Dopamine risk and paternal ADHD symptomatology associated with ADHD symptoms in four and a half-year-old boys
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Objective This study examined the influence of allelic variation in two dopamine genes, the dopamine receptor D4 (DRD4) gene and the dopamine transporter D1 (DAT1) gene, and paternal attention-deficit hyperactivity disorder (ADHD) symptomatology on the level of ADHD symptoms in 96 four and a half-year-old boys.

Method DNA was collected by means of a buccal swab and genotyped for DRD4 and DAT1. Mothers completed the DuPaul ADHD checklist on their sons. ADHD symptomatology ratings for fathers were based on a summed father self-reported and spouse-reported symptoms (Conners Adult ADHD Rating Scale).

Results There were main effects for DAT1 and father symptomatology for the child Total ADHD and Hyperactivity–Impulsivity scores. The main effects for DRD4 were limited to the child Hyperactivity–Impulsivity scores. Child Inattentive scores were influenced only by father symptomatology. Interaction effects between DAT1 and DRD4 and between DAT1 and the father ADHD risk group were found for child Hyperactivity–Impulsivity scores. Boys with the highest level of symptomatology were those with the 10/10 DAT1 genotype and the DRD4-7 genotype or fathers with high symptomatology.

Conclusion The findings of this study indicate that the risk for ADHD, particularly hyperactivity–impulsivity, is exacerbated in the presence of dopamine risk genes and paternal ADHD symptomatology. This study adds to the growing literature on the efficacy of including multiple genetic and environmental risk factors in studies related to the development of psychopathology.

Keywords: child ADHD symptoms, child dopamine risk (DRD4, DAT1), paternal ADHD symptoms

Attention-deficit hyperactivity disorder (ADHD) is a common psychiatric disorder characterized by inattention, impulsivity, and hyperactivity with heritability estimates of 60–91% (Levy et al., 1997; Thapar et al., 2005), although etiologically heterogeneous, family, twin, and adoption studies have shown the disorder to be influenced predominantly by genetic factors (Morrison and Stewart, 1973; Biederman et al., 1995; Thapar et al., 1995; Levy et al., 1997). Molecular genetic studies have targeted genes from the dopamine system as being implicated in the disorder, as many of the neuropsychological deficits associated with the disorder are seen to be the result of a dysfunctioning dopamine system (Swanson et al., 2007).

First-degree relatives of individuals with ADHD are at increased risk for ADHD. Biederman et al. (1995) found that 57% of children of parents with ADHD met criteria for ADHD. Parental ADHD not only increases genetic risk for ADHD, but it also confers exposure to a riskier childrearing environment. Inconsistent use of discipline, poor monitoring of child behavior, and lax parenting have been associated with ADHD symptomatology in parents (Harvey et al., 2003; Murray and Johnston, 2006), although there is some question as to whether an adverse family environment increases child ADHD risk beyond what would be expected from genetic liability (Biederman et al., 2002).

The dopamine receptor D4 gene (DRD4) and the dopamine transporter D1 gene (DAT1) are two genes from the dopamine system that have been associated with ADHD. Both these genes have variations across individuals based on a variable number of tandem repeats (VNTRs) of their base-pair sequences. DRD4 has a 48-bp repeat in exon III. The most frequent variant is the 4-repeat, with the 7-repeat allele the next most frequent. There is substantial evidence that the 7-repeat allele is a risk factor for ADHD. The original report of an association between the 7-repeat allele and ADHD (LaHoste et al., 1996) has been confirmed by many, if not all, subsequent studies and by meta-analyses of association and linkage studies (Swanson et al., 1998; Faraone et al., 1999, 2001; Li et al., 2006). Initially, the association between
ADHD and DRD4 was thought to be through the role dopamine plays in attentional functioning, but studies using laboratory measures of attention failed to confirm such an association (Swanson et al., 2000; Fossella et al., 2002). In fact, both studies found poorer attentional performance for those individuals without the 7-repeat allele.

DAT1 has a 40 bp repeat located in the 3'-untranslated region of the gene. The two most common allelic variants are nine and 10 copies of the repeats. The findings regarding an association between DAT1 and ADHD have been less consistent than those for DRD4, and this inconsistency extends as to which genotype is the risk genotype, although on balance it seems that the 10R genotype confers an increased risk for ADHD (Cook et al., 1995; Waldman et al., 1998). Recent meta-analytic studies of DAT1 reflect the inconsistency of the findings. Li et al. (2006) found no association between 10R DAT1 and ADHD whereas Yang et al. (2007) found a small but significant association between this polymorphism and ADHD. Few studies have examined the DAT1 risk genotype and its association with the specific ADHD subtype. Waldman et al. (1998) found a significant association between 10R DAT1 and the hyperactive–impulsive symptoms of ADHD but not for the inattentive symptoms.

The etiological complexity of psychiatric disorders, including ADHD, has led to increasing recognition of the importance of examining the influence of gene–gene interaction and gene–environment interaction in the development of psychopathology (Rutter, 2006). Interactions between DRD4 and the serotonin transporter gene (5-HTT) have been linked to both variations in temperament in the first year after birth and childhood behavior problems. Auerbach et al. (2001a) found an interaction between DRD4 and 5-HTT and the performance of 12-month-old infants on a measure of duration of looking, with the shortest duration of looking being shown by infants with the long alleles of DRD4 and s/s 5-HTT genotype. Schmidt et al. (2007) found that children with higher internalizing and externalizing scores on a behavior problem checklist were those children with the long DRD4 alleles and at least one short 5-HTT allele.

Both DRD4 and DAT1 have been found to interact with environmental factors to increase the risk of psychopathology. Sheese et al. (2007) found that infants with the 7-DRD4 allele were more affected by the quality of parenting than infants without this allele. Infants with the 7-DRD4 allele who were exposed to poor quality parenting showed the highest levels of sensation-seeking at age 4, whereas infants with that allele who received high quality parenting showed the lowest levels of sensation-seeking. Laucht et al. (2007) found that adolescents homozygous for the 10-repeat DAT1 allele who experienced more psychosocial adversity had significantly more symptoms of inattention and hyperactivity–impulsivity than adolescents with the same homozygous 10-repeat allele but who experience less psychosocial adversity.

The aim of this study is to examine the association of the child DRD4 and DAT1 genotypes and paternal ADHD symptomatology with the level of ADHD symptoms in four and half-year-old boys who have been followed longitudinally since birth. Given earlier findings, we expect all three independent variables (DRD4 risk, DAT1 risk, and risk based on father symptomatology) to be related to ADHD symptoms in the child. In addition to the main effects, interaction effects between DRD4 and DAT1 and between these genes and paternal ADHD symptoms are anticipated.

**Methods**

**Sample**

The sample consists of four and half-year-old boys at familial risk for ADHD. These boys have been followed longitudinally since birth and were enrolled in the Ben-Gurion Infant Development Study based on paternal ADHD symptomatology initially assessed at the birth of the child. Of the 115 boys assessed at 4.5 years, 96 of them are included in this report. These 96 boys had genotype and mother-reported ADHD rating data and their fathers had Conners Adult ADHD Rating Scale (CAARS) (Conners et al., 1999) data.

The research was conducted at the Ben-Gurion University and approved by the ethics committee of the Department of Behavioral Sciences, Ben-Gurion University, Beer Sheva, Israel. After a complete description of the study to the participants, a written informed consent was obtained from them.

**Measures**

**ADHD rating scale – IV (DuPaul et al., 1998)**

This 18-item rating scale assesses the frequency of symptoms exhibited by the child in the last 6 months. Child behavior is rated on a 4-point Likert scale ranging from ‘never or rarely’ to ‘very often’. Three scores are derived from the scale: Inattention, Hyperactivity–Impulsivity, and Total. This study made use of the mother ratings of the child. The Cronbach alphas were 0.79 for the Inattention scale, 0.77 for the Hyperactivity–Impulsivity scale, and 0.86 for the Total ADHD scale.

**Conners adult ADHD rating scale CAARS (Conners et al., 1999)**

CAARS assesses ADHD and associated behavior in adults. The long self-report version (CAARS-S:L) and the observer screening version (CAARS-O:SV) were used in this study. The CAARS-S:L consists of 66 items and nine scales. The CAARS-O:SV consists of 30 items and four scales. Only the Diagnostic and Statistical Manual of Mental Disorders (DSM)-attention-deficit hyperactivity disorder (ADHD) CAARS-S:L and CAARS-O:SV scales were used.
in this study. For both the versions of the CAARS, behavior
is rated on a 4-point Likert scale ranging from 'not at all/
never' to 'very much/very frequently'. The Cronbach alphas
for the CAARS-S:L and CAARS-O:SV DSM-ADHD scales
were 0.84 and 0.8, respectively.

Both the CAARS-S:L and O:SV were completed when
the study children were between 2 and 6 months old.

Child DNA samples
Collection of DNA from the child was carried out using
soft buccal brushes, which were rubbed approximately 20
times on the inside of each cheek. The brushes were then
put into a test tube containing AquaFresh mouthwash.
The DNA samples were sent to Professor Ebstein's
laboratory for analysis. The exon III repeat region of the
DRD4 receptor and the 3–13 bp VNTR of DAT1 were
categorized using PCR amplification procedure with
the following primers:

- **DAT1**: F5'- CTTCCTGACCGCTCATGCTGCTG
  CTCATCTGG-3'

- **DRD4**: F5'- TTGGGTGTAGGGACGGACTCCATGGCG
  GCCTTGG-3'

PCR reactions were performed using 5 µl Master Mix
(Thermo Scientific, Thermo Fisher Scientific Inc., Waltham,
Massachusetts, USA), 2 µl primers (0.5 µmol/l), 0.6 µl
Mg/Cl2 (2.5 mmol/l), 0.4 µl DMSO 5%, and 1 µl of water
to a total of 9 µl total volume and an additional 1 µl of
genomic DNA was added to the mixture. All PCR reac-
tions were carried out on a Biometra T1 Thermocycler
(Biometra, Göttingen, Germany). PCR reaction condi-
tions were as follows: preheating step at 94.0°C for 5 min,
34 cycles of denaturation at 94.0°C for 30 s, reannealing at
55°C for 30 s, and extension at 72°C for 90 s. The reaction
proceeded to a hold at 72°C for 5 min. The reaction
mixture was then electrophoresed on a 3% agarose gel
(AMRESCO) with ethidium bromide to screen for geno-
types. Both the genotype frequencies (i.e. DRD4 and
DAT1) were in Hardy–Weinberg equilibrium in all the
participants. DNA collection was approved by the Helsinki
committee of Soroka Medical Center, Beer Sheva, Israel.
Informed consent for DNA samples were signed by both
the parents.

Risk classification
Child risk for ADHD was based on ADHD symptoms of
the fathers and the DAT1 and DRD4 polymorphisms
associated with ADHD. CAARS scores were used to
classify father ADHD risk. Father self-report CAARS
(S:L) scores and mother reports (CAARS-O:SV) about
ADHD symptoms in the father were added together for
the summed father ADHD scores. Risk classification for
father symptoms was based on the summed scores that
fell into the highest quartile of the summed DSM-ADHD
scales. The father ADHD risk groups were composed of
those fathers whose scores fell in the upper quartile
(> 36).

The DAT1 and DRD4 polymorphisms were grouped by
risk for ADHD. DAT1 was grouped as homozygous 10/10
(risk) versus all other variants. 44.90% of the children had
the 10/10 classification. DRD4 was grouped by the pre-
sence (risk) or absence of the 7-DRD4 allele. 31.25% had
at least one copy of the 7-DRD4 allele.

Statistical analysis
Paternal age was significantly correlated with the child
Total ADHD and Hyperactivity–Impulsivity scales (r =
0.22, P < .05; r = 0.21, P < 0.05, respectively). Therefore,
for analyses involving these scales, paternal age was used
as a covariate. Paternal education, maternal age, and edu-
cation were not correlated with the child ADHD scores.

All analyses included two levels of ADHD risk for each of
the three independent variables: father ADHD (upper
quartile vs. other), child DAT1 (10/10 genotype vs. other),
and child DRD4 (7-DRD4 allele vs. other). The effect of
the father ADHD group and the child genotype groups
(DAT1 and DRD4) on the child Inattentive scores was
analyzed using analysis of variance. Analysis of covariances
with paternal age as a covariate were used for the analysis
of the child Total ADHD and Hyperactivity–Impulsivity
symptoms scores. Three-way interactions were not exam-
ined because there was only one child with all the three
risk indices. Bonferroni post-hoc tests were used to identify
the source of significant interaction effects.

Results
Tables 1 and 2 present the descriptive statistics for the
child ADHD scores by the DAT1 and DRD4 groups
(Table 1) and by the father ADHD groups (Table 2).

Total score
There was a significant main effect for DAT1 [F(1,87) =
5.65, P < 0.05, η² = 0.06]. Children with the homozygous
10/10 had higher Total ADHD scores than children with-
out the homozygous 10/10. There was a significant main
effect for the father DSM-ADHD group [F(1,87) = 8.84,
P < 0.005, η² = 0.09]. Those children whose fathers had
ADHD scores in the upper quartile received higher Total
ADHD scores than children whose fathers’ scores were
not in that quartile. There were no interaction effects.

Inattentive Score
The only significant effect for Inattentive scores was for
the father DSM-ADHD group [F(1,88) = 4.68, P = < 0.05,
η² = 0.06]. Those children whose fathers scored in the
upper quartile received higher scores on the Inattentive
scale than children whose fathers’ scores were not in that quartile. Child genotype was not related to Inattentive scores and there were no interaction effects.

**Hyperactivity–Impulsivity score**

There were significant main effects for the DAT1 [F(1,87) = 12.41, *P* < 0.001, *ηp*² = 0.12], DRD4 [F(1,87) = 4.57, *P* < 0.05, *ηp*² = 0.05], and father DSM-ADHD groups [F(1,87) = 11.51, *P* < 0.001, *ηp*² = 0.12]. For all three main effects, children with the DRD4 and DAT1 risk genotype and paternal DSM-ADHD risk classifications had significantly higher Hyperactivity–Impulsivity scores than the non-risk groups. There were two significant two-way interactions: DAT1 x DRD4 groups [F(1,88) = 10.44, *P* < 0.005, *ηp*² = 0.11] and DAT1 x father DSM-ADHD groups [F(1,87) = 5.87, *P* < 0.05, *ηp*² = 0.05]. Children with both the 7-DRDR and 10/10 DAT1 alleles had significantly higher scores on this scale than children without DRD4 and DAT1 risk (*P* = 0.050) (Fig. 1). Children with homozygous 10/10 DAT1 and father DSM-ADHD risk received significantly higher Hyperactivity–Impulsivity scores than children without DAT1 or father DSM-ADHD risk (*P* = 0.004) (Fig. 2).

**Discussion**

In this sample of boys followed longitudinally from birth, both father ADHD symptomatology and child genotype were associated with ADHD symptoms in the children at the age of four and half years. Of the two dopamine genes examined, DAT1 was more strongly related to the Total ADHD score and the Hyperactivity–Impulsivity score in the children than DRD4. Neither was related to inattentive symptoms in the children. Interaction effects between DAT1 and DRD4 and between DAT1 and the father ADHD risk group were found for the child Hyperactivity–Impulsivity score.

Our findings that father ADHD symptomatology confer ADHD risk for children are in agreement with other family studies of ADHD (Biederman *et al.*, 1995; Faraone *et al.*, 2000). Children with fathers whose ADHD symptom scores fell into the upper quartile had higher ADHD scores than boys with fathers whose scores did not fall into this quartile. Similar to other studies using a quantitative rather than a categorical approach to child ADHD, our
findings show that intergenerational risk for behaviors characteristic of ADHD is high even when parental ADHD classification is not by the DSM criteria (Levy et al., 1997).

Of the two dopamine genes examined, DAT1 had the strongest effect on child ADHD symptoms. Children with the 10/10 DAT1 risk genotype had significantly higher ADHD Total scores and Hyperactivity–Impulsivity scores than children without that genotype. These findings provide further evidence that the 10/10 DAT1 genotype is the DAT1 risk genotype for ADHD. Like Waldman et al. (1998), we found an association between 10/10 DAT1 and hyperactivity–impulsivity symptoms in the children where as there was no association between this genotype and inattentive symptoms. The only main effect for DRD4 was also for symptoms of hyperactivity–impulsivity; the 7-DRD4 allele was associated with more hyperactivity–impulsivity. In a study of temperament in 12-month-old infants (Auerbach et al., 2001b), the 7-DRD4 allele was associated with a higher level of activity, perhaps an early indicator of a behavior that may in certain circumstances, such as in the presence of the 7-DRD4 genotype, progress to hyperactivity. There was no association between the presence or absence of the 7-DRD4 allele and attentional symptoms, which is in contrast to studies of ADHD and of temperament that used laboratory measures of attentional functioning and found DRD4 genotype differences on these measures (Swanson et al., 2000; Fossella et al., 2002). In this study, mothers completed an ADHD questionnaire on their four and half-year-old sons. At this age, parents may be more sensitive to symptoms of hyperactivity and impulsivity than to attentional difficulties that may only become prominent when the children are in a school setting.

Our findings of the effect of a gene–gene interaction on child ADHD symptoms are further proof of how our understanding of psychopathology can be enhanced by examining these interactions. By focusing on the two dopamine genes implicated in ADHD, we found that a child who possesses the risk alleles of both the genes has higher levels of ADHD symptomatology, in particular symptoms of hyperactivity–impulsivity at the age of four and half years, than children who possess other combinations of the variants. In a sample of ADHD patients, Durston et al. (2005) found that the average caudate volume of the 10/10 DAT1 genotype is smaller than that for other DAT1 genotypes, whereas the average prefrontal gray matter volume is smaller for the 7-repeat genotype. Having both the 10/10 DAT1 and the 7-DRD4 genotype, therefore, may act as a double biological hit for increased ADHD susceptibility as was found in this study.

Although the presence of one risk factor may increase the likelihood of risk for ADHD, the presence of two risk factors, either based on gene–gene risk or gene-heritability risk, may identify a subgroup of children who are at particularly high risk. If these interaction effects are replicated in other samples, then very young children with gene–gene risk or gene-heritability risk could be the target of monitoring and perhaps intervention through parent programs, such as those that have been found to be effective in reducing ADHD behavior in preschool children (Sonuga-Barke et al., 2001). Unfortunately, the size of the present sample precluded the possibility of examining three-way interaction effects. Only one child had all the three risk factors. He received the highest Hyperactivity–Impulsivity score and the second highest Total ADHD score.

Although this study cannot make any strong claims regarding gene–environment interaction, the interaction between child genotype (DAT1) and father ADHD symptomatology found in this study may not only serve to increase genetic risk for ADHD but also to increase environmental risk through the less than optimal parenting behavior reported in parents with ADHD symptoms (Harvey et al., 2003; Murray and Johnston, 2006); a risk exacerbated if the parent with ADHD is the father (Psychogiou et al., 2007). Intervention with these families may be particularly challenging because not only is a passive gene–environment correlation in play but the child himself as a result of his risk genotype may evoke negative reactions from the environment, thus making it more difficult to deflect him onto a more normative developmental pathway. Indeed, Sonuga-Barke et al.,
(2002) found that a parent-training program was least effective for 3-year-old children with ADHD who had mothers with high levels of ADHD symptoms. The findings presented here must be viewed with caution until replicated on a larger sample. In this study, ADHD was not determined by clinical diagnosis and neuropsychological evaluation. Instead ADHD in fathers and sons was evaluated using rating scales. These types of scales are widely used in heritability and molecular genetic studies of ADHD. A replication of this study using a mutually exclusive classification into ADHD subgroups is needed. DNA collection was by means of buccal smears rather than by blood samples. This was done to increase the probability of consent to DNA collection, but it carried with it the possibility of a decrease in the quality of DNA and as a result, an increase in the possibility of genotype error. The small size of the current sample prevented us from examining three-way interactions that might have been especially informative in identifying a particularly vulnerable subgroup of children at risk for ADHD. An additional limitation of the study was that the sample was restricted to boys. Overall, the findings of this study indicate that the risk for ADHD, particularly hyperactivity–impulsivity, is exacerbated in the presence of dopamine risk genes and paternal ADHD symptomatology. This study adds to the growing literature on the efficacy of including multiple genetic and environmental risk factors in studies of the development of psychopathology.

Acknowledgement
This study was supported by the Israel Science Foundation, grants 756/98-01 and 869-01.

References


