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Effects of Dopamine Receptor D4 Variation on Alcohol and Tobacco Use and on Novelty Seeking: Multivariate Linkage and Association Analysis

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The dopamine D4 receptor gene contains a polymorphic sequence consisting of a variable number of 48-base-pair (bp) repeats, and there have been a number of reports that this polymorphism is associated with variation in novelty seeking or in substance abuse and addictive behaviors. In this study we have assessed the linkage and association of DRD4 genotype with novelty seeking, alcohol use, and smoking in a sample of 377 dizygotic twin pairs and 15 single twins recruited from the Australian Twin Registry (ATR). We found no evidence of linkage or association of the DRD4 locus with any of the phenotypes. We made use of repeated measures for some phenotypes to increase power by multivariate genetic analysis, but allelic effects were still non-significant. Specifically, it has been suggested that the DRD4 7-repeat allele is associated with increased novelty seeking in males but we found no evidence for this, despite considerable power to do so. We conclude that DRD4 variation does not have an effect on use of alcohol and the problems that arise from it, on smoking, or on novelty seeking behavior.

KEY WORDS: DRD4; novelty seeking; alcohol and tobacco use; multivariate linkage analysis; association analysis

INTRODUCTION

Interest in a possible relationship between the dopamine receptor D4 (DRD4) exon III polymorphism and aspects of personality was sparked by Ebstein et al. [1996] and Benjamin et al. [1996], who reported a significant association between the long repeats of the DRD4 polymorphism and the trait of novelty seeking. Since that time, a number of studies have sought to replicate these results, with mixed but mainly negative outcomes [Malhotra et al., 1996; Ebstein et al., 1997; Gelernter et al., 1997; Ono et al., 1997; Noble et al., 1998; Sullivan et al., 1998; Ekelund et al., 1999; Kuhn et al., 1999; Paterson et al., 1999; Strobel et al., 1999; Tomitaka et al., 1999; Gebhardt et al., 2000; Mitsuyasu et al., 2001; Soyka et al., 2002]. The basis for these conflicting findings may stem from differences in the age range of the subjects since novelty seeking decreases with age and those studies using young subjects report positive associations. Other factors may be (1) differences in the scale used to measure novelty seeking such as the Tridimensional Personality Questionnaire [Cloninger, 1987], Temperament and Character Inventory [Cloninger et al., 1994], Karolinska Scales of Personality [af Klinteberg et al., 1988], and NEO Personality Inventory [Costa and McCrae, 1985], (2) ethnicity of the sample (with association typically non-significant in Continental Europeans), and (3) differences in the gender ratio of samples [see Lusher et al., 2001; Kluger et al., 2002].

In relation to the gender composition of the samples, there have been positive findings of association between DRD4 and novelty seeking in female samples [Ono et al., 1997; Tomitaka et al., 1999] but not in male samples

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[Malhotra et al., 1996; Kuhn et al., 1999], although these studies were potentially confounded in that they sampled different ethnic groups. Working under the assumption that the DRD4 7-repeat allele influences novelty seeking, Harpending and Cochran [2002] have recently proposed that the 7-repeat allele has been positively selected as its apparent phenotypic characteristic of increased impulsiveness and nonconformity may have encouraged human migration or alternatively may have enhanced reproductive success in male-competitive societies. As men tend to display more pronounced novelty seeking (and related behavior) than women, this suggests the potential for gender specific allelic associations.

A meta-analysis of 20 studies (N = 3,907) provided no support for an association between DRD4 polymorphism and novelty seeking as measured by the Tridimensional Personality Questionnaire [Kluger et al., 2002], while another meta-analysis of 21 samples indicated a lack of association between the 7-repeat allele of the DRD4 genotype and novelty seeking [Schinka et al., 2002]. However, in an analysis of 12 samples comparing the presence or absence of long repeat alleles (>5), Schinka and colleagues detected a slight effect. The authors recommended further exploration of the DRD4 genotype and novelty seeking in relation to other personality dimensions and disorders. A number of characteristics such as substance abuse and addictive behaviors have been found to correlate with novelty seeking [Pomerleau et al., 1992; Cloninger et al., 1995; Heath et al., 1995, 1997; Vukov et al., 1995; Howard et al., 1997]. Various researchers have investigated the possibility of DRD4 being associated with alcoholism [George et al., 1993; Sander et al., 1997; Ishiguro et al., 2000], smoking [George et al., 1993; Lerman et al., 1998; Shields et al., 1998], opioid and methamphetamine dependence [Kotler et al., 1997; Li et al., 2000; Tsai et al., 2002], and pathological gambling [Perez de Castro et al., 1997; Comings et al., 1999, 2001].

The mixed history of positive and negative findings of association with DRD4 illustrates the difficulties inherent in gene-hunting in polygenic diseases or quantitative traits, and the need for more powerful methods. Various improvements in methodology are now available. One approach is to analyze linkage and association simultaneously, which enables one to test whether the quantitative trait locus (QTL) is the functional gene or a locus in disequilibrium with the trait locus [Fulker et al., 1999]. In sib-pair designs, if apparently significant associations are found, within-family comparisons can be performed in order to eliminate the possibility that the observed effects are due to population stratification [Fulker et al., 1999; Abecasis et al., 2000]. Also, the use of a replicated or multivariate phenotype may increase power to detect a QTL by reducing errors in phenotype characterization, provided measures are not too strongly positively correlated [Allison et al., 1998; Boomsma and Dolan, 2000; Amos et al., 2001]. This study takes advantage of these improved methods to investigate the linkage and association of the DRD4 genotype to novelty seeking, alcohol-related, and smoking behaviors, thus ensuring maximum confidence in the validity of the results. In light of the suggestion that gender effects should be present for the DRD4 7-repeat allelic association with novelty seeking, a gender × allele interaction effect is tested. The sample includes a large group of dizygotic twin pairs, for many of whom longitudinal data on smoking and drinking habits are available.

METHODS

Sample and Measures

Subjects for this study were originally recruited from the Australian Twin Registry (ATR), a volunteer register begun in 1978. In 1980–1982 a "Health and Lifestyle" survey was mailed to 5,867 twin pairs over the age of 18 years [Jardine and Martin, 1984; Heath et al., 1995], with responses received from 3,808 pairs of twins and 567 single twins. Included in this questionnaire were items regarding smoking and alcohol consumption. If the respondent indicated that they had ever been a smoker, they were asked whether they had quit smoking and how many cigarettes, cigars, or pipes of tobacco they usually smoke/d per day. Alcohol consumption items included frequency of having consumed alcoholic drinks in the past year, and a table for respondents to complete indicating how many glasses of each of beer, wine, spirits, sherry, and other alcoholic drinks the respondent had consumed in the past week. Glass volumes were quoted (7 oz for beer, 4 oz for wine, 1 oz for spirits) in order to standardize quantities of alcohol.

The next wave of data collection occurred in 1988–1989 when a follow-up questionnaire was mailed to all twin pairs who completed the 1980–1982 survey [Heath et al., 1994]. Responses were obtained from 6,327 individuals, including 2,995 twin pairs. Questionnaire items similar to those in the 1980–1982 survey were included, although the frequency of alcohol consumption item included two extra categories: "more than once per day" and "not at all." Additional items included information on current smoking status and average daily cigarette consumption, and a checklist of 14 potential problems associated with alcohol consumption. A short form (54 items) of the Tridimensional Personality Questionnaire [Cloninger, 1987] was also incorporated, from which an 18 item score for the novelty seeking dimension was computed [Heath et al., 1994].

A telephone interview based study using a modified version of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) instrument was conducted during 1992 and 1993 with 5,995 individuals who had participated in the earlier studies outlined above. Details of the interview procedure and instrument are described comprehensively elsewhere [Heath et al., 1997; Statham et al., 1998]. In addition to items similar to those in the mailed questionnaires regarding alcohol use, the interview also enabled diagnosis of alcohol dependence according to DSM-IIIR criteria.

During 1993–1996, blood was collected from 3,300 subjects who had participated in the telephone interviews. In order to obtain further longitudinal data on smoking behavior (which was not covered as part of the

telephone interview) and alcohol consumption in the past week, a questionnaire was filled out by participants on the day that the blood samples were collected. However, the information on smoking behavior is incomplete, with responses not being available for many of the subjects in this study. Where possible, data on smoking behavior for those individuals have been augmented by their responses to a third Health and Lifestyle Questionnaire, which was mailed to twins over the age of 50 between 1993 and 1996 [Kirk et al., 1999]. There was some selection of samples for DRD4 genotyping so that samples were prioritized for analysis if one or both twins had a diagnosis of alcohol dependence or if samples were available from both members of a DZ twin pair.

DRD4 and Flanking Marker Genotyping

DNA extracted from white blood cells was used for the determination of DRD4 48-base-pair (bp) repeat polymorphism status for 377 pairs of DZ twins and 15 single twins (464 women, 305 men). DNA was amplified using the primers 5′GCGACTACGTGGTCTACTCG3′ and 5′AGGACCCTCCATGGCCTTG3′ as described by Ebstein et al. [1996], and the amplified products were separated by electrophoresis in 10% polyacrylamide gels with ethidium bromide detection. The product sizes ranged from 379 bp for the 2-repeat allele to 667 bp for the 8-repeat allele, and repeat numbers were confirmed by sequencing of selected samples. To increase sib-pair identity-by-descent (IBD) information at DRD4 we also typed a flanking marker D11S1984, which had 12 alleles and a polymorphic information content of 0.76. The average meiotic information content of the marker region was estimated at 0.43 in Genehunter 2.1 [Kruglyak et al., 1996].

Zygosity

As we only intended to include DZ twin pairs in the linkage analysis, it was important to confirm twin zygosity in same-sex twin pairs (N = 217 pairs). The zygosity assessment included: (1) a partial 10 cM genome scan of ten chromosomes (1, 2, 6–8, 11, 15–17, 19) which was available for part of the sample. Identity-by-state (IBS) status for the 117 available microsatellite markers was assessed, (2) nine highly polymorphic DNA markers and a segment of the X-Y homologous gene *Amelogenin* using a commercial kit (AmpFLRSTR Profiler Plus, ABI), which co-amplified the repeat regions of the following STR loci: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820, (3) the multipoint IBD probabilities for DRD4 (using the flanking marker, D11S1984); those pairs for whom P (IBD = 2) was less than 0.05 were classified as DZ, (4) blood group results for the ABO, MNS, and Rh systems (Australian Red Cross Blood Service), and (5) self-report questionnaire using items which have been found to give at least 95% agreement with zygosity assignment by genotyping [Eaves et al., 1989]. Of these criteria, 83 pairs were classified using the partial genome scan, 31 pairs were classified using the ABI Profiler Kit, 69 pairs were assigned dizygosity based on their DRD4/D11S1984 IBD probabilities, 28 pairs were assigned dizygosity based on their blood groups, and 7 pairs were classified by self-report.

Statistical Methods

Data. The response categories used in some of the longitudinal measures varied slightly between studies, so in some cases it was necessary to collapse categories in order to obtain consistency. The categories used for the analysis of each item are shown in Table I, with the endorsement frequencies at each wave of data collection. All measures were converted to normal weights scores for use in the Quantitative Transmission Disequilibrium Test (QTDT) program [Abecasis et al., 2000] and for consistency were maintained for data analysis in Mx 1.50 [Neale, 1999], although the raw ordinal method in Mx was found to produce similar estimates of allelic deviations (exemplified for the trait of novelty seeking see "Results").

Linkage and Association Analysis

Sib-pair IBD probabilities were estimated in Genehunter 2.1 [Kruglyak et al., 1996] by using the DRD4 marker and a flanking marker (D11S1984) which was approximated to lie 1 cM from DRD4. Linkage and association analyzes of each of the phenotypes at each available time point were performed on the genotyped sample of DZ twins using Mx and QTDT programs.

Mx Analyses

In Mx, a maximum likelihood (ML) procedure was used to simultaneously estimate parameters in a means model (to test association effect) and variance components model (to test linkage effect). The means model included the fixed effects of age and sex in addition to the allelic effects for which six deviations (alleles 3 through 8) were estimated as free parameters with allele 2 serving as the baseline. The variance components model included familial (F; combined polygenic and shared environmental effects), unique environmental (E) and QTL (Q) effects. The sib-pair covariance included F and Q effects, where Q was conditioned by the estimated proportion of alleles shared IBD (π); the variance–covariance matrix was evaluated in the absence of fixed effects on the mean [see Fulker et al., 1999; Zhu et al., 1999]. This model was fitted to raw continuous data.

The significance of the QTL was tested by comparing the fit of the FEQ and FE models by the likelihood ratio chi-square (χ^2) test (the difference in $-2 \times$ log likelihood between these models is a χ^2 test against one degree of freedom (df)). The joint effect of allelic variation on the trait was similarly tested by fixing the six allelic deviations to zero and comparing this model to one in which all allelic deviations were estimated. We could also estimate the effects of individual alleles (specifically the 7-repeat allele) by fixing all others to zero, and this was further modified to test the effect in only one sex (males).

TABLE I. Distribution (%) of Drinking, Smoking, and Personality Variables in the Genotyped Sample by Gender and Study Wave

Alcohol consumption in last week (standard drinks)	0	1	2	3–4	5–6	7–13	14–20	21 or more
Women								
1980–1982 (n = 368)	14.4	7.6	14.1	12.8	11.1	22.6	8.4	9.0
1988–1989 (n = 425)	28.7	9.4	8.9	13.2	11.5	15.5	8.7	4.0
1993–1995 (n = 462)	38.5	8.2	9.3	10.0	8.4	16.9	6.3	2.4
Men								
1980–1982 (n = 257)	6.2	2.7	3.9	6.6	8.2	17.5	18.7	36.2
1988–1989 (n = 280)	18.2	2.9	6.8	6.1	10.7	18.2	15.4	21.8
1993–1995 (n = 305)	20.0	1.3	4.9	9.5	7.5	20.3	18.4	18.0
Frequency of alcohol consumption	<1–2 times a month	1–2 times a month	1–2 times a week	3–4 times a week	Every day			
Women								
1980–1982 (n = 454)	29.7	15.4	34.4	13.4	7.0			
1988–1989 (n = 438)	28.8	19.6	30.1	12.8	8.7			
1993–1995 (n = 461)	29.1	21.7	24.1	12.4	12.8			
Men								
1980–1982 (n = 294)	13.9	17.3	32.0	26.5	10.2			
1988–1989 (n = 280)	14.6	16.1	27.9	24.6	16.8			
1993–1995 (n = 304)	13.2	13.5	28.0	22.7	22.7			
Symptoms of alcohol-related problems	0	1	2	3	4 or more			
Women								
1988–1989 (n = 378)	43.4	32.3	11.4	6.3	6.6			
1993–1995 (n = 459)	51.0	13.7	13.1	8.7	13.5			
Men								
1988–1989 (n = 226)	17.3	26.1	14.6	14.6	27.4			
1993–1995 (n = 302)	14.2	12.6	17.2	13.9	42.1			
Alcohol dependence diagnosis	No	Yes						
Women								
1993–1995 (n = 459)	88.0	12.0						
Men								
1993–1995 (n = 302)	64.2	35.8						
Novelty seeking score	0–1	2–3	4–5	6–7	8–9	10–11	12+	
Women								
1988–1989 (n = 417)	1.4	5.5	18.7	31.4	30.0	11.0	1.9	
Men								
1988–1989 (n = 260)	1.5	5.0	11.5	23.8	33.8	15.4	8.8	
Smoking status	Never smoked	Ex-smoker	Current smoker					
Women								
1980–1982 (n = 454)	53.5	17.0	29.5					
1988–1989 (n = 445)	54.2	23.1	22.7					
1993–1996 (n = 123)	51.2	29.3	19.5					
Men								
1980–1982 (n = 294)	42.9	20.4	36.7					
1988–1989 (n = 285)	43.9	29.8	26.3					
1993–1996 (n = 88)	38.6	39.8	21.6					
Average daily cigarette consumption[a]	None	1–10	More than 10					
Women								
1980–1982 (n = 454)	53.5	21.8	24.7					
1988–1989 (n = 427)	52.5	21.5	26.0					
1993–1996 (n = 29)	41.4	13.8	44.8					
Men								
1980–1982 (n = 288)	43.8	19.4	36.8					
1988–1989 (n = 267)	42.3	19.1	38.6					
1993–1996 (n = 47)	46.8	19.1	34.0					

[a]This item was added to the 3rd wave questionnaire part way through the study, hence the lower sample size at this time point.

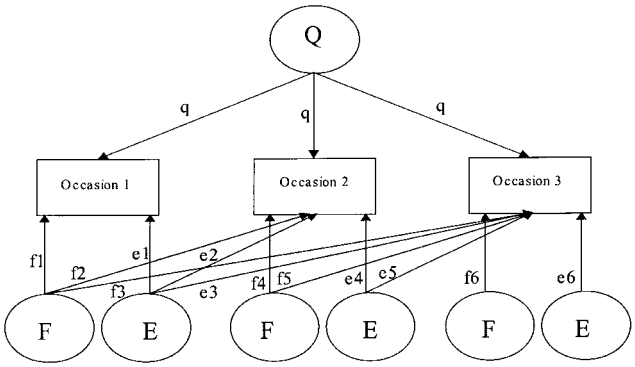

Fig. 1. Path diagram representing familial (F), environmental (E), and QTL (Q) components of variance in the multivariate linkage model for a variable measured on three occasions.

For repeated measures analyses, an FEQ model was parameterized to explain the covariance among the variables (see Fig. 1). While F and E factors assumed the form of a triangular (Cholesky) decomposition with all parameters free to vary, the effect of the QTL was confined to a single factor in which the path coefficients were constrained equal across the time points. Hence, the significance of the QTL was tested against 1 df. Age, sex, and allelic effects in the means model were equated at each time point, although the mean at each time point was free to vary.

A multivariate analysis of usual frequency of drinking and alcohol consumption in the past week, which are strongly related measures, was performed including all time points. In this model the QTL effect was equated across measures and time points and the allelic deviations were similarly constrained equal. However, the fixed effects of age and sex, although equated over time, were free to vary between measures.

QTDT Analyses

The QTDT test of linkage for each of the phenotypes to the DRD4 marker was performed within a model encompassing allelic association [Abecasis et al., 2000]. The Total Association model was also tested. In this model the gene effect on sib-pair differences (or within family component) and the gene effect on the sib-pair means (or between family component) is estimated. Hence, evidence for association will be positively biased in the presence of population stratification. As the population frequencies of the 3-, 5-, 6-, and 8-repeat alleles were $<5\%$ they were combined so that the test of association was based on 3 df (i.e., effects of the combined low frequency alleles, the 2-, and 7-repeat allele) against the baseline 4-repeat allele, which was the most frequently occurring allele.

RESULTS

Sample Characteristics

The distribution of age at each of the data collection waves was similar for females and males, with a higher frequency of younger than older participants. On occasion one the mean age of the sample was approximately 30 years with a standard deviation of 11 years (range: 18–70). On occasions two and three the respective means (and range) were 38 (26–78) and 42 (30–82) years with a standard deviation of 11. For the 392 families in this study there are $4 \times 392 = 1{,}568$ grandparents, for whom we had reported ancestry (8.1% of data were missing). Of those grandparents where ancestry was reported, 85% were from the British Isles and the remainder were from elsewhere in Europe (73% from Northern Europe including France and Russia and 17.3% from Southern Europe including the Middle East; 10.7% of this sample did not have European country of origin specified), with 0.21% originating from either USA or Canada.

Table I shows the frequency of responses for each of the measures. As the genotyped sample was somewhat selected for individuals with alcohol dependence, a comparison of the mean responses (frequencies converted to normal weights) between the full sample (N ranging from 384 to 7,614) and genotyped sample (N ranging from 76 to 752) was performed using a ML procedure in Mx, which accounted for the covariation between twin pairs, separately for MZ and DZ groups. The results confirmed differences between the genotyped subsample and the full sample for all alcohol measures at every time point and for both smoking measures at the first two time points, varying between 0.10 (smoking status at 2nd wave) and 0.25 (alcohol related problems at 3rd wave) of a standard deviation higher than the mean of the full sample. Any such bias would exaggerate the significance of genotypic effects on alcohol and smoking, but since none were even remotely significant (see below) we did not consider that more elaborate analytical methods to allow for such ascertainment were warranted.

The 4- and 7-repeat alleles were by far the most common in this sample, with allele frequencies of 0.66 and 0.17, respectively. Allele frequencies for the 5-, 6-, and 8-repeat alleles were less than 0.01, while the 2- and 3-repeat alleles showed respective frequencies of 0.10 and 0.05.

Linkage and Association Tests

Results of the univariate and multivariate linkage and association tests for each phenotype are shown in Table II. With the exception of the univariate test of association between the 7-repeat allele and smoking status (2nd wave), *P*-values for all linkage and association tests were non-significant, indicating no evidence for linkage or association of DRD4 with any of the phenotypes. The QTDT test of total association showed *P*-values approaching significance for smoking status (1st and 2nd waves) and symptoms of alcohol related problems (2nd wave), but only in the latter variable was this near significance level supported by the Mx association analysis. Although not reported here, the tests of population stratification were in agreement with the total association tests. To examine the robustness of our ML estimates using normal weights (continuous raw data model) we repeated the test for association of DRD4

TABLE II. χ^2 Values for Univariate and Multivariate Tests of Linkage and Association Using Mx and QTDT Programs

	χ^2					
	Mx				QTDT	
Phenotype	QTL effect (1 df)	Association effect of joint alleles under FEQ model (6 df)	Association effect of 7R allele in both sexes (1 df)	Association effect of 7R allele in males only (1 df)	QTL effect (1 df)	Total association (3 df)
TPQ novelty seeking (1988–1989)	0.00	8.80	0.69	0.22	0.00	3.77
Smoking status						
1980–1982	0.09	8.16	2.37	2.19	0.06	7.36
1988–1989	0.32	7.38	4.10*	1.68	0.35	7.57
1993–1996	0.00	10.10	2.13	0.19	0.04	4.38
Multivariate[a]	0.50	4.52				
Cigarette consumption						
1980–1982	0.95	5.66	0.86	0.64	0.70	2.11
1988–1989	1.89	8.76	1.70	0.73	1.79	5.97
1993–1996	1.79	5.98	0.65	0.00	2.39	1.09
Multivariate[a]	0.36	2.96				
Frequency of drinking alcohol						
1980–1982	0.31	6.15	0.05	0.51	0.51	0.54
1988–1989	0.00	2.63	0.00	1.82	0.00	0.36
1992–1993	0.61	7.43	0.24	0.06	0.82	2.60
Multivariate[a]	0.50	3.75				
Alcohol dependence (1993–1995)	0.00	2.83	0.33	1.34	0.00	3.82
Symptoms of alcohol-related problems						
1988–1989	0.71	9.47	0.17	0.06	0.91	1.41
1992–1993	0.15	12.04	0.02	1.41	0.95	6.99
Multivariate[a]	2.52	4.85				
Alcohol consumption in past week						
1980–1982	0.08	7.44	0.04	0.56	1.24	5.50
1988–1989	0.00	4.03	0.01	0.72	0.00	0.81
1992–1993	0.00	8.69	0.39	0.12	0.00	5.10
Multivariate[a]	0.00	6.06				
Multivariate: frequency of drinking & alcohol consumption in past week	0.35	5.59				

[a]Analysis comprises phenotype at each data wave.
*P-value < 0.05.

to novelty seeking using categorical data (multifactorial threshold model). The goodness of fit for the test of association in the threshold model ($\chi^2_6 = 9.17$, $P = 0.16$) was not substantially different from that in which normal weights were used (see Table II).

ML estimates of the percentage of variance accounted for by F, E, and Q factors from the univariate models and the estimate of Q from the multivariate analyses are displayed in Table III. Note that F and Q represents an upper limit to the heritability, although it is also confounded with shared environment. This value is lower for most variables than estimates from much larger samples including MZ twins, and probably reflects the large error variance consequent on relatively small sample size and suboptimal design. However, the sample size is quite large enough to obtain powerful estimates of any association effects, which are the focus of our attention. Also included in Table III is the percentage of variance explained by the joint effect of alleles 3 through 8. To calculate this, the difference in the estimated residual variance of the phenotype from the Mx models in which allelic effects were free to vary and then set to zero, was divided by the residual variance in the latter model and then multiplied by 100. In Table IV, the means and standard errors for the phenotypes, grouped by presence/absence of the 7-repeat allele, are shown. Smoking status at the 2nd wave showed a significant main effect of the 7-repeat allele.

To investigate previous trends of significant association between novelty seeking and DRD4 in young samples only (perhaps because novelty seeking decreases with age), we selected participants on first test occasion who were younger than 40 years (N = 212 twin pairs). The test of association was still not significant, ($\chi^2_6 = 7.35$, $P = 0.29$).

DISCUSSION

Smoking and alcohol dependence are strongly associated, partly because they are affected by some of the same genes [Bierut et al., 1998; Bergen et al., 1999; True et al., 1999], this effect has further been confirmed in a sample from which participants in the present study were drawn [Madden et al., 2000]. Many authors have proposed that the genes which affect both these attributes do so through an association with some aspect of personality, such as the novelty seeking or harm avoidance. One of the most extensively studied genes in this area is the dopamine D4 receptor gene, and in particular the polymorphism in which the number of 48-bp repeats in the DNA leads to variation in the length of the third intracytoplasmic loop.

However, multiple previous reports on associations between the DRD4 48-bp repeat number and novelty seeking, alcoholism and smoking, or other health-related behaviors, have given inconsistent results. This may in part be because the number of subjects in each study has been small, or because the effect size is small. Our results have been obtained on a comparatively large number of subjects, are based on combined linkage and

TABLE III. Mx Estimates of the Percentage of Variance Explained by Familial (F), Unique Environmental (E), and QTL (Q) Effects

	F%	E%	Q%	Joint allelic effects %
TPQ novelty seeking (1988–1989)	19.3	80.7	0	1.51
Smoking status				
1980–1982	15.1	79.8	5.1	1.30
1988–1989	20.9	70	9.1	1.29
1993–1996	19.1	80.9	0	4.80
Multivariate[a]			10.2	
Cigarette consumption				
1980–1982	6.9	68.2	24.9	0.96
1988–1989	14.1	69.7	16.2	1.45
1993–1996	5.7	16.8	77.5	7.94
Multivariate[a]			9.3	
Frequency of drinking alcohol				
1980–1982	24.1	67.5	8.4	0.92
1988–1989	32.4	67.6	0	0.53
1992–1993	6.9	68.2	24.9	1.05
Multivariate[a]			7.7	
Alcohol dependence (1993–1995)	10.9	89.1	0	0.39
Symptoms of alcohol-related problems				
1988–1989	3.5	78	18.5	1.78
1992–1993	20.5	72.6	6.9	2.04
Multivariate[a]			21.4	
Alcohol consumption in past week				
1980–1982	24.5	70.4	5.1	1.37
1988–1989	20.5	79.5	0	0.74
1992–1993	26.2	73.8	0	1.37
Multivariate[a]			0	

The percentage of variance accounted for by the repeat polymorphism under the FEQ model is also shown for univariate models, as a percentage of total residual variance.
[a]Analysis comprises phenotype at each data wave.

TABLE IV. Means (SE) of Phenotypic Normal Weights Grouped by Genotypes Where 0, 1, or 2 Copies of the 7-Repeat Allele Are Present

	Genotype		
	×/× (N = 53–529)	7R/× (N = 22–210)	7R/7R (N = 24–28)
TPQ novelty seeking (1988–1989)	0.01 (0.04)	−0.03 (0.07)	−0.08 (0.18)
Smoking status			
1980–1982	0.06 (0.03)	−0.07 (0.05)	0.01 (0.14)
1988–1989	0.08 (0.03)	−0.07 (0.05)	−0.07 (0.14)
1993–1996	0.08 (0.06)	−0.11 (0.10)	−0.12 (0.40)[a]
Cigarette consumption			
1980–1982	0.05 (0.03)	−0.04 (0.05)	0.04 (0.15)
1988–1989	0.05 (0.03)	−0.04 (0.05)	−0.06 (0.16)
1993–1996	0.06 (0.10)	−0.09 (0.17)	−0.76 (NE)[b]
Frequency of drinking alcohol			
1980–1982	0.03 (0.04)	−0.04 (0.06)	0.17 (0.17)
1988–1989	0.01 (0.04)	0.00 (0.06)	0.11 (0.17)
1992–1993	0.00 (0.04)	0.04 (0.06)	−0.01 (0.18)
Symptoms of alcohol-related problems			
1988–1989	0.02 (0.04)	0.03 (0.07)	0.02 (0.15)
1992–1993	0.01 (0.03)	0.01 (0.06)	0.04 (0.16)
Alcohol dependence (1993–1995)	0.21 (0.02)	0.23 (0.03)	0.21 (0.08)
Alcohol consumption in past week			
1980–1982	−0.01 (0.04)	−0.04 (0.07)	0.08 (0.15)
1988–1989	0.02 (0.04)	0.00 (0.06)	0.05 (0.18)
1992–1993	0.01 (0.04)	0.04 (0.06)	0.19 (0.18)

Alleles other than 7R are denoted "×."
NE, no estimation.
[a]N = 5.
[b]N = 1.

association analyses [Fulker et al., 1999; Zhu et al., 1999], and estimates are confirmed using two statistical programs with somewhat different approaches. Furthermore, multivariate methods were used to give a more accurate assessment of the phenotype and to increase the power of linkage. Gender specific effects of the 7-repeat allele were also investigated.

The findings of our study, one of the largest samples analyzed to date (N = 769), provided no evidence for association of the DRD4 locus with any of the novelty seeking, alcohol use, and smoking measures. Previous association studies of DRD4 and novelty seeking typically sampled around 100 individuals; the maximum sample size formerly reported was 587 individuals and no association was found [Herbst et al., 2000]. In a recent family study of DRD4 and novelty seeking (measured by the Multidimensional Personality Questionnaire), which controlled for population stratification, again no association was detected [Burt et al., 2002]. Our results further concur with two meta-analyses [Kluger et al., 2002; Schinka et al., 2002] each including around 20 studies and indicating no association between DRD4 polymorphism and novelty seeking. Association studies [e.g., Chang et al., 1997; Parsian and Zhang, 1999] of DRD4 with alcoholism, typically diagnosed using DSM-III-R criteria, have also predominantly reported negative findings [e.g., Adamson et al., 1995]. As smoking is strongly related to alcohol use, it too was expected to show an association with DRD4, but this was not supported. A main effect of the 7-repeat allele on smoking status at the 2nd wave was significant, but considering the number of statistical tests performed and the inconsistency of the effect over time, this effect must be attributed to chance. One study has reported an association between smoking indices and presence of a long allele in African-Americans, but in a sample size of only 72 individuals [Shields et al., 1998]. In addition to our main findings, there was no evidence to support the hypothesis of gender differences in the association of the 7-repeat allele to novelty seeking (or any of the related phenotypes).

The percentage of variance explained by the joint allelic effects—although non-significant—was usually less than 1.5% for each individual measure. As there was a slight selection for alcohol dependence in our sample, the true proportion of variance in smoking and alcohol-related behaviors in the population may be even smaller still.

There have been no linkage studies of DRD4 with the measures used in the present study, although Long et al. [1998] have showed highly suggestive linkage (LOD score of 3.1) of D11S1984 to alcohol dependence in a sample of 172 South-western American Indian sib-pairs. Our negative findings of linkage may be a result of insufficient power to detect QTLs of small effect (e.g., 2% of variance), which DRD4 is likely to be [Comings et al., 1999]. With our full sample size we had 80% power to detect a QTL explaining around 40% of the variance against background familial variation of 30%; indeed the upper limit of the confidence interval on Q for the range of measures was typically around 20 or 40%. The low information content of the marker data would have further contributed to the negative linkage results. It was our intention to increase the power for linkage by performing multivariate analyses (i.e., multiple time points & multiple measures), although the gain in power

appeared minimal, with only a slight increase in the chi-square value observed for some measures; there was possibly no genuine linkage to detect.

It has been suggested that the absence of a positive association between novelty seeking (and presumably its related phenotypes) and DRD4 is largely due to methodological differences between studies [Lusher et al., 2001]. However, our study did not differ extensively from others in methodology. For instance, we measured novelty seeking, alcohol dependence, and alcohol use with widely accepted scales that have been used in most previous studies. Although a short form (54 items) of the novelty seeking (TPQ) scale was used in preference to the full 100 item scale, the two forms have similar psychometric properties [see Heath et al., 1994], so it is unlikely that our negative findings are due to use of the shortened version of the TPQ. We controlled for age effects so that decreased novelty seeking in older individuals would not attenuate the association; further, a separate analysis of novelty seeking in participants younger than 40 confirmed that age was not biasing the results. As well, we partialled out the effect of gender, furthermore testing the effect of the 7-repeat allele in males only. Our sample comprised individuals mostly descended from the British Isles, so the results were not negatively biased by the inclusion of Continental Europeans who when sampled usually show a negative association between novelty seeking and DRD4 [e.g., Malhotra et al., 1996; Jönsson et al., 1997].

By using DZ twin pairs and a variance components approach to data analysis we were able to estimate the locus effect, familial effects, and random environmental effects. Another advantage of this approach compared to a population based case-control design is that the effects of population stratification can be tested. Although the total association test results were negative, under certain circumstances population stratification can mask genuine associations [Posthuma et al., in press]. For this reason we also performed the between and within family association tests recommended by Abecasis et al. [2000], and found no evidence for genuine association. Other features of our statistical methods included a joint linkage and association analysis using Mx, and complementary linkage and association tests using QTDT. In contrast to the linkage analyses, tests of association were in general sufficiently powered to detect evidence of association for all measures. Mx and QTDT estimates were consistent.

In future analyses, the possibility of other genes in the region of chromosome 11 affecting novelty seeking, alcohol use, or smoking will be investigated. Association studies are likely to increase in importance with the development and use of single-nucleotide polymorphism (SNP) genotyping. For instance, a recent association between a −521C/T SNP within the promoter region of DRD4 with novelty seeking has been replicated in various ethnic samples [Okuyama et al., 2000; Ronai et al., 2001; Bookman et al., 2002], although negative associations have also been reported [e.g., Ekelund et al., 2001; Jönsson et al., 2002; Strobel et al., 2002]. Association analyses can localize relevant genes to smaller regions than linkage analyses, and have greater power to detect small effects using reasonable numbers of subjects. The disadvantage of requiring very large numbers of association markers for whole of genome studies [Risch and Merikangas, 1996] may be alleviated by analysis of haplotypes if recent suggestions of a limited range of common human haplotypes [Daly et al., 2001; Johnson et al., 2001] are confirmed and extended. Both association and linkage studies will be improved by removal of 'noise' (such as measurement error and short-term biological variation) from the analysis, for example, by using repeated or multiple measures of the same phenotype as was demonstrated in the present study.

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