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MOVEMENT DISORDERS - ORIGINAL ARTICLE

Evidence for epistasis between the 5-HTTLPR and the dopamine D4 receptor polymorphisms in externalizing behavior among 15-year-olds

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Abstract The present study aimed to clarify the functional role of genes in the dopamine and serotonin systems by examining whether polymorphisms in these genes are related to adolescent externalizing behavior either alone or in interaction with each other. Participants were selected from an ongoing prospective study of the outcome of early risk factors. At age 15 years, 298 adolescents (144 males, 154 females) completed the Youth Self Report, 296 primary caregivers the Child Behavior Checklist and 253 teachers the Teacher Report Form. DNA was genotyped for the DRD4 exon III VNTR and the 5-HTTLPR polymorphisms. Results revealed that individuals with the DRD4 7r allele reported significantly more externalizing behavior than carriers of other variants. In addition, a significant interaction emerged, indicating that adolescents carrying two copies of the 5-HTTLPR short allele and the DRD4 7r variant scored highest on aggressive and/or delinquent behavior compared to other genotypes. This result suggests an effect of 5-HTTLPR on externalizing behavior in the presence of DRD4 7r but no effect in its absence.

Keywords Aggression · Externalizing behavior · Adolescence · 5-HTTLPR · DRD4 · Genetics · Gene–gene interaction

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Introduction

Externalizing behaviors among young people such as aggression, violence or delinquency have become a growing public health problem within most industrialized countries. According to recent surveys, the past decade has witnessed a continuous increase in the level of juvenile violence, particularly among the young age group (Bundeskriminalamt 2007; Lösel and Bliesener 2003). Aggressive behavior defined as behavior that causes or threatens physical harm to others (Loeber and Hay 1997) often occurs in the context of other types of antisocial behaviors, such as lying, stealing and truancy, and is an essential component of the DSM IV diagnosis of conduct disorders (CD). Prevalence rates of CD in community samples have been reported to range from 1.8 to 16.0% for boys, and 0.8 to 9.2% for girls (Loeber et al. 2000). Among clinical samples, the rates of externalizing behaviors reached almost 50%, with half of the children and adolescents in inpatient units showing severe aggressive behavior (Döpfner et al. 2002). Longitudinal studies indicate that aggressive behavior in young people is highly predictive of violence in adulthood and is strongly associated with a greater risk of alcohol and drug abuse, accidents, violent crimes, suicide attempts, and depression (Fergusson and Horwood 1998; Nagin and Tremblay 1999).

In recent years, many attempts have been made to uncover the neuropsychological and genetic underpinnings of externalizing behaviors. Family and twin-based heritability studies revealed that a general vulnerability for externalizing behavior is highly heritable, with strong genetic correlations among different types of externalizing disorders such as conduct disorder or substance abuse (Dick et al. 2005; Hicks et al. 2004). When investigating the molecular genetic basis of externalizing behavior, most previous studies focused on variations in genes involved in the regulation of the serotonergic and dopaminergic neurotransmitter systems, as these transmitter systems have been suggested to underlie the behavioral system of approach and inhibition (Auerbach et al. 2001). Dopamine is associated with the activation and intensity of response in situations of reward (Panksepp 1986), while serotonin is assumed to be linked to the inhibition of behavioral and emotional responses (Soubrie 1986).

For a long time, the serotonergic system has been a major target for investigations concerning human aggressive or antisocial behavior. Low levels of 5-HT (achieved, for example, on an experimental basis via tryptophan depletion) are hypothesized to lead to more aggressive behavior and inhibition control problems in children with ADHD (Stadler et al. 2007; Zepf et al. 2008). Most previous research was conducted using polymorphisms of the serotonin transporter promoter region (5-HTTLPR). The serotonin transporter (5-HTT) assumes a key position within the regulation of central serotonergic functioning and is responsible for the reuptake of 5-HT from the synaptic cleft. The 5-HTTLPR polymorphism is located in the transcriptional control region upstream of the 5-HTT coding sequence and consists of a 44 bp insertion or deletion involving six to eight repeat elements (Murphy et al. 2004). Two variants have been frequently described, an insertion variant (long/l-allele) and a short variant (s-allele) caused by a deletion. Lesch et al. (1996) reported that individuals carrying the l-allele of 5-HTTLPR show a basal serotonin transporter activity twice as high as that of carriers of the s-allele, resulting in a lower transcription rate of the s-allele and a lower density of 5-HTT on the plasma membrane of the serotonergic neurons (Champoux et al. 2002). Based on these findings, it was hypothesized that l/l-variants have reduced levels of intrasynaptic 5-HT per time unit compared to s/s and s/l-variants. This genetically induced functional difference in serotonin uptake appears to influence a proportion of phenotypic variation along several human behaviors, including negative emotionality, proneness to anxiety or depression, and aggressiveness (Nobile et al. 2004). The association between a lower density of 5-HTT on neuronal cells and a higher rate of aggressive behavior has recently been demonstrated. In individuals with impulsive and aggressive behavior, Frankle et al. (2005) found a lower density of 5-HTT in neurons of the anterior cingulated cortex (measured by PET). Sekine et al. (2006) reported that a downregulation of 5-HTT within the central nervous system was associated with violent behavior in individuals following methamphetamine abuse.

So far, the results of genetic epidemiological studies assessing the association between the 5-HTTLPR variants and aggressive behavior have been inconsistent. Many groups reported carriers of the s-allele of 5-HTTLPR to exhibit more aggressive or violent behavior than individuals with the s/l or l/l genotype (Haberstick et al. 2006; Hallikainen et al. 1999; Liao et al. 2004; Retz et al. 2004). However, several studies demonstrated an association of the l-allele of 5-HTTLPR with externalizing behavior (Nobile et al. 2007) or severe forms of hyperkinetic disorder (Seeger et al. 2001).

Another neurotransmitter system often discussed in the context of the origin of externalizing behavior is the dopaminergic system. Most research has focused on the dopamine D4 receptor (DRD4), which is a G protein-coupled receptor, exerting an inhibitory effect on the adenylylcyclase and adenosine triphosphate (ATP) production. The DRD4 gene, located on the long arm of chromosome 11, contains a highly polymorphic 48 bp variable number of tandem repeats (VNTR) sequence in exon III. This polymorphism was found to vary between 2 and 11 copies, with the 4- and 7-repeat alleles (4r and 7r) being the most frequent within most populations (Lichter et al. 1993; Vallone et al. 2000). The polymorphic repeated segment codes for the third intracytoplasmatic loop of the receptor, a region which normally couples to the G protein and mediates intracellular signaling (Asghari et al. 1995). However, in in vitro studies, the polymorphism has proved to have no effect on the efficiency of G-alpha-coupling (Kazmi et al. 2000). In association studies, the exon III VNTR of DRD4 has been linked to novelty seeking (Becker et al. 2005; Ebstein et al. 1996), which is closely related to externalizing behavior, such as impulsivity, sensation seeking, and aggression (Battaglia et al. 1996). This observation corresponds well with findings from our study group, indicating that adolescents carrying the 7r variant of DRD4 displayed more excessive drinking behaviors compared to carriers of other alleles (Laucht et al. 2007).

Concerning the association of polymorphisms within DRD4 and 5-HTTLPR with externalizing behavior in children and adolescents, epistasis between these two functionally relevant variants has been postulated to create a specific genotype. This fits with the hypothesis that the serotonergic system functionally regulates dopaminergic neurotransmission and therefore plays an important role in controlling dopamine-mediated externalizing behavior (Oades 2008; Quist and Kennedy 2001). For example,

Nobile et al. (2007) demonstrated that the combination of the 5-HTTLPR l/l genotype with a long variant of the DRD4 exon III VNTR led to more externalizing behavior among children from families with low socioeconomic status. In contrast, Schmidt et al. (2007) reported that, in a group of 7-year-olds, the combination of a long DRD4 variant and the short allele of 5-HTTLPR resulted in more externalizing and internalizing behavior. In a study from our group, Skowronek et al. (2006) found that females carrying the DRD4 7r allele and the long version of the 5-HTTLPR showed significantly increased alcohol abuse compared to individuals with other genotypes. Lakatos et al. (2003) reported that infants with the DRD4 7r allele and at least one l-allele of 5-HTTLPR were more prone to novelty seeking than those with the combination of DRD4 7r and 5-HTTLPR s/s. Interestingly, children with the combination of the s/s genotype and lacking DRD4 7r also exhibited more novelty seeking. Oades and the IMAGE study group (Oades et al. 2008) defined two different forms of impulsivity, the impulsive–aggressive type and the cognitive–impulsive type, and tried to investigate whether there are different genetic variants linked with these two phenotypic variants. They found a significant association between the exon III VNTR of DRD4 and the impulsive–aggressive type, while the results for 5-HTTLPR failed to reach the significance level.

The objective of this study was to further clarify the functional role of the DRD4 exon III and the 5-HTTLPR polymorphisms by investigating their association with externalizing behavior in 15-year-old German adolescents from a high-risk community sample. In particular, we examined whether these polymorphisms are related to adolescent externalizing behavior either alone or in interaction with each other.

Materials and methods

Participants

Participants in this investigation were selected from the Mannheim Study of Children at Risk, an epidemiological cohort study from birth to adolescence on the long-term outcome of early risk factors. The initial sample comprised 384 predominantly (>99.0%) Caucasian infants, born between 1986 and 1988, who were recruited from two obstetric and six children's hospitals of the Rhine-Neckar region of Germany. To be included in the study, parents and infants had to meet criteria intended to enrich and to control the risk status of the sample. Depending on pregnancy and birth history and on family background, infants were assigned to 1 of 9 groups of a 2-factorial design with factor I representing the degree of biological risk (perinatal complications) and factor II the degree of psychosocial risk (family adversity). Each factor was scaled as no, moderate, or high risk. Risk combinations resulting from this 3 × 3 design are, for example, groups characterized by high biological and moderate psychosocial risk or by moderate biological and high psychosocial risk. All groups had about equal size, with a slight oversampling in the high-risk combinations and with sex evenly distributed in all subgroups (full details of the sampling procedure have been reported previously, Laucht et al. 1997, 2000). A psychosocial risk score was determined according to an "enriched" family adversity index as proposed by Rutter and Quinton (1977) measuring 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. An obstetric adversity score was obtained by counting the presence of nine adverse conditions during pregnancy, delivery, and the postnatal period such as preterm labor, asphyxia or seizures. To control for confounding effects of family environment and infant medical status, only firstborn children with singleton birth, German-speaking parents, and no severe physical handicaps, obvious genetic defects or metabolic diseases were included.

Assessments were conducted at the ages of 3 months, and 2, 4.5, 8, 11, and 15 years. The current investigation included 298 adolescents (144 males, 154 females) 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, MQ or IQ <70), 35 (10.4%) refused to participate in blood sampling, and 33 (8.6%) were dropouts or had incomplete data. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

Psychological assessment

To assess behavior problems in adolescents, the Youth Self Report (YSR) was administered to the 15-year-olds (Achenbach 1991a; German version by Döpfner et al. 1994a). The adolescent filled in the YSR during a visit at our laboratory where they participated in a standardized interview and an EEG session. This 120-item scale measures a broad range of adolescent psychopathological behaviors in eight subscales (e.g., depression, somatization). The YSR is a highly reliable questionnaire, discriminating effectively between adolescents referred to the clinic for problem behavior and nonclinical adolescents. It provides scores for internalizing (anxious–depressed, social withdrawal, and somatic complaints) and externalizing behavior problems (delinquent and aggressive behavior), as

well as a total behavior problems score. Good psychometric characteristics have been confirmed for the German version (Walter et al. 1994; Döpfner et al. 1994c, 1995). In addition, the Child Behavior Checklist (CBCL, Achenbach 1991b; German version by Döpfner et al. 1994d) and the Teacher Report Form (TRF, Achenbach 1991c; German version by Döpfner et al. 1994b) were completed by $n = 296$ primary caregivers and $n = 253$ teachers of the participants, respectively. Within this study, our primary interest was on the aggressive and delinquent behavior (externalizing) scales.

Participants completed a substance use inventory including different measures of adolescent alcohol and tobacco use. The inventory is part of the Substance Use Questionnaire (SUQ) designed by Müller and Abbet (1991) in collaboration with the WHO. In addition, the amount of alcohol intake during the last 6 months was assessed with the Lifetime Drinking History LDH (Skinner and Sheu 1982). A measure of the mean amount of alcohol consumed per month was derived. To assess current smoking status reflecting both the frequency and the quantity of smoking, responses were combined into a four-level variable: (1) never used, (2) experimental use (smoked up to once per month), (3) regular use (at least weekly), (4) dependent use (daily smoking >10 cigarettes per day).

Stage of pubertal maturation was assessed by self-ratings of the adolescents using standardized figure drawings depicting Tanner's sexual maturation scale (score range 1–5). Earlier studies have established a reasonable agreement between self-assessments and physicians' ratings (Brooks-Gunn et al. 1987; Duke et al. 1980).

Genotyping

Using the Qiamp (Qiagen, Chatsworth, CA, USA) extraction kit, genomic DNA was isolated from a 5 ml blood sample and stored at −20°C. The genotypes of the DRD4 polymorphisms were determined by polymerase chain reaction (PCR) (Lichter et al. 1993). Genotyping of the regulatory region of the 5-HTT gene was performed as described previously (Seeger et al. 2001). PCR products were visualized by means of UV fluorescence following separation in 2% agarose gels and staining with ethidium bromide. Based on previous results, DRD4 genotypes were classified into two groups according to the presence or absence of the 7r allele (7r vs. non-7r). Of the 298 adolescents studied, 120 (40.3%) had at least one copy of the DRD4 7r allele. The distribution of 5-HTTLPR genotypes was SS = 43 (14.4%), LS = 153 (51.3%), and LL = 102 (34.2%). Genotype frequencies did not deviate from Hardy–Weinberg equilibrium.

Statistical analysis

T-tests or analyses of variance and Chi-square tests, respectively, were performed to test differences in scores and frequencies between different groups. To examine genotype effects on the aggressive and delinquent behavior scales, two-way (DRD4 genotype × 5-HTTLPR genotype) MANCOVAs were performed, controlling for differences in Tanner stage, psychosocial risk, obstetric risk, gender, and substance use. MANCOVAs were followed by univariate analyses and single comparisons where appropriate.

Results

Sample characteristics

Demographic and clinical characteristics of the study sample are presented in Tables 1 and 2. There were no significant differences in YSR, CBCL, and TRF scale scores between gender and region of origin groups, except for a significantly higher YSR aggressive behavior score in females than in males. Furthermore, no significant differences were observed with regard to gender, psychosocial risk, and obstetric risk depending on DRD4 and 5-HTTLPR genotype groups. However, carriers of the DRD4 7r allele were found to have significantly higher Tanner scores than individuals without this allele.

Genotype effects

There was a significant multivariate main effect of the DRD4 genotype on the YSR behavior scores [F (2, 287) = 3.34, $p = 0.037$]. Univariate analyses revealed a significant effect of DRD4 for aggressive behavior [F (1, 288) = 6.69, $p = 0.010$] but not for delinquent behavior [F (1, 288) = 1.88, $p = 0.171$], indicating higher scores in individuals carrying the 7r allele than in those carrying other alleles. No multivariate main effect of 5-HTTLPR genotype was found [F (4, 574) = 0.82, $p = 0.515$]. In addition, a significant multivariate DRD4 × 5-HTTLPR interaction effect emerged [F (4, 574) = 3.11, $p = 0.015$]. Subsequent analyses revealed significant univariate interactions for aggressive behavior [F (2, 288) = 6.28, $p = 0.002$], but not for delinquent behavior [F (2, 288) = 1.91, $p = 0.151$]. Post hoc single comparisons demonstrated that individuals with the DRD4 7r allele and the 5-HTTLPR SS genotype scored highest on YSR aggression with scores that differed significantly from most other groups (Fisher's LSD, $p < 0.05$), except for those

Table 1 Sample characteristics I: YSR, CBCL, and TRF aggressive and delinquent behavior scores by gender and region of origin of parents

	YSR		CBCL		TRF	
	Aggressive behavior	Delinquent behavior	Aggressive behavior	Delinquent behavior	Aggressive behavior	Delinquent Behavior
Total sample ($n = 298$[a])	53.4 (4.8)	55.7 (6.1)	54.7 (6.8)	54.2 (6.2)	54.9 (6.3)	54.2 (6.0)
Males ($n = 144$[b])	52.6 (4.0)	55.4 (5.4)	54.5 (7.1)	53.7 (5.7)	55.1 (6.7)	54.3 (5.9)
Females ($n = 154$[c])	54.2 (5.4)*	56.0 (6.8)	54.9 (6.6)	54.6 (6.7)	54.7 (5.9)	54.1 (6.1)
Region of origin of parents[d]						
Both parents Central European ($n = 264$[e])	53.5 (4.9)	55.8 (6.3)	54.7 (6.9)	54.2 (6.3)	54.8 (6.3)	54.0 (6.0)
One parent Central European, one other European ($n = 27$[f])	53.2 (5.0)	54.9 (5.2)	55.5 (6.5)	54.2 (6.2)	54.6 (5.9)	55.3 (5.9)
Both parents Eastern European ($n = 4$)	51.5 (1.7)	50.0 (0.0)	52.5 (3.0)	50.0 (0.0)	59.8 (9.8)	55.3 (6.2)
One parent Central European, one Non-European ($n = 3$[g])	54.3 (6.7)	58.7 (2.5)	50.0 (0.0)	51.7 (2.9)	56.5 (3.5)	58.5 (3.5)

The values given are mean and SD (in parentheses)

* Significant difference

[a] CBCL ($n = 296$), TRF ($n = 253$)

[b] CBCL ($n = 142$), TRF ($n = 121$)

[c] CBCL ($n = 154$), TRF ($n = 132$)

[d] Region of origin was self-reported by parents: Central European includes German, Austrian, Swiss origin, Eastern European includes Polish, former USSR, former Yugoslavian, former Czechoslovakian origin, Non-European origin includes Afro-American and Asian origin

[e] CBCL ($n = 262$), TRF ($n = 225$)

[f] TRF ($n = 22$)

[g] TRF ($n = 2$)

Table 2 Sample characteristics II: control variables by DRD4 and 5-HTTLPR genotypes

	DRD4		5-HTTLPR			
	7r ($n = 120$)	Non-7r ($n = 178$)	SS ($n = 43$)	LS ($n = 153$)	LL ($n = 102$)	Total ($n = 298$)
Gender (n, % males)	59 (49.2)	85 (47.8)	17 (39.5)	74 (48.4)	53 (52.0)	144 (48.3)
Psychosocial risk (score 1–7)	2.12 (2.04)	1.87 (2.07)	1.65 (1.94)	1.99 (2.15)	2.08 (1.97)	1.97 (2.06)
Obstetric risk (score 1–4)	1.01 (0.98)	1.17 (1.08)	0.95 (0.97)	1.14 (1.11)	1.12 (0.98)	1.11 (1.05)
Tanner stage (score 1–5)	3.98 (0.57)	3.81 (0.64)*	3.78 (0.64)	3.91 (0.62)	3.87 (0.62)	3.87 (0.62)

The values given are mean and SD (in parentheses)

* Significant difference

carrying the DRD4 7r allele/5-HTTLPR LS genotype (Fisher's LSD, $p = 0.205$) and the non-DRD4 7r allele/5-HTTLPR LL genotype (Fisher's LSD, $p = 0.057$) (see Fig. 1).

Roughly similar results were obtained when using the CBCL and TRF, indicating marginally significant interactions for both aggressive [F (2, 286) = 2.99, $p = 0.052$] and delinquent behavior [F (2, 286) = 2.90, $p = 0.057$] in the CBCL, and a significant interaction for delinquent behavior [F (2, 243) = 3.59, $p = 0.029$], but not for aggressive behavior [F (2, 243) = 0.96, $p = 0.384$] in the TRF. Furthermore, adjustment for the potential confounding effect of alcohol and nicotine use did not change the principal findings.

Discussion

The aim of this study was to examine whether aggressive or other externalizing behavior in 15-year-old adolescents was related to known allelic variations of DRD4 and 5-HTT, and whether there is evidence for epistasis between these genes. Our results revealed a significant association of the DRD4 exon III polymorphism with externalizing behavior, but no relationship with the 5-HTTLPR genotype. Carriers of the DRD4 7r allele significantly displayed more aggressive behavior according to YSR self-report than individuals carrying other DRD4 variants. In addition, a significant interaction of the 5-HTTLPR and the DRD4 exon III polymorphism upon aggressive behavior was

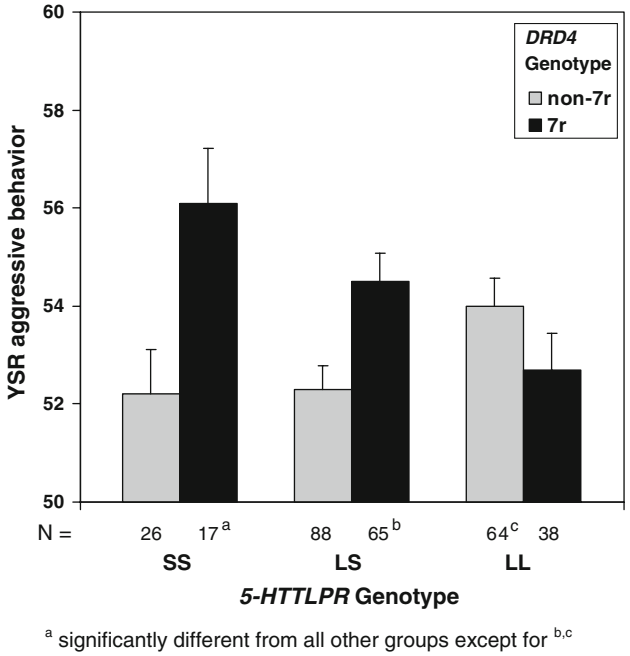

Fig. 1 Mean YSR aggressive behavior scores (SE) in 15-year-olds grouped by 5-HTTLPR and DRD4 genotypes: *T*-scores adjusted for Tanner stage, psychosocial risk, obstetric risk, and gender

observed. Adolescents with two copies of the short allele of the 5-HTTLPR and the 7r variant of DRD4 scored highest on the aggressive behavior scale compared to carriers of other genotypes. This result can be interpreted as indicating an effect of the s/s genotype of 5-HTTLPR in the presence of DRD4 7r but no effect in the absence of DRD4 7r.

Associations between DRD4 7r and externalizing behavior have been widely postulated (Ebstein et al. 1996) and were also observed in previous studies of our group. For example, 15-year-olds carrying the 7r allele of DRD4 were found to score higher on novelty seeking (Becker et al. 2005) and to show higher rates of lifetime smoking and cigarette consumption (Laucht et al. 2005). We also found that individuals with the 7r variant of DRD4 displayed more excessive drinking behavior than carriers of other DRD4 alleles (Laucht et al. 2007).

The role of the 5-HTTLPR-polymorphism in the development of externalizing behavior such as aggression is still unclear. Alterations in the activity of central serotonergic systems have been implicated in impulsive and aggressive behavior (Fishbein et al. 1989) and low levels of serotonin were found to alter aggressive behavior in children with ADHD (Stadler et al. 2007; Zepf et al. 2008). Brown et al. (1989) reported a negative association between 5-HT reuptake in blood platelets and impulsivity. Our findings are in line with those of several studies suggesting an involvement of the 5-HTTLPR s/s genotype in the development of aggression or impulisivity. For example, Hallikainen et al. (1999) found a higher frequency of the s/s genotype of 5-HTTLPR in individuals suffering from alcoholism and antisocial personality disorder and showing habitual impulsive violent behavior. Retz et al. (2004) reported a significant excess of the s-allele and the s/s genotype in patients referred to forensic examination who were characterized by recurrent and overt physical violent behavior. Similarly, Liao et al. (2004) found a higher frequency of the s-allele in a sample of Chinese males convicted for excessively violent crimes compared to normal controls. Based on findings from a family-based study, Haberstick et al. (2006) suggested an association between the s-allele of the 5-HTTLPR and higher aggressive behavior in middle childhood. A possible functional mechanism underlying these observations could be that the reduction of 5-HT uptake caused by the s-allele of 5-HTTLPR leads to an increase in free synaptic serotonin, resulting in a desensitization of various postsynaptic 5-HT receptors, as discussed, for instance, by Hallikainen et al. (1999). As these receptors are said to be involved in the regulation of dopaminergic neurotransmission (Oades 2008; Quist and Kennedy 2001), a dysfunction at this level could lead to a disequilibrium of these two transmitter systems and therefore to a preponderance of the dopaminergic system.

However, in contrast to the research mentioned above, several studies reported evidence implicating the l-allele of 5-HTTLPR in the development of aggressive behavior and externalizing behavior in general. For example, in a study by Seeger et al. (2001), children with hyperkinetic and/or conduct disorder, who generally exhibit more impulsive/aggressive behavior than healthy controls, were found more often to carry the long variant of the 5-HTTLPR. This finding was explained through the assumption that the l-allele leads to a higher transcription rate of the transporter, resulting in a higher density of the protein in the plasma membrane. Accordingly, a more efficient reuptake of serotonin was found in l-allele carriers (Greenberg et al. 1999; Lesch et al. 1996) associated with a decrease in the concentration of the free neurotransmitter in the synaptic cleft.

Concerning the interaction of DRD4 7r with 5-HTTLPR, even more inconsistency has been observed. In accordance with our findings, Schmidt et al. (2007) reported a higher frequency of the combination of DRD4 7r and 5-HTTLPR s/s in 7-year-olds with either externalizing or internalizing behavior as compared to normal controls. In contrast, in a study by Nobile et al. (2007), a higher rate of externalizing behavior was observed in children carrying the DRD4 7r and the 5-HTTLPR l/l genotype as compared to other genotypes when living under conditions of a low socioeconomic status. In infants, Lakatos et al. (2003) found that those with the combination of DRD4 7r and 5-HTTLPR l/l were more open to contact with strangers

than those carrying other genotypes. Using data from our study, Skowronek et al. (2006) reported an association of the combination of the 5-HTTLPR l-allele and DRD4 7r with a higher rate of alcohol and tobacco consumption in 15-year-olds, which was explained by the link of both variants with the temperament trait of novelty seeking.

One reason for the discrepant findings could be that the behaviors investigated were phenotypically different from each other, even though they belong to the spectrum of externalizing behavior. Thus, novelty seeking may be involved in both the development of alcohol and drug consumption as well as in infant behavior toward strangers, but may not play a role in aggressive behavior of adolescents. If this is the case, then different phenotypes were being measured and different results were to be expected. Another possible explanation could lie in considering the DRD4 7r variant as the predominant cause of externalizing behavior, given the consistent findings in the literature regarding the role of this polymorphism. As dopaminergic neurotransmission is supposed to be merely regulated by the serotonergic system, the DRD4 7r variant may only unfold its true potential when accompanied by an alteration in the serotonergic system in any direction. Finally, when attempting to account for the discrepant results, the differences in assessment of externalizing disorders should be taken into account. As most of the cited studies relied on parents' or teachers' reports (mostly due to the younger age of their samples), in our study also self-report was used. A further potential reason for inconsistency was excluded in our study, as we controlled for differences in Tanner stages of the 15-year-olds. Higher levels of sexual hormones (androgens such as testosterone), due to higher Tanner stages, are known to be involved in aggressive and antisocial behavior of adolescents (Maras et al. 2003; Rowe et al. 2004). Thus, the degree of sexual development should be considered when investigating genetic factors in aggression among young people.

Several limitations of this study have to be mentioned. First, because the study sample was enriched with children born at risk, it might not be possible to generalize the results to a general population sample. However, in the present analyses, both obstetric and psychosocial risk factors were included as covariates, adjusting for a possible distorting effect of sample composition. Second, the sample size of the current investigation is relatively small for a genetic association study examining epistasis. Because association studies are prone to false positive results, the results reported here require further validation in independent samples of an adequate size. A third limitation involves the effects of population stratification, such that true associations may be hidden by the population substructure. However, the potential impact of this effect is likely to be minimal here, because all probands were selected from an epidemiological cohort sample of a well-defined region, where 5-HTTLPR and DRD4 allele frequencies in different phenotypes were largely unbiased by geographical variation in proband characteristics. Finally, it has to be considered that Achenbach's psychopathological concepts and instruments used in this study are less clear-cut with respect to the quantification or assessment of different types of aggression. Even though the subscale aggressive behavior claims to measure aggression, obviously the content of the specific items varies greatly. The same applies to the delinquent behavior subscale, which also includes a number of items that appear to measure behavior problems related to aggression.

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