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A Review and Analysis of the Relationship Between Neuropsychological Measures and *DAT1* in ADHD

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Meta-analyses indicate that the gene coding for the dopamine transporter (*DAT1* or *SLC6A3*) is associated with an increased risk for ADHD. The mechanisms of this gene for ADHD are unclear. We systematically reviewed studies linking the VNTR in the 3′ UTR of the *DAT1* to neurophysiological and neuropsychological measures. In addition, a broad set of executive/cognitive and motor tests was administered to 350 children (5–11 years) and adolescents (11–19 years) with ADHD and 195 non-affected siblings. Two VNTRs (in intron 8 and the 3′ UTR) and four SNPs (two 5′ and two 3′) in *DAT1* were genotyped. The effect of the polymorphisms on neuropsychological functioning was studied. The review indicated that the majority of studies did not find a relation between *DAT1* and neurophysiological or neuropsychological measures. In our sample, several of the polymorphisms of *DAT1* were associated with ADHD and ADHD was associated with impaired neuropsychological functioning. However, none of the *DAT1* polymorphisms was convincingly associated with neuropsychological dysfunctioning. This suggests that the effect of *DAT1* on ADHD was not mediated by neuropsychological performance. However, since *DAT1* is mainly expressed in the striatum and not the prefrontal cortex, it may influence striatum-related functions (such as delay aversion) more heavily than prefrontal related functions (such as executive functions). Associations of *DAT1* with ADHD were only found in adolescents, which may suggest that *DAT1* mainly exerts its effect in adolescence, and/or that having a more persistent form of ADHD may mark a more severe or homogeneous genetic form of the disorder. © 2008 Wiley-Liss, Inc.

KEY WORDS: ADHD; *DAT1*; *SCL6A3*; review; non-affected siblings; neuropsychology; endophenotype

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INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) [American psychiatric Association, 1994] is strongly heritable. Based on the results of multiple twin studies conducted worldwide, ADHD has an estimated heritability of approximately 76% [Faraone et al., 2005]. Several reviews and meta-analyses have been published on the involvement of dopaminergic genes in ADHD [Swanson et al., 2000; Curran et al., 2001; Faraone et al., 2001, 2005; Maher et al., 2002; Li et al., 2006; Yang et al., 2007]. Most meta-analyses have shown evidence for the involvement of genes coding for dopamine receptors 4 and 5 (*DRD4* and *DRD5*, respectively), the gene encoding for the dopamine transporter (*DAT1* or *SLC6A3*), and the gene coding for the enzyme dopamine beta-hydroxylase (*DBH*) [Faraone et al., 2005]. We focus here on the role of the *DAT1* gene in ADHD, since it is one of the most studied genes in ADHD [Thapar et al., 2005].

DAT1 is located on chromosome 5p15.3. The most widely studied polymorphism is a 40 base pair variable number of tandem repeats (VNTR) polymorphism located in the 3′ untranslated region (UTR) of the gene [Maher et al., 2002; Faraone et al., 2005; Yang et al., 2007]. The number of repeats ranges between 3 and 13 [Vandenbergh et al., 1992; Nakatome et al., 1996], with 10 and 9, respectively, being the most common [Mitchell et al., 2000]. Although this polymorphism is not located in a translated region of the gene, it may have an effect on gene expression [Mill et al., 2002]. This has been investigated in vitro as well as in vivo, with conflicting results [Madras et al., 2005; Brookes et al., 2007]. It has been suggested that the 10-repeat allele is associated with an abnormally active dopamine transporter, resulting in an increased re-uptake of dopamine and thus in a depletion of dopamine in the synaptic cleft [Mill et al., 2002]. This may lead to hypoactivity of the dopaminergic pathways [Yang et al., 2007]. *DAT1* is mainly expressed in the striatum and to a lesser extent in the prefrontal cortex [Diamond, 2007]. Eliminating *DAT1* gene function in mice increases hyperactivity and disinhibition [Giros et al., 1996]. However, the exact

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mechanisms of the *DAT1* effect on ADHD pathology remain unclear. Several studies have tried to unravel the modes of action of *DAT1* by studying the association of the gene with neurophysiological and neuropsychological measures. Neurophysiological and neuropsychological measures may function as endophenotypes (intermediate phenotypes): heritable, underlying, continuously distributed traits that heighten the risk for developing a disorder and mediate between genotype and phenotype [Gottesman and Gould, 2003]. Endophenotypes are proposed to be more heritable than phenotypes because they are etiologically "closer" to the disease genes than phenotypes and offer the advantage of a quantitative trait instead of dichotomous entities like *DSM* diagnostic categories [Gottesman and Gould, 2003]. Therefore, focusing on neurophysiological and neuropsychological measures in relation to *DAT1* in ADHD may provide insight into the pathways leading from *DAT1* to ADHD.

Review of Studies Linking *DAT1* to Neurophysiological and Neuropsychological Measures

An overview of the studies cited here is provided in Table I. Most studies have compared the 10/10 genotype of the 3′ UTR VNTR with the 9/10 and 9/9 genotypes. The results are inconsistent and tend to suggest no significant association of the VNTR with neurophysiological and neuropsychological measures. With respect to IQ, one study reported that the 10/10 genotype was associated with a lower IQ in two independent samples of ADHD children, but not in controls, suggesting a common genetic basis for ADHD and low IQ [Mill et al., 2006]. However, this finding has not been replicated by a study using a substantially larger sample of ADHD children and their non-affected siblings nor in a study of affected sib-pairs from 251 families [Sonuga-Barke et al., 2008; Loo et al., submitted]. Three other studies using non-ADHD subjects found no relation between *DAT1* and IQ either [Ball et al., 1998; Rueda et al., 2005; Genro et al., 2006]. Therefore, most studies suggest no relation between *DAT1* and IQ.

The three largest studies ($N = 540$, $N = 146$, and $N = 122$) exploring several neuropsychological functions (attention and several executive functions) in relation to *DAT1* in ADHD found no or only few associations [Barkley et al., 2006; Wohl et al., 2008; Loo et al., submitted]. No differences were reported in the largest study between ADHD adolescents and adults with the 10/10 genotype and ADHD adolescents and adults with the 9/10 genotype on 14 of 15 variables [Barkley et al., 2006]. On one variable of inhibition, the 10/10 genotype performed more poorly than the 9/10 genotype. The second largest study did not find differences between the 10 allele and 9 allele on measures of inhibition and cognitive flexibility [Wohl et al., 2008]. The other study did not find any differences between carriers of the 10/10 genotype and others on 14 of 14 variables, except for one interaction (10/10 in combination with ADHD in the mother related to poorer set-shifting) [Loo et al., submitted]. Several other studies, utilizing substantially smaller samples ($N < 100$), also reported mainly negative results. One study found no effect of *DAT1* on 10 of 10 working memory variables, though the 10/10 genotype appeared related to poorer (selective) attention (1 of 4 measures) and inhibition (1 of 1 measure) [Cornish et al., 2005]. Furthermore, no relation was found between *DAT1* and 24 of 25 variables measuring several executive and non-executive functions, except for 1 of 4 measures of inhibition: Adults with the 10/10 genotype contrarily displayed a better inhibition than adults with other genotypes [Boonstra et al., 2007]. Negative or contrary findings were also reported in two other studies, who found no effects of *DAT1* on 2 of 3 measures of vigilance and 15 of 16 measures of attention, except for the one finding that individuals with the 10/10 genotype committed fewer errors than individuals with the 9/10 and 9/9 genotype and less omission errors in the first quarter of a test of attention [Oh et al., 2003; Kim et al., 2006]. In addition, no differences were reported between the 10/10 genotype and other genotypes for regional blood flow during a vigilance task [Szobot et al., 2005].

Similar negative or contrary findings were found when the effect of *DAT1* (10/10 vs. other genotypes) was studied in healthy individuals. One study found no effect on 4 of 4 measures of attention [Fossella et al., 2002]; another study found no effect on 8 of 8 measures of episodic memory, although the 10/10 genotype had less midbrain activation during task performance [Schott et al., 2006]; another study found no effect on 2 of 4 measures of attention and a reversed effect on the other 2 (10/10 performing better on a conflict task and showing stronger ERPs) [Rueda et al., 2005]; another study found no effect of *DAT1* on ERPs (except for a stronger gamma response to target stimuli in 10/10 genotype controls) [Demiralp et al., 2007].

The findings reported above suggest that *DAT1* is not associated with neuropsychological and neurophysiological abnormalities frequently reported in ADHD. However, some studies did report a relation of *DAT1* with these measures. In two studies, the same authors found that ADHD children with the 10/10 genotype displayed an abnormal reduction in attentional asymmetry (i.e., reduced leftward inattention) and increased response variability compared to affected children with other genotypes [Bellgrove et al., 2005a,b]. The same authors also reported that normal children with the 10/10 genotype (or the 3/3 (now called 6/6) genotype in intron 8 which is in moderate linkage disequilibrium with the VNTR in the 3′ UTR) displayed inattention for left-sided stimuli [Bellgrove et al., 2007]. Other researchers found abnormalities in vigilance and EEG activity in response to methylphenidate in ADHD affected children with the 10/10 genotype [Loo et al., 2003]. In addition, one study reported that the 10/10 genotype had no effect on performance on an inhibition task in children with ADHD, their non-affected siblings and controls, yet the 10/10 genotype was associated with lower activation patterns in the striatum during the task in children with ADHD and their non-affected siblings, but not controls [Durston et al., 2008]. The authors suggest that *DAT1* gene effects in the striatum may be involved in translating the genetic risk of ADHD into a neurobiological substrate.

Although the majority of studies report no effects of *DAT1* on neurophysiological and neuropsychological measures, methodological aspects may have contributed significantly to the observed pattern of results. For example, differences in ascertainment (different ADHD subtypes, including controls or not), differences in ADHD measurement methods (interview of questionnaires), sampling (clinically referred or not), participants characteristics (such as age, sex, and comorbidity) and the focus on a single polymorphism are plausibly related to null effects. Given that some effects of *DAT1* on neurophysiological and neuropsychological measures have been found, further research is needed to understand the nature and extent of these effects.

Current Study on the Relation Between *DAT1* and Neuropsychological Measures in ADHD

We sought to improve upon previous studies described above in several respects. First, we recruited a large sample of ADHD subjects ($N = 350$). Most previous studies have utilized much smaller samples, increasing the chance of obtaining spurious results. We further extended our sample with 195 non-affected siblings of ADHD children. Second, we analyzed the effect of *DAT1* separately for children and adolescents, since we expected to find stronger effects of *DAT1* in adolescents. Levels of dopamine decrease with age and the effect of the *DAT1*

TABLE I. Overview of Studies Reporting on the 40-bp VNTR of the 3′ UTR of *DAT1* in Relation to Neurophysiological and Neuropsychological Measures

Authors	N	Age (years)	Measure(s)	Test	Results
Ball et al. [1998]	51 high general cognitive ability	6–15	IQ	10/10 vs. 9/10 vs. 9/9	No differences in allele frequency in high and normal IQ groups
	51 normal general cognitive ability	6–15			
Barkley et al. [2006]	122 ADHD followed longitudinally	12–20 → 19–25	Attention, EF	10/10 vs. 9/10	No differences on 8 of 9 variables when tested in adolescence, except for 1 variable: 10/10 more easily distracted, but only in controls and not in ADHD. No differences on 5 of 6 variables when tested in adulthood, except for 1 variable: 10/10 poorer inhibition (reflected by less earned money)
	67 controls followed longitudinally	12–20 → 19–25			
Bellgrove et al. [2005a]	43 ADHD	6–16	Spatial attention asymmetry	10/10 vs. 9/10 vs. 9/9	For the 1 variable: 10/10 decreased (normal) asymmetry
Bellgrove et al. [2005b]	22 ADHD	M = 12.7	Sustained attention, response variability, spatial attention asymmetry	10/10 vs. 9/10 and 9/9	No difference on 3 of 5 variables. Other 2 variables: 10/10 decreased (normal) asymmetry and increased response variability
	20 controls	M = 11.8			
Bellgrove et al. [2007]	51 normals	9–16	Spatial attention asymmetry	10/10 vs. 9/10 and 9/9 3/3 vs. 2/3 and 2/2 (intron 8) 10-3 haplotype	No main effect on the 1 measure of overall performance, but an interaction was present: 10/10, 3/3 and 10-3 haplotype displayed inattention for left-sided stimuli
Boonstra et al. [2007]	45 ADHD	M = 39.1	EF (fluency, planning, working memory, set shifting, inhibition) and non-EF	10/10 vs. others	No differences on 24 of 25 variables. Other 1 variable: 10/10 better inhibition
Cornish et al. [2005]	58 > 90th percentile SWAN scale	6–11	Selective and sustained attention, inhibition, verbal and visuo-spatial and central executive working memory	10/10 vs. 10/other	No differences for 13 of 15 variables. Other 2 variables: 10/10 poorer for selective attention and inhibition. No differences for working memory
	68 < 10th percentile SWAN scale	6–11			
Demiralp et al. [2007]	50 normals	M = 21.5	Auditory target detection	10/10 vs. others	No differences on 2 of 2 variables. No main effect of genotype on evoked gamma response, but 10/10 showed a specific stronger gamma response to target stimuli compared to standard stimuli
Durston et al. [2005]	26 ADHD	Children	MRI volume analyses of prefrontal gray matter and caudate nucleus	10/10 vs. others	No differences for prefrontal gray matter volume, but 10/10 less volume of caudate nucleus
	26 non-affected siblings	Children			
	20 controls	Children			
Fossella et al. [2002]	200 normals	Adult	Attention	10/10 and 9/10 vs. 9/9	No significant differences for 4 of 4 variables

Genro et al. [2006]	242 normals 100 normals 220 normals	Children Children Adults	IQ	10/10 vs. others	No differences for IQ
Kim et al. [2006]	85 ADHD	M = 9.7	Vigilance and IQ	10/10 vs. 9/10 and 9/9	No differences for 5 of 6 variables. One variable: 10/10 Better vigilance (reflected by less commission errors)
Loo et al. [2003]	27 ADHD	8–13	EEG and vigilance	10/10 vs. 9/10 and 9/9.	On 5 of 5 variables: 10/10 poorer for measures of vigilance. 10/10 abnormal EEG response to methylphenidate
Loo et al. [submitted]	540 ADHD (from 251 families)	6–18	Inhibition, interference control, set-shifting, IQ	10/10 vs. 9/10 and 9/9	No differences for 14 of 14 variables, except for an inter-action: 10/10 in combination with ADHD in mother had poorer set-shifting
Mill et al. [2006]	171 ADHD (sample 1) 1,758 controls (sample 1) 49 ADHD (sample 2) 745 controls (sample 2)	5 5 7, 9, 11, 13 7, 9, 11, 13	IQ (combined score of 4 assessments in sample 2)	10/10 vs. 9/10 and 9/9	In both independent samples, 10/10 lower IQ, but only in ADHD subjects and not in controls
Oh et al. [2003]	44 ADHD	M = 8.6	Attention	10/10 vs. others	No differences for 15 of 16 variables. For one variable: 10/10 better attention (reflected by less omission errors) in first quarter of test
Rueda et al. [2005]	73 normals	4–6	ERP, attention, and IQ	10/10 vs. 9/10 and 9/9	No differences for 5 of 7 variables. For the other 2: 10/10 better attention (i.e., better conflict score) and ERP
Schott et al. [2006]	51 normals	18–31	Episodic memory and fMRI	10/10 vs. 9/10 and 9/9	No differences for 8 of 8 variables of episodic memory, but 10/10 less midbrain activation
Sonuga-Barke et al. [2008]	702 ADHD 694 non-affected siblings	M = 10.8 M = 10.9	IQ	10/10 vs. 9/10 and 9/9	No differences for IQ
Szobot et al. [2005]	34 ADHD	M = 11.6	Vigilance using SPECT	10/10 vs. others	No differences in regional cerebral blood flow during the task
Wohl et al. [2008]	146 ADHD	6–16	Inhibition and cognitive flexibility	10 vs. 9	No difference on 2 of 2 variables

ADHD, attention-deficit/hyperactivity disorder; IQ, intelligence quotient; EF, executive functioning; SWAN, strengths and weaknesses of ADHD symptoms and normal behavior scale; (f)MRI, (functional) magnetic resonance imaging; EEG, ElectroEncephaloGram; ERP, event related potential; SPECT, single photon emission computed tomography.

genotype may, therefore, be stronger in adolescents compared to children [Barkley et al., 2006]. For example, one study reported that the effects of *DAT1* on phenotypical measures of ADHD were stronger, when the longitudinally followed sample was studied in adolescence and adulthood compared to childhood [Barkley et al., 2006]. Third, we applied a broad neuropsychological battery covering not only executive/cognitive functions, but also motor functions. Previous studies have shown that *DAT1* mainly effects dopamine neurotransmission in the basal ganglia and midbrain, but less in the prefrontal cortex [Durston et al., 2005; Schott et al., 2006; Diamond, 2007; Scherk et al., 2007]. Therefore, we expected that the effect of *DAT1* was on motor measures and not (or to a lesser extent) on executive/cognitive measures. Fourth, almost all previous studies except two have studied only the effect of the VNTR in the 3′ UTR of *DAT1* [Bellgrove et al., 2007; Sonuga-Barke et al., 2008]. Although this polymorphism may influence gene expression [Mill et al., 2002], this is not a well established finding [Thapar et al., 2005; Brookes et al., 2007]. The VNTR in the 3′ UTR may also be in linkage disequilibrium with the true functional polymorphism [Brookes et al., 2006; Asherson et al., 2007]. Therefore, we applied a more thorough investigation of the *DAT1* effect on neuropsychological measures, by genotyping 6 polymorphisms (VNTR in the 3′ UTR, VNTR in intron 8, 2 SNPs in the 5′ flanking region, 1 SNP in intron 10 and 1 SNP in intron 13). Two haplotypes (combination of alleles transmitted together) were formed, one from the VNTRs, one from the SNPs, which allowed for robust analyses of the *DAT1* effect, since the haplotypes might tag other variants that are not directly tested [Sklar, 2005] and may be more strongly associated with disease or trait than individual polymorphisms [Barr et al., 2001].

In order to examine the neuropsychological mechanisms of *DAT1* in ADHD, we first confirmed the association between polymorphisms in *DAT1* with ADHD diagnosis in our sample. The association between ADHD diagnosis and neuropsychological dysfunction was confirmed [Rommelse et al., 2007a,b,c, 2008a,b]. Thereafter, we went on to examine the association between risk polymorphisms in *DAT1* and neuropsychological dysfunctions.

MATERIALS AND METHODS

Subjects

Participants were recruited through child psychiatric clinics in the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study that aims to identify genes that increase the risk for ADHD using QTL linkage and association strategies [Brookes et al., 2006]. A total of 238 families with at least one child with the combined subtype of ADHD (proband) and at least one additional sibling (regardless of possible ADHD-status) participated. This resulted in the participation of an additional 112 affected siblings (64 with combined subtype, 28 with inattentive subtype and 20 with hyperactive-impulsive subtype) and 195 non-affected siblings. Two groups were formed: one group of affected participants (N = 350, M age = 12.0, % boys = 75.7, *T*-score ADHD Total Conners' parent = 74.2, *T*-score ADHD Total Conners' teacher = 67.8) and one group of non-affected participants (N = 195, M age = 11.5, % boys = 45.6, *T*-score ADHD Total Conners' parent = 48.1, *T*-score ADHD Total Conners' teacher = 48.1). Non-affected siblings did not differ from control in Conners' ADHD measures [see Rommelse et al., 2007b]. All subjects were between the ages of 5 and 19 years old and were of European Caucasian descent. Participants were excluded, if they had an IQ < 70, a diagnosis of autism, epilepsy, brain disorders or known genetic disorders, such as Down syndrome or Fragile-X-syndrome.

The screening procedures and measures for phenotyping have been described previously [Brookes et al., 2006; Lasky-Su et al., 2007]. Briefly, screening questionnaires (parent and teacher Conners' long version rating scales [Conners, 1996] and parent and teacher Strengths and Difficulties Questionnaires [Goodman, 1997]) were used to identify subjects with ADHD symptoms. Scores were considered clinical if *T*-scores on Conners' ADHD-subscales (*DSM-IV* Inattention, *DSM-IV* Hyperactive-Impulsive, and *DSM-IV* ADHD Total) were ≥63 or scores on the SDQ-hyperactivity scale were >90th percentile. Additionally, the Parental Account of Children's Symptoms (PACS) [Taylor, 1986] was administered to subjects scoring clinically on any of the questionnaires. Impairment was determined as significant if functioning was impaired in home situations and/or at school. For diagnostic purposes, data of the questionnaires and the PACS were subjected to a standardized algorithm to derive each of the 18 *DSM-IV* ADHD symptoms, providing operational definitions for each behavioral symptom [Rommelse et al., 2007a].

Neuropsychological Tasks

The ten neuropsychological tasks used in this study have been described and analyzed elsewhere [Rommelse et al., 2007a,b,c, 2008a,b] and are presented in Table II. Missing data was less than 5% for all variables, except for the Stop task (9%). Based on previous results [Rommelse et al., 2007a,b,c, 2008a,b], the variable for each task, which showed the most optimal results in the endophenotypic analyses, was chosen for

TABLE II. Description of the Neuropsychological Tasks

Task	Aim of measurement	Dependent variable
Executive/cognitive tasks		
Stop task	Inhibition	Stop signal reaction time (SSRT)
Shifting attentional set	Inhibition and cognitive flexibility	Percentage of errors
Time test	Time reproduction	Accuracy (total absolute deviation between stimulus and response)
Visuo-spatial sequencing	Visuo-spatial working memory	Number of correct targets in the correct order
Digit span	Verbal working memory	Digit span backwards
Motor tasks		
Pursuit	Motor control under continuous adaptation	Precision
Tracking	Motor control without continuous adaptation	Precision
Tapping	Self-generated motor output	Variability in tapping rate
Baseline speed	Motor output as response to external cue	Variability in reaction times
Motor timing	Timing of motor output	Variability in reaction times

Full description of the tasks can be found in Rommelse et al. 2007a,b,c, 2008a,b].

analysis. All variables were normalized and standardized using a Van der Waerden transformation (Statistical Package for the Social Sciences version 14). To obtain a robust measure of overall neuropsychological functioning with less error variance than the individual task measures, a principal component analysis was performed on the ten task variables. All ten task measures related to one major component, explaining 47% of the variance in the task measures [see Rommelse et al. 2008c for more detail].

DNA Extraction and DAT1 Genotyping

An elaborate description of DNA extraction and (*DAT1*) genotyping is provided elsewhere [Brookes et al., 2006]. Briefly, DNA was extracted directly from blood samples or cell lines at Rutgers Cell line and DNA repository in the US. Two VNTRs and four single nucleotide polymorphisms (SNPs), which had been genotyped in earlier studies in the IMAGE sample and had shown association with ADHD in this sample were selected for the current study [Brookes et al., 2006; Asherson et al., 2007]. The two VNTRs (40 bp VNTR in the 3′ UTR and 30 bp VNTR in intron 8) had been genotyped in a sample of 1168 IMAGE families, which included 220 of the Dutch families that were part of the current study. Genotyping had been performed using standard polymerase chain reaction (PCR) protocols and visualization of amplified products on 2% agarose gels as described before [Brookes et al., 2005]. The four SNPs (rs2550946, rs11564750, rs3776513, and rs40184) had been genotyped in a sample of 1050 IMAGE families, including 184 Dutch families from this study. Genotyping had been done using the Illumina Golden Gate Assay™ (Illumina, Inc., San Diego, CA) [Brookes et al., 2006], ABI SNPlex (rs3776513) [Tobler et al., 2005] and ABI TaqMan (rs2550946, rs11564750, and rs40184) genotyping platforms (Applied Biosystems, Foster City, CA) [for more details see Brookes et al., 2008]. See Figure 1 for linkage disequilibrium between the polymorphisms.

VNTR genotypes were available for 89.3% of the subjects and we estimated the missing genotypes using haplo.stats [Sinnwell and Schaid, 2005]. Briefly, haplo.stats estimates haplotype frequencies and posterior probabilities of haplotype pairs for a subject, conditional on the observed marker data [Schaid et al., 2002]. Eight different VNTR haplotypes were present in the sample based on the VNTR in the 3′ UTR and the VNTR in intron 8 (Table III). The risk 10-6_10-6 diplotype was present in 52.5% of the sample. A diplotype was defined as a pair of haplotypes from a given participant: one haplotype received from each parent. Thus, a participant has only one diplotype. Genotypes of all four SNPs were available for 344 subjects (63.1%). Since the proportion of missing genotypes may increase with the number of SNPs available in our data set, we decided not to estimate missing data for this set of markers given the fact that the posterior probability for a genotype may be greatly reduced. Twelve different SNP haplotypes were found in the sample based on two SNPs in the 5′ flanking region, one SNP in intron 10 and one SNP in intron 13 (Table III). The haplotype which had increased the risk for ADHD in an earlier study (XXGC_XXGC) [Brookes et al., 2008] was present in 25.6% of the sample.

TABLE III. *DAT1* Haplotype Frequency in the Entire Sample

Haplotype	%
VNTR haplotype	
10-6	72.7
9-5	13.4
9-6	7.2
10-5	4.2
10-9	1.1
11-9	0.7
11-6	0.5
8-6	0.3
	100.0
SNP Haplotype	
GGGC	35.8
AGGT	16.3
GGTT	11.0
AGGC	10.9
GGGT	9.6
AGTT	6.7
ACGC	4.9
ACGT	3.3
GGTC	0.9
ACTC	0.4
AGTC	0.1
GCGC	0.1
	100.0

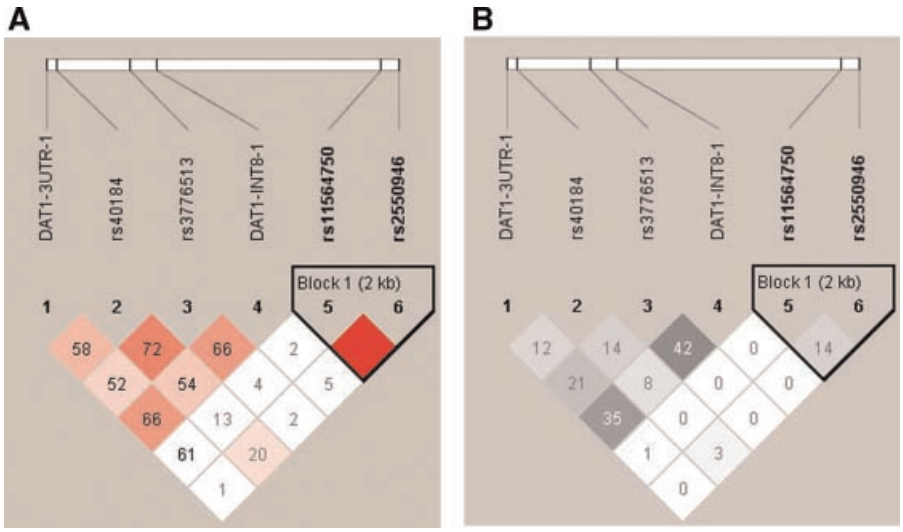

Fig. 1. Linkage disequilibrium values determined by D' (**left panel**) and r^2 (**right panel**). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Data Analysis

Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental *DAT1* genotype information using the Markov–Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3 [Raymond and Rousset, 1995]. No deviations from HWE were detected for any of the polymorphisms (df = 2, *P* values between 0.061 and 0.500).

Association tests for the single markers were based on comparison of the risk genotype with a group consisting of all other genotypes. For this, the risk haplotype was defined as the genotype that had shown association with ADHD in earlier studies with a group of all other genotypes [Brookes et al., 2006, 2008; Asherson et al., 2007]. In order to test the association between *DAT1* and ADHD in our sample, we analyzed whether affected and non-affected siblings differed in proportions of risk and non-risk genotypes on the six individual markers and in the proportion of risk and non-risk diplotype transmission using χ^2 comparisons.

The association between ADHD and poor neuropsychological test performance was analyzed using linear mixed models with diagnosis as between group factor, age as covariate, and family structure as random effect to account for within family correlation [Rommelse et al., 2007a,b,c, 2008a,b]. An aggregated neuropsychological component was used as dependent measure of overall neuropsychological functioning.

The association of *DAT1* with neuropsychological performance was analyzed using a linear mixed model with *DAT1* as factor (risk vs. non-risk genotypes/diplotypes), age as covariate, and family structure as random effect. The aggregated neuropsychological component was used as dependent measure.

All analyses were conducted first for all participants, and repeated after splitting the sample by median age (children <11.5 years and adolescents >11.5 years), since previous studies had shown that the relation of genetic risk markers with neuropsychological functioning as well as ADHD diagnosis is not constant across age [Barkley et al., 2006; Elia and Devoto, 2007]. Correction for multiple comparisons according to the False Discovery Rate (FDR) controlling procedure was applied to the analyses with a *q*-value setting of 0.05 [Benjamini and Hochberg, 1995].

RESULTS

Association of DAT1 With ADHD Diagnosis

As shown in Table IV, significant findings were restricted to the adolescent sample after correction for multiple testing: three single risk markers (10/10 genotype in 3′ UTR VNTR, GG in intron 10 and CC in intron 13) and both risk diplotypes (10-6_10-6 and XXGC_XXGC) were more common in affected adolescents compared to non-affected adolescents. Also, the co-occurrence of the two risk diplotypes together was more common in affected than in non-affected adolescents (Table IV), which was at least partly due to the linkage disequilibrium between the VNTRs and the 3′ SNPs (Fig. 1).

Association of ADHD Diagnosis With Neuropsychological Performance

As previously reported [Rommelse et al., 2007a,b,c, 2008a,b], affected siblings also performed more poorly on the neuropsychological tasks than their non-affected siblings (F (1, 329.4) = 32.90, $P < 0.001$). This result was robust, when analyses were repeated for children and adolescents, separately (F (1, 196.0) = 21.54, $P < 0.001$ and F (1,190.7) = 23.00, $P < 0.001$), indicating ADHD diagnosis to be associated with poorer neuropsychological performance within families.

TABLE IV. Comparison of Frequencies of *DAT1* Risk Markers Between Affected and Non-Affected Siblings

				Overall (O)			Children (C)			Adolescents (A)			% affected/non-affected with risk marker		
Marker	Location	Risk allele(s)	Test	χ^2	df, N	*P*	χ^2	df, N	*P*	χ^2	df, N	*P*	O	C	A
Single marker															
VNTR 3′ UTR	3′ UTR	10	10/10 vs. others	1.76	1, 545	0.09	0.28	1, 267	0.30	**5.77**	**1, 278**	**0.008**	64/58	59/62	**68/53**
VNTR intron 8	Intron 8	6	6/6 vs. others	0.36	1, 545	0.27	4.33	1, 267	0.02	1.54	1, 278	0.11	65/68	60/73	69/62
SNP rs2550946	5′ flanking	G	GG vs. AA and AG	1.13	1, 344	0.14	2.35	1, 165	0.06	0.00	1, 179	0.50	36/31	38/26	35/35
SNP rs11564750	5′ flanking	G	GG vs. CC and CG	0.86	1, 344	0.18	0.10	1, 165	0.38	0.99	1, 179	0.16	84/80	83/80	86/80
SNP rs3776513	Intron 10	G	GG vs. TT and TG	1.09	1, 344	0.15	0.93	1, 165	0.17	**5.67**	**1, 179**	**0.009**	69/63	65/73	**72/54**
SNP rs40184	Intron 13	C	CC vs. TT and TC	3.09	1, 344	0.04	0.04	1, 165	0.42	**5.23**	**1, 179**	**0.01**	30/21	29/28	**31/15**
Haplotype															
VNTR haplotype		10-6	10-6 vs. others	0.36	1, 545	0.28	1.72	1, 267	0.10	**5.10**	**1, 278**	**0.01**	53/51	50/59	**56/42**
SNP haplotype		XXGC	XXGC_XXGC vs. others	3.39	1, 344	0.03	0.00	1, 165	0.49	**7.06**	**1, 179**	**0.004**	29/19	27/28	**30/11**
VNTR and SNP haplotype		10-6 and XXGC	10-6_10-6 with XXGC_XXGC vs. others	3.52	1, 344	0.03	0.00	1, 165	0.48	**6.63**	**1, 179**	**0.005**	24/15	22/22	**26/9**

P values are one-sided. Significant findings corrected for multiple testing in bold.

Association of DAT1 With an Aggregated Neuropsychological Measure

As shown in Table V, no effect on neuropsychological performance was found for any of the single markers or diplotypes, neither in the whole sample nor in the separate analyses in children or adolescents. Thus, the single markers and diplotypes that showed association with the ADHD diagnosis in adolescents were not related to impaired neuropsychological functioning. Moreover, the siblings with the highest possible risk (those with both risk diplotypes) did not differ from other siblings in neuropsychological performance.

Additional Analyses

A clear pattern of results emerged: *DAT1* was associated with ADHD, ADHD was associated with impaired neuropsychological performance, but *DAT1* was not associated with impaired neuropsychological performance. To further substantiate these findings, we sought to reject the hypothesis that we missed a relationship between *DAT1* and neuropsychological performance due to three possibilities.

Possibility 1: DAT1 is only associated with specific neuropsychological measures and not an overall neuropsychological measure. If *DAT1* were associated with specific neuropsychological functions, the overall measure we used might have clouded this relation. Therefore, we repeated the analyses, as described above, for each of ten neuropsychological measures. An additional 270 statistical tests were performed. Of the 90 statistical tests in the entire sample, only two were nominally significant, which is a finding one would expect to find by chance and did not survive multiple testing. Interestingly, though, the two tests showed similar results: a risk genotype (6/6 in intron 8), and the combination of risk diplotypes (10-6_10-6 with XXCG_XXCG) were associated with increased variability of motor timing (F (1, 470.7) = 4.63, $P = 0.03$; F (1, 322.0) = 4.60, $P = 0.03$). In the child subsample, also only two of 90 statistical tests were nominally significant and did not survive multiple testing correction: one risk marker (SNP rs3776513 in intron 10) was associated with a poorer neuropsychological score (Tapping: F (1, 159.0) = 4.55, $P = 0.04$), another risk marker (SNP rs2550946 in the 5′ flanking region) was associated with a better neuropsychological score (Motor Timing: $F(1, 143.9) = 5.48, P = 0.02$). In the adolescent subgroup, two significant and six nominally significant associations emerged. Two of these six findings were described above (i.e., risk markers associated with increased motor timing variability, P values in the adolescent subsample were 0.007 [significant] and 0.04, respectively). Three of the other four associations were for a *DAT1* marker (SNP rs11564750 in the 5′ flanking region) that had not shown association with ADHD in adolescents (Pursuit: F (1, 167.4) = 4.42, $P = 0.04$; Tracking: F (1, 174.2) = 8.43, $P = 0.004$ [significant]; Motor Timing: F (1, 143.4) = 4.20, $P = 0.04$). Therefore, these associations do not shed light on the function of *DAT1* in relation to ADHD. The other nominally significant finding was for the GG genotype in intron 10 and poorer accuracy in Tracking (F (1, 174.3) = 4.15, $P = 0.04$). The results indicate a lack of association between *DAT1* and an aggregated neuropsychological factor was most likely not due to a specific relation between *DAT1* and a neuropsychological functioning that was overlooked in the former analyses.

Possibility 2: DAT1 is only associated with neuropsychological performance in non-affected siblings and not in affected siblings. The non-significant relation between *DAT1* and overall neuropsychological functioning may be attributable to a differential effect of *DAT1* on neuropsychological functioning in affected and non-affected subjects. We reasoned that the effect of a gene may be more purely studied in non-affected siblings than in affected subjects, since this latter group may have accumulated so many risk genes and risk environmental factors that the (small) functional effect of one gene may be obscured. Such a discrepancy in results has indeed been reported in a MRI-based study on brain volume (10/10 genotype at the 3′ UTR VNTR showed smaller caudate nucleus volume only in non-affected siblings and not in affected subjects [Durston et al., 2005]). Therefore, analyses were repeated separately for affected and non-affected siblings. Results revealed some nominal associations between *DAT1* and the aggregated score of neuropsychological functioning in non-affected siblings and not in affected siblings. However, these were present only in children and appeared spurious (data not shown). Thus, the non-significant relation between *DAT1* and the aggregated neuropsychological component reported in the main analyses was not likely due to a differential effect of *DAT1* on neuropsychological functioning in affected and non-affected subjects.

Possibility 3: DAT1 is only associated with neuropsychological performance in subjects without conduct disorder and not in subjects with conduct disorder. Recently, in the larger IMAGE sample we demonstrated that the association between *DAT1* and ADHD was only significant for subjects without conduct disorder as opposed to ADHD subjects with conduct disorder [Zhou et al., 2007]. Since the same may be true for the association between *DAT1* and neuropsychological functioning, we repeated the analyses for subjects without possible conduct disorder (i.e.,

TABLE V. Association of *DAT1* Risk Markers With an Aggregated Neuropsychological Component Score

		Overall		Children		Adolescents	
DAT1	Test	*F*	*P*	*F*	*P*	*F*	*P*
Single marker							
VNTR 3′ UTR	10/10 vs. others	0.67	0.42	0.32	0.57	2.58	0.11
VNTR intron 8	6/6 vs. others	0.03	0.86	0.92	0.34	0.79	0.38
SNP rs2550946	GG vs. AA and AG	1.15	0.29	1.15	0.29	0.40	0.53
SNP rs11564750	GG vs. CC and CG	0.77	0.38	0.01	0.97	2.48	0.12
SNP rs3776513	GG vs. TT and TG	0.12	0.73	0.04	0.83	0.62	0.43
SNP rs40184	CC vs. TT and TC	0.29	0.59	0.14	0.71	1.12	0.29
Haplotype							
VNTR haplotype	10-6_10-6 vs. others	0.24	0.63	0.35	0.55	1.36	0.25
SNP haplotype	XXGC_XXGC vs. others	0.30	0.58	0.12	0.73	1.26	0.26
VNTR and SNP haplotypes	10-6_10-6 with XXGC_XXGC vs. others	1.06	0.30	0.00	0.99	1.62	0.21

Conners' Oppositional Behavior *T*-score parents and teachers $\leq$75). A total of 109 affected siblings (from the original 350) and 10 non-affected siblings (from the original 195) were excluded from analyses. None of the *DAT1* risk markers was associated with the aggregated neuropsychological component score, neither in the overall group nor in the separate subgroups of children and adolescents (data not shown).

DISCUSSION

A review of studies conducted thus far on the neurophysiological and neuropsychological effects of *DAT1* in ADHD (see column 2 and Table I) demonstrated inconsistent results. Most studies have compared the 10/10 genotype with other genotypes of this polymorphism in the 3′ UTR and did not find differences in neurophysiological and neuropsychological measures. In some cases, ADHD patients with the 10/10 genotype performed worse than ADHD patients with other genotypes on measures of attentional asymmetry, response variability, vigilance and EEG activity in response to methylphenidate [Loo et al., 2003; Bellgrove et al., 2005a,b, 2007]. However, contrary findings have also been reported, in which ADHD patients with the 10/10 genotype performed better than ADHD patients with other genotypes [Oh et al., 2003; Kim et al., 2006; Boonstra et al., 2007].

Our study aimed at examining the neuropsychological mechanisms of *DAT1* in ADHD, improving upon previous studies with respect to sample size and sample composition, scope of the neuropsychological battery and number of genotyped polymorphisms in *DAT1*. The most important conclusion that can be drawn from our findings is that *DAT1* is not associated with the neuropsychological measures used in this study, even though several risk markers of *DAT1* were associated with ADHD in this sample and ADHD was strongly related to abnormal neuropsychological functioning. Even the subjects carrying the highest possible risk (2 risk diplotypes) did not differ neuropsychologically from subjects with other diplotypes. The absence of an effect of *DAT1* on neuropsychological measures is in line with the majority of previous studies [Ball et al., 1998; Fossella et al., 2002; Oh et al., 2003; Rueda et al., 2005; Szobot et al., 2005; Barkley et al., 2006; Genro et al., 2006; Kim et al., 2006; Boonstra et al., 2007; Sonuga-Barke et al., 2008; Wohl et al., 2008; Loo et al., submitted]. It may, however, be hypothesized that *DAT1* has an effect on neuropsychological processes not examined in this study or previous studies, like delay aversion. An altered delay aversion has been frequently found in ADHD, in which patients are more motivated to escape or avoid delay than controls [Sonuga-Barke, 2002]. This altered delay aversion appears not related to impaired executive/cognitive functions [Sonuga-Barke, 2002, 2005; Toplak et al., 2005], but is related to reduced striatal activation [Scheres et al., 2007]. Since *DAT1* is mainly expressed in the striatum and to a lesser degree in the prefrontal cortex [Durston et al., 2005; Schott et al., 2006; Diamond, 2007; Scherk et al., 2007], *DAT1* may have an effect on striatum related functions (like delay aversion and motor functions) rather than prefrontal related functions (like executive functions) [Sonuga-Barke, 2002]. The more detailed analysis of individual neuropsychological tests also supported this hypothesis: if there was any association between *DAT1* and neuropsychological functioning in our sample, it was within the domain of motor functioning and not within the executive/cognitive domain.

We did not find differential effects of *DAT1* on neuropsychological functioning after stratification of the sample into affected and non-affected siblings. An effect in non-affected siblings only, was previously found for *DAT1* on the nucleus caudatus volume [Durston et al., 2005] and for the *DRD4* gene on ADHD in our own studies [Altink et al., 2008]. We also did not find effects of *DAT1* on neuropsychology after stratification according to the presence or absence of conduct disorder, an analysis inspired by our findings in the larger IMAGE sample that showed association of *DAT1* with ADHD only in the absence of conduct disorder [Zhou et al., 2007].

Interestingly, splitting the sample into children and adolescents resulted in nominally significant findings predominantly in the adolescent group. This may suggest that the effect of *DAT1* on ADHD is not constant across development, but becomes apparent in late childhood and adolescence [Elia and Devoto, 2007]. This may be related to the finding that dopamine levels decrease with increasing age, resulting in a relatively larger effect of an "overactive" dopamine transporter on ADHD [Diamond, 2007]. Some support for this hypothesis has also been reported by Barkley et al. [2006]. They followed children through adolescence and later through adulthood and reported that the effect of *DAT1* on phenotypic measures of ADHD increased substantially with increasing age. Given that the genotype did not differ between measurement moments in childhood, adolescence, and adulthood, the study of Barkley et al. [2006] provides preliminary evidence that the effect of *DAT1* on ADHD may be stronger in older subjects with ADHD compared to younger subjects. However, an alternative explanation is also possible. It may be that adolescents and adults with ADHD carry a stronger genetic load or form a genetically more pure subgroup of ADHD patients than preadolescent children with ADHD. That is, having a persistent form of ADHD that continues into adolescence and adulthood may be more heavily related to genetic factors than a remitting form of ADHD [Faraone et al., 2000].

Limitations

A limitation of this study was that we had SNP data available for only 63% of the sample (though 89% of the VNTR data was available). It could be argued that this may have a profound effect on the power of the study to detect effects of *DAT1* and may explain the negative results of *DAT1* on neuropsychological measures. However, if that would be true, then also no associations would be expected between the SNPs and ADHD. This was not the case, two SNPs were associated with ADHD. Moreover, in theory, the effect of *DAT1* on neuropsychological functioning should be more readily detectable than the effect of *DAT1* on ADHD: neuropsychological measures may act as endophenotypes, which are hypothesized as stronger linked to individual genes [Almasy and Blangero, 2001; Castellanos and Tannock, 2002]. It thus seems implausible that the absence of a relation between *DAT1* and neuropsychological functioning was attributable to limited power. However, it may be feasible that *DAT1* is associated either with other neuropsychological traits, or with neuropsychological functioning only in the presence of particular environmental conditions not accounted for in the current study. For example, it has been reported that *DAT1* genotype only has an effect on ADHD symptomatology if the child was exposed to prenatal smoking or if the child grew up in the context of great psychosocial adversity [Laucht et al., 2007; Becker et al., 2008]. Not taking into account such moderating factors may explain the null findings in the review and analyses described in this article.

CONCLUSION

Several polymorphisms and haplotypes of *DAT1* were associated with ADHD in this subsample of IMAGE. ADHD was also associated with abnormal neuropsychological functioning. In contrast, *DAT1* had no relation to neuropsychological dysfunction. This suggests that the effect of *DAT1* on the ADHD phenotype is not mediated by neuropsychological performance. However, since *DAT1* is mainly

expressed in the striatum and not the prefrontal cortex, *DAT1* may influence striatum related functions (like delay aversion and motor functions) more heavily than prefrontal related functions (like executive functions). An effect of age seemed present with several *DAT1* risk markers and diplotypes nominally associated with ADHD in adolescents. This suggests that the effect of *DAT1* on ADHD is not constant across development, but only becomes apparent in adolescence, and/or that having a persistent form of ADHD that continues into adolescence may mark a more severe or homogeneous genetic form of the disorder.

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