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Perinatal Risk Factors Interacting With Catechol O-Methyltransferase and the Serotonin Transporter Gene Predict ASD symptoms in Children With ADHD

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Abstract

Background—Symptoms of Autism Spectrum Disorder (ASD) and Attention-Deficit/Hyperactivity Disorder (ADHD) often co-occur. Given the previously found familiarity of ASD symptoms in children with ADHD, addressing these symptoms may be useful for genetic association studies, especially for candidate gene findings that have not been consistently replicated for ADHD.

Methods—We studied the association of the catechol o-methyltransferase (*COMT*) Val158Met polymorphism and the serotonin transporter (*SLC6A4/SERT/5-HTT*) 5-HTTLPR insertion/deletion polymorphism with ASD symptoms in children with ADHD, and whether these polymorphisms would interact with pre- and perinatal risk factors, i.e., maternal smoking during pregnancy and low birth weight. Analyses were performed using linear regression in 207 Dutch participants with combined type ADHD of the International Multicenter ADHD Genetics (IMAGE) study, and repeated in an independent ADHD sample (n = 439) selected from the TRacking Adolescents' Individual Lives Survey (TRAILS). Dependent variables were the total and subscale scores of the Children's Social Behavior Questionnaire (CSBQ).

Results—No significant main effects of *COMT* Val158Met, 5-HTTLPR, maternal smoking during pregnancy and low birth weight on ASD symptoms were found. However, the *COMT* Val/Val genotype interacted with maternal smoking during pregnancy in increasing stereotyped behavior in the IMAGE sample (p = 0.008); this interaction reached significance in the TRAILS sample after correction for confounders (p = 0.02). In the IMAGE sample, the 5-HTTLPR S/S genotype interacted with maternal smoking during pregnancy, increasing problems in social interaction (p = 0.02), and also interacted with low birth weight, increasing rigid behavior (p =

0.03). Findings for 5-HTTLPR in the TRAILS sample were similar, albeit for related CSBQ subscales.

Conclusions—These findings suggest gene-environment interaction effects on ASD symptoms in children with ADHD.

Keywords

Attention-deficit/Hyperactivity Disorder; Autism Spectrum Disorder; gene environment; *5-HTT*; *COMT*

Many children with attention deficit/hyperactivity disorder (ADHD) show symptoms of Autism Spectrum Disorders (ASD), especially problems in reciprocal social interaction (Nijmeijer et al., 2008b). Studies in community twin samples ADHD and ASD have shown that a substantial part of genetic influences are shared between ADHD and ASD (Reiersen, Constantino, Grimmer, Martin, & Todd, 2008; Ronald, Simonoff, Kuntsi, Asherson, & Plomin, 2008); our own study confirmed the familiarity of ASD symptoms in children with ADHD and their siblings (Nijmeijer et al., 2008a). Moreover, genetic linkage studies have identified overlapping sets of suggestive disease loci for ADHD and ASD, (e.g., Faraone et al., 2005; Waldman & Gizer, 2006; Yang & Gill, 2007).

Addressing ASD symptoms in children with ADHD may be particularly useful for genetic association studies, especially for candidate gene findings that have not been consistently replicated for ADHD. That is, non-replicated findings may be due to the heterogeneity of ADHD, which may partly stem from presence or absence of ASD symptoms (Mulligan et al., 2009; Nijmeijer et al., 2008). The serotonin transporter gene (*SLC6A4/SERT/5-HTT*) and catechol O-methyltransferase (*COMT*) are interesting candidate genes for the study of ASD symptoms in children with ADHD, as both have been implicated not only in ADHD (e.g., Gizer, Ficks, & Waldman, 2009), but also in autism (e.g., Huang & Santangelo, 2008; James et al., 2006).

SLC6A4 contains a 44 bp deletion/insertion functional polymorphism in the promoter region, called 5-HTTLPR. In a recent meta-analysis for 5-HTTLPR in ADHD, a significant association was found between the long (L) allele and ADHD (Gizer et al., 2009). On the other hand, a slight majority of studies has indicated the short (S) allele as the ASD risk allele (for reviews see Devlin et al., 2005; Huang & Santangelo, 2008). Animal studies have shown that serotonin is important in the regulation of attention and response control (Gainetdinov et al., 1999; Wistanley et al., 2005). Deficiencies herein are reported for both ADHD and ASD (e.g., Geurts et al., 2004; Happé et al., 2006). Involvement of serotonin in ASD is further supported by its role in early neurodevelopment (Whitaker-Azmitia, 2001), by findings of elevated platelet serotonin levels (Anderson et al., 2002; Mulder et al., 2004), the effectiveness of selective serotonin reuptake inhibitors in the treatment of ASD symptoms (Kolevzon, Mathewson, & Hollander, 2006), and the association between 5-HTTLPR and cortical gray matter volumes (Wassink et al., 2007).

COMT contains a single nucleotide polymorphism (SNP) resulting in either valine or methionine encoding alleles (Val158Met). A decreased *COMT* activity is associated with the Met-allele. In the prefrontal cortex (PFC), this reduced enzyme activity is thought to increase dopamine levels. Research in ADHD patients suggests that the PFC functions inefficiently in the presence of the Val allele (high activity *COMT*; Blasi et al., 2005; Boonstra et al., 2008), and some studies found that the Val158Met polymorphism was associated with stimulant response in children with ADHD (e.g., Kereszturi et al., 2008). Despite the potential relevance of this polymorphism to the etiology of ADHD, two meta-analyses found no association between ADHD and the Val158Met SNP (Cheuk & Wong,

2006; Gizer et al., 2009). In a recent study, Caspi et al. (2008) concluded that the association may apply only to those children with ADHD who show comorbid antisocial behavior. Pálmason et al. (2010) could not confirm this association, although they did find an association between the *COMT* Val158Met SNP and ADHD. *COMT* may be an interesting candidate gene for ASD, as inefficient PFC functioning is also implicated in ASD (e.g. Geurts et al., 2004), and dopamine antagonists, such as risperidone, improve some aspects of autism, such as irritability (e.g., McCracken et al., 2002) and executive functioning (Troost et al., 2006). Only two studies to date have addressed the association between *COMT* and ASD (James et al., 2006; Yirmiya et al., 2001). Yirmiya et al. found no association, whereas James et al. reported the overrepresentation of the *COMT* Val-allele in children with autism as compared to normal control children.

Apart from genetic influences, a range of environmental factors are important in the etiology of ADHD, of which maternal smoking during pregnancy and low birth weight have been the most consistently replicated (Bhutta, Cleves, Casey, Cradock, & Anand, 2002; Langley, Rice, van den Bree, & Thapar, 2005; Linnert et al., 2003). Both are also found to be autism risk factors (Hultman, Sparen, & Cnattingius, 2002; Klevzon, Gross, and Reichenberg, 2007). Not taking different levels of exposure to these risk factors into account may, in addition to clinical heterogeneity, explain inconsistent association study results for *SLC6A4* and *COMT* in ADHD. This follows from the notion of gene environment (GxE) interactions, i.e., only in the presence of a certain environmental risk factor may a genotype contribute to an increased risk for a disorder. One previous study has reported significant interaction between *COMT* and birth weight, i.e., for antisocial behaviour in children with ADHD (Thapar et al., 2005). Sengupta et al. (2006), though, could not replicate this finding. The interaction of low birth weight and maternal smoking during pregnancy with *SLC6A4* was investigated by Langley et al. (2008), who did not find significant effects on a diagnosis of ADHD, nor on associated antisocial behavior. Clearly, addressing GxE interactions in ADHD and ASD has only recently been started and requires much more study.

In the current study, we investigated main and interaction effects of *COMT*, *SLC6A4*, maternal smoking during pregnancy, and low birth weight on ASD symptoms in children with ADHD in the Dutch subsample of the International Multicenter ADHD Genetics (IMAGE) study. We continued previous work on *SLC6A4* and *COMT* in the IMAGE sample, in which no association between 5-HTTLPR and ADHD (Xu et al., 2008), and between *COMT* Val158Met and ADHD (Brookes et al., 2006) was found. ASD symptoms were measured with the Children's Social Behavior Questionnaire (CSBQ; Hartman, Luteijn, Serra, & Minderaa, 2006), that was only available for the Dutch subsample of IMAGE. The CSBQ is an instrument particularly suited to measure the continuous distribution of ASD traits in the population as well as in clinical groups other than ASD (Hartman et al., 2006; Luteijn et al., 2000). All analyses were repeated in an independent Dutch ADHD sample for replication, consisting of children who participated in the TRacking Adolescents' Individual Lives Survey (TRAILS; Huisman et al., 2008).

Methods

This is an abbreviated version of the methods section. A more elaborate methods section, including genotyping information and detailed sample characteristics, is provided online: ([link to website](#)).

Participants

The first sample consisted of 207 Dutch European-Caucasian ADHD-affected probands aged 5-17 who participated in the IMAGE project (Kuntsi et al., 2006; Brookes et al., 2006). For the second sample, 439 European-Caucasian children aged 10-13 participating in

TRAILS were selected who on the attention deficit/hyperactivity problems scale of the Child Behavior Checklist (CBCL) had scores higher than the 93rd percentile as defined in the CBCL norms (Achenbach, Dumenci, & Rescorla, 2003; Achenbach & Rescorla, 2001). All families gave written informed consent prior to participation.

Measures

CSBQ—The CSBQ was used to assess ASD symptoms (Hartman, Luteijn, Serra, & Minderaa, 2006). It has six subscales entitled “not optimally tuned to the social situation” (tuned; addressing emotional overreacting and stubbornness/disobedience), “reduced contact and social interest” (social), “orientation problems in time, place, or activity” (orientation), “difficulties in understanding of social information” (understanding), “fear of and resistance to changes” (change), and “stereotyped behavior” (stereotyped).

Pre-/ perinatal risk factors—Information regarding birth weight and maternal smoking during pregnancy was collected using a questionnaire completed by the mother for both samples. Smoking was divided into two categories, i.e., mothers who had and had not smoked during pregnancy. Birth weight was dichotomized as well: i.e., birth weight less than 2500 grams (the standard clinical cut-off) versus birth weight equal to or more than 2500 grams.

Potential confounders—Socioeconomic status (SES) was divided in three categories: low, intermediate, and high based on parental occupation (IMAGE) or education (TRAILS). Other confounders included the anxious-shy, the oppositional, and the DSM-IV total scale of the Conners' parent rating scale, long form (Conners, 1996; IMAGE), and the CBCL Diagnostic and Statistical Manual-oriented scales anxiety problems, oppositional defiant problems, and attention deficit/hyperactivity problems (TRAILS).

Statistical Analyses

GxE effects on CSBQ scales were assessed with linear regression. Risk alleles for the polymorphisms in this study were the S allele for 5-HTTLPR, and the Val allele for *COMT*. A recessive model was used for *COMT* (in accordance with Caspi et al., 2008), and both a recessive and a dominant model for 5-HTTLPR. In the first block of the regression analyses the genetic data and the environmental factor were entered. In the second block, the interaction term was added. Any analysis with a significant interaction was subsequently adjusted for the following covariates: gender, age, SES (for the analyses with smoking only), oppositional and anxious behavior, and ADHD symptom count. We used an alpha of 0.05 to indicate statistical significance.

Results

Significant regression results are reported in Table 1 and Table 2.

IMAGE Study

We found no significant main effects of *COMT* Val158Met, 5-HTTLPR, maternal smoking during pregnancy, or birth weight on any CSBQ scale. Multiple regression analyses for GxE effects showed a significant interaction between the *COMT* Val/Val genotype and maternal smoking during pregnancy for the CSBQ stereotyped subscale under the recessive model ($B = 5.16$, $SE\ B = 1.92$, $\beta = 0.32$, $p = 0.008$), and a significant, negative effect of *COMT* Val158Met (i.e., this effect is significant only in children whose mothers did not smoke during pregnancy; $B = -2.35$, $SE\ B = 0.87$, $\beta = -0.28$, $p = 0.008$). Figure 1a shows the direction of the effect. Significant effects for 5-HTTLPR were found for the recessive model. Significant interaction was found between the 5-HTTLPR S/S genotype and

maternal smoking during pregnancy, increasing scores on the CSBQ social scale ($B = 6.17$, $SE\ B = 2.65$, $\beta = 0.19$, $p = 0.021$). Furthermore, our analyses showed a significant interaction between the 5-HTTLPR S/S genotype and birth weight for the CSBQ change subscale ($B = 4.65$, $SE\ B = 2.09$, $\beta = 0.18$, $p = 0.028$). For the directions of the effects, see Figures 1c and 1d.

All three significant interactions described above remained significant after correction for potential confounders, see the online version of Table 1.

TRAILS Study

The interaction between the *COMT* Val/Val genotype and maternal smoking during pregnancy for the stereotyped scale reached trend level significance in the TRAILS sample ($B = 1.26$, $SE\ B = 0.71$, $\beta = 0.11$, $p = 0.077$), and became significant after correction for confounders ($B = 1.58$, $SE\ B = 0.68$, $\beta = 0.14$, $p = 0.02$), see Table 2. Also, we found significant interaction effects between birth weight and the 5-HTTLPR S/S genotype for both the tuned and the understanding subscales ($B = 4.93$, $SE\ B = 2.107$, $\beta = 0.13$, $p = 0.020$, and $B = 3.11$, $SE\ B = 1.44$, $\beta = 0.12$; $p = 0.032$, respectively). Directions of the significant interaction effects were similar to those found for the IMAGE sample, also see Figure 1b. No significant effects were found for 5-HTTLPR interactions when confounders were included in the analyses, see the online version of Table 2.

Discussion

The current study examined main and interaction effects of two ADHD candidate genes and two perinatal risk factors on ASD symptoms within the context of ADHD in two independent samples. While we did not find significant main effects of *COMT* Val158Met and 5-HTTLPR, maternal smoking during pregnancy, and low birth, our results did indicate that *COMT* and *SLC6A4* interacted with maternal smoking during pregnancy and low birth weight. The simultaneous presence of the *COMT* Val/Val genotype and maternal smoking during pregnancy was associated with increased stereotyped behaviors in children with combined type ADHD. We replicated this result in an independent ADHD sample which combined clinically referred children and children from the general population. Furthermore, being homozygous for the 5-HTTLPR S allele was associated with raised levels of ASD symptoms in children with ADHD whose mothers had smoked during pregnancy, and in children with a birth weight below 2500 grams. The interaction between smoking and the 5-HTTLPR was associated with increased impaired social interaction in the IMAGE sample, while low birth weight in interaction with 5-HTTLPR raised levels of rigid behavior in the IMAGE sample, and communication problems and emotional overreacting/stubbornness in the TRAILS sample.

The significant GxE interactions in the IMAGE sample and the interaction between *COMT* and maternal smoking during pregnancy in the TRAILS sample were robust against correction for potential confounders (including anxiety, shyness, ADHD severity, gender, and SES), which underlines the ASD-specificity of these results. Notably, Caspi and colleagues (2008) recently found in three independent samples that among children with ADHD the *COMT* Val homozygous children showed more antisocial behavior than did Met allele carriers. We therefore also included oppositional behavior as a covariate, and found the interaction between *COMT* and maternal smoking during pregnancy to remain significant.

All findings in this study were for specific CSBQ subscales rather than for an overall measure of ASD. This is in line with evidence of distinct genetic influences for the different ASD symptom domains (for a review, see Ronald & Happé, 2008). With regard to the 5-

HTTLPR interaction with maternal smoking during pregnancy, we found effects in the social interaction domain. Interestingly, 5-HTTLPR S allele carriers among children with autism were previously shown to be rated as more severe on the autism diagnostic interview-revised subdomain “failure to use nonverbal communication to regulate social interaction” (Brune et al., 2006). Similarly, the 5-HTTLPR S allele by itself has been shown to not convey a risk for autism, but, rather, to increase the severity of autistic behaviors in the social and related communication domains (Tordjman et al., 2001). Likewise, a study by Sutcliffe et al. (2005) suggested that allelic heterogeneity at the *SLC6A4* locus confers a risk for autism and rigid compulsive behavior in particular, which fits well with the interaction effects between the 5-HTTLPR S/S genotype and low birth weight for rigid behavior observed in the current study. Even though the mentioned studies refer to genetic main effects rather than interaction effects, the correspondence to our outcome domains is remarkable.

The relation between environmental risk factors and offspring behavior is complicated (Knopik, 2009). For instance, in addition to having direct detrimental effects, maternal smoking during pregnancy is also associated with parental (e.g., IQ, ADHD, antisocial behavior, substance abuse) and parenting characteristics, and physical health (Ernst, Moolchan, and Robinson, 2001). We controlled for some of the associated factors by including SES in our analyses, but cannot preclude the influence of other smoking-associated factors in the present study. Notably, future studies should consider taking parental ADHD into account when investigating smoking as a risk factor for ASD symptoms in children with ADHD, given the likely genetic overlap between ADHD and ASD. Birth weight appears to be a less complex environmental risk factor than maternal smoking, as it has been shown to mainly index environmental factors in utero rather than genetic influences (Brooks, Johnson, Steer, Pawson, and Abdalla, 1995).

Interestingly, all significant interactions we found indicated that children with the high risk genotypes (i.e., *COMT* Val/Val or 5-HTTLPR S/S) were not only the most likely children to have increased levels of ASD symptoms when exposed to maternal smoking during pregnancy or when they had a low birth weight, but were also the children who had the lowest levels of ASD symptoms when not exposed to maternal smoking during pregnancy or low birth weight (see also figure 1). The finding that those most likely to be adversely affected by adverse environments are the same as those most likely to benefit from a healthy environment is consistent with Belsky's differential-susceptibility hypothesis (Belsky et al., 2004), and suggests that *COMT* Val158Met and 5-HTTLPR may be regarded as “plasticity genes” rather than “vulnerability genes”.

A limitation of our study may have been that the most severe ASD cases were excluded from participation in the IMAGE sample. However, since ASD-symptoms are generally considered to represent a continuous trait (Constantino and Todd, 2003; Spiker, Lotspeich, Dimiceli, Myers, and Risch, 2002), this is not likely to be a serious shortcoming. Nevertheless, we did not actually investigate the most severe end of the ASD spectrum, and therefore cannot be sure whether our findings pertain to narrowly defined autism as well. Furthermore, no formal ASD diagnostic instruments were available for the replication sample, and as a consequence it is unknown how many children actually fulfilled criteria for ASD. Ideally, subsequent studies would use samples with extremes of both the ADHD and ASD spectra represented, in which parent reports as well as a detailed developmental history and observational data would be collected to assess autistic and ADHD symptomatology. Ratings by experienced clinicians are essential in future studies, especially for their expertise in discriminating ASD- from ADHD-related social and communication problems (Clark et al., 1999). Another methodological issue is the small number of children in the clinical sample with a birth weight below 2500 grams, which warrants extra caution in interpreting

the interaction we found between 5-HTTLPR and birth weight. Also, additional gene variants that influence the expression of the serotonin transporter (Hranilovic et al., 2004; Wendland, Martin, Kruse, Lesch, and Murphy, 2006) and additional SNPs in *COMT* could be investigated (see for instance Hallelund et al., 2009). Doing so in future studies may define GxE interactions with more precision.

To our knowledge, our findings represent the first attempt to identify genes and GxE interaction effects underlying the occurrence of ASD symptoms in children with ADHD. A major strength of this study is the fact that two independent samples were analyzed, i.e., a carefully and thoroughly investigated clinical ADHD sample, and a sample with ADHD at varying levels of severity, used as a replication sample. Excitingly, this resulted in replication of the findings for the interaction between *COMT* Val/Val and maternal smoking during pregnancy, and similar results for the interaction between 5-HTTLPR and low birth weight. These findings, together with the interaction we found between 5-HTTLPR S/S and maternal smoking during pregnancy, have important implications insofar that they suggest the modifying effects of GxE interaction on ASD symptoms in children with ADHD.

Textbox

- Symptoms of Autism Spectrum Disorder (ASD) and Attention-Deficit/Hyperactivity Disorder (ADHD) often co-occur.
- ADHD and ADHD share both genetic and environmental risk factors.
- Using GxE interaction models, we found significant interactions of the *COMT* Val/Val genotype with maternal smoking during pregnancy, and of the 5-HTTLPR S/S genotype with maternal smoking during pregnancy and with low birth weight, increasing ASD symptoms in two independent samples.
- Our findings suggest modifying effects of GxE interaction on ASD symptoms in children with ADHD.

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Figure 1.

Children's Social Behavior Questionnaire (CSBQ) Subscale Scores according to Genotype and Presence or Absence of Perinatal Risk Factors in Children with Combined Type ADHD.

Figure 1a: Significant Interaction between *COMT* Val/Val and Maternal Smoking during Pregnancy ($p = 0.008$) – IMAGE sample

Figure 1b: Trend significant Interaction between *COMT* Val/Val and Maternal Smoking during Pregnancy ($p = 0.077$) – TRAILS sample

Figure 1c: Significant Interaction between 5HTTLPR S/S and Maternal Smoking during Pregnancy ($p = 0.021$) – IMAGE sample.

Figure 1d: Significant Interaction between 5HTTLPR S/S and Birth Weight < 2500 grams ($p = 0.028$) – IMAGE sample.

Note. ADHD = Attention-Deficit/Hyperactivity Disorder; *COMT* = Catechol O-Methyltransferase; IMAGE = International Multicenter ADHD Genetics project; TRAILS = Tracking Adolescents' Individual Lives Survey.

Table 1

Regression results for the IMAGE sample- interaction effects between *COMT* Val158Met / SLC6A4 5-HTTLPR and maternal smoking during pregnancy/low birth weight predicting ASD symptoms

CSBQ subscale	Regression variables	B	SE B	β	p-value
Stereotyped	Maternal smoking during pregnancy	-1.10	1.00	-0.12	0.27
	<i>COMT</i> Val158Met	-2.35	0.87	-0.28	0.008
Social	Smoking $\times$ <i>COMT</i> Val158Met	5.17	1.92	0.32	0.008
	Maternal smoking during pregnancy	0.03	0.86	0.002	0.98
	5-HTTLPR	-1.73	1.10	-0.12	0.12
Change	Smoking $\times$ 5-HTTLPR	6.17	2.65	0.19	0.02
	Birth weight	-0.55	0.74	-0.06	0.46
	5-HTTLPR	-0.36	0.45	-0.06	0.42
	Birth weight $\times$ 5-HTTLPR	4.65	2.09	0.18	0.03

Note. ASD = Autism Spectrum Disorders; *COMT* = Catechol O-Methyltransferase; CSBQ = Children's Social Behavior Questionnaire; IMAGE = International Multicenter ADHD Genetics project

Table 2

Regression results for the TRAILS sample -Interaction effects between *COMT* Val158Met / SLC6A4 5-HTTLPR and maternal smoking during pregnancy/low birth weight predicting ASD symptoms

CSBQ subscale	Regression variables	B	SE B	β	p-value
Stereotyped	Maternal smoking during pregnancy	0.04	0.33	0.01	0.90
	<i>COMT</i> Val158Met	-0.72	0.41	-0.10	0.08
Tuned	Smoking $\times$ <i>COMT</i> Val158Met	1.26	0.71	0.11	0.08
	Birth weight	-0.49	0.82	-0.03	0.55
	5-HTTLPR	-0.04	0.65	0.00	0.95
	Birth weight $\times$ 5-HTTLPR	4.93	2.11	0.13	0.02
Understanding	Birth weight	0.25	0.56	0.02	0.66
	5-HTTLPR	-0.25	0.45	-0.03	0.57
	Birth weight $\times$ 5-HTTLPR	3.11	1.44	0.12	0.03

Note. ASD = Autism Spectrum Disorders; *COMT* = Catechol O-Methyltransferase; CSBQ = Children's Social Behavior Questionnaire; TRAILS = TRacking Adolescents Individual Lives' Survey