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The dopamine D4 receptor and the hyperactivity phenotype: a developmental-epidemiological study

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Attention-deficit hyperactivity disorder (ADHD) affects 2–6% of school-age children and is a precursor of behavioural problems in adolescence and adulthood. Underlying the categorical definition of ADHD are the quantitative traits of activity, impulsivity, and inattention which vary continuously in the population. Both ADHD and quantitative measures of hyperactivity are heritable, and influenced by multiple genes of small effect. Several studies have reported an association between clinically defined ADHD and the seven-repeat allele of a 48-bp tandem repeat polymorphism in the third exon of the dopamine D4 receptor gene (DRD4). We tested this association in a large, unselected birth cohort (*n* = 1037) using multiple measures of the hyperactivity phenotype taken at multiple assessment ages across 20 years. This longitudinal approach allowed us to ascertain whether or not DRD4 has a general effect on the diagnosed (*n* = 49) or continuously distributed hyperactivity phenotype, and related personality traits. We found no evidence to support this association.
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Introduction

Attention-deficit hyperactivity disorder (ADHD) is characterised by hyperactivity, impulsivity, and impairments in attention. It affects between 2–6% of school-age children, with a strong male sex-bias. The long-term prognosis of children diagnosed with ADHD is poor, with an increased risk of persistent psychopathology in adolescence and adulthood, often manifesting itself in a range of disruptive and antisocial behaviours. Childhood hyperactivity has been associated with drug and alcohol abuse, poor educational achievement, peer-relationship problems, crime and delinquency.[1,2]

ADHD, as defined by operational criteria, is a dichotomous trait making up a distinct diagnostic category. However, measures of activity, impulsivity, and inattention have been shown to vary quantitatively through the population, suggesting that clinical ADHD should be regarded as the extreme of a continuously-distributed trait rather than as a discrete category.[3] Twin and adoption studies have demonstrated that the aetiology of ADHD has a large genetic component, with heritability estimates ranging from ~0.5 to ~0.9.[4] Genetic influences appear to be the same for both males and females, and are consistent from early childhood to adolescence.[5] It is likely that ADHD is determined by the additive action of numerous genes, or quantitative trait loci (QTLs), of relatively small effect.[6]

Several candidate loci have been studied in clinical ADHD samples, with most association studies focusing on loci involved in the brain's mesolimbic dopamine system. This links regions of the brain involved in emotional/affective processing and executive functioning—the very functions which are postulated to be dysfunctional in ADHD patients. There is considerable biochemical and pharmacological evidence to suggest that the dopamine system plays a major role in ADHD.[7] Stimulant medications that have a strong therapeutic effect on hyperactivity act by inhibiting the reuptake of catechoholamines such as dopamine at the synapse. Furthermore, PET imaging studies of the brain suggest there is dopaminergic dysfunction at the level of dopaminergic nuclei in children with ADHD.[8] Several dopamine-related candidate genes have been investigated to date, including the dopamine transporter gene (*DAT1*), genes encoding the numerous dopamine receptors (*DRD1–DRD5*), and loci coding for proteins involved in the metabolism of dopamine including *DBH* and *MAOA*.

The most consistent findings have been with the

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dopamine D4 receptor (*DRD4*) gene. The *DRD4* gene contains a 48 base-pair variable number of tandem repeats (VNTR) polymorphism in its third exon, encoding a portion of the third intracellular loop region of the transcribed protein that spans the nerve cell membrane and mediates interaction with secondary signalling proteins. The number of repeats ranges from 2 to 11, and although the functional significance of this polymorphism is yet to be ascertained, evidence suggests that different D4 receptor variants may display different pharmacological properties.[9] This polymorphism was first reported to be associated with novelty seeking and impulsivity,[10,11] two personality traits that are correlated with ADHD, and subsequently it has been assayed in relation to a broad range of clinical disorders. Twelve published studies have shown association or association and linkage between the 7-repeat allele of *DRD4* and ADHD,[12–23] whereas seven have found no association.[24–30] A recent meta-analysis of published and unpublished data calculated an odds ratio (OR) of 1.9 (95% CI: 1.4–2.2, $P = 0.00000008$) from seven case-control studies and 1.4 (95% CI: 1.1–1.6, $P = 0.02$) from 14 family-based studies.[31]

Difficulties in replicating QTL findings in psychiatry have prompted a call for well-characterised samples with a wealth of phenotypic data.[32] To examine the relationship between DRD4 and hyperactivity, this article uses data from a developmental-epidemiological study of psychiatric disorders. The Dunedin Study offers several methodological strengths. First, it is an investigation of a complete birth cohort. Such a sampling frame avoids biases inherent in identifying cases and recruiting volunteer controls for clinical studies.[33] Second, in the Dunedin Study data about the hyperactivity phenotype have been collected at multiple ages during childhood, adolescence, and adulthood. Such repeated testing offers the opportunity to test whether the association between DRD4 and hyperactivity is developmentally robust and obtains at different periods in development. Third, data have been collected via psychiatric interviews with the Study members, dimensional rating scales obtained from parents and teachers, and using self-reports of personality scales of impulsivity. This multimethod-multisource data-collection strategy offers the opportunity to test whether or not the association between DRD4 and hyperactivity is robust to different methods of phenotypic assessment.[34]

Materials and methods

Participants are members of the Dunedin Multidisciplinary Health and Development Study, a longitudinal investigation of health and behaviour in a complete birth cohort.[35] The study members were born in Dunedin, New Zealand between April 1972 and March 1973. Of these, 1037 children (91% of eligible births; 52% male) participated in the first follow-up assessment at age 3, and they constitute the base sample for the remainder of the study. Cohort families represent the full range of socio-economic status in the general population of New Zealand's South Island and are primarily white. Follow-ups have been carried out at ages 5 ($n = 991$), 7 ($n = 954$), 9 ($n = 955$), 11 ($n = 925$), 13 ($n = 850$), 15 ($n = 976$), 18 ($n = 993$), 21 ($n = 992$), and most recently at 26 years when 980 (96%) of the 1019 Study members still alive were assessed. The basic procedure involves bringing participants to the research unit within 60 days of their birthday for a full day of individual data collection. The various research topics are presented as standardised modules, each administered by a different trained examiner. At each assessment, interview data are supplemented by questionnaires completed by persons who know the subject well (eg parents, teachers).

Each of the phenotypic measures described in this article has been published earlier in the course of the longitudinal study. All have reliabilities >0.70. For each measure, we cite a methodological paper from the Dunedin study that may be consulted for further details about the reliability and validity of each measure.

Parent reports of hyperactivity

At ages 7, 9, and 11 parents completed the Rutter Child Scale (RCS)[36,37] questionnaire that inquires about a child's symptoms of behavioural and emotional disorder during the past year. Parents rate each behaviour on the RCS as 'does not apply' (0), 'applies somewhat' (1), or 'certainly applies' (2). To measure hyperactivity, we used scores from the RCS hyperactivity scale, supplemented with items concerning inattention, impulsivity, and hyperactivity that were derived from the Diagnostic and Statistical Manual of Mental Disorders (DSM-III) diagnostic criteria for Attention Deficit Disorder (ADHD).[38] At ages 13 and 15, parents completed the Revised Behavior Problem Checklist (RBPC),[39] which contains more extensive and age-appropriate items than the RCS. Items were scored 0, 1, or 2, as they were for the RCS.[40]

Teacher reports of hyperactivity

At ages 7, 9, 11, and 13 teachers completed the teacher version of the Rutter Child Scale,[32] supplemented with DSM-III ADHD items and scored in the same way as its parent counterpart.[38]

Psychiatric diagnosis of ADHD

At ages 11, 13, and 15, Study members were interviewed using the Diagnostic Interview Schedule for Children—Child version (DISC-C),[41] with a reporting period of 12 months at each age.[42] Interviews were conducted by a psychiatrist or clinical psychologist in private, standardised sessions. Diagnoses were made via computer algorithms following the then-current version of the Diagnostic and Statistical Manual of Mental Disorders.[43] Diagnoses were corroborated by parents' and/or teachers' reports of current symptoms, and symptom onset by age 7 was established using parent and teacher reports from assessments at ages 5 and 7.[44,45] Clinical assessment and treatment was reported by parents for 64% of the diagnosed children. In this article, we report on Study members who met diagnos-

tic criteria for ADHD between ages 10–15 ($n = 49$, 5.8% of sample, 80% males).

Personality reports of impulsivity
At ages 18 and 26, Study members completed the Multidimensional Personality Questionnaire (MPQ), a self-report personality instrument designed to assess a broad range of individual differences in affective and behavioural style, and whose phenotypic structure is strongly related to the genetic components of personality.[46] In this article, we focus on Constraint, a highly reliable personality superfactor which is a combination of the primary traits of Traditionalism, Harm-Avoidance, and Self-Control. Individuals high on Constraint tend to endorse social norms, act in a cautious and restrained manner, and avoid thrills; individuals who score low on this superfactor are more likely to exhibit disorders of impulse control.[47] Individual differences in Constraint are highly correlated with personality measures of novelty-seeking and impulsivity that have been explored in previous studies of DRD4.[48,49]

At age 26, we also asked Study members to nominate someone who knew them well and mailed 'informants' the Big Five Inventory which assesses the Five-Factor Model of personality,[50] including the Conscientiousness superfactor which has been examined in previous studies of DRD4.[51] Conscientiousness describes the extent and strength of impulse control; individuals high on Conscientiousness are planful, reliable, and able to delay gratification.[52] There is good evidence for the convergence of self and informant reports of Conscientiousness.[53]

DNA extraction and genotyping
At age 26, DNA was obtained from 953 Study members (97.3% of those assessed at that age); 93% of the DNA samples were obtained via blood drawn by a registered nurse and 7% were obtained using buccal swabs where Study members did not wish to undergo phlebotomy.[54] DNA was extracted from blood samples using standard procedures.[55,56] A modified procedure was used to extract DNA from buccal cells.[57] The exon 3 VNTR was amplified on an MJ PTC-225 thermal cycler (MJ Research, MA, USA) with an initial 9-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′-GGT CTG CGG TGG AGT CTG-3′ and 5′-GCG ACT ACG TGG TCT ACT-3′. Reactions were performed in 22-μl volumes and included 50 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (incorporating a 50/50 deaza dGTP/dGTP mix), 10% DMSO, 10 mM GeneAmp 10 × PCR Gold Buffer (PE Applied Biosystems, Foster City, USA) and 1 unit of AmpliTaq Gold (PE Applied Biosystems). PCR products were run out on a 2% agarose gel stained with ethidium bromide and analysed under UV light. Reactions for 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 from the common 2, 3 and 4 repeat alleles, has been examined in our laboratory by comparison with fluorescently tagged products visualised on an ABI 310 genetic analyser (PE Applied Biosystems) and found to be sufficiently sensitive to unambiguously detect the 7-repeat allele.

Allele and genotype frequencies
To avoid potential problems of population stratification, individuals of Maori origin were not included in this analysis; 880 Caucasian individuals were used. Caucasian study members reported the ethnicity of all four grandparents, and only 4% reported one or two non-European grandparents. Table 1 shows the allele frequencies observed among non-Maori members of the the Dunedin Study. The four-repeat allele was the most common (65.0%), followed by the seven-repeat (19.4%) and two-repeat (8.8%) alleles. Figure 1 compares the frequencies observed in the Dunedin Study to those reported for various non-clinical Caucasian samples and various ethnic groups world-wide. Whilst DRD4 allele frequencies can be seen to fluctuate considerably between continents, the allele frequencies in the Dunedin cohort match those of their North European ancestor populations very closely.

Statistical analysis
Because we had an *a priori* hypothesis that the postulated risk conferred by DRD4 was associated with the 7-repeat allele, the sample was split into three groups based on their genotype: those homozygous for allele 7 (7/7; $n = 38$, 4.3% of the sample, 53% male), those heterozygous for allele 7 and any other allele (7/N7; $n = 266$, 30.2% of the sample, 49% male), and those without allele 7 (N7/N7; $n = 576$, 66% of the sample, 52% male). Because different quantitative hyperactivity phenotypes were collected at different ages and from different sources, we standardised each of the measures using the z-score transformation; each phenotype thus had a mean of 0 and a standard deviation (SD) of 1. We present the means standardised for the full sample, as well as the results standardised within sex. One-way analyses of variance, with genotype as the grouping variable, were calculated for males and females, separately. Using the reported z-scores, it is also possible to calculate the effect sizes between the groups (in SD units; d), where $d = 0.2$ is a small effect size, $d = 0.5$ is a medium effect size, and $d = 0.8$ is a

Table 1 DRD4 exon 3 VNTR allele frequencies in non-Maori members of the Dunedin birth-cohort

Repeat number	*Allele frequency (%)*
2	8.8
3	4.6
4	65.0
5	0.9
6	0.6
7	19.4
8	0.6

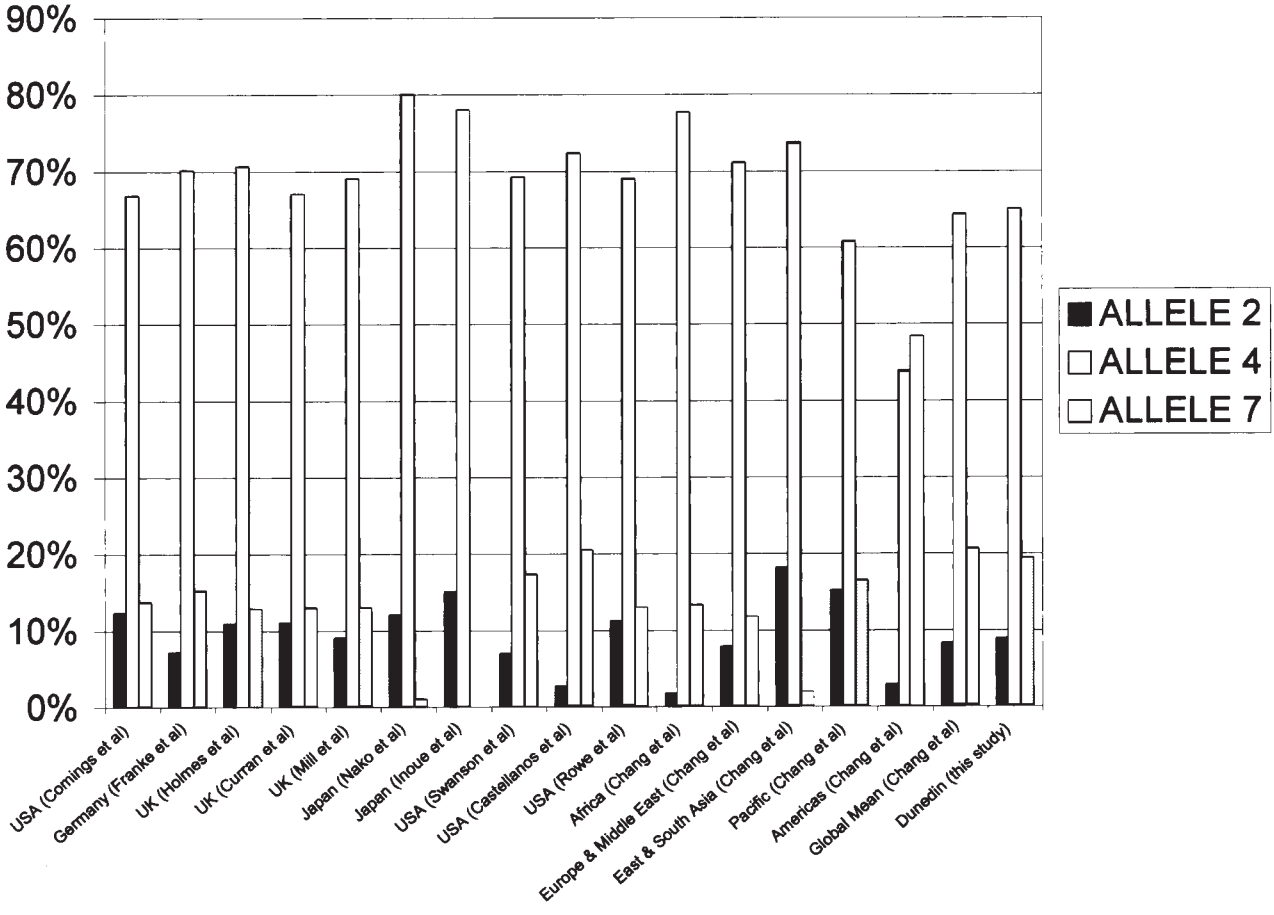

Figure 1 Worldwide allele frequencies for the DRD4 exon three VNTR polymorphism.[13,15,20–22,24,72–76]

large effect size.[58] The personality variables were normally distributed. However, the parent- and teacher-reports of hyperactivity were positively skewed; sensitivity analyses were thus performed using a logarithmic transformation of the scales, and the results remained unchanged in terms of substantive meaning and statistical significance. The association between DRD4 and a diagnosis of ADHD was examined via a 3 (genotype) × 2 (disorder) χ^2 analysis.

Results

Quantitative measures of hyperactivity

Table 2 shows standardised mean scores for the three genotype groups on quantitative measures of parent-rated hyperactivity, at ages 7, 9, 11, 13, and 15. There was no statistically significant association between hyperactivity and the seven-repeat allele at any of the assessment stages. Table 3 shows the standardised mean scores for the three genotype groups on quantitative measures of teacher-rated hyperactivity, at ages 7, 9, 11, and 13. Here too there was no evidence of a statistically significant association between hyperactivity and the seven-repeat allele.

Clinically-defined ADHD

Overall 5.8% of the sample met diagnostic criteria for ADHD between the ages of 10–15. Males were four times (95% CI: 1.97–8.14) more likely than females to meet diagnostic criteria for ADHD. There was no statistically significant association between the DRD4 genotype and ADHD in the full sample ($\chi^2 = 1.092$, $P = 0.579$) or among males ($\chi^2 = 1.562$, $P = 0.458$). (There were too few ADHD females to conduct separate analyses for them.) Figure 2 shows the prevalence rate of ADHD in males, according to genotype. There was no evidence that the DRD4 7-repeat allele was associated with ADHD (OR = 0.84; 95% CI: 0.41–1.7). However the 7/7 genotype conferred a small but non-significant risk for ADHD relative to the other genotype groups (OR = 1.85; 95% CI: 0.52–6.6). As a further check, we used information from the DISC-C to construct three quantitative symptom scales by summing children's responses to symptoms specific to impulsivity, inattention, and hyperactivity. Comparisons of the genotype groups on these three symptom scales revealed no significant statistical associations ($P \geq 0.85$).

Table 2 Standardised mean scores by genotype group on quantitative measures of hyperactivity at ages 7, 9, 11, 13, and 15: Parent ratings[a]

Age	*Males*			*Females*			*Total sample*			*F-ratio*[b]	*P value*
	N7/N7	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*		
7	0.015 (*n* = 275)	−0.045 (*n* = 119)	−0.11 (*n* = 19)	−0.026 (*n* = 255)	0.057 (*n* = 119)	−0.22 (*n* = 17)	−0.0035 (*n* = 530)	0.00054 (*n* = 238)	−0.16 (*n* = 36)	M: 0.271 F: 0.704 T: 0.444	0.763 0.495 0.641
9	0.033 (*n* = 268)	−0.17 (*n* = 121)	0.26 (*n* = 19)	0.0011 (*n* = 247)	0.0047 (*n* = 119)	0.13 (*n* = 18)	0.018 (*n* = 515)	−0.095 (*n* = 240)	0.20 (*n* = 37)	M: 2.67 F: 0.161 T: 1.983	0.071 0.852 0.138
11	−0.038 (*n* = 261)	−0.070 (*n* = 120)	−0.030 (*n* = 18)	−0.030 (*n* = 244)	−0.020 (*n* = 120)	0.086 (*n* = 18)	−0.033 (*n* = 505)	−0.050 (*n* = 240)	0.018 (*n* = 36)	M: 0.051 F: 0.119 T: 0.087	0.951 0.888 0.917
13	−0.023 (*n* = 245)	−0.046 (*n* = 108)	−0.17 (*n* = 17)	−0.037 (*n* = 229)	−0.0011 (*n* = 112)	0.22 (*n* = 17)	−0.028 (*n* = 474)	−0.029 (*n* = 220)	0.0051 (*n* = 34)	M: 0.192 F: 0.586 T: 0.020	0.825 0.557 0.98
15	−0.037 (*n* = 277)	−0.067 (*n* = 125)	0.18 (*n* = 20)	−0.017 (*n* = 262)	0.060 (*n* = 127)	0.012 (*n* = 17)	−0.027 (*n* = 539)	−0.0072 (*n* = 252)	0.11 (*n* = 37)	M: 0.592 F: 0.249 T: 0.362	0.553 0.779 0.696

[a]Measures are standardised using the z-score transformation (mean = 0, SD = 1).
[b]F-ratios are provided separately for males (M), females (F), and the full sample (T).

Table 3 Standardised mean scores by genotype group on quantitative measures of hyperactivity at ages 7, 9, 11, and 13: Teacher ratings[a]

Age	*Males*			*Females*			*Total sample*			*F-ratio*[b]	*P value*
	N7/N7	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*		
7	−0.031 (*n* = 278)	−0.052 (*n* = 122)	−0.14 (*n* = 19)	−0.045 (*n* = 254)	−0.045 (*n* = 119)	0.021 (*n* = 17)	−0.036 (*n* = 532)	−0.055 (*n* = 241)	−0.069 (*n* = 36)	M: 0.115 F: 0.039 T: 0.044	0.892 0.962 0.957
9	−0.033 (*n* = 272)	−0.039 (*n* = 122)	0.20 (*n* = 18)	−0.014 (*n* = 253)	0.032 (*n* = 118)	0.16 (*n* = 17)	−0.024 (*n* = 525)	−0.015 (*n* = 240)	0.17 (*n* = 35)	M: 0.498 F: 0.300 T: 0.664	0.608 0.741 0.515
11	−0.036 (*n* = 266)	−0.034 (*n* = 122)	0.11 (*n* = 19)	−0.042 (*n* = 244)	0.064 (*n* = 119)	−0.15 (*n* = 18)	−0.035 (*n* = 510)	−0.0021 (*n* = 241)	0.012 (*n* = 37)	M: 0.207 F: 0.610 T: 0.111	0.813 0.544 0.895
13	−0.046 (*n* = 233)	−0.026 (*n* = 107)	−0.14 (*n* = 16)	−0.0021 (*n* = 221)	−0.050 (*n* = 111)	−0.12 (*n* = 17)	−0.025 (*n* = 454)	−0.043 (*n* = 218)	−0.13 (*n* = 33)	M: 0.094 F: 0.162 T: 0.193	0.911 0.851 0.825

[a]Measures are standardised using the z-score transformation (mean = 0, SD = 1).
[b]F-ratios are provided separately for males (M), females (F), and the full sample (T).

Quantitative measures of impulsive personality traits
Table 4 shows standardised mean scores on the MPQ Constraint scale for the three genotype groups. Although there was no statistically significant association between Constraint and the seven-repeat allele, it is of interest that those individuals homozygous for allele 7 scored noticeably lower than those in other genotype groups on Constraint (ie, they were more impulsive) at both ages 18 and 26 years. This trend was more marked among males than females. In addition, Table 4 shows no significant association between informants' reports of Conscientiousness using the Five-Factor Model of personality and the seven-repeat allele.

Discussion

The present study provides little support for the hypothesis that the seven-repeat allele of the DRD4 exon 3 VNTR is associated with either clinical ADHD

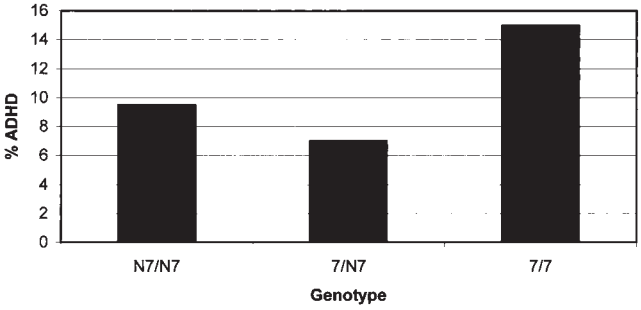

Figure 2 DMS-III ADHD prevalence rates in males, according to genotype.

or the quantitative trait of hyperactivity. Individuals with a 7/7 genotype did score higher on quantitative measures of hyperactivity at some assessment ages. Likewise, we observed a trend toward an association of the seven-repeat allele with personality traits of impulsivity. However, these effects were small and non-significant. Most important, the effects did not replicate across different ages and reporters within the study.

Several studies have replicated the original association reported between DRD4 and hyperactivity, but there have also been some non-replications and a few ambiguous results. To our knowledge, the present study is the most comprehensive investigation to date of the role of DRD4 in the hyperactivity phenotype. In terms of design, it is the first study to investigate this association in an unselected birth cohort avoiding ascertainment bias in either cases or controls, and thereby providing accurate estimates of effect sizes in the population. In terms of measurement, it is the first study to use multimethod and multisource assessments of the hyperactivity phenotype at multiple developmental periods, spanning a period of 20 years. The Dunedin Study ADHD phenotype measures have good psychometric reliability, and their construct validity is demonstrated by the Study's prior contributions to the ADHD literature.[59–64] Across multiple assessment ages (7, 9, 11, 13, 15, 18, and 26 years) and across different methods of measurement and ascertainment (parent-reports, teacher-reports, psychiatric interviews, personality self-reports, and personality informant-reports), we failed to find a clear-cut and consistent association between DRD4 and hyperactivity.

Our findings appear to contradict the results of a recent meta-analysis by Faraone *et al*.[31] Interestingly, the meta-analysis found much stronger evidence for the association of DRD4 with ADHD in case-control studies than in family-based studies. These results are mirrored in at least three recent clinical studies[20,21,23] where evidence of association was found using case-control analyses but not by family-based analyses. These findings suggest the hypothesis that data from case-control studies may be spurious. Such studies are, by definition, prone to population stratification artefacts. Family-based study designs, on the other hand, are exempt from admixture effects, and are thus less likely to give spurious findings. As will be discussed below, there is reason for confidence that the sample used for this analysis is homogenous and unaffected by population heterogeneity. The validity of case-control studies can be further confounded by the use of non-representative control groups. The selection of virtually any control group will be biased in some respect, a problem discussed in Berkson's Fallacy.[65] The problem of an unrepresentative control group is overcome in our study by simply using the total cohort in analyses of quantitative variables, and by comparing individuals affected with clinically defined ADHD against the non-affected remainder of the cohort.

It is possible that low power compromised our ability to detect an association with clinically-defined ADHD. But this does not fully account for the pattern of findings in our longitudinal investigation. In the Dunedin Study, with n = ~1000 and prevalence rates of 5% for an exposure (eg homozygous for allele 7) and 5% for a psychiatric outcome (ADHD), respectively,

Table 4 Standardised mean scores on quantitative measures of impulsive personality traits[a]

Age	*Males*			*Females*			*Total sample*			*F-ratio*[b]	*P value*
	N7/N7	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*	*N7/N7*	*7/N7*	*7/7*		
18 self-report	0.042 (n = 283)	–0.062 (n = 122)	–0.18 (n = 18)	–0.018 (n = 260)	0.14 (n = 131)	–0.16 (n = 17)	0.0059 (n = 543)	0.059 (n = 253)	–0.16 (n = 35)	M: 0.732 F: 1.478 T: 0.803	0.482 0.229 0.448
26 self-report	0.011 (n = 299)	0.0073 (n = 131)	–0.34 (n = 20)	–0.022 (n = 276)	0.14 (n = 135)	–0.024 (n = 18)	–0.013 (n = 575)	0.078 (n = 266)	–0.20 (n = 38)	M: 1.120 F: 1.278 T: 1.551	0.327 0.280 0.213
26 informant-report	–0.025 (n = 287)	0.067 (n = 123)	–0.057 (n = 20)	–0.00022 (n = 271)	0.047 (n = 134)	0.14 (n = 17)	–0.013 (n = 560)	0.073 (n = 257)	0.026 (n = 37)	M: 0.405 F: 0.222 T: 0.669	0.667 0.801 0.513

[a]Measures are standardised using the z-score transformation (mean = 0, SD = 1). Self-reports of Constraint using the MPQ; informants reports of Conscientiousness using the five-factor model of personality.
[b]F-ratios are provided separately for males (M), females (F), and the full sample (T).

the minimum detectable odds ratio ($1 - \beta = 0.80$; $\alpha = 0.05$) is 3.3. If the prevalence rate for an exposure is 35% (eg those with allele 7), the minimum detectable odds ratio is 2.1. Moreover, if, as hypothesised, DRD4 has an effect on the underlying quantitative trait of hyperactivity, this association should have been observed in this large sample which has the power to detect small-to-moderate correlations. Nonetheless, we found no significant associations between DRD4 with continuous measures of hyperactivity nor with personality traits of impulsivity.

As mentioned, population stratification can be a problem in association studies of complex diseases, especially when looking at genes that confer a small effect. The effect of population heterogeneity, which can produce false-negative results as well as false-positive results, can be confounded even further if the risk allele being studied is not actually the causal polymorphism, but is instead in linkage disequilibrium (LD) with a functional variant at another locus. The functional significance of the VNTR in exon three of DRD4 is still unclear. Whilst Asghari *et al* concluded that different repeat lengths conferred pharmacological properties to the D4 receptor, with the 7-repeat acting to dull the response of dopaminergic cells to dopamine,[9] such findings are not ubiquitous and more recent studies do not concur in suggesting an important functional role for the repeat region.[66–68] It is thus possible that in some, but not all populations, the 7-repeat is in LD with another, functional variant, such as the –521 (C/T) DRD4 promoter polymorphism reported by Okuyama *et al* shown to reduce transcriptional activity by ~40%.[69] Evidence to support the theory that the effect of DRD4 on hyperactivity is mediated not by the exon 3 VNTR but by a functional variant in the promoter region of the gene comes from a recent study by McCracken *et al*.[28] They found a highly significant association with the presence of a 120-bp duplication in the promoter of DRD4, but no significant association with the exon three VNTR. However, Barr *et al*[70] failed to replicate this finding. Although it is possible that LD relationships may be the cause of discrepancies seen in association studies of the 7-repeat allele, population stratification can probably be ruled out as a confounding factor in this study. Participants have all given information about the number of Caucasian grandparents they have, and subjects who are clearly of Maori origin were excluded from the analyses. Furthermore, the allele frequencies observed in the Dunedin sample match closely those observed in other Caucasian samples (see Figure 1). As a final check for stratification we adopted a genomic control approach based on latent class analysis similar to Satten *et al*.[71] One hundred individuals were selected at random from the sample and typed for 40 unlinked microsatellite markers. In a stratified sample one would expect to observe Hardy–Weinberg disequilibrium and linkage disequilibrium across the unlinked markers: our genomic control approach aims to identify subpopulations (latent classes) such that within each there is Hardy–Weinberg and linkage equilibrium. In the current sample, however, there was no support for having more than one latent class, which is consistent with the sample being homogeneous.

In summary, data from this birth cohort that have contributed to the ADHD literature for some time, provide little evidence to support the association of the seven-repeat allele of DRD4 with quantitative measures of hyperactivity from ages 7 to 15; nor with the presence of clinically-defined ADHD; nor with personality trait measures of impulsivity in adolescence or adulthood. Future work should focus on ascertaining the functional significance of the exon three repeat polymorphism, and identifying new variants within the gene that may account for the effects seen in other studies whilst explaining why such results have not been consistently replicated.

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