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ORIGINAL PAPER

DRD3 Gene and ADHD: A Pharmaco-Behavioural Genetic Study

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Abstract

Results of candidate gene investigations in ADHD have been difficult to replicate. The complexity of the phenotypes and their underlying determinants, and the relatively small effect sizes of genetic variants may, in part, be contributing to these inconsistencies. The objective of this study is to conduct an exploratory analysis using a comprehensive approach to investigate the role of candidate genes. This approach combines a dimensional behavioural approach akin to Research Domain Criteria (RDoC), a pharmaco-dynamic evaluation of behaviours relevant to ADHD, together with association and linkage testing in a large sample of children with ADHD. Parents, teachers, and research staff evaluated children with ADHD under three experimental conditions (EC): 1 week of baseline observation, followed by 1 week of methylphenidate (MPH) and 1 week of placebo, administered in a double-blind crossover order. Several quantitative behavioural and cognitive dimensions relevant for ADHD were also assessed. We combined family-based (FBAT) and quantitative trait genetic analyses ($n = 575$ probands with members of their nuclear families) to investigate the role of *DRD3* (Ser-9-Gly) in ADHD and its relevant behavioural dimensions. Comparing the behaviours of children with different genotypes under the three EC showed a nominal association between the *T* allele and poorer behavioural scores during the MPH week (as assessed by teachers), particularly in boys. With the family-based analysis, the *T* allele showed a nominal association with increased risk for ADHD, response to placebo and MPH as assessed by research staff, and the modulation of other behavioural and cognitive dimensions. These results provide convergent, albeit preliminary evidence for the implication of the *DRD3* (Ser-9-Gly) polymorphism in the aetiology of ADHD and the modulation of its various behavioural dimensions, including RDoC cognitive constructs and response to pharmacological probes. This illustrative example suggests that this research paradigm might help to reliably uncover the role of other candidate genes in ADHD.

Keywords Attention-deficit/hyperactivity disorder (ADHD) · *DRD3* · Pharmaco-dynamic · RDoC · RCT

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders, affecting 5.9–7.1% of children (Willcutt 2012). Its mean heritability is estimated at 70–80% (Franke et al. 2012), placing ADHD as one of the most heritable psychiatric disorders. Various lines of evidence indicate that the brain dopamine systems play a major role in the pathophysiology of ADHD. Indeed, dopamine (DA) has a major role in modulating several aspects of human behaviour highly relevant for ADHD including saliency (Bromberg-Martin et al. 2010), attention (Nieoullon 2002), and motor behaviours (Joshua et al. 2009). Consequently, genes coding for proteins implicated in synaptic DA transmission have been popular candidate genes in association studies. However, these studies have fallen out of favour, as they lead to inconsistent results.

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Notwithstanding the challenge of complex behavioural genetics, we believe that candidate gene approaches can hold promise in the field, provided that (1) phenotypes are decomposed into clinically relevant and neurobiological pertinent dimensions. This dimensional analysis is akin to the Research Domain Criteria (RDoC) approach, a research framework that represents a novel approach to classification of psychiatric disorders for research purposes, where the core symptoms of any mental disorder (termed "constructs") may be grouped in one or more of five major domains that are encapsulated within the framework (Iacono 2016). (2) Whenever possible, these behaviours are assessed under different experimental conditions, including pharmacological probing that bears relevance to these behaviours and the genes under investigation. (3) Finally, different statistical approaches are used to control for different sources of bias. When the results from different approaches converge, the results gain greater credibility. A major advantage of the candidate gene approach compared to Genome Wide Association Studies is that it investigates genes with already substantial knowledge relevant to their physiology, which make them amenable to clinical translation.

In ADHD, the *DRD3* gene has been suggested as a promising candidate for several reasons. A knockout mouse model of *Drd3* shows increased locomotor activity (Accili et al. 1996). Furthermore, while DRD3 antagonists induce hyperactivity, administration of dopaminergic agonists (such as of 7-OH-DPAT) that bind preferably to DRD3 receptors reduces locomotor activity (Daly and Waddington 1993; Svensson et al. 1994). In addition, DRD3 receptor is predominantly localized in the mesolimbic areas of the brain. These areas are critical for cognitive and emotional functions, novelty seeking, as well as expression of reward (Wise and Bozarth 1984), all of which have been implicated in ADHD (Barkley 2010; Martinez et al. 2016; Willcutt et al. 2005).

The Ser-9-Gly polymorphism (rs6280*)* in *DRD3* has been extensively studied. This polymorphism encodes an amino acid substitution at position 9 towards the amino terminal end of the receptor; the *C* allele encodes a glycine, while the *T* allele encodes a serine. In vitro studies have shown that the variant encoded by the *C* allele has a higher affinity for DA, and displays stronger cAMP and MAPK signalling compared to the T allele (Jeanneteau et al. 2006).

Previous studies have investigated the association between *DRD3* and ADHD. Barr et al. conducted family-based analysis in children with ADHD ($n = 100$), investigating the role of rs6280 and another polymorphism located in intron 5, but negative results were obtained (Barr et al. 2000). Despite this initial negative result, several studies have been conducted to test for the association between different polymorphisms in *DRD3* and ADHD (Brookes et al. 2006; Kirley et al. 2002; Muglia et al. 2002; Payton et al. 2001; Wu et al. 2012), but lack of association was reported. Notwithstanding these negative findings, we proposed to investigate the association in a more comprehensive study with a relatively large sample.

Synaptic DA is not only regulated by a large number of synaptic proteins but it is also modulated by environmental factors, such as salient environmental cues (Schultz 2010, 2016), stress (Mizrahi et al. 2012), and pharmacological agents such as methylphenidate (MPH) (Wilens 2008) (the main molecule used for the treatment of ADHD symptoms) and placebo (acting at least partly through DA signalling) (de la Fuente-Fernandez et al. 2006). The study of DA candidate genes in the context of such complexity may lead to inconsistent results if no efforts are directed to control at least part of this complexity. Consequently, we have been recruiting and comprehensively evaluating a large number of children with ADHD for the purpose of identifying genetic variations modulating the risk for ADHD or its phenotypic variability. In view of the importance of DA in modulating attention, motor activity, and emotional regulation, all of these children were invited to participate in a study that examines a range of their motor and cognitive characteristics (aligned with RDoC) while they are not taking any pharmacological agent and subsequently in response to placebo and methylphenidate (0.5 mg/kg per day), given for a 1 week period each according to a randomized crossover design. These three experimental conditions (EC) are theoretically associated with different synaptic levels of Dopamine: baseline (lowest DA levels) (Volkow et al. 2007), placebo (partial increase in DA levels) (de la Fuente-Fernandez 2009; Murray and Stoessl 2013), and MPH (highest increase in DA levels). The primary target behaviours were assessed by parents and teachers using the Conners' Global Index, well-validated scales to assess the child's behaviour. Other secondary measures are also collected (see "Methods" section).

We have used two independent approaches in our genetic analyses. The first is the transmission disequilibrium test encapsulated in the Family-based Association Tests (FBAT), which specifically examines the over-transmission of a risk allele to the affected child. These family-based analyses have the major advantage of controlling for population stratification, an important confounding factor in association studies. In addition, the presence of a significant family-based association is indicative of association in the presence of genetic linkage, which can strengthen the inference of genetic causality. The second approach is based on the comparison of behavioural dimensions between children with different genotypes under different EC. Both approaches were applied to the total sample and subsequently restricted to boys, as sex discrepancy in many ADHD characteristics is significant (with a higher prevalence rate of ADHD in boys) (Polanczyk et al. 2014). In fact, accounting for sex in studying the role of candidate genes in the pathophysiology of ADHD could

make clinical research more rigorous. Our main premise in this work is that convergent evidence from both of these genetic approaches may help to further investigate this gene for association with ADHD.

Methods

Participants

This study is based on a sample of 575 children with ADHD (496 Caucasians), with ages between 6 and 12 (mean = 9.03, SD = 1.81). Children and their parents were interviewed by a child psychiatrist and a structured interview (DISC-IV) was conducted with parents (Shaffer et al. 2000). All children met DSM-IV criteria for ADHD. Children with an IQ less than 70 on the Weschler Intelligence Scale for Children IV (WISC-IV) or with a history of Tourette's syndrome, autism, or psychosis were excluded from the study. The study protocol was approved by the Research Ethics Board of the Douglas Mental Health University Institute (DMHUI), Montreal, Canada. All parents signed an informed consent and all children gave verbal assent to participate.

Study Procedure

This is an ongoing randomized pharmaco-genetic behavioural study (started in 1999) using placebo and MPH as pharmacological probes to dynamically study the genetics of ADHD, rather than a classical trial of response to medication. However, the trial is registered in clinicaltrials.gov, number NCT00483106. All participants were evaluated while they were not taking any medication for at least one week (baseline week). Subsequently, they were given methylphenidate (MPH) at the dose of 0.5 mg/kg/day for 1 week and placebo for another week, both administered twice a day. The treatments were administered in a random order under double-blind crossover conditions and the study design was explained in the same manner to both parents and teachers.

Information from parents and teachers was obtained to ensure a comprehensive assessment of the child's behaviour. Both parents and teachers were asked to evaluate the behaviour of the child at home and in the classroom using the Conner's Global Index scale (Conners et al. 1999) (Conners'-P and Conners'-T, respectively) at baseline, and at the end of the placebo and MPH weeks. Conner's-T is completed at the end of each week on Friday by the teacher at school. The medication is continued on the weekend and parents were asked to complete the Conner's-P evaluation on Sunday. Raw scores of Conner's are transformed into standardized *T* scores, with a population mean of 50. Scores greater than 65 are considered clinically significant. On the third day of each testing week, the children were invited to participate in the Continuous Performance Task (CPT) (CPT; attention, response inhibition, and impulse control) (Conners 1995) before and 1 h after administration of MPH/PBO. This test provides a number of quantitative measures relevant to ADHD, including reaction time variability (RTV). Increased RTV is one of the most consistent findings in children with ADHD (Kofler et al. 2013), and was proposed as a construct within the "cognitive systems" domain of functioning proposed by the RDoC initiative of the National Institute of Mental Health (NIMH) (Garvey et al. 2016) to probe the neurobiology of ADHD. Clinical staff completed the Clinical Global Impression (CGI)-severity and overall improvement scale based on their half day of behavioural observation (Fig. 1). The flow of participants through the study is summarized in Figure S1 (available online).

Masking and Randomization

Methylphenidate and placebo were encapsulated into opaque gelatin capsules in weekly blister packs by a pharmacist not otherwise affiliated with the study. Their order of administration was determined by counterbalanced random assignment.

Genotyping

Peripheral blood/saliva samples were obtained from affected children, their parents, and at least one of the siblings. Genomic DNA was extracted from the lymphocytes with a commercial method (QiAmp DNA Blood Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. *DRD3* rs6280 was amplified using Sequenom (now Agena Bioscience) iPLEX Gold Assay technology at McGill University and Genome Quebec Innovation Center (Quebec, Canada) according to the standard protocol (Reagents). In order to estimate genotyping error, each plate included duplicates of two reference samples. Genotypes for these samples were read with 100% accuracy on each of the plates.

Statistical Analysis

Statistical analyses to test the effect of the *DRD3* genotypes on behavioural outcomes under the three experimental conditions were conducted using ANOVAs where *DRD3* genotypes was the between subject factors and behavioural outcomes under the three experimental conditions (baseline, placebo, and MPH weeks) were the within-subject factors. Chi-square statistics and ANOVAs were used to compare the clinical and demographic characteristics between the three *DRD3* genotype groups, as appropriate. SPSS version 20 was used for all these analyses. The transmission disequilibrium test (TDT) of nuclear families was performed using the Family-Based Association Test [FBAT version 2.0.3 (Horvath et al.

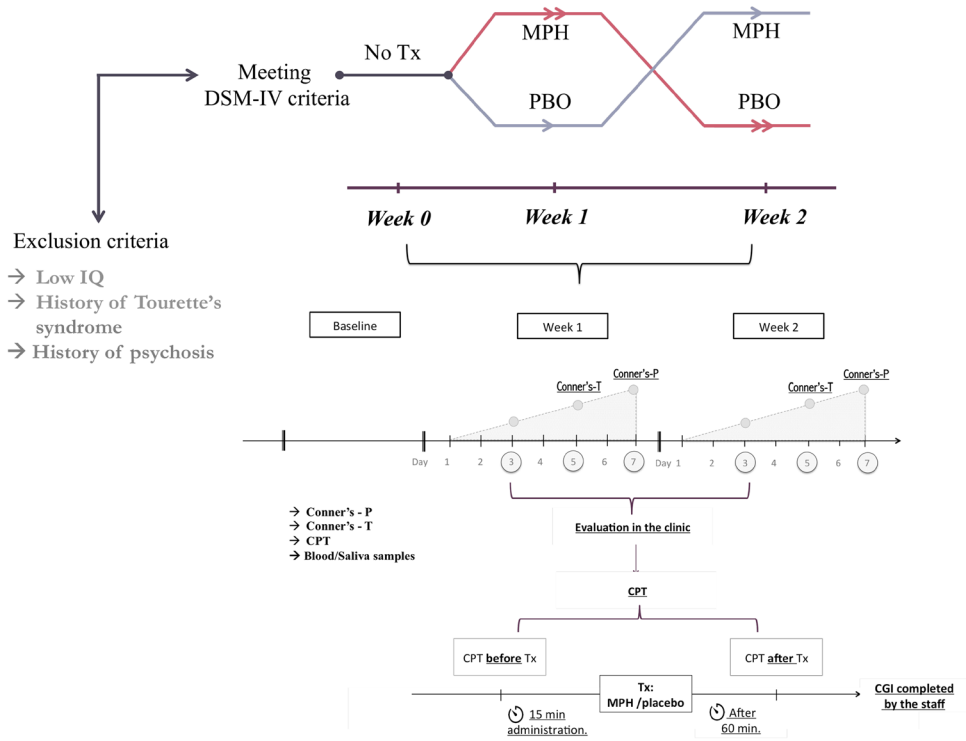

Fig. 1 Timeline of intervention during the study. Treatment order (placebo or MPH) was randomized

2001)]. Tests were first conducted in the total sample, and subsequently restricted to males (the sample restricted to females was too small to allow reliable analyses). Based on the total number of tests performed ($n = 25$), the threshold for statistical significance was set at $p < 0.002$, after Bonferroni correction (0.05/25).

Results

Demographic Characteristics of Participants

Clinical characteristics of the three genotype groups (total sample 575 patients) of children are presented in Table 1. The three genotype groups did not differ with regard to sex, age, ADHD subtypes, and IQ. The *C/C* genotype group was

Table 1 Demographic and baseline characteristics of children with ADHD separated according to their *DRD3* rs6280 genotypes

DRD3 rs6280				
	C/C ($n=69$)	*C/T* ($n=236$)	*T/T* ($n=268$)	Statistic and p value
M/F (% males)	52/17 (75.4%)	174/62 (73.7%)	218/50 (81.3%)	$\chi^2=4.3$, df = 2, $p=0.112$
Ethnicity (% caucasians)	43 (62.3%)	210 (89.3%)	243 (90.7%)	$\chi^2=40.49$, df = 2, $\mathbf{p=0.00}$
Age, years (SD)	9.13 (1.79)	9.12 (1.81)	9.00 (1.72)	$F_{2,572}=.31$, $p=0.74$
Income (%< $30,000 per year)	22 (35.5%)	83 (38.1%)	91 (35.5%)	$\chi^2=0.359$, df = 2, $p=0.835$
Previous treatment (%yes)	40 (58.8%)	141 (62.1%)	170 (65.1%)	$\chi^2=1.1$, df = 2, $p=0.58$
WISQ full-scale IQ	96.94 (13.27)	97.07 (12.6)	96.04 (13.42)	$F_{2,534}=0.396$, $p=0.673$
CBLC total T score	68.8 (8.17)	69.12 (7.8)	67.99 (9.11)	$F_{2,567}=1.14$, $p=0.321$
Total DISC ADHD items	12.66 (3.34)	12.67 (3.59)	12.50 (3.73)	$F_{2,565}=0.154$, $p=0.86$
DISC inattention items	7.12 (1.87)	7.09 (2.12)	7.05 (2.16)	$F_{2,565}=0.032$, $p=0.97$
DISC hyperactivity items	5.54 (2.64)	5.57 (2.62)	5.45 (2.65)	$F_{2,565}=0.15$, $p=0.86$
DISC impulsivity items	2.1 (1.02)	2.09 (.97)	1.99 (.99)	$F_{2,565}=0.74$, $p=0.48$

Significant values are given in bold at $p < 0.05$

M male, *F* female, *Income ≤ $30,000* low income family, *WISC-full scale IQ* Wechsler intelligence scale for children–III, *CBCL* child behavioral checklist, *DISC* diagnostic interview schedule for children. Values are mean (SD), counts or proportions

significantly less represented in non-Caucasians ($p < 0.001$). Thus, we restricted the repeated measures ANOVA, comparing the levels of behaviours under different EC, to Caucasians ($n = 496$).

Pharmaco-Behavioural Genetic Approach

We compared the three *DRD3* rs6280 genotype groups with respect to their behaviours as assessed by teachers and parents under the three EC. These analysis were restricted to Caucasian boys ($n = 327$).

Effect of *DRD3* Ser-9-Gly Polymorphism on Conners' Teacher Scores

Repeated measures analysis of variance revealed a nominal 2-way interaction between *DRD3* genotype and EC ($F_{(3.9,\ 631.9)} = 3.15$; $p = 0.015$). Importantly, this nominal association does not pass the threshold for statistical significance if a stringent Bonferroni correction for multiple tests is applied ($p < 0.002$), and is discussed as a limitation of the study. Since Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated [χ^2 (2) = 8.14, $p = 0.017$], a Greenhouse-Geisser correction was used. Post hoc power analysis, performed using G*Power software at $p < 0.05$, showed that the effect size was 0.014. Power calculation indicated that at $n = 288$, there was a 95% chance of rejecting the null hypothesis. Post hoc analysis showed that the three genotype groups behave similarly at baseline and on placebo week ($p > 0.05$) but show nominally significant differences given treatment with MPH. With MPH, the *C*/*C* genotype display lower scores (indicating greater improvement) compared to the *C*/*T* and *T*/*T* genotypes ($\text{Mean}_{C/C} = 50.79$, SD = 9.04; $\text{Mean}_{C/T} = 55.58$, SD = 10.69; $\text{Mean}_{T/T} = 54.99$, SD = 11.4) (Fig. 2a). Since the *C*/*T* and *T*/*T* genotypes were indistinguishable with regard to their response to MPH, we grouped these two genotypes and tested a dominant model. Carriers of the *T* had worse scores under MPH compared to the *C*/*C* group ($F_{(1.9,\ 633.3)} = 4.91$, $p = 0.008$) (Fig. 2b). Similar analyses were carried out without gender stratification and the results were similar but less significant (data not shown).

Effect of *DRD3* Ser-9-Gly Polymorphism on Conners' Parents Scores

There was no statistically significant interaction ($F_{(3.8,\ 633.3)} = 2.11$, $p = 0.08$) or main effects ($F_{(2,\ 329)} = 0.38$, $p = 0.64$) of *DRD3* according to parents' Conners' scores. However, using the *T* allele dominant model revealed a nominal significant effect ($F_{(1.9,\ 635.8)} = 3.31$, $p = 0.039$). Unlike the observations by teachers, parental Conners' scores were similar at baseline and on MPH, but tended to be statistically different under placebo (Fig. 3b), with carriers of the T allele responding better to placebo.

Family-Based Association Approach

Based on the significant results obtained with the pharmaco-behavioural genetic approach and to further investigate the role of the *T* allele, family-based association tests were conducted. As depicted in Table 2, nominal associations were observed with several behavioural and cognitive dimensions of ADHD in the total sample. Notably, the most significant results were observed when FBAT analysis was restricted to boys, though they do not pass the threshold for statistical significance after Bonferroni correction. In boys, the *T* allele is over-transmitted from parents to affected children,

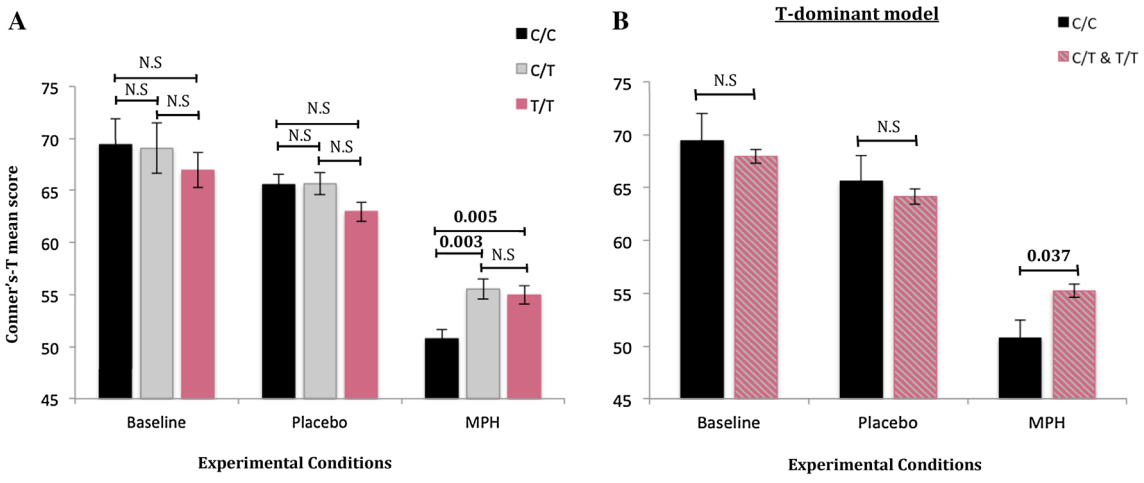

Fig. 2 Conners' Global Index Teachers (Conner's-T ± SEM) scores separated according to child's *DRD3* genotypes at baseline, placebo, and MPH weeks. Error bars represent standard error of the mean

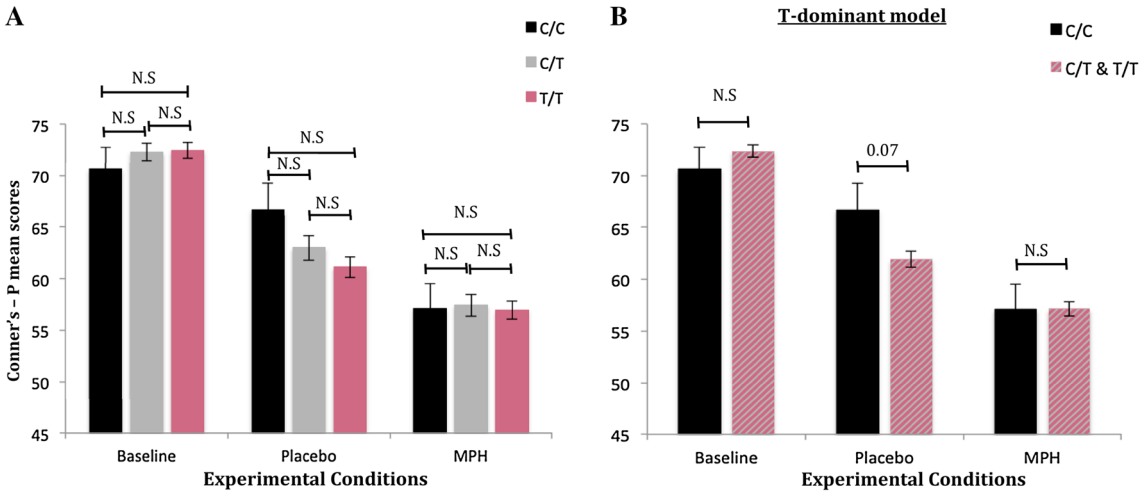

Fig. 3 Conners' Global Index Parents (Conner's-P ± SEM) scores separated according to child's *DRD3* genotypes at baseline, placebo, and MPH weeks. Error bars represent standard error of the mean

suggesting that this allele, or a genetic variant in linkage disequilibrium with this allele, is the risk allele for ADHD ($Z = 2.4$, $p = 0.016$). In the quantitative FBAT analysis, the *T* allele was over-transmitted to children with higher number of total ADHD ($p = 0.027$), hyperactivity ($p = 0.024$), impulsivity ($p = 0.023$), and oppositional defiant disorder items ($p = 0.003$) on the DISC-IV. In terms of cognitive function, nominal association was observed with several behavioural dimensions evaluated in the CPT. In particular, an association was noted with commission errors ($p = 0.015$), hit reaction time (RT) standard error (SE) ($p = 0.023$), variability of SE ($p = 0.011$), and RT by Inter-Stimulus Interval (hit RT ISI) change ($p = 0.025$). The risk allele was over-transmitted to children with worse performance. The risk allele was also associated with a lower score on WISC verbal IQ ($p = 0.03$). The risk allele was also associated with lower CGI overall improvement on placebo ($p = 0.003$) and MPH ($p = 0.002$) as assessed by the staff.

Discussion

Investigating the role of candidate genes in increasing the risk for complex psychiatric disorders has been criticized, due to the plethora of inconsistent results. Several methodological approaches can be implemented to reduce the problems associated with this approach. First, the complexity of psychiatric phenotypes has been considered as one of the sources of inconsistencies and it has been suggested that using behavioural dimensions at various levels of analyses (behavioural, neurocognitive, and neurophysiological) as implemented in the NIMH RDoC initiative may help in facilitating biological discoveries in psychiatric disorders. Second, the small effect of individual genetic variants on behavioural outcomes is now believed to be the rule. Thus, relatively large sample sizes are required to be able to uncover these effects. Third, behavioural assessments are highly sensitive to various environmental factors such as the setting of the observation, the expectations of different observers, and medications that can be used to influence these behaviours. Evaluating behaviours under different conditions, including the use of pharmacological agents that modulate these behaviours under controlled conditions, can also help to reduce the noise in genetic association studies.

Here we present an integrated pharmaco-behavioural genetic approach to investigate candidate genes implicated in ADHD. In this approach, in addition to a comprehensive behavioural and cognitive evaluation, we also probed several of these dimensions using placebo and MPH, each given for a period of 1 week according to a double-blind crossover design. This approach is particularly pertinent for the investigation of dopamine-related genes, given the role placebo and MPH have in modulating synaptic dopamine and its relevant behaviours. By applying this pharmaco-behavioural genetic approach in a sample of 494 patients, we garnered multiple lines of evidence implicating the *DRD3* gene (rs6280) in ADHD and its phenotypic variability. Our approach builds on the *a priori* implication of *DRD3* as a strong candidate gene in ADHD and the convergence of the results from different lines of investigation. First, analysing quantitative behaviours and response of these behaviours to treatment, in relation to rs6280, provide evidence on the role played by *DRD3* in ADHD. The association was observed between rs6280 and behavioural response to MPH as assessed by the CGI-teachers, particularly under a dominant model. Second, a joint analysis using FBAT adds another line of evidence to our results as it revealed an over-transmission of the *T* allele from parents to the affected child, indicating that this locus

Table 2 Association of rs6280 (*T* allele) with ADHD and clinical/behavioural dimensions

Trait	Total sample				Boys			
	Number of families	*Z* statistic	*p* value	Effect size	Number of families	*Z* statistic	*p* value	Effect size
Total number DISC ADHD items	223	1.70	0.08	0.11	169	2.2	0.027	0.17
Number of DISC inattention items	220	1.41	0.15	0.10	167	1.84	0.065	0.14
Number of DISC hyperactivity items	213	1.79	0.07	0.12	161	2.24	0.024	0.18
Number of DISC impulsivity items	204	2.09	0.03	0.15	156	2.73	0.023	0.22
Number of DISC CD items	112	0.64	0.5	0.06	86	0.715	0.47	0.08
Number of DISC ODD items	185	2.31	0.02	0.17	138	2.95	0.003	0.25
CBCL total score								
CGI overall improvement on placebo	205	2.48	0.01	0.17	156	2.88	0.003	0.023
CGI overall improvement on MPH	207	2.69	0.007	0.19	158	3.07	0.002	0.024
CPT								
Omission errors	143	0.281	0.7	0.02	107	2.4	0.6	0.23
Commission errors	170	1.892	0.058	0.15	129	2.40	0.015	0.21
Hit reaction time	170	0.449	0.6	0.03	129	−0.30	0.7	0.03
Hit reaction time standard error	170	2.226	0.025	0.17	129	2.26	0.02	0.20
Variability of standard error	170	2.722	0.006	0.21	129	2.52	0.011	0.22
Detectability	170	1.34	0.17	0.10	129	1.85	0.06	0.16
Response style	170	0.81	0.41	0.06	129	0.97	0.33	0.09
Perseveration	143	1.61	0.10	0.13	107	1.49	0.13	0.14
Hit reaction time block change	170	1.19	0.23	0.09	129	0.71	0.47	0.06
Hit SE block change	170	0.05	0.95	0.00	129	−0.32	0.74	0.03
Hit RT ISI change	170	2.20	0.027	0.17	129	2.23	0.025	0.20
Hit SE ISI change	170	2.56	0.010	0.20	129	1.65	0.98	0.15
IQ								
WISC full-scale IQ	202	−2.08	0.036	0.15	157	−1.7	0.07	0.14
WISC verbal IQ	100	−2.33	0.019	0.23	155	−2.16	0.03	0.17

ADHD ADHD diagnosis, *ADHD items* number of ADHD items as assessed by the Diagnostic Interview Schedule for Children fourth edition (DISC-IV), *Conners'* Conners' Global Index, *CBCL* child behavioural checklist T score. Results passing the threshold for statistical significance ($p < 0.05$) have been highlighted. *CGI* clinical global impression scale overall improvement score, *WISC* Wechsler intelligence scale for children 3rd/4th edition standardized scores, *CPT* continuous performance test *t* scores, *SE* standard error, *RT* reaction time, *ISI* inter-stimulus interval. Effect size (ES) was calculated using the number of informative families and the *Z* statistic. ES of 0.1, 0.3, and 0.5 are considered small, medium, and large, respectively

might be linked to ADHD. Interestingly, the risk allele was associated with higher total number of ADHD, hyperactivity, impulsivity, oppositional defiant disorder items on the DISC-IV, and overall improvement on placebo and MPH as observed by the clinical staff.

It may also be noted that we have identified significant association with a cognitive construct that has been previously suggested to be pertinent for ADHD. Indeed, several studies reviewed by Baroni and Castellanos (Baroni and Castellanos 2015) and a recent meta-analysis concluded that increased RTV is consistently observed in children, adolescents, and adults with ADHD, suggesting that it may be a "stable" feature of ADHD (Kofler et al. 2013), and has been suggested as part of the neurocognitive construct domains of ADHD as conceptualized by the RDoC approach. In this study, we have identified an association between the risk allele and higher *T* scores on CPT measures, indicating a slowing in reaction time to the "target" and "non-target", as well as a loss of consistency, which together suggest a loss of vigilance and inattentiveness (Conners 2000).

Our results are consistent with previous work demonstrating that the *C* allele yields a significantly higher binding affinity for DA and shows a stronger intracellular signalling (Jeanneteau et al. 2006). Therefore, from a biological standpoint, it is possible that children who are carriers of the *T* allele are less sensitive to the effects of dopamine, and are therefore more at risk to ADHD and dysfunction in its associated behavioural dimensions. It is also plausible that these children may have a lower response to MPH treatment. The consistency of these biological observations with our genetic association studies adds important credence to our results.

While the results are consistent with the effect of this polymorphism on ADHD and many of the behavioural dimensions pertinent to ADHD, other behavioural dimensions that we have probed do not show statistically significant association with this gene. Although this could reflect smaller effects which are undetectable given the present sample size, it is also possible that these dimensions are not under the control of this gene, which is expected in such complex phenotypes. The differential association of this gene with Conners' Teachers and Conners' Parents is also an intriguing observation. However, here again it is conceivable that the effects of this gene are different in different environments and according to different observers, illustrating again the complexity of gene–behaviour relationships.

Study's Strengths and Limitations

To the best of our knowledge, this is the first study that comprehensively investigates the role of *DRD3* rs6280 (Ser-9-Gly) polymorphism in children with ADHD. Family-based association analyses are a robust method that are not affected by population stratification and have greater statistical power (Haldar and Ghosh 2011). Moreover, because the non-transmitted parental alleles are the control alleles, this method controls for other possible sources of bias, such as socioeconomic status. Our study has almost double the sample size compared to previous family-based association studies. One other major strength of our study is its pharmaco-genetic approach. To our knowledge, this study is one of the largest using the double-blind, placebo-controlled, crossover design for evaluation of behavioural response to MPH and placebo in children with ADHD. Nevertheless, sample size is relatively limited, and the positive association results must be taken with caution, particularly since false-positive findings are rampant in association studies.

Analysing behaviours dynamically by using active medication or placebo is a major strength of this study as it may increases the variance between subjects, thus improving our capacity to identify gene effects. Finally, one other major strength of this study is the detailed phenotyping of children with respect to behaviour (at home, school, and clinic) and cognitive dimensions aligned with RDoC constructs, as well as short-term response to placebo and MPH.

Several limitations to this study need to be acknowledged. The major limitation is the exploratory nature of this study. If a stringent Bonferroni correction for total number of tests performed was to be applied, none of the nominal associations reported here would be significant. Second, although we garnered evidence of the implication of the *DRD3* gene in ADHD using a convergent approach, a confirmation sample was not available. Therefore, increasing the sample size and replicating these results in independent research groups is important to establish their generalizability. It is also important to genotype multiple other polymorphisms in this gene to better understand the genetic determinant of these observations. Notwithstanding these limitations, results have been presented as preliminary findings as they may provide insight into aetiology of ADHD. Further work is required to confirm these associations in large samples with deep phenotyping before firm conclusions can be reached.

Conclusion

This study demonstrates, for the first time, that the functional polymorphism in the DRD3 gene might play a role in childhood ADHD. The results of this study strongly suggest that children with the *T/T* genotype may be characterized with differential profiles of response to MPH in the context of a short-term clinical trial. However, confirmation of these findings is awaited in independent studies with different ethnic populations. These results may have important clinical and pharmacological implications for the treatment of ADHD, and may help in the design of better interventions that are tailored for the specific needs of each of these groups of patients.

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Author Contributions RJ and NG designed the study and wrote the protocol. WF and SS performed the statistical analyses. WF and RJ performed the literature search and wrote the manuscript. AL provided expert statistical review. All authors contributed to and have approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest Dr. S.M. Sengupta and Weam Fageera report no biomedical financial interests or potential conflicts of interest. Dr. Aurelie Labbe receives research support from the Natural Sciences and Engineering Research Council of Canada and from the Canadian Institute for Health Research (CIHR). Dr. Natalie Grizenko reports receiving research funding from CIHR and is a member of the advisory board for Purdue and Shire. Dr. Ridha Joober reports having received research funding from CIHR. He is on the advisory boards and speakers' bureau of Pfizer, Janssen Ortho, BMS, Sunovion, Otsuka, Lundbeck, Perdue, and Myelin. He has received grant funding from them and from AstraZeneca and HLS. He has received honoraria from Janssen Canada, Shire, Lundbeck, Otsuka, Pfizer and from Perdue for CME presentations and royalties for Henry Stewart talks.

References

Accili, D., Fishburn, C. S., Drago, J., Steiner, H., Lachowicz, J. E., Park, B. H., … Fuchs, S. (1996). A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice.

Proceedings of the National Academy of Sciences of the United States of America, 93(5), 1945–1949.

Barkley, R. A. (2010). Differential diagnosis of adults with ADHD: The role of executive function and self-regulation. *The Journal of Clinical Psychiatry, 71*(7), e17. https://doi.org/10.4088/JCP.9066tx1c.

Baroni, A., & Castellanos, F. X. (2015). Neuroanatomic and cognitive abnormalities in attention-deficit/hyperactivity disorder in the era of 'high definition' neuroimaging. *Current Opinion in Neurology, 30*, 1–8. https://doi.org/10.1016/j.conb.2014.08.005.

Barr, C. L., Wigg, K. G., Wu, J., Zai, C., Bloom, S., Tannock, R., … Kennedy, J. L. (2000). Linkage study of two polymorphisms at the dopamine D3 receptor gene and attention-deficit hyperactivity disorder. *American Journal of Medical Genetics, 96*(1), 114–117.

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: Rewarding, aversive, and alerting. *Neuron, 68*(5), 815–834. https://doi.org/10.1016/j.neuron.2010.11.022.

Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N., … Asherson, P. (2006). The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: Association signals in DRD4, DAT1 and 16 other genes. *Molecular Psychiatry, 11*(10), 934–953. https://doi.org/10.1038/sj.mp.4001869.

Conners, C. K. (1995). *Conners' continuous performance test computer program*. Toronto: Multi-Health Systems Incorporated (MHS).

Conners, K. (2000). *Conners' continuous performance test II (CPT II V.5)*. North Tonawanda: Multi-Health Systems Incorporated (MHS).

Conners, C. K., Erhardt, D., & Sparrow, E. (1999). *Conner's adult ADHD Rating scales: Technical manual*. North Tonawanda: Multi-Health Systems Incorporated (MHS).

Daly, S. A., & Waddington, J. L. (1993). Behavioural effects of the putative D-3 dopamine receptor agonist 7-OH-DPAT in relation to other "D-2-like" agonists. *Neuropharmacology, 32*(5), 509–510.

de la Fuente-Fernandez, R. (2009). The placebo-reward hypothesis: Dopamine and the placebo effect. *Parkinsonism & Related Disorders, 15*(3), 72–74. https://doi.org/10.1016/s1353-8020(09)70785-0.

de la Fuente-Fernandez, R., Lidstone, S., & Stoessl, A. J. (2006). Placebo effect and dopamine release. *Journal of Neural Transmission, 70*, 415–418.

Franke, B., Faraone, S. V., Asherson, P., Buitelaar, J., Bau, C. H., & Ramos-Quiroga, J. A., …, International Multicentre Persistent, A. C. (2012). The genetics of attention deficit/hyperactivity disorder in adults, a review. *Molecular Psychiatry, 17*(10), 960–987. https://doi.org/10.1038/mp.2011.138.

Garvey, M., Avenevoli, S., & Anderson, K. (2016). The national institute of mental health research domain criteria and clinical research in child and adolescent psychiatry. *Journal of the American Academy of Child and Adolescent Psychiatry, 55*(2), 93–98. https://doi.org/10.1016/j.jaac.2015.11.002.

Haldar, T., & Ghosh, S. (2011). Power comparison between population-based case-control studies and family-based transmission-disequilibrium tests: An empirical study. *Indian Journal of Human Genetics, 17*(Suppl 1), 27–31. https://doi.org/10.4103/0971-6866.80355.

Horvath, S., Xu, X., & Laird, N. M. (2001). The family based association test method: Strategies for studying general genotype–phenotype associations. *European Journal of Human Genetics, 9*(4), 301–306. https://doi.org/10.1038/sj.ejhg.5200625.

Iacono, W. G. (2016). Achieving success with the research domain criteria (RDoC): Going beyond the matrix. *Psychophysiology, 53*(3), 308–311. https://doi.org/10.1111/psyp.12584.

Jeanneteau, F., Funalot, B., Jankovic, J., Deng, H., Lagarde, J. P., Lucotte, G., & Sokoloff, P. (2006). A functional variant of the dopamine D3 receptor is associated with risk and age-at-onset of essential tremor. *Proceedings of the National Academy of Sciences of the United States of America, 103*(28), 10753–10758. https://doi.org/10.1073/pnas.0508189103.

Joshua, M., Adler, A., & Bergman, H. (2009). The dynamics of dopamine in control of motor behavior. *Current Opinion in Neurology, 19*(6), 615–620. https://doi.org/10.1016/j.conb.2009.10.001.

Kirley, A., Hawi, Z., Daly, G., McCarron, M., Mullins, C., Millar, N.,... . Gill, M. (2002). Dopaminergic system genes in ADHD: Toward a biological hypothesis. *Neuropsychopharmacology, 27*(4), 607–619. https://doi.org/10.1016/S0893-133X(02)00315-9.

Kofler, M. J., Rapport, M. D., Sarver, D. E., Raiker, J. S., Orban, S. A., Friedman, L. M., & Kolomeyer, E. G. (2013). Reaction time variability in ADHD: A meta-analytic review of 319 studies. *Clinical Psychology Review, 33*(6), 795–811. https://doi.org/10.1016/j.cpr.2013.06.001.

Martinez, L., Prada, E., Satler, C., Tavares, M. C., & Tomaz, C. (2016). Executive dysfunctions: The role in attention deficit hyperactivity and post-traumatic stress neuropsychiatric disorders. *Frontiers in Psychology, 7*, 1230. https://doi.org/10.3389/fpsyg.2016.01230.

Mizrahi, R., Addington, J., Rusjan, P. M., Suridjan, I., Ng, A., Boileau, I., … Wilson, A. A. (2012). Increased stress-induced dopamine release in psychosis. *Biological Psychiatry, 71*(6), 561–567. https://doi.org/10.1016/j.biopsych.2011.10.009.

Muglia, P., Jain, U., & Kennedy, J. L. (2002). A transmission disequilibrium test of the Ser9/Gly dopamine D3 receptor gene polymorphism in adult attention-deficit hyperactivity disorder. *Behavioural Brain Research, 130*(1–2), 91–95.

Murray, D., & Stoessl, A. J. (2013). Mechanisms and therapeutic implications of the placebo effect in neurological and psychiatric conditions. *Pharmacology & Therapeutics, 140*(3), 306–318.

Nieoullon, A. (2002). Dopamine and the regulation of cognition and attention. *Progress in Neurobiology, 67*(1), 53–83.

Payton, A., Holmes, J., Barrett, J. H., Hever, T., Fitzpatrick, H., Trumper, A. L., … Thapar, A. (2001). Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: A family-based study. *American Journal of Medical Genetics, 105*(5), 464–470.

Polanczyk, G. V., Willcutt, E. G., Salum, G. A., Kieling, C., & Rohde, L. A. (2014). ADHD prevalence estimates across three decades: An updated systematic review and meta-regression analysis. *International Journal of Epidemiology, 43*(2), 434–442. https://doi.org/10.1093/ije/dyt261.

Reagents, i. SNP Genotyping with iPLEX Reagents and the Massarray® System.

Schultz, W. (2010). Dopamine signals for reward value and risk: Basic and recent data. *Behavioral and Brain Functions, 6*, 24. https://doi.org/10.1186/1744-9081-6-24.

Schultz, W. (2016). Dopamine reward prediction-error signalling: A two-component response. *Nature Reviews Neuroscience, 17*(3), 183–195. https://doi.org/10.1038/nrn.2015.26.

Shaffer, D., Fisher, P., Lucas, C. P., Dulcan, M. K., & Schwab-Stone, M. E. (2000). NIMH diagnostic interview schedule for children version IV (NIMH DISC-IV): Description, differences from previous versions, and reliability of some common diagnoses. *Journal of the American Academy of Child and Adolescent Psychiatry, 39*(1), 28–38. https://doi.org/10.1097/00004583-200001000-00014.

Svensson, K., Carlsson, A., & Waters, N. (1994). Locomotor inhibition by the D3 ligand R-(+)-7-OH-DPAT is independent of changes in dopamine release. *Journal of Neural Transmission/General Section, 95*(1), 71–74.

Volkow, N. D., Wang, G. J., Newcorn, J., Telang, F., Solanto, M. V., Fowler, J. S., … Swanson, J. M. (2007). Depressed dopamine activity in caudate and preliminary evidence of limbic involvement in adults with attention-deficit/hyperactivity disorder.

Archives of General Psychiatry, 64(8), 932–940. https://doi.org/10.1001/archpsyc.64.8.932.

Wilens, T. E. (2008). Effects of methylphenidate on the catecholaminergic system in attention-deficit/hyperactivity disorder. *Journal of Clinical Psychopharmacology, 28*(3 Suppl 2), S46–S53. https://doi.org/10.1097/JCP.0b013e318173312f.

Willcutt, E. G. (2012). The prevalence of DSM-IV attention-deficit/hyperactivity disorder: A meta-analytic review. *Neurotherapeutics, 9*(3), 490–499. https://doi.org/10.1007/s13311-012-0135-8.

Willcutt, E. G., Doyle, A. E., Nigg, J. T., Faraone, S. V., & Pennington, B. F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: A meta-analytic review. *Biological Psychiatry, 57*(11), 1336–1346. https://doi.org/10.1016/j.biopsych.2005.02.006.

Wise, R. A., & Bozarth, M. A. (1984). Brain reward circuitry: Four circuit elements "wired" in apparent series. *Brain Research Bulletin, 12*(2), 203–208.

Wu, J., Xiao, H., Sun, H., Zou, L., & Zhu, L. Q. (2012). Role of dopamine receptors in ADHD: A systematic meta-analysis. *Molecular Neurobiology, 45*(3), 605–620. https://doi.org/10.1007/s12035-012-8278-5.