Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking

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Human personality traits which can be reliably measured by a number of rating scales, show a considerable heritable component1,2. The tridimensional personality questionnaire (TPQ) is one such instrument and was designed by Cloninger to measure four distinct domains of temperament — Novelty Seeking, Harm Avoidance, Reward Dependence and Persistence — that are hypothesized to be based on distinct neurochemical and genetic substrates. Cloninger proposed that individual variations in the Novelty Seeking trait are mediated by genetic variability in dopamine transmission2. Individuals who score higher than average on the TPQ Novelty Seeking scale are characterized as impulsive, exploratory, fickle, excitable, quick-tempered and extravagant, whereas those who score lower than average tend to be reflective, rigid, loyal, stoic, slow-tempered and frugal. We now show that higher than average Novelty Seeking test scores in a group of 124 unrelated Israeli subjects are significantly associated with a particular exonic polymorphism, the 7-repeat allele in the locus for the D4 dopamine receptor gene (D4DR). The association of high Novelty Seeking and the 7-repeat allele was independent of ethnicity, sex or age of the subjects. This work, together with the accompanying confirmations in this issue3, provides the first replicated association between a specific genetic locus involved in neurotransmission and a normal personality trait.

Evidence that Novelty Seeking behaviours are related to dopamine come from studies of experimental animals, Parkinson's disease patients and the effects of dopamine agonists such as amphetamines, cocaine and alcohol compared to dopamine blockers such as haloperidol4. The D4DR gene was a particularly attractive candidate as a quantitative trait locus for Novelty Seeking because of several singular characteristics: (i) D4DR contains an unusually polymorphic 16-amino acid repeat region in the putative third cytoplasmic loop5,6; (ii) physiological differences in ligand binding have been observed between the commonest short receptor containing 4 repeats and the commonest long receptor containing 7 repeats5,6; (iii) D4DR receptor mRNA shows a distinct neuro-anatomical distribution in comparison to D2 and D3 mRNAs with a concentration in limbic areas associated with cognitive and emotional behaviours5; and (iv) D4DR may be a site of action of the atypical neuroleptic, clozapine6.

D4DR exon III genotypes and TPQ questionnaires were analysed for 124 normal adult male and female volunteers. The frequencies of the D4DR exon III repeat polymorphisms in this group were similar to those observed in other populations; the most frequently observed alleles were the 4 repeat and the 7 repeat (Fig. 1) and the most common genotypes were 4/4 and 4/7. Analysis of variance (ANOVA) revealed that the group of subjects with the 7-repeat allele exhibit significantly elevated Novelty Seeking scores in comparison to subjects lacking the 7-repeat allele (Table 1). The effect size for the 7-repeat allele was 0.5 standard deviation units. By contrast, the scores for Reward Dependence, Persistence and Harm Avoidance were statistically indistinguishable in the two groups of subjects. Similar results were obtained when the data

<table>
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<th>Table 1 TPQ personality factor scores in subject groups sorted by D4DR allele and genotype*</th>
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<tr>
<td><strong>Subject group</strong></td>
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<td>7 allele present</td>
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*TPQ results are reported as mean raw scores ± S.E.M.

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were grouped by 4,4 versus 4,7 genotype (Table 1) or according to the content of short versus long alleles (see Methods). As population heterogeneity can confound association studies, we investigated the possible influence of demographic variables on the association between D4DR genotype and Novelty Seeking. Possible effects of ethnicity, sex and age were analysed by one-way analysis of covariance (ANCOVA). The presence of allele 7 had a significant main effect on the dependent variable, Novelty Seeking (NS) whereas membership in the Ashkenazi (n = 90) or various non-Ashkenazi (n = 34) groups (NS: F,1,121 = 6.43, P = 0.012; ethnicity: F,1,121 = 0.33, R = 0.092, P = 0.57), or membership in the male (n = 69) or female (n = 55) groups (NS: F,1,121 = 5.54, P = 0.02; sex: F,1,121 = 0.92, R = 0.08, P = 0.34), or age (NS: F,1,119 = 6.32, P = 0.013; age: F,1,119 = 0.77, R = 0.08, P = 0.38) did not have significant effects. Similarly, a summary of all effects' design showed no significant effect of ethnicity, sex or age (covariates: F,1,117 = 0.61, multiple R = 0.12, P = 0.66; NS: F,1,117 = 5.98, P = 0.016). Although there was the expected tendency for Novelty Seeking scores to decline with age, this trend was not significant in our relatively young group of subjects (R = -0.11, F = 1.38, P = 0.242).

In summary, we have conducted an association study that employed a self-report questionnaire in order to reveal a contribution of common polymorphisms in the D4DR gene to a normal aspect of human behaviour, Novelty Seeking. As predicted by Cloninger, this association was trait-specific in that D4DR genotype had no significant effects on the orthogonal personality factors of Reward Dependence, Persistence and Harm Avoidance. Moreover, the association did not appear to be due to population stratification since it was independent of the ethnicity, sex or age of the subjects.

The observed association raises the question of the causal relationship between the structure of the D4 receptor and its function in Novelty Seeking. The longer 7-repeat allele can be distinguished from the shorter 2- and 4-repeat alleles by their salt-dependent binding to agonists and antagonists. Clozapine competition for receptor binding shows that the D4.2 and D4.4 receptors have lower dissociation constants for clozapine in the absence than in the presence of sodium chloride whereas the D4.7 receptor does not exhibit the same sodium sensitivity. Further studies of the D4 receptor have confirmed that ligand binding is subtly influenced by both exon III repeat number and salt concentration. Although these observed differences are small, the repeat sequence might confer other properties to the receptor that are undetected in binding studies. For example, three naturally occurring polymorphisms in the human β3-adrenergic receptor display normal agonist binding and functional coupling to Gs, but markedly alter the degree of agonist-promoted down regulation of receptor expression. Similar studies on the effects of the exon III repeat polymorphism on D4 receptor activities would be informative.

In addition to the possible effect of repeat length in exon III on the function of D4DR, the effect of amino acid variations within particular repeat lengths may also have a role in receptor functioning. At least 25 unique haplotypes were observed for the different allelic configurations of this protein. The two most common haplotypes for the 4- and 7-repeat alleles account for 90% and 81%, respectively, of the observed sequence variants and the second most common 4 and 7 haplotypes respectively account for only 3% and 5% of the total. Due to the infrequency of the additional haplotypes, it seems unlikely that they contribute significantly to the observed association between Novelty Seeking and allelic type. However, one particular sequence variant of the 7- or 4-repeat allele could be responsible for the effect of one or both of these alleles on Novelty Seeking behaviour.

At the present time, allelic association studies are the strategy of choice for detecting quantitative trait loci such as those involved in normal personality as they provide the statistical power needed to detect relatively small gene effects that contribute to complex behavioural traits. Association studies are most meaningful when they employ candidate genes that a priori make "biological sense" and have functional significance in the determination of the trait. Examples of quantitative trait loci that have been detected by association studies include a deletion polymorphism in the angiotensin-converting enzyme that is related to cardiovascular disease and apolipoprotein E point substitutions that are correlated with both longevity and late-onset Alzheimer's disease.

This report is an early example of an association between a common polymorphism and a normal human personality trait. As with any newly proposed association between specific alleles and a complex behaviour, independent confirmation of our results is necessary. The accompanying paper also demonstrates an association of the longer repeat D4DR alleles with personality characteristics related to the TPQ-Novelty Seeking factor. This corroboration is especially impressive since (i) a different personality questionnaire was employed; (ii) an ethnically distinct population sample was examined; and (iii) the association was evident within members of the same family as well as between unrelated individuals. Given the significant heritability of many human behaviours and the rapid progress of the human genome project, it is likely that additional genes that influence normal and abnormal psychological characteristics will be found in the future.

**Methods**

**Subject selection and test administration.** Normal volunteers were recruited from students and staff at Ben-Gurion University and Beersheva Mental Health Center. Volunteers gave informed consent and the protocol was approved by the Ben-Gurion University Helsinki Committee. There were 69 males and 35 females and the average age was 29.8 ± 8.9 (mean ± S.D.) years. The ethnic composition was 90 Ashkenazi Jews, 25 Sephardic Jews, 5 mixed Ashkenazi/Sephardic Jews, 1 Arab, 1 Druze, and 2 Jews of unknown ethnic background. The volunteers filled out a Hebrew version of the TPQ, which consists of 100 questions with yes–no answers, and donated 20 cc of blood by venipuncture.

The validity of the four factor structure of the Novelty Seeking scale (NS total = NS1+NS2+NS3+NS4) was supported by the highly significant correlations obtained by regression analysis comparing NS vs NS1 (R = 0.63, P < 0.0001), NS vs NS2 (R = 0.80, P < 0.0001), NS vs NS3 (R = 0.49, P < 0.0001) and NS vs NS4 (R = 0.74, P < 0.0001). Similarly Harm Avoidance (HA) was significantly correlated with HA1 (R = 0.80, P <
Genotyping. DNA was extracted using a Qiaamp kit (Qiagen). PCR amplification was carried out by a laboratory technician blind to subject identity using Vent polymerase (New England Biolabs) and a high denaturing temperature (98 °C for 1 min) with a combined annealing and extension reaction for 5 min at 70 °C (ref. 17). The primers employed were: D4-3: 5'-GCGACTCTGGTTTCTACCG-3' and D4-4: 5'-AGGACCCCTATGGCTTC-3'. The reaction mixture contained the following components: 1 X Vent buffer (New England Biolabs), 1 μM primers, 62.5 ng genomic DNA, 400 μM dNTPs and 0.25 U of Vent DNA polymerase in a total volume of 12.5 μl. Thirty cycles were employed in a Perkin-Elmer Cetus 9600 thermal cycler. The reaction mixture was electrophoresed on a 2% Metaphor gel (FMC) with ethidium bromide to screen for genotypes.

Statistical analysis. Associations between TPC test scores and D4DR genotype were assessed by ANOVA, and corrected for demographic variables by ANCOVA. TPC results are reported as raw scores. D4DR genotypes were classified on the basis of the absence or presence of allele 7, or by 4,4 versus 4,7 genotype, because i) the 4 and 7 alleles account for the majority of all alleles in the population (Fig. 1), and ii) physiological differences between the D4-4 and D4-7 receptors have been reported. When genotypes were inventoried according to various different methods, including the "short" (s, 2-5 repeats) and "long" (l, 6-8 repeats) allele classification used by the accompanying article, the following ANOVA results were obtained for Novelty Seeking scores: for both allele 7 versus allele 8, F = 6.34, P = 0.013; for s/s versus l/s or l/l, F = 6.63, P = 0.011; for s/s versus s/l versus l/l, F = 3.95, P = 0.022. The distinction made in the accompanying paper between long and short repeats of the repeat sequence is not completely arbitrary but is based on the original observation that the 2,4 and 4,4 genotypes could be distinguished by their physiological response from the longer 4,7 genotype. It is also worth noting that combining alleles of different lengths in the comparison of allelic distributions is not an uncommon strategy in association studies, even when data is lacking on the physiological effect of length size. Such a procedure was employed to demonstrate an association between a dinucleotide repeat of variable length at the MAOA gene with early onset alcoholism and the association between the class I (600-bp modal length) INS VNTR and insulin dependent diabetes mellitus.

No correction was made in the obtained P values for multiple testing. Based on Cloninger's theory of personality, we had an a priori hypothesis about the association of Novelty Seeking scores with this polymorphism in a dopaminergic gene, although clearly no prediction could be made about the direction of such an association, that is, the 7-repeat allele could have been associated with increased or decreased scores. The ANOVA was carried out considering only two allelic types, presence and absence of the 7 allele since the 4 and 7 repeats represent 90% of the detected alleles in this Israeli population. Almost no information is lost by restricting the analysis to these most frequently observed alleles, considering the low frequencies of the other alleles. Even if we correct for the number of alleles tested (presence or absence of the 7 repeat), corrected P values remain in the P < 0.05 significance range.

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