Research in Developmental Disabilities 92 (2019) 103430

Contents lists available at ScienceDirect

Research in Developmental Disabilities

journal homepage: www.elsevier.com/locate/redevdis

Dopamine transporter genotype modulates brain activity during a working memory task in children with ADHD

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ARTICLE INFO

Number of completed reviews is 2

Keywords:
Attention deficit/hyperactivity disorder (ADHD)
fMRI
Working memory
DAT1 VNTR polymorphism
Caudate nucleus
Cerebellum

ABSTRACT

Dopamine active transporter gene (DAT1) is a candidate gene associated with attention-deficit/hyperactivity disorder (ADHD). The DAT1 variable number tandem repeat (VNTR)-3' polymorphism is functional and 9R carriers have been shown to produce more DAT than 10R homozygotes. We used functional magnetic resonance imaging (fMRI) to investigate the effects of this polymorphism on the neural substrates of working memory (WM) in a small but selected population of children with ADHD, naïve of any psychotropic treatment and without co-morbidity. MRI and genotype data were obtained for 36 children (mean age: 10,36 +/− 1,49 years) with combined-type ADHD (9R n = 15) and 25 typically developing children (TDC) (mean age: 9,55 +/− 1,25 years) (9R n = 12). WM performance was similar between conditions. We found a cross-over interaction effect between gene (9R vs. 10R) and diagnosis (TDC vs. ADHD) in the orbito-frontal gyrus, cerebellum and inferior temporal lobe. In these areas, WM-related activity was higher for 9R carriers in ADHD subjects and lower in TDC. In ADHD children only, 10R homozygotes exhibited higher WM-related activity than 9R carriers in a network encompassing the parietal and the temporal lobes, the ventral visual cortex, the orbito-frontal gyrus and the head of the caudate nucleus. There was no significant results in TDC group. Our preliminary findings suggest that DAT1 VNTR polymorphism can modulate WM-related brain activity ADHD children.

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https://doi.org/10.1016/j.ridd.2019.103430
Received 24 May 2018; Received in revised form 4 June 2019; Accepted 23 June 2019
Available online 12 July 2019

What this paper adds

We discuss working-memory (WM) related brain activation depending on DAT1 VNTR, comparing 9R carriers and 10R homozygotes in ADHD and TDC. It's the first article to study such effect in ADHD on a WM task. The main interests of this exploratory study are the medication-naïve subjects, the different WM-related activation pattern depending on brain localisation that highlight complex compensatory mechanisms and endophenotypic heterogeneity in ADHD subjects.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental disorder in children and adolescents, with a worldwide prevalence rate between 5.3 and 7.1% (Polanczyk & Rohde, 2007). Its features include extreme levels of motor activity, impulsivity and inattention. Individuals with ADHD may present with predominantly inattentive or hyperactive symptoms or, more commonly, a combination of both (ADHD-combined type). These symptoms may persist into adulthood in about 30–60% of cases and they are associated with lowered academic functioning, an increased risk for drug abuse and negative consequences for family and peer relations (Wilens et al., 2011).

ADHD is thought to have a complex genetic determinism with many small effect size associated genes that interact with the environment (Hawi et al., 2015). Most of the candidate genes associated with ADHD (*DAT1, DRD4, DRD5, 5HTT, HTR1B, et SNAP25*) (Gizer, Ficks, & Waldman, 2009) belong to the dopaminergic system, but only explain a small part of phenotypic variance (Smith, Mick, & Faraone, 2009). DAT1, which codes for the dopamine transporter (DAT), the primary protein responsible for clearing dopamine from the synaptic space, is one of the most extensively studied candidate genes in ADHD. DAT activity varies considerably in different brain regions, but is more pronounced in the nucleus accumbens and dorsal striatum and within the striatum, density gradients are detected from superior to inferior, medial to lateral and anterior to posterior regions, particularly in the caudate nucleus (Jucaite, Fernell, Halldin, Forssberg, & Farde, 2005; Kaufman & Madras, 1992). DAT1 is of particular interest to ADHD because DAT is the principal target of methylphenidate, the most effective pharmacological treatments for the disorder (Madras, Miller, & Fischman, 2005). Besides, the DAT1 knockout mouse is an animal model that seems to have a high validity for ADHD (Kooij et al., 2008). DAT1 contains 15 exons, and is located on chromosome 5p15.3. It has a polymorphic variable number tandem repeat (VNTR) which is a repeated length of 40 bp located in the 3′ untranslated transcribed region (UTR) of exon 15. The 10-repeat (10R) and nine-repeat (9R) alleles of this VNTR are the most frequently occurring (Vandenbergh et al., 1992). Although several studies have reported a significant association between the DAT1 3′ UTR 10R allele and ADHD (Cook et al., 1995; Faraone et al., 2005; Yang et al., 2007), others have suggested that it may be the 9R allele that is preferentially transmitted in individuals with ADHD (Franke et al., 2008) and a meta-analysis found no evidence of association with ADHD and the DAT1 VNTR polymorphism (Li, Sham, Owen, & He, 2006). The functional effect of DAT1 polymorphism was also unclear. Many studies suggested that 10R homozygotes produced more DAT than 9R (Brookes et al., 2007; Brown et al., 2010; Cheon, Ryu, Kim, & Cho, 2005), while others suggested the opposite (Giessen et al., 2009; van Dyck et al., 2005). However, a recent meta-analysis of positron emission tomography (PET) studies and single photon emission tomography (SPECT) suggested that 9R carriers produce more DAT than 10R in the striatum, independently of any psychiatric disease (Faraone, Spencer, Madras, Zhang-James, & Biederman, 2014). Plus, in the PET studies analyses, the affected group were only ADHD patients and the results were consistent with the 9R allele predicting greater DAT binding in human. They also argued that the heterogeneous results of previous studies were due to important differences in study methodology, age and medication status. It suggests that 9R carriers who produce more DAT could have a decrease in dopaminergic transmission that has been linked to ADHD (Volkow et al., 2007), because of the higher dopamine clearance in the synaptic space.

At the cognitive level, different models have been proposed to explain the symptoms. Alteration of executive functions has been the most described and studied model in ADHD (Barkley, 1997) and some coherent results have been found in several functional magnetic resonance imaging (fMRI) studies. They show that neuronal processes differ between typically developing children (TDC) and ADHD in attention, inhibition and working memory tasks (Hart, Radua, Nakao, Mataix-Cols, & Rubia, 2013), even if this is neither necessary nor sufficient to explain all the symptoms (Nigg, Willcutt, Doyle, & Sonuga-Barke, 2005; Thissen et al., 2014). In this model, working memory (WM), attention and inhibition are the most altered executive functions, and WM can be used as an endophenotype for ADHD (Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). Noticeably, a positive correlation was found between dopaminergic function measured by TEP in the striatum, fMRI activation and WM load in healthy subjects (Landau, Lal, O'Neil, Baker, & Jagust, 2009). Neurodevelopmental studies suggest that the WM neuronal process differs between children and adults. In children, it can involve additional areas such as the premotor cortex, the parietal lobe, the insula, the striatum and the cerebellum (Ciesielski, Lesnik, Savoy, Grant, & Ahlfors, 2006).

Few fMRI studies have investigated executive functions-related effects of DAT1 polymorphism in children with ADHD. Two studies have found effects on brain regions previously implicated in ADHD, including the striatum during a task of motor inhibition (Bedard et al., 2010; Durston et al., 2008a). They found opposite results concerning DAT1 polymorphism related brain activation in the striatum despite the use of the same Go/No-Go task. Another fund hypo-activation for a 10R-homozygotes ADHD adult group compared to a 9R-carrier ADHD adult group in the dorsal anterior cingulate cortex and the prefrontal cortex, in an interference task (Brown et al., 2010). Studies conducted in the resting state reported a trend for significance for a relation between DAT1 VNTR and increased task-related suppression in the default mode network (DMN) in adults with ADHD 9R-carriers as compared to 10R-homozygotes (Ariel Beth Brown et al., 2011). In children with ADHD, decreased anti-correlation and altered functional connectivity were found in the DMN (Broyd et al., 2009; Sun et al., 2012). However, results in these studies might be biased by differences in developmental stage, presence of comorbidities, and in particular the medication status. As for the latter, recent studies suggested that methylphenidate (MPH) may exert both neurofunctional and structural long-term effects (Hart et al., 2013), as well as long term effects on striatal dopamine transporter (Fusar-Poli, Rubia, Rossi, Sartori, & Balottin, 2012). To the best of our knowledge, no study investigated yet the effects of DAT1 VNTR on brain activity during working memory in non-medicated children with ADHD.

In this study, we hypothesized that DAT1 VNTR polymorphism modulates working memory-related brain activity in a selected population of never-treated children with ADHD, regardless of intelligence quotient (IQ), gender, economic status and WM performance. Assuming the decreased dopaminergic transmission in 9R carriers, we hypothesized hypo/underactivity in brain regions are usually activated in WM in children, including the parietal lobe, the striatum and the cerebellum (Ciesielski et al., 2006; Owen, McMillan, Laird, & Bullmore, 2005). We also examined the caudate as a region of interest (ROI) since it's a place of high DAT concentration (Jucaite et al., 2005; Kaufman & Madras, 1992) and that it has been more recently shown to be involved in WM tasks (Moore, Li, Tyner, Hu, & Crosson, 2013; Murty et al., 2011; Vance et al., 2007).

2. Material and methods

2.1. Participants

Sixty-one children aged 7,8 to 12,9 years participated in this study. Thirty-six children fulfilling DSM-IV-R criteria for ADHD combined type (mean age 10,36 +/− 1,49 years) were recruited from the neuropediatric outpatient clinic in Erasme Hospital, Université Libre de Bruxelles (ULB), Belgium. Twenty-five typically developing children (TDC) (mean age 9,55 +/− 1,25 years) were recruited from local schools in Brussels or via personal requests to professionals working at Erasme Hospital. Parental socio-economic status (SES) was assigned to one of three categories (unskilled/qualified worker, clerk/ commercial occupation, and graduate occupation) by considering the profession of the most qualified parent (Sterzer, Stadler, Poustka, & Kleinschmidt, 2007). IQ estimates were obtained with the age-appropriate Wechsler Abbreviated Scale of Intelligence (WASI). Diagnosis for ADHD was based on clinical features including typical history and behavioral report. The Kiddie Schedule for Affective Disorders and Schizophrenia for School Aged Children-Present and Lifetime Version (K-SADS-PL) (Endicott & Spitzer, 1978) was completed at screening for each participant (children with ADHD and TDC) to establish the diagnosis according to DSM-IV-R criteria and to ensure that children presented no psychiatric condition. Severity of symptoms was measured using the ADHD rating scale parent form (Pappas, 2006).

Exclusion criteria for all children were presence of a psychiatric condition other than ADHD, presence of learning disability, history of prematurity, current or past medical or neurological disorder, current or past psychotropic drug, contraindication to MRI, and IQ estimate under 85. Each child and her/his parents gave their written consent to participate in this study approved by the Ethics Committee of the ULB (reference: P2007/332 / B40620072950) and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. For comparisons between groups, Student T-tests were performed for age, IQ and WM performance, and χ^2 tests were performed for gender and SES.

2.2. Genotyping

Saliva samples were collected using Oragene DNA sample collection kits OG-500 (DNA Genotek), and DNA was extracted according to the manufacturer's protocol (http://www.dnagenotek.com). The VNTR polymorphic region 3' of DAT1 was amplified using the sense primer: 5'-TGTGGTGTAGGGAACGGCCTGAGA3' and the antisense primer: 5'-TGTTGGTCTGCAGGCTGCCTGCAT3'. The polymerase chain reaction (PCR) amplification under standard conditions was performed in a final volume of 15 µL with 50 µM of deoxyribonucleotides, 50 pmol of sense and antisense primers, 1X buffer, 1.5 mM MgCl2 and 5 units of Invitrogen Taq polymerase. Each tube contained 30 ng of genomic DNA. They were placed in the thermal cycler and the PCR was performed according to the standard program: denaturation: 95 °C for 7 min, then 40 cycles: 75 s at 95 °C, 90 s at 63 °C, 35 s at 72 °C and then final extension: 72 °C for 7 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel with ethidium bromide DNA revelation. Based on these results, 4 groups have been made. Among 36 ADHD children, 15 were 9R carriers (mean age 10,57 +/−1,64) and 21 were 10R homozygotes (mean age 10,22 +/−1,4). Among 25 TDC, 13 were 9R carriers (mean age 9,72 +/−1,09) and 12 were 10R homozygotes (mean age 9,32 +/−1,47).

2.3. Working memory task

WM performance and underlying cerebral activity were measured using a verbal N-back task with two different conditions as described in Massat et al. (Massat et al., 2012) see also (Gevins & Smith, 2000) (Owen et al., 2005). In both conditions, stimuli were black numbers (Arial font, size 74) displayed on a white background on the center of the screen, successively presented in pseudo-random order. In the vigilant/ control 0-back (N0) condition, subjects had merely to press a button with the right hand whenever the number "2" was displayed. In the WM 2-back (N2) condition, subjects had to press the button when the displayed number was identical to the number displayed two trials before. During the fMRI session, subjects were administered 5 blocks in the N0 condition alternated with 5 blocks in the N2 condition. Each block consisted of a sequence of 30 trials (including 10 targets) each displayed for 1750 ms with an interstimulus interval of 250 ms. Each block was followed by a resting period of random duration ranging between 11 and 16 s, during which the instruction relative to the forthcoming condition was displayed (i.e. either "number 2" [N0] or "same than two numbers before" [N2]). A fixation cross replaced the instruction 2.5 s before the start of a novel series of 30 numbers. Corrected accuracy scores (hits - false detections/2) were obtained in the N2 and N0 conditions. Before scanning, all participants performed the whole task once outside the MRI environment to ensure a clear understanding of the instructions. During the fMRI session, stimuli were projected on a translucent screen that was seen via a mirror fixed to the head coil and located in front of the subject, and responses were made with the right hand on a commercially available MRI compatible keypad system (fORP; Current Design, Vancouver) connected to a PC. The timing of MR images acquisition and stimuli presentation was synchronized using the clock signal of the MRI scanner. Head stabilization was achieved using a head-restraining foam and MR scanner noise was attenuated using foam earplugs and headphones.

2.4. Image acquisition

Participants were scanned using a 3-Tesla Philips Achieva MRI scanner (Philips Healthcare, Best, The Netherlands) with an 8 channel SENSE head coil. A high-resolution, 3D T1-weighted structural scan was acquired using a sagittal TFE sequence with the following parameters: 160 slices; TR =1960 ms; TE = 4,60 ms; TI =1040 ms; flip angle = 8°; field of view = 250 mm x 250 mm; matrix size = 320 × 320 × 160; reconstruction interpolated voxel size = 0,78 × 0,78 × 1,0 mm. Functional data were acquired using a T2* sensitive gradient echo (EPI) sequence (TR = 2130 ms, TE =40 ms, FA 90°, SENSE acceleration factor 2,5, matrix size 64 × 64 × 32; voxel size: 3,06 × 3,06 × 3 mm3). Thirty-two contiguous transverse slices were acquired, covering the whole brain. The MR scanner was equipped with the Quasar imaging gradients (maximum amplitude and slew rate: 30 m T/m and 200 m T/m/ms) and an 8-channel SENSE head coil.

2.5. Image processing

Functional MRI data were pre-processed and analyzed using the Statistical Parametric Mapping software SPM8b (Wellcome Department of Cognitive Neurology, London) implemented in MATLAB 7.8 (Mathworks Inc., Sherbom, MA). The first five functional volumes in the acquisition were discarded to avoid transient spin saturation effects. Preprocessing included realignment and adjustment for movement related effects, co-registration of functional and anatomical data, spatial normalization into standard stereotactic MNI space and spatial smoothing using a Gaussian kernel of 8 mm full width at half maximum (FWHM). 9 subjects showing excessive scan-to-scan head motion (4 mm) were excluded from the analyses. This 4 mm threshold can seems a bit high in pediatrics ADHD population that tends to move during the task. We chose it because of the low sample size. Even if some authors used a 3 mm threshold (Brown et al., 2010), or a 4 mm threshold (Bedard et al., 2010) in similar studies, larger studies should/could decrease this movement threshold. The magnitude of individual head motion at each time point for translation and rotation parameters was computed to enter as confound covariate in statistical analyses (see above). For our groups, mean head motion was calculated and it was not different between children with ADHD and TDC (*ps* > 0,8).

2.6. Brain imaging data analyses

Data were analyzed using a mixed-effects model aimed at showing a stereotypical effect in the population from which the subjects were drawn (Penny, Holmes, & Friston, 2003). For each subject, a first-level intra- individual analysis aimed at modelling data to partition observed neurophysiological responses into components of interest, confounds and error, using a general linear model (Friston, 2003). The regressors of interest were built using box cars positioned at each block (N2 and N0) presentation. These regressors were secondarily convolved with the canonical hemodynamic response function. Movement parameters derived from realignment of the functional volumes (translations in x, y and z directions and rotations around x, y and z axes) were included as covariates of no interest in the design matrix. High-pass filtering was implemented in the matrix design using a cut-off period of 256 s to remove low drift frequencies from the time series. Serial correlations were estimated with a restricted maximum likelihood (ReML) algorithm using an intrinsic first order autoregressive model during parameter estimation. Effects of interest were then tested by linear contrasts, generating statistical parametric maps [SPM(T)]. Here, the contrast of interest was the difference of activation between N2 and N0 conditions (N2 vs. N0) as the best approximation of WM-related neural activity. Summary statistic images were then further spatially smoothed (6 mm FWHM Gaussian kernel) and entered in a second-level analysis in which subjects were treated as a random effect (RFX). We used a dual smoothing approach to reduce the inter-individual variability at the preprocessing level, as ADHD are generally subjects that tends to move during image acquisition and then to accommodate inter-subject variability in group analyses (Mikl et al., 2008; Rowe et al., 2002).

One-sample t tests were used to assess the N2 vs. N0 contrast in the 9R carriers and 10R homozygotes group separately in both ADHD and control subjects. A conjunction null analysis identified the brain areas commonly activated in the 9R carriers and 10R homozygotes in ADHD in contrasts of interest (Friston, Penny, & Glaser, 2005). To compare the N2 vs. N0 contrast between the 9R carriers and 10R homozygotes group in both ADHD and TDC subjects we ran a 2 × 2 ANOVA with diagnosis and genotype as fixed factors. Restricted maximum likelihood estimates of variance components were used to allow possible departure from the sphericity assumptions in RFX conjunction analyses (Penny, Holmes, & Friston, 2003).

Percentage changes in blood-oxygen-level-dependant (BOLD) signal from baseline levels in N2 and N0 conditions at specific coordinates was obtained using RFX Plot software (Gläscher, 2009) both for the 9R carriers and 10R homozygotes groups, in TDC and ADHD children.

In all the analyses presented in this study, the resulting set of voxel values for each contrast constituted a map of the t statistic [SPM(T)], thresholded at p < 0001 (uncorrected for multiple comparisons). Statistical inferences were then obtained after corrections at the voxel level using Gaussian random field theory (Worsley et al., 1996). For conjunction analysis, correction level was $p_{FWE} < 0,05$ after correction for multiple comparison in whole brain volume. For comparisons between 9R carriers and 10R homozygotes, correction level was pcorr < 0,001 uncorrected for multiple comparisons in the whole brain volume with a minimal cluster size of 20 voxels. This threshold was chosen according to (Ramani, 2019; Woo, Krishnan, & Wager, 2014), because of the low sample size in each group, the small effect size attempted for this genetic risk variant, and the exploratory nature of this study. For ROI analysis, correction level was psvc < 0,05 corrected in a small spherical volume (radius 6–10 mm). As mentioned in the introduction, ROI were taken from previous fMRI studies examining the DAT1 genotype effect in N-back task in children and adults with ADHD (Moore et al., 2013; Murty et al., 2011; Vance et al., 2007).

3. Results

3.1. Sample characteristics and behavioral performance

The distributions of genotypes of both ADHD and TDC samples were under Hardy-Weinberg equilibrium. Analyses revealed no significant differences between children with ADHD and TDC for age, gender and IQ (Table 1). In the ADHD group (Table 2) and in the TDC group (Table 3) there was also no difference between the 9R carriers and the 10R homozygous groups for age, gender and IQ. For the parental SES, there was no difference between the ADHD subjects and the TDC (χ^2 = 0,897, ddl = 2, p = 0,63). There was also no difference between 9R carriers and 10R homozygotes in the ADHD group (χ^2 = 1,49, ddl = 2, p = 0,56) and in the TDC group (χ^2 = 1,37, ddl = 2, p = 0,49). WM performance reflecting the updating process (UP = N0–N2 corrected accuracy scores) was similar between 9R carriers and 10R homozygotes in the ADHD group (Table 2) and TDC group (Table 3).

Table 1
Characteristics of participants.

Total participants	ADHD (n = 36)	TDC (n = 25)	*p value*
Age (sd)	10,36 (1,49)	9,55 (1,25)	0,34
Performance (sd)	8,18 (9,1)	6,37 (3,9)	0,377
IQ (sd)	106,7 (9,4)	110,5 (7,8)	0,115
Gender	Male : 58%	Male 60%	0.86 [a]

Table 2
Characteristics of ADHD participants based on DAT VNTR genotype.

ADHD	9R (n = 15)	10R (n = 21)	*p value*
Age (sd)	10,57 (1,64)	10,22 (1,4)	0,51
Performance (sd)	7,17 (8,44)	8,90 (9,8)	0,58
IQ (sd)	109,2 (9,1)	105 (9,4)	0,207
Gender	Male 60%	Male 58%	0.86 [a]

Table 3
Characteristics of TDC based on DAT VNTR genotype.

TDC	9R (n = 13)	10R (n = 12)	*p value*
Age (sd)	9,72 (1,09)	9,325 (1,47)	0,46
Performance (sd)	7,12 (3,6)	5,40 (4,3)	0,313
IQ (sd)	110 (9,1)	111,4 (6)	0,683
Gender	Male 60%	Male 57%	0.66 [a]

Abbreviations: ADHD, Attention-deficit/Hyperactivity Disorder; Performance = Hits minus False alarms (max 10); IQ, Intelligence quotient; SD, Standard deviation; TDC, Typically developing children; Data were analyzed using one-way ANOVAs; a: Pearson χ^2 test. Age, IQ and performance were analyzed using Student t-tests.

3.2. Conjunction analysis

As expected, we found WM-related activity (i.e., higher activity in the N2 than in the N0 condition) in the superior parietal lobe, the cerebellum (VIIa), the right middle frontal gyrus, the supplementary motor area and the right insula (Table 4) (Fig. 1).

Table 4
WM-related brain activation in 9R carriers group and 10R homozygous group among ADHD patients (conjunction analysis, N2 > N0).

Anatomical area	x y z (mm)	T	K
Right superior parietal sulcus	+28 −60 +53	8,46	1951
Left superior parietal sulcus	−26 −60 +51	7,94	1062
Right mid frontal gyrus	+31 +7 +59	7,85	434
Left supplementary motor area	0 +18 +48	8,35	333
Right cerebellum (lobule VIIa crus I)	+33 −66 −30	7,67	161
Left cerebellum (lobule VIIa crus I)	−40 −68 −31	6,68	138
Right Insula	+38 +22 −3	7,48	124

Brain areas in which BOLD response (N2 > N0) is common for ADHD 9R carriers and 10R homozygous groups. Coordinates x y z (mm) in MNI standard stereotactic space. T = t-statistic value. K = Cluster extent. All results significant at the voxel level $p_{FWE} < 0,05$ after correction for multiple comparison in whole brain volume.

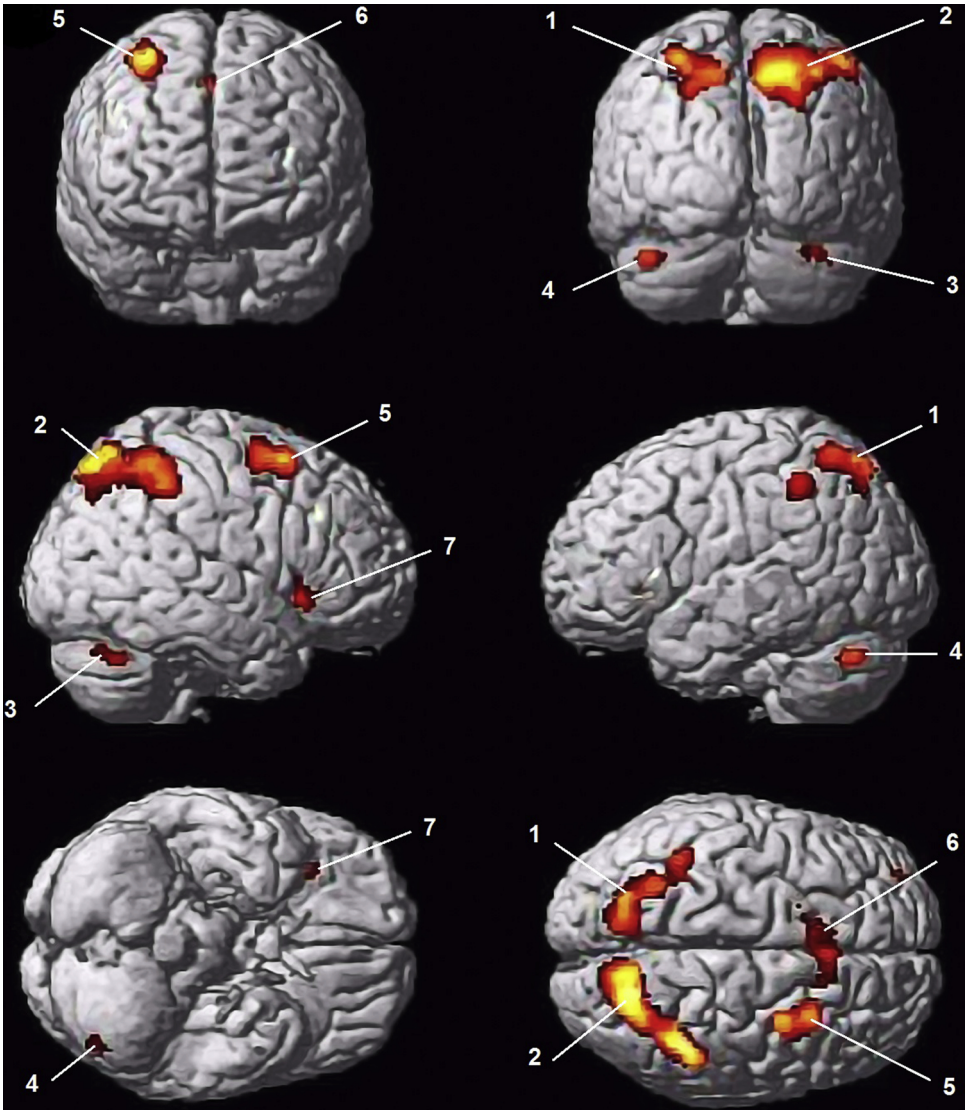


Fig. 1. Statistical map showing common (N2-N0) BOLD response of 9R carrier group and 10R homozygous group among ADHD and TDC subjects in the anatomical areas mentioned in the Table 4. 1: Left superior parietal sulcus, 2: Right superior parietal sulcus, 3: Right cerebellum (lobule VIIa crus I), 4: Left cerebellum (lobule VIIa crus I), 5: Right mid frontal gyrus, 6: Left supplementary motor area, 7: Right Insula.

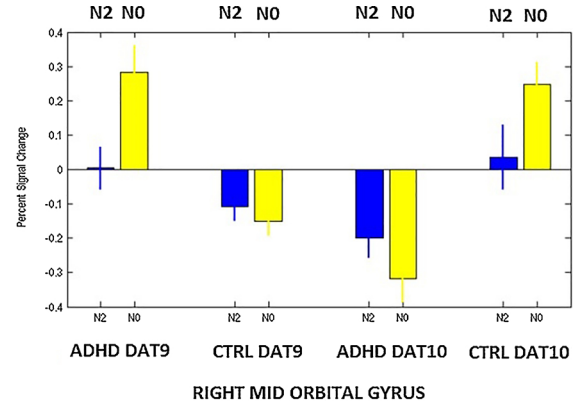
3.3. Effect of genotype and diagnosis in N-back task

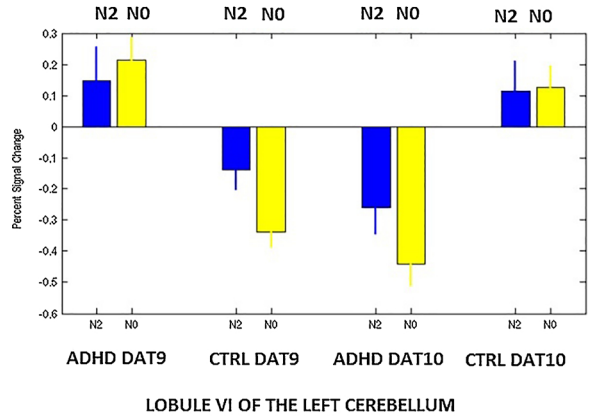
The ANOVA evidenced an interaction effect between genotype (9R vs 10R) and diagnosis (ADHD vs TDC) in the lobule VI of the left cerebellum, the right inferior temporal lobe and the right middle orbital gyrus (Table 5). In these regions, the ADHD 9R carriers showed an increased activation during the WM task compared to the 10R homozygotes. It's the opposite pattern for the TDC group (Fig. 2). Besides, we had no significant main effect: it's consistent with a cross over interaction and the opposite pattern of the neuronal activation we found in the interaction analysis.

Table 5
Interaction between genotype and diagnosis.

Anatomical area	x y z (mm)	T	K
right mid orbital gyrus	+22 +40 −8	4,11	37
lobule VI of the left cerebellum	−6 −72 −18	3,82	63
right inferior temporal lobe	+54 +8 −6	3,59	30

Interaction between genotype and diagnosis effect in WM-related (N2 > N0) effects. Coordinates x y z (mm) in MNI standard stereotactic space. T = t-statistic value. K = cluster extent. All results significant at the voxel level $p < 0{,}001$ uncorrected.





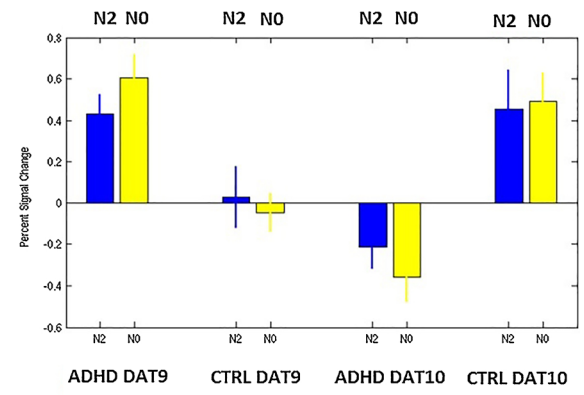


Fig. 2. Percent BOLD signal changes from baseline levels in N2 and N0 condition in brain areas showing a different WM-related activity when analyzing the effect of genotype (9R carriers and 10R homozygotes) and diagnosis in N-back task. The ADHD 9R carriers showed an increased activation during the WM task compared to the 10R homozygotes. It's the opposite pattern for the TDC group.

3.4. WM-related effects in 9R carriers vs. 10R homozygotes in ADHD group

Because we had no main effect results and were initially interested in the DAT1 genotype neuronal modulation in ADHD, we conducted post-hoc analysis in each group. In ADHD subjects only, a whole brain analysis revealed increased in WM-related brain activation (i.e. N2 vs. N0) in 10R homozygotes as compared to 9R carriers in the left parietal superior, left fusiform (temporal lobe) and middle orbital gyrus and the hOC4v area (Fig. 3). The exploration of the caudate nucleus as region of interest (ROI) also revealed higher WM-related activation in 10R than 9R carriers in the head of the right caudate nucleus (Fig. 4) (Table 6).

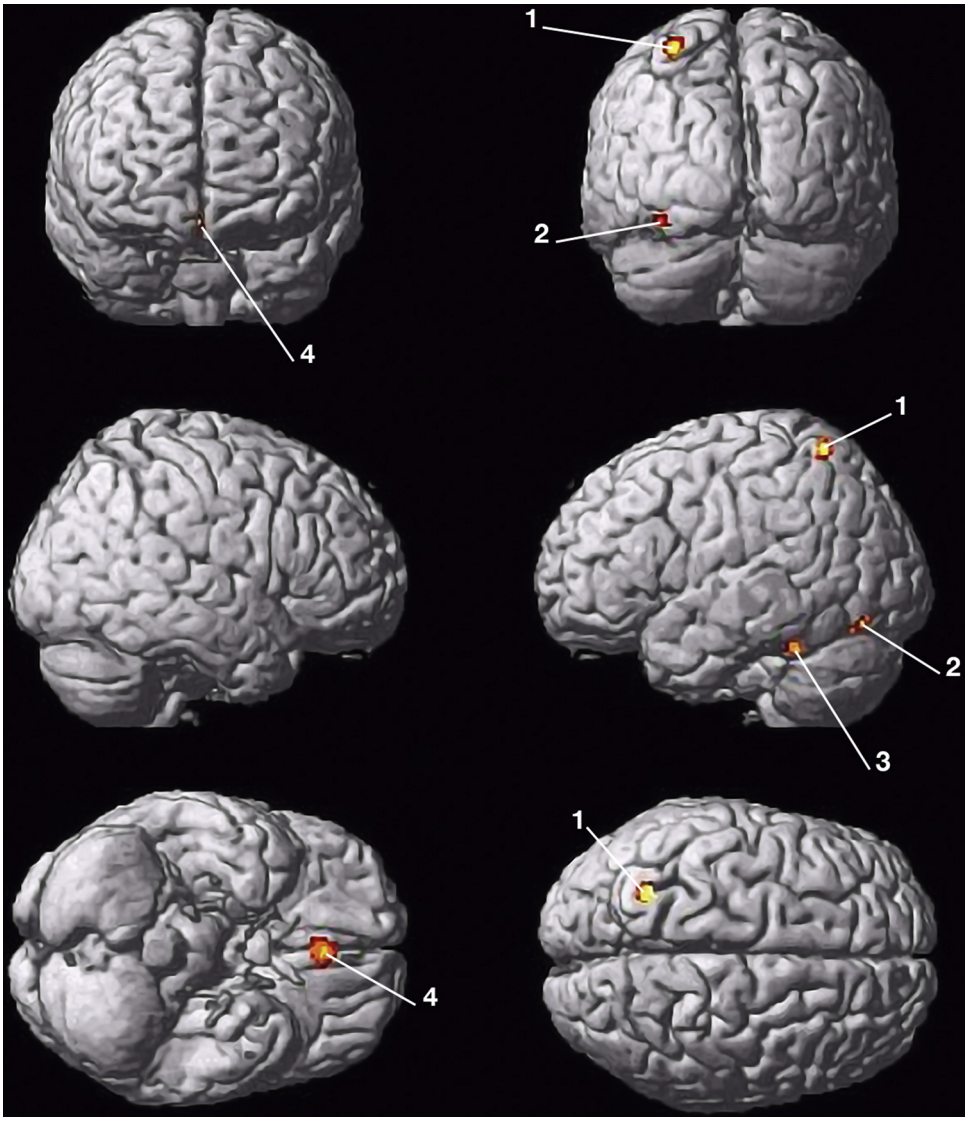


Fig. 3. statistical map showing different (N2-N0) BOLD response of 9R carrier group compared to 10R homozygous group among ADHD subjects in the anatomical areas mentioned in the Table 5. 1: left superior parietal sulcus, 2: Left fusiform gyrus (hOC4v), 3: Left fusiform gyrus, 4: Right mid orbital gyrus.

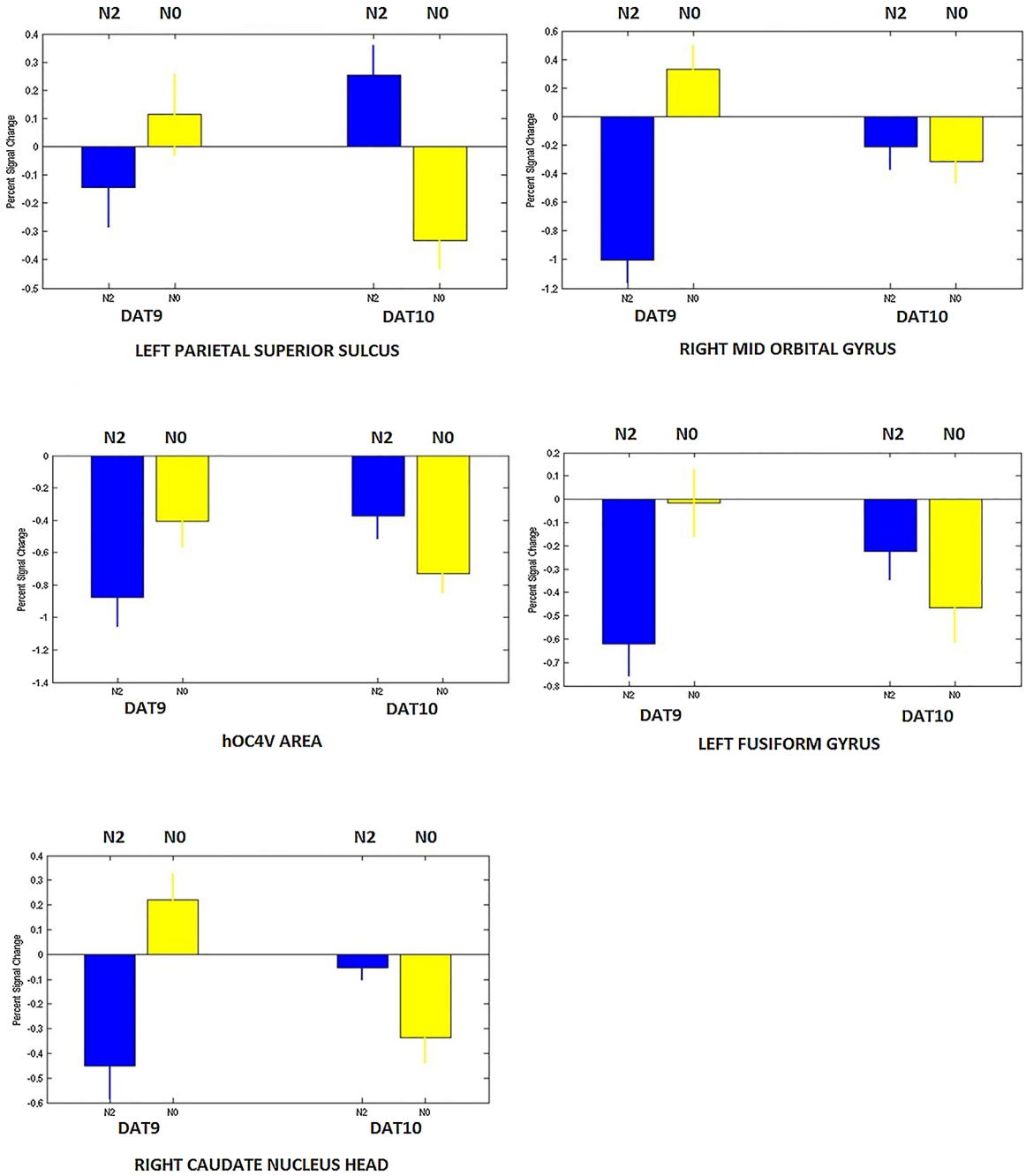


Fig. 4. Percent BOLD signal changes from baseline levels in N2 and N0 condition in brain areas showing a different WM-related activity when comparing 9R carriers and 10R homozygous ADHD subjects. There is a higher WM-related activation in 10R when compared to 9R carriers.

Table 6
WM-related brain activation comparing 9R carriers and 10R homozygous ADHD subjects.

Anatomical area	x y z (mm)	T	K
Left superior parietal sulcus	−28 −59 +65	4,30	57
Right mid orbital gyrus	+1 +35 −12	3,80	83
Left fusiform gyrus (hOC4v)	−32 −74 −14	3,68	31
Left fusiform gyrus	−43 −45 −24	3,79	36
Head of right caudate nucleus*	−12 +20 −2	3,11	24

Brain areas in which BOLD response (N2 > N0) is different in ADHD 9R carriers compared to 10R homozygous groups. Coordinates x y z (mm) in MNI standard stereotactic space. T = t-statistic value. K = cluster extent. All results significant at the voxel level $p < 0{,}001$ uncorrected. *after ROI analysis, result significant at $p^{svccorr} < 0{,}05$.

3.5. WM-related effects in 9R carriers vs. 10R homozygotes in TDC group

We found no significant differences in WM-related activation between the 9R carriers and the 10R homozygotes in the TDC group

4. Discussion

In this fMRI study, we investigated the modulatory effect of DAT1 VNTR on brain activity during a WM task in children with ADHD and TDC. Importantly, our population of ADHD children was carefully selected, naïve for any medication and devoid of the often-present co-morbidity. Also, behavioral performance of 9R carriers and 10R homozygotes was at the same level in both the ADHD and the TDC groups, thus ruling out the hypothesis that highlighted brain activity differences could be due to a confounding behavioral parameter. On the other hand, it also means that DAT1 polymorphism did not modulate WM performance in our small sample. A conjunction analysis evidenced the expected, well-known pattern of WM-related cerebral activity both in 9R carriers and 10R homozygous children, in a network encompassing the superior parietal lobe, the VIIa cerebellum lobules, the right middle frontal gyrus, the supplementary motor area and the right insula (Massat et al., 2012).

In ADHD children, the comparison between 9R carriers and 10R homozygotes disclosed a modulatory effect of DAT1 VNTR polymorphism on WM-related brain activity in expected brain regions such as the parietal lobe or the caudate nucleus (Ciesielski et al., 2006; Massat et al., 2012), but also in the fusiform gyrus and the ventral visual cortex. The fusiform gyrus, located at the inferior part of the temporal lobe, is known to be involved in attention to spatial position and verbal stimuli. In the context of a WM task, fusiform activation might help focusing attention despite the presence of distracting stimuli, and to execute quickly the responses required by the task (Mirsky & Duncan, 2001). In the ADHD, a similar hypothesis was developed based on studies that sought to investigate and discuss deficits other than in the fronto-striatal loop (Rubia, Smith, Brammer, & Taylor, 2007; Shafritz, Marchione, Gore, Shaywitz, & Shaywitz, 2004) which reported differences in functional activity in the right temporal lobe between ADHD and TDC. More precisely, the authors proposed that their findings extended the fronto-striatal deficit hypothesis of ADHD in the context of executive/inhibitory functions to temporal lobe abnormalities during perceptive attention allocations (Rubia et al., 2007). Then, differences of WM related activation in the inferior temporal gyrus were evidenced in children with ADHD as compared to TDC, suggesting that more attention should be paid to the role of this region in ADHD than it was done until now (Kobel et al., 2010). Finally, hOC4v is part of the ventral visual cortex, reflecting the links between WM and visual and attentional strategies (Pessoa, Gutierrez, Bandettini, & Ungerleider, 2002). As a matter of fact, WM manipulation and maintenance is assumed to involve the prefrontal cortex and secondary brain regions to perform supporting processes such as verbal rehearsal, attention allocation and/or visual-spatial processing (Rämä, Sala, Gillen, Pekar, & Courtney, 2001). ADHD children may use visual memory more than TDC when performing an N-back task (Fassbender et al., 2011).

In children with ADHD, there was a higher WM-related activity in all brain areas mentioned above in 10R homozygotes than in 9R carrier participants (Fig. 4), which is in line with our assumption that 10R homozygotes having a lower intracerebral DAT concentration, the stock of intra-synaptic dopamine is larger than in carriers of 9R (Faraone et al., 2014). Similarly, the ROI analysis in the caudate nucleus (i.e. the brain area where the DAT1 gene is preferentially expressed) evidenced higher WM-related activity in the head of the right caudate nucleus in 10R homozygotes than 9R children. Considering our initial assumption, for a task performed with the same level of performance, it is possible that the 9R group showed higher efficiency (Landau et al., 2009). Indeed, the effects of DAT may depend on the initial balance of the dopaminergic system (Waldman et al., 1998): brain function is impaired when dopamine levels are too high or too low. A second hypothesis would be the presence of a compensatory activation through a different functional activation pattern. This hypothesis is reinforced by our gene-diagnosis interaction results. In fact, analysis including TCD showed an interaction between gene and diagnosis in the lobule VI of the left cerebellum, in the right inferior temporal lobe and in the right mid orbital gyrus, and in these areas, in ADHD subjects, 9R carriers showed an increased WM-related brain activity compared to 10R homozygotes. The cerebellum seems to be substrate of the fixed anatomical changes that underlie ADHD (Mackie et al., 2007) and it's activity is modulate by the DAT1 genotype in ADHD subjects during a response inhibition task (Durston et al., 2008b). Besides, recent research suggested that the cerebellum is activated in WM task mainly for the regulation of interferences (Bomyea, Taylor, Spadoni, & Simmons, 2018). It could explain why, in this area, 9R carriers exhibit a higher activation to compensate the lack of dopamine in other areas and maintain the same level of performance.

So far, the few studies combining DAT1 VNTR polymorphism and functional imaging in ADHD have found different, sometimes

opposite patterns in terms of brain activity. In these studies, the clinical heterogeneity of the participants was often questioned but as mentioned before, in our study, the ADHD population is carefully selected. Additionally, ADHD is supposed to be associated with a high number of genes with minor effects interacting with environmental factors (Faraone & Mick, 2010; Hawi et al., 2015): some combinations may have different effects on brain activity, and some genetic variants may also be associated with compensatory mechanisms. For instance in Parkinson's disease, DAT1 can autoregulate its expression in early disease to compensate the lack of dopamine (Greenbaum et al., 2013).

Finally, we are willing to acknowledge the limitations of this exploratory study, which includes, from a genetic point of view, the limitations of a gene candidate study for a multigenic disorder such as ADHD: a small effect size of this genetic risk variant on a complex phenotype like brain activation, as well as on a complex phenotype like ADHD. Indeed, a deep understanding of neurobiology, neuroanatomy, psychiatric genetics, clinical contexts, as well as management of complex data would be required to perfectly integrate the genetic heterogeneity of ADHD. On the one hand, we should continue to link some specific brain alteration with risk genetic variants in selected populations, within exploratory studies which require replication in a larger sample. But on the other hand, genome-wide association studies (hypothesis free) would be also very promising, using brain imaging variables as phenotypes. However, these "whole brain imaging/genome wide analysis (GWAS)" still face statistical analysis and computational power issues, especially in GWAS multivariate approaches (Hibar, Kohannim, Stein, Chiang, & Thompson, 2011) (Mufford et al., 2017).

5. Conclusion

In this exploratory study, we found that the VNTR polymorphism of the DAT1 gene modulates brain activity during a WM task in children with ADHD, independently of any prior psychotropic medication and psychiatric comorbidity and level of performance. To the best of our knowledge, this is the first study to explore the effect of DAT1 VNTR on brain activity during a WM task in ADHD children. We found different pattern of WM-related activation in ADHD subjects when comparing 9R carriers and 10R homozygotes, depending on the brain area, arguing for complex compensatory mechanisms. These data might contribute to elucidate the neurofunctional consequences of a risk gene in ADHD, and how this variation may produce endophenotypic heterogeneity within the disorder.

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