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D4 Dopamine-Receptor (DRD4) Alleles and Novelty Seeking in Substance-Dependent, Personality-Disorder, and Control Subjects

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Summary

Two reports have been published suggesting an association between the personality trait of novelty seeking and the DRD4*7R allele at the D4 dopamine-receptor locus (with heterozygotes or homozygotes for DRD4*7R having higher novelty seeking). We studied novelty seeking and four coding-sequence polymorphisms affecting protein structure in the D4 dopamine-receptor gene (DRD4) in a sample of 341 American subjects, of whom 224 are of primarily European ancestry and 117 are of primarily African ancestry. These subjects had diagnoses of substance dependence or personality disorder (PD) or were screened to exclude major psychiatric diagnosis. We found that, although the substance-dependent subjects had significantly higher novelty seeking than the control and PD subjects, they did not differ in DRD4*7R allele frequency. There was no association between any DRD4 polymorphism and novelty seeking in any population or diagnostic group, except for a significant association between the DRD4*7R allele and *lower* novelty seeking among European American females and African American substance abusers. The novelty seeking of subjects heterozygous for a null mutation did not differ from that of subjects with two functional alleles. We conclude that the most likely explanation of these results is that the DRD4 VNTR does not influence directly the trait of novelty seeking, in these samples.

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Introduction

Dopamine is one of the most important neurotransmitters in the brain, and dopamine-receptor genes have been the subject of numerous candidate-gene studies in psychiatry. The D4 dopamine-receptor gene, DRD4 (Van Tol et al. 1991), has been one of the most studied, owing to the protein product's interesting ligand-binding properties (Van Tol et al. 1991), its location in limbic brain areas (Van Tol et al. 1991; Meador-Woodruff et al. 1994), the presence of several coding-region polymorphisms (Van Tol et al. 1992; Catalano et al. 1993; Nöthen et al. 1994; Seeman et al. 1994), and the effects of those polymorphisms on ligand binding (discussed below). DRD4 maps to distal chromosome 11p (Gelernter et al. 1992), proximal to HRAS (Petronis et al. 1993).

We have reported linkage disequilibrium between the seven-repeat allele at a DRD4 VNTR polymorphism (DRD4*7R) and Tourette syndrome, using the Spielman transmission-disequilibrium test (Grice et al. 1996). DRD4 alleles recently have been reported to be associated with personality measures, specifically novelty seeking (Benjamin et al. 1996; Ebstein et al. 1996). Persons with high novelty seeking are described as "impulsive, exploratory, excitable, disorderly and distractible" (Cloninger 1987, p. 411). According to one article (Benjamin et al. 1996), allelic variation at DRD4 may account for 10% of the genetic variation of novelty seeking. Ebstein et al. (1996) studied 124 unrelated Israeli subjects; the DRD4*7R allele was found to be associated with higher novelty seeking, independent of ethnicity, sex, or age. Benjamin et al. (1996) studied 315 siblings, most of whom were males, family members, and unrelated individuals from the United States and found an association of so-called long alleles (DRD4*7R and DRD4*8R) with novelty seeking; stratification was excluded as an explanation, on the basis of the sibling-pair data. Although these two reports were consistent, the findings could not be replicated in Finnish (Malhotra et al. 1996) or Swedish (Jönsson et al. 1997) subjects.

Several factors suggested, beforehand, that the findings might be difficult to replicate. Patients with type 2

alcoholism (a subtype that is thought to be particularly heritable) and substance dependence are expected to have relatively high novelty seeking (Cloninger 1987). Since alcohol- and drug-dependent subjects have high novelty seeking, the allele associated with novelty seeking (DRD4*7R) should be observed at an increased frequency in a sample of alcohol- and drug-dependent subjects. Previously published results (George et al. 1993; Adamson et al. 1995; Muramatsu et al. 1996) suggest, however, that there is no association of the DRD4*7R allele with alcohol dependence.

Also, a moderate degree of assortative mating exists for certain features of personality (Feng and Baker 1994), so random mating does not apply for genetic loci affecting these personality features, and, if the effects are strong enough, there consequently should be a deviation from Hardy-Weinberg equilibria (HWE) expectations at such loci. If DRD4 alleles really affected personality to a significant degree, there should be an increase in matings of persons with like genotypes and increased observed homozygosity for the operant alleles, with an increase in observed DRD4*7R homozygotes. However, to our knowledge, no deviation from HWE expectations has been reported at this locus. It was with these considerations in mind that we studied DRD4 allele frequencies in subjects dependent on alcohol, cocaine, or heroin; in subjects with personality disorders (PDs); and in normal control subjects.

In addition to the exon 3 VNTR, which is the most studied variant at this locus, there exist at least three additional coding-sequence variations that also affect the amino acid sequence. Two are in exon 1 (a 12-bp duplication of unknown functional effect but reported to be associated with psychosis [Catalano et al. 1993] and a 13-bp deletion resulting in a null allele [Nöthen et al. 1994]). An additional single-base mutation, $D4_{194Glycine}$, in exon 3 was observed previously only in individuals of African ancestry; the protein product exhibits activity that is two orders of magnitude lower than that of the normal D4 receptor (Seeman et al. 1994; Liu et al. 1996); therefore, it is nearly a null mutation also.

Published studies (including Benjamin et al. 1996; Ebstein et al. 1996; Malhotra et al. 1996) have considered primarily the exon 3 VNTR polymorphism (Van Tol et al. 1992; Asghari et al. 1994, 1995). This polymorphism consists of a variable number of 48-bp tandem repeats located in the third cytoplasmic loop of the corresponding protein (Van Tol et al. 1991; Lichter et al. 1993). The initial expression study of cDNA for cloned receptor variants and two subsequent studies reported different properties for the alleles with seven repeats (DRD4*7R), compared with the two other most common alleles (DRD4*2R and DRD4*4R), with respect to clozapine and spiperone binding or to other functional measures (Van Tol et al. 1992; Asghari et al. 1994, 1995). Rarer VNTR alleles, for example, DRD4*8R, were not studied. It is possible that, for any of these studies, the observed effects were conveyed by sequence differences other than those at the identified exon 3 site (e.g., one of the other three known coding-region variants discussed above). We studied all four known DRD4 coding-region variants but did not distinguish between different VNTR alleles of identical length but of differing sequence.

Subjects and Methods

Subjects

Subjects were assessed at one of three sites, as described below. Informed consent was obtained from all subjects. Assessment at the different sites was as follows:

1. University of Connecticut Health Center (UCHC), Farmington (162 substance-dependent subjects, 5 PD subjects, and 48 controls). Control subjects were assessed by use of the SCID (Structured Clinical Interview from the *Diagnostic and Statistical Manual of Mental Disorders,* 3d ed., rev. [DSM-III-R] [American Psychiatric Association 1987]) (Spitzer et al. 1992) and the SCID II (First et al. 1995) and were evaluated to exclude those with a major axis I psychiatric disorder. The PD subjects were evaluated in the same manner as that used for the controls but were found to have PDs. Substance-dependent subjects were diagnosed, by use of SCID, with either alcohol or drug dependence or both; schizophrenia was an exclusion criterion. Novelty seeking was assessed by use of the Temperament and Character Inventory, in which the Tridimensional Personality Questionnaire (TPQ) (Cloninger et al. 1991) is embedded.

2. Medical College of Pennsylvania–Hahnemann School of Medicine (MCP) (87 subjects [63 with PDs and 24 controls]). Diagnostic procedures are summarized elsewhere (Coccaro et al. 1996). In brief, diagnoses were made by use of DSM-III-R and were assigned by a best-estimate process based on information obtained by interviews using the Schedule for Affective Disorders and Schizophrenia (SADS) (Spitzer and Endicott 1975) and the Structured Interview for the Diagnosis of Personality Disorder, revised (SIDP-R) (Pfohl et al. 1989), by clinical interviews by a research psychiatrist, and by review of all other available clinical data. Novelty seeking was assessed by use of the TPQ (Cloninger et al. 1991).

3. Mt. Sinai School of Medicine (MSSOM), New York (39 subjects, all with PDs). Subjects were evaluated by use of the SADS (Spitzer and Endicott 1975). All subjects with PDs were interviewed by one rater, with the SIDP-R (Pfohl et al. 1989); a second rater administered an independent SIDP to an individual close to the subject. Consensus diagnoses were reached in a meeting of

all raters, with an expert diagnostician. Novelty seeking was assessed by use of the TPQ (Cloninger et al. 1991).

A total of 341 subjects, of whom 204 (59.8%) were male, participated in the study. A significantly greater proportion of the PD patients were male (72.9%), compared with that of the controls (52.8%) and that of the substance-dependent patients (54.3%) ($\chi^2 = 11.13$, 2 df, $P = .004$). The subject group was 65.7% European American and 34.3% African American. A significantly greater proportion of controls were African American (54.2%), compared with both substance-dependent patients (30.2%) and PD patients (27.1%) ($\chi^2 = 16.25$, 2 df, $P = .0003$). The mean (SD) age of all subjects was 35.4 (9.8) years, with the controls being significantly younger (32.6 [9.6] years) than either the substance-dependent patients (36.4 [9.4] years) or the PD patients (35.7 [10.2] years) ($F[2, 338] = 3.83$, $P = .023$). Among the 162 patients with a lifetime DSM-III-R substance-use disorder (American Psychiatric Association 1987), 132 patients had alcohol dependence, 93 patients had cocaine dependence, and 55 patients had opiate dependence (some subjects had more than one substance-dependence diagnosis). Among the 107 PD patients, breakdown by diagnosis is as follows (some subjects received more than one PD diagnosis, including more than one diagnosis within a cluster). For the MCP subjects, there were 21 dramatic-cluster diagnoses (antisocial, histrionic, narcissistic, or borderline), 20 anxious-cluster diagnoses (obsessive-compulsive, passive-aggressive, dependent, or avoidant), and 18 odd-cluster diagnoses (paranoid, schizoid, or schizotypal), and 23 subjects received a diagnosis of PD not otherwise specified (NOS). For the MSSOM subjects, there were totals of 39 dramatic-cluster diagnoses, 39 anxious-cluster diagnoses, 26 odd-cluster diagnoses, and 4 NOS diagnoses. For the UCHC subjects, there were 2 dramatic-cluster diagnoses, 2 anxious-cluster diagnoses, 1 odd-cluster diagnosis, and 2 NOS diagnoses.

Preparation of DNA and Polymorphisms Studied

DNA was extracted from whole blood by standard methods. Genotyping was accomplished at the VA Connecticut Healthcare System, West Haven Campus. A Perkin Elmer Cetus model 9600 PCR cycler was used for all reactions; all reactions used the hot-start technique.

Exon 3 VNTR.—The conditions used have been described elsewhere (Grice et al. 1996).

Exon 3 single-base substitution (glycine 194).—The primers were ex3-1f and ex3-1r, as reported by Seeman et al. (1994). The PCR product was digested by use of *Acc*I (New England Biolabs), to recognize the polymorphism (Seeman et al. 1994).

Exon 1 13-bp deletion and 12-bp insertion/deletion.—We amplified a region of exon 1 containing both polymorphisms, using the 5′ primer (Ex1S) described by Catalano et al. (1993) and the 3′ primer (519) described by Nöthen et al. (1994) as Chang and Kidd (1997), but we digested the PCR product with *Pst*I (New England Biolabs), which cleaves the product into fragments of ~100 bp and ~200 bp. The 13-bp deletion occurs in the shorter fragment, the 12-bp duplication in the longer fragment.

Statistical Analyses

Hierarchical multiple regression was used to examine the correlation of novelty-seeking scores with demographic features, psychiatric diagnostic group, the DRD4 VNTR genotype, and two-way and three-way interactions involving the VNTR genotype. In this analysis, independent variables were entered in five sets, in the following order: (1) demographics (sex, race, and age); (2) diagnostic groups (controls, substance-dependent patients, and PD patients); (3) VNTR genotype (DRD4*7R allele absent vs. one or two alleles present); (4) all two-way interactions involving the VNTR genotype; and (5) the three-way interactions involving the VNTR genotype (with the exception that the two orthogonal diagnostic-group predictors were not allowed to interact). Demographic variables were centered or effect coded (−1, 1), and two orthogonal dummy variables were created from the three-level diagnosis. Two orthogonal contrasts (controls vs. patients and substance-dependent patients vs. PD patients) were used to examine the difference in novelty-seeking scores among the diagnostic groups.

This analytic approach made it possible to control for the effects of each of the demographic and diagnosis-predictor variables prior to examination of the effect of genotype on novelty-seeking scores. The subsequent hierarchical entry of the two-way and then the three-way interactions served to clarify further the main effects that were observed, while insuring that the higher-order interactions were orthogonal to all lower-order antecedent effects.

A similar analytic approach was used to examine the correlation of the exon 1 12-bp insertion and the null mutations, with novelty-seeking scores. Each of these genotypes was examined in separate regression analyses. In both cases, however, the unacceptably small cell sizes (table 1) that would have resulted from the use of interaction terms as predictor variables led to the examination of only the main effects of (1) demographic variables, (2) diagnostic groups, and (3) genotype on novelty-seeking scores. Analysis of the exon 1 12-bp insertion was performed by use of the presence or absence of this variant alone. The exon 1 13-bp deletion is a literal null mutation, and the other exon 3 mutation, $D4_{194Glycine}$, substantially decreases ligand binding. Be-

Table 1

Allele Frequencies for DRD4 Polymorphisms

Polymorphism and Subject Group	2*n*	Allele Frequency							
		2 Repeats	3 Repeats	4 Repeats	5 Repeats	6 Repeats	7 Repeats	8 Repeats	10 Repeats
Exon 3 VNTR:									
European American:									
Normal controls	66	.045	.000	.697	.045	.000	.182	.030	.000
PD	156	.096	.032	.615	.000	.006	.250	.000	.000
Substance dependent	210	.090	.052	.671	.010	.014	.152	.010	.000
Total	432	.086	.037	.655	.012	.009	.192	.009	.000
African American:									
Normal controls	78	.038	.013	.628	.038	.000	.218	.051	.013
PD	58	.069	.000	.724	.034	.000	.138	.034	.000
Substance dependent	98	.061	.000	.602	.051	.020	.245	.020	.000
Total	234	.056	.004	.641	.043	.009	.209	.034	.004
		Duplication Present				Duplication Absent			
Exon 1 12-bp duplication:									
European American:									
Normal controls	58	.914				.086			
PD	156	.936				.064			
Substance dependent	218	.931				.069			
Total	432	.931				.069			
African American:									
Normal controls	72	.944				.056			
PD	58	.931				.069			
Substance dependent	92	.967				.033			
Total	222	.950				.050			
		Polymorphism Absent				Polymorphism Present			
Exon 1 13-bp deletion:									
European American:									
Normal controls	54	1.000				.000			
PD	156	.987				.013			
Substance dependent	216	.977				.023			
Total	426	.984				.016			
African American:									
Normal controls	72	1.000				.000			
PD	58	1.000				.000			
Substance dependent	92	1.000				.000			
Total	222	1.000				.000			
		$D4_{194Glycine}$				$D4_{194Valine}$			
Exon 3 $D4_{194Glycine}$ mutation:									
European American:									
Normal controls	58	.000				1.000			
PD	156	.000				1.000			
Substance dependent	222	.005				.995			
Total	436	.002				.998			
African American:									
Normal controls:	78	.064				.936			
PD	58	.034				.966			
Substance dependent	94	.032				.968			
Total	230	.043				.957			

Note.—For some individuals, genotypes for all four systems were not available.

cause of the similarity of the predicted effect of these two mutations, in the reduction or the elimination of protein function, and the relative rarity of the inactive or the much less active variant forms, we considered together the groups having either of these mutations.

For comparison of DRD4 allele frequency by group, 2×2 χ^2 was used. To ascertain possible deviation from HWE expectations for DRD4*7R observations, we used the program HWsim (described in Cubells et al. 1997).

Results

Multivariate Linear Regression with the VNTR Polymorphism

The demographic independent-variable set accounted for a significant proportion of the variance in novelty-seeking scores ($F[3, 329] = 5.96$, $R^2 = .052$, $P = .001$). Two of the three demographic variables were significant in the final regression equation. Sex was not a significant predictor ($F[1, 319] = 0.67$, $P = .41$). However, race was a significant predictor ($F[1, 319] = 6.67$, $P = .010$), with European Americans having higher novelty-seeking scores (mean 20.3, SD 6.7) than African Americans (mean 18.6, SD 5.7). Age also was a significant predictor ($F[1, 319] = 15.65$, $P < .001$), with younger subjects having higher novelty-seeking scores (standardized $\beta = -.19$).

The diagnostic independent-variable set also accounted for significant variance ($F[2, 327] = 44.85$, $R^2 = .204$, $P < .001$). The two patient groups had higher novelty-seeking scores (mean 20.5, SD 6.5) than controls (mean 16.8, SD 5.1) ($F[1, 319] = 22.26$, $P < .001$). Similarly, substance-dependent patients had higher novelty-seeking scores (mean 22.7, SD 6.0) than PD patients (mean 17.1, SD 5.9) ($F[1, 319] = 40.71$, $P < .001$).

Although the variance accounted for by the single-variable genotype set was significant ($F[1, 326] = 6.69$, $R^2 = .015$, $P = .010$), it accounted for only ~1.5% of the variance in novelty-seeking scores. Interestingly, subjects without the DRD4*7R allele had higher novelty-seeking scores (mean 20.4, SD 6.5) than subjects with one or two copies of that allele (mean 18.5, SD 6.2) ($F[1, 319] = 5.36$, $P = .021$).

The variance accounted for by the two-way-interaction set was not significant ($F[5, 321] = .66$, $P = .66$). Nonetheless, it was retained in the equation, because the set of higher-order (i.e., three-way) interactions accounted for significant variance ($F[2, 319] = 5.96$, $R^2 = .026$, $P = .003$). Of the eight three-way interactions analyzed, only two were significant. The sex, by race and by VNTR-genotype interaction, was statistically significant ($F[1, 319] = 6.37$, $P = .012$). Decomposition of the interaction revealed that, among European American females, the absence of the DRD4*7R allele was associated with significantly higher novelty-seeking scores (mean 22.8, SD 6.5), compared with the presence of the DRD4*7R allele (mean 19.6, SD 7.0) ($F[1, 208] = 4.66$, $P = .032$). The presence versus the absence of the DRD4*7R allele had no significant impact on novelty-seeking scores for any of the other three race-by-sex groups. The other three-way interaction that was statistically significant ($F[1, 319] = 8.40$, $P = .004$) involved the second diagnostic variable (substance-dependent vs. PD patients), by race and by VNTR genotype. Decomposition of the interaction revealed that, among African American patients with substance dependence, the absence of the DRD4*7R allele was associated with significantly higher novelty-seeking scores (mean 23.6, SD 5.1), compared with the presence of the DRD4*7R allele (mean 19.1, SD 3.6) ($F[1, 109] = 5.89$, $P = .017$). The presence versus the absence of the DRD4*7R allele had no significant impact on novelty-seeking scores for any of the other three race-by-diagnostic groups (fig. 1).

Repetition of the entire analysis, by use of genotype as a continuous independent variable (i.e., 0, 1, or 2 copies of the DRD4*7R allele), produced nearly identical results. Furthermore, examination of the study site (UCHC, MCP, or MSSOM) as a main effect as well as the interaction of the study site and the VNTR genotype revealed that these were not significant predictors of novelty-seeking scores, so it was removed from the analysis.

Multivariate Linear Regression with the Null Alleles

A total of 18 subjects heterozygous for the null or the very low activity allele were observed. (For the African American subjects, all of the heterozygotes are heterozygotes for glycine 194, whereas, for the European American subjects, all but one are heterozygous for the 13-bp deletion.)

As in the first analysis, the demographic independent-variable set accounted for a significant proportion of the variance in novelty-seeking scores ($F[3, 316] = 6.58$, $R^2 = .059$, $P < .001$). Although sex was not a significant predictor ($F[1, 312] = 2.03$, $P = .16$), both race ($F[1, 312] = 6.887$, $P = .009$) and age ($F[1, 312] = 11.44$, $P = .001$) were significantly correlated with novelty-seeking scores. Consistent with the results of the first analysis, European Americans and younger subjects had higher novelty-seeking scores.

The diagnostic independent-variable set also accounted for significant variance ($F[2, 314] = 41.16$, $R^2 = .195$, $P < .001$). The two patient groups had higher novelty-seeking scores than controls (mean 16.8, SD 5.1) ($F[1, 312] = 13.29$, $P < .001$). Similarly, substance-dependent patients had higher novelty-seeking scores than PD patients ($F[1, 312] = 63.59$, $P < .001$).

The variance accounted for by the single-variable genotype set was not significant ($F[1, 313] = .24$, $P =$

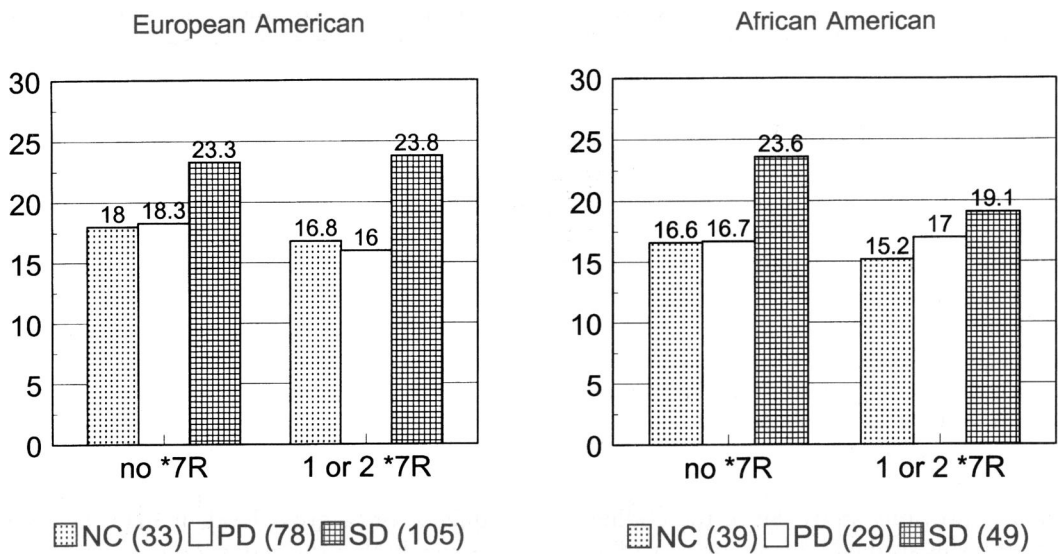

Figure 1 DRD4 exon 3 genotype and novelty seeking, by TPQ. NC = normal control; PD = personality disordered; and SD = substance dependent.

.63). Between subjects with one of the null alleles (n = 18; mean 19.1, SD 5.5) and those without a null allele (n = 302; mean 19.9, SD 6.5), there was no difference in novelty-seeking scores.

Multivariate Linear Regression with the Exon 1 12-bp Insertion

As in the other analyses, the demographic independent-variable set accounted for a significant proportion of the variance in novelty-seeking scores (F[3, 323] = 6.16, R^2 = .054, P < .001). Two of the three demographic variables also were significant in the final regression equation. Sex was not a significant predictor (F[1, 319] = 2.75, P = .098). Both race (F[1, 312] = 6.01, P = .015) and age (F[1, 319] = 10.63, P = .001) were significant predictors, with European Americans and younger subjects having higher novelty-seeking scores.

The diagnostic independent-variable set also accounted for significant variance (F[2, 321] = 39.25, R^2 = .186, P < .001). Patients had higher novelty-seeking scores than controls (F[1, 319] = 12.95, P < .001), with substance-dependent patients having higher novelty-seeking scores than PD patients (F[1, 319] = 60.28, P < .001).

The variance accounted for by the single-variable genotype set was not significant (F[1, 320] = 2.44, P = .12). Between subjects with the exon 1 12-bp insertion (n = 38; mean 21.2, SD 6.9) and those without that variant (n = 289; mean 19.6, SD 6.4), there was no difference in novelty-seeking scores.

HWE and Allele-Frequency Comparisons

If the DRD4*7R allele affects novelty seeking, it also might influence assortative mating, and this could cause a deviation from HWE. However, the expected number of DRD4*7R homozygotes (under HWE) was observed in each population: for European American subjects, the observations for DRD4*7R homozygotes and heterozygotes and for other genotypes were 7, 69, and 140, respectively; for deviation from HWE expectations, χ^2 = 0.18 (1 df) (not significant). For African American subjects, the corresponding figures were 7, 35, and 75; χ^2 = 1.09 (1 df) (not significant). There were no significant allele-frequency differences between the high novelty-seeking (substance dependent) and low novelty-seeking (normal control + PD) groups, for any of the four polymorphic systems.

Discussion

We observed no significant increase in DRD4*7R allele frequency in any group, despite considerable variability in the level of novelty seeking among subgroups; the only significant associations seen were *lower* novelty seeking in European American women and in substance-dependent African Americans with a DRD4*7R allele. (Interestingly, Malhotra et al. [1996] reported a significant association between the DRD4*7R allele and decreased novelty seeking in Finnish alcoholic offenders.) This suggests that the DRD4*7R allele is not associated with increased novelty seeking in these subject groups, as reported elsewhere for other subjects (Benjamin et al.

1996; Ebstein et al. 1996). We used the same measure of novelty seeking as that used by Ebstein et al. (1996), so a direct comparison with their results is possible. No other DRD4 polymorphism was associated with novelty seeking in any subgroup of either population. Power analyses based on the reported figures of Benjamin et al. (1996) suggested that a sample size of 328 (a threshold that we slightly exceeded) would have been adequate to detect an effect of the magnitude that they reported for the VNTR (0.4 SD) (two-tailed test), with $\alpha = .05$ and 90% power (Brent et al. 1993).

We studied groups of subjects for whom genetic determinants of novelty seeking could differ (substance dependent and non–substance dependent, Americans of European ancestry and of African ancestry, and males and females), but, overall, our sample size was larger than that for any previous study. Since the coding-sequence polymorphisms studied here are presumed to affect function directly, population stratification should not be a major issue. Furthermore, the data analytic approach employed made it possible to control for subgroup differences in novelty seeking, prior to examination of the impact of a DRD4 genotype.

We examined all known coding-sequence DRD4 polymorphisms, some of which clearly have a greater effect on function than the exon 3 VNTR. Heterozygotes for DRD4 exon 1 or exon 3 null mutations either should express approximately one-half the usual amount of D4 dopamine-receptor protein (exon 1 null) or should have one-half nearly nonfunctional D4 receptors (exon 3 null). For D4 receptor–related phenotypes, these individuals with reduced D4 function should show some variation, compared with individuals with two alleles encoding a functional receptor. However, we found that there was no difference in novelty seeking, for the small observed number of null heterozygotes. This should provide a good test of the hypothesis of an association of DRD4 alleles with novelty seeking, unless the DRD4*7R allele represents a large gain of function, that is, unless the difference between the DRD4*7R allele and the DRD4*4R allele (for example) is quantitatively greater than the difference between the DRD4*4R allele and zero function. Our results with the exon 1 12-bp duplication argue against the hypothesis that it is this variant that conveys the primary phenotypic effect.

There are several possible explanations for this failure to observe higher novelty seeking in subjects with the DRD4*7R allele, as observed by Ebstein et al. (1996) and by Benjamin et al. (1996). A true association could be present only in certain populations or in certain ranges of the phenotype. The DRD4*7R allele thus could have a small effect on increasing novelty seeking only in the normal range, in the context of other genetic and environmental factors that are more important. However, our results do not support such an association, even in the normal range. Another possibility is that the association could be present—but for a phenotype that is not ideally measured by personality measures such as the TPQ and that is interacting with those measures. Consistent with this hypothesis, our previous data (Grice et al. 1996) do support a physiological role for DRD4 variation. In general, this hypothesis is also consistent with the fact that we did observe a significant relationship between novelty seeking and the DRD4*7R allele, in our African American substance-dependent subjects and European American female subjects, but in the direction opposite that observed by Ebstein et al. (1996) and by Benjamin et al. (1996). This could be explained by an unidentified interaction between DRD4 alleles and personality.

Alternatively, since the exon 3 VNTR affects protein sequence on a gross level, it may have been assumed incorrectly that this variation contributes directly to the protein differences observed: it also could be in linkage disequilibrium with a still unknown polymorphism that has a greater effect on function. In different populations (e.g., African American and European American), the DRD4*7R VNTR allele could be in linkage disequilibrium with different alleles of this putative polymorphism actually conveying a phenotypic effect. This could account for our observation and the Malhotra et al. (1996) observation of the DRD4*7R allele being associated with low novelty seeking in African American and Finnish subjects with substance-use disorders and for the Ebstein et al. (1996) and Benjamin et al. (1996) observations of the same allele being associated with high novelty seeking in European American or in White Middle Eastern subjects. This still could not explain why we saw no association in our normal control and PD subjects, and the lack of an effect of the DRD4 mutations either eliminating or greatly reducing D4 dopamine-receptor function would remain problematic.

This failure to replicate an association between the DRD4*7R allele and *higher* novelty seeking may simply be a chance event, but the possibility that the present results represent a false negative should be viewed in the context of the absence of previous data showing an association between the DRD4*7R allele and diagnoses associated with high novelty seeking, such as alcoholism. We note that our finding, to a limited extent, is consistent with the earlier observations that DRD4 alleles are associated with novelty seeking. We conclude that, if genetic variation at the DRD4 locus exerts an effect on human novelty seeking, it is likely to be through a mutation in linkage disequilibrium with the exon 3 VNTR rather than as a direct consequence of that variation.

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