Studies of the 48 bp Repeat Polymorphism of the 
**DRD4** Gene in Impulsive, Compulsive, Addictive 
Behaviors: Tourette Syndrome, ADHD, Pathological 
Gambling, and Substance Abuse

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Prior studies have reported an association 
between the presence of the 7 repeat allele 
of the 48 bp repeat polymorphism of the 
third cytoplasmic loop of the dopamine D4 
receptor gene (**DRD4**) and novelty seeking 
behaviors, attention deficit hyperactivity 
disorder (ADHD), Tourette syndrome (TS), 
pathological gambling, and substance abuse. However, other studies have failed to 
replicate some of these observations. To de-
termine whether we could replicate these 
associations we genotyped 737 individuals 
from four different groups of control sub-
jects, and 707 index subjects from four dif-
ferent groups of impulsive, compulsive ad-
dictive behaviors including substance 
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INTRODUCTION

The most commonly studied polymorphism of the do-
pamine D4 receptor gene (**DRD4**) is the 48 bp repeat in 
the portion of the gene coding for the third intracyto-
plasmic loop [Van Tol et al., 1992]. This results in three
major alleles representing 2, 4, and 7 repeats, and minor alleles of 3, 5, 6, and 8 repeats. Expression studies have shown that the major alleles differ moderately in the interaction of the D4 receptor with the antipsychot-ic clozapine [Van Tol et al., 1992]. Despite the evidence for a functional correlation with the major alleles, as with many behavioral genetic investigations, the results have been variable from study to study. These associations cluster into a group of impulsive, compulsive, and addictive behaviors, listed as follows.

Novelty Seeking

Ebstein et al. [1996] and Benjamin et al. [1996] independently reported an association between the presence of the 7 allele with higher novelty seeking scores in individuals in the general population using Cloninger’s Temperament and Character Inventory (TCI). Subsequent to this Malhotra et al. [1996] reported a failure to confirm this finding in Finnish subjects. Using a different instrument, Jonsson et al. [1997] found no association between the 7 allele and several personality traits in a Swedish population. Noble (personal communication) observed a modest association between the 7 allele and novelty seeking behavior that was more significant when the association was made with the presence of either the 7 allele of the DRD4 gene and/or the Taq A1 allele of the DRD2 gene. Using the TCI, Ono et al. [1997] reported an association between the 7 allele and novelty seeking in 153 normal Japanese students. Also using the TCI, Vandenbergh et al. [1997] found no association between the 7 allele and novelty seeking in older subjects, Pogue-Geile et al. [1998] found no association between with novelty seeking in 281 male and female twins, and Sander et al. [1997] found no association with either novelty seeking or alcoholism. Gelernter et al. [1997] found no association between the DRD4 gene and novelty seeking, testing several polymorphisms including the DRD4 7 allele.

Recognizing the discrepancy in results, Ebstein and Belmaker [1997] proposed a set of guidelines to maximize the association with novelty seeking. These included always using Cloninger’s TPC or TCI, or the NEO-PI-R, restricting studies the younger subjects (18 to 32) with a high school or college education, individual analysis of men and women, and the exclusion of individuals with psychiatric disease. Using these criteria, Epstein et al. [1997] examined an additional 94 subjects. Although there was no significant association with novelty seeking when grouped by the presence or absence of the 7 allele, a significant difference in the range of novelty seeking scores was noted using a non-parametric test (P = .01). The effect was significant in female but not male subjects. Epstein et al. [1997] concluded all the results combined support a modest role for the long alleles in novelty seeking at least in some population groups.

Tourette Syndrome (TS) and Attention Deficit Hyperactivity Disorder (ADHD)

Grice et al. [1996] reported an association between the presence of the 7 allele and TS, using the transmis-
sion-disequilibrium test (TDT) in 64 family trios. However, all but 12 of the trios came from four large families and the positive results came from only two of these families, suggesting genetic heterogeneity. In Mexico, Nicolini et al. [1997] reported an association between the 7 allele in 12 probands with chronic tics and obsessive compulsive disorder (OCD) (TS + OCD) but not in 49 OCD probands without tics. While Barr et al. [1996] did not find evidence for linkage between the DRD4 gene and TS using either lod scores or nonparametric tests, these approaches may lack the power to detect the role of genes with small effects [Risch and Merikangas, 1996]. Hebebrand et al. [1997] evaluated 102 TS probands and their parents using the TDT and four different polymorphisms of the DRD4 gene that altered the amino acid composition and had a frequency in Caucasians of >1%. All TDT tests were negative including that for the 7 repeat allele in exon 3. Lahoste et al. [1996] reported an association between the presence of the 7 allele and ADHD.

Pathological Gambling

Perez de Castro et al. [1997] reported an association between the 7 alleles and pathological gambling. They examined 68 Caucasian pathological gamblers and 68 controls. The presence of the 7 allele was most common in female pathological gamblers (p = .033).

Alcoholism

George et al. [1993] reported a significant increase in the frequency of the 3 and 6 alleles in severe alcoholics. In a Japanese population, Muramatsu et al. [1966] observed a modest but significant increase in the frequency of the 5 allele in alcoholics that also carried the aldehyde dehydrogenase 2 gene. Adamson et al. [1995] found no association between any alleles or genotypes of the 48 bp repeat alleles in Finnish alcoholics versus controls, and Geijer et al. [1997] found no association in Swedish alcoholics. Parsian et al. [1997] found no significant differences in DRD4 allele frequencies between 176 Caucasian controls versus 322 alcoholics, and no significant differences in transmitted alleles in 58 family trios. Genotype frequencies were not reported. Others also found no association between the DRD4 gene and alcoholism [Chang et al., 1997].

Drug Dependence

Kotler et al. [1997] reported an association between the 7 allele and opioid dependence. In Chinese subjects, Li et al. [1997] found a borderline association between the 7 allele and heroin abuse (P = .07). When the long alleles (5–7) were compared with the short alleles (2–4), the association was just significant (P = .046, two-tailed). Mel et al. [see Ebstein et al., 1997] also found an association between the DRD4 gene and heroin abuse in non-Ashkenazi Israelis.

Most studies of the possible association between the 7 allele and schizophrenia [Barr et al., 1994; Hong et al., 1997; Kohn et al., 1997; Macciardi et al., 1994; Nanko et al., 1993; Petronis et al., 1995; Sommer et al., 1993; Tanaka et al., 1995] or manic depressive disorder-
While such a history of successes and failures in the confirmation of initial association studies is common in psychiatric genetics, for the DRD4 gene it is reasonable to ask why. There are a number of possibilities. (1) The most pessimistic is that the DRD4 gene is not associated with any behavioral disorders and all the positive results are type I errors. However, it seems reasonable to conclude that with so many positive results some are probably real. (2) Since these are polygenic disorders and since the effects of one gene such as the DRD4 gene are minor and account for only 1 to 2% of the variance for the different phenotypes, for some populations the DRD4 gene may play a role in a given disorder or personality variable while in different populations other genes are more predominant and the association will be negative. Thus, through a combination of population heterogeneity and small effect size, variations from study to study would be the expected outcome [Comings, 1998c]. (3) Examining subjects predominately by the presence or absence of the 7 allele may not be the most sensitive approach and other strategies may be more sensitive across multiple diagnoses and multiple sets of controls. (4) In a complex polygenic system, certain alleles or genotypes may play a role in one phenotype while other alleles or genotypes may play a role in other phenotypes. For example, in our studies of the role of many different genes in subjects with TS and ADHD, specific genotypes of gene A and B may be additive for one phenotype but not additive, or even subtractive, in their effect on another phenotype. The 48 bp polymorphism of the DRD4 gene is uniquely suited to testing the possibility that different alleles or genotypes may have different effects in different phenotypes.

Over the past three years we have genotyped many subjects for the 48 bp repeat of the DRD4 gene. The positive studies reviewed above suggest that the DRD4 gene is involved in subjects that in general have impulsive, compulsive, and addictive behaviors. The positive associations reported for the DRD2 gene have also involved impulsive, compulsive, and addictive behaviors [Blum et al., 1995, 1996]. This is consistent with animal studies showing an important role of dopamine in rewarding impulsive, hyperreactive, and stereotyped behaviors [DiChiara et al., 1988; LeMoal and Simon, 1991]. Since most of the probands we have tested fall into this same category (TS, ADHD, pathological gambling, and substance abuse), and since we have also examined multiple sets of controls, we felt that this wide range of individuals would allow us to examine the following questions that may clarify the role of the DRD4 gene in behavior: (1) Is the increase in frequency of the 7 allele a characteristic of subjects across a wide range of impulsive, compulsive, and addictive behaviors? (2) Is the presence or absence of the 7 allele the best parameter to examine in studies of the DRD4 gene or are other alleles or allele groupings, such as the 5 to 8 alleles or the 2 allele, also associated with behavioral disorders? (3) Does the phenomenon of molecular heterosis, that we have observed for other dopamine receptor genes (DRD1 [Comings et al., 1997a], DRD2 [Comings and MacMurray, 1997a; Comings, 1998b, 1998c], DRD3 [Comings et al., 1997a; Comings and MacMurray, 1997a]), also occur at the DRD4 gene? (4) Are alleles or genotypes more important in identifying the role of the DRD4 gene in behavior? (5) Are there differences in the effects of different alleles or genotypes at the 48 bp polymorphism for different phenotypes?

**METHODS**

**Control Subjects**

In this study we used as controls four different groups of individuals with either no disorder or nonpsychiatric disorders. These include the following. (1) One hundred and twenty-nine controls consisting of staff members, including both professional and nonprofessional personnel, from the City of Hope Medical Center and the Jerry L. Pettis Veterans Administration Medical Center at Loma Linda, CA, screened to exclude drug and alcohol abuse/dependence [Comings et al., 1991]. These were termed COH controls. (2) One hundred and thirty-six college students from the California State University at San Bernardino screened to exclude drug and alcohol abuse/dependence [Comings et al., 1997b; Comings et al., 1997a]. These were termed SB controls. (3) Three hundred and sixty-eight random population-based volunteers for a study of childbearing motivation, performed in cooperation with Dr. R. Miller. DNA from these subjects was obtained from buccal smear kits mailed to the subjects already involved in prior studies for the purpose of potentially identifying genes associated with childbearing behaviors [Miller et al., 1999b; Miller et al., 1999]. These were termed RM controls. (6) One hundred and four subjects with multiple sclerosis from the Neurological Disease Brain Bank (Dr. Wallace Tourtellotte, West Los Angeles V.A. Medical Center, Los Angeles, CA). These were termed the MS controls. In total there were 737 nonpsychiatric non-Hispanic Caucasian control subjects.

**Index Subjects**

We have used as index subjects four different groups of individuals with a range of impulsive, addictive behaviors, all from previous studies. (1) One hundred and sixty-five subjects from a prior study of the role of the DRD2 gene in pathological gambling [Comings et al., 1996b]. These were termed gamblers. (2) Two hundred and twenty-three subjects with TS from prior studies of the role of the DRD1, DRD2, DRD3, DBH, DAT1, TDO2, MAOA, and other genes in TS [Comings et al., 1991, 1993b, 1996a, 1996c, 1997a]. These were termed TS. (3) Fifty-two subjects with ADHD from prior studies of the role of the DRD2 and TDO2 genes in ADHD [Comings et al., 1991, 1996a]. These were termed ADHD. (4) Two hundred and sixty-seven subjects with alcohol and drug abuse/dependence from the Addiction Treatment Unit (ATU) of the Jerry L. Pettis Veterans Administration Hospital that were part of earlier studies of the molecular genetics of substance abuse [Comings et al., 1991, 1994, 1997a, 1997b; Gade et al., 1996; Johnson et al., 1997]. These subjects were obtained
over a period from 1989 to 1997. The subjects obtained since 1994 were administered several psychological tests after they were thoroughly detoxified.

Quantitative Scores

The latter set of ATU subjects were administered the Addiction Severity Index, Fifth Edition [Hodgins and Guebaly, 1992]. This involved many different variables to assess the severity of drug and alcohol abuse. To reduce the problem of the analysis of multiple tests, these were summed to a quantitative “drug score” [Comings et al., 1997b]. All TS subjects were given the Human Behavioral Questionnaire. This questionnaire, based on the Diagnostic Interview Schedule [Robins et al., 1981] and Diagnostic and Statistical Manual of Mental Disorders (DSM-IIIR and DSM IV) criteria, allowed the compilation of 24 different quantitative traits relating to a range of impulsive, compulsive, affective, cognitive, and other behaviors [Comings, Dowow et al., 1991]. All the genotyping was done in the Department of Medical Genetics at the City of Hope Medical Center. All samples were identified only by a random number.

Personality Tests

All the SB subjects and all the ATU individuals obtained since 1994 were administered two personality tests, the NEO Revised [Costa and McCrae, 1996], and the TCI of Cloninger et al. [1993] and Svrakic et al., [1993].

Allele Detection

The DRD4 48 bp repeat alleles were determined by the polymerase chain reaction technique of Nanko et al., [1993]. We found that because of the GC rich nature of the polymorphic region, unless 5-azaguanadine completely replaced guanadine, some heterozygotes were missed, producing pseudohomozygosity. This required the identification of the alleles by silver staining [Buddele et al., 1991]. All the genotyping was done in the Department of Medical Genetics at the City of Hope Medical Center. All samples were identified only by a random number.

Genotype Groupings

Several methods of genotype analysis were used. For each variable except D22-77, the genotype group anticipated to have the lowest scores was set to 0, and the other genotypes were scored as 1 or higher. These are summarized below.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>1+</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7non7</td>
<td>non 7</td>
<td>7</td>
</tr>
<tr>
<td>D4non44</td>
<td>44</td>
<td>non-44</td>
</tr>
<tr>
<td>Dhomohet</td>
<td>homozygotes</td>
<td>heterozygotes</td>
</tr>
<tr>
<td>D22-77</td>
<td>22</td>
<td>1 = 24, 2 = 44, 3 = 45-47, 4 = 77</td>
</tr>
<tr>
<td>D5–8</td>
<td>non 5–8</td>
<td>5–8</td>
</tr>
</tbody>
</table>

To emphasize the 7 allele, subjects were divided into those carrying genotypes that contained the 7 allele (27, 47, 77) versus all other genotypes. For this variable, D7non7, those with non-7 genotypes were scored 0 and those with a genotype containing the 7 allele were scored 1. To emphasize the 44 genotype, subjects were divided into those with the 44 genotype versus all others. For this variable, D4non44, those with the 44 genotype were scored 0, and those with all other genotypes were scored 1. To emphasize homozygosity versus heterozygosity, subjects were divided into those homozygous for any allele versus those heterozygous for different alleles. For this variable, Dhomohet, those who were homozygotes (22, 33, 44, 55, 66, 77) were scored 0, and those who were heterozygotes were scored 1. One of us has suggested that the size of repeat alleles themselves may play a direct role in gene regulation [Comings, 1998a]. To test this hypothesis the D22-77 group scored genotypes by increasing length of the alleles. This involved the following scoring: 22 = 0, 24 = 1, 44 = 2, 45-47 = 3, 77 = 4. Since these represented the majority of subjects, and since it was not clear how to handle some of the minor genotypes such as 26, 27, 36, and 37, these other genotypes were left out of these analyses. While the use of multiple classifications of alleles and genotypes can be criticized on statistical grounds [Goldman, 1996], a failure to examine more than one classification also carries the risk of missing important phenotypic effects.

Statistics

The potential differences between the frequencies of alleles or genotypes in the total control group versus the individual or total subject group was examined by chi-square analysis using the asymptotic Pearson chi-square test of the statistical package StatXact3 for Windows® from Cytel Software Corporation (Cambridge, MA). A Bonferroni corrected α of .05/4 or .0125, was used. Analysis of variance was used to examine the relationship between DRD4 genotype and quantitative scores.

RESULTS

Table I summarizes the control and subject groups, the mean age, and the percentage of males in the sample. Clinical descriptions of the groups can be found in prior publications [Comings et al., 1991, 1994, 1996b, 1996c, Miller et al., 1997a]. The most notable difference between the control subjects versus the index subjects was the sex ratio. Most of the index cases were males, while the control cases tended to show a slight excess of females.

Total Allele Distributions

Table II lists the frequencies of the 2–8 repeat alleles in the four groups of control subjects, and four groups of index subjects with various impulsive and addictive behaviors. The number and percentage of each allele in the eight groups are shown. This allows an examination of the variation in gene frequency that can occur when there is relatively small numbers subjects the control groups. For example, the frequency of the 2
alleles ranged from 7.4 to 15.4% and the frequency of the 4 allele ranged from 62.5 to 74.5% in the controls. All of the control groups were in Hardy-Weinberg equilibrium. Since there were more males in the index groups than the control groups, it was reasonable to ask whether there was a significant difference in the distribution of the alleles or genotypes in male versus female controls. There were not. For alleles \( \chi^2 = 4.75 \), d.f. = 6, \( p = .57 \). For the genotypes \( \chi^2 = 13.61 \), d.f. = 16, \( p = .63 \).

### All Alleles

When the total set of alleles was compared in a \( 2 \times 7 \) chi-square test of total controls versus index groups, the distributions were significant at \( \alpha \leq .0125 \) for the gamblers (\( p < .0001 \)), TS (\( p = .0003 \)), ADHD (\( p < .0003 \)), and the total index group (\( p \leq .0002 \)).

#### 7 Allele Frequencies

The frequencies of the 7 alleles in all groups are shown in Table IIIA. The frequencies of the 7 allele ranged from .087 to .190 in the control subjects and from .143 to .183 in the index subjects. The overall frequency was .137 for the 737 total control subjects and .138 for the 707 total index subjects. Individually none of the subject groups were significant at a Bonferroni corrected \( \alpha = .0125 \).

### 5–8 Allele Frequencies

Since many authors examine the effect of the \( DRD4 \) gene by pooling the 5, 6, 7, and 8 alleles we also used this approach (Table IIIIB). With a Bonferroni corrected \( \alpha = .0125 \) the results were significant for the gamblers (\( P < .0001 \)), ADHD (\( \leq .010 \)), and the total group (\( P < .0004 \)).

#### Homozygosity Versus Heterozygosity

The frequencies of the genotype groups for the total control subjects and total index subjects are summarized in Figure 1. This showed a trend for index subjects to have a decrease in the frequency of homozygous genotypes (22, 44) and an increase in heterozygous genotypes involving the 5–7 alleles (45, 46, 47). This suggested that positive heterosis at the \( DRD4 \) genes was occurring in subjects with impulsive and addictive behaviors. To examine this the frequencies of homozygotes versus heterozygotes, and the homozygote/heterozygote ratios, are shown in Table IV. In the control groups the homozygote/heterozygote ratios ranged from 1.091 to 1.419 with a mean of 1.213. In the subject groups the ratios ranged from .726 to 1.00 with a mean of .867. The ratios were significant using a Bonferroni corrected \( p = .0125 \) for the gamblers (\( p \leq .0031 \)) and the total group (.0015).

#### Quantitative Traits

While the above results suggest that the 7 versus non-7 allele comparison is not the most powerful approach to examining the phenotypic effect of this \( DRD4 \) polymorphism, it does not address the relative power of the different genotype variables. To continue to explore the possible role of heterosis, in the following figures, the genotypes are arranged into three homozygous genotypes (22, 44, 77) interspersed with two groupings of heterozygotes (23, 24) and (45, 46, 47). These results for the drug score for Caucasians only and for the total ATU group (Caucasians + Blacks + Hispanics) are shown in Figure 2. There was a U-shaped distribution with individuals with the 44 genotype having the lowest scores, the 23-24 and 45-46-47 heterozygotes

### TABLE I. Summary of Groups Tested for \( DRD4 \) Alleles

<table>
<thead>
<tr>
<th>Groups*</th>
<th>N</th>
<th>Mean age (S.D.)</th>
<th>% males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COH</td>
<td>129</td>
<td>46.3 (15.3)</td>
<td>42</td>
</tr>
<tr>
<td>SB</td>
<td>136</td>
<td>34.2 (7.9)</td>
<td>52</td>
</tr>
<tr>
<td>RM</td>
<td>368</td>
<td>30.7 (4.3)</td>
<td>49</td>
</tr>
<tr>
<td>MS</td>
<td>104</td>
<td>57.5 (13.0)</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>737</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index subjects:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamblers</td>
<td>165</td>
<td>43.9 (10.1)</td>
<td>77</td>
</tr>
<tr>
<td>TS</td>
<td>223</td>
<td>18.2 (13.4)</td>
<td>78</td>
</tr>
<tr>
<td>ADHD</td>
<td>52</td>
<td>14.9 (12.2)</td>
<td>74</td>
</tr>
<tr>
<td>ATU</td>
<td>267</td>
<td>41.5 (7.5)</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>707</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*COH, City of Hope Medical Center and Jerry L. Pettis V.A. Medical Center Staff; SB, students from California State University at San Bernadino; RM, volunteers working with Dr. R. Müller; MS, subjects with multiple sclerosis; TS, Tourette’s syndrome; ADHD, attention deficit hyperactivity disorder; ATU, Addiction Treatment Unit.

### TABLE II. Allele Counts for the 48 bp \( DRD4 \) Repeat Polymorphism—N (%)

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Group</th>
<th>T</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>COH controls</td>
<td>258</td>
<td>19 (7.4)</td>
<td>8 (3.1)</td>
<td>175 (67.8)</td>
<td>3 (1.2)</td>
<td>3 (1.2)</td>
<td>49 (19.0)</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SB controls</td>
<td>272</td>
<td>26 (9.6)</td>
<td>4 (1.5)</td>
<td>194 (71.3)</td>
<td>6 (2.2)</td>
<td>2 (0.7)</td>
<td>40 (14.7)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM controls</td>
<td>736</td>
<td>113 (15.4)</td>
<td>39 (5.3)</td>
<td>460 (62.5)</td>
<td>17 (2.3)</td>
<td>8 (1.1)</td>
<td>95 (12.9)</td>
<td>4 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MS controls</td>
<td>208</td>
<td>23 (11.0)</td>
<td>8 (3.8)</td>
<td>155 (74.5)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>18 (8.7)</td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1474</td>
<td>181 (12.3)</td>
<td>59 (4.0)</td>
<td>984 (66.8)</td>
<td>27 (1.8)</td>
<td>14 (0.9)</td>
<td>202 (13.7)</td>
<td>7 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects</td>
<td>Gamblers</td>
<td>330</td>
<td>20 (6.1)</td>
<td>7 (2.1)</td>
<td>214 (64.8)</td>
<td>14 (4.2)</td>
<td>8 (2.4)</td>
<td>60 (18.2)</td>
<td>7 (2.1)</td>
<td>36.96</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Tourette synd</td>
<td>446</td>
<td>35 (7.8)</td>
<td>15 (3.4)</td>
<td>311 (69.7)</td>
<td>3 (7)</td>
<td>16 (3.6)</td>
<td>64 (14.3)</td>
<td>2 (4)</td>
<td>25.09</td>
<td>.0003</td>
</tr>
<tr>
<td></td>
<td>ADHD</td>
<td>104</td>
<td>11 (10.6)</td>
<td>9 (8.7)</td>
<td>56 (53.8)</td>
<td>8 (7.7)</td>
<td>0 (0.0)</td>
<td>19 (18.3)</td>
<td>1 (1)</td>
<td>25.50</td>
<td>.0003</td>
</tr>
<tr>
<td></td>
<td>ATU</td>
<td>534</td>
<td>57 (10.7)</td>
<td>35 (6.6)</td>
<td>329 (61.6)</td>
<td>10 (1.9)</td>
<td>10 (1.9)</td>
<td>92 (17.2)</td>
<td>1 (2)</td>
<td>14.83</td>
<td>.021</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1414</td>
<td>123 (8.7)</td>
<td>66 (4.7)</td>
<td>910 (64.4)</td>
<td>35 (2.5)</td>
<td>34 (2.4)</td>
<td>235 (16.6)</td>
<td>11 (8)</td>
<td>25.85</td>
<td>.0002</td>
</tr>
</tbody>
</table>

*Chi-square analysis versus the total controls, d.f. = 6. Abbreviations as in Table I.
higher scores, and the 22 and 77 homozygotes the highest scores. Since the distribution for the total group (all races) was virtually identical and had more power, it was included and was significant at $P < 0.0064$.

### Personality Traits

A multivariate analysis of variance (MANOVA) of the five subscales of the NEO personality inventory was performed using the D22-77 variable. This variable was chosen because it provided an assessment of a wider range of genotypes than the variables that simply divided the genotypes into two groups. The results are shown in Table V. The most significant association was with openness ($P = 0.06$). The total MANOVA was significant ($p < 0.001$). The distribution of the scores for openness and conscientiousness across the versus genotype groups is shown in Figure 3. For both, the scores were lowest for those carrying the 22 genotype. Note that for these two scores, individuals with low values are considered to have the most severe psychopathology. Since the scores for openness increased and stayed high for the remaining genotypes, the result for the D22-77 scoring variable was significant. By contrast the scores for conscientiousness peaked for the 44 genotype then decreased across the remaining genotypes, and the result for the D22-77 scoring was of borderline significance. This again illustrates that to assess fully the association between $DRD4$ genotypes and different phenotypes may require more complex assessment that specifically includes the 2 alleles and 22, 23, and 24 genotypes. By MANOVA, none of the seven factors of the TCI, including novelty seeking, were significant using any of the $DRD4$ variables.

### Quantitative Traits in TS and ADHD Subjects

To examine further the relationship between $DRD4$ genotypes and quantitative traits, a totally different set of subjects was examined, the COH controls, TS, and ADHD subjects. When all those that had completed the Human Behavioral Questionnaire and had $DRD4$ genotyping were included, the total $n = 362$. To
minimize the problem of multiple comparisons, only those scores were chosen that were most relevant to the results in the literature, i.e., the ADHD, ODD, CD, and tic scores. In previous studies [Comings et al., 1996c] we have found that using the entire range of quantitative trait variables can be a powerful method of examining the effects of genes on behavior. Since both controls and TS and ADHD subjects completed the same structured questionnaire, since the range of scores in these groups tended to overlap, and since a broader range of scores provided more power, the scores for controls and TS and ADHD subjects were combined. These results are shown in Figure 4. The 22 genotype often had the highest scores whereas the 77 homozygotes often had the lowest scores. Using the D22-77 variable, the only score that was significant using a Bonferroni corrected $\alpha$ of .0125 was the conduct or CD score ($P = .006$). This was significant because the score for the 22 genotype was one of the highest, and the score for the 77 genotype was one of the lowest. For the ADHD and ODD scores the higher scores were for the 45–47 group, providing some support for positive heterosis in that the scores were higher than for the 44 or the 77 homozygotes. By contrast, the situation for the 22, 23, and 24 genotypes was the opposite, providing some support for negative heterosis in that the score for the 24 genotypes were lower than for the 22 or 44 homozygotes.

**DISCUSSION**

Several conclusions are apparent from these studies of four different control groups and four different groups of subjects with impulsive, addictive behaviors.

1. **The division of the alleles into groupings other than 7 non-7 may be superior.** Benjamin et al. [1996] recommended a division of the alleles into short (2–4) versus long (5–8 repeats). For all four of our index groups and the total index group this gave more significant results. Thus, using an $\alpha$ of .0125 none of the groups were significant for the 7 versus non-7 analysis while the gamblers, ADHD, and total groups were significant using the 5–8 versus 2–4 analysis. As discussed below, other methods of dividing the genotypes and alleles may also be relevant.

2. **Heterosis also plays a role in the DRD4 gene.** In regard to populations, heterosis refers to a situation where the progeny (hybrid) has a significantly greater phenotypic effect than for either parental strain [Gardner and Snustad, 1981; Stern, 1973]. The vigor of hybrid corn is a common example. We have used the term

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**TABLE IV. Homozygosity Versus Heterozygosity for the 48 bp DRD4 Repeat Polymorphism**

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COH</td>
<td>70</td>
<td>.543</td>
<td>59</td>
<td>.457</td>
<td>1.186</td>
<td></td>
<td></td>
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<tr>
<td>SB</td>
<td>78</td>
<td>.574</td>
<td>58</td>
<td>.426</td>
<td>1.345</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM</td>
<td>192</td>
<td>.522</td>
<td>176</td>
<td>.478</td>
<td>1.091</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>61</td>
<td>.587</td>
<td>43</td>
<td>.413</td>
<td>1.419</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>404</td>
<td>.548</td>
<td>333</td>
<td>.452</td>
<td>1.213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Index subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gamblers</td>
<td>69</td>
<td>.421</td>
<td>95</td>
<td>.579</td>
<td>0.726</td>
<td>8.73</td>
<td>.0031</td>
</tr>
<tr>
<td>TS</td>
<td>111</td>
<td>.500</td>
<td>111</td>
<td>.500</td>
<td>1.00</td>
<td>1.59</td>
<td>.207</td>
</tr>
<tr>
<td>ADHD</td>
<td>23</td>
<td>.423</td>
<td>28</td>
<td>.549</td>
<td>0.821</td>
<td>1.81</td>
<td>.178</td>
</tr>
<tr>
<td>ATU</td>
<td>123</td>
<td>.464</td>
<td>142</td>
<td>.536</td>
<td>0.866</td>
<td>5.52</td>
<td>.019</td>
</tr>
<tr>
<td>Total</td>
<td>326</td>
<td>.464</td>
<td>376</td>
<td>.536</td>
<td>0.867</td>
<td>10.10</td>
<td>.0015</td>
</tr>
</tbody>
</table>

*Chi-square analysis against the total of all controls, d.f. = 1. Abbreviations as in Table I.

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**TABLE V. Multivariate Analysis of Variance of the Five Factors of the NEO Personality Inventory Versus the D22-77 Variable ($F$ and $P$)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreeableness</td>
<td>2.86</td>
<td>.025</td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>2.47</td>
<td>.047</td>
</tr>
<tr>
<td>Extraversion</td>
<td>0.97</td>
<td>.427</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>1.63</td>
<td>.168</td>
</tr>
<tr>
<td>Openness</td>
<td>3.74</td>
<td>.006</td>
</tr>
<tr>
<td>Total (Wilks)</td>
<td>10.10</td>
<td>.001</td>
</tr>
</tbody>
</table>

*($N = 167$, Caucasians Only).
molecular heterosis to refer to a situation where a polymorphism for a single gene shows a significantly greater effect in the heterozygote (usually 12) than either homozygote (11, 22). We have observed this phenomenon in many genes including other dopamine receptor genes (DRD1, DRD2, and DRD3) [Comings, 1998b, 1998c; Comings et al., 1997a; Comings and MacMurray, 1997b; Lynch and Walsh, 1998]. This can be either positive heterosis with heterozygotes having higher scores on quantitative traits than homozygotes, or negative heterosis with lower means in heterozygotes. This raises the possibility that heterosis may play a role in the phenotypic effect of all the dopamine receptor genes. The observation of a significant increase in the frequency of the heterozygous versus homozygous genotypes are summarized in Table IV. This was significant with $\alpha = .0125$ for the gamblers and the total index groups. However, this conclusion is complicated by the finding that the quantitative scores for severity of drug dependence (drug score) did not follow the pattern expected for heterosis, i.e., highest scores for the heterozygous genotypes (24, and 45 to 47), and lowest scores for the 22, 44, and 77 genotypes. Instead, the results for the drug score were consistent with a recessive effect, with the highest scores for the 22 and 77 genotypes, or a codominant effect with higher scores for all non-44 genotypes.

3. Both positive and negative heterosis may occur at the DRD4 gene. In our studies of the dopamine receptor genes heterosis was positive for the DRD2 gene [Comings, 1998b, 1998c; Comings and MacMurray, 1997b] and negative for the DRD1 [Comings et al., 1997a] and DRD3 [Comings et al., 1993a, 1996b] genes. One of the characteristics of heterosis was that it could be phenotype specific and gender specific [Comings and MacMurray, submitted]. However, in each of these cases and those in the literature, only bi-allelic polymorphisms were studied. The more complex 48 bp polymorphism of the DRD4 gene, with 7 alleles and multiple major genotype groups, allowed the potential detection of more complex types of heterosis. Thus, the ADHD and ODD scores (Fig. 4) were consistent with positive heterosis for the longer alleles with the scores for the 45 to 47 heterozygous genotypes being higher than for the 44 and 77 homozygous genotypes, and with negative heterosis for the shorter alleles with the scores for the 23 and 24 genotypes being lower than for the 22 and 44 homozygous genotypes. This clearly is a tentative conclusion that will require additional studies in other populations.

4. The shorter alleles of the 48 bp play a role in the phenotypic effect of the DRD4 gene. The present studies show that both heterozygosity and homozygosity for the 2 repeat allele also play significant roles in determining the phenotypic effect. For example, the drug score and the alcohol score for all races was higher for 22 homozygotes than for 77 homozygotes, and the scores for the 23 and 24 heterozygotes were comparable to those for the 45 to 47 heterozygotes. The NEO scores for conscientiousness and openness were lowest for the 22 homozygotes. Despite the small number of 22 homozygotes, the low value for this genotype was the major reason there was a significant association between the DRD4 gene and openness. Finally, scores for the 22 homozygotes were highest for all four of the scores examined, ADHD, ODD, CD, and tics.
5. Some phenotypes may show a U-shaped association with the DRD4 genotypes. While it may seem counterintuitive that some phenotypes such as the drug score would show a U-shaped pattern, we have observed a similar result with some other repeats. For example, using a variable number of tandem repeats (VNTR) at the MAOA gene, we observed that both the drug score and the ADHD score showed the lowest scores for the most frequent median sized alleles, and increased scores for the rarer shorter and longer alleles [Gade et al., 1998]. This is understandable if the repeats themselves play a role in gene regulation. One of us has previously postulated that micro- and minisatellites play such a direct role through their effect on the formation of Z-DNA [Comings, 1998a]. Since the amount of Z-DNA formed is very sensitive to variations in both sequence and the length, different sized alleles, and alleles with different sequences, could have a significant affect on the phenotype. The 48 bp DRD4 allele is known to show a significant degree of microheterogeneity [Lichter et al., 1993]. Thus, mutations that produce alleles that are either larger or smaller than the most common alleles may have similar positive or negative affects on the phenotype.

For the quantitative traits, the genotype patterns could be classified into U-shaped (for the drug score and conscientiousness score), a mixed heterosis pattern (for the ADHD and ODD scores), and a 22 predominant pattern (for the openness, CD, and tics scores). Given the number of variables involved, the small percentage of the variance explained, the number of cases, variations in both sequence and the length, different sized alleles, and alleles with different sequences, could have a significant affect on the phenotype. The 48 bp DRD4 allele is known to show a significant degree of microheterogeneity [Lichter et al., 1993]. Thus, mutations that produce alleles that are either larger or smaller than the most common alleles may have similar positive or negative affects on the phenotype.

In summary, when the examination of the role of the DRD4 gene is limited to the frequency of the 7 allele, and when the effect of the other repeat alleles and genotypes is ignored, a significant portion of the phenotypic effect of the DRD4 gene may be missed.

REFERENCES


Comings DE. 1998b. Molecular heterosis as the explanation for the controversy about the effect of the DRD2 gene on dopamine D2 receptor density. Mol Psychiatry (in press)


