

# 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

Sarah Curran,<sup>1,2\*</sup> J. Mill,<sup>1</sup> P. Sham,<sup>1</sup> F. Rijdsdijk,<sup>1</sup> K. Marusic,<sup>1</sup> E. Taylor,<sup>1,2</sup> and P. Asherson<sup>1</sup>

<sup>1</sup>Social, Genetic, and Developmental Psychiatry Research Centre, Institute of Psychiatry, London, United Kingdom

<sup>2</sup>Department of Child and Adolescent Psychiatry, GKT Medical School, London, United Kingdom

Current developments in molecular genetics have led to a rapid increase in research aimed at the identification of genetic variation that influences complex human phenotypes. One phenotype that has aroused a great deal of interest is the behavioral trait hyperactivity and the related clinical disorder attention-deficit hyperactivity disorder (ADHD). The driving force behind the molecular genetic research in this area is the overwhelming evidence from quantitative genetic studies that show high heritability ( $h^2 = 0.7\text{--}0.9$ ) for the behaviors characterizing the diagnosis of ADHD, whether the disorder is viewed as a categorical entity or a continuous trait. To date, molecular studies have aimed at identifying susceptibility genes for ADHD, defined using operational diagnostic criteria, and have focused on variation within genes that regulate dopamine neurotransmission. Several studies report ADHD to be associated with the 7-repeat allele of a 48 bp repeat polymorphism (DRD4-7) in exon 3 of the dopamine D4 receptor gene (DRD4). In this study, we take a dimensional perspective of ADHD and examine the relationship of this DRD4 polymorphism in a sample of children selected from the general population on the basis of high and low scores on the five ADHD items of the Strengths and Difficulties Questionnaire (SDQ) as rated by their parents. We found a significant relationship between DRD4-7 and high-scoring individuals [ $\chi^2 = 8.63$ ;  $P = 0.003$ ; OR = 2.09

(95% CI 1.24 < OR < 3.54),  $F$ -statistic = 7.245;  $P = 0.008$ ]. © 2001 Wiley-Liss, Inc.

**KEY WORDS:** QTL; quantitative trait; ADHD; DRD4 receptor gene, association study, childhood behavior

## INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a disorder prevalent in 2%–5% of school-age children and clinically defined as the persistent behavior patterns of overactivity, inattention, and impulsivity that are pervasive across social situations and accompanied by substantial social impairments. The long-term outcome of the categorically defined clinical disorder is poor [Taylor et al., 1996; Mannuzza and Klein, 2000] with an increased risk of social isolation and persistent psychopathology in adolescence and adulthood affecting up to 60% of cases [Barkley, 1996; Cantwell, 1996; Hill and Schoener, 1996]. However, there is some evidence that dimensional methods of representing these behavioral variations may prove to have even greater predictive validity [Fergusson et al., 1995]. Sex differences are probably overestimated in clinical samples, but more subtle differences do exist. In epidemiological samples, the male:female ratio is approximately 3:1, whereas in clinical samples the male:female ratio varies from 5–9:1, probably reflecting referral bias and comorbidity with other externalizing disorders such as conduct disorders and aggression [Gaub and Carlson, 1997].

Twin studies examining the relative importance of genetic and environmental influences have consistently shown ADHD to be among the most highly heritable behaviors in childhood, whether assessed dichotomously as a categorical disorder or dimensionally as a continuum, with heritability in the order of 70%–90% [Cantwell, 1972; Goodman and Stevenson 1989a, 1989b; Biederman et al., 1990, 1992; Gillis et al., 1992; Stevenson, 1992; Thapar, 1995; Gjone et al., 1996;

\*Correspondence to: Dr. Sarah Curran, Social, Genetic, and Developmental Psychiatry Research Centre, Institute of Psychiatry, De Crespigny Park, London, SE5 8AF, U.K.  
E-mail: s.curran@iop.kcl.ac.uk

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Levy et al., 1996, 1997; Silberg et al., 1996; Eaves et al., 1997; Sherman et al., 1997]. Although ADHD is diagnosed using operational criteria to define diagnostic categories, measures of activity and attention are continuously distributed in the general population and many studies have found a high level of correspondence between quantitative measures of hyperactivity and the categorical diagnosis [Edelbrock, 1986; Bird et al., 1987; Biederman et al., 1993, 1996; Chen et al., 1994; Boyle et al., 1997; Hudziak, 1997]. In these studies, there does not appear to be any obvious bimodality that separates ADHD children from non-ADHD children, suggesting that ADHD may be at one extreme of a quantitative dimension. In fact, twin studies have generally applied dimensional rating scales of hyperactivity, with clinical cutoffs applied when diagnostic categories were required. These studies all show high heritabilities regardless of where these cutoffs have been made and regardless of whether diagnostic or continuous criteria have been applied. Furthermore, mathematical modeling applied to such studies supports the hypothesis that ADHD is the extreme of a behavior that varies genetically throughout the entire population [Gillis et al., 1992; Stevenson, 1992; Levy et al., 1997]. Taken together, these findings suggest strongly that a dimensional perspective on hyperactivity is a valid approach to the identification of quantitative trait loci (QTLs) and should provide a powerful complementary strategy to the study of clinically defined subtypes.

Though the evidence for a large genetic component in ADHD has come from twin studies based on questionnaire-derived symptom scores, molecular genetic studies have to date focussed on ADHD diagnosed using categorical criteria. As far as we are aware, there have been no published studies taking a QTL perspective for the ascertainment and selection of cases. QTL methods are statistically powerful approaches, whereby continuous traits may be genetically mapped to multiple discrete chromosomal locations (known as QTLs), by examining the relationship between genetic markers and the relative amount of a given trait. While originally developed to study quantitative phenotypes in plants and animals, the approach has been successfully applied to continuous traits in humans. Recent examples in rodents include the localization of loci that contribute to hypertension, diabetes, obesity, and atherosclerosis [Aitman et al., 1999; Mu et al., 1999], as well as behavioral phenotypes such as alcohol consumption [Crabbe et al., 1999] and learning [Caldarone et al., 1997]. Moisan et al. [1996] mapped a major QTL to rat chromosome 8 for hyperactivity scores. In humans, chromosomal synteny between the mouse model and humans was used to map a gene for the complex trait of obesity, and QTL linkage scans have shown promising results for type 2 diabetes mellitus and hypertension [Lembertas et al., 1997; Hsueh et al., 2000; Watanabe et al., 2000]. Applications of QTL techniques have been used to study reading ability [Fisher SE et al., 1998, 1999; Gayan et al., 1999] and intelligence [Fisher PJ et al., 1999; Hill et al., 1999] and are likely to be useful in conditions such as ADHD, in

which quantitative measures of the phenotype can be applied.

We therefore set out in this study to establish whether the reported association between the 7-repeat allele of the variable number tandem repeat polymorphism in exon 3 of DRD4 (DRD4-7) and diagnosed cases of ADHD would replicate using a QTL approach in an epidemiological sample. A dopamine hypothesis of ADHD and hyperactivity has long been held, based first on studies of the neurochemistry of attention in animals and humans and secondly on the dramatic amelioration of ADHD symptoms by stimulant medications such as methylphenidate, whose main mode of action is via the dopamine system, though their specific site of action is unclear [Shaywitz et al., 1977; Shekim et al., 1979; Shen and Wang 1984; Zametkin et al., 1984; McCracken, 1991; Goldman-Rakic, 1992; Castellanos, 1997]. It is therefore of considerable interest that genetic studies from several independent groups have found evidence for association and linkage with dopamine system genes, including the D4 and D5 receptor genes (DRD4 and DRD5) and the dopamine transporter gene (DAT1) in samples of individuals meeting diagnostic criteria for ADHD. The most robust of these findings so far is an association between ADHD and DRD4-7, with nine studies providing evidence for this association [LaHoste et al., 1996; Rowe et al., 1998; Smalley et al., 1998; Swanson et al., 1998; Faraone et al., 1999; Holmes et al., 2000; Muglia et al., 2000; Tahir et al., 2000; Mill et al., 2001; Sunohara et al., 2000], whereas four found no association [Castellanos et al., 1998; Eisenberg et al., 2000; Hawi et al., 2000; Kotler et al., 2000]. A recent meta-analysis of published and unpublished data suggests a respectable but modest odds ratio of 1.9 (95% CI=1.4-2.2;  $P=0.00000008$ ) from 7 case control studies and 1.4 (95% CI=1.1-1.6;  $P=0.02$ ) from 14 family-based studies [Faraone et al., 2001]. The functional significance of the DRD4 polymorphism is still uncertain [Asghari et al., 1995; Jovanovic et al., 1999; Watts et al., 1999; Kazmi et al., 2000]. It remains possible that the 7-repeat is in linkage disequilibrium (LD) with a functional variant, which may confound association studies and lead to discrepant association findings. The possibility that functional promoter polymorphisms explain the findings has been suggested, but is perhaps unlikely following negative findings for three such polymorphisms, in a series of trio samples that showed linkage and association to DRD4-7 [Barr et al., 2001].

## MATERIALS AND METHODS

### Sample Ascertainment

In this study, we screened an epidemiological sample of 5- to 15-year-old children from southern England, using the parent version of the Strengths and Difficulties Questionnaire (pSDQ) [Goodman, 1997]. The SDQ was chosen as our primary measure for several reasons. It is a brief 30-item questionnaire that can be easily administered by post to the parents of individuals in the age group selected. The SDQ is currently being used in several epidemiological surveys and has shown good

predictive and discriminative validity against psychiatric diagnoses and good concurrent validity with the Rutter and Achenbach Screening Questionnaires. Furthermore, parent-rated SDQ scores of hyperactivity are known to be highly heritable [Martin, 1999]. Parent-rated SDQ has a sensitivity of 33.3% (5–10 years) and 45% (11–15 years) to predict any hyperkinetic disorder according to ICD-10, and a sensitivity of 29.9% (5–10 years) and 41% (11–15 years) to predict any ADHD disorder according to DSM-IV [Goodman et al., 2000]. The sensitivities for a combination of parent- and teacher-rated questionnaires to predict these clinical disorders are much higher, ranging from 75% to 85%, with predictions from teacher-rated questionnaires alone marginally better than parents alone.

Children were recruited to this study by contacting parents who were taking part in an unrelated study being carried out at the SGDP Research Centre. A large database of adults unselected for any phenotype had been established through a collaborative project with the Medical Research Council–funded GP Research framework [Sham et al., 2001]. The ethnicity of this population is 95% white Caucasian. Adults were contacted if they indicated they had children and agreed to take part in another study. Through this database, 12,000 adults were identified, asked whether they agreed to participate in the study, and, if so, to complete pSDQs on each of their children aged 5–15 years. The response rate was 30%, with 33% of responders willing to participate in this study. A total of 3,097 completed pSDQ scores were returned.

**Sample Selection**

Obtaining adequate statistical power to detect linkage to or association with genes for complex disorders can be difficult. One power-enhancing strategy that considers restraints such as genotyping and has been adopted in several QTL studies is the selective

sampling of phenotypically extreme individuals [Risch and Zang 1995; Van Gestel et al., 2000]. However, for a given population size, optimal selection criteria must take into account the range of possible underlying genetic models and balance out the effects of extreme selection with the requirement for large sample size. Furthermore, simulations by Allison et al. [1998] show that the *ceteris paribus* assumption (i.e., the more extreme the selection, the more power one achieves) is not always true, in particular where biallelic QTLs have relatively symmetric allele frequencies and smaller mean displacement among genotypes. Using simulations, we have investigated the optimum selection criteria for QTL association studies under a range of likely genetic models and find that a 20%–27% cutoff at both ends of the distribution provides the best solution (data not shown).

In our study, we decided a priori to select cases for DNA studies and further phenotypic measurement, using thresholds of the 10-point scale derived from the five ADHD items within the pSDQ. High scorers included all individuals scoring 7 and above out of the possible 10, and low scorers included those scoring 0 or 1. Teacher-rated SDQ questionnaires (tSDQ) were requested for selected children and mouth swabs taken for DNA extraction.

The frequency distribution for the five ADHD items from the pSDQ, for the total sample of 3,097 individuals, is shown in Figure 1. The frequencies of the various scores in this study are compatible with the results of a large epidemiological sample of approximately 10,300 U.K. children in the same age range [Goodman et al., 2000]. As can be seen, 17% of the sample scoring 7/10 and above were selected as high scorers, and 31% scoring less than or equal to 1/10 were low scorers. In total, DNA was obtained from 224 individuals (133 high scorers and 91 low scorers) for genotyping studies. Among the high scorers, the male:female ratio was 2:1, which is much lower than the male:female ratio found in clinical samples. In

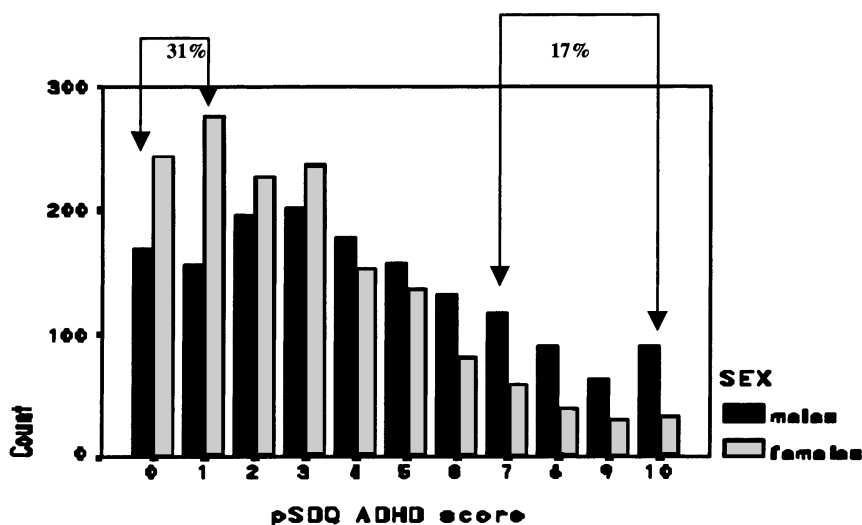


Fig. 1. Frequency distribution of parent ratings on the SDQ ADHD items in a population sample of 3,097 individuals.

contrast, the ratio was reversed for low scorers on the pSDQ scorers with male:female of 2:3.

### DNA Collection and Amplification of DRD4 Exon 3 VNTR

DNA was collected by mail using cotton wool buds and two 15 ml tubes containing 2.5 ml of a storage/preservative solution [STE buffer (100 mM NaCl, 10 mM Tris-HCl, pH 8, 10 mM EDTA, pH 8), with 0.2 mg/ml proteinase K and 0.5% SDS] [Freeman et al., 1997]. The average yield per individual was 120 µg DNA.

The exon 3 VNTR was amplified with an initial 5-min denaturing step at 95°C, followed by 35 cycles of 93°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. Primers used were 5'-GGTCTGCGGTGGAGTCTG-3' and 5'-GCGACTACGTGGTCTACT-3'. Reactions were performed in 22 µl volumes and included 50 ng of genomic DNA, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs (incorporating a 50/50 deaza dGTP/dGTP mix), 10% DMSO, 10 mM GeneAmp 10 × PCR Gold Buffer (PE Applied Biosystems), and 1 unit of AmpliTaq Gold (PE Applied Biosystems). PCR products were run out on a 2% agarose gel stained with ethidium bromide and analyzed under UV light. Homozygous genotypes were repeated if clear and strong bands were not observed. The ability of this protocol to detect the long 7-repeat allele in heterozygotes, which shows marked differential amplification with the common 2-, 3-, and 4-repeat alleles, has been examined in our laboratory by comparison with fluorescently tagged products visualized on an ABI 310 (PE Applied Biosystems) and found to be sufficiently sensitive. Each individual was genotyped twice, and discrepancies were rechecked with two further PCRs.

### Association Analysis With pSDQ Data

DRD4 allele frequencies for the high- and low-scoring groups are shown in Table I. In the comparison of the two groups, we found a significant difference between the frequencies of DRD4 alleles (chi-square = 14.37; df = 5;  $P = 0.013$ ). We then tested the specific hypothesis suggested by previous studies, i.e., DRD4-7 is the associated allele, by comparing the frequency of the 7-repeat allele to the other alleles combined [chi-square = 8.63; df = 1;  $P = 0.003$ ; OR = 2.09 (95% CI 1.24 < OR < 3.54)]. Genotype frequencies for the 7-repeat versus the rest were found to be in Hardy-Weinberg equilibrium (chi-square = 1.28; df = 1;  $P = 0.24$ ).

Further analysis performed using simple linear regression in SPSS, with the presence of the 7-repeat allele as the independent variable and pSDQ scores as

the dependent variable, was also significant ( $F$ -statistic = 7.245;  $P = 0.008$ ; standardized coefficient for the 7-repeat allele as beta = 0.178;  $t = 2.692$ ;  $P = 0.008$ ). Controlling for sex as a confounding variable, the  $P$  value was 0.009 and showed that female sex had a slightly negative influence with a standardized coefficient of -0.262. Using all alleles in a stepwise multiple regression, the only solution found to be significant was with DRD4-7 alone, rather than in combination with any of the other alleles.

We used a maximum-likelihood method implemented in Mx [Neale, 1997] to estimate the correlation between pSDQ scores in the complete data set of 3,000 individuals, with the presence of DRD4-7. This allowed for more accurate estimation of the correlation taking into account variance differences in measures due to selection on pSDQ. We found the correlation coefficient ( $r$ ) = 0.13, and therefore the variance ( $r^2$ ) in pSDQ scores accounted for by the association with DRD4-7 to be 2%.

### Association Analysis With Parent and Teacher SDQ Data

A maximum-likelihood method was again employed to estimate the correlation between pSDQ and tSDQ ADHD scores using MX ( $r = 0.48$ ). Parent and teacher SDQ data were available on a total of 143 out of the 226 individuals that were genotyped. The variance of the parent scores (1.67) was not greatly different from that of the teacher scores (1.74) and therefore the same weighting was given for both scores.

Taking individuals scoring  $\geq 7/10$  on the pSDQ ADHD scale and  $\geq 4/10$  on the tSDQ ADHD scale (we were not stringent here as we had selected the sample on parent scores), we categorized individuals into pervasively high or pervasively low (pSDQ  $\leq 1$ ; tSDQ < 4) groups. There were 51 individuals in the high pervasive category and 57 individuals in the low pervasive category. The association of DRD4-7 with scores in the high pervasive versus low pervasive subgroups did not reach nominal significance levels, but showed a trend in the expected direction [chi-square = 2.64;  $P = 0.1$ ; OR = 1.78 (95% CI 0.84 < OR < 3.82)] and gave rise to DRD4-7 frequencies that were proportionally similar to those in the total pSDQ sample (Table II).

## DISCUSSION

With consistent evidence from the field of behavior genetics that ADHD has an important genetic component, there is now much international interest in trying to identify susceptibility genes for the disorder.

TABLE I. DRD4 Allele Counts and Frequencies (in Brackets) in High and Low Scorers on pSDQ ADHD Items\*

	2-repeat	3-repeat	4-repeat	5-repeat	7-repeat	8-repeat
High-score group	16 (16.0%)	8 (3.0%)	170 (63.9%)	1 (0.4%)	70 (26.3%)	1 (0.4%)
Low-score group	18 (9.9%)	8 (4.4%)	124 (68.1%)	2 (1.1%)	26 (14.3%)	4 (2.2%)

\*The high group score  $\geq 7$ , the low group  $\leq 1$  on pSDQ. No 6-repeats were found in this sample.

TABLE II. DRD4–7 Allele Count and Frequencies (in Brackets) in Pervasively High- and Pervasively Low-Group Scorers on Parent and Teacher SDQ Items

	7-repeat allele	Non-7-repeat alleles
High pervasive group	23 (22.5%)	79 (77.5%)
Low pervasive group	16 (14.0%)	98 (86.0%)

Although molecular studies to date have focused on clinical samples of ADHD, there is substantial support for a dimensional perspective on ADHD, viewing it as the extreme of a continuously distributed trait, and therefore good reason to adopt potentially powerful quantitative trait methods. In this study, we provide further support for the QTL approach by replicating the finding of association between DRD4–7 and clinical ADHD, in a sample of children selected on the basis of high (top 17%) and low (bottom 31%) parent-rated ADHD scores, from a population sample unselected for phenotype. The selection criteria used approximations to the cutoffs suggested by statistical simulations to maximize power. The significance of the result was similar using a simple chi-square test to compare the low- and high-scoring groups as for a linear regression test using continuous trait scores (though of course not all data from the screened population were available for this analysis).

The main limitations to this study are, first, that it is difficult to rule out population stratification as a reason for the positive findings. Although our genotypes are in Hardy-Weinberg equilibrium, we cannot systematically eliminate the possibility of recent population admixture. Secondly, the sample is selected on the basis of scores on a parent scale that only moderately predicts clinical ADHD, even though it does show high heritability, and thus the significance of the findings to the clinical disorder remains uncertain. However, combining parent and teacher ratings gives improved prediction of the clinical disorder. It is therefore notable that although the association did not reach nominal significance when parent and teacher scores were combined ( $P = 0.1$ ), the size of the effect observed in the subsample used for this analysis was of the same order found for the analysis of pSDQ data alone (pervasive subsample being 50% of total sample). A power calculation showed that in the pervasive subsample, we only had 80% power to replicate a genotypic association with an odds ratio of 4, at an alpha level of 0.05. Taken together, these findings and the previous well-replicated association between DRD4–7 and clinical ADHD support the notion that our QTL approach has the ability to detect a finding that is clinically relevant.

Although the QTL approach to ADHD appears to be a promising one, there remain some problem areas that we need to concern ourselves with at the outset. Phenotypic measurement remains a controversial issue. The task here is considerably more challenging than a simple measurement of blood pressure to investigate QTLs for hypertension. Findings from behavioral genetic studies show considerable variation depending on the source of information (parent, teacher, indivi-

dual), or the type and number of assessment instruments, all of which have modest correlations.

Two recent sets of twin data exemplify the important issues. Thapar et al. [2000] found heritability for ADHD considered as a dimensional trait to be considerably higher when estimated by parent ratings on the Rutter scale (0.84) than when estimated by parent ratings on the Du Paul scale (0.47). These results contrasted with another [Martin, 1999] recent twin study, in which heritability estimates for parent ratings of ADHD items from the SDQ were 0.58, while those for parent ratings on the short Conners scale were 0.78. In this study, bivariate analysis of parent SDQ and Conners ratings showed a large shared genetic component (50%) between the two rating scales, and that all of the genetic variance for the SDQ scale was included in this, but that the Conners scale detected a further 24%. A similar result was found for teacher ratings on the two questionnaires, suggesting that for ADHD, scales containing more items capture a greater proportion of genetic variance. Both studies appear to show reduced or absent contrast effects with the longer Du Paul and Conners rating scales. Thapar et al. [2000] formally tested for this and showed rater contrast effects when using the Rutter, but not the Du Paul scale (apart from inattention items alone on the Du Paul).

The use of questionnaire rating scales versus clinical interviews in behavior genetic studies may introduce further variation; questionnaires belonging to the psychometric tradition that assumes disorders are extremes on the same underlying continuum that describes variation in the normal range, whereas clinical interviews derive from the medical tradition for categorizing disorders. Genetic and environmental effects might differ, because the individuals fitting into the clinical extremes may have a different balance of environmental and genetic risk factors.

Finally, behavior genetic studies of ADHD have in general used parent ratings alone. However, estimates of heritability that combine parent and teacher ratings [Thapar et al., 2000] or use structural equation modeling to create a latent trait from multiple raters and rating scales [Simonoff et al., 1998] are as high or better (in the case of the latent trait) than parent- and teacher-rated scales alone. Given that parent-only-rated scales predict a clinical diagnosis of ADHD much less often than when combined with a teacher rating and are more subject to rater bias, the most robust method may be to use multiple measures and informants to create a heritable latent trait.

In conclusion, we have replicated the DRD4–7 association with ADHD using a QTL approach in a general population sample. This suggests that DRD4–7 or a functional variant in LD with that allele is a QTL that exerts influence over the range of hyperactivity scores in a population, rather than a risk factor for narrow diagnostic subtypes. We further suggest that these findings support the use of QTL approaches to the identification of novel QTLs for ADHD. With the problem of nonreplication of association findings already evident, we suggest that a unified approach to phenotypic measurement should be considered.

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## REFERENCES

- Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majali KM, Trembling PM, Mann CJ, Shoulders CC, Graf D, St Lezin E, Kurtz TW, Kren V, Pravenec M, Ibrahimi A, Abumrad NA, Stanton LW, Scott J. 1999. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* 21:76–83.
- Allison DB, Heo M, Schork NJ, Wong S-L, Elston RC. 1998. Extreme selection strategies in gene mapping studies of oligogenic quantitative traits do not always increase power. *Hum Hered* 48:97–107.
- Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH. 1995. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J Neurochem* 65:1157–1165.
- Barkley RA. 1996. Attention-deficit/hyperactivity disorder. In: Mash EJ, Barkley RA, editors. *Child psychopathology*. New York: Guilford Press. p 63–112.
- Barr CL, Feng Y, Wigg KG, Schacher R, Tannock R, Robert W. 2001. 5'-untranslated region of the dopamine D4 receptor gene and attention deficit hyperactivity disorder. *Am J Med Genet (Neuropsychiatr Genet)* 105:84–90.
- Biederman J, Faraone SV, Keenan K, Knee D, Tsuang MT. 1990. Family-genetic and psychosocial risk factors in DSM-III attention deficit disorder. *J Am Acad Child Adolesc Psychiatry* 29:526–533.
- Biederman J, Faraone S, Keenan K, Benjamin J, Krifcher B, Moore C, Sprich-Buckminster S, Ugalia K, Jellinek MS, Steingard R, Spencer T, Norman D, Kolodny R, Kraus I, Perrin J, Keller MB, Tsuang MT. 1992. Further evidence for family-genetic risk factors in attention deficit hyperactivity disorder: patterns of comorbidity in probands and relatives in psychiatrically and paediatrically referred samples. *Arch Gen Psych* 49:728–738.
- Biederman J, Faraone SV, Doyle A, Lehman BK, Kraus I, Perrin J, Tsuang MT. 1993. Convergence of the Child Behaviour Checklist with structured interview-based psychiatric diagnoses of ADHD children with and without comorbidity. *J Child Psychol Psychiatry* 34:1241–1251.
- Biederman J, Faraone SV, Mick E, Moore P, Lelon E. 1996. Child behaviour checklist (CBCL) findings further support comorbidity between ADHD and major depression in a referred sample. *J Am Acad Child Adolesc Psychiatry* 35:734–742.
- Bird H, Canino G, Gould M, Ribera J, Rubio-Stipec M, Woodbury M, Huertas-Goldman H, Sesman M. 1987. Use of the child behaviour checklist as a screening instrument for epidemiological research in child psychiatry: results of a pilot study. *J Am Acad Child Adolesc Psychiatry* 26:207–213.
- Boyle M, Offord D, Racine Y, Szatmari P, Sanford M, Fleming J. 1997. Adequacy of interviews vs checklists for classifying childhood psychiatric disorder based on parent reports. *Arch Gen Psychiatry* 54:793–799.
- Caldarone B, Saavedra C, Tartaglia K, Wehner JM, Dudek BC, Flaherty L. 1997. Quantitative trait loci analysis affecting contextual conditioning in mice. *Nat Genet* 17:335–337.
- Cantwell DP. 1972. Psychiatric illness in families of hyperactive children. *Arch Gen Psychiatry* 27:728–738.
- Cantwell DP. 1996. Attention deficit disorder: a review of the past 10 years. *J Am Acad Child Adolesc Psychiatry* 35:978–987.
- Castellanos FX. 1997. Toward a pathophysiology of attention deficit hyperactivity disorder. *Clin Pediatr* 36:381–393.
- Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL, Sidransky E. 1998. Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. *Mol Psychiatry* 3:431–434.
- Chen W, Faraone S, Biederman J, Tsuang M. 1994. Diagnostic accuracy of the child behaviour checklist scales for attention deficit hyperactivity disorder: a receiver operating characteristic analysis. *J Consult Clin Psychol* 62:1017–1025.
- Crabbe JC, Phillips TJ, Buck KJ, Cunningham CL, Belknap JK. 1999. Identifying genes for alcohol and drug sensitivity: recent progress and future directions. *Trends Neurosci* 22:173–179.
- Eaves LJ, Silberg JL, Meyer JM, Maes HH, Simonoff E, Pickles A, Rutter M, Neale MC, Reynolds CA, Erickson MT, Heath AC, Loeber R, Truett KR, Hewitt JK. 1997. Genetics and developmental psychopathology. II. The main effects of genes and environment on behavioural problems in the Virginia Twin Study of Adolescent Behavioural Development. *J Child Psychol Psychiatry* 38:965–980.
- Edelbrock C. 1986. Behavioural ratings of children diagnosed for attention deficit disorder. *Psychiatr Ann* 16:36–40.
- Eisenberg J, Zohar A, Mei-Tal G, Steinberg A, Tartakovsky E, Gritsenko I, Nemanov L, Ebstein RP. 2000. A haplotype relative risk study of the dopamine D4 receptor (DRD4) exon III repeat polymorphism and attention deficit hyperactivity disorder (ADHD). *Am J Med Genet* 96:258–261.
- Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J. 1999. Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am J Psychiatry* 156:768–770.
- Faraone SV, Doyle AE, Mick E, Biederman J. 2001. Meta-analysis of the association between the dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. *Am J Med Genet (Neuropsychiatr Genet)* (in press).
- Fergusson DM, Horwood LJ, Jensen PS. 1995. Predictive validity of categorically and dimensionally scored measures of disruptive childhood behaviours. *J Am Acad Child Adolesc Psychiatry* 34:477–485.
- Fisher PJ, Turic D, Williams NM, McGuffin P, Asherson P, Ball D, Craig I, Eley T, Hill L, Chorney K, Chorney MJ, Benbow CP, Lubinski D, Plomin R, Owen MJ. 1999. DNA pooling identifies QTLs on chromosome 4 for general cognitive ability in children. *Hum Mol Genet* 8:915–922.
- Fisher SE, Stein JF, Monaco AP. 1998. A genome-wide search strategy for identifying quantitative trait loci involved in reading and spelling disability (developmental dyslexia). *Eur Child Adolesc Psychiatry* 8 (Suppl 3):47–51.
- Fisher SE, Marlow AJ, Lamb J, Maestrini E, Williams DF, Richardson AJ, Weeks DE, Stein JF, Monaco AP. 1999. A quantitative-trait locus on chromosome 6p influences different aspects of developmental dyslexia. *Am J Hum Genet* 64:146–156.
- Freeman B, Ball D, Powell J, Craig I, Plomin R. 1997. DNA by mail: the usefulness of cheek scrapings. *Behav Genet* 27:251–257.
- Gaub M, Carlson CL. 1997. Gender differences in ADHD: a meta-analysis and critical review. *J Am Acad Child Adolesc Psychiatry* 36:1036–1044.
- Gayán J, Smith SD, Cherny SS, Cardon LR, Fulker DW, Brower AM, Olson RK, Pennington BF, DeFries JC. 1999. Quantitative-trait locus for specific language and reading deficits on chromosome 6p. *Am J Hum Genet* 64:157–164.
- Gillis JJ, Gilger JW, Pennington BF, DeFries JC. 1992. Attention deficit disorders in reading disabled twins: evidence for a genetic aetiology. *J Abn Child Psychol* 20:303–315.
- Gjone H, Stevenson J, Sundet JM. 1996. Genetic influence on parent-reported attention-related problems in a Norwegian general population twin sample. *J Am Acad Child Adolesc Psychiatry* 35:691–709.
- Goldman-Rakic PS. 1992. Topography of cognition: parallel distribution networks in primate association cortex. *Ann Rev Neurosci* 11:137–156.
- Goodman R. 1997. The strengths and difficulties questionnaire: a research note. *J Child Psychol Psychiatry Appl Discip* 38:581–586.
- Goodman R, Stevenson J. 1989a. A twin study of hyperactivity. I. An examination of hyperactivity scores and categories derived from Rutter teacher and parent questionnaires. *J Child Psychol Psychiatry* 30:671–689.
- Goodman R, Stevenson J. 1989b. A twin study of hyperactivity. II. The aetiological role of genes, family relationships and perinatal adversity. *J Child Psychol Psychiatry* 30:691–709.
- Goodman R, Ford T, Simmons H, Gatward R, Meltzer H. 2000. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. *Br J Psychiatry* 177:534–539.
- Hawi Z, McCarron M, Kirley A, Daly G, Fitzgerald M, Gill M. 2000. No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. *Am J Med Genet* 96:268–272.
- Hill JC, Schoener EP. 1996. Age dependent decline of ADHD. *Am J Psychiatry* 153:1143–1146.

- Hill L, Craig IW, Asherson P, Ball D, Eley T, Ninomiya T, Fisher PJ, Turic D, McGuffin P, Owen MJ, Chorney K, Chorney MJ, Benbow CP, Lubinski D, Thompson LA, Plomin R. 1999. DNA pooling and dense marker maps: a systematic search for genes for cognitive ability. *Neuroreport* 10:843–848.
- Holmes J, Payton A, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Thapar A. 2000. Family-based and case control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. *Mol Psychiatry* 5:523–530.
- Hsueh WC, Mitchell BD, Schneider JL, Wagner MJ, Bell CJ, Nanthakumar E, Shuldiner AR. 2000. QTL influencing blood pressure maps to the region of PPH1 on chromosome 2q31–34 in old order Amish. *Circulation* 101:2810–2816.
- Hudziak JJ. 1997. Identification of phenotypes for molecular genetic studies of common childhood psychopathology. In: Blum K, Noble EP, editors. *Handbook of psychiatric genetics*. Boca Raton, FL: CRC Press. p 201–217.
- Jovanovic V, Guan HC, Van Tol HH. 1999. Comparative pharmacological and functional analysis of the human dopamine D4.2 and D4.10 receptor variants. *Pharmacogenetics* 9:561–568.
- Kazmi MA, Snyder LA, Cypess AM, Graber SG, Sakmar TP. 2000. Selective reconstitution of human D4 dopamine receptor variants with Gi alpha subtypes. *Biochemistry* 39:3734–3744.
- Kotler M, Manor I, Sever Y, Eisenberg J, Cohen H, Ebstein RP, Tyano S. 2000. Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. *Am J Med Genet* 96:278–281.
- LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, et al. 1996. Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. *Mol Psychiatry* 1:121–124.
- Lembertas AV, Perusse L, Chagnon YC, Fislis JS, Warden CH, Purcell-Huynh DA, Dionne FT, Gagnon J, Nadeau A, Lussis AJ, Bouchard C. 1997. Identification of an obesity quantitative trait locus on mouse chromosome 2 and evidence of linkage to body fat and insulin on the human homologous region 20q. *J Clin Invest* 100:1240–1247.
- Levy F, Hay D, McLaughlin M, Wood C, Waldman I. 1996. Twin sibling differences in parental reports of ADHD, speech, reading and behaviour problems. *J Child Psychol Psychiatry* 37:569–578.
- Levy F, Hay DA, McStephen M, Wood C, Waldman I. 1997. Attention-deficit hyperactivity disorder: a category or a continuum? genetic analysis of a large-scale twin study. *J Am Acad Child Adolesc Psychiatry* 36:737–744.
- Mannuzza S, Klein RG. 2000. Long-term prognosis in attention-deficit/hyperactivity disorder. *Child Adolesc Psychiatr Clin N Am* 9:711–726.
- Martin N. 1999. A twin study of the genetics of childhood attention deficit hyperactivity disorder and its comorbidity with conduct disorder. PhD thesis. University College of Wales, College of Medicine.
- McCracken J. 1991. A two-part model of stimulant action on attention deficit hyperactivity in children. *J Neuropsychiatry* 3:201–209.
- Mill J, Curran S, Kent L, Richards S, Gould A, Virdee A, Hockett L, Sharp J, Batten C, Fernando S, Simonoff E, Thompson M, Zhao J, Sham P, Taylor E, Asherson P. 2001. Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. *Mol Psychiatry* (in press).
- Moisan MP, Courvoisier H, Bihoreau MT, Gauguier D, Hendley ED, Lathrop M, James MR, Mormede P. 1996. A major quantitative trait locus influences hyperactivity in the WKHA rat. *Nat Genet* 14:471–473.
- Mu JL, Naggert JK, Svenson KL, Collin GB, Jung Han Kim, McFarland C, Nishina PM, Levine DM, Williams KJ, Paigen B. 1999. Quantitative trait loci analysis for the differences in susceptibility to atherosclerosis and diabetes between inbred mouse strains C57BL/6J and C57BLKS/J. *J Lipid Res* 40:1328–1335.
- Muglia P, Jain U, Macciardi F, Kennedy JL. 2000. Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene. *Am J Med Genet* 96:273–277.
- Neale MC. 1997. *Mx: statistical modelling*. Richmond, VA: Department of Human Genetics.
- Risch N, Zhang H. 1995. Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science* 268:1584–1589.
- Rowe DC, Stever C, Giedinghagen LN, Gard JM, Cleveland HH, Terris ST, Mohr JH, Sherman S, Abramowitz A, Waldman ID. 1998. Dopamine DRD4 receptor polymorphism and attention deficit hyperactivity disorder. *Mol Psychiatry* 3:419–426.
- Sham PC, Sterne A, Purcell S, Cherny SS, Webster M, Rijdsdijk F, Asherson P, Ball D, Craig I, Eley T, Goldberg D, Gray J, Mann A, Owen M, Plomin R. 2001. GENESIS: creating a community-based sample of sibships for QTL analysis of anxiety and depression. *Twin Research* (in press).
- Shaywitz BA, Cohen DJ, Bower NB. 1977. CSF amine metabolites in children with minimal brain dysfunction (MBD): evidence of involvement of brain dopamine. *J Paediatr* 90:67–71.
- Shekim WO, Dekirmenjian H, Chapel JL. 1979. Urinary MHPG excretion in minimal brain dysfunction and its modification by d-amphetamine. *Am J Psychol* 136:667–671.
- Shen YC, Wang YF. 1984. 3-methoxy-4-hydroxyphenylglycol-sulfate excretion in 73 school children with minimal brain-dysfunction syndrome. *Biol Psychiatry* 19:861–870.
- Sherman DK, Iacono WG, McGue M. 1997. Attention-deficit hyperactivity disorder dimensions: a twin study of inattention and impulsivity-hyperactivity. *J Am Acad Child Adolesc Psychiatry* 36:745–753.
- Silberg J, Rutter M, Meyer J, Maes H, Hewitt J, Simonoff E, Pickles A, Loeber R. 1996. Genetic and environmental influences on the covariation between hyperactivity and conduct disorder in juvenile twins. *J Child Psychol Psychiatry* 37:803–816.
- Simonoff E, Pickles A, Hervas A, Silberg JL, Rutter M, Eaves L. 1998. Genetic influences on childhood hyperactivity: contrast effects imply parental rating bias, not sibling interaction. *Psychol Med* 28:825–837.
- Smalley SL, Bailey JN, Palmer CG, Cantwell DP, McGough JJ, Del'Homme MA, Asarnow JR, Woodward JA, Ramsey C, Nelson SF. 1998. Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. *Mol Psychiatry* 3:427–430.
- Stevenson J. 1992. Evidence for a genetic aetiology in hyperactivity in children. *Behav Genet* 22:337–344.
- Sunohara GA, Roberts W, Malone M, Schachar R, Tannock R, Basile V, Wigal T, Wigal SB, Schuck S, Moriarty J, Swanson JM, Kennedy JL, Barr CL. 2000. Linkage of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 39:1537–1542.
- Swanson JM, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, LaHoste GJ, Wigal S. 1998. Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. *Mol Psychiatry* 31:38–41.
- 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.
- Taylor E, Chadwick O, Heptinstall E, Danckaerts M. 1996. Hyperactivity and conduct problems as risk factors for adolescent development. *J Am Acad Child Adolesc Psychiatry* 35:1213–1226.
- Thapar A. 1995. Childhood hyperactivity scores are highly heritable and show sibling competition effects: twin study evidence. *Behav Genet* 25:537–543.
- Thapar A, Harrington R, Ross K, McGuffin P. 2000. Does the definition of ADHD affect heritability? *J Am Acad Child Adolesc Psychiatry* 39:1528–1536.
- Van Gestel S, Houwing-Duistermaat JJ, Adolfsson R, Van Duijn CM, Van Broeckhoven C. 2000. Power of selective genotyping in genetic association analyses of quantitative traits. *Behav Genet* 30:141–146.
- Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow J Jr, Birznieks G, Chang J, Eldridge W. 2000. The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. II. An autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–2000.
- Watts VJ, Vu MN, Wiens BL, Jovanovic V, Van Tol HH, Neve KAS. 1999. *Psychopharmacology (Berl)* 141:83–92.
- Zametkin AJ, Karoum F, Rapoport JL, Brown GL, Wyatt RJ. 1984. Phenylethylamine excretion in attention deficit disorder. *J Am Acad Child Adolesc Psychiatry* 23:310–314.