No Association Between Novelty Seeking and the Type 4 Dopamine Receptor Gene (*DRD4*) in Two New Zealand Samples

Patrick F. Sullivan, M.D., F.R.A.N.Z.C.P., Wendy J. Fifield, B.Sc. (Hons.), Martin A. Kennedy, Ph.D., Roger T. Mulder, F.R.A.N.Z.C.P., J. Douglas Sellman, F.R.A.N.Z.C.P., and Peter R. Joyce, F.R.A.N.Z.C.P., Ph.D.

Objective: *In 1986 and 1987, Cloninger postulated the existence of the heritable behavioral trait of novelty seeking and its putative underpinnings in the dopaminergic systems of the ventral midbrain. Two widely reported studies found significant associations between novelty seeking and the type 4 dopamine receptor gene (*DRD4*), although a more recent study did not. The authors' objective was to investigate this association in two New Zealand samples.* *Method:* *The authors studied two nonoverlapping samples: subjects in a depression treatment trial (N=86) and subjects from 14 pedigrees dense with alcoholism (N=181).* DRD4 *genotyping was based on a standard protocol.* *Results:* *Novelty seeking and* DRD4 *were not statistically associated.* *Conclusions:* *In these samples, there was no suggestion that the* DRD4 *polymorphism contributed to individual differences in the behavioral trait of novelty seeking.*

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A decade ago, Cloninger (1, 2) postulated the existence of the heritable behavioral trait of novelty seeking and its putative underpinnings in the dopaminergic systems originating in the ventral midbrain. Large twin studies supported the heritability of novelty seeking (about 50%) (3, 4). There have been two reports of significant associations between novelty seeking and a polymorphism of the type 4 dopamine receptor (*DRD4*) (5, 6). This polymorphism—a 48 base-pair sequence repeated 2–8 times—codes for an expressed sequence associated with differential pharmacological characteristics (7), and the presence of the 7-repeat allele (5) or 6 or more repeats (6) was associated with higher novelty seeking scores. Subsequent studies in Finland (8) and Sweden (9) did not replicate these findings. These reports generated substantial interest in scientific and lay circles.

The objective of our report was to attempt to confirm or refute an association between novelty seeking and *DRD4* in two separate samples in New Zealand.

METHOD

Subjects

We studied two groups of subjects: 1) 86 subjects with a current major depressive illness of at least moderate severity who were studied in the context of a randomized clinical trial that compared the long-term efficacy of the antidepressants fluoxetine and nortriptyline and 2) 181 subjects who were members of 14 multiplex alcoholic pedigrees who were ascertained from clinical sources and studied according to a protocol designed to maximize comparability to a larger-scale set of American studies. Both studies were ethically reviewed and approved in advance, and all subjects provided written informed consent.

Measures

Psychiatric diagnoses were determined by using structured diagnostic interviews (10, 11) administered by trained raters. All subjects completed the self-report Temperament and Character Inventory (12, 13). This instrument contains essentially the same 34 items used by Ebstein et al. (5) and Malhotra et al. (8). (Benjamin et al. [6] computed novelty seeking scores from another personality measure.) To maximize comparability to the previous reports, the total novelty seeking scores reported here were summed across these 34 items.

Received Feb. 26, 1997; revision received July 11, 1997; accepted July 31, 1997. From Virginia Commonwealth University/Medical College of Virginia, Richmond; the Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics; and Cytogenetics and Molecular Oncology, Department of Pathology, and University Department of Psychological Medicine, Christchurch School of Medicine, Christchurch, New Zealand. Address reprint requests to Dr. Sullivan, Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, P.O. Box 980126, Richmond, VA 23298-0126; sullivan@psycho.psi.vcu.edu (e-mail).

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TABLE 1. Demographic, Diagnostic, and *DRD4* Genotypic Characteristics of Subjects From a Depression Clinical Trial and From Multiplex Alcoholic Pedigrees

Characteristic	Depression Clinical Trial (N=86)					Multiplex Alcoholic Pedigrees (N=181)				
	Mean	*SD*				*Mean*	*SD*			
Age (years)	32.0	11.0				39.7	14.1			
Total score on 34-item novelty seeking scale	15.9	5.4				16.1	5.3			
	N	*%*				*N*	*%*			
Sex										
Male	34	39.5				90	49.7			
Female	52	60.5				91	50.3			
Lifetime alcohol dependence	—	28.6				79	43.6			
Lifetime major depression	86	100.0				63	34.8			
	DRD4 *Frequency*					DRD4 *Frequency*				
4-repeat allele	0.608					0.596				
7-repeat allele frequency	0.228					0.251				
7-repeat allele present	0.388					0.453				
	Mean	*SD*	*Effect Size*	*p*	*Adjusted p*[a]	*Mean*	*SD*	*Effect Size*	*p*	*Adjusted p*[a]
Relation of *DRD4* 7-repeat allele and novelty seeking scale score										
Novelty seeking score										
DRD4 7-repeat allele present	16.0	6.0				16.4	5.5			
DRD4 7-repeat allele absent	15.7	5.1				15.7	5.3			
Analysis			0.06	0.78[b]	0.85[c]			0.13	0.38[d]	0.77[e]

[a]Adjusted for age and sex. [b]F=0.08, df=1, 83. [c]F=0.03, df=1, 81.
[d]F=0.79, df=1, 177. [e]F=0.08, df=1, 175.

DNA Extraction

Material from subjects in the 14 pedigrees dense with alcoholism (N=181) was in the form of lymphoblastoid cell lines generated by Epstein-Barr virus transformation of white blood cells. DNA was extracted from 2×10^6 frozen cells of each cell line (14). A different method was used to extract DNA from peripheral blood samples of subjects from the depression treatment trial (N=86). Peripheral blood (5 ml in an EDTA or lithium-heparin tube) was mixed with 45 ml of lysis buffer (0.32 M sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$, 1% Triton X-100). Leukocytes were recovered by centrifugation and resuspended in lysis solution (4 M guanidine isothiocyanate, 25 mM sodium acetate, 0.84% β-mercaptoethanol) to release DNA. An equal volume of isopropanol was added to precipitate the DNA, which was recovered by centrifugation and washed three times in cold 70% ethanol. The DNA was then resuspended and dissolved in 10 mM Tris pH 8.0–1 mM EDTA (0.5 ml) and stored at 4°C.

DRD4 *Genotyping*

Primers for genotyping of *DRD4* were D4-3 and D4-42 (15). Polymerase chain reaction was carried out in 25 µl buffer (as supplied with the enzyme) containing 200 µM each of the four deoxynucleoside triphosphates, 1.5 mM $MgCl_2$, 0.5 µM of each primer, 10% dimethyl sulfoxide, and approximately 50 ng of genomic DNA. A "hot-start" strategy was used in which reactions were heated to 99°C for 1 minute, then cooled to 95°C before the addition of 0.5 units of eLONGase (Life Technologies, Gaithersburg, Md.). Temperature cycles (35 in total) were 95°C for 30 seconds (beginning with the enzyme addition step), 60°C for 30 seconds, and 72°C for 40 seconds. A final step of 72°C for 4 minutes completed the reactions. All products were resolved by electrophoresis on 2% agarose gels, stained with ethidium bromide, and sized by comparison with a 123 base-pair ladder (Life Technologies, Gaithersburg, Md.).

Statistical Analysis

For maximal comparability to previous reports (5, 6, 8), we stratified our samples by the presence or absence of the *DRD4* 7-repeat allele. All p values reported are two-tailed.

Because novelty seeking is negatively correlated with age and may have gender differences (13), age and gender were included as covariates in analysis of variance models. Effect sizes were calculated according to the method of Cohen (16): an effect size of 0.2 was considered a small effect and an effect size of 0.5 was considered a medium effect.

To take into account the genetic relationships in the subjects from the 14 pedigrees dense with alcoholism (N=181), we used a sibling pair approach. We constructed a data set consisting of all possible pairs of siblings (N=133) and then selected the 72 pairs discordant for the presence of the *DRD4* 7-repeat allele. We compared the novelty seeking scores of these discordant sibling pairs by using Student's t test. In these analyses, a given sibling may be included more than once. Such an inclusion, however, is "anticonservative" in that the p value obtained tends to be smaller than the true p value.

RESULTS

Primary Results

As shown in table 1, substantial proportions of each sample had lifetime DSM-III-R diagnoses of major depression and alcohol dependence. The mean novelty seeking total score for each sample was similar to that reported by Ebstein et al. (5). The *DRD4* 7-repeat allele was more prevalent in these two New Zealand samples

than in the Israeli sample of Ebstein et al. (7-repeat allele frequency=0.274) (5) or in the U.S. sample of Benjamin et al. (7-repeat allele frequency=0.167) (6).

For *DRD4*, there was no statistically significant association in either sample between the 7-repeat allele and the behavioral trait of novelty seeking. Moreover, the effect sizes (16) we observed were considerably less than those of Ebstein et al. (effect size=0.51) (5) or Benjamin et al. (effect size=0.39) (6).The analyses in table 1 for the subjects from 14 pedigrees dense with alcoholism (N=181) consider each subject as an independent observation even though they are genetically related. To take these relationships into account, we compared the novelty seeking scores of the 72 sibling pairs discordant for the presence of the *DRD4* 7-repeat allele (from 133 possible sibling pairs). Again, there was no significant association between novelty seeking and the presence or absence of the *DRD4* 7-repeat allele (paired t test=0.569, p=0.51; mean novelty seeking score with 7-repeat allele present=16.2, SD=4.5, with 7-repeat allele absent=16.7, SD=6.0). These analyses ignore the fact that a given sibling may be included more than once in these discordant pairs. However, these analyses are "anticonservative" in that the p value obtained tends to be smaller than the true p value. Given that p=0.52 for the t test above, it is unlikely that these data contain a significant relationship between novelty seeking and the *DRD4* 7-repeat allele.

Secondary Results

We also conducted several preplanned secondary analyses. 1) There were no significant associations between any of the four novelty seeking subscales and the presence or absence of the *DRD4* 7-repeat allele in either of the two samples (p>0.20 for all eight comparisons). 2) For the depressed sample, novelty seeking scores for each subject were available from an informant and from the subject 6 months after the initial determination of novelty seeking. The use of multiple informants and longitudinal measurements is an important way to distinguish the "stable" part of novelty seeking from the error inherent in self-reported behavioral traits (17). We used principal components analysis of these three different novelty seeking scores (i.e., from the subject initially, from the subject after 6 months, and from an informant) to extract an index of "stable" novelty seeking; the first principal component accounted for 79.2% of the variance in novelty seeking scores. Similar to the analyses reported in table 1, the presence or absence of the *DRD4* 7-repeat allele was not significantly associated with the stable component of novelty seeking (p=0.54, controlling for age and sex). 3) Clinical samples such as the ones in the present report are likely to be heterogeneous. It is possible that such heterogeneity could mask a true association of *DRD4* with novelty seeking. When we stratified each of the two samples by the presence of certain "impulsive" traits (i.e., the presence of DSM-III-R alcohol dependence, bulimia nervosa, childhood conduct disorder, and adult antisocial personality disorder), there were again no significant associations between novelty seeking and the *DRD4* 7-repeat allele.

DISCUSSION

Our results are in contrast to findings in the initial reports by Ebstein et al. (5) and Benjamin et al. (6) that associated the behavioral trait of novelty seeking with the *DRD4* 7-repeat allele, but they are consistent with the negative results of Malhotra et al. (8). In the main and secondary analyses, no comparison approached statistical significance. Consequently, in our samples, there was no evidence—or even a subtle trend—that this *DRD4* polymorphism had any impact on the behavioral trait of novelty seeking as determined by self-report. From these results, *DRD4* would not appear to be a plausible candidate gene for novelty seeking.

There are two important caveats to our findings. First, the composition of clinical samples is widely recognized to be influenced by any number of potential biases. Such biases could have altered or masked a true association between novelty seeking and *DRD4*. For example, the symptoms of major depression and alcoholism themselves might bias an individual's self-report of novelty seeking. Second, the *DRD4* 48 base-pair repeat polymorphism we studied varies in the sequence and order of the repeats (15). As in the previous reports (5, 6, 8, 9), we studied only the number of repeats—but not their sequence or order—and cannot exclude the possibility that individual variation in these two factors is relevant to novelty seeking.

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