Molecular Psychiatry (2000) 5, 96–100

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms: dopamine receptor D4 (DRD4), serotonin transporter promoter region (5-HTTLPR) and catechol O-methyltransferase (COMT)

J Benjamin[1], Y Osher[1], M Kotler[1], I Gritsenko[2], L Nemanov[2], RH Belmaker[1] and RP Ebstein[2]

[1]*Faculty of Health Sciences, Ben Gurion University of the Negev;* [2]*Research Laboratory, S Herzog Memorial Hospital, Israel*

Keywords: personality; polymorphism; tridimensional personality questionnaire; catechol O-methyltransferase; dopamine D4 receptor; serotonin transporter promoter

Dopamine D4 receptor (DRD4), serotonin transporter promoter regulatory region (5-HTTLPR) and catechol O-methyltransferase (COMT) polymorphisms were examined for association with TPQ personality factors in 455 subjects. Significant interactions were observed by multivariate analysis, (COMT × 5-HTTLPR: Hotelling's Trace = 2.3, $P = 0.02$) and by subsequent univariate 3-way ANOVA when Novelty Seeking (NS) was the dependent variable: 5-HTTLPR × D4DR ($F = 6.18$, $P = 0.03$) and COMT × 5-HTTLPR ($F = 4.42$, $P = 0.03$). In the absence of the short 5-HTTLPR allele and in the presence of the high enzyme activity COMT val/val genotype, NS scores are higher in the presence of the DRD4 seven-repeat allele. The effect of these three polymorphisms on NS was also examined using a within-families design. Siblings who shared identical genotype groups for all three polymorphisms (COMT, DRD4 and 5-HTTLPR) had significantly correlated NS scores (intraclass coefficient = 0.39, $F = 2.26$, $P = 0.008$, $n = 49$) whereas sibs with dissimilar genotypes in at least one polymorphism showed no significant correlation for NS scores (intraclass coefficient = 0.177, $F = 1.43$, $P = 0.09$, $n = 110$). Similar interactions were also observed between these three polymorphisms and Novelty Seeking when the 150 independently recruited and non-related subjects were analyzed. The current results are consistent with two earlier reports in which we demonstrated an interaction between the 5-HTTLPR and DRD4 polymorphisms in 2-week-old neonates, in the same children assessed again at 2 months of age and in adults. *Molecular Psychiatry* (2000) **5,** 96–100.

Twin studies demonstrate that personality traits measured by several self-report questionnaires including the TPQ[1] and NEO-PI-R[2] are partially inherited and between 30–60% of the observed variance can be accounted for by genes. Only recently, however, have common genetic polymorphisms especially DRD4 and 5-HTTLPR, been provisionally assigned to particular temperament factors.[3–5] It seems reasonable that as additional genetic information is inventoried for various cohorts, associations between DRD4, 5-HTTLPR and other candidate genes and complex phenotypes will be further clarified. We,[6–8] and others,[9–11] have begun to examine multiple interactions between common genetic polymorphisms and complex behavioral traits. The catechol O-methyltransferase (COMT) gene contains a common, functional polymorphism in which a val-to-met amino acid substitution markedly reduces enzyme activity to about 20% of wild-type (val) levels.[12] Recent studies have suggested a role for COMT in behaviors often associated with impulsivity and disinhibition,[13–15] although to our knowledge, this gene has yet to be examined for a role in normal human personality.

In the current study we initially examined using multivariate ANOVA four TPQ personality factor scores for 455 subjects grouped by three polymorphisms: DRD4, COMT and 5-HTTLPR. A significant multivariate interaction of COMT × 5-HTTLPR (Hotelling's Trace = 2.3, $P = 0.02$) was observed. Significant interactions were also observed in subsequent analysis when NS was the dependent variable and the three polymorphisms were the independent variables (univariate 3-way ANOVA): 5-HTTLPR × D4DR ($F = 6.18$, $P = 0.03$), COMT × 5-HTTLPR ($F = 4.42$, $P = 0.03$). P values are adjusted by the Bonferroni correction for multiple testing as explained in the Methods section. Descriptive statistics for NS and HA grouped by these three polymorphisms are presented in Table 1. Neither main effects nor interactions between these three polymorphisms and HA and Reward dependence were observed (data not shown). See below for further analysis of HA. A significant interaction between the COMT and 5-HTTLPR polymorphisms and TPQ Persistence (RD2) was observed and these results are reported elsewhere.[16]

To illustrate these interactions, SPSS marginal mean NS scores (adjusted for age and sex effects) are plotted in Figure 1. In the absence of the short 5-HTTLPR allele and in the presence of COMT homozygosity for the high enzyme val/val genotype, NS scores are higher in the presence of the DRD4 seven-repeat allele (Figure

Table 1 Descriptive statistics. The 5-HTTLPR long = L/L genotype and the 5-HTTLPR short = L/S and S/S genotypes. DRD4 (–7) are subjects without the 7-repeat allele and DRD4 (+7) with the 7-repeat allele

	COMT	*5-HTTLPR*	*DRD4*	*Mean*	*SD*	*n*
HA	val/val	long	–7	13.59	6.11	22
			7	12.10	6.17	10
		short	–7	13.19	5.80	68
			7	13.43	6.75	37
	val/met	long	–7	12.36	6.06	36
			7	12.19	6.22	26
		short	–7	12.89	5.92	101
			7	13.99	6.13	67
	met/met	long	–7	11.90	6.79	10
			7	12.50	3.70	4
		short	–7	14.22	6.10	50
			7	10.87	5.42	23
NS	val/val	long	–7	16.91	4.22	22
			7	20.71	6.34	10
		short	–7	16.21	4.35	68
			7	15.43	4.76	37
	val/met	long	–7	15.86	4.34	36
			7	14.92	4.02	26
		short	–7	16.14	4.88	101
			7	15.90	5.36	67
	met/met	long	–7	15.20	4.73	10
			7	18.25	4.11	4
		short	–7	16.08	4.74	50
			7	14.48	4.55	23

1). If subjects are selected for the COMT val/val genotype (n = 136), and NS scores grouped by two independent variables (DRD4 and 5-HTTLPR), then a significant interaction by 2-way ANOVA is observed between DRD4 × 5-HTTLPR (F = 5.33, P = 04). A significant main effect of the 5-HTTLPR polymorphism on NS (F = 6.69, P = 0.02, n = 137) is also observed. Similarly, if subjects are selected for both COMT (val/val) and 5-HTTLPR (long/long) genotypes, then subjects with the DRD4 seven-repeat allele show marginally significant higher NS scores than subjects without the DRD4 seven-allele (independent samples t = 2.02, P = 0.05, df = 30).

If subjects are selected for the met/met COMT genotype (n = 87), no significant main effects or interactions are observed when NS is the dependent variable. Moreover, when subjects are selected for both polymorphisms (COMT = met/met and 5-HTTLPR = L/L), no significant effect of the DRD4 seven-repeat allele on NS scores is observed (t = 1.13, P>0.1, df = 12). Finally, in the presence of the heterozygous COMT val/met genotype no significant main effects or interactions are observed between the 5-HTTLPR and the DRD4 seven-repeat on NS.

We have examined a large number of subjects using the TPQ questionnaire (not all have been genotyped) and have observed a small but highly significant negative correlation between the TPQ personality factors NS and HA (r = –0.123, P = 0.0001, n = 1492, controlling for age and sex). This negative correlation between HA and NS prompted us to examine more closely the relationship between the two polymorphisms that purportedly contribute to these personality factors. We therefore analyzed HA and 5-HTTPLR separately for each COMT group. In the presence of the met/met COMT genotype and presence of the short 5-HTTLPR alleles (see shaded cells in Table 1), subjects without the DRD4 seven-allele had elevated Harm Avoidance scores compared with subjects with the DRD4 seven-allele (t = 2.49, P = 0.02, n = 73). No effect of the DRD4 seven-repeat allele is observed when subjects are selected for the val/val COMT genotype (t = 0.18, P = 0.8, n = 105).

When the 150 non-related subjects were analyzed by univariate 3-way ANOVA (NS scores grouped by 5-HTTLPR, DRD4 and COMT), essentially similar results were obtained as for the extended data set. (Main effects: DRD4 F = 4.7, P = 0.09; COMT F = 4.92, P = 0.027. Interactions: 5-HTTLPR × DRD4 F = 3.37, P = 0.021; 5-HTTLPR × COMT F = 8.26, P = 0.001 and DRD4 × COMT × 5-HTTLPR F = 4.46, P = 0.09.)

About 70% of these subjects were independently recruited siblings, allowing us to examine the effect of these three polymorphisms on NS using a within-families strategy. Firstly, intraclass correlation coefficients (for NS scores) were calculated for siblings who shared identical genotype groupings for each of the three polymorphisms (COMT val/val, val/met or met/met; 5-HTTLPR short or long genotypes; and DRD4 short or long alleles). A significant intraclass correlation was

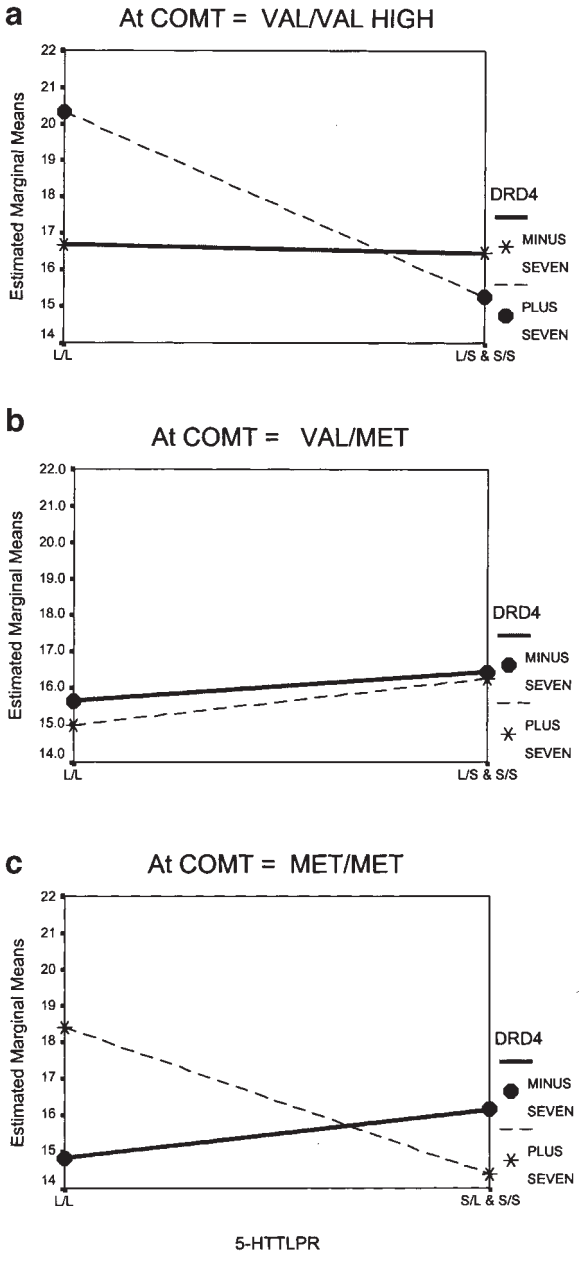

Figure 1 TPQ NS scores grouped by three polymorphisms: COMT, DRD4 and 5-HTTLPR. (a) COMT = VAL/VAL HIGH; (b) COMT = VAL/MET; (c) COMT = MET/MET.

observed for NS scores within sib pairs (coefficient = 0.39, $F = 2.26$, $P = 0.008$, $n = 49$). Contrariwise, siblings who shared at least one dissimilar of the three examined genotypes showed no significant correlation for NS scores (coefficient = 0.177, $F = 1.43$, $P = 0.09$, $n = 110$).

When sibling groups sharing either of the two homozygous (val/val *vs* met/met) COMT genotypes (identical for 5-HTTLPR and DRD4) were examined, sib pairs with the val/val genotype had significantly correlated NS scores (coefficient = 0.67, $F = 5.11$, $P = 0.009$, $n = 13$). However, no significant difference in mean NS scores by paired *t*-test ($t = 0.07$, $P > 0.1$) was observed. Conversely, siblings with the COMT met/met genotype (identical for 5-HTTLPR and DRD4) showed no correlation in NS scores (coefficient = 0.16, $F = 1.36$, $P = 0.9$, $n = 10$) but displayed a marginally significant difference in mean NS scores by paired *t*-test (15.00 ± 1.49 SD *vs* 18.70 ± 4.62 SD and $t = 2.55$, $P = 0.09$). Similar results regarding the permissive role of the val/val COMT genotype were also observed by a between families analysis, as discussed above.

The current results are consistent with an earlier study[8] in which we demonstrated an interaction between the 5-HTTLPR and DRD4 seven-repeat on Brazelton Orientation scores (perhaps a putative antecedent temperament of adult NS) in 2-week-old neonates. At 2 months of age, these children were again assessed[7] using the Infant Behavioral Questionnaire[17] and a significant interaction between the DRD4 seven-repeat × 5-HTTLPR promoter region polymorphisms on Negative Emotionality and Distress to Limitations was observed. In those infants lacking the DRD4 seven-repeat, the effect of 5-HTTLPR S/S genotype was to significantly raise scores for Negative Emotionality and Distress to Limitations. These infants also reacted more negatively when presented with sudden or novel stimuli. In both infants and adults short alleles of 5-HTTLPR and long alleles of DRD4 oppose each other's effects; ie approach behaviors (Orientation to environmental stimuli in infants and NS in adults) are promoted by long DRD4 alleles, whereas short alleles of 5-HTTLPR increase avoidance behaviors. These results are psychologically intuitive since common sense dictates that individuals characterized by a drive for new experiences and sensations are less likely to be also characterized by high HA (= fearful, shy, fatigable people).

Although we fail to observe a direct association between 5-HTTPLR and TPQ HA in the total cohort of 455 ($F = 1.69$, $P = 0.19$, $n = 455$), in a previous cohort,[18] and in a combined analysis ($n = 836$, $t = 0.58$, $P > 0.1$; mean HA score for 5-HTTLPR L/L = 13.34 ± 0.41 L/S and S/S = 13.08 ± 0.22), the current results (in those subjects with the met/met genotype), and our previous studies in neonates,[7,8] nevertheless provide indirect evidence for an association between 5-HTTLPR and HA. We also note that in a subset of 148 subjects recruited from the Beersheva region (by JB and YO) a significant association between HA and 5-HTTLPR is observed in the absence of any additional genetic information.[19] In our expanded set of subjects two additional genes, the DRD4 and COMT, modify the contribution of the serotonin promoter region polymorphism to HA.

In light of this modulation of D4DR on NS by at least two other functional polymorphisms, COMT and 5-HTTLPR, we suggest that failure to replicate associations between personality factors and some genes may be partially due to the presence of additional modifying common polymorphisms. Replication is especially difficult across ethnic categories when allele frequencies often differ. It should be considered that in a population with an increased frequency of COMT

val/val genotype and the 5-HTTLPR short allele the lack of effect of DRD4 on NS might be reported as a 'false negative.'

To summarize, we have found that NS and antecedent behaviors are most strongly expressed in subjects lacking the 5-HTTLPR short allele and possessing long DRD4 alleles. An additional gene, COMT, which metabolizes catecholamines but not serotonin, plays a further role in our sample in modulating the effect of both the 5-HTTLPR and DRD4 genes on personality. Most intriguingly the interaction between 5-HTTLPR and DRD4 is observable from early infancy[7,8] to adulthood, spanning the entire developmental period of human temperament. Moreover, these findings are consistent with some animal and human studies suggesting that dopamine and serotonin dually mediate NS and impulsive behaviors.[20,21]

Methods

Subject groups

There were 182 males and 273 females; average age was 26.95 ± 10.4 years SD. Subjects were recruited from many sources (high schools, villages, kibbutz members, university students and staff) and paid for their participation in the study. No psychiatric screening was carried out but some of the sib pairs were asked about a history of mental illness and substance abuse (data will be presented elsewhere). About 70% of the subjects were related sib pairs. The Ben-Gurion University Helsinki Human Subjects Committee approved the study.

Genotyping

DNA was extracted and genotypes determined as previously described: DRD4,[4] 5-HTTLPR[18] and COMT.[22]

Statistical analysis

The effect of all three independent variables (DRD4, 5-HTTLPR and COMT) on the dependent variables: TPQ personality factors including Harm Avoidance (HA), NS, Reward dependence (RD134) and Persistence (RD2) were analyzed by multivariate and subsequent univariate ANOVA (SPSS for Windows) in the complete data set. Sex and age were entered as covariates. We had a prior hypothesis regarding the interaction between DRD4 $\times$ 5-HTTLPR and NS based on previous studies from our group[7,8] and as we discussed in the Introduction, the COMT polymorphism has also been associated with impulsive behaviors.[13,15] We therefore used a 'rule of thumb' Bonferroni correction for multiple testing in the univariate 2- and 3-way ANOVA analyses and multiplied *P* values (significance levels) by the number of polymorphisms examined when NS was the dependent variable. Validation of initial findings in independently recruited cohorts may be more convincing than debate about nominal *vs* corrected significance levels. No correction was made for *t*-test levels of significance since only one independent variable was examined. Note that the SPSS program does not calculate post-hoc significance levels for main effects when the independent variable has less than three levels eg DRD4 (long *vs* short) or 5-HTTLPR (long *vs* short), for interactions between independent variables or for main effects when covariates are entered in the model.

The sib pairs were tested by paired *t*-test for mean differences. Intraclass correlation coefficients (SPSS) were calculated for concordant and discordant sibling's Novelty Seeking scores and corrected for multiple testing.

The D4DR exon III repeat polymorphism genotypes were grouped first by either the presence or absence of the seven-repeat allele, and in separate second analysis, by the short (2–5 repeats) *vs* the long (6–8 repeats) classification scheme.[3,4] Almost no differences were observed between results using the two methods. Principal analyses of the effect of the serotonin promoter region polymorphism were performed after grouping the genotypes according to S/S and S/L *vs* L/L, ie assuming a dominant effect of the short allele as originally demonstrated by Lesch and his collaborators.[5]

Acknowledgements

This research was supported by The Israel Science Foundation founded by the Israel Academy of Sciences and Humanities (RPE and MK) and by grant No. 95–58 of the United States–Israel Binational Science Foundation (BSF), Jerusalem, Israel (JB).

References

1 Heath AC, Cloninger CR, Martin NG. Testing a model for the genetic structure of personality: a comparison of the personality systems of Cloninger and Eysenck. *J Pers Soc Psychol* 1994; **66**: 762–775.
2 Bergeman CS *et al*. Genetic and environmental effects on openness to experience, agreeableness, and conscientiousness: an adoption/twin study. *J Pers* 1993; **61**: 159–179.
3 Benjamin J *et al*. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat Genet* 1996; **12**: 81–84.
4 Ebstein RP *et al*. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nat Genet* 1996; **12**: 78–80.
5 Lesch KP *et al*. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527–1531.
6 Ebstein RP *et al*. 5-HT2C (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. *Am J Med Genet* 1997; **74**: 65–72.
7 Auerbach J *et al*. Dopamine D4 receptor (D4DR) and serotonin transporter promoter (5-HTTLPR) polymorphisms in the determination of temperament in 2-month-old infants. *Mol Psychiatry* 1999; **4**: 369–373.
8 Ebstein RP *et al*. Dopamine D4 receptor and serotonin transporter promoter in the determination of neonatal temperament. *Mol Psychiatry* 1998; **3**: 238–246.
9 Comings DE. Polygenic inheritance and micro/minisatellites. *Mol Psychiatry* 1998; **3**: 21–31.
10 Cloninger CR *et al*. Anxiety proneness linked to epistatic loci in genome scan of human personality traits. *Am J Med Genet* 1998; **81**: 313–317.
11 Blum K *et al*. Association of polymorphisms of dopamine D2 receptor (DRD2), and dopamine transporter (DAT1) genes with

schizoid/avoidant behaviors (SAB). *Mol Psychiatry* 1997; **2**: 239–246.
12 Lachman HM *et al*. Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996; **6**: 243–250.
13 Lachman HM, Nolan KA, Mohr P, Saito T, Volavka J. Association between catechol O-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. *Am J Psychiatry* 1998; **155**: 835–837.
14 Strous RD, Bark N, Parsia SS, Volavka J, Lachman HM. Analysis of a functional catechol-O-methyltransferase gene polymorphism in schizophrenia: evidence for association with aggressive and antisocial behavior. *Psychiatry Res* 1997; **69**: 71–77.
15 Kotler M *et al*. Homicidal behavior in schizophrenia associated with a genetic polymorphism determining low catechol O-methyltransferase (COMT) activity. *Am J Med Genet (Neuropsych Genet*) 1999; (in press).
16 Benjamin J *et al*. An interaction between the catechol O-methyltransferase (COMT) and serotonin promoter region (5-HTTLPR) polymorphisms contributes to Tridimensional Personality Questionnaire (TPQ) Persistence scores in normal subjects. *Neuropsychobiology* (in press).
17 Rothbart MK. Measurement of temperament in infancy. *Child Develop* 1981; **52**: 569–578.
18 Ebstein RP *et al*. No association between the serotonin transporter gene regulatory region polymorphism and the Tridimensional Personality Questionnaire (TPQ) temperament of harm avoidance. *Mol Psychiatry* 1997; **2**: 224–226.
19 Osher Y, Hamer D, Benjamin J. Association and linkage of anxiety-related traits with a functional polymorphism of the serotonin transporter gene regulatory region in Israeli sibling pairs. *Mol Psychiatry* (in press).
20 Netter P, Hennig J, Roed IS. Serotonin and dopamine as mediators of sensation seeking behavior. *Neuropsychobiology* 1996; **34**: 155–165.
21 Puumala T *et al*. Behavioral and pharmacological studies on the validation of a new animal model for attention deficit hyperactivity disorder. *Neurobiol Learn Mem* 1996; **66**: 198–211.
22 Eisenberg J *et al*. A haplotype relative risk study of catechol-O-methyltransferase (COMT) and attention deficit hyperactivity disorder (ADHD): association of the high-enzyme activity val allele with ADHD impulsive-hyperactive phenotype. *Am J Med Genet (Neuropsych Genet)* 1999; (in press).

Correspondence: Professor RP Ebstein, Research Laboratory, S Herzog Memorial Hospital, PO Box 35300, Jerusalem 91351, Israel. E-mail: ebstein@netmedia.net.il
Received 13 May 1999; revised and accepted 7 July 1999