exceptions: (1) ADHD cases were obtained from the MGHPPC, the child psychiatry clinic at Children's Hospital in Boston, or by referrals from individual child psychiatrists throughout the community; (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. Individuals 18 years of age or older provided written informed consent, mothers provided written informed consent for minor children and children provided written assent to participate in this study.

2.2. ADHD diagnostic assessment

We collected psychiatric information from children using the K-SADS-E (Epidemiologic Version), a widely used semi-structured psychiatric diagnostic interview, with established psychometric properties (Orvaschel & Puig-Antich, 1987). The interview inquired about the child's lifetime history of psychopathology. This included information on the affection status of ADHD and the age at which each child onset with the disorder, which were the primary variables of importance in this analysis. The K-SADS-E provides a standardized method of obtaining and recording symptoms necessary for the assessment of most Axis I categories. For all children including siblings, psychiatric data were collected from the mother. In addition, children 12 and older were directly evaluated. Discrepancies between the child and the parent interview were resolved by the diagnostic procedures discussed below. We did not directly interview children younger than 12 because they are limited in their expressive and receptive language abilities, they lack the ability to map events in time, and they have limited powers of abstraction. Given these limitations, there is a real question about whether the young child's self-perceptions, memories, feelings and reported behavior can be reliably assessed through self-report. Although limited, studies on the use of interview techniques among young children show that their replies are unreliable (Achenbach & McConaughy, 1987; Breton et al., 1995; Edelbrock, Costello, Dulcan, Kalas, & Conover, 1985; Schwab-Stone, Fallon, Briggs, & Crowther, 1994).

Final diagnostic assignment was made after a blind review of all available information by a diagnostic committee chaired by Dr. Joseph Biederman and 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 notes and the structured interview data were reviewed by the diagnostic committee so that the committee could make a best estimate diagnosis as described by Leckman, Sholomskas, Thompson, Belanger, and Weissman (1982). Definite diagnoses were assigned to subjects who meet all diagnostic criteria. Subthreshold diagnoses were assigned to those subjects who meet most, but not all, required criteria. Diagnoses presented for review were considered definite only if a consensus is achieved that criteria are met to a degree that would be considered clinically meaningful.

By "clinically meaningful" we mean that the data collected from 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, we used the most severe diagnosis from either source as the consensus diagnosis, unless the diagnosticians suspect that the source was not supplying reliable information. Interviewers of subjects were blind to all prior data collected from that subject and his or her family members.

2.3. Drug use screening inventory

Drug frequency information was collected from the Drug Use Screening Inventory (DUSI) (Tarter & Hegedus, 1991). The DUSI is a self-report instrument that quantifies adolescent involvement with drug and alcohol use. The validity of the DUSI was found to be good, with strong correlations between this and the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS) and DSM-III-R substance abuse symptoms (Tarter & Kirisci, 1997). In this study drug frequency information was collected on the following drugs: cigarettes, alcohol, cocaine/crack, marijuana/pot, stimulants/uppers, LSD/mescaline, tranquilizers/benzodiazapenes, pain killers, heroin/opiates, PCP, sniff gases/fumes. Subjects reported whether they used these drugs 0, 1–2, 3–9, 10–20, or more than 20 times in the last month.

2.4. Genotyping methods

Thirty-five single nucleotide polymorphisms (SNPs) were selected across *DAT* and flanking regions at a density of approximately 1 SNP/2.6 kb. To evaluate SNP assay quality and characterize the linkage disequilibrium (LD) relationships we screened the SNPs in 12 multigenerational CEPH pedigrees. SNPs were selected for testing in the ADHD family sample if they met the pre-specified quality control metrics. Twelve multigenerational CEPH families were used to generate haplotype blocks of LD. The EM algorithm and the haplotype block criteria of Gabriel et al. (2002) were used to determine the LD structure, as implemented in the program Haploview (Barrett, Fry, Maller, & Daly, 2005). Genotyping of the SNPs was done by MALDI-TOF mass spectrometry (Buetow et al., 2001).

2.5. Family-based association test-principal components (FBAT-PC)

Family-based association tests use genetic data from family members to evaluate the possible association of a disease phenotype and a genetic variant. FBAT-PC is a data reduction technique that generates one univariate trait from several inputted phenotypes and then uses the generated trait in a family-based association test (Lange et al., 2004). The approach that FBAT-PC uses to create the univariate trait maximizes the genetic effect of the multiple phenotypes that are inputted into the analysis. FBAT-PC uses genotypic information that is not used in the subsequent association analysis (e.g. the non-informative families and the expected offspring genotypes given the parents phenotypes of the informative families) to generate an estimate of the genetic effect for a given SNP (Lange et al., 2004). A maximally heritable univariate trait can then be generated by using this genetic effect estimate to generating a set of weights for the phenotypes that maximize the overall genetic effect. These weighted phenotypes are then added together to construct the final univariate trait. Because none of the genetic information that will be used in the subsequent genetic analyses was used to generate the univariate phenotype, the subsequent association analyses remain completely independent of the trait construction.

We apply the FBAT-PC methodology to 11 drug frequency variables taken from the DUSI. For each SNP a univariate trait was constructed by generating a weight for each of the 11 drug frequency variables and summing the weighted drug frequency variables together. As stated above, these weights were determined by using the SNP genotype information to find the set of weights that maximize the genetic effect.

Upon finding a significant association with the univariate trait in a family based association test, it is important to have a clinical interpretation for the univariate trait that was used in the analysis. This interpretation can best be determined by looking at the correlation of each drug frequency variable with the generate univariate trait (personal communication, Christoph Lange). If there is no significant association between a given SNP and the univariate trait, then no clinical interpretation of the univariate trait is made. We looked at all genetic models (additive dominant, recessive, and heterozygous advantage) and required the minimum number of informative families to be 20. All of the statistical tests for the single SNP analysis were then adjusted using the false discovery rate (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001). For any SNP that achieved nominal significance in the single SNP analysis, a haplotype analysis using sliding windows of 3 was performed on the corresponding haplotype block. All haplotype analyses were adjusted for using multiple comparisons using the false discovery rate (Benjamini & Hochberg, 1995).

3. Results

Of the 229 available families in these datasets, 189 had sufficient information to be used in this analysis. Descriptive information on these families is listed in Table 1. The distribution of drug frequency responses is listed in Table 2.

Using the various genetic models and the 35 SNPs encompassing DAT resulted in a total of 127 different statistical tests. After adjusting for all of these tests using FDR, there were no significant findings; however there were six SNPs that were significant prior to this adjustment using the heterozygous advantage model. These SNPs are listed in Table 3 and a summary of the nominal findings are listed in Table 4.

Table 1
Descriptive statistics on individuals used in the FBAT-PC analysis

Number of people	438
Number of families	189
Number of individuals with drug frequency information in each family	
1	70
2	35
3	43
4	37
5	3
6	1
Ever used Drugs (percentage)	
Yes	141 (39.6)
No	215 (60.4)
Missing	82

Table 2
Drug frequency use in the past month

	0 times	1–2 times	3–9 times	10–20 times	>20 times
Cigarettes	322	13	7	6	76
Alcohol	216	55	44	27	82
Cocaine/crack	395	7	8	4	9
Marijuana/pot	342	22	9	13	38
Stimulants/uppers	390	9	7	3	14
LSD/mescaline	397	13	6	5	2
Tranquilizers/benzos	406	8	6	2	1
Pain killers	373	20	11	5	14
Heroin/opiates	413	9	0	1	0
PCP	416	5	2	0	0
Sniff gases or fumes	414	6	0	3	4

The haplotype algorithm divided the SNPs into 5 haplotype blocks. Four of the five haplotype blocks contain the gene (position 1,445,908–1,498,543, UCSC Genome Browser). Details of the linkage disequilibrium structure are provided in Table 3. Haplotype analyses were only performed on the haplotype block where at least one of the constituent SNPs achieved nominal significance in the single SNP analysis. Therefore, the haplotype analysis was restricted to haplotype block four. Analyses were performed using a sliding window of three throughout haplotype block four. In the sliding window analysis, the haplotype blocks using the marginally significant in the single SNP analysis were also marginally significant in the haplotype analysis; however these findings did not remain significant after the multiple comparison adjustment.

4. Discussion

This paper utilized a new methodology that generates maximally heritable phenotypes at each SNP throughout *DAT*. In this analysis, we did not find any results that remained significant after adjusting for multiple comparisons. This may be related to several factors.

Table 3
Markers examined to define linkage disequilibrium around *DAT*

SNPs	Haplotype block	
rs246995, rs2963257, rs4975544	No block assigned	3′
rs2113328, hCV2854696	1	
hCV2854700, rs1472617, hCV2854709	No block assigned	
hCV2854710, hCV2960969	2	
rs3776513, rs3776510, rs2245660, rs2550936	3	
rs6347, rs4975640	No block assigned	
rs2042449, rs2975292, rs464049, rs365663, rs456082, rs464528, rs461753, rs459141, rs460000, rs457702	4	
rs462523, rs2617605	No block assigned	
rs6350, rs2963238, rs2652510, rs4738, rs8352, rs2911487, rs2277007	5	5′

Table 4
FBAT-PC results from the SNPs on *DAT*

Marker	# of info. families	Physical location (BP)*	Function	Unadjusted *p*-value	Adjusted *p*-value
rs456082	77	1,483,265	Intron	0.0033	0.12
rs464528	80	1,483,873	Intron	0.0015	0.12
rs461753	78	1,483,964	Intron	0.0123	0.31
rs459141	81	1,484,792	Intron	0.0039	0.12
rs460000	81	1,485,575	Intron	0.0039	0.12
rs457702	78	1,487,180	Intron	0.0381	0.80

* Locations taken from UCSC Genome Browser, May 2004 freeze.

There is a well-documented association between bipolar disorder and SUDs. A prospective study of children and adolescents with and without ADHD found that early-onset bipolar disorder predicted subsequent SUD independently of ADHD (Biederman et al., 1997). West et al. (1996) reported that 40% of inpatient adolescents with BPD suffered from SUDs and Wilens and colleagues have shown in two independent datasets that juvenile BPD is a risk for SUD (Wilens et al., 1999; Wilens et al., 2004). Because of the high comorbidity between adolescent bipolar disorder and ADHD, ADHD is also comorbid with SUDs. Due to the comorbidity between ADHD and SUDs, the rate at which we observe substance abuse in this dataset is more frequent that what would be observed in the general population; however, the frequency of drug use is less than what would be observed in a sample ascertained on drug use. Therefore, there are a notable number of individuals who used none or a few substances. Although the number of informative families in the analysis seems sufficient, the low variability in the frequency of drug use reduces the overall power of the analysis. Another clear limitation of this study is the age of the subjects, as it is likely that several of the individuals will initiate substance use later in life and this information is was not used in the analysis. Therefore, one explanation for no positive associations is an insufficient number of substance users in this sample. Sample heterogeneity represents also a limitation for this study, as the ADHD families used in this analysis were ascertained through different studies and had slightly different diagnostic systems (DSM-III-R or DSM-IV).

Because this study could be underpowered to detect genetic associations, future studies may want to reexamine the six adjacent SNPs in *DAT* that achieved nominal significance in this sample, as they may achieve statistical significance in a sample with more drug users.

Another possible explanation for the null results is that DAT may not affect those who use drugs comorbid with ADHD but may affect other drug users, as there is evidence that ADHD drug abusers form a relatively homogeneous subgroup of SUDs (Kaminer, 1992; Lambert, 1988; Levin & Kleber, 1995; Schubiner et al., 1995; Tarter et al., 1977; Wilens et al., 1994). Finally it is possible that this is a true null result and the univariate phenotype generated in this paper is not association with DAT.

This study represents a new analytic technique in which a maximally heritable phenotype is generated for each SNP throughout a candidate gene. Psychiatric epidemiology often uses instruments to collect detailed information on various psychiatric disorders. Information gathered from these instruments ranges from basic symptomatology to complex neuropsychiatric assessments. As the field of psychiatric genetics is emerging, quantitative phenotypes/endophenotypes have been used increasingly in analyses to detect genetic effects. Often these variables are generated using data reduction techniques such as

principle components analysis, factor analysis, or item response theory. Twin studies have succeeded in using these techniques to generate quantitative traits and heritability estimates. The FBAT-PC methodology presented in this paper represents another strategy that uses a data reduction technique to generate a phenotype. Using this approach, genetic data are used to generate a univariate trait that is maximally heritable. Generating a maximally heritable phenotype using family data in this way could prove valuable in future genetics research.

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