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Quantitative Trait Locus Analysis of Candidate Gene Alleles Associated With Attention Deficit Hyperactivity Disorder (ADHD) in Five Genes: *DRD4, DAT1, DRD5, SNAP-25*, and *5HT1B*

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It has been widely postulated that the categorical diagnosis of attention deficit hyperactivity disorder (ADHD) should be seen as the extreme end of a set of traits quantitatively distributed in the general population. A consequence of this is that the genes associated with DSM-IV ADHD should also influence these underlying traits in non-affected individuals. The aim of this study was to examine if specific candidate loci previously shown to be associated with DSM-IV ADHD, also act as quantitative trait loci (QTLs) for ADHD-symptoms in the general population. We have genotyped five candidate markers in a population-based sample of male dizygous twin-pairs (n = 329 pairs). We found little evidence to support a role for the previously-nominated alleles of a *DRD4* VNTR, a *5HT1B* SNP, or a microsatellite marker near to *DRD5*, in the distribution of ADHD-symptoms scores; however, we found some evidence to suggest that the *DAT1* 3′UTR VNTR and weak evidence that a microsatellite in *SNAP-25* may have a role in continuous measures of ADHD-symptoms hyperactivity above and beyond their role in clinical ADHD. © 2005 Wiley-Liss, Inc.

KEY WORDS: attention-deficit hyperactivity disorder (ADHD); genetics; association; quantitative-TDT (Q-TDT); quantitative trait loci (QTL); *DRD4*; *DRD5*; *DAT1*; *5HT1B*; *SNAP-25*

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a common and highly heritable pervasive disorder characterized by hyperactivity, inattention, and impulsivity. ADHD is one of the most prevalent forms of child psychopathology affecting between 2% and 5% of school-age children, with a strong male sex-bias in clinical samples. ADHD, as defined by operational criteria, is a dichotomous trait making up a distinct diagnostic category. Measures of activity, impulsivity, and inattention, are however continuously distributed in the general population, and many studies have found an excellent correspondence between quantitative measures of these traits and the categorical diagnosis of ADHD [e.g., Biederman et al., 1993; Boyle et al., 1997]. These studies do not report any obvious bimodality separating ADHD children from non-ADHD children and it has thus been argued that clinical ADHD should be regarded as the extreme end of these quantitative traits rather than as a discrete category [Levy et al., 1997]. This has implications not only for the classification, diagnosis, and treatment of ADHD, but is also important in the context of identifying aetiologies associated with the disorder.

ADHD is a complex, multifactorial disorder and the precise aetiology is not yet known [Spencer et al., 2002]. Behavioral genetic analyses have highlighted the strong role of genes on individual differences in ADHD symptom scores [reviewed by Thapar, 2003]. Heritability estimates from most studies estimate additive genetic variance to be in the range of 60–90%, and a figure of 70% seems a reasonable average [Eaves et al., 1997]. It is likely that susceptibility is mediated by the effect of numerous genes, or quantitative trait loci (QTL), of small effect interacting both with the environment and each other. Most twin studies examine variations in symptom scores across the entire range and find them to be almost equally heritable when assessed by parents or teachers. Several studies have employed the DeFries and Fulker extremes method of analysis [DeFries and Fulker, 1988] and find that the genetic contribution to symptoms at the upper end of the distribution is generally the same as for the entire distribution [Thapar, 2003]. Furthermore, it appears that genetic influences are similar for both males and females, and do not change much from early childhood to adolescence [Gjone et al., 1996]. Price et al. [2001] studied early continuous-ratings of ADHD symptoms in 6,000 UK twin pairs and found ADHD symptoms measured at ages 2–4 to be highly heritable (~90%), regardless of twin age or the measurement cut-off used. Furthermore, it was found that group heritability was equally high at both the low and high ends of the distribution (Price et al., unpublished data). The notion that heritability is constant across the trait distribution has interesting consequences in terms of finding genes that influence ADHD symptom scores. A good test of this conclusion would be to examine whether specific genetic risk factors, known to have a role in clinical ADHD, correlate with continuous-ratings of ADHD symptoms in the general population. To date, however, there have been few studies investigating the role of ADHD candidates in causing variation in the normal distribution of underlying phenotypes.

Considerable progress has been made in identifying genes that are associated with clinically diagnosed ADHD. Most

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focus has been on genes involved in dopaminergic, and to a lesser extent serotonergic, neurotransmission in the brain [Swanson et al., 2001]. Evidence that these systems play a role in the aetiology of ADHD comes from animal models, pharmacological studies, and brain imaging. The goal of this study was *not* to discover novel ADHD QTLs, but to focus on markers (and more precisely specific alleles of these markers) that have been previously associated with DSM-IV ADHD. The aim was to investigate whether associations discovered in clinical samples can be extrapolated to continuous-ratings of ADHD symptoms in the general population. We have taken a hypothesis-driven approach and focussed on polymorphisms in five candidate genes (*DRD4, DRD5, DAT1, 5HT1B*, and *SNAP-25*) for which there is already strong a priori evidence to suggest an association with DSM-IV ADHD. Further information about these candidate genes, their postulated role in ADHD, and the polymorphisms genotyped are listed in Table I.

MATERIALS AND METHODS

Sample

The sample consisted of 329 pairs of male dizygotic (DZ) twins (658 individuals), which were selected from a larger community sample called the Twins' Early Development Study (TEDS): a large ongoing longitudinal twin study [Trouton et al., 2002]. The TEDS sampling frame consists of all twins born in England and Wales in 1994-1996, although only a subset of twins from the first two cohorts were available for this study. Families giving birth to twins were identified and contacted by the Office of National Statistics. Background information on pregnancy, birth, and general family details were collected at age 18 months. The parents of twins' completed questionnaires on early ADHD symptoms for ages 2, 3, 4, and 7 years and additionally by the twins' teachers for age 7. ADHD symptom scores were assessed using the subscale from the Revised Rutter Parent Scales for Preschool Children questionnaire (RRPSPC) at ages 2 and 3, and with the hyperactivity subscale of the Strengths and Difficulties Questionnaire (SDQ) additionally at ages 4 and 7 [Goodman, 1997]. Mothers completed the majority of the parent rated questionnaires. At age 7 years, the twins' teachers also completed the SDQ questionnaire. For the main analyses presented here, we derived a composite index of early ADHD symptoms by taking total ADHD-symptom score averaged across the parents 2-, 3-, 4- and 7-year data, plus the teacher 7-year data. Additional analyses completed for the individual time-point data and for various combinations of these data are available online.

Genotyping and Analysis

DNA was extracted from buccal swabs as described in Freeman et al. [2003]. Candidate-gene polymorphisms were genotyped using standard PCR conditions described previously (see Table I). Family-based association analyses were performed using two methods. First, the quantitative transmission disequilibrium test (QTDT) was used [Abecasis et al., 2000], which is available online at http://www.hgmp.mrc.ac.uk/Registered/Option/qtdt.html. It can be used to analyze quantitative traits in nuclear families with or without parental genotypes. Several association models can be used within QTDT. In this study, we firstly employed the 'orthogonal model' of association (AO) that considers the correlation between within-pair differences in phenotype and genotype within a variance component framework. We also utilized the 'total association' model (AT) that evaluates the total evidence for association from both within-pair differences and the between pair sums that correlates the sum of the phenotypic score with the sum of the number of risk alleles for each pair. The AO test is robust to stratification effects, whereas the overall AT test of association that includes the between pairs component is not and can therefore produce misleading results in the presence of population stratification. Population stratification was evaluated using the AP test from the QTDT program that compares the between and within family components of association.

In addition to QTDT analysis, we verified the QTDT test by correlating the differences and the sums of the trait value for the two twins in a pair with the difference and the sums of the postulated risk/protective alleles for the two twins in a pair. A statistic for the between-pairs component of association alone is not provided in the summary output from QTDT. These tests are also provided in a simple way to estimate the proportion of trait variance explained by associated alleles. SPSS (version 10.0) was used to carry out these analyses.

Hypothesis-Driven Allele-Specific Tests

This study incorporated several measures of early ADHD symptoms, and involved genotyping several polymorphic genetic markers. In order to overcome problems of multiple

TABLE I. Five Polymorphisms Genotyped in This Study

Gene	Description	Polymorphism	Association with ADHD	Reference
DRD4 (11p15.5)	Member of the D2 receptor family; inhibits adenylate cyclase, decreases C-AMP, and is inhibitory	Exon 3 48-bp VNTR	Meta-analysis suggests association of 7-repeat allele with ADHD	Faraone et al. [2001]
DRD5 (4p15.3)	Member of the D1 receptor family; activates adenylate cyclase, increases cyclic AMP, and is excitatory	(CA)n microsatellite	Meta-analysis suggests association of allele 9 (148 bp) with ADHD	Lowe et al. [2004]
DAT1 (5p15.3)	Mediates uptake of dopamine into neurons; site of action of methylphenidate	3′UTR 40-bp VNTR	Meta-analysis suggests association of 10-repeat allele with ADHD	Curran et al. [2001]
5HT1B (6q13)	Member of the 5-HT-1 receptor family; high homology to 5-HT-1D	G861C SNP	Multi-centre study found association of 861G allele with ADHD	Hawi et al. [2002]
SNAP-25 (20p11.2)	Presynaptic plasma membrane protein; plays an important role in the synaptic vesicle membrane docking and fusion pathway	Intron 1 (TAAA)n microsatellite	Allele 5 found to be associated with ADHD and allele 2 found to be protective in a UK ADHD sample	Mill et al. [2002b]

testing and to ensure that data were analyzed within a biologically plausible framework, only prior hypotheses about specific 'risk' alleles were tested in any analyses performed. These hypotheses were deduced from the current published literature of association studies with clinical ADHD (see Table I). The specific alleles tested, with their postulated direction of effect, were: (1) *DRD4* exon 3 VNTR: 7-repeat allele (postulated risk allele), (2) *DRD5* microsatellite: allele 9 (148-bp) (postulated risk allele), (3) *DAT1* 3′UTR VNTR: 10-repeat allele (postulated risk allele), (4) *5HT1B*: allele 861G (postulated risk allele), and (5) *SNAP-25* intron 1 microsatellite: allele 2 (postulated protective allele); allele 5 (postulated risk allele).

Power of the Sample

Using the Genetic Power Calculator for variance-component QTL association for sibships (http://statgen.iop.kcl.ac.uk/gpc/), we estimated the power of this 329 sibling pairs to identify QTL associations with two-tailed tests of significance. Assuming that the markers investigated have the same allele frequency with and are in complete linkage disequilibrium with a QTL for ADHD symptoms, the sample can detect QTLs contributing as little as 1.3% to trait variance with a nominal alpha-value of 0.05 and 80% power.

RESULTS

Allele Frequencies

The allele frequencies of the candidate risk alleles closely match those seen for these markers in other studies of Caucasian populations. Frequencies of the specific alleles tested for association were: *DRD4* VNTR allele 7, 19.2%; *DAT1* VNTR allele 10, 73.1%; *DRD5* microsatellite allele 9 (148-bp), 43.7%; *5HT1B* SNP allele G, 74.0%; *SNAP-25* microsatellite allele 2, 10.9%; *SNAP-25* microsatellite allele 5, 59.7%.

QTDT Global Tests of Significance

Global tests of association were used before specific candidate alleles were investigated, primarily to investigate any potential stratification effects at each locus (using the AP test of association), and to see if any of the markers was exerting strong, global effects for any of the measures. The *P*-value from such global tests relate to an average of all alleles with a frequency greater than 5%. Stratification effects were seen for both *DRD5* and *DAT1*, but not for any of the other markers (AP statistic Table II). Where stratification effects are present, the total association model (AT) may be misleading, and only the orthogonal model (AO) should be used to test for association. Using the AO statistic, we found global evidence of association for *DAT1* (AO: $P = 0.002$) and *DRD5* (AO: $P = 0.002$), but not *SNAP-25, DRD4*, or *5HT1B*.

TABLE II. Global QTDT Tests of Association With a Composite Measure of Hyperactivity

	QTDT analysis		
	AP	AT	AO
DRD4	ns	ns	ns
DRD5	17.82 ($P = 0.003$)	ns	16.89 ($P = 0.002$)
DAT1	7.76 ($P = 0.02$)	ns	11.18 ($P = 0.002$)
5HT1B	ns	ns	ns
SNAP-25	ns	ns	ns

AP tests for population stratification, and when significant the AT test of association may give misleading results, and only the orthogonal model (AO) should be used.
ns = $P > 0.05$.

Allele-Specific Tests of Association

We found no evidence to indicate that allele-7 of the *DRD4* VNTR or the G-allele of the *5HT1B* SNP were associated with early ADHD-symptom scores in this sample. For both markers, all QTDT subtests were non-significant.

Analysis of the *DAT1* 3′UTR VNTR allele-10 showed evidence of population stratification (AP: $P = 0.006$) and thus the AT analyses should be treated with caution. It can be seen however that the within-pair AO test of association did show significant association (AO: $P = 0.0009$).

Analysis of the *DRD5* microsatellite allele-9 (148-bp) also showed evidence for population stratification (AP: $P = 0.005$), although the designated risk allele (allele 9) was associated with the composite index of ADHD symptoms using the within-pair AO test of association (AO: $P = 0.003$). However, the within-pair differences correlation between genotype and phenotype was in the opposite direction to that predicted from prior analysis of this polymorphism in clinical ADHD samples, with the 9-repeat risk allele being correlated with lower hyperactivity scores.

For the *SNAP-25* intron 1 microsatellite, no evidence was found to suggest that the putative risk allele (allele 5) was associated with increased hyperactivity scores. However, we did find significant evidence to suggest that the putative protective allele (allele 2) was associated with lower hyperactivity scores. Results from QTDT analysis for this allele showed some evidence for population stratification (AP: $P = 0.03$) and significant evidence for association using the within-pairs AO test (AO: $P = 0.02$). The within-pair differences correlation was in the predicted direction ($r = -0.18$).

DISCUSSION

In this study, we tested for association between specific ADHD risk-alleles that have previously shown association with DSM-IV ADHD, and quantitative measures of early ADHD symptoms at ages 2, 3, 4, and 7 years, in a sample of 329 male DZ twin-pairs. Two major advantages of using the TEDS twin sample was that all twin-pairs have the same age (an important asset for the investigation of developmental disorders) and family-based association methods could be used that allow both within-pair and between-pair tests of association. Furthermore, by having multiple measures of early ADHD symptoms taken at four different time points, it was possible to create a composite index that is likely to reduce the measurement error inherent in single time-point measures of behavior and thereby increase the power of the analysis. The composite index reflects a stable tendency to show a certain level of ADHD symptoms during early childhood. We have previously shown the importance of genetic influences on the stability of early ADHD symptoms in the TEDS sample, with a common factor model indicating that 90% of the stability of the ADHD symptoms scores is mediated by genetic influences (Price et al., unpublished manuscript).

A further advantage of the methods used in this study was that by using previously nominated polymorphisms within candidate genes, and having prior hypotheses about the risk/protective alleles, association-based designs could be used which should have the power to detect small effects. We found evidence to support the a priori hypotheses that the *DAT1* 3′UTR VNTR allele-10 associated with ADHD is correlated with high levels of early ADHD symptoms, and weak evidence that allele 2 of the *SNAP-25* intron 1 microsatellite is a protective allele that is correlated with lower levels of early ADHD symptoms. No evidence was found to support a role for either the 7-repeat allele of the *DRD4* exon 3 VNTR or the G-allele of a SNP in *5HT1B* in this sample. Finally, significant associations were found for allele-9 (148-bp) of the *DRD5* microsatellite, but the direction of effect was opposite to that

expected from studies on clinical ADHD samples and is therefore unlikely to represent a true finding.

Overall, population data for several of the markers, most notably *DRD4*, appear to be negative according to currently available data. Other QTL studies of *DRD4* have concurred with our finding that the *DRD4* 7-repeat allele shows no relation to quantitative measures of ADHD symptoms in population samples [Todd et al., 2001; Mill et al., 2002c; Curran et al., personal communication). Given the fairly strong overall evidence for association of this polymorphism with DSM-IV ADHD [Faraone et al., 2001], these replicated negative findings between *DRD4* and quantitative measures of ADHD symptoms are somewhat surprising, suggesting that these studies may be underpowered or that *DRD4* is not in fact a QTL for ADHD symptoms despite its role as a risk factor for ADHD. A number of factors may be involved. First, it is possible that the rating scales used in these studies are poorly correlated with an underlying distribution of genetic liability. Second, the *DRD4* VNTR may be associated with aspects of the clinical disorder not strictly related to ADHD symptoms alone. For example, it has been suggested that the association between *DRD4* and ADHD may be mediated by co-morbidity with conduct disorder [Holmes et al., 2002]. Third, it is possible that specific environmental factors, over-represented in clinical samples, may play an important role (e.g., through gene-environment interactions) and that these need to be taken into account in population-based analyses. Fourth, the sample sizes used for QTL studies to date may lack power to detect genotype-phenotype correlations, particularly if there are sources of phenotypic measurement error.

There is, however, some evidence to suggest that alleles of *DAT1* and *SNAP-25*, both previously associated with DSM-IV ADHD, may be associated with a dimensional ADHD-like phenotype. The *DAT1* 3′UTR VNTR was found to give some overall evidence for association with continuous measures of ADHD-symptoms, with the 10-repeat allele associated with higher hyperactivity scores. The within-pair differences correlation for this finding was 0.26, providing an estimate for proportion of variance of 6.8%. It is however possible for sibling-interaction effects, known to operate on parent rated SDQ measures of ADHD-symptoms, to alter the QTL variance as estimated from between- and within-sibship components of association. Any interaction, which reduces the sibling correlation, will inflate within-sibship differences and therefore also the within-sibship estimate of the QTL effect size. The significance test of the QTL effect will still be valid, however (Purcell and Sham, personal communication).

QTL association between the *DAT1* 10-repeat allele and ADHD symptom score was first suggested by Waldman et al. [1998] among unaffected siblings of ADHD probands. This allele has also been associated with DSM-IV ADHD by a number of groups worldwide and, although the association is not ubiquitous, recent meta-analyses suggest a small overall effect with evidence of heterogeneity between datasets [Curran et al., 2001; Waldman et al., personal communication]. Whilst the exact mechanism behind this association is still unknown, there is mounting evidence to suggest that this polymorphism may have direct functional effects on gene expression, or be in strong LD with another variant that confers such an effect [Madras et al., 2002; Mill et al., 2002a].

We also found some evidence for an association between allele 2 of the *SNAP-25* microsatellite marker and early ADHD-symptom scores. The association with allele 2 was only found with the composite measure of the ADHD-symptom scores that took into account each child's hyperactivity levels at ages 2, 3, 4, and 7, and not the single time-point data (data available online), therefore reflecting the stable tendency to show a certain level of ADHD symptoms. The prior hypothesis that this allele has a protective role on ADHD symptoms was supported by the results of within-pair differences analysis, which showed a significant negative correlation between the presence of the allele and hyperactivity scores ($r = -0.18$, trait variance explained = 3.2%). However, no evidence was found for an association with any other alleles, including allele 5, which appeared to be a risk allele in a clinical ADHD sample [Mill et al., 2002b].

Analyses of the *DRD5* microsatellite in the TEDS sample also highlighted some interesting results, with allele-9 (148-bp) of this highly polymorphic dinucleotide repeat appearing to be associated with the composite index of ADHD symptoms. This allele has, however, been recently nominated as a risk allele for DSM-IV ADHD in a large meta-analysis incorporating data from numerous groups [Lowe et al., 2004]. Unlike studies on clinical samples, however, we found that allele-9 was negatively associated with ADHD symptoms scores in the TEDS population sample. Such a discrepancy is currently hard to explain given that little is known about the mechanisms behind the purported association with allele-9 of this microsatellite. Assuming that the (CA)n repeat is unlikely to be a functional polymorphism itself, it is possible that different linkage disequilibrium relationships with a nearby functional variant in various sample groups are producing the discrepant findings for allele-9. However, it is more likely that this is merely a chance finding.

An interesting observation from these data is that the within-pair association analyses, which are exempted from stratification effects, show the highest degree of association between a gene and ADHD symptoms when there is stratification. In the data presented here, we found significant evidence of stratification for all three associated markers using the AP test from QTDT. Furthermore, the between-pair analyses for these marker-trait associations were all negative. However, evidence for stratification in this dataset depends on the phenotypic measure used, rather than the genotype data. This is illustrated using the *DAT1* data (Table III), where measures that provide no evidence of stratification, also find evidence of the association from the between pairs sums analysis. Similar data are seen for the other associated markers (data available online). This suggests that in this dataset, stratification effects may be acting to mask real associations.

In this dataset, the stratification effect does not appear to be genetic stratification that is usually thought to be a confounding factor in genetic association studies, but rather error in the measurement of the behavioral phenotype. The parent rated SDQ scale of ADHD symptoms used in this sample may be particularly prone to measurement error, since rater-bias giving rise to near zero DZ twin correlations is well documented for this scale. In contrast, longer rating scales that include all 18 of the ADHD DSM-IV items usually give rise to DZ correlations of around 0.25–0.3 [Thapar et al., 1999; Martin et al., 2002]. When using the SDQ scale, parents appear to contrast their DZ twins, one child against the other within a family, rather than compare a child to some precalculated childhood norm.

An additional complication is that twin studies of parent and teacher ratings of ADHD symptoms correlate only to a modest degree, around 0.3 in most studies. Twin analyses using ADHD items from the Conners' scales indicated that only 31% of the variance in teacher and parent ratings of ADHD symptoms were due to genetic effects common to both ratings, whereas 41% of the variance in parent ratings and 50% of the variance in teacher ratings were due to additional genetic effects that were unique to each [Martin et al., 2002]. Therefore, the ratings by parents and teachers reflect only partially overlapping phenotypes and genotypes. In this dataset, however, there was no evidence for any of the associations from the teacher ratings of ADHD-symptoms when considered alone (data available online). Despite this, the importance of teacher

TABLE III. Association Analysis of the 10-Repeat *DAT1* VNTR Allele Using QTDT and Sums and Differences Correlations

	QTDT analysis			Sums and differences correlations	
Measure	AP	AT	AO	Sums	Differences
1	ns	8.09 ($P = 0.002$)	5.05 ($P = 0.01$)	0.11 ($P = 0.03$)	0.13 ($P = 0.01$)
2	ns	4.11 ($P = 0.02$)	5.94 ($P = 0.007$)	0.07 (ns)	0.14 ($P = 0.008$)
3	ns	ns	ns	0.04 (ns)	0.03 (ns)
4	ns	8.04 ($P = 0.002$)	7.01 ($P = 0.004$)	0.13 ($P = 0.02$)	0.17 ($P = 0.004$)
5	6.14 ($P = 0.01$)	ns	5.29 ($P = 0.01$)	−0.07 (ns)	0.15 ($P = 0.01$)
6	ns	ns	ns	0.02 (ns)	0.11 ($P = 0.05$)
7	ns	ns	4.58 ($P = 0.02$)	−0.01 (ns)	0.15 ($P = 0.01$)
8	7.68 ($P = 0.006$)	ns	9.89 ($P = 0.0009$)	0.01 (ns)	0.26 ($P = 0.0005$)

For the main analyses presented in this study, we restricted analysis to the composite index derived by averaging across all data-points: 1 = 2-year RRPSPC, 2 = 3-year RRPSPC, 3 = 4-year SDQ, 4 = age 2-4-years composite, 5 = 7-year SDQ, 6 = 7-year teacher SDQ, 7 = 7-year parent and teacher composite, 8 = 2-, 3-, 4-, 7-year parent and teacher composite.

ratings is further supported by the observation that performance on a cognitive-experimental task associated with ADHD correlates strongly ($r > 0.5$) with teacher ratings of ADHD symptoms in a general population sample, but not with parent ratings (Kuntsi et al., unpublished data). This raises the possibility that other continuous variables, such as cognitive-experimental and other types of endophenotypes, may provide a better representation of underlying genetic liability and relate more strongly to ADHD genetic risk alleles. One potential example is the recent report of a significant association among the *DAT1* 10-repeat allele, poor performance on a sustained attention task, EEG changes, and drug response [Loo et al., 2003].

There are a number of limitations to this study. The measures of ADHD symptoms used for this study are brief and do not equate to the length or description of the diagnostic tools used to assess ADHD status, or to more in depth assessment of ADHD-symptom scores using scales such as DSM-IV checklists. The sample only contained male DZ twins and therefore provides no information on the association in females. The sample was relatively small and may therefore be underpowered when phenotypic measurement error is taken into account. Furthermore, there may be factors that cause referral biases in clinical samples that may affect comparisons with samples selected from the general population. The findings presented here thus require replicating in other population samples that have used more detailed or precise measures of ADHD symptoms and that include females before any firm conclusions can be made.

Another limitation of this study is the fact that only single markers were used to represent each gene, although we focused here on specific alleles for which there were strong a priori hypotheses. This approach assumes that the variants typed confer actual functional effects, or are in strong LD with other functional variants within the gene. In reality, however, it is unlikely that LD extends across each of the candidate genes, and thus the role of other polymorphisms within each of the genes cannot be discounted. As discussed above, there is some evidence to suggest that the *DAT1* VNTR and perhaps the DRD4 7-repeat allele have direct functional effects but it is likely that the other markers genotyped in this study are anonymous. For example, although it is often stated that the *DRD4* 7-repeat allele exerts a direct functional effect [Asghari et al., 1995], subsequent studies have failed to confirm this finding and the functionality of this allele remains uncertain [Jovanovic et al., 1999; Watts et al., 1999; Kazmi et al., 2000]. Other *DRD4* polymorphisms such as 120-bp promoter duplication may be risk variants for ADHD [McCracken et al., 2000] and further QTL studies of *DRD4* should therefore include such additional markers.

To conclude, we have used a hypothesis-driven candidate gene approach to examine the relationship between risk alleles and continuous measures of early ADHD symptoms in a general population sample of male DZ twin pairs. Our data suggest that alleles of polymorphisms in both *DAT1* and *SNAP-25* may be associated with continuous measures of hyperactivity in this sample, but we found no evidence to support a role for ADHD-risk alleles in *DRD4*, *DRD5*, and *5HT1B*.

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