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Investigation of the dopamine D5 receptor gene (*DRD5*) in adult attention deficit hyperactivity disorder

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Abstract

Several lines of evidence from neuroimaging, pharmacology and genetics support the involvement of the dopaminergic system in the etiology of Attention Deficit Hyperactivity Disorder (ADHD). Previous candidate gene studies have investigated the association between a dinucleotide $(CA)_n$ repeat polymorphism, located 18.5 kb from the start codon of the DRD5 gene, and ADHD. Association between the 148 bp allele and ADHD has been reported in some studies, however replication of the finding has not been consistent. We tested for an association between the $(CA)_n$ repeat and adult ADHD in a sample comprised of 119 families with adult ADHD probands and 88 unrelated adult ADHD cases with a corresponding number of controls matched for age, ethnicity and sex. In the family sample we found a non-significant trend for association between the 148 bp allele and ADHD ($Z = 1.91$, $p = 0.055$). An excess of non-transmissions was detected for the 150 and 152 bp alleles ($Z = -2.26$, $p = 0.023$; $Z = -2.20$, $p = 0.028$). Quantitative analysis performed using the Brown Attention Deficit Disorder Scale (BADDS) showed association between the 150 bp allele and lower total score ($p = 0.011$), and lower effort ($p = 0.008$), activation ($p = 0.008$) and attention ($p = 0.01$) cluster scores. We did not replicate association findings in the case–control group, likely due to the lack of statistical power of this sample. Our findings add to the literature suggesting *DRD5* $(CA)_n$ repeat has a modest effect in modulating susceptibility to adult ADHD but further studies are required.

Keywords: ADHD; DRD5; STR; FBAT; Association

Several lines of evidence support the involvement of dopaminergic system dysfunction in the etiology of ADHD. The efficacy of psychostimulants, such as methylphenidate (MPH) and dextroamphetamine, in ameliorating ADHD symptoms is consistent with the dopamine dysfunction hypothesis. Faraone et al. confirmed the efficacy of methylphenidate in adults, providing evidence for involvement of the dopamine pathways in the persistent form of ADHD [9]. Positron emission tomography (PET) studies have shown decreased frontal cortical activity in adults with ADHD and have indicated an increased extracellular dopamine level in subjects treated with methylphenidate, which acts by blocking the Dopamine Transporter [6,25,26]. Although environmental factors play an unknown role in the etiology of the disorder, a strong genetic component has been demonstrated in several studies [13,24]. The adult form of ADHD may have an even stronger genetic determination than child ADHD. Faraone et al. found a significantly higher relative risk to relatives of an adult ADHD proband than relatives of a child ADHD proband [8].

Mutations in DNA sequences may negatively affect proteins important in dopamine pathways, leading to dysfunction in dopaminergic neurons. Candidate gene studies have reported positive association between dopamine system genes and ADHD [4,18,19,27]. The DRD5 receptor gene, mapped to chromosome 4p15.1–p15.3, belongs to a group of dopamine receptors that stimulate the activation of adenylate cyclase through the coupling of G-proteins. A number of studies have investigated the association between a dinucleotide $(CA)_n$ repeat polymorphism, located 18.5 kb from the 5′ end of the DRD5 gene, and ADHD. Positive association for the 148 bp allele in

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ADHD subjects has been shown in some studies [5,11,17]. However, other investigations of the same polymorphism have failed to replicate the positive result for the 148 bp allele [1,2,21,23]. In a joint analysis, Lowe et al. collected genotypic information from 14 samples of probands and their parents [15]. The analysis of the combined dataset showed strong association of small effect for the 148 bp allele. In a meta-analysis that combined all the published studies of European and Asian populations up to October 2005, Li et al. also showed a strong association between the 148 bp allele and ADHD [14]. The intent of our study was to test the association between the $(CA)_n$ repeat and ADHD in our sample comprised of 119 nuclear families and 88 adult cases with age- and sex-matched controls.

The sample used for this study has been extensively described in previous papers [19,20]. Here we report a brief description. The family sample was comprised of 338 individuals in 57 complete trios, 41 trios with a missing parent, 17 families with 1 affected and 1 unaffected child, 3 families with 2 affected children, and 1 family with 1 affected and 2 unaffected children. All probands within the sample were patients diagnosed with adult ADHD [(mean age: 34.7; S.D. ± 12.7; males/females: 97(69%)/44(31%)].

The case–control sample consisted of 88 cases with controls matched for age, ethnicity and sex [(mean age for cases: 34.2; S.D. ± 11.2; males/females: 59(67%)/39(33%); mean age for controls: 34.8; S.D. ± 11.5].

The diagnosis of ADHD was based on fulfilling the ADHD DSM-IV criteria both at the time of the interview and during childhood, as recalled by the patient and by at least one first-degree relative involved in the study. Most of the patients met criteria for the ADHD inattentive or combined subtypes, with few patients meeting criteria for the hyperactive/impulsive subtype. For broad assessment of psychiatric conditions, the Structured Clinical Interview for DSM-IV (axis I) was administered.

Patients diagnosed with other psychiatric conditions, with the exception of dysthymia, were removed from the study. Approximately 30% of referrals were not included in our sample because of the presence of comorbid conditions or because they did not meet the criteria for the disorder.

The majority of participants were of mixed European Caucasian ancestry (94%). The remaining 6% was composed of probands of African or Chinese descent.

The Brown Attention Deficit Disorder Scale (BADDS) adult version [3] and the Wender Utah Rating Scale (WURS) [28] were performed on all patients.

This study was approved by the Centre for Addiction and Mental Health (CAMH) ethical review committee. Patients were invited to participate in the study after a clinician-referral to the Adult and Adolescent ADHD Research Program of CAMH, an affiliate teaching hospital of the University of Toronto.

Blood samples were collected from patients and relatives and the DNA was extracted following standard high salt procedures. The $(CA)_n$ repeat was amplified using the method provided by Sherrington et al. [22]. The forward primer was labeled with HEX (Applied Biosystems Inc., Ontario, Canada). PCR products were separated using an ABI 3100 Avant Automated Genetic Analyzer (Applied Biosystem Inc., Ontario, Canada). Allele assignment was performed using Genotyper 3.7^{TM}. Lab technicians that worked in the genotype procedures were blind to the diagnosis and to the family structure of all samples.

Table 1
Family-based analysis of *DRD5* $(CA)_n$ in adult ADHD families

Allele	Frequency	*S*	*E*	*Z*	*p*
136	0.08	13	10	1.09	0.275
138	0.07	13	11	0.89	0.371
146	0.08	14	11	1.28	0.201
148	0.42	55	47	1.91	0.055
150	0.12	10	16.5	−2.26	0.023
152	0.09	7	12.5	−2.20	0.028
Others	0.14				

Alleles with a frequency lower then 5% were grouped together.
Global chi-square 14.73, d.f. = 6, $p = 0.022$.
S represents the test statistic and expresses the count of alleles observed in the affected offspring.
E is the value expected for *S* under the null hypothesis of no association.

The Family Based Association Test (FBAT) was used to investigate the presence of biased transmission of alleles to probands. Secondly, transmissions were weighted by quantitative scores from the BADDS and WURS scales [12]. For each allele the expected transmissions under the null hypothesis (*E*), observed transmissions (*S*), *Z* statistic and two-sided *p*-value are computed. Families in which one parental genotype is missing are included in the analysis when transmission can be determined unambiguously. A global chi-square and associated *p*-value are also calculated.

To assess the significance of differences in allele frequencies between cases and controls the χ^2 test was used. The power of our sample was calculated using the PS-Power and sample size calculation program, version 2.1.30 [7]. Considering a frequency of 0.40 in the general population (from our control sample) for the 148 bp allele and a significance level of 0.05, our sample has a power of 85% to detect an odds ratio (OR) of 2.5.

Probands of the family and the case–control samples did not differ in terms of gender, mean age or ethnic background. The FBAT test showed a global chi-square of 14.73, d.f. = 6, $p = 0.02$ (Table 1). In the single allele analysis we found a non-significant trend for association between the 148 bp allele and ADHD ($Z = 1.91$, $p = 0.055$). An excess of non-transmissions was detected for the 150 and 152 bp alleles, showing a possible protective effect against ADHD ($Z = -2.26$, $p = 0.023$; $Z = -2.20$, $p = 0.028$). The quantitative analysis performed using Brown ADD scale showed association between allele 150 bp and lower scores (lower severity of the disease) on effort ($p = 0.008$), activation ($p = 0.008$), attention ($p = 0.01$) clusters and the BADDS total score ($p = 0.01$) (Table 2). Evidence for a protective effect of the 150 bp allele was also found when the WURS score was considered. The case–control analysis failed to replicate the results found in the family-based test (data not shown).

Results from the family sample provide additional support for an association between the *DRD5* $(CA)_n$ repeat and ADHD. We

Table 2
Family-based analysis of quantitative phenotypes of adult ADHD families

Quantitative measure	Allele	*S*	*E*	*Z*	*p*
Activation	148	664.00	595.00	1.001	0.317
	150	96.00	217.50	−2.617	0.008
	152	81.00	129.50	−1.362	0.173
Attention	148	675.00	610.00	0.915	0.359
	150	107.00	227.50	−2.491	0.012
	152	81.00	131.00	−1.369	0.170
Effort	148	540.00	540.00	1.246	0.213
	150	92.00	210.00	−2.637	0.008
	152	82.00	119.50	−1.186	0.235
Affect	148	422.00	370.00	1.174	0.240
	150	58.00	130.00	−2.493	0.012
	152	53.00	89.50	−1.509	0.131
Memory	148	407.00	368.00	0.895	0.371
	150	68.00	120.50	−1.947	0.051
	152	41.00	84.00	−1.727	0.084
BADDS total	148	2788.00	2483.00	1.055	0.291
	150	421.00	905.50	−2.523	0.011
	152	338.00	553.50	−1.431	0.152
WURS total	148	1966.00	1736.50	1.135	0.256
	150	312.00	618.50	−2.267	0.023
	152	206.00	375.50	−1.655	0.098

The quantitative phenotype is derived from the Brown Attention Deficit Disorder Scale adult version (BADDS) [3] and from the Wender Utah Rating Scale (WURS) [28].
S represents the test statistic and expresses the count of alleles observed in the affected offspring weighted to the phenotype measures.
E is the value expected for *S* under the null hypothesis of no association.

show a trend for an over-transmission of the 148 bp allele. The 148 bp allele has been previously reported to be associated with ADHD in a number of papers, and a meta-analysis of the data confirmed the result [14]. On the other hand, lack of association for the 148 bp allele has also been reported. We also report a significant under-transmission for alleles 150 and 152 bp. In the paper by Barr et al. [2] the authors did not show an association for the 148 bp allele but reported a significant under-transmission of alleles 136 and 146 bp, suggesting a protective effect linked to these alleles. Different patterns of association might be due to the different structure of samples. In addition, the association of $(CA)_n$ repeat alleles with ADHD is unlikely due to a functional activity of the microsatellite, that is located 18.5 kb from the start codon of the DRD5 gene. Alleles at $(CA)_n$ are likely in linkage disequilibrium with one or more functional polymorphisms involved in the modulation of the pathophysiology of the disorder and further studies are needed in order to identify these variants.

One limitation of our study was the focus on a single polymorphism, as a map of SNPs covering a broader region of the gene would be helpful for investigating the haplotype structure. Haplotype analysis increases the power to detect an association for a susceptibility locus. Hawi et al. [10], reported significant association for a haplotype of the 148 bp allele and another microsatellite. The association generates more support to the hypothesis that functional variants within *DRD5* are involved in ADHD etiology and linkage disequilibrium is responsible for our positive association.

Results showed in the family-based test were not confirmed in our case–control sample.

Discordant findings are unlikely due to clinical and demographic characteristics of the two samples, as probands did not differ in terms of gender, mean age, ethnicity or clinical features. The lack of association in the case–control sample could be due to the low power of our sample in detecting association for a gene of small effect. In the meta-analysis by Li et al. [14] the overall OR for the strongly associated 148 bp allele in the nine studies included in the analysis was reported to be ∼1.3. An OR of similar strength (OR = 1.24) was reported in the joint analysis by Lowe et al. [15] confirming an association of small effect for the DRD5 microsatellite and ADHD in different datasets. Finally, in the meta-analysis by Maher et al. [16] performed on data collected from five studies, the pooled OR for the 148 bp allele was 1.57. Our sample was estimated to have a power of 85% to detect an OR of 2.5 and 30% to detect on OR of 1.5, but the observed average OR was 1.2 (1.1 for the 148 bp).

In conclusion, even though our findings provide additional support for the involvement of the *DRD5* $(CA)_n$ repeat in adult ADHD, we cannot completely discard the results of no association reported in the case–control sample. Future studies using denser marker maps and sequencing approaches will be required to clarify the role of the *DRD5* $(CA)_n$ repeat in ADHD.

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References

[1] S.C. Bakker, E.M. van der Meulen, N. Oteman, H. Schelleman, P.L. Pearson, J.K. Buitelaar, R.J. Sinke, DAT1, DRD4, and DRD5 polymorphisms are not associated with ADHD in Dutch families, Am. J. Med. Genet. (Neuropsych. Genet.) 132 (2005) 50–52.
[2] C.L. Barr, K.G. Wigg, Y. Feng, G. Zai, M. Malone, W. Roberts, R. Schachar, R. Tannock, J.L. Kennedy, Attention-deficit hyperactivity disorder and the gene for the dopamine D5 receptor, Mol. Psychiatry 5 (2000) 548–551.
[3] T.E. Brown, Brown ADD Scale, The Psychological Corporation, San Antonio, Texas, Harcourt Brace & Company, 1996.
[4] D.E. Comings, B.G. Comings, D. Muhleman, G. Dietz, B. Shahbahrami, D. Tast, E. Knell, P. Kocsis, R. Baumgarten, B.W. Kovacs, D.L. Levy, M. Smith, R.L. Borison, D. Evans, L.M. Klein, J. MacMurray, J.M. Tosk, G. Sverd, R. Gysin, S.D. Flanagan, The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders, JAMA 266 (1991) 1793–1800.
[5] G. Daly, Z. Hawi, M. Fitzgerald, M. Gill, Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children, Mol. Psychiatry 4 (1999) 192–196.
[6] D. Dougherty, A. Bonab, T. Spencer, S. Rauch, B. Madras, A. Fischman, Dopamine transporter density in patients with attention deficit hyperactivity disorder, Lancet 354 (1999) 2132–2133.

[7] W.D. Dupont, W.D. Plummer, PS power and sample size program available for free on the Internet, Controlled Clin. Trials 18 (1997) 274.
[8] S.V. Faraone, J. Biederman, J.A. Feighner, M.C. Monuteaux, Assessing symptoms of attention deficit hyperactivity disorder in children and adults: which is more valid? J. Consult Clin. Psychol. 68 (2000) 830–842.
[9] S.V. Faraone, T. Spencer, M. Aleardi, C. Pagano, J. Biederman, Meta-analysis of the efficacy of methylphenidate for treating adult attention-deficit/hyperactivity disorder, J. Clin. Psychopharmacol. 24 (2004) 24–29.
[10] Z. Hawi, N. Lowe, A. Kirley, F. Gruenhage, M. Nöthen, T. Greenwood, J. Kelsoe, M. Fitzgerald, M. Gill, Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci, Mol. Psychiatry 8 (2003) 299–308.
[11] Z. Hawi, R. Segurado, J. Conroy, K. Sheehan, N. Lowe, A. Kirley, D. Shields, M. Fitzgerald, L. Gallagher, M. Gill, Preferential transmission of paternal alleles at risk genes in attention-deficit/hyperactivity disorder, Am. J. Hum. Genet. 77 (2005) 958–965.
[12] S. Horvath, X. Xu, N.M. Laird, The family based association test method: strategies for studying general genotype-phenotype associations, Eur. J. Hum. Genet. 9 (2001) 301–306.
[13] F. Levy, D.A. Hay, M. McStephen, C. Wood, I. Waldman, Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large scale twin study, J. Am. Acad. Child Adolesc. Psychiatry 36 (1997) 737–744.
[14] D. Li, P.C. Sham, M.J. Owen, L. He, Meta-analysis shows significant association between dopamine system genes and attention deficit hyperactivity disorder (ADHD), Hum. Mol. Genet. 15 (2006) 2276–2284.
[15] N. Lowe, A. Kirley, Z. Hawi, P. Sham, H. Wickham, C.J. Kratochvil, S.D. Smith, S.Y. Lee, F. Levy, L. Kent, F. Middle, L.A. Rohde, T. Roman, E. Tahir, Y. Yazgan, P. Asherson, J. Mill, A. Thapar, A. Payton, R.D. Todd, T. Stephens, R.P. Ebstein, L. Manor, C.L. Barr, K.G. Wigg, R.J. Sinke, J.K. Buitelaar, S.L. Smalley, S.F. Nelson, J. Biederman, S.V. Faraone, M. Gill, Joint analysis of the DRD5 marker concludes association with attention-deficit/hyperactivity disorder confined to the predominantly inattentive and combined subtypes, Am. J. Hum. Genet. 74 (2004) 348–356.
[16] B.S. Maher, M.L. Marazita, R.E. Ferrell, M.M. Vanyukov, Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis, Psychiatry Genet. 12 (2002) 207–215.
[17] I. Manor, M. Corbex, J. Eisenberg, I. Gritsenkso, R. Bachner-Melman, S. Tyano, R.P. Ebstein, Association of the Dopamine D5 Receptor With Attention Deficit Hyperactivity Disorder (ADHD) and Scores on a Continuous Performance Test (TOVA), Am. J. Med. Genet. (Neuropsych. Genet.) 127 (2004) 73–77.
[18] V.L. Misener, P. Luca, O. Azeke, J. Crosbie, I. Waldman, R. Tannock, W. Roberts, M. Malone, R. Schachar, A. Ickowicz, J.L. Kennedy, C.L. Barr, Linkage of the dopamine receptor D1 gene to attention-deficit/hyperactivity disorder, Mol. Psychiatry 9 (2004) 500–509.
[19] P. Muglia, U. Jain, F. Macciardi, J.L. Kennedy, Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene, Am. J. Med. Genet. (Neuropsych. Genet.) 96 (2000) 273–277.
[20] P. Muglia, U. Jain, B. Inkster, J.L. Kennedy, A Quantitative Trait Locus Analysis of the Dopamine Transporter Gene in Adults with ADHD, Neuropsychopharmacology 27 (2002) 655–662.
[21] A. Payton, J. Holmes, J.H. Barrett, T. Hever, H. Fitzpatrick, A.L. Trumper, R. Harrington, P. McGuffin, M. O'Donovan, M. Owen, W. Ollier, J. Worthington, A. Thapar, Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: a family-based study, Am. J. Med. Genet. (Neuropsych. Genet.) 105 (2001) 464–470.
[22] R. Sherrington, B. Mankoo, J. Attwood, G. Kalsi, D. Curtis, K. Buetow, S. Povey, H. Gurling, Cloning of the human dopamine D5 receptor gene and identification of a highly polymorphic microsatellite for the DRD5 locus that shows tight linkage to the chromosome 4p reference marker RAF1P1, Genomics 18 (1993) 423–425.
[23] E. Tahir, Y. Yazgan, B. Cirakoglu, F. Ozbay, I. Waldman, P.J. Asherson, Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children, Mol. Psychiatry 5 (2000) 396–404.
[24] A. Thapar, J. Holmes, K. Poulton, R. Harrington, Genetic basis of attention deficit and hyperactivity, Br. J. Psychiatry 174 (1999) 105–111.
[25] N.D. Volkow, G.J. Wang, J.S. Fowler, G. Logan, B. Angrist, R. Hitzemann, J. Lieberman, N. Pappas, Effects of methylphenidate on regional brain glucose metabolism in humans: relationship to dopamine D2 receptors, Am. J. Psychiatry 154 (1997) 50–55.
[26] N.D. Volkow, L. Chang, G.J. Wang, J.S. Fowler, Y.S. Ding, M. Sedler, J. Logan, D. Franceschi, J. Gatley, R. Hitzemann, A. Gifford, C. Wong, N. Pappas, Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex, Am. J. Psychiatry 158 (2001) 2015–2021.
[27] I.D. Waldman, C. Rowe, A. Abramowitz, T. Kozel, H. Mohr, S.L. Sherman, H.H. Cleveland, M.L. Sanders, J.M. Gard, C. Stever, Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity, Am. J. Hum. Genet. 63 (1998) 1767–1776.
[28] M.F. Ward, P.H. Wender, F.W. Reimherr, The Wender Utah Rating Scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder, Am. J. Psychiatry 150 (1993) 885–890.