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Polymorphisms in the Dopamine D5 Receptor (*DRD5*) Gene and ADHD

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There is considerable evidence to support a role of dopamine-related genes in the molecular aetiology of attention-deficit hyperactivity disorder (ADHD). A microsatellite located near the dopamine D5 receptor (*DRD5*) gene has been associated with ADHD in a number of studies, but other polymorphisms within the vicinity of this gene have not been examined. In this study we genotyped three microsatellites spanning the DRD5 region in a large clinical sample. Overall, we found little evidence to support a role for DRD5 in ADHD. We found no evidence of association with either the previously associated DRD5 marker, or a repeat in the promoter region of the gene. We did, however, find significant association for an allele of D4S615, a dinucleotide repeat located 131 kb 3′ of DRD5 that has been previously associated with schizophrenia. A global test incorporating all alleles of this marker, however, was not significant and thus this finding needs replication before any conclusions can be made.

KEY WORDS: attention-deficit hyperactivity disorder (ADHD); dopamine D5 receptor (DRD5); genetics; association study; haplotypes; D4S615

INTRODUCTION

There is considerable evidence to suggest a role of dopaminergic genes in the molecular aetiology of attention-deficit hyperactivity disorder (ADHD) [Faraone and Biederman, 1998; Kirley et al., 2002]. Whilst the most replicated findings have been with polymorphisms in the dopamine D4 receptor (*DRD4*) gene and the dopamine transporter gene (*DAT1*), there is mounting evidence that the dopamine D5 receptor (*DRD5*) gene is also associated with ADHD. DRD5 has been cloned and mapped to 4p16.1-p15.3 and is a member of the guanine nucleotide-binding (G-protein) receptor family. The structure of the *DRD5* gene is relatively simple, comprising of a single exon, and it shows high structural and functional homology with the dopamine D1 receptor (DRD1) [Sunahara et al., 1991]. Like the D1 receptor, DRD5 stimulates adenyl cyclase activity via G-protein coupling, but D5 receptors have a much higher affinity for dopamine than D1 receptors. Several non-functional DRD5 pseudogenes are known to exist [Grandy et al., 1991], and these have complicated the precise genetic characterisation of this gene.

Several repeat polymorphisms within the vicinity of the *DRD5* gene have been described. Sherrington et al. [1993] identified a highly polymorphic microsatellite $(CT/GT/GA)_n$ located ~19 kb from the 5′ end of the *DRD5* gene (Kathryn Evans, personal communication). This has been examined for association with ADHD in four clinical samples. Two found a significant association with the 148 bp allele [Daly et al., 1999; Tahir et al., 2000], while two have found a trend for association of this allele [Barr et al., 2000; Payton et al., 2001]. A recent meta-analysis of five family-based studies of this DRD5 polymorphism gave a positive pooled odds ratio (OR) of 1.57 (95% CI 1.25–1.96, $P = 0.000083$) [Maher et al., 2002]. Another meta-analysis, using data from 15 published and unpublished samples, gives a slightly more modest OR of 1.25 (Gill et al., personal communication). The 5′ flanking and promoter region of DRD5 was characterised by Beischlag et al. [1995], who found that the major transactivation domain was 119–182 bp upstream of the transcriptional start site. Within this region they discovered a small dinucleotide repeat $(TC)_n$, for which to our knowledge, no published association studies with ADHD exist. Interestingly, this marker is much closer to the actual *DRD5* gene than the

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more widely studied $(CT/GT/GA)_n$ microsatellite. Other informative polymorphisms have been mapped around the DRD5 locus including D4S615, which is located ~131 kb 3′ of the *DRD5* gene and has been putatively associated with schizophrenia [Muir et al., 2001]. Support for a possible role of DRD5 in the aetiology of ADHD comes from a recent genomewide linkage scan [Fisher et al., 2002]. *DRD5* was one of only 3 candidate genes (out of 36 analysed) that coincided with sites of positive linkage identified by the screen. In this study, we have genotyped each of these polymorphisms in a large clinically-ascertained ADHD sample and their immediate family, and looked for association of specific alleles of each of the markers individually, and of haplotypes of all three markers together, by searching for biased transmission to affected offspring.

MATERIALS AND METHODS

Sample

In total, DNA from 188 probands and their families were used for this study. DNA was available from both parents for 121 of the families, and from only the mother in 64 families. The 113 of the affected families had at least 1 sibling who was also genotyped. Cases were referred for assessment if they were thought by experienced clinicians to have a diagnosis of the combined subtype of ADHD under DSM-IV criteria, with no significant Axis I co-morbidity apart from oppositional defiant disorder (ODD) and conduct disorder (CD). Parents of referred cases were interviewed with a modified version of the child and adolescent psychiatric assessment (CAPA) [Angold et al., 1995]. Information on ADHD symptoms at school were obtained using the long form of the Conners [1995] questionnaire. Following assessments HYPESCHEME data sheets were completed using data gathered from the research interview, questionnaire and where necessary review of case notes. HYPESCHEME is an operational criteria checklist for ADHD and hyperkinetic disorders, which summarises and applies DSM-IV and ICD-10 operational criteria [Curran et al., 2000]. HYPESCHEME diagnoses were checked against researcher applied DSM-IV criteria and discrepancies reviewed by two researchers (P.A. and S.R.). Where consensus could not be reached, cases were brought to case conference and final consensus agreement made with a senior clinical researcher (E.T.). All the subjects used in this study had an IQ above 70, were free of neurological disease and damage, and did not have any congenital disorders known to cause hyperactivity. Cases were included in this study if they had a diagnosis of ADHD under DSM-IV criteria. Out of 188 cases included in this study, 176 had the combined subtype, 8 had the hyperactive/impulsive subtype and 4 the inattentive subtype. DNA was obtained using buccal swabs and extracted as described in Freeman et al. [2003].

Genotyping

The $[(CT)_6(GT)_{21}(GA)_{13}]$ microsatellite was genotyped using the primers 5′-FAM CGT GTA TGA TCC CCT GCA G-3′ and 5′-GCT CAT GAG AAG AAT GGA GTG-3′ with an initial 5 min denaturing step at 95°C followed by 35 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension phase of 72°C for 10 min. The D4S615 microsatellite polymorphism was amplified using the primers 5′-FAM CTA TAC ATC ACC ATT TGT CTG TGG C-3′ and 5′-GCT AAG CTA TTG CAG TAA TTT GCT AC-3′ with an initial 5 min denaturing step at 95°C followed by 35 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension phase of 72°C for 10 min. The DRD5 promoter dinucleotide was amplified using the primers 5′-FAM ATC CAC CCA CCT CGG CCT CCC AAA-3′ and 5′-ATG CAA GGT CTT TTC CTC ATA TTG-3′ using a hot-start PCR protocol: an initial 5 min denaturing step at 95°C then 2 min at 85°C at which time Taq polymerase was added, followed by 35 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension phase of 72°C for 10 min. Fluorescently-tagged products for each of the markers were separated on an ABI 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) and analysed using GENOTYPER (PE Applied Biosystems) software.

Analysis

Family genotype data was analysed using David Clayton's program TRANSMIT (version 5.4) which is available for download from the web at http://www.gene.cimr.cam.ac.uk/clayton/software/. TRANSMIT tests for association between genetic markers and disease by examining the transmission of markers from parents to affected offspring. The main features of TRANSMIT, which differ from other similar programs are that it can deal with transmission of multi-locus haplotypes, even if phase is unknown, and that parental genotypes may be unknown. The tests are based on a score vector, which is averaged over all possible configurations of parental haplotypes and transmissions consistent with the observed data. Data from unaffected siblings (or siblings whose disease status is unknown) may be used to narrow down the range of possible parental genotypes that need to be considered, thus maximising the power of our sample to detect an association with any of the DRD5 markers tested. Although the markers genotyped in this study are separated by fairly large physical distances, they all map to the same general area of chromosome 4 spanning DRD5, and so we decided to investigate the levels of linkage disequilibrium (LD) between them. LD relationships were assessed in the parental samples using the program 2LD, written by Jing Hua Zhao, and available online at http://www.iop.kcl.ac.uk/iop/Departments/PsychMed/GepiBSt/software.html. Two standardised measures of LD were calculated—D′ and Φ^2. D′ is the most-widely used measure of LD, and is a standardised, pairwise disequilibrium value that has the advantage of being independent of allele frequency. Φ^2 takes into account allele frequency and gives more information about how well a genotype at one marker location predicts that at another. Two markers can be in strong LD, as shown by a high D′ value, but still be uninformative about each other's genotype, because of large differences in allele frequency.

RESULTS

DRD5 $(CT/GT/GA)_n$ Microsatellite

In total 13 alleles were detected in this sample. Parental allele frequencies were found to be closely similar to those seen in other studies of this marker in Caucasian populations. Allele 9 (148 bp), the most common allele, was found to have a frequency of 47% in our parental sample. We found no overall evidence for biased transmission of any of the alleles of this polymorphism to individuals with ADHD (global $\chi^2 = 9.86$, 12 df, $P = 0.63$). Previous studies have found biased transmission of allele 9 (148 bp) to affected probands, although we found no evidence to support this (TRANSMIT output: observed transmissions = 168, expected transmissions = 170.6, untransmitted = 173.2, $\chi^2 = 0.16$, $P = 0.69$).

DRD5 Promoter Dinucleotide

In total six alleles of this polymorphism were detected. Allele 5 was the most frequent with a frequency of 83% in our parental sample, followed by allele 4 (9%) and allele 6 (6%). The other alleles were very rare (<3%). Again there was no significant evidence of biased transmission of any of the alleles of this marker to ADHD probands (global $\chi^2 = 4.03$, 4 df, $P = 0.40$).

D4S615

In total nine alleles of D4S615 were observed in this sample. Allele frequencies were found to reflect those of Muir et al. [2001] very closely. The most common allele was allele 5 (242 bp) which had a frequency of 27% in parent and sibling samples. Overall there was no evidence to suggest this marker is associated with ADHD (global $\chi^2 = 11.77$, 8 df, $P = 0.16$). As can be seen in Table I, however, we found some evidence for biased under-transmission of allele 6 (244 bp) to ADHD probands (allele-specific $\chi^2 = 6.3341$, 1 df, $P = 0.01$) suggesting that this may be a protective allele. Interestingly, this is the same allele that was found to be associated with an increased risk of schizophrenia by Muir et al. [2001].

Haplotype Analysis and Calculation of LD Between Markers

As described above, TRANSMIT can look for biased transmission of multi-locus haplotypes. We examined all possible marker haplotype combinations and found no evidence for biased transmission of any haplotype. Levels of LD between the three markers were all relatively low, although in each case the relationship was significant. LD relationships between the three markers are given in Table II. A large number of haplotypes were identified in the sample, all having a frequency <10%. This is not surprising given the large number of alleles at each locus, the large physical distances between markers, and the incomplete levels of LD across them.

DISCUSSION

In this study we examined three polymorphisms located in the vicinity of DRD5 for evidence of biased transmission in a clinical ADHD sample. Taken together, our data suggests there is little evidence to support an association between DRD5 and ADHD. None of the markers gave significant overall χ^2 values when tested for biased allelic transmission to affected offspring. Using allele-specific tests we did find evidence for biased under-transmission of allele 6 (244 bp) of D4S615 to ADHD probands. This finding should be treated with caution, however, because allele-specific tests involve a degree of multiple testing and this association needs to be replicated before any conclusions can be made. There was no evidence to suggest that specific haplotypes comprising of alleles from all three markers were individually associated with ADHD; although this is not particularly surprising given the large physical distances between the three markers and the low levels of LD observed between them.

Previous studies of DRD5 in ADHD have focussed singly upon the $(CT/GT/GA)_n$ microsatellite repeat [identified by Sherrington et al., 1993]. Although we found no evidence to implicate this marker in ADHD, results from other published studies on this marker have been largely positive. Tahir et al. [2000] replicated the original findings of Daly et al. [1999], while Barr

TABLE I. Output From TRANSMIT for Individual Alleles of D4S615*

Allele (bp)	Observed	Expected	Untransmitted	Var (O−E)	χ^2
(1) 234	2	1.0831	0.1661	0.49667	1.69
(3) 238	0	0.54153	1.0831	0.24831	1.18
(5) 242	105	103.33	101.65	34.682	0.08
(6) 244	46	58.24	70.48	23.653	6.33*
(7) 246	68	64.905	61.809	24.613	0.39
(8) 248	6	6.002	6.004	2.7329	0.00
(9) 250	65	62.074	59.148	24.363	0.35
(10) 252	82	76.745	71.49	29.52	0.95
(11) 254	0	1.0831	2.1661	0.49667	2.36

*The global test of association taking into account all alleles gave a non-significant χ^2 value of 11.772 (8 df, $P = 0.16$).

TABLE II. LD Relationships Between the Three DRD5 Markers

Marker 1	Marker 2	D′	Φ^2
$(CT/GT/GA)_n$ microsatellite	Promoter repeat	0.44	0.86
$(CT/GT/GA)_n$ microsatellite	D4S615	0.33	0.49
Promoter repeat	D4S615	0.22	0.12

et al. [2000] and Payton et al. [2001] have found non-significant trends for association. There are a number of reasons why we may have failed to replicate this finding in our study. It is possible that there is not enough power in our sample to detect what is likely to be a small effect. The meta-analysis of Maher et al. [2002] gives an overall OR of ~1.6. While our sample is fairly large (188 affected probands and their families), we have estimated that to detect an association with an OR of 1.6, assuming 80% power and an alpha level of 0.05, would require a sample size of about 300 complete trios. A larger, unpublished, meta-analysis of this marker gives a more modest OR of 1.25 (Gill et al., personal communication), and to detect such a small effect would require an even larger sample. Another possible confounding factor between studies is clinical heterogeneity. Our sample, however, appears to be fairly representative of those used in other studies with a predominance of combined-type diagnosis and the vast majority of affected individuals being male.

None of the polymorphisms genotyped in this study are likely to be directly functional. Although no functional studies have been performed on the $(CT/GT/GA)_n$ marker reported by Sherrington et al. [1993], its location quite a distance from the coding region of DRD5 suggests it is not involved in gene expression and does not alter DRD5 protein structure, receptor binding or signalling. In many respects the dinucleotide $(TC)_n$ repeat identified by Beischlag et al. [1996] in the promoter region of DRD5 may be a better candidate for psychopathology being much closer to the coding region of the gene, although this polymorphism has been shown not to affect D5 promoter-mediated luciferase activity. Finally, it is unlikely that variation at D4S615 has any direct bearing on DRD5 expression, given that it is an anonymous marker located ~140 kb from DRD5 [Evans et al., 2001]. It is, therefore, likely that any association made with any of these DRD5 markers results from LD with another, functional variant that has yet to be characterised. Variations in LD relationships are known to exist in different populations, and these are a potential confound in any association study that does not look directly at the causal, functional variant. A number of variants within the coding region of DRD5 have been described, and some of these have been shown to cause differences in dopamine binding affinities [Cravchik and Gejman, 1999]. Future work on this gene should thus focus on examining additional markers located within, or close to, the coding region of the gene that may have a more direct bearing on gene function.

To conclude, our data do not support the association between allele 9 (148 bp) of a $(CT/GT/GA)_n$ microsatellite located in the region of DRD5 and ADHD, which has been replicated in a number of other studies. Furthermore, we found no overall evidence to support an association between two additional DRD5 markers although we did find a possible association with an allele of D4S615. Allele 6 (244 bp) of this marker was found to be significantly under-transmitted to affected probands, suggesting it may confer some form of protective effect, although this finding needs further replication in other samples before any conclusions can be made.

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REFERENCES

Angold A, Prendergast M, Cox A, Harrington R, Simonoff E, Rutter M. 1995. The child and adolescent psychiatric assessment (CAPA). Psychol Med 25(4):739–753.

Barr CL, Wigg KG, Feng Y, Zai G, Malone M, Roberts W, Schachar R, Tannock R, Kennedy JL. 2000. Attention-deficit hyperactivity disorder and the gene for the dopamine D5 receptor. Mol Psychiatry 5(5):548–551.

Beischlag TV, Marchese A, Meador-Woodruff JH, Damask SP, O'Dowd BF, Tyndale RF, Van Tol HH, Seeman P, Niznik HB. 1995. The human dopamine D5 receptor gene: Cloning and characterization of the 5′-flanking and promoter region. Biochemistry 34(17):5960–5970.

Beischlag TV, Nam D, Ulpian C, Seeman P, Niznik HB. 1996. A polymorphic dinucleotide repeat in the human dopamine D5 receptor gene promoter. Neurosci Lett 205(3):173–176.

Conners CK. 1995. The conners rating scales: Instruments for assessments of childhood psychopathology. Durham: Duke University Press .

Cravchik A, Gejman PV. 1999. Functional analysis of the human D5 dopamine receptor missense and nonsense variants: Differences in dopamine binding affinities. Pharmacogenetics 9(2):199–206.

Curran S, Newman S, Taylor E, Asherson P. 2000. Hypescheme: An operational criteria checklist and minimum data set for molecular genetic studies of attention deficit and hyperactivity disorders. Am J Med Genet 96(3):244–250.

Daly G, Hawi Z, Fitzgerald M, Gill M. 1999. Mapping susceptibility loci in attention deficit hyperactivity disorder: Preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 4(2):192–196.

Evans KL, Le Hellard S, Morris SW, Lawson D, Whitton C, Semple CA, Fantes JA, Torrance HS, Malloy MP, Maule JC, Humphray SJ, Ross MT, Bentley DR, Muir WJ, Blackwood DH, Porteous DJ. 2001. A 6.9-mb high-resolution BAC/PAC contig of human 4p15.3-P16.1, a candidate region for bipolar affective disorder. Genomics 71(3):315–323.

Faraone SV, Biederman J. 1998. Neurobiology of attention-deficit hyperactivity disorder. Biol Psychiatry 44(10):951–958.

Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, Newbury DF, Crawford LR, Palmer CG, Woodward JA, Del'Homme M, Cantwell DP, Nelson SF, Monaco AP, Smalley SL. 2002. A genomewide scan for loci involved in attention-deficit/hyperactivity disorder. Am J Hum Genet 70(5):1183–1196.

Freeman B, Smith N, Curtis C, Huckett L, Mill J, Craig IW. 2003. DNA from buccal swabs recruited by mail: Evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 33(1):67–72.

Grandy DK, Zhang YA, Bouvier C, Zhou QY, Johnson RA, Allen L, Buck K, Bunzow JR, Salon J, Civelli O. 1991. Multiple human D5 dopamine receptor genes: A functional receptor and two pseudogenes. Proc Natl Acad Sci USA 88(20):9175–9179.

Kirley A, Hawi Z, Daly G, McCarron M, Mullins C, Millar N, Waldman I, Fitzgerald M, Gill M. 2002. Dopaminergic system genes in ADHD: Toward a biological hypothesis. Neuropsychopharmacology 27(4):607–619.

Maher BS, Marazita ML, Ferrell RE, Vanyukov MM. 2002. Dopamine system genes and attention deficit hyperactivity disorder: A meta-analysis. Psychiatr Genet 12(4):207–215.

Muir WJ, Thomson ML, McKeon P, Mynett-Johnson L, Whitton C, Evans KL, Porteous DJ, Blackwood DH. 2001. Markers close to the dopamine D5 receptor gene (*DRD5*) show significant association with schizophrenia but not bipolar disorder. Am J Med Genet 105(2):152–158.

Payton A, Holmes J, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, O'Donovan M, Owen M, Ollier W, Worthington J, Thapar A. 2001. Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: A family-based study. Am J Med Genet 105(5):464–470.

Sherrington R, Mankoo B, Attwood J, Kalsi G, Curtis D, Buetow K, Povey S, Gurling H. 1993. 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(2):423–425.

Sunahara RK, Guan HC, O'Dowd BF, Seeman P, Laurier LG, Ng G, George SR, Torchia J, Van Tol HH, Niznik HB. 1991. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. Nature 350(6319):614–619.

Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ. 2000. Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 5(4):396–404.