Research Paper

COMT and *DAT1* genes are associated with hyperactivity and inattention traits in the 1993 Pelotas Birth Cohort: evidence of sex-specific combined effect

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Background: Attention-deficit/hyperactivity disorder (ADHD) symptoms are dimensionally distributed in the population. This study aimed to assess the role of the catechol-O-methyltransferase (*COMT*) and of the dopamine transporter (*DAT1*) genes on ADHD symptoms in the general population. **Methods:** We investigated 4101 individuals from the 1993 Pelotas Birth Cohort Study using the parent version of the Strengths and Difficulties Questionnaire (SDQ) at ages 11 and 15 years. The SDQ hyperactivity/inattention scores were the main outcomes. **Results:** Linear regression analyses demonstrated that the increasing number of *COMT*^{158}Val and *DAT1* 10R alleles significantly predicted increasing SDQ hyperactivity/inattention scores in boys at both 11 and 15 years of age (β coefficient = 0.049, t = 2.189, p = 0.029, R^2 = 0.012, and β coefficient = 0.064, t = 2.832, p = 0.005, R^2 = 0.008, respectively). The presence of both *COMT*^{158}Val and *DAT1* 10R alleles was also associated with full categorical ADHD diagnosis at 18 years of age in boys (χ^2 = 4.561, p = 0.033, odds ratio 2.473, 95% confidence interval 1.048–5.838) from this cohort. We did not observe these associations in girls. **Limitations:** Our analyses of SDQ hyperactivity/inattention scores were not corrected for SDQ scores of conduct problems because these variables were highly correlated. **Conclusion:** This study demonstrates a role for *COMT* and *DAT1* genes on hyperactivity/inattention symptoms and provides further support for ADHD as the extreme of traits that vary in the population. It also confirms previous evidence for sexual dimorphism on *COMT* and *DAT1* gene expression.

Introduction

Symptoms of hyperactivity/impulsivity and inattention are dimensionally distributed in the population. Evidence supporting this idea comes from the study of twin pairs from the general population, which demonstrate high heritability estimates for these behavioural traits, ranging from 0.60 to 0.91.[1–3] The low end of the distribution would be represented by few behavioural problems and better cognitive function than the high and symptomatic end. This extreme and impairing end would probably be represented by the categorical diagnosis of attention-deficit/hyperactivity disorder (ADHD).[4] A study on inhibition, an executive function whose impairments are part of the cognitive deficits seen in individuals with ADHD, demonstrated that performance on inhibition-related tasks were positively associated with ADHD-like traits in a large sample of healthy adults who did not have a first-degree relative with ADHD.[5]

The heritability estimates for ADHD are essentially the same for both continuous and categorical approaches, consistent with a dimensional view of ADHD and a strong genetic component.[1–3,6] Based on the normal distribution of ADHD traits in the general population, the identification and understanding of ADHD susceptibility genes may benefit from studies of this dimensional characteristic of ADHD in nonclinical samples.[2] Despite high heritability estimates, the identification of ADHD genetic susceptibility markers has been difficult, with few replicable findings described so far.[7,8] The main candidates for ADHD molecular genetic studies have been genes involved in the dopamine pathway, given considerable evidence supporting the dopaminergic hypothesis. According to this theory, an

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underlying dopamine deficit would be responsible for at least part of the ADHD phenotype spectrum.[9,10]

The catechol-O-methyltransferase (COMT) enzyme is involved in catecholamine's clearance from the synaptic cleft in an extraneuronal degradation process. It is one of the mechanisms involved in dopamine signalling termination, which is particularly important to control frontal lobe dopamine levels.[11,12] The *COMT* gene has a common functional polymorphism, a valine to methionine change at codon 158 (Val[158]Met, rs4680). Val allele homozygosity determines a 3- to 4-fold increase in enzyme activity, resulting in faster catecholamine catabolism.[13,14] Three meta-analyses have failed to detect an association between this polymorphism and ADHD.[7,15,16] Discrepancies may be attributed to sexual dimorphism given that the *COMT* ADHD susceptibility allele may differ according to sex.[17,18]

The dopamine transporter gene (*DAT1* or *SLC6A3*) codes for the dopamine transporter protein (DAT) that is responsible for the reuptake of dopamine from the synaptic cleft back into the presynaptic neuron. Similarly to COMT, DAT is involved in control of the strength and duration of the dopamine signal. However, DAT is the main mechanism of dopamine regulation in other brain regions: the striatum and nucleus accumbens.[19] The most investigated *DAT1* polymorphism is a 40 base pairs (bp) variable number of tandem repeats (VNTR) located at the gene's 3′-untranslated region (3′UTR). Ten (10R) and 9 (9R) repeat alleles are the most common.[20] A study has demonstrated that DAT messenger RNA (mRNA) expression in postmortem midbrain tissue is higher for homozygous 10R carriers.[21] However, 2 meta-analyses of neuroimaging studies detected increased DAT activity for 9R carriers in striatal brain regions.[22,23] This result is intriguing, given that 3 meta-analyses have reported a small but significant association between the 10R allele and genetic susceptibility to ADHD.[6,7,24]

Despite all data from the literature demonstrating that ADHD traits are normally distributed in the population, a recent study suggested a different genetic architecture for ADHD and ADHD traits.[25] In this context, genes identified as risk factors for a full categorical diagnosis of ADHD would not necessarily be associated with ADHD traits in the general population. Thus, to explore this hypothesis, we aimed to investigate the role of the ADHD candidate genes *COMT* and *DAT1* on hyperactivity/inattention traits in a large birth cohort. There is no evidence to support sex differences regarding the influence of genetic and environmental factors acting on the ADHD continuum,[2,3] but because the dopaminergic system seems to be particularly sensitive to estrogen,[26] we investigated the role of these genes separately for boys and girls.

Methods

The Institutional Review Board of the School of Medicine from Universidade Federal de Pelotas approved this study. Parents or legal guardians signed an informed consent form authorizing their own participation and that of the children in the study.

Participants

The individuals included in this study were born in 1993 in Pelotas, Brazil. The data collection methodology and demographic data from this birth cohort are fully described elsewhere.[27,28] Of the children born alive (n = 5249), 87.5%, 85.7%, and 81.4% were reassessed at ages 11, 15 and 18 years, respectively. During the 15-year assessment, 4101 participants provided a saliva sample for DNA investigations; they were included in the present study.

Phenotypic assessments

To evaluate hyperactivity/impulsivity and inattention symptoms, the primary caregiver answered the validated Brazilian Portuguese version of the Strengths and Difficulties Questionnaire (SDQ).[29,30] The SDQ subscale of hyperactivity and inattention problems allows computation of a score ranging from 0 to 10. We used these scores as the main outcome measures for the present study. The data were collected at ages 11 and 15 years.

A general psychiatric assessment was performed at 18 years of age using the validated Brazilian Portuguese version of the MINI International Neuropsychiatric Interview (M.I.N.I.), a short semistructured diagnostic interview for the DSM-IV, and the International Classification of Diseases, tenth revision (ICD-10) codes for psychiatric disorders, which provided prevalence estimates of the most common anxiety (generalized anxiety disorder and social phobia) and mood disorders (bipolar disorder and major depressive disorder).[31,32] The ADHD assessment was performed using a structured interview based on DSM-5.[33] Further details are available elsewhere.[34,35]

DNA collection and genotyping

We obtained DNA samples from saliva using an Oragene OG-250 DNA self-collection kit following the manufacturer's recommended protocol (DNA Genotek Inc.). We genotyped the *COMT* Val[158]Met polymorphism using the TaqMan allelic discrimination system following the manufacturer's recommended protocol (Applied Biosystems Inc.). The *DAT1* 3′UTR VNTR was genotyped as previously described.[36,37]

Statistical analysis

Allele and genotype frequencies were estimated by counting. We tested Hardy–Weinberg equilibrium using Genepop 4.0 software.[38] In this investigation we chose *COMT*-[158]Val as the reference allele based on functional studies demonstrating higher enzymatic activity of this allele,[13,14] consistent with a dopamine deficit hypothesized to underlie at least part of ADHD symptoms.[9,10] We chose the *DAT1* 10R allele as the reference based on the results of previous meta-analyses.[6,7,24] Other alleles (3R, 5R, 6R, 7R, 8R, 9R, 11R, and 12R) were pooled owing to low frequency.

We assessed possible confounders using a χ^2 test for categorical variables and a t test for continuous variables. Covariates were included in the models if they were associated

with study factors and outcomes at $p \leq 0.20$. The potential confounders evaluated were anxiety disorders, IQ, mood disorders and skin colour as a marker of race. These data were obtained from the mothers following the Brazilian census method of classification based on ethnoracial self-classification, which includes 5 groups: white, mixed, black, Asian, and indigenous. A study of genomic ancestry involving the 1982 Pelotas Birth Cohort reported statistically significant associations between ancestry and the phenotype of self-classified ethnoracial group, both at population and individual levels. The study also demonstrated that European ancestry is predominant in Pelotas (85.3%).[39] In our analyses, we dichotomized the variable skin colour as white (66.8%) and others (33.2%).

Sample power was estimated based on sample size and a small effect size using G*Power version 3.1 software.[40] We performed linear regression analyses to verify the association between *COMT* and *DAT1* independently and to determine whether the increasing number of *COMT*^{158}Val and *DAT1* 10R alleles predicted increasing hyperactivity/inattention symptoms at the 11- and 15-year assessments. Two-way analyses of variance were performed to test for a possible interaction between *COMT* and *DAT1* genes and hyperactivity/inattention symptoms at the 11- and 15-year assessments. To test whether the presence of *COMT*^{158}Val and *DAT1* 10R alleles were associated with full ADHD diagnosis at 18 years of age, we used the χ^2 test. We performed these analyses separately for boys and girls using SPSS for Windows, version 18.0 (IBM Corp.). All tests were 2-tailed. We considered results to be significant at $p < 0.05$ in all analyses.

Results

The prevalence of a full ADHD diagnosis assessed at 18 years of age was 3.5%. Of these cases, 33.1% and 31.3% presented SDQ hyperactivity/inattention scores of 8 or higher at the 11- and 15-year assessments, respectively. This observation is similar to that in a previous report of adult ADHD from another birth cohort in which the majority of cases lacked childhood history of ADHD.[41] The *COMT*^{158}Val allele and *DAT1* 10R allele were the most frequent alleles in both male (57.4% and 71.2%, respectively) and female participants (57.2% and 71.4%, respectively). The genotype frequencies did not significantly deviate from those expected according to Hardy–Weinberg equilibrium. The SDQ scores at the 11- and 15-year assessments for both boys and girls deviated significantly from normality (Kolmogorov–Smirnov test all $p < 0.05$). However, skewness indicated an approximately normal distribution of the variables (values ranging from 0.014 to 0.56).[42] No evidence of heteroscedasticity (Levene test p values ranging from 0.12 to 0.84) or deviation from linearity (p values ranging from 0.22 to 0.82) was observed. Therefore, considering these results and the large sample size, we opted to conduct linear regression analyses on untransformed SDQ scores.

We performed linear regression analyses to verify *COMT* and *DAT1* main effects independently, but no significant results were detected for boys or girls (Table 1 and Table 2). As COMT and DAT have a synergistic effect on dopamine clearance from the synaptic cleft, we divided the sample into 3 groups according to the presence of *COMT*^{158}Val and *DAT1* 10R alleles as follows: 1) no *COMT*^{158}Val or *DAT1* 10R allele, 2) presence of *COMT*^{158}Val or *DAT1* 10R allele, or 3) presence of both *COMT*^{158}Val and *DAT1* 10R allele. The linear regression analyses were performed with skin colour as a covariate given its association with study factors and outcome at a significance level of $p \leq 0.20$. These analyses demonstrated that the number of *COMT*^{158}Val and *DAT1* 10R alleles significantly predicted an increase in SDQ hyperactivity/inattention scores in boys at both the 11- and 15-year assessments (β coefficient = 0.049, $t = 2.189$, $p = 0.029$, $R^2 = 0.012$ and β coefficient = 0.064, $t = 2.832$, $p = 0.005$, $R^2 = 0.008$, respectively; Table 3). The results remained significant for both the 11- and 15-year assessments after we excluded the smallest subgroup of individuals not

Table 1: Linear regression analyses for *COMT* Val^{158}Met polymorphism on hyperactivity/inattention scores from the Strengths and Difficulties Questionnaire according to sex

Sex	Age, yr	Genotype	*n*	SDQ score, mean ± SD	β coefficient	*t*	*p* value	R^2
Male	11	Met/Met	363	4.45 ± 3.15	0.043	1.908	0.06	0.012
		Met/Val	937	4.84 ± 3.08				
		Val/Val	653	4.95 ± 3.08				
	15	Met/Met	364	3.87 ± 3.04	0.030	1.305	0.19	0.005
		Met/Val	938	4.37 ± 3.13				
		Val/Val	654	4.27 ± 3.19				
Female	11	Met/Met	396	3.86 ± 2.94	–0.004	–0.167	0.87	0.002
		Met/Val	962	3.80 ± 3.02				
		Val/Val	687	3.85 ± 3.07				
	15	Met/Met	395	3.22 ± 2.72	0.013	0.609	0.54	0.003
		Met/Val	963	3.47 ± 2.97				
		Val/Val	688	3.40 ± 2.92				

SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire.

carrying *COMT*^{158}Val or *DAT1* 10R (β coefficient = 0.049, t = 2.132, p = 0.033, R^2 = 0.012 and β coefficient = 0.045, t = 1.974, p = 0.047, R^2 = 0.005, respectively).

Two-way analysis of variance (ANOVA) was performed to assess if these positive associations reflected a gene × gene interaction. There was no evidence of interaction between *COMT* and *DAT1* ($F_{4,1931}$ = 1.072, p = 0.37 and $F_{2,1934}$ = 1.086, p = 0.36) for hyperactivity/inattention scores in boys at both 11 and 15 years of age, respectively (Table 4). As no covariates were identified for the analyses of full ADHD diagnosis at age 18 years, χ^2 tests were performed. These analyses demonstrated that the presence of both *COMT*^{158}Val and *DAT1* 10R alleles was associated with ADHD in boys (χ^2 = 4.561, p = 0.033, odds ratio [OR] 2.473, 95% confidence interval [CI] 1.048–5.838; Table 5). No significant associations were observed for girls.

In order to ensure that the results observed herein were not influenced by population substructure, even though skin colour was included as a covariate in both linear regression analyses and 2-way ANOVAs, we repeated all analyses restricting the sample to white participants (66.8%). Despite the decrease in power, we observed results in the same direction (Appendix 1, Tables S1 to S5, available at jpn.ca).

Discussion

Our study suggests that the increasing number of *COMT*^{158}Val and *DAT1* 10R alleles predicts increasing symptoms of hyperactivity/inattention in boys from the general population assessed at 11 and 15 years of age. The presence of both alleles was also associated with full ADHD diagnosis at age 18 years in boys from this cohort. We did not observe these associations in girls, confirming previous evidence of sexual dimorphic effect of these genes.

The idea that ADHD is an extreme of behavioural traits comes to some extent from the observation that hyperactivity/impulsivity and inattention symptoms are present in the general population. Twin studies demonstrated that ADHD as a trait as well as a category is substantially influenced by genetic factors.[1–3] Similar heritability estimates were found

Table 2: Linear regression analyses for *DAT1* 3′UTR VNTR polymorphism on hyperactivity/inattention scores from the Strengths and Difficulties Questionnaire according to sex

Sex	Age, yr	Genotype*	*n*	SDQ score, mean ± SD	β coefficient	*t*	*p* value	R^2
Male	11	_R/_R	149	4.59 ± 3.02	0.016	0.726	0.47	0.010
		_R/10R	829	4.82 ± 3.13				
		10R/10R	963	4.83 ± 3.08				
	15	_R/_R	150	3.80 ± 3.13	0.035	1.548	0.12	0.005
		_R/10R	831	4.25 ± 3.17				
		10R/10R	963	4.31 ± 3.12				
Female	11	_R/_R	132	4.25 ± 3.09	–0.032	–1.445	0.15	0.003
		_R/10R	891	3.85 ± 3.00				
		10R/10R	1013	3.76 ± 3.03				
	15	_R/_R	132	3.70 ± 2.91	–0.038	–1.719	0.09	0.004
		_R/10R	891	3.47 ± 2.94				
		10R/10R	1014	3.30 ± 2.87				

SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire; UTR = untranslated region; VNTR = variable number of tandem repeats.
*_R = 3R, 5R, 6R, 7R, 8R, 9R, 11R, or 12R alleles.

Table 3: Linear regression analyses of the number of *COMT*^{158}Val and *DAT1* 10R alleles on the hyperactivity/inattention scores from the Strengths and Difficulties Questionnaire according to sex

Sex	Age, yr	Group	*n*	SDQ score, mean ± SD	β coefficient	*t*	*p* value	R^2
Male	11	No *COMT*^{158}Val or *DAT1* 10R	36	4.39 ± 3.19	0.049	2.189	0.029	0.012
		COMT^{158}Val or *DAT1* 10R	438	4.52 ± 3.10				
		COMT^{158}Val and *DAT1* 10R	1467	4.91 ± 3.08				
	15	No *COMT*^{158}Val or *DAT1* 10R	37	3.05 ± 3.04	0.064	2.832	0.005	0.008
		COMT^{158}Val or *DAT1* 10R	438	3.99 ± 3.06				
		COMT^{158}Val and *DAT1* 10R	1469	4.35 ± 3.16				
Female	11	No *COMT*^{158}Val or *DAT1* 10R	22	3.77 ± 2.50	-0.023	-1.060	0.29	0.003
		COMT^{158}Val or *DAT1* 10R	482	3.97 ± 3.02				
		COMT^{158}Val and *DAT1* 10R	1532	3.79 ± 3.03				
	15	No *COMT*^{158}Val or *DAT1* 10R	22	2.82 ± 2.08	0.012	0.522	0.60	0.003
		COMT^{158}Val or *DAT1* 10R	481	3.38 ± 2.82				
		COMT^{158}Val and *DAT1* 10R	1534	3.41 ± 2.95				

SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire.

when ADHD was analyzed in both ways, suggesting a continuous distribution of genetic liability. The fact that the heritability estimates did not rise with increasing symptom severity or categorical ADHD diagnosis is consistent with its dimensional characteristic.[1,3] Results from twin studies are further supported by neurobiological data, as slower cortical thinning during adolescence was associated with hyperactivity and impulsivity symptoms in both typically developing children and children with ADHD.[43] In addition, deficits in basic information processing were found to be linearly associated with ADHD severity that ranged from asymptomatic to clinical ADHD.[44]

A twin study provided evidence of an additive genetic pattern of ADHD inheritance because the observed concordance rates between dizygotic twins were around half of those observed in monozygotic twins.[3] This is consistent with the idea that genetic variants of small effect, either common or rare, together contribute to build a genetic risk for a given psychiatric disorder.[45] Recently, it was demonstrated that common variants of the dopamine/norepinephrine, serotonin and neurite outgrowth pathways are associated with quantitative measurements of hyperactivity/impulsivity symptoms in children with ADHD.[46] In studies involving the general population, polygenic risk scores derived from common molecular variants for categorical ADHD were found to predict attentional and hyperactive/impulsive traits in the general population.[47] On the other hand, polygenic risk scores derived from ADHD traits in the general population sample predicted ADHD categorical diagnosis and symptom severity.[48] Our results are in agreement with those findings. *COMT* and *DAT1* in combination were associated with ADHD symptoms in the general population.

Sex differences reported in this study are consistent with previous reports of sexual dimorphism described for both genes. *COMT* sexual dimorphism is largely attributed to estrogen, which impacts *COMT* expression through 2 estrogen response elements present in the promoter region. *COMT* mRNA concentrations are lower in cells expressing estrogen receptors, suggesting that the expression is different in boys than in girls, mainly due to downregulation by estrogen.[49–51] Despite this experimental evidence, a meta-analysis failed to detect sex as a moderator of the association between *COMT* and ADHD in clinical samples.[15] The absence of positive findings could be due to heterogeneity across studies and lack of power. Even with greater sample sizes, we were able to detect *COMT* sex-specific effects only when analyzed in combination with *DAT1*, which indicates that the effect size is very small. In this sense, the study of quantitative traits in larger population samples may help increase power.

In our analyses, the *COMT*^{158}Val allele was associated with both higher ADHD symptom scores and ADHD diagnosis in boys, contrary to the evidence from early family-based and case–control studies that suggested sex-specific effects for *COMT*. In these studies, the *COMT*^{158}Met allele was associated with ADHD in boys from some clinical samples.[17,18] However, a large population study on executive functioning, which is known to be impaired in individuals with ADHD, demonstrated that boys carrying the *COMT*^{158}Met allele performed better in a series of tasks than boys who were homozygous *COMT*^{158}Val carriers. There were no discernible effects in girls.[52] Moreover, in agreement with our results, the *COMT*^{158}Val allele was associated with ADHD comorbid with conduct disorders in several studies of both clinical and population samples.[53–59] The discrepancies concerning the definition of the *COMT* risk allele may be attributed to other variants of the gene. Evidence suggests that COMT enzymatic activity is in fact determined by haplotype blocks, whose structure may vary across populations and could explain conflicting results.[60]

Some early evidence also suggested sexual dimorphism for *DAT1*. It has been reported that estrogen has an antagonistic effect on DAT activity,[61] which may protect against ADHD by delaying dopamine reuptake. This is somewhat confirmed by a report that demonstrated an interaction between prenatal smoke exposure and *DAT1* genotype in humans. The 10R allele homozygous boys exposed to maternal smoke had higher hyperactivity/impulsivity symptoms than boys carrying other genotypes. This interaction was not observed in girls.[62] A study on delinquency reported a male-specific association with *DAT1*. Individuals who were 10R allele homozygous and 10R/9R heterozygous presented trajectories of serious delinquency about twice as high as those observed for 9R homozygous individuals.[63] *DAT1* was also associated with continuous measures of ADHD in boys from the general population.[64,65] Recently, *DAT1* was reported to be associated with ADHD symptoms in a nonclinical adult population.[66] It was also associated with the executive function of inhibition, which is impaired in adults with ADHD from the general population.[67]

Evidence from animal models demonstrates that the dopaminergic function is essentially different in males and females. In rats, estrogen and progesterone modulate dopamine activity in the striatum and nucleus accumbens. This activity varies

Table 4: Two-way analyses of variance of *COMT* and *DAT1* genes and the hyperactivity/inattention scores from the Strengths and Difficulties Questionnaire according to sex

Sex	Age, yr	Category	df	*F*	*p* value
Male	11	*COMT*	2	0.852	0.43
		DAT1	2	0.385	0.68
		COMT × *DAT1*	4	1.072	0.37
		Error	1931	—	—
	15	*COMT*	2	3.166	0.042
		DAT1	2	2.270	0.10
		COMT × *DAT1*	4	1.086	0.36
		Error	1934	—	—
Female	11	*COMT*	2	0.076	0.93
		DAT1	2	0.763	0.47
		COMT × *DAT1*	4	0.181	0.95
		Error	2026	—	—
	15	*COMT*	2	1.479	0.23
		DAT1	2	1.010	0.36
		COMT × *DAT1*	4	0.413	0.80
		Error	2027	—	—

df = degrees of freedom; SDQ = Strengths and Difficulties Questionnaire.

Table 5: The presence of full categorical attention-deficit/hyperactivity disorder diagnosis at 18 years of age according to the presence of both *COMT*^{158}Val and *DAT1* 10R alleles according to sex

Sex	Genotype	With ADHD	Without ADHD	χ^2	*p* value	OR (95% CI)
Male	*COMT*^{158}Val / *DAT1* 10R carriers	45	1319	4.561	0.033	2.473 (1.048–5.838)
	Others	6	435	—	—	—
Female	*COMT*^{158}Val / *DAT1* 10R carriers	62	1407	0.008	0.93	0.978 (0.590–1.622)
	Others	21	466	—	—	—

ADHD = attention-deficit/hyperactivity disorder; CI = confidence interval; OR = odds ratio.

in an estrous cycle–dependent way and is attenuated by oophorectomy. Unlike in females, dopamine activity in males is not affected by estrogen or absence of testicular hormones.[68] Accordingly, estrogen was associated with an attenuated methamphetamine-evoked dopamine output in mice, while this effect was not observed with testosterone.[69]

In humans, as in animals, the dopamine system seems to be strongly affected by estrogen.[26] This effect may be partially attributed to *COMT* and its regulation by estrogen.[50,51] Dopamine release in the striatum, putamen and caudate following an amphetamine challenge is significantly higher in men than in women.[70] Sex differences, however, are not restricted to the dopamine system, but rather involve the whole brain. A longitudinal neuroimaging study showed that cortical and subcortical grey matter development occurs earlier in girls.[71] One study of ADHD reported an overall reduction on the surface area of the prefrontal cortex only in girls, whereas only boys showed overall reductions in the surface area of the total premotor cortex.[72] Another study demonstrated that cortical thinning is associated with symptom persistence from childhood into adulthood, and it has been observed that ADHD persistence was greater in girls.[73] The marked difference of ADHD prevalence in childhood seen in clinical samples in itself suggests sex differences, as the ratio of boys to girls with ADHD varies from 3:1 to 9:1.[74] However, the scenario may be different for adult samples from the general population. In the present sample of 18-year-olds with diagnosed ADHD, there is a preponderance of girls,[35] a finding that is in agreement with a previous report.[75]

Limitations

The results presented herein must be interpreted in the context of some limitations. First, SDQ scores of conduct problems were not considered as covariates since hyperactivity/inattention scores and conduct scores were highly correlated ($r_s = 0.553$, $p \leq 0.001$ and $r_s = 0.503$, $p \leq 0.001$ at the 11- and 15-year assessments, respectively). Second, The SDQ scores do not follow a normal distribution, but the deviation is not extreme. We tried several transformations, including log, reciprocal and square root. None of them produced a significant increase in the approximation to the normal distribution. However, we observed no heterogeneity of variances, which is an assumption that has high impact on *p* values. Moreover, we compared the Akaike information criterion (AIC) statistics between regression models using the untransformed and the square root transformed scores. We observed lower AIC values with the transformed scores, but the results did not differ from those of untransformed scores. Therefore, we decided to maintain the original SDQ values to make the interpretation of the effects more amenable. Third, no genomic control was performed; therefore, our findings could have been biased by hidden genetic heterogeneity present in our specific sample of the southern Brazilian population. But, since skin colour showed a high correlation with genomic ancestry in the same population,[39] we considered that it was a good proxy for genetic substructure. Fourth, from the analyses performed herein, it is not possible to determine the exact effect size of each variant since no significant main effects were detected despite 99% power to detect small effects. The observed effect of these variants combined was very small, possibly owing to the combination of only 2 genes. However, small effect sizes are to be expected in multifactorial traits such as ADHD.

Conclusion

Our results confirm a role for *COMT* and *DAT1* on symptoms of hyperactivity/inattention, adding evidence to the idea that ADHD represents the end of a continuum of behavioural traits that vary in the population. Furthermore, the present results support a sexual dimorphic effect of these genes on ADHD traits. Future research on ADHD genetic susceptibility should take into account the possible heterogeneity that arises from sex differences.

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