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Brief report

Analysis of catechol-*O*-methyltransferase and 5-hydroxytryptamine transporter polymorphisms in patients at risk for suicide

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Abstract

Genotype frequencies of functional polymorphisms in the genes encoding the serotonin transporter (5-HTT) and the enzyme catechol-*O*-methyltransferase (COMT) were not different in 51 suicidal inpatients compared to 51 control subjects. Within the patient group, increased hopelessness and suicide ideation were associated with homozygosity of the 5-HTT high promotor activity allele.

Keywords: Hopelessness; Serotonin; Major depression

1. Introduction

Suicidal behavior appears to have a genetic component (Mann et al., 1999a; Roy et al., 1999). The 5-hydroxytryptamine (5-HT) transporter (5-HTT, SERT, SLC6A4) may be a candidate gene for suicidal behavior based upon various lines of evidence linking serotonin and suicide (Åsberg et al., 1986), and, more specifically, serotonin uptake binding sites in platelets and brain to affective illness and suicide (Roy et al., 1999). The 5-HTT gene has two common alleles which differ in length by 44 base pairs and transcriptional activity; the long allele, *l*, has higher transcriptional activity than the short allele, *s* (Heils et al., 1996). The *s* allele has been associated with affective illness in some studies (Collier et al., 1996; Fur-

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long et al., 1998; Gutiérrez et al., 1998), but not in others (Kunugi et al., 1997; Ohara et al., 1998). Ohara et al. (1998) reported that number of suicide attempts was not related to allele frequency. Du et al. (1999), however, recently found that the *l* allele was more common in 24 depressed suicide victims compared to normal control subjects.

Another gene, catechol-*O*-methyltransferase (COMT), which is involved in catecholamine degradation, is of potential interest because of data linking catecholamines to suicidal behavior (Castrogiovanni et al., 1994; Ordway et al., 1994; Klinteberg et al., 1987; von Knorring et al., 1987). COMT enzyme activity is governed by a common genetic polymorphism at codon 158 (Weinshilboum and Raymond, 1977; Aksoy et al., 1993; Lotta et al., 1995; Lachman et al., 1996). An allele encoding a valine residue at amino acid 158 is associated with relatively high activity (*H* allele), whereas the allele encoding a methionine residue is associated with relatively low activity (*L* allele). Although there is no evidence for an association between this polymorphism and the core diagnosis in patients with either bipolar disorder or schizophrenia (Daniels et al., 1996; Biomed European Bipolar Collaborative Group, 1997; Lachman et al., 1997; Strous et al., 1997a; Gutiérrez et al., 1998), it appears that COMT may modify the clinical phenotype of these conditions. Schizophrenic patients homozygous for the *L* allele, for example, show an increased propensity for violence (Strous et al., 1997b; Lachman et al., 1998).

Given these findings, as well as the data relating aggressive/impulsive behavior to suicide risk (Mann et al., 1999b), we tested the hypothesis that the functional polymorphisms in the COMT and 5-HTT genes may be associated with suicidal ideation and/or behavior.

2. Methods

2.1. Subjects

Fifty-one patients (32 female, 19 male; mean age ± S.D., 38 ± 15; 41 Caucasian, 6 African-American, 2 Hispanic, 1 Indian, 1 Asian) participated in this IRB-approved study. Subjects consisted of newly hospitalized patients who were considered to be at high risk of suicide (principal reason for admission) based on the clinical assessment by the admitting physician. Patients were diagnosed by the admitting physician using DSM-IV criteria (DSM-IV, American Psychiatric Association, 1994). Axis I diagnoses included major depression without psychosis ($n = 22$), major depression with psychosis ($n = 12$), bipolar disorder, depressed, without psychosis ($n = 9$), and bipolar disorder, depressed, with psychosis ($n = 3$), schizoaffective disorder ($n = 4$), schizophrenia ($n = 3$), anxiety disorder ($n = 7$), substance abuse ($n = 7$), and post-traumatic stress disorder ($n = 3$). Twenty-six patients (50%) had more than one Axis I diagnosis. Of the patients 84% had a history of making at least one suicide attempt.

Control subjects were 51 individuals (28 females and 23 males; mean age ± S.D., 41 ± 13) matched for race from the same general geographic area as the clinical sample. These subjects were not formally screened for psychiatric disorders, but did deny a history of serious psychiatric illness.

2.2. Research design

Patients meeting criteria for participation completed interview and self-report instruments 24 h after admission to assess the severity of suicidal ideation [Scale for Suicide Ideation (SSI), Beck et al. (1979), mean ± S.D. = 17.8 ± 8.6], depression [Beck Depression Inventory (BDI), Beck and Steer (1987), mean ± S.D. = 32.4 ± 12.8], hopelessness [Hopelessness Scale (HS), Beck and Steer (1988), mean ± S.D. = 13.2 ± 5.6], and general psychopathology [Brief Psychiatric Rating Scale (BPRS), Overall and Gorham (1962), mean ± S.D. = 29.2 ± 7.5]. Patients were also interviewed to assess factors associated with suicide risk. Given the small sample size, we limited statistical analyses to factors most likely to have genetic contributions: family history of suicide (20 yes, 28 no, 3 unknown) and lifetime number of suicide attempts (mean ± S.D. = 2.5 ± 4.5).

2.3. COMT and 5-HTT genotype determinations

The codon 158 polymorphism was identified by a PCR-based restriction fragment length polymorphism analysis as previously described in detail (Lachman et al., 1996). The 5-HTT promoter polymorphism was detected by PCR using primers CCGCTCTGAATGCCAGCACCTAAC and AGAGGGACTGAGCTGGACAACCAC. The PCR reaction was carried out in a 20-μl volume containing approximately 100 ng of genomic DNA with the Expand Long Template PCR system (Boehringer Mannheim, Germany) in Expand buffer 1. After an initial denaturation step of 94°C for 2 min, the cycling parameters were 30 cycles consisting of 94°C for 15 s, 68°C for 2 min, and then a final extension at 68°C for 5 min. The PCR fragment was radio-labeled by including 5 μCi ^{32}P-dCTP in the reaction mix. The PCR product is a 479/523-bp fragment that was resolved by electrophoresis through a 4% non-denaturing acrylamice gel and visualized by autoradiography.

2.4. Data analysis

COMT and 5-HTT genotype frequencies were compared between groups using χ^2 analysis. Within the patient group, exploratory χ^2 analyses, *t*-tests, and one-way analyses of variance (ANOVA) were performed to examine possible associations between genotype and our clinical measures. We assumed a dominant-recessive effect for the 5-HTT gene and grouped the *ss* and *ls* genotypes for statistical analyses. This assumption is based on the work of Lesch et al. (1996) who found that mRNA concentrations, [^{125}I]RTI-55 binding, and [^{3}H]5-HT uptake are significantly higher in lymphoblast cell lines associated with the *ll* genotype compared to either the *ls* or *ss* genotypes. We also performed these analyses without assuming a dominant-recessive effect for the 5-HTT gene. Where appropriate, *P* values were adjusted using a Bonferroni correction. The genotypes of both control and patient groups were in Hardy–Weinberg equilibrium.

3. Results

There were no significant differences between patient and control groups with respect to COMT or 5-HTT genotype frequencies (Table 1). However, the genotype frequency for 5-HTT was significantly related to HS and SSI scores. The HS score for the *ll* genotype (16.6 ± 2.3) was significantly higher than for the grouped *ss* and *ls* genotypes (11.8 ± 5.9; $t(46) = 2.9$, $P = 0.005$, 95% CI = 1.5–8.1). Likewise, the SSI score for the *ll*

Table 1
Genotype frequencies for COMT and 5-HTT gene polymorphisms, patient vs. control groups[a]

COMT	Patients ($n = 49$)	Controls ($n = 49$)	χ^2	d.f.	*P*
LL	9	7	0.90	2	0.64
LH	28	26			
HH	12	16			
L frequency	0.47	0.41			
H frequency	0.53	0.59			
5-HTT	Patients ($n = 51$)	Controls ($n = 51$)			
ss	9	10	0.36	2	0.84
ls	28	25			
ll	14	16			
s frequency	0.45	0.44			
l frequency	0.55	0.56			

[a] *L*, COMT low enzyme activity allele; *H*, COMT high enzyme activity allele; *s*, low promotor activity allele; *l*, 5-HTT high promotor activity allele.

genotype (22.6 ± 7.1) was significantly higher than for the grouped *ss* and *ls* genotypes (16.0 ± 8.5; $t(28) = 2.7$, $P = 0.01$, 95% CI = 1.7–11.4).

Without assuming a dominant-recessive effect for the 5-HTT gene, there continued to be a significant group effect for the HS score (ANOVA, $F_{2,47} = 4.2$, $P = 0.021$). The HS score for the *ll* genotype was significantly higher compared to the *ls* genotype (11.7 ± 5.9, $P = 0.02$, 95% CI = 0.6–9.2), but not compared to the *ss* genotype (12.2 ± 6.5, $P = 0.20$, 95% CI = −1.4–10.1). In addition, significant group effects were observed for SSI ($F_{2,50} = 3.9$, $P = 0.03$) and BPRS scores ($F_{2,50} = 3.1$, $P = 0.05$). Mean scores for the *ll*, *ls*, and *ss* genotypes for the SSI were 22.6 ± 7.1, 15.2 ± 8.5, and 18.6 ± 8.6, respectively (*ll* vs. *ls*, $P = 0.03$, 95% CI = −0.7 to −14.0, other post hoc comparisons, minimum $P = 0.76$). Mean scores for the *ll*, *ls*, and *ss* genotypes for the BPRS were 30.4 ± 7.2, 27.1 ± 6.0, and 33.7 ± 10.2, respectively (*ss* vs. *ls*, $P = 0.065$, 95% CI = −13.4–0.3, other post hoc comparisons, minimum $P = 0.58$).

4. Discussion

Although suicidal patients did not differ from normal control subjects with respect to the frequency of functional polymorphisms in the COMT and 5-HTT genes, the observed relationship between the 5-HTT *ll* genotype and increased mean HS and SSI scores is of interest. Hopelessness is among the most consistently observed factors associated with long-term suicide risk in depressed patients (Beck et al., 1985, 1990; Fawcett et al., 1990). Moreover, hopelessness may be both a state and trait dimension (Beck et al., 1985, 1990). With respect to the latter, there is evidence that many patients suffer from chronic hopelessness even after other symptoms of depression have remitted, and that these patients are at particularly high risk of engaging in suicidal behavior (Rifai et al., 1994; Young et al., 1996; Szanto et al., 1998). Although our findings seem to be discrepant with the those of Furlong et al. (1998) whose meta-analysis revealed a significant association between the *s* allele and both unipolar and bipolar depression, it is possible that different genotypes may be associated with hopelessness and suicidal behavior on the one hand, and depressive illness on the other. The findings of Du et al. (1999) are consistent with this view. Alternatively, this discrepancy may be explained by sampling error.

Our results are consistent with the possibility that homozygosity for the *l* allele is related to diminished post-synaptic serotonergic activity and heightened suicide risk (Mann et al., 1999b) in a subgroup of patients with the highest hopelessness scores. This study must be viewed as preliminary, given its many limitations including small sample size (132 patients and 132 control subjects would be needed to achieve 80% power, $\alpha = 0.05$, OR = 2.0), lack of structured diagnostic assessments of patients and control subjects, and diagnostic heterogeneity in the patient sample. Nevertheless, our findings suggest that future studies exploring the role of the 5-HTT promoter polymorphism in suicide risk and hopelessness are warranted.

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References

American Psychiatric Association, 1994. Diagnostic and Statistical Manual of Mental Disorders, 4th ed. American Psychiatric Press, Washington, DC.

Aksoy, S., Kelner, J., Weinshilboum, R.M., 1993. Catechol-*O*-methyltransferase pharmacogenetics: photoaffinity labeling and western blot analysis of human liver samples. Pharmacogenetics 3, 116–122.

Åsberg, M., Nordström, P., Träskman-Bendz, L., 1986. Cerebrospinal fluid studies in suicide: an overview. Annals of the New York Academy of Sciences 487, 243–255.

Beck, A.T., Kovacs, M., Weissman, A., 1979. Assessment of suicidal intention: the Scale for Suicide Ideation. Journal of Consulting and Clinical Psychology 47, 343–352.

Beck, A.T., Steer, R.A., Kovacs, M., Garrison, B., 1985. Hopelessness and eventual suicide: a 10-year prospective study

of patients hospitalized with suicidal ideation. American Journal of Psychiatry 142, 559–563.

Beck, A.T., Steer, R.A., 1987. Manual for revised Beck Depression Inventory. Psychological Corporation. San Antonio, TX.

Beck, A.T., Steer, R.A., 1988. Manual for Beck Hopelessness Scale. Psychological Corporation. San Antonio, TX.

Beck, A.T., Brown, G., Berchick, R.J., Stewart, B.L., Steer, R.A., 1990. Relationship between hopelessness and ultimate suicide: a replication with psychiatric outpatients. American Journal of Psychiatry 147, 190–195.

Castrogiovanni, P., Capone, M.R., Maremmani, I., Marazziti, D., 1994. Platelet serotonergic markers and aggressive behaviour in healthy subjects. Neuropsychobiology 29, 105–107.

Collier, D.A., Stober, G., Li, T., Heils, A., Catalano, M., DiBella, D., Arranz, M.J., Murray, R.M., Vallada, H.P., Bengel, D., Muller, C.R., Roberts, G.W., Smeraldi, E., Kirov, G., Sham, P., Lesch, K.P., 1996. A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Molecular Psychiatry 1, 453–460.

Daniels, J.K., Williams, N.M., Williams, J., Jones, L.A., Cardno, A.G., Murphy, K.C., Spurlock, G., Riley, B., Scambler, P., Asherson, P., McGuffin, P., Owens, M.J., 1996. No evidence for allelic association between schizophrenia and a polymorphism determining high or low catechol-*O*-methyltransferase activity. American Journal of Psychiatry 153, 268–270.

Du, L., Faludi, G., Palkovits, M., Demeter, E., Bakish, D., Lapierre, Y.D., Sotonyi, P., Hrdina, P.D., 1999. Frequency of long allele in serotonin transporter gene is increased in depressed suicide victims. Biological Psychiatry 46, 196–201.

Fawcett, J., Scheftner, W.A., Fogg, L., Clark, D.C., Young, M.A., Hedeker, D., Gibbons, R., 1990. Time-related predictors of suicide in major affective disorder. American Journal of Psychiatry 147, 1189–1194.

Furlong, R.A., Ho, L., Walsh, C., Rubinsztein, J.S., Jain, S., Paykel, E.S., Easton, D.F., Rubinsztein, D.C., 1998. Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. American Journal of Medical Genetics 81, 58–63.

Gutiérrez, B., Pintor, L., Gasto, C., Rosa, A., Bertranpetit, J., Vieta, E., Fananas, L., 1998. Variability in the serotonin transporter gene and increased risk for major depression with melancholia. Human Genetics 103, 319–322.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation in human serotonin transporter gene expression. Journal of Neurochemistry 66, 2621–2624.

Klinteberg, B., Schalling, D., Edman, G., Oreland, L., Åsberg, M., 1987. Personality correlates of platelet monoamine oxidase (MAO) activity in female and male subjects. Neuropsychobiology 18, 89–96.

Kunugi, H., Hattori, M., Kato, T., Tatsumi, M., Sakai, T., Sasaki, T., Hirose, T., Nanko, S., 1997. Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Molecular Psychiatry 2, 457–462.

Lachman, H.L., Papolos, D., Saito, T., Yu, Y.M., Szumlanski, C.L., Weinshilboum, R.M., 1996. Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6, 243–250.

Lachman, H.M., Kelsoe, J., Moreno, L., Katz, S., Papolos, D.F., 1997. No evidence for linkage of COMT polymorphism in bipolar disorder. Psychiatric Genetics 7, 13–17.

Lachman, H.M., Nolan, K.A., Mohr, P., Saito, T., Volavka, J., 1998. Association between catechol-*O*-methyltransferase genotype and violence in schizophrenia and schizoaffective disorder. American Journal of Psychiatry 155, 835–837.

Lesch, K.-P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., Taskinen, J., 1995. Kinetics of human soluble and membrane-bound catechol *O*-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry 34, 4202–4210.

Mann, J.J., Waternaux, C., Haas, G.L., Malone, K.M., 1999a. Toward a clinical model of suicidal behavior in psychiatric patients. American Journal of Psychiatry 156, 181–189.

Mann, J.J., Oquendo, M., Underwood, M.D., Arango, V., 1999b. The neurobiology of suicide risk: a review for the clinician. Journal of Clinical Psychiatry 60 (2), 7–11.

Ohara, K., Nagai, M., Tsukamoto, T., Tani, K., Suzuki, Y., Ohara, K., 1998. Functional polymorphism in the serotonin transporter promotor at the SLC6A4 locus and mood disorders. Biological Psychiatry 44, 550–554.

Ordway, G.A., Smith, K.S., Haycock, J.W., 1994. Elevated tyrosine hydroxylase in the locus coeruleus of suicide victims. Journal of Neurochemistry 62, 680–685.

Overall, J.E., Gorham, D.R., 1962. The Brief Psychiatric Rating Scale. Psychological Reports 10, 799–812.

Rifai, A.H., George, C.J., Stack, J.A., Mann, J.J., Reynolds, C.F., 1994. Hopelessness in suicide attempters after acute treatment of major depression in late life. American Journal of Psychiatry 151, 1687–1690.

Roy, A., Nielsen, D., Rylander, G., Sarchiapone, M., Segal, N., 1999. Genetics of suicide in depression. Journal of Clinical Psychiatry 60 (2), 12–17.

Szanto, K., Reynolds, C.F., Conwell, Y., Begley, A.E., Houck, P., 1998. High levels of hopelessness persist in geriatric patients with remitted depression and a history of attempted suicide. Journal of the American Geriatric Society 46, 1401–1406.

Strous, R., Bark, N., Woerner, M., Parsia, S.S., Lachman, H.M., 1997a. Lack of association of COMT codon 158 polymorphism in schizophrenics. Biological Psychiatry 41, 493–495.

Strous, R.D., Bark, N., Parsia, S.S., Volavka, J., Lachman, H.M., 1997b. Analysis of a functional catechol-*O*-methyl-

transferase gene polymorphism in schizophrenia: evidence for association with aggressive and antisocial behavior. Psychiatry Research 69, 71–77.

von Knorring, L., Oreland, L., von Knorring, A.L., 1987. Personality traits and platelet MAO activity in alcohol and drug abusing teenage boys. Acta Psychiatrica Scandinavica 75, 307–314.

Young, M.A., Fogg, L.F., Scheftner, W., Fawcett, J., Akiskal, H., Maser, J., 1996. Stable trait components of hopelessness: baseline and sensitivity to depression. Journal of Abnormal Psychology 105, 155–165.

Weinshilboum, R.M., Raymond, F.A., 1977. Inheritance of low erythrocyte catechol-*O*-methyltransferase activity in man. American Journal of Human Genetics 29, 216–218.