

# Interacting Effects of the Dopamine Transporter Gene and Psychosocial Adversity on Attention-Deficit/Hyperactivity Disorder Symptoms Among 15-Year-Olds From a High-Risk Community Sample

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**Context:** Recent evidence suggests that gene  $\times$  environment interactions could explain the inconsistent findings of association studies relating the dopamine transporter (*DAT1*) gene with attention-deficit/hyperactivity disorder (ADHD).

**Objective:** To examine whether psychosocial adversity moderated the effect of genetic variation in *DAT1* on ADHD symptoms in adolescents from a high-risk community sample.

**Design:** Prospective cohort study.

**Setting:** Data were taken from the Mannheim Study of Children at Risk, an ongoing longitudinal study of the long-term outcomes of early risk factors followed up from birth on.

**Participants:** Three hundred five adolescents (146 boys, 159 girls) participated in a follow-up assessment at age 15 years.

**Main Outcome Measures:** Measures of ADHD symptoms according to *DSM-IV* were obtained using standardized structural interviews with adolescents and their parents. Psychosocial adversity was determined accord-

ing to an "enriched" family adversity index as proposed by Rutter and Quinton. DNA was genotyped for the common *DAT1* 40-base pair (bp) variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region; 3 previously described single nucleotide polymorphisms in exon 15, intron 9, and exon 9; and a novel 30-bp VNTR polymorphism in intron 8.

**Results:** Adolescents homozygous for the 10-repeat allele of the 40-bp VNTR polymorphism who grew up in greater psychosocial adversity exhibited significantly more inattention and hyperactivity-impulsivity than adolescents with other genotypes or who lived in less adverse family conditions (significant interaction,  $P = .013-.017$ ). This gene  $\times$  environment interaction was also observed in individuals homozygous for the 6-repeat allele of the 30-bp VNTR polymorphism and the haplotype comprising both markers.

**Conclusions:** These findings provide initial evidence that environmental risks as described by the Rutter Family Adversity Index moderate the impact of the *DAT1* gene on ADHD symptoms, suggesting a *DAT1* effect only in those individuals exposed to psychosocial adversity.

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**N**UMEROUS FAMILY, TWIN, and adoption studies have provided compelling evidence that attention-deficit/hyperactivity disorder (ADHD) is highly heritable, with an estimated genetic contribution of approximately 80%.<sup>1</sup> Molecular genetics studies have implicated several genes as mediating the susceptibility to ADHD. Genes in dopamine pathways have attracted particular interest, since both pharmacological and genetic findings suggest that dopamine plays an important role in attentional, motivational, and exploratory neurobehavioral processes. Over the past decade, a great deal of research has focused on the dopamine transporter (*DAT1*), located presynaptically on dopaminergic neurons, which regulates the

reuptake of dopamine into presynaptic terminals. The *DAT1* gene has been a prime candidate for ADHD, since psychostimulant medication used to treat this disorder is theorized to inhibit the reuptake function of the dopamine transporter, leading to an increase in the quantity and duration of dopamine available in the synaptic cleft.<sup>2</sup> To date, most studies have examined a polymorphic 40-base pair (bp) variable number of tandem repeats (VNTR) sequence located in the 3' untranslated region (3'UTR) of the gene,<sup>3</sup> varying between 3 and 11 copies, of which only the 9-repeat (9R) and the 10-repeat (10R) alleles are common.<sup>4</sup> Functional relevance for this polymorphism has been demonstrated by in vivo brain single-photon emission computed tomography scan studies that indicated significantly

higher *DAT1* availability among probands with ADHD<sup>5</sup> and among individuals homozygous for the 10R allele as compared with 9R carriers,<sup>6</sup> suggesting that the 10R allele may be associated with an increased production or turnover of the dopamine transporter. However, conflicting results have been reported from other studies that have either found no influence of the VNTR polymorphism on *DAT1* density<sup>7</sup> or found an opposite effect.<sup>8</sup> In animal studies, *DAT1* knockout mice were shown to exhibit a dramatic increase in motor activity, with dopamine remaining in the synaptic cleft 100 times longer than in heterozygous and wild-type mice.<sup>9</sup>

Since the first report by Cook et al,<sup>10</sup> many studies have examined the role of the *DAT1* 40-bp VNTR polymorphism in ADHD. So far, however, results have been contradictory. While several studies reported a significant association of this polymorphism with ADHD, others have failed to replicate this finding. First meta-analyses based on 9 and 11 studies, respectively, yielded trends for association (odds ratio [OR], 1.16;  $P = .06$  and OR, 1.27;  $P = .06$ ).<sup>11,12</sup> However, in a more recent meta-analysis surveying 13 family-based studies, no significant association ( $P = .21$ ) between ADHD and the 40-bp VNTR polymorphism was established.<sup>13</sup>

A number of reasons have been discussed that may account for the inconsistent findings. One possible explanation may be derived from the fact that the heritability coefficient from behavioral genetics studies indexes both the direct effect of genes and the effects of interactions between genes and shared environments.<sup>14</sup> Different levels of environmental risk exposure could act to moderate the genetic risk for ADHD in a way such that the genetic effect may only become apparent among the subgroup of individuals exposed to the environmental risk and not among those without risk exposure. Thus, gene  $\times$  environment interactions could explain the fact that not all individuals carrying a vulnerable *DAT1* genotype develop a disorder. However, although the significance of gene  $\times$  environment interactions in developmental psychopathology has been highlighted by various researchers,<sup>15</sup> few such interactions have been successfully characterized in relation to ADHD.<sup>16</sup>

Gene  $\times$  environment interactions may be of particular importance in ADHD, as research has implicated several aspects of the biological and psychosocial environment as potential risk factors for ADHD. Many studies have provided evidence that psychosocial adversity factors raise the risk for ADHD.<sup>17,18</sup> These factors include characteristics of adverse family environments as described by the Rutter Family Adversity Index,<sup>19</sup> such as marital discord, parental psychopathology, low maternal education, and single parenthood. However, since adversity factors have emerged as predictors of a variety of child maladaptive behavior, they may be considered as nonspecific triggers of an underlying predisposition or as modifiers of the course of illness.<sup>20</sup>

Another possible source of heterogeneity may be that the 40-bp VNTR polymorphism is not directly associated with ADHD but has an influence only through linkage disequilibrium with some other polymorphisms that are functionally responsible for the association. Several findings indicating stronger associations with haplotypes containing the 10R allele of the VNTR polymor-

phism in combination with alleles of markers located nearby support the assumption that this polymorphism is acting as a tagging marker for an alternative functional site. Barr et al<sup>21</sup> found biased transmission of a common haplotype consisting of the 10R allele and 2 polymorphisms in exon 9 and intron 9. Similar findings with several nearby genetic markers were reported by Feng et al.<sup>22</sup> Recently, Brookes et al<sup>23</sup> replicated the association of the 40-bp VNTR polymorphism with ADHD, demonstrating associations with a haplotype including the 10R allele of this polymorphism and a 6-repeat (6R) allele of a 30-bp VNTR polymorphism within intron 8, earlier described by Vandenberg et al,<sup>24</sup> and named "allele 3" by Brookes et al.<sup>23</sup> In addition, this haplotype showed significant interactions with maternal prenatal alcohol use.

In conclusion, there is evidence to suggest that gene  $\times$  environment interactions may underlie the inconsistent findings regarding the association between *DAT1* and ADHD. The present study examined the independent and combined effects of the *DAT1* 40-bp VNTR polymorphism and psychosocial adversity on ADHD symptoms in 15-year-olds from a high-risk community sample. To make use of the information of linkage disequilibrium in this region, we studied not only the 40-bp VNTR polymorphism, but also included 4 further markers reported earlier. Similar to Brookes et al,<sup>23</sup> we specifically set out to investigate a possible gene  $\times$  environment interaction between the 2 VNTR markers (and their haplotype) and psychosocial adversity acting toward the manifestation of ADHD symptoms.

## METHOD

### SAMPLE

This investigation was conducted as part of the Mannheim Study of Children at Risk, a large-scale prospective longitudinal study of the outcome of early risk factors from infancy into adulthood.<sup>25</sup> The initial sample consisted of 384 children of predominantly European descent born between 1986 and 1988, who were recruited from 2 obstetric and 6 children's hospitals of the Rhine-Neckar Region of Germany. Infants were included consecutively into the study according to a 2-factorial design intended to enrich and control the risk status of the sample (full details of the sampling procedure have been reported previously).<sup>26</sup> As a result, approximately two thirds of the study sample had experienced moderate to severe obstetric complications such as preterm birth, while about two thirds of the families had psychosocial adversities such as marital discord or chronic difficulties. To control for confounding effects of family environment and infant medical status, only firstborn children with singleton births and German-speaking parents were enrolled in the study. Furthermore, children with severe physical handicaps, obvious genetic defects, or metabolic diseases were excluded. Assessments were conducted at regular intervals throughout childhood, most recently at age 15 years. The current investigation included 305 adolescents (146 boys, 159 girls) who participated in the 15-year assessment and for whom genetic data were available. Of the original sample of 384 participants, 18 (4.7%) were excluded because of severe handicaps (neurological disorder or  $IQ < 70$ ), 28 (7.3%) were dropouts, and 33 (9.8%) refused to participate in blood sampling. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

**Table 1. Characteristics of the 147 Participants Exposed to Higher Psychosocial Adversity and Definition of Adversity Items**

Item	Definition	%
Low educational level of a parent	Parent without completed school education or without skilled job training	35.2
Overcrowding	>1.0 Person a room or size of housing $\leq 50$ m <sup>2</sup>	15.7
Parental psychiatric disorder	Moderate to severe disorder according to <i>DSM-III-R</i> criteria (interviewer rating, $\kappa = 0.98$ )	40.9
History of parental broken home or delinquency	Institutional care of a parent/>2 changes of parental figures until the age of 18 years or history of parental delinquency	34.0
Marital discord	Low quality of partnership in 2 of 3 areas (harmony, communication, emotional warmth) (interviewer rating, $\kappa = 1.00$ )	22.0
Early parenthood	Age of a parent $\leq 18$ y at child's birth or relationship between parents lasting <6 mo at time of conception	48.4
1-Parent family	At child birth	20.8
Unwanted pregnancy	An abortion was seriously considered	26.4
Poor social integration and support of parents	Lack of friends and lack of help in child care (interviewer rating, $\kappa = .71$ )	8.2
Severe chronic difficulties	Affecting a parent lasting >1 y (interviewer rating, $\kappa = 0.93$ )	51.6
Poor coping skills of a parent	Inadequate coping with stressful events of the past year (interviewer rating, $\kappa = 0.67$ )	73.6

## ASSESSMENT

Psychosocial adversity was determined according to an "enriched" family adversity index as proposed by Rutter and Quinton.<sup>19</sup> Information was derived from a standardized parent interview conducted at the 3-month assessment. The index assesses the presence of 11 adverse family factors, covering characteristics of the parents, the partnership, and the family environment during a period of 1 year prior to birth. Families with a score of 0 or 1 on the index (ie, median) formed the group with lower exposure to adversity and those with a score of 2 or more, the group with higher exposure to adversity. A description of the higher-adversity group and definitions of the index items are presented in **Table 1**. As is evident, inadequate parental coping with stressful events was by far the most frequent item in this group.

Psychiatric assessment of adolescents at age 15 years was conducted with the Schedule for Affective Disorders and Schizophrenia in School-Age Children.<sup>27,28</sup> The Schedule for Affective Disorders and Schizophrenia in School-Age Children is a widely used structured diagnostic interview completed independently with parents and adolescents, for which a considerable body of reliability and validity data has been published. Informants were asked about symptoms during the 12-month period prior to assessment. A symptom was defined as present when criteria were met in either the parent or adolescent interview. Symptoms of ADHD were assigned to domains as given in *DSM-IV* and the number of symptoms present was calculated. Two symptom scores indexing severity of inattention (9 items,  $\alpha = .90$ ) and hyperactivity-impulsivity (9 items,  $\alpha = .88$ ) were formed, which were highly correlated ( $r = 0.75$ ;  $P < .001$ ). Because the distribution of symptom scores was markedly skewed, with the majority of participants receiving values of zero (as is usually the case in community samples), scores were dichotomized (no vs any symptom present). Twenty adolescents (6.6%) fulfilled criteria for a current diagnosis of ADHD (13 boys [8.9%]; 7 girls [4.4%]) and 52 (17.0%) received a lifetime diagnosis of ADHD (33 boys [22.6%]; 19 girls [11.9%]) according to *DSM-IV*.

## GENOTYPING

EDTA anticoagulated venous blood samples were collected from 305 individuals. Leukocyte genomic DNA was isolated with the Qiaamp DNA extraction kit (Qiagen, Chatsworth, Calif). A total of 5 markers were genotyped: a 40-bp VNTR polymorphism in the 3'UTR, a 30-bp VNTR polymorphism in intron 8, and 3 single nucleotide polymorphisms (SNPs) with dbSNP (SNP database) numbers rs6347 in exon 9, rs8179029 in intron 9, and rs27072 in exon 15 (3'UTR). The 40-bp VNTR polymor-

phism was genotyped with the primers and reaction conditions of Sano et al.<sup>29</sup> Polymerase chain reaction was carried out using a nucleotide mix consisting of 7.4mM deoxyadenosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate and 3.7mM deoxyguanosine triphosphate and 7-deaza-2'-deoxyguanosine 5'-triphosphate (Amersham Biosciences, Piscataway, NJ). After an initial denaturation step, 35 cycles of amplification of 1 minute at 94°C, 1 minute at 63°C, and 35 seconds at 72°C were performed. The 30-bp intron 8 VNTR polymorphism described previously<sup>23,24</sup> was genotyped according to the procedure by Vandenberg et al.<sup>24</sup> Genotyping of the SNPs was completed using TaqMan SNP Genotyping Assays (7900HT Fast Real-Time-PCR-System; Applied Biosystems, Foster City, Calif). All genotypes were scored independently by 2 individuals who were blind to the diagnostic data.

## STATISTICAL ANALYSIS

Genotypes were tested for deviation from Hardy-Weinberg equilibrium using standard  $\chi^2$  goodness-of-fit tests. The pairwise linkage disequilibrium estimates  $D'$  and the Cramer V were calculated using COCAPHASE version 2.404 of the UNPHASED program suite (<http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased/>).<sup>30</sup> COCAPHASE was also used for estimating haplotype frequencies for the 5-marker haplotype and the 2-marker haplotype of the 30-bp VNTR and 40-bp VNTR polymorphisms. For the latter, individual haplotypes were constructed using PHASE version 2 (<http://www.stat.washington.edu/stephens/software.html>).<sup>31</sup>

To examine gene  $\times$  environment effects on ADHD symptoms, logistic regression was conducted. Models were fit for the main effects of the 30-bp VNTR and 40-bp VNTR genotypes and haplotype, respectively, and the main effect of psychosocial adversity with subsequent addition of the interaction term. All models included sex as a covariate. For these analyses, genotypes and the 2-marker haplotype of the 30-bp VNTR and 40-bp VNTR polymorphisms were classified according to homozygosity for the risk alleles (6R for the 30-bp VNTR, 10R for the 40-bp VNTR) and haplotype (6R-10R), respectively.

## RESULTS

### SAMPLE CHARACTERISTICS

Marker groups did not differ significantly with regard to sex, age, nonverbal IQ (Culture Fair Test 20 assessed at

**Table 2. Pairwise Linkage Disequilibrium Estimates Using D' and the Cramer V**

D' by Marker	Cramer V by Marker				
	30-bp VNTR	rs6347	rs8179029	rs27072	40-bp VNTR
30-bp VNTR		0.73	0.91	0.13	0.41
rs6347	0.85		0.76	0.09	0.44
rs8179029	0.92	0.99		0.17	0.62
rs27072	0.46	0.12	0.70		0.24
40-bp VNTR	0.63	0.46	0.73	0.80	

Abbreviations: bp, base pair; VNTR, variable number of tandem repeats.

age 11 years), psychosocial adversity score, obstetric risk score (obstetric adversity index counting the presence of 9 adverse conditions during pregnancy, delivery, and the postnatal period, such as preterm labor, asphyxia, or seizures<sup>25</sup>), prematurity, and maternal smoking during pregnancy (any regular maternal tobacco use during pregnancy assessed at the 3-month interview). In addition, no significant differences according to sex were observed on any of these variables.

### GENETIC ANALYSIS

In the overall sample of 305 adolescents, allele frequencies for the 5 markers typed were as follows: 30-bp VNTR polymorphism: 19.5% (5-repeat [5R]), 78.7% (6R), and 1.8% (7-repeat [7R]); rs6347: 29% (C) and 71% (T); rs8179029: 81% (C) and 19% (T); rs27072: 80% (C) and 20% (T); and 40-bp VNTR polymorphism: 0.5% (<9R), 25.3% (9R), 73.9% (10R), and 0.3% (>10R). For the haplotype consisting of the 30-bp VNTR and the 40-bp VNTR polymorphisms, individual frequencies were as follows: 68% (6R-10R), 15% (5R-9R), 10% (6R-9R), 4% (5R-10R), 2% (7R-10R), 0.5% (6R->10R), and 0.5% (6R-<9R). Genotypes were in Hardy-Weinberg equilibrium: 30-bp VNTR polymorphism ( $\chi^2_3=0.48$ ;  $P=.92$ ); rs6347 ( $\chi^2_1=0.0$ ;  $P=.96$ ); rs8179029 ( $\chi^2_1=0.21$ ;  $P=.65$ ); rs27072 ( $\chi^2_1=1.17$ ;  $P=.28$ ); and 40-bp VNTR polymorphism ( $\chi^2_6=9.64$ ;  $P=.14$ ). Linkage disequilibrium estimates for all pairs of markers are presented in **Table 2**. Estimates are comparable to those reported by Brookes et al.<sup>23</sup>

### ADHD SYMPTOMS BY DAT1 MARKERS AND PSYCHOSOCIAL ADVERSITY

For the 5 *DAT1* variants, no main effect on ADHD symptoms was observed. This was true for single markers as well for haplotype analyses including 2-, 3-, 4-, and 5-marker windows (all  $P>.50$ ; data not shown). However, significant interactions of the 30-bp VNTR and 40-bp VNTR genotypes and haplotype with psychosocial adversity on ADHD symptoms emerged (**Table 3**). Subsequent analysis demonstrated that rates of inattention and hyperactivity-impulsivity were significantly elevated in adolescents exposed to higher adversity who were homozygous for the 10R allele of the 40-bp VNTR polymorphism as compared with all other groups (inattention,  $P=.015-.001$ ; hyperactivity-impulsivity,  $P=.011-$

.001). Similar results were obtained for the 6R allele of the 30-bp VNTR polymorphism and the 6R-10R haplotype (**Figure 1** and **Figure 2**). Cochran-Armitage trend tests suggested a “moderated” gene dose effect for the 6R-10R haplotype, indicating that heterozygotes had intermediate rates of inattention (1-sided  $P=.054$ ) and hyperactivity-impulsivity (1-sided  $P=.034$ ) in the higher-adversity group, but not in the lower-adversity group. In addition, there were significant main effects of psychosocial adversity on inattention and hyperactivity-impulsivity ( $P=.012-.003$ ), with adolescents from adverse family backgrounds exhibiting higher symptom rates than those from less disadvantaged environments.

### COMMENT

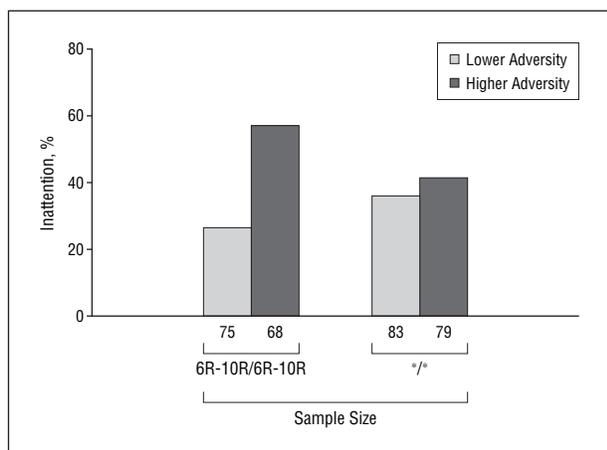
The current study sought to examine whether a gene  $\times$  environment interaction could explain the inconsistent findings of recent molecular genetics research associating the *DAT1* gene with ADHD. Our results provide initial evidence that environmental risks as described by the Rutter Family Adversity Index moderate the impact of the *DAT1* gene on the development of ADHD symptoms, revealing a *DAT1* effect only in those individuals exposed to psychosocial adversity. In detail, we found that 15-year-olds who grew up in greater adversity and were homozygous for the 10R allele of the 40-bp VNTR polymorphism in the 3'UTR and for the 6R allele of the 30-bp VNTR polymorphism in intron 8 as well as for their haplotype exhibited significantly more inattention and higher hyperactivity-impulsivity than those with other genotypes/haplotypes or living in less adverse family conditions. In addition, there were no significant main effects of genetic variants of *DAT1* on ADHD symptoms, suggesting that, to some extent, the *DAT1* risk operates through its effect on susceptibility to risk environments.

The present investigation is one of a small number of studies in humans to date that have examined whether environmental moderation plays a role in the association between the *DAT1* gene and ADHD. While previous studies focused on single environmental risks, such as exposure to maternal use of alcohol or tobacco during pregnancy,<sup>16,23</sup> the present study used a composite measure of family adversity as a potential moderator of genetic risk. However, caution must be exercised in the interpretation of such composite measures, since it is difficult to separate the effect of environmental factors from the genetic

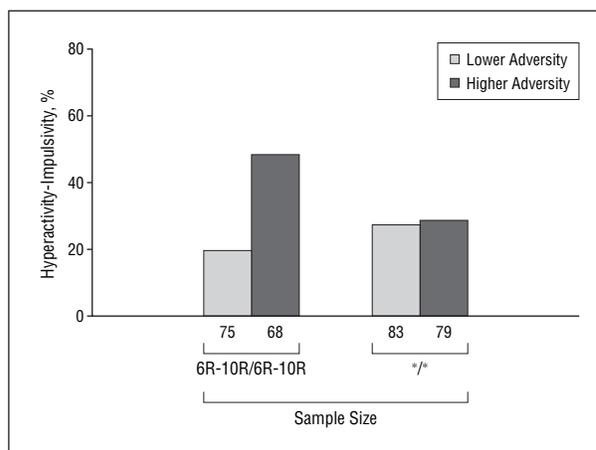
**Table 3. Inattention and Hyperactivity-Impulsivity Grouped by *DAT1* Markers (G) and Exposure to Psychosocial Adversity (E)**

	No./Total No. (%)		G × E Interaction, OR (95% CI)	P Value
	Lower Exposure to Psychosocial Adversity (n = 158)	Higher Exposure to Psychosocial Adversity (n = 147)		
<b>Inattention</b>				
40-bp VNTR				
*/* (n = 137)	25/72 (34.7)	25/65 (38.5)	3.28 (1.24-8.68)	.017
10R/10R (n = 168)	25/86 (29.1)	47/82 (57.3)		
30-bp VNTR				
*/* (n = 118)	24/61 (39.3)	24/57 (42.1)	3.51 (1.25-9.82)	.017
6R/6R (n = 187)	26/97 (26.8)	48/90 (53.3)		
Haplotype				
*/* (n = 162)	30/83 (36.1)	33/79 (41.8)	3.33 (1.27-8.78)	.015
6R-10R/6R-10R (n = 143)	20/75 (26.7)	39/68 (57.4)		
<b>Hyperactivity-impulsivity</b>				
40-bp VNTR				
*/* (n = 137)	19/72 (26.4)	17/65 (26.2)	3.70 (1.32-10.40)	.013
10R/10R (n = 168)	19/86 (22.1)	39/82 (47.6)		
30-bp VNTR				
*/* (n = 118)	20/61 (32.8)	18/57 (31.6)	2.91 (1.10-7.72)	.032
6R/6R (n = 187)	18/97 (18.6)	38/90 (42.2)		
Haplotype				
*/* (n = 162)	23/83 (27.7)	23/79 (29.1)	3.92 (1.41-10.92)	.009
6R-10R/6R-10R (n = 143)	15/75 (20.0)	33/68 (48.5)		

Abbreviations: bp, base pair; CI, confidence interval; OR, odds ratio; VNTR, variable number of tandem repeats; 6R, 6-repeat allele; 10R, 10-repeat allele; \*/\*, all other genotypes/haplotypes.



**Figure 1.** Percentage of inattention in adolescents grouped by the presence or absence of the *DAT1* 6-repeat allele–10-repeat allele (6R-10R) haplotype and exposure to psychosocial adversity. The 6R-10R/6R-10R haplotype exposed to higher adversity is significantly different from all other groups. \*/\* indicates all other genotypes/haplotypes.



**Figure 2.** Percentage of hyperactivity-impulsivity in adolescents grouped by the presence or absence of the *DAT1* 6-repeat allele–10-repeat allele (6R-10R) haplotype and exposure to psychosocial adversity. The 6R-10R/6R-10R haplotype exposed to higher adversity is significantly different from all other groups. \*/\* indicates all other genotypes/haplotypes.

liability imparted by parents. Studies using genetically sensitive designs have indicated that many supposed environmental effects actually, in part, reflect genetic factors.<sup>32</sup> Several of our psychosocial adversity factors may be proxies for heritable influences. For example, the adverse consequences of low education or psychopathology of a parent might well be due to genetic variation. If the association between psychosocial adversity and ADHD was entirely genetically mediated (gene × environment correlation), then the gene × environment interaction identified in this study could actually reflect interactions between the *DAT1* gene and other genes that were not

measured (gene × gene interaction). Although there were no significant differences between genotype groups regarding psychosocial adversity (data not shown), the present study cannot rule out the potential confound of genetic mediation of environmental risk. In an attempt to control for possible genetic mediation, we reanalyzed the association between *DAT1*, psychosocial adversity, and ADHD symptoms, adjusting for parental history of psychiatric disorder prior to birth and low parental education. Adjustment for these major candidates for genetically mediated environmental risks did not change the principal finding, indicating a significant *DAT1* × adversity

interaction effect on inattention and hyperactivity-impulsivity.

Several other confounders of psychosocial adversity have been discussed in the literature. These include environmental risk factors for ADHD, which are mediated by biological adversity such as obstetric complications, prematurity, and smoking during pregnancy.<sup>33</sup> The latter has been recently identified as moderating the *DAT1* effect on ADHD.<sup>16</sup> When results were adjusted for these biological adversity factors, the *DAT1* × psychosocial adversity interaction remained significant.

Although the present investigation further supports the role for gene × environment interaction in the etiology of ADHD, our results provide no evidence for the exact mechanism by which this interaction confers risk for the disorder. In particular, our findings leave open the question as to how environmental adversity influences pathophysiological pathways by which this effect is mediated. Further research is needed to identify the proximal environmental risk factors, such as characteristics of disturbed parent-child relationships, which lend themselves to biologically plausible hypotheses about their impact on neurobiological systems that underlie psychiatric symptoms.

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