

Association Between Catechol O-methyltransferase (*COMT*) Haplotypes and Severity of Hyperactivity Symptoms in Adults

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It has been suggested that symptoms of attention-deficit/hyperactivity disorder (ADHD) is related to low dopamine levels in the prefrontal cortex. The enzyme catechol O-methyltransferase (*COMT*), which degrades dopamine and other catecholamines, is important for monoamine signaling in this brain-region, but genetic studies of the functional Val158Met (rs4680) polymorphism in ADHD have been inconsistent. However, recently it was shown that also common synonymous *COMT* variants modulate total *COMT* enzymatic activity by affecting the expression of the gene [Nackley et al. (2006); *Science* 314(5807):1930–1933]. We therefore hypothesized that analysis of haplotypes could reveal more about the association between *COMT* and ADHD symptoms than the Val158Met polymorphism alone. SNPs rs6269, rs4633, rs4818, and rs4680, tagging the common putative functional *COMT* haplotypes, were genotyped in 435 adult subjects with a clinical diagnosis of ADHD and 383 controls and analyzed for association with ADHD and the hyperactivity/impulsivity and inattention dimensions from the Adult ADHD Self-Report Scale (ASRS). All markers showed a trend for association with the hyperactivity/impulsivity scale, peaking at marker rs6269 ($P = 0.007$). Haplotype analysis revealed that the rs6269 risk allele tags the suggested high *COMT*-activity haplotype, which is associated with the highest hyperactivity/impulsivity score in our sample ($P = 0.01$). Our results also suggest that there is a stepwise decreased hyperactivity/impulsivity score associated with the proposed mid and low activity haplotypes described previously. In conclusion, we suggest that *COMT* haplotype variation is associated primarily with the hyperactivity/impulsivity dimension of ADHD and point to the importance of testing this hypothesis in future studies. © 2008 Wiley-Liss, Inc.

Key words: Adult ADHD; hyperactivity; *COMT*; ASRS; dopamine

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a common condition in school aged children world-wide. Moreover, the

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majority of the affected children will have persisting symptoms as adults [Biederman and Faraone, 2005]. According to DSM-IV, ADHD can be separated in three subtypes: predominantly inattentive type, predominantly hyperactive/impulsive type and combined type [American Psychiatric Association, 2000].

Studies are inconclusive with regard to heritability and aetiology for the different subtypes. Todd et al. [2001] reported high heritability for DSM-IV combined type and inattentive type in female twins with ADHD, whereas the hyperactive-impulsive type was substantially less heritable. In contrast, in the Australian Twin ADHD Project, Levy et al. [2001] found that the hyperactive-

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Abbreviation used: ADHD, attention-deficit/hyperactivity disorder; *COMT*, catechol O-methyltransferase; ASRS, adult ADHD self-report scale; SNP, single nucleotide polymorphism; LD, linkage disequilibrium. Grant sponsor: Research Council of Norway; Grant sponsor: Western Norway Regional Health Authority.

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impulsive type was more heritable than the inattentive type. These results suggest that the aetiology of the hyperactive-impulsive type may be different from the aetiology of the combined and inattentive types. It has also been discussed whether the subtypes of ADHD should be separated, or if they have a common biological foundation [Lubke et al., 2007]. McLoughlin et al. [2007] found that many genes that were associated with the hyperactivity-impulsivity dimension, were also associated with the inattentive dimension. However, there was significant genetic heterogeneity. They concluded that their results provide genetic support for combining the two dimensions that define ADHD, but suggested that some symptom-specific genes probably will be identified. However, further research is needed to gain more knowledge about specific genetic influence on ADHD subtypes.

A number of genetic variants have been presented as susceptibility factors for ADHD. As ADHD is believed to depend on a dysfunctional and hypoactive dopamine system [Solanto, 2002; Sagvolden et al., 2005], several dopamine related genes have been suggested as candidate genes for ADHD. One is the COMT gene, encoding an enzyme that participates in the degradation of catecholamine transmitters [Mannisto and Kaakkola, 1999; Haavik et al., 2008]. COMT is an attractive candidate gene since it might be especially important for modulating dopamine levels in the frontal cortex, a region which is strongly implicated in ADHD aetiology [Diamond, 2007]. This brain region has low levels of the dopamine transporter (DAT) [Chen et al., 2004], which could imply that dopamine levels are more dependent on catabolic enzyme such as COMT.

The most well studied single nucleotide polymorphism is the G/A transition at codon 158 of the membrane bound COMT (codon 108 of the soluble COMT). This results in a valine to methionine substitution and a three to fourfold decrease in enzyme activity [Lachman et al., 1996]. Due to its high enzyme activity, the Val variant of this polymorphism degrades prefrontal dopamine more quickly than the Met variant [Palmatier et al., 1999; Chen et al., 2004; Goldberg and Weinberger, 2004]. Therefore, it is suggested to contribute to the proposed hypodopaminergic state in ADHD, and is possibly implicated in frontal lobe function. Kereszturi [2008] found that the Val allele was more frequent in a group of ADHD patients than in a healthy population. A recent study also showed that the Val158Met was associated with phenotypic variation in ADHD [Caspi et al., 2008]. The Val/Val homozygotes had more symptoms of conduct disorder, were more aggressive and more likely to be involved in criminal offences compared with Met carriers. Several studies have also shown that individuals with the Val/Val variant show impaired performance on some cognitive tasks [Egan et al., 2001; Bilder et al., 2002; Goldberg et al., 2003]. For example, Frank et al. [2007] found that participants homozygous for Met could more rapidly adapt behavior on a trial- to trial basis. However, studies have also shown an opposite pattern [Bellgrove et al., 2005]. Reuter et al. [2006] found higher scores on hyperactivity/impulsivity, inattention and total symptom score on the World Health Organization Adult ADHD Self Report Scale (ASRS) [Kessler et al., 2005] in individuals with the Met/Met variant. Overall, most studies show weak, inconsistent or no results with regard to this polymorphism and ADHD [Cheuk and Wong, 2006; Retz et al., 2008].

Nackley et al. [2006] have suggested that COMT haplotypes (combinations of SNPs) modulate protein expression by altering mRNA secondary structure. According to them, Val158Met interacts with other SNPs and this determines the functional expression of the gene. They demonstrated that COMT haplotypes varied with respect to messenger RNA stem-loop structures, and that the most stable structure was associated with the lowest protein levels and enzymatic activity. Haplotypes divergent in synonymous changes exhibited the largest difference in COMT enzymatic activity. Since COMT degrades dopamine, it is reasonable to hypothesize that the haplotype with the proposed highest COMT activity is related to the lowest prefrontal dopamine level, and that the haplotype with the proposed lowest COMT activity is related to the highest prefrontal dopamine level. Nackley et al. [2006] defined the haplotypes using four SNPs: one located in the soluble COMT promoter region (A/G; rs6269) and three in the soluble and membrane bound COMT coding region at codons his62his (C/T; rs4633), leu136leu (C/G; rs4818), in addition to the common Val158Met (A/G rs4680).

Inspired by Nackley et al. [2006], we examined the SNPs from their study; rs6269, rs4633, rs4818, and rs4680 in the COMT gene. A priori we hypothesized that there is a relationship between COMT haplotypes and dimensional symptom scores of ADHD, measured with the ASRS, that could not be detected by only the Val158/108Met polymorphism. Thus, we expected that the participants with haplotypes and SNPs that may cause high COMT activity, assumed to result in a low dopamine level, would report the highest symptom scores on the ASRS.

MATERIALS AND METHODS

Subjects

The participants in the present study were adults (>18 years) with a clinical diagnosis of ADHD, recruited from all parts of Norway [Johansson et al., 2007]. Most of them were originally referred by their primary physician to psychiatric out-patient clinics where they were diagnosed by psychiatrists or clinical psychologists. About 6% were diagnosed at psychiatric hospitals. The patients received the ADHD diagnosis as adults (82.3%) or during childhood (17.7%). However, all the participants were also officially diagnosed as having persistent ADHD as adults. The criteria used for diagnosing ADHD followed the national guidelines from the Norwegian regional ADHD expert committees; the ICD-10 research criteria. Those are compatible with the DSM-IV criteria, requiring that six or more of nine symptoms of inattention and/or hyperactivity must have been present for at least 6 months and must be disruptive and inappropriate for developmental level. Some hyperactive, impulsive or inattentive symptoms that cause impairment should have been present before 7 years of age, and must be present in two or more settings [American Psychiatric Association, 2000]. Information about subtype of diagnosis was not collected in this study. There were no formal exclusion criteria.

Approximately 2,000 patients from the address registry of the Norwegian regional ADHD expert committees were invited by letter to join the project, in addition to patients being recruited directly from psychiatrists nationwide. An informed consent form

and checklists of current symptoms, including the ASRS, were filled in by the participants. A questionnaire containing information about diagnosis, current medical treatment and treatment response was filled in by their present psychiatrist or general practitioner [Johansson et al., 2007]. Saliva or venous blood samples were collected at the time of inclusion in the project. The first 435 patients responding with both questionnaires and biological samples were included in the present study.

A total of 383 ethnically matched comparison cases (138 students and 245 randomly selected persons aged 18–40 years from the Norwegian Medical Birth Registry) were recruited as a control group. No screen for ADHD was undertaken in the control group before entering the study. Although this might have led to reduced power, the prevalence of ADHD in this group is assumed to be lower than 5%. This assumption is supported by results in a recent population based study of 7–9 years old Norwegian children (the Bergen Child Study), where the prevalence rate of ADHD was estimated to be 1.7% [Heiervang et al., 2007].

The project was approved by the Regional Committee for Medical Research Ethics of Western Norway and the Norwegian Social Science Data Services (NSD).

Methods

Severity of ADHD symptoms. ASRS was used to determine the levels of ADHD symptoms during the past six months. ASRS is a screening scale for use in the general population [Kessler et al., 2005]. The Norwegian version of the ASRS is a translation of the original scale and consists of 18 questions that follow the DSM-IV-TR criteria for ADHD [American Psychiatric Association, 2000]. Nine questions address the frequency of inattentive symptoms, and nine address the frequency of hyperactivity/impulsivity symptoms. The participants indicate how often the symptoms occur by using a Likert scale from 0 to 4 (0 = never, 1 = seldom, 2 = sometimes, 3 = often, 4 = very often). Reuter et al. [2006] used a method based on continuously varying traits when examining ASRS scores in relation to Val158Met. This method has been shown to be well suited for investigating the genetics of ADHD symptoms [Stevenson et al., 2005], and a dimensional approach was therefore chosen in the present study.

Use of medication. Medication status and symptoms were recorded at the time of patient inclusion. If no information about ADHD medication status was obtained at the time of filling out the ASRS questionnaire, the patients were most likely not using medication against ADHD. In our analysis, we have chosen a conservative approach where every patient without confirmed information about medication at the time of scoring on the ASRS is categorized as non-medicated. Sixty-four percent of the patients used medication at inclusion into the study, of whom 91.7% used central stimulants.

SNP-selection. SNPs were selected to tag all three common putative functional haplotypes as described in Nackley et al. [2006]. In addition to the Val158Met (A/G; rs4680), one SNP was located in the S-COMT promoter region (A/G; rs6269) and two in the S- and MB-COMT coding region at codons his62his (C/T; rs4633) and leu136leu (C/G; rs4818).

Genotyping. DNA was extracted either from whole blood, or from saliva using the Oragene™ DNA Self-Collection Kit from

DNA Genotek (DNA Genotek, Inc., Ontario, Canada) at the HUNT biobank (Levanger, Norway). DNA from cases and controls were mixed on 96-well plates with a minimum of two internal controls and two blank samples on each plate. Genotyping of SNPs rs4680 and rs4818 was performed using the MassARRAY® iPLEX™ System (SEQUENOM, Inc., San Diego, CA) and the TaqMan allelic discrimination assay was used for SNPs rs6269 and rs4633. Total genotyping concordance rate was 100% (86/86 identical duplicate genotypes among 11 subjects with duplicate DNA spread over all assay plates). No SNP deviated from Hardy–Weinberg equilibrium ($P > 0.01$).

Statistical analysis. SPSS version 14.0.2 was used for one-way ANOVA and chi-square test to compare age and gender between the groups. One-way ANOVA was used to explore if group status and medication were related to difference in scores on ASRS. Allele frequencies and single marker tests for each marker were calculated using the PLINK software [Purcell et al., 2007b]. For the quantitative analysis (ASRS total score, hyperactivity/impulsivity score and inattention score), we performed linear regression both with the PLINK and SPSS 14.0.2 software, using gender, ADHD and current medication as cofactors (only results from the PLINK-analyses are presented in the text, but very similar results were obtained using both program). No significant deviations from Hardy–Weinberg equilibrium were detected for any marker. Haplotype analyses were performed using the WHAP [Purcell et al., 2007a] and PLINK [Purcell et al., 2007b] software with the same covariates as described above (similar results were obtained with conditional logistic regression as implemented by the UNPHASED software [Dudbridge, 2006], data not shown). *P* values are presented without correction for multiple testing.

RESULTS

Mean age at inclusion into the study was 34.4 years (SD = 10.5) in the ADHD group and 27.8 years (SD = 6.7) in the control group. A one-way ANOVA showed that this difference was statistically significant ($P < 0.001$). There were 53.3% men in the ADHD group and 41.4% in the control group. Chi-square test showed that the gender distribution were different between the groups ($P = 0.001$). A total of 338 persons (77.7%) in the ADHD group scored above cut-off for ADHD on the ASRS scale, and 8.6% scored above cut-off in the control-group. Cut off was defined as 21 points on one or both sub-scales [Kessler et al., 2005]. A majority (63.9%) of the participants in the ADHD-group were on medication for ADHD when filling in the questionnaires.

Clinical characteristics are shown in Table I. Control subjects generally reported lower scores on all ASRS subscale measures. Scores on ASRS-subcales for non-medicated and medicated participants in the ADHD-group are shown in Table II. Interestingly, the mean ASRS scores in the medicated subgroup were significantly lower than for the patients who did not report current use of medication ($P < 0.001$). Furthermore, stepwise regression analysis indicated that gender, in addition to current medication and ADHD status, are important predictors for ASRS scores. Hence, these covariates were included in the regression analysis in all further statistical tests.

TABLE I. Comparison Between Mean Scores on ASRS-Subscales in 435 ADHD-Patients and 383 Controls

ASRS subgroup	ADHD	Ctrls	F	P
	(N = 435)	(N = 383)		
Hyperactivity/impulsivity	Mean [SD] 22.1 [6.8]	Mean [SD] 10.3 [5.2]	757.3	<0.001
Inattention	23.7 [6.6]	12.2 [4.8]	793.6	<0.001
Total, ASRS	45.8 [12.2]	22.5 [8.9]	949.4	<0.001

Genetic Analyses

All four SNPs were in strong pair wise linkage disequilibrium (LD) with almost perfect correlation ($r^2 > 0.98$) between rs6269-rs4818 and rs4633-rs4680 (Supplementary Fig. 1). Consequently, only three common haplotypes were detected in the Norwegian sample. Table III shows the single point association results for the ADHD diagnosis, and for ASRS quantitative scores using linear regression analysis adjusted for gender, current medication and ADHD status. None of the four tested SNPs showed an association with ADHD as a categorical, dependent variable. However, all four SNPs showed tendency for association with the hyperactivity/impulsivity dimension of the ASRS. The strongest association, $P = 0.007$, was seen for rs6269, corresponding to a 0.81 increased mean hyperactivity/impulsivity score per A-allele compared to the G-allele after controlling for gender, current medication and ADHD status. Mean scores for the different genotypes are shown in Figure 1. Stratification of the subjects into three groups; individuals with ADHD (1) on current medication, (2) not on current medication, and (3) controls, showed consistent results for the hyperactivity/impulsivity scale with the strongest effect in the group who used medication at the time of study (Table IV). Results for the inattentive symptoms were less consistent, with opposite patterns of association for the medicated and unmedicated group of patients.

It has been suggested that COMT haplotypes may have more impact on COMT activity than single SNPs [Nackley et al., 2006]. We therefore tested for association between ASRS hyperactivity/impulsivity scores and four marker haplotypes (rs6269-rs4633-rs4818-rs4680) using linear regression analyses with the WHAP and PLINK software and controlling for the same covariates (Table V). Three haplotypes were present at frequency of more than 1%. The proposed high COMT activity haplotype, G-C-G-G/Val, was associated with higher hyperactivity/impulsivity scores, $P = 0.01$, com-

pared to the other haplotypes. Although not nominally significant, there was also a tendency of stepwise decreased hyperactivity/impulsivity score associated with the proposed mid (A-T-C-A/Met) and low (A-C-C-G/Val) activity haplotypes. Stratification analysis revealed that the haplotype association was primarily caused by the group on current medication ($P = 0.03$ for the G-C-G-G/Val-haplotype, Supplementary Table I). For the inattentive symptoms, results were again different between patients on current medication and patients who did not use central stimulants (data not shown).

DISCUSSION

In the present study, all four COMT polymorphisms showed a trend for association with the ASRS hyperactivity/impulsivity rating scale, peaking at marker rs6269 located in the S-COMT promoter region. Haplotype analysis revealed that this polymorphism segregates the suggested high COMT activity haplotype from the others. It further showed that only two of the four SNPs are needed to tag these haplotypes. The results also suggested that all three common haplotypes individually are associated with different hyperactivity/impulsivity-score levels and corresponding high, average and low COMT activity, consistent with the results from Nackley et al. [2006]. Importantly, the Val158Met (rs4680) polymorphism did not show such an effect on its own.

Our results for Val158Met do not confirm the findings of Reuter et al. [2006], but are in line with other studies that show weak or no association between this polymorphism and a diagnosis of ADHD [Cheuk and Wong, 2006; Retz et al., 2008]. Such apparently conflicting results are also found for the Val158Met in other psychiatric disorders [Craddock et al., 2006]. Different study designs and relatively small sample sets may partly explain the inconsistencies. However, different results could also be expected if LD in different populations varies between Val158Met and other nearby SNPs which potentially could affect expression of the gene. This has also been suggested by Nackley et al. [2006], who demonstrated that COMT haplotypes have stronger effects on total enzymatic activity than the Val158Met polymorphism itself. Hence, we argue that the Val158Met polymorphism needs to be considered in the context of haplotypes, a view that is supported by several other studies [Handoko et al., 2005; Meyer-Lindenberg et al., 2006].

Based on the results in the present study, we propose that the haplotype that is predicted to give the highest enzymatic COMT-activity could be associated with lower dopamine levels, in particular in the prefrontal cortex, where the dopamine transporter is expected to be less important. This could, if true, be important with

TABLE II. Comparison Between Mean Scores on ASRS-Subscales for Medicated and Non-Medicated ADHD-Patients

ASRS subgroup	Medicated (N = 278)	Non-medicated (N = 157)	F	P
	Mean [SD]	Mean [SD]		
Hyperactivity/impulsivity	21.3 [7.0]	23.5 [6.0]	11.0 [1,432]	<0.001
Inattention	23.1 [6.9]	24.8 [6.0]	6.3 [1,433]	0.013
Total, ASRS	44.4 [12.9]	48.3 [10.2]	10.6 [1,431]	<0.001

TABLE III. Comparison of COMT SNP Allele Frequencies in All 435 ADHD Patients and 383 Matched Controls and Quantitative Linear Regression Analysis for the Different ASRS-Subscales Controlling for Gender, Current Medication, and ADHD

SNP	Position	Alleles (minor/major)	Case freq.	Ctrls freq.	OR	P	Quantitative traits (ASRS score)					
							Hyperactivity		Inattention		ASRS total	
							Beta	P	Beta	P	Beta	P
rs6269	18329952	G/A	0.38	0.37	1.05 [0.86–1.29]	0.62	0.83	0.007	-0.15	0.61	0.68	0.21
rs4633	18330235	C/T	0.44	0.43	1.05 [0.86–1.27]	0.66	0.55	0.06	-0.30	0.30	0.24	0.65
rs4818	18331207	G/C	0.38	0.37	1.09 [0.89–1.33]	0.42	0.73	0.02	-0.29	0.34	0.44	0.42
rs4680	18331271	G/A	0.44	0.43	1.05 [0.86–1.28]	0.62	0.47	0.1	-0.46	0.10	0.00	0.99

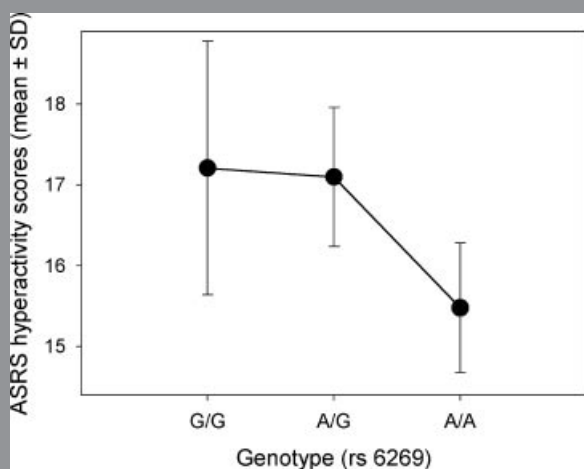


FIG. 1. Mean scores on the ASRS-hyperactivity scale for the different genotypes of SNP rs6269. The different alleles of the SNP are proposed to lead to differences in cellular COMT activity.

respect to ADHD since several studies have reported that low dopamine level in prefrontal cortex is related to ADHD symptoms [Sagvolden et al., 2005]. It is interesting that we only find consistent results for the hyperactivity/impulsivity-scale, but not for the inattentive dimension. This is in contrast to the study by Reuter et al. [2006], who found that all ASRS-subscales were related to COMT genotypes in their sample. However, Congdon and Canli [2005] suggest that it may be possible to relate subtypes of ADHD to different activation patterns in the prefrontal cortex. Both clinical and experimental studies have suggested a relationship between dopamine transmission and motor hyperactivity. Some researchers have suggested that hypodopaminergic functioning in prefrontal cortex results in excessive self-stimulation (hyperactivity) in order to maintain an optimal level of activation [Zentall and Zentall, 1983]. Sikstrom and Soderlund [2007] have proposed that abnormally low tonic extracellular dopamine in ADHD up-regulates the autoreceptors so that stimuli-evoked phasic dopamine is boosted. Stimuli that evoke moderate arousal in the brain lead to well-functioning performance, but too little or too much stimuli attenuate cognitive performance. Strong, salient stimuli may easily

TABLE IV. Stratified Linear Regression Analysis Between Common Genetic Variants in the COMT Gene and ASRS Quantitative Scores

SNP	Hyperactivity		Inattention		ASRS-total	
	Beta	P	Beta	P	Beta	P
Cases on medication, N = 267						
rs6269	1.62	0.011	1.43	0.02	3.05	0.009
rs4633	0.82	0.18	0.57	0.34	1.39	0.21
rs4818	1.24	0.05	1.05	0.10	2.29	0.05
rs4680	0.68	0.25	0.40	0.49	1.08	0.32
Cases not on medication, N = 154						
rs6269	0.09	0.91	-2.28	0.002	-2.17	0.08
rs4633	0.15	0.84	-1.99	0.003	-1.93	0.10
rs4818	0.31	0.68	-2.26	0.002	-1.93	0.12
rs4680	0.25	0.71	-2.00	0.002	-1.83	0.11
Controls, N = 383						
rs6269	0.54	0.15	-0.41	0.24	0.12	0.85
rs4633	0.41	0.26	-0.30	0.38	0.10	0.88
rs4818	0.52	0.17	-0.41	0.24	0.10	0.88
rs4680	0.31	0.39	-0.50	0.13	-0.20	0.75

TABLE V. Genetic Association of Common COMT Haplotypes as Determined by rs6269, rs4633, rs4818, and rs4680 on Quantitative Scores on the ASRS Hyperactivity-Scale in All 838 Subjects (Controlling for Gender, Current Medication, and ADHD)

Haplotype	Val/Met	Expression level ^a	COMT activity ^a	Freq. (n = 828)	Correlation coeff. (95%CI) ^{b,c}	<i>P</i> _{adj} ^{c,d}
G-C-G-G	Val	High	High	0.38	0.74 [0.14, 1.34]	0.01
A-T-C-A	Met	High	Intermediate	0.56	0	0.07
A-C-C-G	Val	Low	Low	0.06	-0.60 [-1.82, 0.62]	0.19
<i>P</i> _{overall} = 0.02						

^aSuggested levels as described in Nackley et al. [2006].

^bRepresents per haplotype increase of symptom score relative to the effect of reference haplotype "A-T-C-A."

^cAdjusted for covariates: Current medication, gender, ADHD.

^d*P*-value represents test haplotype versus all others combined.

disrupt attention, whereas an environment with impoverished stimuli causes low arousal, something that is typically compensated for by hyperactivity, according to Siktstrom and Soderlund. It is also possible that COMT may be related to specific phenotypes within or across the ADHD subtypes, and it has been suggested that dopamine affects measures of impulsivity [Congdon and Canli, 2005]. However, we did not find any evidence for a stronger association with rs6269 for the questions that specifically address the impulsivity dimension than the other questions in the hyperactivity/impulsivity scale. Our sample though, probably has little power to detect such an effect, and further studies are needed to explore the relation between DSM-IV ADHD subtypes and COMT.

Pharmacological studies also support that low dopamine level in prefrontal cortex is implicated in ADHD. Methylphenidate, that blocks the dopamine transporter [Volkow et al., 1998] and increases the brain's extracellular dopamine levels [Volkow et al., 2001], has been shown to reduce symptoms of motor activity [Rappoport et al., 1985; Borchering et al., 1989; van der Meere et al., 1995]. A recent study of 90 responders versus 32 non-responders showed an association between the Val-allele or Val/Val genotype and good methylphenidate response [Kereszturi et al., 2008]. When symptom severity was analyzed as a continuous trait, a significant interaction of COMT genotype and methylphenidate was found on the hyperactivity-impulsivity scale. The symptom severity scores for all three genotype groups also decreased following the methylphenidate administration, but Val/Val homozygote children had less severe symptoms than those with Met/Met genotype after treatment. Kereszturi et al. [2008] propose that the interaction might reflect that the regulatory effect of COMT dominates dopamine transmission prefrontally relative to subcortical dopamine systems. One limitation of our study is that most of the patients filled in the ASRS while using medication for ADHD. Accordingly, our data clearly show that patients who currently use medication for ADHD generally score lower on the ASRS ($P < 0.001$). Although we tried to compensate for this by modeling current medication as a cofactor in the analysis, it probably restricts the power of our analyses. We therefore also analyzed the results using stratification for use of medication. The relationship between hyperactivity-symptoms and rs6269 remained significant in the medicated group and there was a similar, but non significant tendency in controls. However, we found no association with hyperactivity for the group of patients who did not report use of medication at the time of study. For the inattentive symptoms,

results were contradictory between medicated and unmedicated patients: Patients on medication again showed an association between increased score and alleles for the high expression (low dopamine) haplotype while the same alleles in unmedicated patients were associated with significantly lower symptom score (Table IV). It is possible that a relatively large proportion of the participants in the group without medication are non-responders and that the groups therefore represent qualitatively different samples. McLoughlin et al. [2007] concluded that their results provide genetic support for combining the two dimensions that define ADHD, but suggested that some symptom-specific genes probably will be identified. One hypothesis may be that also COMT has some symptom specific influence. If that is true, this may partly explain the conflicting results that have been presented concerning the Val158Met polymorphism. Our findings also illustrate the importance of considering medication status and that this could explain the different results regarding COMT and ADHD.

Our results are presented without correction for multiple testing. We minimized the number of statistical tests by restricting our primary hypothesis to the idea that haplotypes are better determinants for the final COMT activity than single SNPs [Nackley et al., 2006] and to test them against three phenotypes: the clinical ADHD-diagnosis, and the hyperactivity/impulsivity and inattention dimensions as measured by the ASRS. Furthermore, we showed that only two of the four SNPs are needed to tag the variation in our sample (but present data from all SNPs for comparison with other studies). Hence, in light of the primary hypothesis, the omnibus haplotype test for the hyperactivity/impulsivity quantitative trait could by traditional standards be considered border-line significant, $P = 0.05$ using a standard Bonferroni correction for three tests. Thus, the results should be treated as explorative until confirmed in future studies.

CONCLUSION

The present study showed an association between COMT haplotypes and hyperactivity. The strongest association was found for rs6269, which segregates the high-hyperactivity/impulsivity-score haplotype from the others. However, a haplotype analysis suggested that there is in fact three distinct haplotypes which individually are associated with different hyperactivity-score levels. The suggested

rank order, from highest hyperactivity/impulsivity score to lowest, is related to the proposed low, average and high dopamine haplotypes [Nackley et al., 2006]. Our results support the hypothesis by Nackley et al. [2006], which suggests that a particular combination of common synonymous SNPs along a haplotype can have stronger effects on gene function than non-synonymous variations. Our results are also consistent with theories that suggest that low dopamine level in prefrontal cortex contribute to the symptoms of ADHD.

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