

**FAMILY-BASED ASSOCIATION STUDY OF DAT1 AND DRD4 POLYMORPHISM IN
KOREAN CHILDREN WITH ADHD**

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ABSTRACT

Although the etiology of Attention Deficit/Hyperactivity Disorder (ADHD) is not well understood, evidence from the family and twin studies suggests that ADHD is familial and highly heritable. The aim of the study was to test whether dopamine transporter gene (*DAT1*) and dopamine receptor D4 gene (*DRD4*) polymorphisms are in linkage disequilibrium with ADHD in Korean children, using a family-based association study. 126 trios were studied and 87% of probands were boys (mean age = 8.2 years, mean IQ = 104). ADHD NOS was the most common subtype and comorbidity rates were low. Descriptive analysis, TDT test, logistic regression and QTDT were performed. The 10-repeat allele and 4-repeat allele were the most frequent for *DAT1* and *DRD4*. TDT test for *DAT1* and *DRD4* did not show preferential transmission. Based on logistic regression and QTDT, the 5-repeat allele of *DRD4* may confer protection for hyperactive-impulsivity symptom severity compared to the 4-repeat allele. The negative TDT finding between *DAT1* and *DRD4* VNTR polymorphisms and ADHD should be interpreted with caution; partly due to lack of power caused by low heterozygosity in the study population. Future studies are necessary to test the hypothesis generated in this study that the 5-repeat allele of *DRD4* is protective for hyperactive-impulsivity symptom severity compared to the 4-repeat allele.

Key Words: TDT, *DRD4* 5 repeat allele, Korean

Word count: 214

INTRODUCTION

ADHD is a complex, childhood-onset, neuropsychiatric disorder with an estimated prevalence of 3-5% in the US school-aged children and 2-7.6% in Korean children (APA 1994; Cho and Shin 1994; Kim et al. 1999; Pyo et al. 2001). Although the etiology of ADHD is not well understood, evidence from family and twin studies suggests that ADHD is familial and highly heritable (Heiser et al. 2004).

Based on animal models, imaging studies and the efficacy of stimulant treatment, the dopamine transporter gene (*DAT1*) and the dopamine D4 receptor gene (*DRD4*) have been proposed as candidate genes for ADHD. *DAT1* is localized to chromosome 5p15.3 and has a variable number of tandem repeat (VNTR) polymorphism in the 3' untranslated region (UTR) ranging from 3 to 11 copies (Vandenbergh et al. 1992). *DRD4* maps to chromosome 11p15.5 and displays a high degree of variability at the 48-bp VNTR on exon 3, which can be repeated 2 to 11 times (Ding et al. 2002; Gelernter et al. 1992). The 10-repeat allele of *DAT1* and 7-repeat allele of *DRD4* were identified as risk alleles and have been studied in association studies, with both positive and negative reports (Faraone et al. 2001; Maher et al. 2002).

However, the majority of the studies have been conducted in European children. There were few studies conducted in Chinese ADHD children, also with contradictory findings (Chen et al. 2003; Qian et al. 2004). And there is a single case-control study that reported the increased frequency of *DAT1* 9-repeat allele in Korean children with ADHD compared to historical controls (Shin et al. 2002). Replication of the genetic linkage disequilibrium or an association of dopamine system genes with ADHD in different populations is crucial for establishing a role of dopamine system genes in the etiology of ADHD across population.

The aim of this study is to explore the possibility that *DAT1* and *DRD4* polymorphisms

may be in linkage disequilibrium with ADHD in Korean children using a family-based association study design.

MATERIALS AND METHODS

Subjects

Consecutive subjects consenting to participate in a family-based association study for ADHD and their biological parents were studied between September 2000 and August 2002 at four, university-based, South Korean child psychiatry outpatient clinics (Hallym University, Yonsei University, Seoul National University and Gyeongsang National University.) Included in the study were children between the ages of 6 and 12, with a full scale IQ above 70, who met DSM-IV diagnostic criteria for ADHD on the Diagnostic Interview Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime-Korean Version (K-SADS-PL-K.) Good to excellent validity and reliability of K-SADS-PL-K in ADHD and other disruptive behavioral disorders were reported in Korean children (Kim et al. 2004). Individuals with neurological disorders, seizure disorder, pervasive developmental disorder, Tourette disorder or chronic tic disorders, bipolar mood disorder and psychotic disorders were excluded. Written informed consent was obtained as approved by the institutional review board at the Hallym University Sacred Heart Hospital.

Genotyping

DNA was extracted from whole blood using a standard DNA isolation procedure. For each locus, PCR was carried out in a 10 μ l volume containing 50 ng of genomic template, 0.5 μ M of each primer, one of which was 5' fluorescently labeled, 200 μ M of each dNTP, 1 x PCR buffer, 1.5 mM MgCl₂, and 0.3 units of DyNAzyme™ EXT DNA polymerase (Finnzymes Oy,

Espoo, Finland), with 0.5 M GC-melt (Clontech, Palo Alto, CA, USA). Primer sequences were as follows: *DAT1*, 5'-NED-TGTGGTGTAGGGAACGGCCTGAG-3' and 5'-CTTCCTGGAGGTCACGGCTCAAGG-3'; For *DRD4*, 5'-FAM-GCGACTACGTGGTCTACTCG-3' and 5'-GGTCTGCGGTGGAGTCTG-3'

Samples were amplified on an Applied Biosystems 9700 thermal cycler (Foster City, CA) with an initial 12 minute step to heat-activate the enzyme at 96 °C, followed by 45 cycles consisting of a denaturation step of 96 °C for 30 s, an annealing step for 45 s at 68 °C for *DAT1*, 55 °C for *DRD4*, and an extension step of 72 °C for 3 min, with final extension step at 72 °C for 10min. Post PCR products were purified with Sephadex G-50 gel filtration system, and then added to 10 µl of deionized formamide and 0.5 µl of ROX labeled size standard. PCR products were injected and detected by laser-induced fluorescence on an ABI PRISM 3700 Genetic Analyzer at the University of Chicago DNA Sequencing and Genotyping Core. Electropherograms were processed with Genescan software and alleles were called with Genotyper software, blind to all but a number that is consecutively assigned and is not related to whether the subject is a child, father or mother and without any indication of pedigree relationship to adjacent numbers.

Statistical Analysis

Descriptive statistics were used to examine clinical characteristics, distribution of genotypes and allele frequencies in Korean children with ADHD. Genotypic incompatibilities were identified with Pedcheck and trios with incompatible genotypes were zeroed out (O'Connell and Weeks 1998). Sib_tdt test was used for TDT (ASPEX version 2.2). To test for an interaction between *DRD4* and *DAT1*, we created a “pseudohaplotype” of these two genes to look for preferential transmission of a particular combination of *DAT1* and *DRD4* alleles. TDTPHASE

was used for this analysis (Dudbridge et al. 2000). For the quantitative analysis, symptom severity was computed from each ADHD items in K-SADS-PL-K. Each item was scored as 2 for threshold symptoms, 1 for sub-threshold symptoms and 0 for no symptom. Inattention severity was computed from summing of 9 items (2 from screening module and 7 from supplement module) and hyperactivity-impulsivity from 12 items (2 from screening module and 10 from supplement module). Each subscale severity score was then transformed into a z-score for the comparison between subscales. Z-score indicates how far and in what direction, that item deviates from its distribution's mean, expressed in units of its distribution's standard deviation, and is calculated by (individual score-group mean)/standard deviation. ANOVA were performed to explore the mean differences of the quantitative measures of inattention and hyperactive-impulsivity amongst the most common genotypes of *DAT1* and *DRD4*. The associations between those common genotypes of *DAT1* and *DRD4* and the quantitative measures were tested using logistic regression. This method is similar to that described in Waldman et al (Waldman et al. 1999). The quantitative traits were the independent variables and the dependent variable was either the genotype an individual carried (case-control) or the allele that was transmitted from a heterozygous parent (TDT). Sex and age were entered as covariates. Additionally, quantitative transmission disequilibrium test (QTDT) was performed to examine the association between *DAT1* and *DRD4* and the ADHD subscale severity scores. QTDT incorporates variance components methodology in the analysis of family data and includes exact estimation of p-values for analysis of small samples and non-normal data (Abecasis et al. 2000). Sex and age were entered as covariates. For the alleles of which p-value was significant in QTDT, empirical significance levels were provided.

RESULTS

The study subjects consisted of 136 families: 10 families were dropped from the final analysis due to exclusion criteria including low IQ, the presence of tics, and the failure to meet the K-SADS-PL-K threshold diagnostic criteria for ADHD. Among the final 126 families, 19 families did not have complete genotyping because one or two family members did not complete blood collection (13 families with one missing parent, 3 families with two missing parents and 3 families with missing genotyping.) There was not a significant difference between completely genotyped families (N=107) and incompletely genotyped families (N=19) in clinical characteristics such as age, sex, IQ and diagnostic profile. Of the probands, 87% were boys. Mean age of the probands was 8.3 years (S.D.=1.8) and the mean IQ was 104 (S.D.=16). The DSM IV diagnoses of ADHD subtypes included inattentive type 28%, hyperactive-impulsive type 8%, combined type 29% and not otherwise specified (NOS) 36%. Comorbidity in the study subjects was low, with mostly depressive disorders (n=6) and anxiety disorders (n=4) (table 1).

Genotypic frequencies of *DAT1* and *DRD4* were consistent with those expected under Hardy-Weinberg equilibrium (HWE). χ^2 test statistics and p-value of HWE for *DAT1* and *DRD4* were 24.44 (p=0.222) and 82.302 (p=0.185), respectively. The allele and genotype frequencies of *DAT1* and *DRD4* in the study population were presented in table 2. The most frequent allele and genotype of *DAT1* in the parents and probands were 10-repeat allele (91.7 and 91.2%) and 10/10 (84.3 and 82.4%). The most frequent allele and genotype of *DRD4* for parents and probands were 4-repeat allele (76.4 and 78.8%) and 4/4 (56.7 and 63.2%). The frequencies of alleles and genotypes in the *DAT1* and *DRD4* in this study population are compatible with the previous reports in the Korean population (Choi et al. 1999; Kim et al. 2001; Lee et al. 2003a; Lee et al. 2003b; Shin et al. 2002).

TDT test for *DAT1* and *DRD4* did not show preferential transmission of any allele (table 3). Pseudohaplotype analyses to examine gene-gene interaction between *DAT1* and *DRD4* also failed to show preferential transmission (data not shown.)

The results of the one-way ANOVA to explore variability in quantitative measures of inattention and hyperactive-impulsivity among most common genotypes were summarized in figure 1. The hyperactive-impulsive severity showed a significant difference between 4/4 and 4/5 genotypes of *DRD4*. Compared to children who have 4/4 genotype, children with 4/5 genotype had a lower hyperactivity-impulsivity score ($p = 0.023$). The mean z-scores of hyperactivity-impulsivity subscale for children with 4/5 and 4/4 genotypes were -0.8240 and 0.1654, respectively. Post-hoc logistic regression showed that the 4/5 and 4/2 genotypes of *DRD4* were associated with lower hyperactivity-impulsivity scores as compared with the 4/4 genotype. Odds ratios (ORs) for 4/5 and 4/2 genotypes were 0.18 ($p=0.012$) and 0.60 ($p=0.043$), respectively, after controlling for sex and age (table 4). Logistic TDT failed to show preferential transmission of any allele to the probands by symptom severity. On the other hand, the QTDT analysis showed a preferential transmission of 5-repeat allele of *DRD4* to the probands with lower hyperactive-impulsive subscale score. Empirical p-value was 0.020 (table 5).

DISCUSSION

Unique features of Korean genetics and Korean ADHD

This is the first study conducted in the Korean population to examine the relationship between genes involved in dopamine system activity and ADHD, using family-based association study design. Korea is a promising and interesting site for a genetic epidemiological study since evidence suggests that those Koreans who reside in Korea may constitute a relatively

homogeneous genetic population. Anthropologists trace the origins of Koreans to a single population which occupied Manchuria until about 1,000 B.C. when nomadic Koreans migrated down to the Korean peninsula (Kim 1982). Thus, Koreans of today originated from a relatively ethnically homogeneous ancestral group. Unlike China and Japan, Korea does not contain national minorities, or aboriginals. Cultural and historical factors, such as minimal immigration of non-Koreans into Korea, and strong social pressures against marriage to non-Koreans reinforce Korean genetic homogeneity.

The profiles of subtype diagnoses of ADHD in this study population showed that NOS is the most common (35.7%), followed by the combined, inattentive and hyperactive-impulsive subtypes. This rather unusual profile is due to cultural difference: Korean parents have a tendency to underreport negative outcomes (Ha et al. 1998). If the diagnostic criteria are expanded to include sub-threshold diagnoses of ADHD, including sub-threshold symptoms in K-SADS-PL-K as positive symptoms, the diagnostic agreement between clinical diagnoses and K-SADS-PL-K diagnoses in ADHD improved from good to excellent (Kim et al. 2004). The profile of the subtype of sub-threshold ADHD in this study population is: combined type 78.6%, inattention type 18.3%, hyperactive-impulsive type 1.6% and NOS 1.6%.

Interestingly, the study population in this sample has unique features compared to those in other studies: 1) the study population is composed of single ethnicity – Koreans; 2) the study population is composed based on a strict inclusion criteria with K-SADS-PL-K diagnoses, excluding conditions that are commonly accompanied with ADHD such as tic disorders, mental retardation, developmental disorders and other neurological disorders; and 3) this study population has very low prevalence of other comorbid disruptive behavioral disorders. Low comorbidity in Korean children with ADHD is of particular interest. The method that was used

to identify children with ADHD in this study is very similar to those used in other studies. However, the prevalence and the profile of comorbidity are different from those in other studies (APA 1994). From the findings in our previous study of validity and reliability of K-SADS-PL-K, comorbidity in ADHD children was also very low and had a similar profile. Among the 43 children diagnosed with ADHD using K-SADS-PL-K, (inattentive 11, hyperactive-impulsive 4, combined type 12 and NOS 16), 8 children (11.6%) had comorbid diagnoses – 6 with tic disorders, 1 with separation anxiety disorder and 1 with conduct disorder (unpublished data). In two Korean studies that examined the prevalence of ADHD and comorbid conditions, 2.3-3.4% of conduct disorders and 13.6-25.8% of oppositional defiant disorder were reported in the children with ADHD (Cho and Shin 1994; Pyo et al. 2001). However, these studies used parents' rating scales to identify children with ADHD and disruptive behavioral disorders, which might have led to the overestimation of the rates of these disorders. The low comorbidity in this study population may come from the strict inclusion criteria, resulting in a narrow definition of ADHD with low comorbidity. On the other hand, this may also suggest that Korean ADHD has different etiological origin from ADHD with high prevalent comorbid conditions if these comorbid conditions share same etiological origin with underlying ADHD. Only further detailed study will lead to understanding of these differences.

Novel *DRD4* allele in Korean population

During the course of *DRD4* genotyping, two unrelated subjects were found to have an allele of 428 bp. This allele was classified as 4.5 repeats because of its size between 4 and 5 repeats. One of the two subjects was the mother in one family and the other subject was a male proband from an unrelated family in which father was not genotyped. This allele was confirmed by independent PCR and genotyping. To our knowledge, this allele has not been reported

previously. No specific characteristics were identified in these two subjects. The implication of this allele, however, may be investigated further with sequencing.

TDT analysis of *DAT1* and *DRD4*

The high frequency of the 10-repeat allele of *DAT1* and extremely low frequency of 7-repeat allele in *DRD4* in the study population are consistent with previous findings in the Korean population, which supports the conclusion that there was no significant sampling bias in this study population.

This study finds that there is no linkage or association for *DAT1* and *DRD4* in a well-characterized Korean population, using TDT. Two previous studies of *DAT1* in Chinese children with ADHD resulted in two contradictory findings. One case-control study reported a positive association between *DAT1* and ADHD in Taiwanese children (Chen et al. 2003). However, this study has following limitations: The study participants were composed of children with mental retardation (13%) and pervasive developmental disorders (6%), leading to a strong possibility of inclusion of phenocopies. Also, the study's case-control design may suffer from population stratification. The second study, using family-based association design and TDT analysis reported no evidence of association between Chinese children with ADHD and *DAT1* 6-12 repeat alleles (Qian et al. 2004). With regard to the association between *DRD4* and ADHD, there are two previous reports on the Chinese children with ADHD. One recent case-control study reported the increased prevalence of the 2-repeat allele in Han Chinese children with ADHD (Leung et al. 2004). However, this study suffers from severe methodological limitation by using a historical control group, in addition to the possibility of population stratification confounding. Also, the number of cases was very small (n=32). The second study that used family-based control reported no evidence of association between *DRD4* 2-6 repeat alleles and Chinese

children with ADHD (Qian et al. 2004). Our finding is consistent with previous negative findings in Chinese population using family-based association design and TDT analyses. Nonetheless, for the current study, the negative TDT analysis of findings between *DAT1* and *DRD4* and ADHD in the TDT analysis needs to be interpreted with caution. First, a lack of power may make it difficult to detect association. Our sample size has 80% power to detect an OR of 2 for *DRD4* and 4 for *DAT1*, whereas previous studies reported ORs between 0.9-2.1 in *DRD4* 7-repeat allele and 0.62-2.67 in *DAT1* 10-repeat allele, thus it is very likely to be underpowered (Faraone et al. 2001; Maher et al. 2002). In addition, low heterozygosity in *DAT1* (15.7%) also contributed to a decrease in the power of this study. Second, the 10-repeat allele of *DAT1* may be the susceptibility allele, but the presence or absence of other susceptibility genes or environmental factors may determine susceptibility because most Koreans carry this susceptibility allele. It is possible there may be polymorphisms in linkage disequilibrium with these variants that would be more informative in the Korean population. However, we must consider that *DAT1* or *DRD4* may not be a susceptibility gene in the Korean population.

Quantitative analysis

The 7-repeat allele has been identified as a risk allele for ADHD in Caucasian children. However, this 7-repeat allele is rarely present in Asian population. No 7-repeat allele has been found in the Chinese children with ADHD in previous studies (Leung et al. 2004; Qian et al. 2004). The present study also did not find a proband with a 7-repeat allele (although it was present in one parent.) The post-hoc quantitative analysis of symptom severity according to the common genotypes in this Korean population showed a tendency that the 4/5 and 4/2 genotypes compared to 4/4 genotype of *DRD4* are protective factors. Along with the results from QTDT analysis, this finding suggests that 5-repeat allele of *DRD4* compared to 4-repeat allele may be a

protective factor for the hyperactive-impulsive symptoms in Korean children. This finding will merit further investigation since there have been no previous reports on the association between the 5-repeat allele of *DRD4* and ADHD. In addition, the comparative pharmacological and functional analysis of the human *DRD4* 2-, 4- and 7-repeat alleles reported no major differences among those alleles, however, no functional research has been reported on the *DRD4* 5-repeat allele (Jovanovic et al. 1999). It is of note that the composition of the repeats is not identical and therefore, a simple correlation between repeat length and ADHD risk would not necessarily be predicted (Ding et al. 2002). If confirmed, the finding of protective role of the 5-repeat allele relative to the 4-repeat allele may establish the 4-repeat allele as an intermediate risk allele between the more protective 5-repeat allele and higher risk 7-repeat allele. Given the absence of overlap in the same population of the 5 and 7-repeat alleles, this would be a difficult prediction to test, except in populations with Asian and Caucasian admixture. Also, this finding emphasizes the importance of population structure in the search of candidate genes or susceptibility genes for the complex diseases. However, the present finding could have been a false positive. The statistical significance in the ANOVA and the OR in the logistic regression were not corrected for multiple comparisons, and the results of the QTDT were corrected only for the number of alleles tested with empirical significance.

Strength and Limitations

This study has several strengths: 1) a well-established standardized diagnostic tool which has been validated in Korean population was used to identify children with ADHD and other comorbid conditions; 2) strict inclusion and exclusion criteria were used to minimize an inclusion of phenocopies and to maximize the homogeneity of the study population; and 3) TDT that was used in this study has an advantage over case-control study design to avoid population

stratification. However, the low prevalence of heterozygous parents markedly decreased the study power to detect small gene effects in the TDT analysis of preferential transmission of risk alleles to children with ADHD.

In conclusion, TDT analyses of *DAT1* and *DRD4* alleles did not show preferential transmission to the Korean children with ADHD. However, quantitative analyses showed that compared to the 4-repeat allele, the *DRD4* 5-repeat allele was relatively protective for hyperactivity-impulsivity symptom severity. This post-hoc result suggests a new hypothesis that merits testing in the future studies of different study population.

To ensure study power to detect small gene effects, larger number of sample sizes or meta-analyses will be required. Gene-gene interaction and gene-environment interactions, as well as phenotypic characteristics in different populations should be examined in future studies to help understand the role of *DAT1* and *DRD4* in the etiology of ADHD. Also other candidate genes including other dopamine receptor gene(s) and genes involved in the noradrenergic system may provide opportunities for further research.

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Table 1 Clinical characteristics of study subjects

Characteristics		Number (%)
Sex	Male	110 (87.3)
	Female	16 (12.7)
Age	Mean (SD)	8.3 years (1.8)
IQ	Mean (SD)	
	Total IQ	104 (16)
	Verbal IQ	105 (16)
	Performance IQ	103 (15)
K-SADS-PL ADHD Diagnoses		
	Inattentive type	35 (27.8)
	Hyperactive-impulsive type	10 (7.9)
	Combined type	36 (28.6)
	ADHD NOS	45 (35.7)
K-SADS-PL Comorbid Diagnoses		
	Depressive disorders	6 (60.0)
	Anxiety disorders	4 (40.0)

SD; standard deviation, ADHD; attention deficit/hyperactivity disorder, NOS: Not Otherwise Specified, K-SADS-PL: Diagnostic Interview Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime

Table 2 Frequencies of *DAT1* and *DRD4* alleles and genotypes in parents and probands with ADHD.

	Parents	Probands with ADHD
	N (%)	N (%)
DAT1 allele frequencies		
6R (328 bp)	2 (0.4)	2 (0.8)
7R (365 bp)	12 (2.6)	7 (2.8)
9R (444 bp)	14 (3.0)	7 (2.8)
10R (483 bp)	422 (91.7)	228 (91.2)
11R (521 bp)	10 (2.2)	6 (2.4)
DAT1 genotype frequencies		
6/10	2 (0.9)	2 (1.6)
7/7	2 (0.9)	0 (0.0)
7/10	8 (3.5)	7 (5.6)
9/10	14 (6.1)	7 (5.6)
10/10	194 (84.3)	103 (82.4)
10/11	10 (4.3)	6 (4.8)
DRD4 allele frequencies		
2R (318 bp)	76 (16.4)	38 (15.2)
3R (364 bp)	8 (1.7)	2 (0.8)
4R (410 bp)	353 (76.4)	197 (78.8)
4.5R (428 bp)	1 (0.2)	1 (0.4)
5R (458 bp)	17 (3.7)	9 (3.6)
6R (502 bp)	6 (1.3)	0 (0.0)
7R (546 bp)	1 (0.2)	3 (1.2)
DRD4 genotype frequencies		
2/2	2 (0.9)	3 (2.4)
2/3	2 (0.9)	1 (0.8)
2/4	66 (28.6)	28 (22.4)
2/4.5	0 (0.0)	1 (0.8)
2/5	3 (1.3)	1 (0.8)
2/6	1 (0.4)	1 (0.8)
3/4	6 (2.6)	1 (0.8)
4/4	131 (56.7)	79 (63.2)
4/4.5	1 (0.4)	0 (0.0)
4/5	14 (6.1)	8 (6.4)
4/6	4 (1.7)	2 (1.6)
6/7	1 (0.4)	0 (0.0)

N: number of subjects, ADHD; attention deficit/hyperactivity disorder, R: repeat, bp: base pair

Table 3 TDT Test for *DAT1* and *DRD4* alleles in Korean families with ADHD probands

Allele	Number	%	Transmitted	Non-transmitted	χ^2
DAT1					
6R	2	0.4	0	2	2.00
7R	12	2.6	4	3	0.14
9R	14	3.1	7	7	0.00
10R	420	91.7	17	16	0.03
11R	10	2.2	5	5	0.00
					Global p-value 0.81
DRD4					
2R	75	16.3	31	34	0.14
3R	8	1.7	2	6	2.00
4R	352	76.5	45	37	0.78
4.5R	1	0.2	0	0	0.00
5R	17	3.7	8	8	0.06
6R	6	1.3	3	3	0.00
7R	1	0.2	0	1	1.00
					Global p-value 0.67

R: repeat

Table 4 Association between quantitative measures of ADHD symptoms and common genotypes of *DAT1* and *DRD4*

	Inattention Severity		Hyperactive-impulsive Severity	
	OR (CI)	p-value	OR (CI)	p-value
Genotype Logistic Regression				
DAT1 (reference 10/10 genotype)				
7/10	1.02 (0.45-2.32)	0.962	2.07 (0.84-5.10)	0.114
9/10	0.87 (0.40-1.88)	0.723	1.71 (0.73-4.03)	0.218
10/11	1.95 (0.67-5.66)	0.219	0.75 (0.30-1.88)	0.537
DRD4 (reference 4/4 genotype)				
4/2	0.87 (0.56-1.34)	0.521	0.60 (0.36-0.98)	0.043
4/5	0.83 (0.41-1.68)	0.600	0.18 (0.05-0.69)	0.012
Logistic TDT				
DAT1				
7R	2.91 (0.30-28.11)	0.356	1.16 (0.10-13.25)	0.903
9R		Non convergent		
10R	0.73 (0.33-1.62)	0.443	0.64 (0.23-1.76)	0.385
11R		Non convergent		
DRD4				
2R	1.38 (0.89-1.38)	0.369	0.81 (0.41-1.60)	0.544
3R	0.28 (0.18-2.55)	0.557	0.53 (0.04-7.72)	0.643
4R	0.73 (0.74-1.11)	0.339	1.59 (0.85-2.97)	0.145
5R		Non convergent		
6R		Non convergent		

OR indicates an odds ratio adjusted for sex and age. CI indicates 95% confidence interval. p indicates p-value. Non-convergent means a maximum likelihood estimate could not be found, probably due to lack of data.

Table 5 QTDT analysis of the quantitative measures of ADHD symptoms subscales and DAT1 and DRD4 polymorphisms individual alleles

Tested Allele	Inattention severity		Hyperactive-impulsive severity	
	F	p-value	F	p-value
DAT1				
6R			not tested	
7R	0.25	0.715	0.19	0.775
9R	0.05	0.829	2.44	0.083
10R	1.62	0.269	1.30	0.223
11R	3.49	0.061	0.16	0.570
DRD4				
2R	0.31	0.552	0.00	0.936
3R	0.00	0.957	0.08	0.736
4R	0.00	0.949	1.31	0.261
5R	1.98	0.170	7.62	0.007*
6R	0.38	0.324	1.43	0.450

*: Empirical significance level: $p=0.020$

Not tested due to small sample size.

QTDT: quantitative transmission disequilibrium test

Figure 1 Mean z-scores of ADHD subscale by common genotypes of *DAT1* and *DRD4*