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5-HT_{2C} (*HTR2C*) Serotonin Receptor Gene Polymorphism Associated With the Human Personality Trait of Reward Dependence: Interaction With Dopamine D4 Receptor (*D4DR*) and Dopamine D3 Receptor (*D3DR*) Polymorphisms

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We recently reported an association between the long repeat allele of the dopamine D4 exon III receptor polymorphism and a human personality dimension, novelty seeking, as measured by the tridimensional personality questionnaire (TPQ), a personality instrument designed by Cloninger to reflect heritable facets of human temperament. The D4 receptor polymorphism (*D4DR*) accounts for only a small percent of the variance for this trait, suggesting that additional genes influence both novelty seeking as well as the other temperaments that are inventoried by the Cloninger TPQ. In the current investigation, we examined, in the original cohort of 120 normal volunteers, two additional coding region polymorphisms, a glycine to serine substitution in the dopamine D3 receptor (*D3DR*) and a cysteine to serine substitution in the 5-HT_{2C} serotonin receptor (*HTR2C*). Three-way analysis of variance (TPQ score grouped by D4DR, D3DR and 5-HT_{2C}) demonstrated that reward dependence and persistence scores were significantly reduced by the presence of the less common 5-HT_{2Cser} polymorphism. The effect of the serine substitution in this X-linked serotonin receptor polymorphism on reward dependence was also observed when male and female subject groups were separately analyzed. There was also a significant interaction between the two dopamine receptor polymorphisms and the serotonin polymorphism on reward dependence. In particular, the effect of the 5-HT_{2C} polymorphism on reward dependence was markedly accentuated in individuals who had the long version of the D4DR exon III repeat polymorphism. When present in the same individual, the 5-HT_{2C} and dopamine receptor polymorphisms account for 30% of the observed variance for persistence (RD2) and 13% of the variance for reward dependence scores (RD134). However, the number of subjects with both less common D4DR and 5-HT_{2C} polymorphisms is small, underscoring the importance of verifying this interaction in a larger cohort. *Am. J. Med. Genet. 74:65–72, 1997.*

KEY WORDS: 5-HT_{2C} serotonin receptor; *D3DR* and *D4DR* dopamine receptor polymorphism; personality; reward; TPQ

INTRODUCTION

We recently reported an association between a human personality trait, novelty seeking, and the long repeat allele of the D4 dopamine receptor exon III polymorphism [Ebstein et al., 1996; Cloninger et al., 1996]. Our results were confirmed and extended by another laboratory which employed a different personality questionnaire in an ethnically distinct population [Benjamin et al., 1996]. These two reports constitute the first demonstrated association between a normal

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personality dimension and a specific genetic polymorphism. Novelty seeking is one of four distinct domains of temperament that are measured by Cloninger's tridimensional personality questionnaire (TPQ) [Cloninger, 1987; Cloninger et al., 1991, 1993; Svrakic et al., 1993]. No association was observed between the *D4DR* exon III repeat polymorphism and the other three domains of personality (harm avoidance, reward dependence, and persistence).

The likelihood that a number of additional genes are involved in the determination of novelty seeking, as well as the other normal personality dimensions, prompted us to examine two additional polymorphisms that are of interest in both normal and abnormal behavior. The dopamine D3 receptor (*D3DR*) has recently attracted considerable interest as a site of action of antipsychotic drugs and for its potential role in the pathogenesis of schizophrenia [Crocq et al., 1992; Ebstein et al., 1996a]. The human *DRD3* gene has been localized to chromosome 3q13.3 by in situ hybridization [Le Connait et al., 1991] and contains a polymorphic site in the first exon that gives rise to a glycine to serine substitution in the N-terminal extracellular domain [Lannfelt et al., 1992]. This point mutation can be detected through changes in the restriction site of MscI. D3 receptors readily bind classical and also atypical antipsychotic drugs but differ from most other dopamine receptor subtypes in that they are primarily localized to limbic regions which may be particularly important in the regulation of emotions [Sokoloff et al., 1990; Zahm and Brog, 1992]. Finally, this receptor is also of interest due to its role in rodent cocaine self-administration [Caine and Coob, 1993].

A second polymorphism of interest is the serotonin receptor 5-HT_{2C} subtype (*HTR2C*) which is involved in the regulation of endocrine responses, including the production and secretion of adrenocorticotropin hormone (ACTH) [King et al., 1989], oxytocin [Bagdy et al., 1992], and prolactin [Aulakh et al., 1992]. A cys_{23}-ser_{23} point mutation has recently been identified in the human 5-HT_{2C} serotonin receptor [Lappalainen et al., 1995], which has been mapped to the long arm of chromosome X. Recently it has been shown that proportion of time spent in hospital since the first admission was significantly greater in schizophrenic patients hemi- or homozygous for the less common 5-HT_{2Cser} allele than in patients carrying other genotypes [Segman et al., 1996].

In the current study, we examined the original cohort, in which an association had been demonstrated between the long repeat *D4DR* allele with increased novelty seeking scores [Ebstein et al., 1996b; Benjamin et al., 1996; Cloninger et al., 1996], for two additional coding region polymorphisms located in the 5-HT_{2C} serotonin receptor and the *D3DR* receptor. We demonstrate that the less common 5-HT_{2Cser} allele significantly reduces reward dependence scores and, furthermore, that the serotonergic effect on reward dependence appears to be modulated by both dopamine receptor polymorphisms.

METHODS

Subject Selection and Test Administration

Normal volunteers were recruited from students and staff at the Ben-Gurion University Department of Behavioral Sciences and the 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 55 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. Four subjects from the original study were not included in the analysis of the serotonin polymorphism due to loss of the individual DNA samples.

Genotyping

DNA was extracted using a Qiamp kit (Qiagen, Germany). Polymerase chain reaction (PCR) amplification of the *D4DR* exon III polymorphism was carried out by a laboratory technician blind to subject identity using Vent polymerase (New England Biolabs, Beverly, MA) and a high denaturing temperature (98°C for 1 min) with a combined annealing and extension reaction for 5 min at 70°C [Sommer et al., 1993]. The primers employed were [Lichter et al., 1993]: D4–3:GCGACTACG TGGTCTACTCG and D4–42:AGGACCCTCATGGCCTTG. The reaction mixture contained the following components: 1 × 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.

PCR amplification of the 5-HT_{2C} polymorphism was carried out using the following primers (M. Noethen, personal communication): 2CF 5′-GGCCTATTGGTTTGGCCAT-3′ and 5′-CCATGATACAAGGATG3′. One hundred ng of genomic DNA were added to a reaction mixture containing 2.5U Taq polymerase, 2 μl 10× Taq buffer, 10 pmol of each primer, 200 μM dNTPs in a total volume of 20 μl. Following initial incubation at 94°C for 5 minutes, thirty cycles were performed with a profile of 94°C for 30 seconds, 57°C for 30 seconds and 72°C for 30 seconds. This profile was followed by extension at 72°C for 4 min. The PCR products were then digested twice with 10 units of NlaIII restriction endonuclease (New England Biolabs) with the supplied buffer for 2.5 hours at 37°C. The digestion products were analyzed in ethidium bromide-stained 2.5% agarose gel under uv light. This polymorphism reveals a two-allele system with reported frequencies of 0.13 and 0.87, respectively [Lappalainen et al., 1995].

PCR amplification of the *D3DR* polymorphism was carried out with the following primers, flanking exon 1 of the D3 receptor gene: 5′-GCTCTATCTCCAACTCTCACA-3′ and 5′-AAGTCTACTCACCTCCAGGTA-3′ [Ebstein et al., 1996a]. One hundred nanograms of DNA were diluted to 12.5 μl using water and heated to 99°C for 3 min. Then a reaction mixture was added containing 1.5 U Taq polymerase, 1× Taq polymerase buffer, 0.5 μM of each primer, 160 μM dNTPs, and 0.01% gelatin in a total volume of 25 μl. Thirty-five cy-

cles were performed with a profile of 95°C for 20 seconds, 56°C for 20 seconds, and 72°C for 20 seconds. This profile was followed by a 72°C chase for 4 min. The PCR products were then digested with MscI restriction enzyme overnight and subsequently the digestion products were analyzed in 3.5% agarose ge. This polymorphism reveals a two-allele system with frequencies 0.68 and 0.32, respectively [Lannfelt et al., 1992].

Statistical Analysis

All statistical tests were carried out using SPSS for Windows.

RESULTS

TPQ scores, which measure four temperament dimensions (harm avoidance, novelty seeking, reward dependence and persistence), and 5-HT$_{2C}$, *D3DR* and *D4DR* genotypes were compared for 120 normal adult male and female volunteers. The original reward domain, which originally consisted of 4 subscales (RD1–4), was subsequently modified by Cloninger. RD2 (persistence) was separated from RD134 (reward) due to empirical evidence [Cloninger et al., 1996] which suggested that there are environmental influences that sometimes induce a weak correlation between persistence and reward dependence but no shared genetic factors. We have included both the new reward dependence (RD134) category consisting of the 3 subscales as well as the divided domain, persistence (RD2), in our analyses.

Male and female subjects. Examination of both male and female subjects by simple factorial analysis of variance (ANOVA) revealed that RD134 scores were significantly reduced (group A, Table I and (Fig. 1) by the less common 5-HT$_{2Ccys}$ polymorphism, when the three 5-HT$_{2C}$ receptor genotypes (1,1 = cys-cys; 1,2 = cys-ser, and 2,2 = ser-ser) were simultaneously compared by three-way (RD134 by 5-HT$_{2C}$, *D4DR* and *D3DR*, with sex, ethnicity and age as covariates) ANOVA (5-HT$_{2C}$: F = 6.54, DF$_{2,115}$, P = 0.002; D4DR: F = 5.47, P = 0.021). Significant two-way interactions were observed for *D4DR* × *D3DR* (F = 4.46, DF$_{2,115}$, P = 0.037) and *D3DR* × 5-HT$_{2C}$ (F = 3.83, DF$_{2,115}$, P = 0.025). No significant effect of age or ethnicity was observed. A significant effect was also observed for the less common 5-HT$_{2C}$ polymorphism on RD2 or persistence (5-HT$_{2C}$: F = 4.64, DF$_{2,115}$, P = 0.012). A significant interaction was observed between *D4DR* × 5-HT$_{2C}$ (F = 3.82, P = 0.025). No significant effect of the 5-HT$_{2C}$ genotype was observed on novelty seeking (5-HT$_{2C}$: F = 2.05, DF$_{2,116}$, P = 0.135) or harm avoidance (5-HT$_{2C}$: F = 1.303, DF$_{2,116}$, P = 0.276).

RD134 scores were also significantly reduced (group A, Table I and Fig. 1) by the less common 5-HT$_{2Ccys}$ polymorphism, when two 5-HT$_{2C}$ receptor genotypes (1,1 = cys-cys, and 2,2 = ser-ser) were compared by three-way (RD134 by 5-HT$_{2C}$, *D4DR* and *D3DR*) ANOVA (5-HT$_{2C}$: F = 8.77, DF$_{1.101}$, P = 0.004; *D4DR*: F = 4.39, P = 0.039) with sex, ethnicity and age as covariates. No significant effect of age or ethnicity was observed. This group consisted of 11 "hemizygote" males (2 = ser) and 4 homozygote females (2,2 = ser-ser). A significant effect was also observed for the less common 5-HT$_{2C}$ polymorphism on RD2 or persistence (F = 7.67, DF$_{1,101}$, P = 0.007) when two 5-HT$_{2C}$ receptor genotypes (1,1 = cys-cys, and 2,2 = ser-ser) were compared.

There is a dose effect of the 5-HT$_{2Cser}$ allele which is reflected in a reduction of the mean scores of RD2 and RD134 scales such that $\bar{x}_{score}$ 1,1 cys-cys > $\bar{x}_{score}$ 1,2cys-ser > $\bar{x}_{score}$ 2,2 ser-ser (Table I, group A and Fig. 1). Trend analysis (one-way ANOVA) shows that the effect of the 5-HT$_{2Cser}$ allele on RD134 (value = −1.86, t = −1.99, P = 0.048) is significant.

Since population stratification can confound association studies, we examined the distribution of the three genotypes in the two principal ethnic groups in the Israeli population (Ashkenazi and non-Ashkenazi). Chi-square analysis revealed no significant differences in frequency of the 5-HT$_{2C}$ (1,1; 1,2; 2,2) genotypes (χ^2 = 3.58,P = 0.16), the *D4DR* long-short genotypes (χ^2 = 0.02, P = 0.87) and the *D3DR* (1,1; 1,2 and 2,2) genotypes (χ^2 = 1.27, P = 0.53). Although no differences in genotype frequency were observed, differences in harm avoidance ($\bar{x}_{ASH}$ = 13.4 ± 0.59 vs. $\bar{x}_{NON}$ = 10.21 ± 0.1.00, F = 6.48, DF = $_{1,120}$, P = 0.012) and persistence ($\bar{x}_{ASH}$ = 4.88 ± 0.20 vs. $\bar{x}_{NON}$ = 5.711 ± 0.37, F = 4.15, DF = $_{1,119}$, P = 0.044) scores between the Ashkenazi and non-Ashkenazi groups were observed. In the non-Ashkenazi group, a highly significant effect of 5-HT$_{2C}$ on persistence was observed (F = 12.986, DF = 1, 31, P = 0.001). In the Ashkenazi population, a significant effect of 5-HT$_{2C}$ on RD134 was observed (F = 7.397, DF = 1, 83, P = 0.008).

The effect of the less common 5-HT$_{2Ccys}$ polymorphism, comparing the 1,1 (n = 91) vs. 2,2 (n = 15) genotypes on reward dependence, is also observed in the absence of additional genotype information as shown by two-way ANOVA: RD2 or persistence by 5-HT$_{2C}$ (5-HT$_{2C}$: F = 8.15, DF = $_{1,101}$, P = 0.005). The effect on RD134 did not quite attain statistical significance: (5-HT$_{2C}$: F = 3.55, DF$_{1,101}$, P = 0.062), with sex, age and ethnic group as covariates.

Since the 5-HT$_{2C}$ polymorphism is located on the X chromosome, we also examined the simultaneous effects of the 3 polymorphisms on the four temperament domains in both the male and female groups.

Female subjects. RD134 scores (Group F, Table I) were significantly reduced by the presence of the less common 5-HT$_{2Ccys}$ polymorphism, when the serotonin receptor genotypes (1,1; 1,2; 2,2) were compared by three-way (5-HT$_{2C}$, *D3DR* and *D4DR*) ANOVA (5-HT$_{2C}$: F = 6.09, P = 0.005; *D4DR*:F = 4.57, P = 0.039; *D3DR*: F = 7.04, P = 0.011).

The effect of the less common 5-HT$_{2Ccys}$ polymorphism on reward in female subjects is not observed in the absence of additional genotype information as shown by two-way ANOVA: RD2 and RD134 by 5-HT$_{2C}$, when 1,1 1,2 and 2,2 genotypes are compared (Table I, group F). The failure to observe the effect of the serotonin polymorphism in females is most likely due to the weaker effect of the heterozygote genotype (1,2 = cys-ser) on reward in comparison to the stronger effect of the "hemizygote" (males)/homozygote (females) genotypes. However, when the female subjects are grouped by the presence or absence of the 5-HT$_{2Ccys}$ polymorphism (1,1 = cys-cys vs. 1,2 = cys-ser and 2,2 = ser-

TABLE I. Effect of Genotype on TPQ Scores

Group	Genotype	N	Novelty seeking	Reward 134	Persist RD2	Harm
A						
Male and female	1,1 cys	91	16.31 (.57)[a]	14.05 (.35)	5.23 (.22)	12.59 (.63)
5-HT_{2C}	1,2 cys-ser	14	14.57 (.90)	13.57 (.87)	4.78 (.59)	12.64(1.29)
	2,2 ser	15	16.20 (.99)	12.20 (.80)	4.33 (.45)	12.00(1.54)
B						
Male and female	Short	86	15.38 (.48)	13.87 (.34)	5.03 (.22)	12.52 (.59)
D4DR	Long	38	17.84 (.94)	13.71 (.62)	5.15 (.33)	12.97(1.08)
C						
Male and female	1,1 gly	60	16.16 (.64)	13.75 (.42)	5.40 (.19)	11.61 (.74)
D3DR	1,2 gly-ser	57	16.08 (.70)	14.07 (.46)	4.67 (.31)	13.49 (.78)
	2,2 ser	7	16.28(1.61)	12.42(1.19)	5.42(1.10)	14.85(1.99)
D						
Male and female	gly	60	16.16 (.64)	13.75 (.42)	5.40 (.19)	11.61 (.74)
D3DR-All	ser	64	16.10 (.64)	13.88 (.43)	4.76 (.30)	13.64 (.72)
E						
Male	1 cys	55	15.6 (.70)	13.32 (.46)	5.47 (.27)	11.90 (.80)
5-HT_{2C}	2 ser	11	15.54(1.26)	12.27 (.94)	4.45 (.56)	12.09(1.85)
F						
Female	1,1 cys	35	17.41 (.95)	15.2 (.52)	4.85 (.36)	13.63(1.02)
5-HT_{2C}	1,2 cys-ser	14	14.57 (.90)	13.57 (.87)	4.78 (.59)	12.64(1.29)
	2,2 ser	4	18.00(1.08)	12.00(1.77)	4.00 (.82)	11.75(3.19)
G						
Male and female	1,1 cys	30	18.26(1.12)	14.10 (.65)	5.43 (.39)	12.33(1.31)
5-HT_{2C}	1,2 cys-ser	3	15.66 (.88)	14.00(2.64)	3.33 (.33)	12.33(2.66)
D4DR-Long	2,2 ser	3	15.33(3.71)	8.66(2.92)	3.66 (.33)	18.66 (.88)
Male	2 ser	2	13.00(5.00)	8.00(2.00)	3.50 (.50)	9.46(1.86)
Female	2,2 ser	1	20.00	10.00	4.00	17.00
H						
Male and female	1,1 cys	60	15.36 (.62)	14.03 (.42)	5.13 (.26)	12.72 (.70)
5-HT_{2C}	1,2 cys-ser	11	14.27(1.12)	13.45 (.92)	5.18 (.55)	12.72(1.54)
D4DR-Short	2,2 ser	12	16.41 (.97)	13.08 (.77)	4.50 (.71)	10.33(1.58)
Male	2 ser	9	16.11(1.24)	13.20 (.79)	4.66 (.67)	10.44(1.84)
Female	2,2	3	17.33(1.20)	12.66(2.33)	4.00(1.15)	10.00(3.78)
I						
Male and female	1,1 cys	42	16.64 (.81)	14.14 (.50)	5.64 (.22)	11.23 (.88)
D3DR-All	1,2 cys-ser	5	11.20(1.39)	11.00(1.04)	5.20 (.92)	13.00(2.86)
gly	2,2 ser	9	16.22(1.22)	12.66(1.04)	4.44 (.56)	10.33 (.20)
J						
Male and female	1,1 cys	49	16.04 (.80)	13.97 (.51)	4.87 (.35)	13.75 (.88)
D3DR-All	1,2 cys-ser	9	16.44 (.55)	15.00 (.94)	4.55 (.80)	12.44(1.39)
ser	2,2 ser	6	16.16(1.83)	11.50(1.33)	4.16 (.83)	14.50(2.24)

[a] Numbers in parentheses are S.E.M.

ser), then a significant effect of the serotonin receptor on RD134 is observed (ANOVA: F = 4.19, DF = 1,51, $P = 0.046$) even in the absence of additional genotype information. Similar results are obtained by comparing the mean scores of female subjects with and without the polymorphism (RD134: z = −2.07, $P = 0.038$, Mann-Whitney U).

Male subjects. RD134 scores were significantly reduced in male subjects (group E, Table I) by the presence of the less common 5-HT_{2Ccys} allele when the three genotypes (5-HT_{2C}, *D3DR* and *D4DR*) were compared by three-way ANOVA (5-HT_{2C}: F = 4.75, $DF_{1,58}$, $P = 0.034$). Similar results were observed for RD2 (5-HT_{2C}: F = 9.56, $DF_{1,63}$, $P = 0.003$). The effect of the less common 5-HT_{2Ccys} polymorphism, comparing the 1,1 (n = 55) vs. 2,2 (n = 11) genotypes on RD2 or persistence, is also observed in the absence of additional genotype information as shown by two-way ANOVA: RD2 by 5-HT_{2C} (5-HT_{2C}: F = 7.10, $DF_{1,63}$, $P = 0.01$), with sex, age and ethnic group as covariates.

Interactions. In order to better understand the roles played by the three polymorphisms in determining reward dependence, the effect of the 5-HT_{2C} polymorphism in male and female individuals having the long and short form of the *D4DR* exon III repeat polymorphism was examined (groups G and H, Table I). Both male and female subjects were included in the same analysis since the number of individuals having the less common forms of both the 5-HT_{2Ccys} and the *D4DR* exon III long repeat is small. The presence of the long *D4DR* repeat strongly potentiates the effect of the 5-HT_{2Ccys} allele. Three subjects (two "hemizygote" males and one female) were homozygous for the cys polymorphism and three subjects were heterozygote (cys-ser).

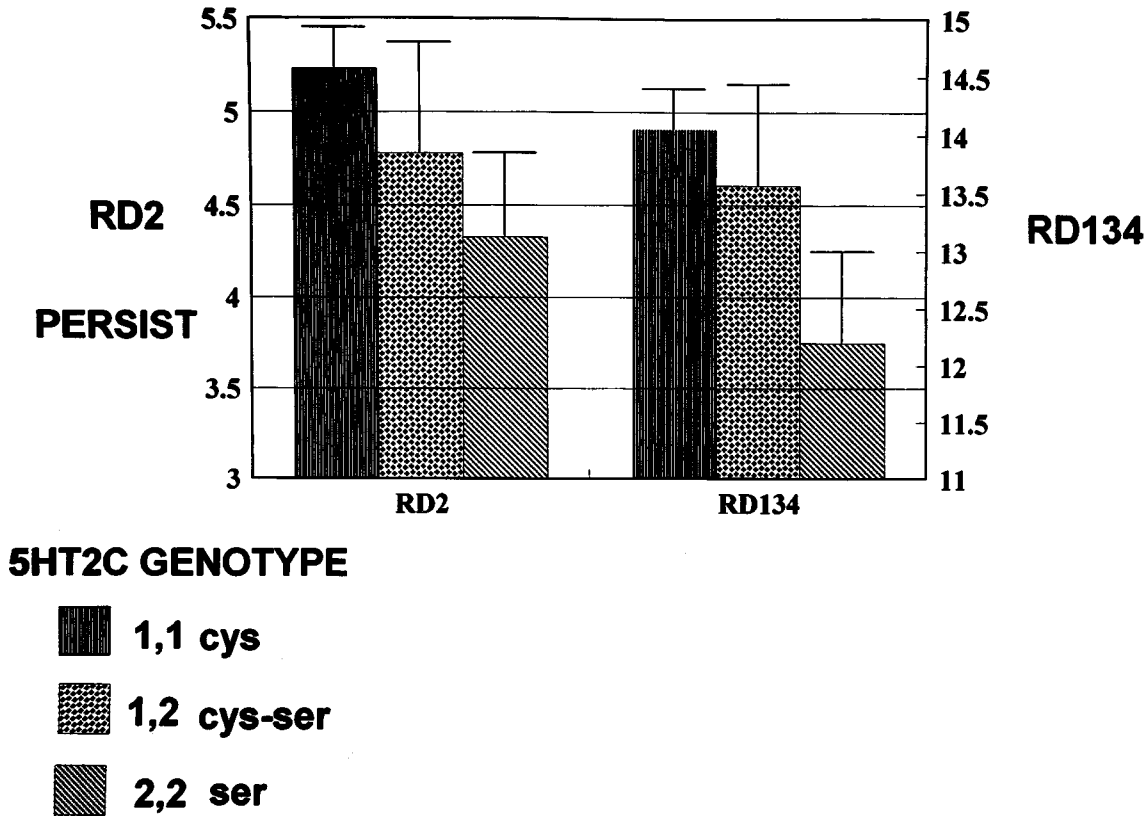

Fig. 1. The effect of the 5-HT$_{2Cser}$ serotonin receptor polymorphism on reward dependence (RD134) and persistence (RD2) in male and female subjects.

Although the number of subjects were small, a significant difference in mean RD2 (t = 3.45, P = 0.006, Student's t test) and RD134 (t = 3.66, P = 0.034) was observed between subjects with the 1,1 (n = 30) vs. the 2,2 genotype (n = 3). A significant difference in mean values is also observed when the 1,1 (n = 30) vs. the 1,2 and 2,2 (n = 6) genotypes are compared (RD2: z = −2.57, P = 0.01 Mann-Whitney U). Moreover, in subjects having the short form of the *D4DR* exon III receptor repeat polymorphism, no significant differences (RD2: t = 1.03, P = 0.32; RD134: t = 1.08, P = 0.30) were observed between individuals having the 5-HT$_{2Ccys}$ (n = 60) vs. the 5-HT$_{2Cser}$ (n = 12) polymorphisms (group H, Table I).

DISCUSSION

It is a genuine challenge to relate specific genes to temperament due both to the difficulties involved in measuring and defining heritable personality traits as well as due to the inherent complexities of the neurochemical and neuroanatomical substrates of behavior which involve multiple interactions among several neurotransmitter systems. Temperament is a complex trait characterized by multifactorial patterns of inheritance in which numerous genes interact with environmental factors to determine the personality dimensions [Belmaker and Biederman, 1994; Bouchard, 1994; Loehlin, 1992; Mann, 1994; Plomin, 1990; Plomin et al., 1994a]. Genetic influences, which contribute to complex behavior, are presumably due to the interaction of multiple genes of differing effect size. These multiple gene effects can contribute additively to temperament and any single gene contributing to such involved traits is neither necessary nor sufficient to its expression. Genes that determine temperament are predicted to show small effects, and are similar to other quantitative trait loci (QTLs) that determine complex traits and which have now been characterized in many higher organisms including plants, rodents and man [Belnap et al., 1993; Clarke et al., 1995; Davies et al., 1994; East, 1915; Haley, 1995; Hofstetter et al., 1995; Lander and Schork, 1994; Markel and Corley, 1994; McCouch and Doerge, 1995; Paterson et al., 1991; Plomin et al., 1994b].

The current study is one of the few investigations in which several polymorphisms have been simultaneously examined for their association with a human behavioral trait. The noteworthy analysis of the interaction of three dopaminergic genes (DRD2, DβH and DAT1) and Tourette Syndrome by Comings et al., [1996] is not only especially relevant to the present study but also to the overall role played by polygenic inheritance in normal and abnormal behaviors. Comings' insightful discussion of the implications of polygenic inheritance in psychiatric genetics, and the general guidelines he suggests for further studies of multiple gene effects, merits considerable attention.

In the current investigation, unrelated subjects were given the TPQ, a self-report psychological questionnaire that measures four personality temperaments, and genotyped for three receptor polymorphisms, 5-HT_{2C}, *D3DR* and *D4DR*. Those subjects having the less common 5-HT_{2Cser} polymorphism have significantly lower reward dependence and/or persistence scores than individuals having the more common allele. This restraining effect of the less common 5-HT_{2C} allele on reward dependence and/or persistence was observed when both male and female subjects were considered as a single group as well as when male and female subjects were analyzed separately. The effect was observed in the two principal ethnic groups (Ashkenazi and non-Ashkenazi) represented in the Israeli population. Finally, the effect of the 5-HT_{2C} genotype on reward dependence and/or persistence was also observed in the absence of additional genotype information.

In addition to the effect of the 5-HT_{2C} polymorphism, the presence of the *D4DR* receptor polymorphism appeared to modulate the effect of the serotonin receptor on reward dependence and/or persistence scores. The consequence of having both the long repeat *D4DR* and the 5-HT_{2Cser} alleles was marked on reward dependence (RD134), with an effect size on mean scores of >2 standard deviation units. When the combined group of male and female subjects is examined by comparing partial correlation coefficients (controlling for age, sex and ethnicity) the variance (r^2) "explained" by the 5-HT_{2C} genotype (comparing 11 vs. 12 and 22) is 3.2% ($P = 0.06$) for RD2 or persistence and 4.2% for RD134 ($P = 0.03$). However, when the same analysis is carried out in those subjects having only the long repeat *D4DR* allele, the variance explained by the 5-HT_{2C} genotype is 29% for RD2 or persistence ($P = 0.001$) and 12.8% for RD134 ($P = 0.04$). We emphasize that these results should be interpreted cautiously since only a small number of individuals (n = 6) have both the less common 5-HT_{2C} polymorphism and the long repeat *D4DR* allele, suggesting that a larger cohort must be employed in order to substantiate the role played by multiple polymorphisms in the determination of behavioral traits.

We examined two dopaminergic genotypes and one serotonergic genotype for their association with human personality dimensions. The number of known genetic polymorphisms exhibited by behaviorally relevant genes is continually expanding and although many of these are of potential interest in the context of the present investigation, especially the dopamine D2 receptor polymorphism which has in some studies been linked to alcoholism [Blum et al., 1990], practical considerations restricted our initial choice to *D4DR* exon III, the 5-*HT_{2C}* and the *D3DR* genes. Future studies by ourselves and other investigators will undoubtedly enlarge the number of additional genotypes examined for their association with personality dimensions.

In the current study, three genotypes were examined for their effect on 4 personality domains. Although the results were not corrected for multiple comparisons, the significance levels of the 5-HT_{2C} effect on reward dependence and/or persistence after deflating for multiple testing by a factor of 12 (4 personality dimensions $\times$ 3 genotypes = 12) for the combined male and female cohort is RD134 $P = 0.024$ (12×0.002). Similarly, the level of significance for the effect of 5-HT_{2C} on RD134 scores in female when each cohort is analyzed separately, is $P = 0.06$ (0.005×12). The level of significance for male subjects for RD2 or persistence after deflating for multiple comparisons is $P = 0.036$ (12×0.003).

Although the criteria for establishing significance in association studies are far from clear [Lander and Kruglyak, 1995], the approach taken by Thomson [1994 and see also Davies et al., 1994] in trying to unravel the role of multiple genes in insulin-dependent diabetes mellitus (IDDM) is worth considering. In early studies, IDDM was linked by both association and affected sib-pair studies to the HLA locus at 6p21 (the so-called *IDDM1* locus). Other loci, e.g., *IDDM2* at 11p15, playing a role in IDDM, were also established by case-control design and then confirmed by nuclear family-based association studies. Although linkage with affected sib-pair data has also been demonstrated, it is not consistently observed, as might be expected when a number of loci are involved in a complex trait, and each loci has a small effect. In such circumstances, linkage will be difficult to detect and replicate. The non-HLA IDDM genes clearly illustrate these phenomena. The expected norm in monogenic diseases (based on lod score significance levels of $P < 0.001$) could clearly miss a signal in complex diseases from a gene even having a moderate effect, and so this requirement may be too stringent. In summary, what criteria are desirable for establishing linkage for complex traits or diseases? Thomson has proposed the following guidelines for provisional assignment of linkage to a marker region: (i) weak ($P < 0.05$) evidence for linkage and/or association in at least three independent data sets; (ii) moderate ($P < 0.01$) evidence in at least two independent data sets; or (iii) strong ($P < 0.001$) evidence in one, or the overall, data set. Some of these preliminary linkages will later turn out to be false (type I errors) but as Thomson [1994, p. 109] emphasizes "with complex diseases it is surely preferable to err on the side of false linkage which will later be refuted than to pass over a genuine linkage."

Finally, as discussed by Wang et al. [1995] in their recent report of a susceptibility locus for schizophrenia on chromosome 6pter-p22, if a gene of small effect size is associated with susceptibility to schizophrenia, possibly interacting additively with other loci, then theoretical analyses indicate that hundreds of additional families may be required for replication [Suarez et al., 1994]. We agree with the preference of both Thomson [1994] and Wang et al., [1995] to present highly cautious reports of association or linkage findings for complex traits, as we have attempted to do in the current report, and with clear recognition of the problems associated with defining stringent criteria for significance levels.

In our first study, a significant effect of the long repeat *D4DR* allele was observed on novelty seeking [Ebstein et al., 1996b] and our results were confirmed and extended by a second group [Benjamin et al., 1996] em-

ploying both a different population group and a different personality instrument, the NEO-PI-R [Costa and McCrae, 1992]. Although the NEO-PI-R does not explicitly define the four personality traits measured in the TPQ instrument, it contains multiple items that are clearly related to the TPQ. It is therefore possible to "translate" the NEO-PI-R scores into the TPQ domains with the caveat that the correspondence between the two questionnaires is only partial. For novelty seeking, the correlation has been estimated at 70% [Cloninger et al., 1996]. In contrast to the quick validation by Benjamin et al. [1996] of our observed effect of the *D4DR* polymorphism on novelty seeking, the effect of the serotonin polymorphism on reward dependence is not seen in the American cohort (Hamer, personnel communication).

Several possible explanations for the failure of Benjamin et al. [1996] to observe an effect of the 5-HT$_{2C}$ genotype on reward dependence are worth considering. First of all, in contrast to the complex genetic architecture of the NEO instrument, the facets of the TPQ scale used in the current study were designed by Cloninger to be genetically homogenous and independent of other temperament dimensions. The simple genetic architecture of the TPQ dimensions has recently been replicated in quantitative genetic analyses of large samples of twins [discussed in Cloninger et al., 1996]. Together, these results suggest that the use of the TPQ has distinct advantages when genetic aspects of human behavior are the primary focus of the investigation, and may prove a more effective instrument for unravelling the role of heredity in personality in comparison to other tests such as the NEO. Correspondence between investigations employing two separate instruments for assessing personality domains may, therefore, not always be expected.

A study in tomato plants is intriguing for its possible pertinence to our findings. Paterson et al., [1991] described 29 QTLs in tomato plants that affect fruit size, soluble solids concentration and pH. The QTLs were detected in three environments (two in California and one in Israel). They were located on 11 chromosomes and accounted for 5–42% of the phenotypic variance in a trait, and showed different types of gene action. Among these 29 QTLs, 4 were detected in all three environments, 10 in two environments, and 15 only in a single environment. The two California environments were most similar, sharing 44% (11/25) QTLs, while the Israeli environment was quite different, sharing 35% (7/20) and 19% (5/26) QTLs with the respective California environments. The authors point out that QTLs may be characterized by their sensitivity to environmental factors, suggesting that studies done in a single environment are likely to ignore or underestimate the number of QTLs which can influence a trait.

How does the presently described role of the 5-HT$_{2C}$ receptor polymorphism on reward dependence and persistence relate to the known functions of serotonergic pathways in animal and human behavior? Serotonin is implicated in many physiological functions (sleep, appetite, pain, sexual behavior) as well as in many pathological disorders (anxiety, depression, schizophrenia, migraine, alcoholism, emesis) [Benkalfat, 1993]. Moreover, at least 12 serotonin receptors including subtypes are known [Bockaert, 1994] and each of these subtypes may be involved in the mediation of different behaviors. In spite of the complexity of serotonin's effects, a general theory of serotonin's role in behavior has been proposed that is consistent with animal and human studies [Depue and Spoont, 1986; Soubrie, 1986]. Reduced serotonergic transmission is suggested to involve a shift from the inhibition of behavior to its facilitation, a switch more commonly described in humans in terms of decreased impulse control. Cloninger's original supposition that serotonin is the principal neurotransmitter involved in harm avoidance or behavioral inhibition is apparently related to the general concept that inhibition of serotonergic pathways leads to behavioral facilitation.

We observed in the current study an effect of both serotonergic and dopaminergic receptor polymorphisms on reward and persistence behavior. In the rat, the ventral tegmental area of the brain and its projection to the nucleus accumbens, including the ascending mesolimbic dopaminergic system, comprises the principal component of the brain's reward circuitry that is preferentially activated by intracranial self-stimulation [discussed in Olds, 1995]. The role of serotonin in the brain's reward mechanism is less clear, but the current view is that serotonergic neurons that synapse on mesolimbic dopamine neurons can regulate intracranial self-stimulation, and hence reward behavior, by modulating dopamine transmission. Apparently, the relationship between 5-HT and dopaminergic activity presumed to exist for the regulation of drug-induced motor activity extends to the regulation of brain stimulation-induced reward. This explanation is consistent with the view that serotonergic transmission exerts inhibitory regulation of behaviors, some of which like intracranial self-stimulation models of reward, have been shown to be primarily dopamine-dependent. It should be noted that other neurotransmitter systems also impinge on reward, especially the opioid peptides [Bozarth and Wise, 1984]. In summary, animal studies show linkage between serotonergic transmission, dopamine and reward and provide a neurochemical explanation for the current findings of an interaction between dopamine and serotonergic genetic polymorphisms in the determination of reward dependence in man.

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