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Association of DRD4 in Children With ADHD and Comorbid Conduct Problems

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Recent family and twin study findings suggest that ADHD when comorbid with conduct problems may represent a particularly familial and heritable form of ADHD. Although several independent groups have shown association between the DRD4 7 repeat allele and ADHD, others have failed to replicate this finding. Previous TDT analyses of UK and Eire samples had also been negative. We set out to further examine the role of DRD4 but selecting a subgroup of children with ADHD and comorbid conduct problems. Families were recruited from Manchester, Ireland, Birmingham and London clinics. From these, 67 children who fulfilled diagnostic criteria for ADHD and who displayed conduct disorder symptoms were selected. TDT analysis, which had previously yielded negative results for the total sample, showed evidence of association between DRD4 and "ADHD with conduct problems" (7 repeat allele-24 transmissions, 13 non-transmissions; one-tailed $P = 0.05$). These results provide further support for the role of DRD4 in ADHD. Furthermore, these results when considered together with family and twin study findings, suggest that those children with ADHD and comorbid conduct problems may be particularly informative for molecular genetic studies of ADHD. Further work is needed to examine these phenotype issues.

KEY WORDS: attention deficit hyperactivity disorder; conduct disorder; genetics; DRD4; dopamine

INTRODUCTION

Findings from genetic epidemiology have now established that attention deficit hyperactivity disorder (ADHD) is a highly familial and heritable condition [Thapar et al., 1999]. Molecular genetic studies of ADHD represent the next phase of research and results are beginning to emerge, with particular attention focused on genes involved in dopamine neurotransmitter pathways. To date, molecular genetic studies of ADHD have employed either case-control or family-based association approaches, such as the transmission disequilibrium test (TDT) [Spielman et al., 1993].

There have now been seven published studies, utilizing family-based designs, showing positive linkage and association of the DRD4 7 repeat allele and ADHD [Swanson et al., 1998; Faraone et al., 1999; Smalley et al., 1999; Barr et al., 2000; Muglia et al., 2000 (trend using TDT); Sunohara et al., 2000; Tahir et al., 2000]. Three groups, however, have shown association using case-control analysis but not when using family-based methods [Rowe et al., 1998; Holmes et al., 2000; Mill et al., 2000] (the Rowe et al. study found significant linkage disequilibrium with inattentive symptoms), and others have failed to find evidence of association for DRD4 [Castellanos et al., 1998; Eisenberg et al., 2000; Hawi et al., 2000; Kotler et al.,

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2000]. It is unclear as to whether non-replication may be due to differences in sample ascertainment, genetic or diagnostic heterogeneity, or inadequate statistical power or reflect true differences between populations. A recent meta-analysis of all these studies, however, suggests that there is an association of DRD4 with ADHD although the effect size is small particularly when utilizing data from family-based studies [Faraone et al., 2001].

Recently, it has been proposed that molecular genetic studies may yield greater success by examining more heritable subtypes of the ADHD phenotype and that ADHD when comorbid with conduct disorder may represent such a phenotype [Faraone et al., 2000]. Clinical studies have shown that ADHD plus conduct disorder is a clinically more severe condition and has a worse outcome than ADHD alone [Barkley et al., 1990; Jensen et al., 1997; Kuhne et al., 1997]. Family research has also suggested that ADHD with comorbid conduct disorder may represent a more strongly familial sub-type [Faraone et al., 1998]. A recent report of prevalence rates of ADHD in the parents and siblings of children with ADHD showed that relative risks varied from 4–5.4 amongst relatives of those with ADHD alone but rose to 4.8–9.5 for relatives of probands with ADHD and conduct disorder [Faraone et al., 2000]. Furthermore new findings from a twin study have suggested that children who display both ADHD and conduct symptoms, even when this category is defined broadly, may represent a group with greater genetic loading than children with ADHD symptoms alone [Thapar et al., 2001]. This study was based on a population-based sample of twins and the broad category of "ADHD and conduct problems" was defined used a cut-point on parent-rated questionnaire measures. Given these findings, in this present study we sought to re-examine the association between DRD4 and ADHD in the combined U.K. and Ireland samples, in the subgroup of children with ADHD and comorbid conduct problems. Previous TDT analyses of these samples had failed to demonstrate association with DRD4 [Hawi et al., 2000; Holmes et al., 2000; Mill et al., 2000].

MATERIALS AND METHODS

Families of children with suspected ADHD were recruited from Child and Adolescent Psychiatry Clinics in the UK and Eire by four research groups (Manchester/Cardiff, London, Birmingham, Dublin). Written informed consent and assent were obtained from parents and children. All four centres used the same assessment instrument, the Child and Adolescent Psychiatric Assessment (CAPA) [Angold et al., 1995] and the same exclusion criteria that were: IQ test score below 70/no mental retardation, Tourette syndrome, pervasive developmental disorder, major medical or neurological condition or fragile X syndrome.

Mothers were interviewed about symptoms of ADHD, oppositional defiant disorder and conduct disorder using the Child and Adolescent Psychiatric Assessment (CAPA) [Angold et al., 1995], a semi-structured, investigator-based diagnostic interview. In the London sample, information on conduct disorder symptoms was obtained from parent and child rated questionnaires rather than the CAPA. Interviews were carried out by trained psychologists and psychiatrists. Three of the research teams (Manchester, Eire, Birmingham) were trained in assessment by the same person (AT) who in turn had originally been trained at London.

Interviews were audiotaped and supervised by an experienced child and adolescent psychiatrist at each centre (MF (Eire), ET (London), AT (Manchester and Birmingham) with good reported inter-rater reliability [Holmes et al., 2000]. Information was obtained from teachers using a teacher telephone interview that entails asking the class teacher about all DSM-IV and ICD-10 symptoms of ADHD and hyperkinetic disorder [Holmes et al., 2000] or Conners questionnaire [Conners, 1998]. Teacher ratings of ADHD symptoms at school were used to determine whether the criterion of symptom pervasiveness was met.

Diagnoses were assigned on the basis of ICD-10, DSM-IV and DSM-III-R criteria using information from the parent CAPA and teacher reports. Children were included in the present study if they fulfilled the diagnostic criteria for ICD-10 Hyperkinetic Disorder or DSM-IV/DSM-III-R ADHD *and* displayed conduct problems. Given that just 21 children in the sample fulfilled diagnostic criteria for conduct disorder, it was necessary to use a broader definition of conduct disturbance. This approach is supported by the twin study findings that had suggested that children with a broader definition of ADHD and conduct disturbance represented a more heritable subgroup [Thapar et al., 2001]. Children with "conduct problems" were defined as those children who fulfilled all criteria for ODD and displayed at *least* one symptom of conduct disorder within the past 6 months, with associated impairment in functioning. A more stringent definition of "conduct problems" (endorsement of four or more conduct symptoms by parent or child) was adopted for the London cases, given that assessment of conduct symptoms was based on child or parent questionnaire ratings.

On the basis of these criteria, 67 children (65 males, 2 females) aged between 6 and 18 years (mean = 9.65, SD = 2.86) were classified as "ADHD with co-morbid conduct disturbance" (DSM-IV ADHD combined type, $n = 55$; hyperactive-impulsive type, $n = 9$; DSM-III-R ADHD, $n = 3$).

Association and linkage of DRD4 with ADHD and conduct disturbance was examined using an extended transmission disequilibrium test (ETDT) [Sham and Curtis, 1995]. This method examines transmissions from heterozygous parents to affected offspring and tests for an overall departure from the pattern expected under the null hypothesis. As some alleles were rare, Monte-Carlo methods were used to assess significance instead of the χ^2 distribution that is only applicable for large sample sizes. Given the a priori hypothesis of involvement of the seven repeat allele, a one-tailed binomial test was conducted to examine for excess transmission of this allele. Finally the

Mann-Whitney test was used to compare the age, total ADHD scores and total hyperactivity, impulsive, inattention and hyperactive-impulsive scores of the subgroup of children with ADHD and conduct problems ($n = 67$) with the remaining sample ($n = 204$).

DNA was extracted from blood samples and mouth swabs, and genotyping of a 48 bp VNTR in exon 3 of the DRD4 receptor gene was carried out according to standard laboratory protocols that have been described in full previously [Hawi et al., 2000; Holmes et al., 2000; Mill et al., 2000].

RESULTS

Fifty-one parent-child transmissions were informative for TDT analysis. Evidence of linkage and association between DRD4 and ADHD was found with observed preferential transmission of allele 7 (see Table I). Given that conduct symptoms were assessed differently in London, we also separately examined the Manchester, Eire, and Birmingham cases alone that resulted in no difference to the results (22 transmissions vs. 11 non-transmissions; $P = 0.04$).

Children with ADHD and conduct problems when compared to the remaining sample were younger (mean age 9.4 vs. 10.5; $Z = -3.006$, $P = 0.003$), showed higher hyperactivity scores (mean score 15 vs. 14.5; $Z = -3.543$, $P < 0.0001$) and higher hyperactive-impulsive scores (mean score 8.05 vs. 7.28; $Z = -2.744$, $P = 0.006$). There were, however, no differences between the groups in terms of total ADHD symptom scores, impulsive scores or inattention scores.

DISCUSSION

Our results suggest an association between the DRD4 7 repeat allele and ADHD with comorbid conduct problems. These findings provide further confidence in the previously reported association between DRD4 and ADHD. As mentioned earlier, there have so far been seven published family-based association studies that report an association between DRD4 and ADHD [Swanson et al., 1998; Faraone et al., 1999; Smalley et al., 1999; Barr et al., 2000; Muglia et al., 2000; Sunohara et al., 2000; Tahir et al., 2000]. The U.K. and Eire ADHD research groups had previously been unable to replicate these findings when using TDT analysis.

The analyses presented in this study suggest that our initial negative findings that differed from many other groups, may in part have been due to sample differences and that when a more strongly familial or heritable group is identified, positive association can be detected. Moreover these molecular genetic findings lend support to the suggestion from previously reported family and twin study findings that selecting those with ADHD and conduct problems may yield a group with greater genetic loading. Nevertheless given 'conduct problems' had to be broadly defined, our findings clearly needed to be tested in larger samples that will allow for identifying a sufficient number of those with ADHD who fulfill all the diagnostic criteria for conduct disorder. It is possible that these findings, together with those from family and twin studies could be accounted for by a factor other than conduct problems. We can not rule out the possibility that age, hyperactivity (and hyperactive-impulsive symptoms) and other features such as parental psychopathology that may characterize children with comorbid conduct problems may be more important indices of heterogeneity. Our subsample of children with ADHD and conduct problems showed no difference from the rest of the collected sample in terms of total ADHD symptom severity, inattention or impulsiveness scores. Those with ADHD and conduct problems, however, showed increased hyperactivity (and hyperactive-impulsive) scores and were younger. The age difference may reflect referral bias given that children with ADHD and conduct problems may be detected and referred at a younger age. Our sample size did not allow for subdividing the sample further to test out additional hypotheses but clearly the possibility of an alternative explanation for our findings and distinguishing between the effects of comorbid conduct problems and high hyperactivity need to be considered in future larger studies.

In conclusion these results add to support for the involvement of DRD4 in ADHD and further suggest that those with ADHD and conduct disorder problems may be a particularly suitable group to focus on in molecular genetic studies of ADHD.

TABLE I. Transmission for DRD4 Alleles*

	2	3	4	5	7
Passed	4	0	23	0	24
Not passed	6	5	24	3	13

*χ^2 for allele-wise TDT = 13.63, 4df, $P = 0.009$; adjusted P-value using Monte Carlo methods = 0.02. One-sided binomial test for transmission of allele 7: $P = 0.05$.

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