Am. J. Hum. Genet. 74:348–356, 2004

Report

Joint Analysis of the *DRD5* Marker Concludes Association with Attention-Deficit/Hyperactivity Disorder Confined to the Predominantly Inattentive and Combined Subtypes

Naomi Lowe,[1] Aiveen Kirley,[1] Ziarih Hawi,[1] Pak Sham,[2] Harvey Wickham,[2] Christopher J. Kratochvil,[3] Shelley D. Smith,[3] Saretta Y. Lee,[4] Florence Levy,[4] Lindsey Kent,[5] Fiona Middle,[6] Luis A. Rohde,[7] Tatiana Roman,[7] Eda Tahir,[8] Yanke Yazgan,[8] Philip Asherson,[2] Jonathan Mill,[2] Anita Thapar,[9] Antony Payton,[9] Richard D. Todd,[10] Timothy Stephens,[10] Richard P. Ebstein,[11] Iris Manor,[12] Cathy L. Barr,[13] Karen G. Wigg,[13] Richard J. Sinke,[14] Jan K. Buitelaar,[15] Susan L. Smalley,[16] Stan F. Nelson,[16] Joseph Biederman,[17] Stephen V. Faraone,[17] and Michael Gill[1]

[1]Departments of Genetics and Psychiatry, Trinity College, Dublin; [2]Social, Genetic and Developmental Research Centre, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London; [3]Psychopharmacology Research Center, University of Nebraska Medical Center, Omaha; [4]School of Psychiatry, University of New South Wales Prince of Wales Hospital, Sydney; [5]Developmental Psychiatry, University of Cambridge, Cambridge, United Kingdom; [6]Department of Psychiatry, University of Birmingham, Birmingham, United Kingdom; [7]ADHD Outpatient Program and Department of Genetics, Federal University of Rio Grande do Sul, Federal School of Medical Sciences of Porto Alegre, Porto Alegre, Brazil; [8]TUBITAK, Marmara Research Centre, Research Institute of Genetics and Biotechnology, Kocaeli, Turkey; [9]Department of Psychological Medicine, University of Wales College of Medicine, Cardiff; [10]Departments of Psychiatry and Genetics, Washington University School of Medicine, St. Louis; [11]Department of Psychology, Hebrew University and Herzog Hospital, Jerusalem; [12]Geha Mental Health Center, Petak Tikvah, Israel; [13]Cell and Molecular Biology Division, Toronto Western Research Institute, University Health Network, and Brain and Behaviour Programme, The Hospital for Sick Children, Toronto; [14]Department of Biomedical Genetics, University Medical Center Utrecht, Utrecht; [15]Department of Psychiatry, University Medical Center Nijmegen, Nijmegen, The Netherlands; [16]Center for Neurobehavioral Genetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles; and [17]Harvard Medical School Department of Psychiatry, Massachusetts General Hospital, Boston

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable, heterogeneous disorder of early onset, consisting of a triad of symptoms: inattention, hyperactivity, and impulsivity. The disorder has a significant genetic component, and theories of etiology include abnormalities in the dopaminergic system, with *DRD4*, *DAT1*, *SNAP25*, and *DRD5* being implicated as major susceptibility genes. An initial report of association between ADHD and the common 148-bp allele of a microsatellite marker located 18.5 kb from the *DRD5* gene has been followed by several studies showing nonsignificant trends toward association with the same allele. To establish the postulated association of the $(CA)_n$ repeat with ADHD, we collected genotypic information from 14 independent samples of probands and their parents, analyzed them individually and, in the absence of heterogeneity, analyzed them as a joint sample. The joint analysis showed association with the *DRD5* locus ($P = .00005$; odds ratio 1.24; 95% confidence interval 1.12–1.38). This association appears to be confined to the predominantly inattentive and combined clinical subtypes.

Attention-deficit/hyperactivity disorder (ADHD [MIM 143465]) is a common disorder of childhood onset that manifests itself as a combination of inattentive, hyperactive, and impulsive symptoms. It is known to affect 3%–9% of school-aged children worldwide (Faraone et al., in press) and may often persist into adulthood (Faraone et al. 2000). The heritability of ADHD is estimated to be 70%–90% (Levy et al. 1997; Thapar et al. 1999). Children with this disorder often develop severe problems with personal relationships and academic development. Compared with control subjects, ADHD probands exhibit lower grades, fail more courses, have worse performance on standardized tests, have fewer friends, and are rated less adequate in psychosocial adjustment (Man-

Received July 3, 2003; accepted for publication November 19, 2003; electronically published January 19, 2004.

Address for correspondence and reprints: Dr. Naomi Lowe, Department of Genetics, Trinity College, Dublin 2, Ireland. E-mail: lowen@tcd.ie

0002-9297/2004/7402-0016$15.00

Table 1

Information about Individual Groups

Group Number	Primary Investigator	No. of Cases	Publication Status[a]	Origin of Sample	Instrument of Diagnosis[b]
1	C. J. Kratochvil	41	U	United States	KSADS
2	F. Levy	66	U	Australia	ATBRS
3	L. Kent	69	U	United Kingdom	CAPA
4	S. V. Faraone	82	U	United States	K-SADS-E
5	L. A. Rohde	85	U	Brazil	K-SADS-E
6	E. Tahir	100	P[c]	Turkey	KSADS
7	P. Asherson	103	U	United Kingdom	CAPA
8	A. Thapar	106	P[d]	United Kingdom	CAPA
9	R. D. Todd	161	U	United States	MAGIC
10	M. Gill	168	P[e] + U	Ireland	CAPA
11	R. P. Ebstein	176	U	Israel	Clinical interview
12	C. L. Barr	178	P[f] + U	Canada	PICS-IV
13	R. J. Sinke	207	U	Holland	DISC
14	S. L. Smalley	438	P[g]	United States	K-SADS-PiL

[a] U = unpublished data; P = previously published data; P + U = previously published data with the addition of new unpublished data.

[b] KSADS = Kidi schedule for affective disorders and schizophrenia (Kaufman et al. 1997). ATBRS = Australian Twin Behavior Rating Scale (Hay et al. 2001). CAPA = Child and Adolescent Psychiatric Assessment (Angold et al. 1995). K-SADS-E = Schedule for Affective Disorders and Schizophrenia for School-Age Children–Epidemiologic Version (Orvaschel et al. 1987). MAGIC = Missouri Assessment of Genetics Interview for Children (Reich 2000). PICS-IV = Parent Interview for Child Symptoms (A. Schachar, A. Ichowicz, unpublished data). DISC = Diagnostic Interview Schedule for Children (Shaffer et al. 2000). K-SADS-PiL = Schedule for Affective Disorders and Schizophrenia for School-Age-Children Present and Lifetime version (Kaufman et al. 1997).

[c] Tahir et al. (2000).

[d] Payton et al. (2001).

[e] Daly et al. (1999).

[f] Barr et al. (2000).

[g] Kustanovich et al. (in press).

nuzza and Klein 2000). In addition, individuals with this disorder are at greater risk for substance abuse (Flory et al. 2003). Furthermore, individuals with ADHD are reported to have increased driving problems; as part of a case-control study, individuals with ADHD self-reported more traffic citations—including, speeding, vehicular crashes, and license suspensions—than their counterparts in the control group (Barkley et al. 2002).

The exact etiology of ADHD is unknown, but it is widely recognized to have a significant genetic component, as demonstrated by family (Biederman et al. 1992), twin (Silberg et al. 1996), and adoption (Cadoret and Stewart 1991) studies. Although both the serotonergic and noradrenergic systems have been implicated in ADHD, the dopaminergic system is by far the most extensively explored to date. Neuropharmacological studies have demonstrated that methylphenidate and dexamphetamine—stimulant medications that are effective in ~70% of patients with ADHD (Spencer et al. 1996)—block the reuptake of dopamine by the dopamine transporter DAT1 (Amara and Kuhar 1993; Krause et al. 2000). Neuroimaging studies have shown abnormalities in the frontal lobe and subcortical structures, regions that are known to be rich in dopaminergic neurotransmission and important in the control of attention and response to organization (Lou et al. 1990; Zametkin et al. 1990; Rubia et al. 1997). Multiple animal models have been produced, including the DAT1 knockout mouse, which expresses spontaneous hyperactivity and difficulty with learning tasks (Davids et al. 2003). In addition, molecular genetic studies have implicated susceptibility genes, including those for the dopamine D4 receptor (*DRD4*), the dopamine transporter (*DAT1*), dopamine β hydroxylase (*DBH*), synaptosomal associated protein 25 (*SNAP25*), and the dopamine D5 receptor (*DRD5*).

To date, the most extensively examined genes of the dopaminergic system are *DRD4* and *DAT1* (Hawi et al. 2003*a*). The primary focus of attention at *DAT1* has been the 480-bp allele of a 40-bp repeat located in the 3′ UTR of the gene. In the case of *DRD4*, the majority of studies have reported on a 48-bp microsatellite located in exon 3 of the gene and translated to the third intracellular loop of the protein. Recently, meta-analyses have been performed on both of these markers, in an attempt to definitively confirm or refute association with ADHD (Faraone et al. 2001; Maher et al. 2002).

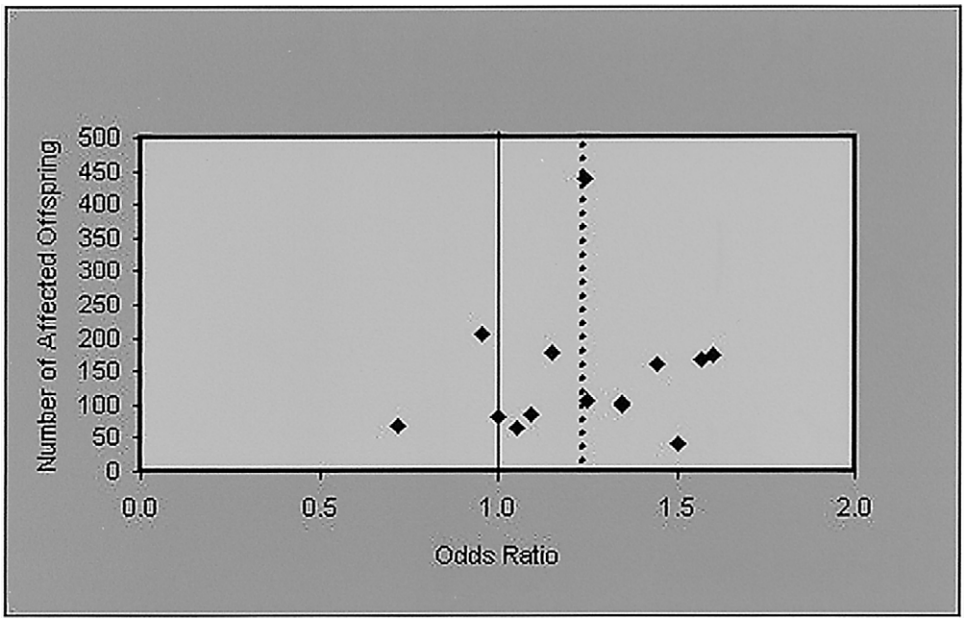

Figure 1 Funnel plot showing the number of affected offspring versus OR. The inverted funnel shape of this plot depicts results consistent with the absence of publication bias. The solid line represents OR = 1, and the dotted line represents OR = 1.24.

In 1999, Daly et al. reported a significant association between ADHD and the 148-bp allele of a microsatelite marker located 18.5 kb 5′ to the *DRD5* gene on chromosome 4. Subsequent studies of this $(CA)_n$ repeat marker have shown nonsignificant trends toward association with the same allele (Barr et al. 2000; Tahir et al. 2000; Payton et al. 2001). To avoid the premature conclusion of the presence or absence of an association with ADHD (and guided by the meta-analyses of *DRD4* and *DAT1*), a joint analysis of *DRD5* was proposed. Although there is no evidence to suggest that the D5 microsatellite is itself functional, the association reported by Daly et al. (1999) is too strong to be ignored. Therefore, we hypothesized that if the association with ADHD were true, the microsatellite may be in linkage disequilibrium (LD) with one or more functional variants. To this end, we invited all known groups with samples based on parent-proband trios to genotype their sample for the marker and present us with their data for analysis (table 1).

In the event of an overall significant association between the variant and ADHD, we proposed that, owing to the unique opportunity presented by a data set of this magnitude, we would further examine the association in relation to a number of different subdivisions. Three times more males than females receive a diagnosis of ADHD (Anderson et al. 1987), and it has been reported by teachers that affected boys are more inattentive and more hyperactive/impulsive than affected girls (Hartung et al. 2002). Furthermore, Clarke et al. (2003) have reported results indicating that girls with ADHD also exhibit abnormalities in their electroencephalograms; however, there is far less variance in their profiles than appears in boys. We therefore proposed analysis by the sex of the affected child. In addition, we planned to examine the sex of the transmitting parent, because the literature has suggested the possibility of imprinting at the *DRD2* (Kirley et al. 2002) and *SNAP-25* loci (Brophy et al. 2002) in conjunction with multiple known imprinted genes in various other areas of psychiatric genetics (Davies et al. 2001). Finally, recent attention has focused on more homogeneous diagnostic symptoms and subtypes. Waldman et al. (1998) reported that, in between-family association analyses, levels of predominantly hyperactive-impulsive symptoms were related to the number of *DAT1* high-risk alleles but that levels of the inattentive symptoms were not, whereas within-family analysis showed association between *DAT1* and the combined subtype. We therefore also proposed to analyze the joint sample in relation to diagnostic subtype.

All children in the present study were given a diagnosis of one of the three clinical subtypes (predominantly inattentive, predominantly hyperactive/impulsive, or combined) of ADHD, according to criteria of the *Diagnostic and Statistical Manual,* fourth edition (DSM-IV) (American Psychiatric Association 1994). To receive a diagnosis of predominantly inattentive or predominantly hyperactive/impulsive ADHD, children must display a minimum of six of the nine symptoms from the inattentive or hyperactive/impulsive sections of the DSM-IV in a minimum of two settings (e.g., home and school). If a child has

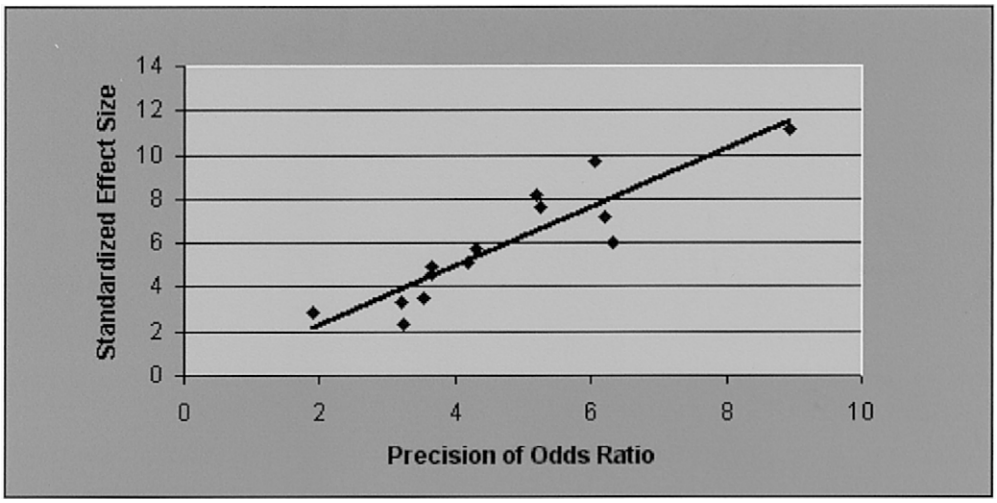

Figure 2 Regression of the standardized effect size versus the precision of the OR. The intercept of the line will occur close to 0, implying no bias in the samples.

six or more symptoms in both sections, then he or she receives a diagnosis of combined-type ADHD. Inclusion criteria for the present study were the presence of a DSM-IV diagnosis of childhood-onset ADHD and the genotyping of one or both parents. Exclusion criteria were the presence of pervasive developmental disorders, fragile X syndrome, major neurological disorders, fetal alcohol syndrome, Tourette syndrome, psychosis, and a full-scale IQ score <70. Information on the sex of the children was provided along with the clinical subtype. Genotypic data for the microsatellite marker were collected from each group, along with at least three DNA samples, which were genotyped at the Neuropsychiatry Genetics Laboratory, Dublin, to ensure consistent allele calling between centers. The data received from each group consisted of genotypes from affected probands and either or both of their parents.

To obtain as complete a sample as possible—and to avoid potential bias—we contacted all members of the ADHD collaborative network (Faraone et al. 2003) and invited their participation. We also searched PubMed using the keywords "ADHD" and "*DRD5*." This revealed an earlier meta-analysis (Maher et al. 2002) that contained data from four published studies, which we have included, and from an additional unpublished sample, which we excluded because it did not meet our inclusion criteria. We are aware of five additional samples that have not yet been genotyped for this marker. This approach to sample collection should provide adequate power to confirm a gene of small effect and to minimize publication bias. A funnel plot (Egger et al. 1997) was produced to examine for evidence of sample bias in the study. This was achieved by plotting the number of affected children for each sample against its equivalent odds ratio (OR), in conjunction with the OR for the combined sample (fig. 1). The larger samples should result in ORs that are closer to the true OR than the smaller samples. In addition, we regressed the normal SD of the OR against the precision of the OR, according to the methods used by Egger et al. (1997) through use of STATA 6.0 (StataCorp 1999). This analysis works on the same basis as the funnel plot, in that the precision of the OR increases with sample size. Egger et al. (1997) showed that, in the absence of bias, the regression would run through the origin (fig. 2). This method produces an R^2 (measure of the fit of the regression to the data) and a value for the intercept of the line, along with corresponding statistics.

Initially, association analyses were performed on each individual group's sample, using the family-based extended transmission/disequilibrium test (ETDT) (Sham and Curtis 1995). This test was designed to avoid population stratification by using parental genotypes as internal controls and to disregard duos whose inclusion could potentially lead to bias (Curtis and Sham 1995). It is adapted from the transmission/disequilibrium test (TDT) and is used to examine for linkage in the presence of association between multiallelic markers and disease phenotypes. The analysis involves the comparison of transmissions and nontransmissions of the risk allele from heterozygous parents to affected offspring.

To test for heterogeneity among the samples, we used the transmission and nontransmission information of the 148-bp allele from the TDT analysis. Two different logistic regression models were compared. The first model assumed homogeneity (i.e., that the regression coefficients were the same across studies, so only one regression coefficient was estimated); the second model assumed het-

erogeneity (i.e., that all the regression coefficients were different, so that as many regression coefficients were estimated as there were studies). Both models produced a −2 log likelihood value, a measure that attempts to assess the suitability of the statistical method used for a given set of data. Using the formula $(-2 \log \text{likelihood}_{\text{individual}}) - (-2 \log \text{likelihood}_{\text{combined}}) = \chi^2$, we tested for evidence of a significant difference between the two models, which would be indicative of heterogeneity among the samples.

For the sensitivity analysis, each group's data were removed in turn from the combined total, and the remaining data were reanalyzed. This was performed to ensure that no individual group was biasing the combined result.

The attributable fraction (Levin 1953) for the marker was calculated according to the formula $f(\text{RR} - 1)/[f(\text{RR} - 1) + 1]$, where f is the frequency of the 148-bp allele in the sample of nontransmitted alleles and RR is the relative risk. The value was calculated as $\text{RR} = [a/(a + c)]/[b/(b + d)]$, where a is the number of transmissions of the risk allele, b is the number of nontransmissions of the risk allele, c is the number of transmissions of the nonrisk alleles, and d is the number of nontransmissions of the nonrisk alleles.

To test the combined sample for significant differences between the diagnostic subtype, the sex of the child, and the sex of the transmitting parent, we first performed TDT analyses on the subsets of each group (e.g., a male subset and a female subset for the group defined by sex), using the ETDT. The ORs calculated for each were then weighted according to the number of cases in each subset and were compared with each other by use of logistic regression.

TDT analyses on the individual samples tested showed a significant association between the 148-bp allele and two of the ADHD samples. The majority of the rest displayed an excess of transmission of the 148-bp allele but did not attain statistical significance. Two of the samples showed a level of transmission of the 148-bp allele that was less than the expected transmission (table 2; fig. 3).

Despite these variations, the heterogeneity test failed to detect the presence of heterogeneity between the 14 samples ($\chi^2 = 11.98$ with 13 df; $P = .53$). They were therefore combined, and the joint analysis on this data set (1,980 probands and 3,072 parents) indicated a strongly significant association of small effect ($\chi^2 = 16.45$ with 1 df; $P = .00005$; OR = 1.24; 95% CI 1.12–1.38) between ADHD and the 148-bp allele of the microsatellite marker. Sensitivity analysis (table 3) showed strong significance, regardless of the data set removed, with the P value never >.001 and the OR never fluctuating >0.04 in either direction from the combined result. The attributable fraction was calculated using the frequency of the 148-bp allele among nontransmitted parental alleles as a conservative estimate of population frequency and was found to be 0.07.

Table 2

Individual Group Results

Group Number	No. of Affected Cases	No. of Parents	Allele Range[a]	Frequency of Allele 10 (%)	OR	*P*
1	41	63	6–17	49.2	1.50	.44
2	66	82	7–17	35.1	1.05	.88
3	69	121	4–18	50.8	.72	.29
4	82	126	5–18	46.5	1.00	1.00
5	85	138	7–19	39.7	1.09	.77
6	100	193	4–17	39.8	1.34	.21
7	103	179	6–18	44.9	1.35	.28
8	106	233	6–18	43.2	1.25	.42
9	161	271	6–19	44.5	1.45	.05
10	168	325	6–17	43.6	1.57	.02
11	176	333	1–16	36.3	1.60	.004
12	178	268	5–17	46.4	1.15	.38
13	207	295	6–19	44.6	.95	.75
14	438	445	5–20	38.5	1.24	.05
Total	1,980	3,072	1–20	43.1	1.24	.00005

[a] Allele range: allele 1 = 166 bp, allele 20 = 128 bp, and allele 10 = 148 bp.

In the funnel plot (fig. 1), the larger samples displayed ORs closer to that of the combined OR than did the smaller samples, resulting in a visual representation of an inverted funnel that is consistent with the absence of sample bias. The method described by Egger et al. (1997) produced a scatter plot of the data with an R^2 value of 0.8036 (fig. 2), implying a good fit of the data to the line. The intercept was calculated to be 0.179 (SE = 1.100245; $t = 0.16$; $P = .874$; 95% CI −2.218645 to 2.575809). According to Egger et al. (1997), in the absence of publication bias, the intercept of this line should be 0. If bias exists, then the intercept should be significantly >0. Our intercept of 0.179 is not significant and is therefore consistent with the funnel plot in its implication that publication bias is absent from the sample.

We examined the association in relation to the sex of the child, which was negative, and the sex of the transmitting parent, which showed a trend toward a maternal effect ($\chi^2 = 2.6$; with 1 df; $P = .11$) and the diagnostic subtype. The three clinical subtypes—predominantly inattentive ($n = 445$), combined ($n = 1{,}106$), and predominantly hyperactive/impulsive ($n = 122$)—were examined separately and found to have ORs of 1.5, 1.3, and 0.9, respectively, with corresponding P values of .0001, .0001, and .6. Comparison between the individual ORs (table 4) indicated a significant difference ($P = .035$) between the inattentive and hyperactive/impulsive subtypes.

Loci conferring risk with OR <1.5 require large samples for detection and replication (Dahlman et al. 2002). In the field of psychiatric genetics, this is difficult to attain;

consequently, there are multiple small studies of markers without conclusive results. This has led to the introduction of the combined, or meta-analysis, approach to this area of genetics. The principal concerns in combining multiple data sets are the possibility of heterogeneity between samples and the possibility of publication bias. In the present study, we found no evidence of heterogeneity among the samples and no publication bias, and we therefore combined the data from all 14 groups.

Using the large combined sample, we have determined that the OR for the DRD5 148-bp variant is 1.24; however, because the allele is common in the population, it has an attributable fraction of 0.07. For complex disorders involving the interaction of multiple genes and environmental factors, it is difficult to attribute an individual case to any single etiological factor. Nevertheless, the attributable fraction is still a useful index of potential public health importance, because it is defined to be the fractional reduction in the frequency of disease if the risk factor were eliminated from the population. Sensitivity analysis showed that the omission of any of the groups—including that with the smallest *P* value (group 11), that with the largest *P* value (group 13), or that containing the original findings (group 10)—did little to alter the overall results, implying that no one group's data are responsible for the combined result.

The existence of a single published result showing significant association between the 148-bp allele and ADHD (Daly et al. 1999), coupled with a number of nonsignificant trends in the same direction (Barr et al. 2000; Tahir et al. 2000; Payton et al. 2001), follows a pattern familiar to researchers and others engaged in association studies with small samples. It could have been

Table 3

Sensitivity Analysis

Subtracted Group	OR	95% CI	*P*
1	1.24	1.11 – 1.38	.00007
2	1.25	1.12 – 1.39	.00004
3	1.26	1.14 – 1.40	.00002
4	1.25	1.12 – 1.39	.00004
5	1.24	1.12 – 1.38	.00007
6	1.24	1.11 – 1.38	.00011
7	1.24	1.11 – 1.38	.00009
8	1.24	1.12 – 1.38	.00007
9	1.22	1.10 – 1.37	.00027
10	1.22	1.09 – 1.36	.00041
11	1.20	1.08 – 1.34	.00103
12	1.25	1.12 – 1.40	.00007
13	1.28	1.15 – 1.43	.00001
14	1.24	1.10 – 1.40	.00039
Total[a]	1.24	1.12 – 1.38	.00005

NOTE.—Results are shown for the joint data set, with the subtraction of each group in turn.

[a] Totals indicate results when no group is subtracted.

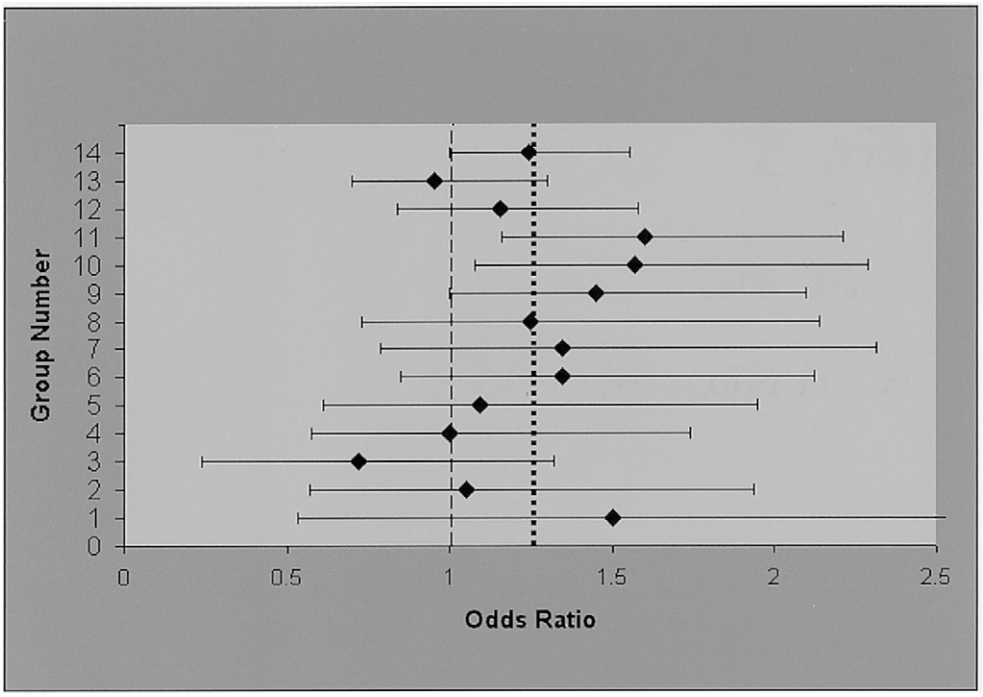

Figure 3 ORs and respective 95% CIs for each sample, displayed in order of size, with the largest sample at the top. The solid line represents an OR of 1, and the dotted line represents an OR of 1.24

Table 4

Logistic Regression Analysis Comparing ORs of the Diagnostic Subgroups

Comparison	χ^2	P	95% CI
Predominantly inattentive vs. combined	1.287	.257	.902–1.471
Predominantly hyperactive/impulsive vs. combined	2.654	.103	.659–1.039
Predominantly inattentive vs. predominantly hyperactive/impulsive	4.468	.035	.367–.963

concluded, incorrectly, that the original result was a false-positive finding. The present joint analysis, however, suggests that the original is a true finding of association between ADHD and the polymorphism and that individual sample sizes lacked sufficient power for confirmation.

Subtype analyses indicate that the association of the 148-bp allele is selective for predominantly inattentive and combined subtypes. It should be noted that although the sample size for the predominantly hyperactive/impulsive group is relatively small, the power of this sample to detect association with the disorder was >75% ($\alpha = 0.05$) and 85% ($\alpha = 0.1$). Whether the DRD5 association is restricted to inattentive symptoms awaits further study using QTL (Curran et al. 2001) or alternative subtyping approaches (Todd et al. 2001; Rasmussen et al. 2002).

The microsatellite marker is located 18.5 kb from the transcription start site of the *DRD5* gene, and no functional role has been reported to date. This makes it an unlikely, although not impossible, functional variant. It is more probable that the microsatellite is in LD with the true functional variant (or variants) located in (or closer to) the *DRD5* gene. In support of this theory, a recent publication reported a significant haplotype composed of the 148-bp allele of this marker and another microsatellite located at the 3′ UTR of the gene ($\chi^2 = 14.208$; $P = .00017$) with a significant LD between the markers ($D' = 0.519$) (Hawi et al. 2003*b*).

In conclusion, the present study has shown that the 148-bp allele of this DRD5 microsatellite marker is associated with ADHD in a large combined homogeneous international sample. It has also been indicated that the association may be confined to particular subtypes or symptom traits, although we acknowledge that this finding requires additional confirmation. We believe that the results of this joint analysis will encourage efforts to identify the functional variant or variants of this gene and will promote further collaboration in the field of psychiatric genetics.

Acknowledgments

Acknowledgments are given in order of authors' affiliations, and numbers in parentheses correspond to affiliation numbers at the beginning of this article. We gratefully acknowledge the following: (1) In Dublin, the generous support of the Health Research Board, Dublin, The Wellcome Trust (support to A.K. and Z.H.), and the Hyperactive and Attention Disorder (HAD) Group Ireland, and the assistance of our clinical colleagues, Celine Mullins, Michael Fitzgerald, Mary McCarron, and Grainne Daly. (2) In London, The Wellcome Trust, Action Research, Sparks, Jane Worthington, Bill Ollier, University of Manchester, Michael O'Donovan, Michael Owen, Darko Turic, and Kate Langley. (3) In Omaha, the assistance of Cynthia R. Ellis, Daryl Bohac, Jodi Polaha, Karen Dittmer, Tina Benedict-Vest, Judy Kenyon, Denise Hoover, Brigette Vaughan, Andrea Steenson, and Holly Zumpfe; research in Omaha was supported by funding from the Weibe Foundation, an Edna Ittner pediatric research grant, and the University of Nebraska Medical Center seed grant program. (4) In Australia, Professor Peter Schofield at the Garvan Institute, Sydney, for his support of the study, and Dr. Chris Ingall, Lismore, for contributing to the sample. (5) In Cambridge, The Wellcome Trust for funding the sample collection. (6) In Birmingham, the UK Medical Research Council. (7) In Porto Alegre, Professor Mara Helena Hutz at the ADHD outpatient program and the Department of Genetics at the Federal University of Rio Grande do Sul. At the ADHD outpatient program, the work was supported by research grant GPG 98201 from the University Hospital and by scholarships from Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS/02/60016-0) and the National Council for Scientific and Technological Development. (8) In Kocaeli, the European Molecular Biology Organisation, the UK Medical Research Council, TUBITAK, and Eczacibasi Scientific Research and Award Fund. (10) In St. Louis, participants were T. Stephens, B Lobos, and R Neuman; the work was supported by National Institutes of Health (NIH) grants R01 MH52813 and R01 NS43762 (to R.D.T.). (11 and 12) In Jerusalem and Petak Tikvah, research was partially supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities (grant to R.P.E.). (13) In Toronto, participants were J. Kennedy, A. Ickowicz, T. Pathare, W. Roberts, M. Malone, R. Tannock, and R. Schachar; the research was supported by The Hospital for Sick Children Psychiatric Endowment Fund grant MT14336 and by Canadian Institutes of Health Research grant MOP-14336. (14 and 15) In the Netherlands, participants were S. C. Bakker and E. M. van der Meulen. (15) In Los Angeles, collaborators were Stanley Nelson, James McGough, James McCracken, Deborah Lynn, May Yang, Vlad Kustanovich, and Matt Ogdie; the research was supported by NIH grant MH58277. (17) In Boston, the work was supported by NIH grants R01 HD37999 and R01 HD37694 (to S.V.F.) and R01 HD036317 and R01 MH050657 (to J.B.); the activities of the ADHD Molecular Genetics Network are supported by NIH grant R13 MH59126 (to S.V.F.). In addition, the authors thank all the families who participated in the study.

Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for ADHD)

References

Amara SG, Kuhar MJ (1993) Neurotransmitter transporters: recent progress. Annu Rev Neurosci 16:73–93

American Psychiatric Association (1994) Diagnostic and statistical manual of mental disorders, 4th ed. Washington, DC

Anderson JC, Williams S, McGee R, Silva PA (1987) DSM-III disorders in preadolescent children: prevalence in a large sample from the general population. Arch Gen Psychiatry 44:69–76

Angold A, Prendergast M, Cox A, Harrington R, Simonoff E, Rutter M (1995) The Child and Adolescent Psychiatric Assessment (CAPA). Psychol Med 25:739–753

Barkley RA, Murphy KR, Dupaul GI, Bush T (2002) Driving in young adults with attention deficit hyperactivity disorder: knowledge, performance, adverse outcomes, and the role of executive functioning. J Int Neuropsychol Soc 8:655–672

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 5:548–551

Biederman J, Faraone SV, Keenan K, Benjamin J, Krifcher B, Moore C, Sprich-Buckminster S, et al (1992) Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder: patterns of comorbidity in probands and relatives psychiatrically and pediatrically referred samples. Arch Gen Psychiatry 49:728–738

Brophy K, Hawi Z, Kirley A, Fitzgerald M, Gill M (2002) Synaptosomal-associated protein 25 (SNAP-25) and attention deficit hyperactivity disorder (ADHD): evidence of linkage and association in the Irish population. Mol Psychiatry 7:913–917

Cadoret RJ, Stewart MA (1991) An adoption study of attention deficit/hyperactivity/aggression and their relationship to adult antisocial personality. Compr Psychiatry 32:73–82

Clarke AR, Barry RJ, McCarthy R, Selikowitz M, Clarke DC, Croft RJ (2003) EEG activity in girls with attention-deficit/hyperactivity disorder. Clin Neurophysiol 114:319–328

Curran S, Mill J, Sham P, Rijsdijk F, Marusic K, Taylor E, Asherson P (2001) QTL association analysis of the DRD4 exon 3 VNTR polymorphism in a population sample of children screened with a parent rating scale for ADHD symptoms. Am J Med Genet 105:387–393

Curtis D, Sham PC (1995) A note on the application of the transmission disequilibrium test when a parent is missing. Am J Hum Genet 56:811–812

Dahlman I, Eaves IA, Kosoy R, Morrison VA, Heward J, Gough SC, Allahabadia A, Franklyn JA, Tuomilehto J, Tuomilehto-Wolf E, Cucca F, Guja C, Ionescu-Tirgoviste C, Stevens H, Carr P, Nutland S, McKinney P, Shield JP, Wang W, Cordell HJ, Walker N, Todd JA, Concannon P (2002) Parameters for reliable results in genetic association studies in common disease. Nat Genet 30:149–150

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 4:192–196

Davids E, Zhang K, Tarazi FI, Baldessarini RJ (2003) Animal models of attention-deficit hyperactivity disorder. Brain Res Brain Res Rev 42:1–21

Davies W, Isles AR, Wilkinson LS (2001) Imprinted genes and mental dysfunction. Ann Med 33:428–436

Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple graphical test. BMJ 315:629–634

Faraone SV (2003) Report from the Fourth International Meeting of the Attention Deficit Hyperactivity Disorder Molecular Genetics Network. Am J Med Genet 121B:55–59

Faraone SV, Biederman J, Spencer T, Wilens T, Seidman LJ, Mick E, Doyle AE (2000) Attention deficit hyperactivity disorder in adults: an overview. Biol Psychiatry 48:9–20

Faraone SV, Doyle AE, Mick E, Biederman J (2001) Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 158:1052–1057

Faraone SV, Sergeant J, Gillberg C, Biederman J. The worldwide prevalence of ADHD: is it an American condition? World Psychiatry (in press)

Flory K, Milich R, Lynam DR, Leukefeld C, Clayton R (2003) Relation between childhood disruptive behavior disorders and substance use and dependence symptoms in young adulthood: individuals with symptoms of attention-deficit/hyperactivity disorder and conduct disorder are uniquely at risk. Psychol Addict Behav 17:151–158

Hartung CM, Willcutt EG, Lahey BB, Pelham WE, Loney J, Stein MA, Keenan K (2002) Sex differences in young children who meet criteria for attention deficit hyperactivity disorder. J Clin Child Adolesc Psychol 31:453–464

Hawi Z, Kirley A, Lowe N, Fitzgerald M, Gill M (2003*a*) Recent genetic advances in ADHD and diagnostic and therapeutic prospects. Expert Rev Neurother 3:453–464

Hawi Z, Lowe N, Kirley A, Gruenhage F, Nothen M, Greenwood T, Kelsoe J, Fitzgerald M, Gill M (2003*b*) Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. Mol Psychiatry 8:299–308

Hay D, McStephen M, Levy F (2001) The diagnostic genetics of ADHD symptoms and subtypes. In: Levy F, Hay D (eds) Attention genes and ADHD. Hove, United Kingdom, Brunner-Routledge, pp 35–37

Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, Williamson D, Ryan N (1997) Schedule for affective disorders and schizophrenia for school-age children-present and lifetime version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 36:980–988

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 27:607–619

Krause KH, Dresel SH, Krause J, Kung HF, Tatsch K (2000) Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methyl-

phenidate as measured by single photon emission computed tomography. Neurosci Lett 285:107–110

Kustanovich V, Ishii J, Crawford L, Yang M, McGough JJ, McCracken JT, Smalley SL, Nelson SF. Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. Mol Psychiatry (in press)

Levin M (1953) The occurrence of lung cancer in man. Acta Union International Contra Cancrum 9:531–541

Levy F, Hay DA, McStephen M, Wood C, Waldman I (1997) Attention deficit hyperactivity disorder: a category or continuum? Genetic analysis of a large-scale twin study. J Am Acad Child Adolesc Psychiatry 36:737–744

Lou HC, Henriksen L, Bruhn P (1990) Focal cerebral dysfunction in developmental learning disabilities. Lancet 335:8–11

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

Mannuzza S, Klein RG (2000) Long-term prognosis in attention-deficit/hyperactivity disorder. Child Adolesc Psychiatr Clin N Am 9:711–726

Orvaschel H, Puig-Antich J (1987) Schedule for Affective Disorder and Schizophrenia for School-Age Children-Epidemiological, 4th version. Nova University Center for Psychological Study, Fort Lauderdale

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 105:464–470

Rasmussen ER, Neuman RJ, Heath AC, Levy F, Hay DA, Todd RD (2002) Replication of the latent class structure of attention-deficit/hyperactivity disorder (ADHD) subtypes in a sample of Australian twins. J Child Psychol Psychiatry 43:1018–1028

Reich W (2000) Diagnostic interview for children and adolescents (DICA). J Am Acad Child Adolesc Psychiatry 39:59–66

Rubia K, Overmeyer S, Taylor E, Bullmore E, Brammer M, Williams S, Simmos A, Andrew C (1997) Inhibitory control of hyperactive adolescents in FMRI. In: Toga AW, Frackowiak RSJ, Mazziotta JC (eds) Neuroimage: proceedings of the Third International Conference on Functional Mapping of the Human Brain (May 19–23, 1997, Copenhagen). Academic Press, New York

Shaffer D, Fisher P, Lucas CP, Dulcan MK, Schwab-Stone ME (2000) NIMH Diagnostic Interview Schedule for Children, version IV (NIMH DISC-IV): description differences from previous versions, and reliability of some common diagnoses. J Am Acad Child Adolesc Psychiatry 39:28–38

Sham PC, Curtis D (1995) An extended transmission disequilibrium test (TDT) for multi-allele marker loci. Ann Hum Genet 59:323–336

Silberg J, Rutter M, Meyer J, Maes H, Hewitt J, Simonoff E, Pickles A, Loeber R, Eaves L (1996) Genetic and environmental influences on the covariation between hyperactivity and conduct disturbance in juvenile twins. J Child Psychol Psychiatry 37:803–816

Spencer T, Biederman J, Wilens T, Harding M, O'Donnell D, Griffin S (1996) Pharmacotherapy of attention-deficit hyperactivity disorder across the life cycle. J Am Acad Child Adolesc Psychiatry 35:409–432

StataCorp (1999) Reference manual for Stata, version 6.0. StataCorp, College Station, TX

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 5:396–404

Thapar A, Holmes J, Poulton K, Harrington R (1999) Genetic basis of attention deficit and hyperactivity. Br J Psychiatry 174:105–111

Todd RD, Rasmussen ER, Neuman RJ, Reich W, Hudziak JJ, Bucholz KK, Madden PA, Heath A (2001) Familiality and heritability of subtypes of attention deficit hyperactivity disorder in a population sample of adolescent female twins. Am J Psychiatry 158:1891–1898

Waldman ID, Rowe DC, Abramowitz A, Kozel ST, Mohr JH, Sherman SL, Cleveland HH, Sanders ML, Gard JM, Stever C (1998) Association and linkage of the dopamine transporter gene and attention deficit hyperactivity disorder in children: owing to diagnostic subtype and severity. Am J Hum Genet 63:1767–1776

Zametkin AJ, Nordahl TE, Gross M, King AC, Semple WE, Rumsey J, Hamburger S, Cohen RM (1990) Cerebral glucose metabolism in adults with hyperactivity of childhood onset. N Engl J Med 323:1361–1366