**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 behavioral traits, ranging from 0.60 to 0.91. The low end of the distribution would be represented by few behavioral 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). 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.

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. 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. Despite high heritability estimates, the identification of ADHD genetic susceptibility markers has been difficult, with few replicable findings described so far. The main candidates for ADHD molecular genetic studies have been genes involved in the dopaminergic hypothesis. According to this theory, an

**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<sup>Val158Val</sup> and DAT1<sup>10R</sup> 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<sup>2</sup> = 0.012, and β coefficient = 0.064, t = 2.832, p = 0.005, R<sup>2</sup> = 0.008, respectively). The presence of both COMT<sup>Val158Val</sup> and DAT1<sup>10R</sup> alleles was also associated with full categorical ADHD diagnosis at 18 years of age in boys (χ<sup>2</sup> = 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.

**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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underlying dopamine deficit would be responsible for at least part of the ADHD phenotype spectrum.\textsuperscript{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.\textsuperscript{11,12} The COMT gene has a common functional polymorphism, a valine to methionine change at codon 158 (Val\textsuperscript{158}Met, rs4680). Val allele homozygosity determines a 3- to 4-fold increase in enzyme activity, resulting in faster catecholamine catabolism.\textsuperscript{13,14} Three meta-analyses have failed to detect an association between this polymorphism and ADHD.\textsuperscript{15,16} Discrepancies may be attributed to sexual dimorphism given that the COMT ADHD susceptibility allele may differ according to sex.\textsuperscript{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.\textsuperscript{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.\textsuperscript{20} A study has demonstrated that DAT messenger RNA (mRNA) expression in postmortem midbrain tissue is higher for homozygous 10R carriers.\textsuperscript{21} However, 2 meta-analyses of neuroimaging studies detected increased DAT activity for 9R carriers in striatal brain regions.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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,\textsuperscript{2,3} but because the dopaminergic system seems to be particularly sensitive to estrogen,\textsuperscript{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.\textsuperscript{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).\textsuperscript{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).\textsuperscript{31,32} The ADHD assessment was performed using a structured interview based on DSM-5.\textsuperscript{33} Further details are available elsewhere.\textsuperscript{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\textsuperscript{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.\textsuperscript{26,37}

**Statistical analysis**

Allele and genotype frequencies were estimated by counting. We tested Hardy–Weinberg equilibrium using Genepop 4.0 software.\textsuperscript{28} In this investigation we chose COMT-Val\textsuperscript{158}Met as the reference allele based on functional studies demonstrating higher enzymatic activity of this allele,\textsuperscript{13,14} consistent with a dopamine deficit hypothesized to underlie at least part of ADHD symptoms.\textsuperscript{9,10} We chose the DAT1 10R allele as the reference based on the results of previous meta-analyses.\textsuperscript{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 \(\chi^2\) test for categorical variables and a \(t\) test for continuous variables. Covariates were included in the models if they were associated.
Evidence of sex-specific combined effect

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 ethnic-racial 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 ethnic-racial group, both at population and individual levels. The study also demonstrated that European ancestry is predominant in Pelotas (85.3%). 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. We performed linear regression analyses to verify the association between COMT and DAT1 independently and to determine whether the increasing number of COMT158Val 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. Two-way analyses of variance were performed to test for the association between COMT and DAT1 independently and to determine whether the increasing number of COMT158Val and DAT1 10R alleles predicted increasing hyperactivity/inattention symptoms at the 11- and 15-year assessments. To test whether the presence of COMT158Val and DAT1 10R alleles were associated with full ADHD diagnosis at 18 years of age, we used the \( \chi^2 \) 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. The COMT158Val 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). 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 DAT1 have a synergistic effect on dopamine clearance from the synaptic cleft, we divided the sample into 3 groups according to the presence of COMT158Val and DAT1 10R alleles as follows: 1) no COMT158Val or DAT1 10R allele, 2) presence of COMT158Val or DAT1 10R allele, or 3) presence of both COMT158Val 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 COMT158Val and DAT1 10R alleles significantly predicted an increase in SDQ hyperactivity/inattention scores in boys at both the 11- and 15-year assessments (\( \beta \) coefficient = 0.049, \( t = 2.832, \) \( p = 0.005, R^2 = 0.008 \), 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 Val158Met polymorphism on hyperactivity/inattention scores from the Strengths and Difficulties Questionnaire according to sex |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sex | Age, yr | Genotype | n | SDQ score, mean ± SD | \( \beta \) 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 | 3.84 ± 3.08 | 0.030 | 1.265 | 0.19 | 0.005 |
| | | Val/Val | 653 | 4.95 ± 3.08 | 0.030 | 1.265 | 0.19 | 0.005 |
| | 15 | Met/Met | 364 | 3.87 ± 3.04 | 0.030 | 1.265 | 0.19 | 0.005 |
| | | Met/Val | 938 | 4.37 ± 3.13 | 0.030 | 1.265 | 0.19 | 0.005 |
| | | Val/Val | 654 | 4.27 ± 3.19 | 0.030 | 1.265 | 0.19 | 0.005 |
| Female | 11 | Met/Met | 396 | 3.86 ± 2.94 | –0.004 | –0.167 | 0.87 | 0.002 |
| | | Met/Val | 962 | 3.80 ± 3.02 | –0.004 | –0.167 | 0.87 | 0.002 |
| | | Val/Val | 687 | 3.85 ± 3.07 | –0.004 | –0.167 | 0.87 | 0.002 |
| | 15 | Met/Met | 395 | 3.22 ± 2.72 | 0.013 | 0.609 | 0.54 | 0.003 |
| | | Met/Val | 963 | 3.47 ± 2.97 | 0.013 | 0.609 | 0.54 | 0.003 |
| | | Val/Val | 688 | 3.40 ± 2.92 | 0.013 | 0.609 | 0.54 | 0.003 |

SD = standard deviation; SDQ = Strengths and Difficulties Questionnaire.
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, $\chi^2$ tests were performed. These analyses demonstrated that the presence of both COMT$^{158}$Val and DAT1$^{10R}$ alleles was associated with ADHD in boys ($\chi^2 = 4.561, p = 0.033$, 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.
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.\textsuperscript{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.\textsuperscript{43} In addition, deficits in basic information processing were found to be linearly associated with ADHD severity that ranged from asymptomatic to clinical ADHD.\textsuperscript{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.\textsuperscript{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.\textsuperscript{45} Recently, it was demonstrated that common variants of the dopamine/norepinephrine, serotonin and norepinephrine outgrowth pathways are associated with quantitative measurements of hyperactivity/impulsivity symptoms in children with ADHD.\textsuperscript{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.\textsuperscript{47} On the other hand, polygenic risk scores derived from ADHD traits in the general population sample predicted ADHD categorical diagnosis and symptom severity.\textsuperscript{48} Our results are in agreement with those findings. COMT and DAT\textsubscript{1} 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.\textsuperscript{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.\textsuperscript{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 DAT\textsubscript{1}, 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\textsuperscript{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\textsuperscript{158}Met allele was associated with ADHD in boys from some clinical samples.\textsuperscript{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\textsuperscript{158}Met allele performed better in a series of tasks than boys who were homozygous COMT\textsuperscript{158}Val carriers. There were no discernible effects in girls.\textsuperscript{52} Moreover, in agreement with our results, the COMT\textsuperscript{158}Val allele was associated with ADHD comorbid with conduct disorders in several studies of both clinical and population samples.\textsuperscript{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.\textsuperscript{60}

Some early evidence also suggested sexual dimorphism for DAT\textsubscript{1}. It has been reported that estrogen has an antagonistic effect on DAT activity,\textsuperscript{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 DAT\textsubscript{1} 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.\textsuperscript{62} A study on delinquency reported a male-specific association with DAT\textsubscript{1}. 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.\textsuperscript{63} DAT\textsubscript{1} was also associated with continuous measures of ADHD in boys from the general population.\textsuperscript{64,65} Recently, DAT\textsubscript{1} was reported to be associated with ADHD symptoms in a nonclinical adult population.\textsuperscript{66} It was also associated with the executive function of inhibition, which is impaired in adults with ADHD from the general population.\textsuperscript{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

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df = degrees of freedom; SDQ = Strengths and Difficulties Questionnaire.
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.30,33 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,30 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 ($r = 0.553, p \leq 0.001$ and $r = 0.503, p \leq 0.001$ 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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Evidence of sex-specific combined effect

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