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ORIGINAL RESEARCH ARTICLE

Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior

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A common 44-base pair insertion/deletion polymorphism in the promoter region of the human serotonin transporter (5-HTT) gene has been observed to be associated with affective illness and anxiety-related traits. This biallelic functional polymorphism, designated long (L) and short (S), affects 5-HTT gene expression since the S promoter is less active than the L promoter. Since there is strong evidence of a disturbance in brain serotonergic transmission among antisocial, impulsive, and violent type 2 alcoholic subjects, we decided to test the hypothesis that the frequency of the S allele, which is associated with reduced 5-HTT gene expression, is higher among habitually violent type 2 alcoholics when compared with race and gender-matched healthy controls and non-violent late-onset (type 1) alcoholics. The 5-HTT promoter genotype was determined by a PCR-based method in 114 late onset (type 1) non-violent alcoholics, 51 impulsive violent recidivistic offenders with early onset alcoholism (type 2), and 54 healthy controls. All index subjects and controls were white Caucasian males of Finnish origin. The S allele frequency was higher among type 2 alcoholics compared with type 1 alcoholics ($\chi^2 = 4.86$, $P = 0.028$) and healthy controls ($\chi^2 = 8.24$, $P = 0.004$). The odds ratio for SS genotype *vs* LL genotype was 3.90, 95% CI 1.37–11.11, $P = 0.011$ when type 2 alcoholics were compared with healthy controls. The results suggest that the 5-HTT 'S' promoter polymorphism is associated with an increased risk for early onset alcoholism associated with antisocial personality disorder and impulsive, habitually violent behavior.

The human serotonin (5-HT) transporter (5-HTT or SERT) is believed to be essential in regulating the magnitude and duration of serotonergic responses.[1] The human 5-HTT is coded by a single gene (SLC6A4) on chromosome 17q12.[1–3] A variable number tandem repeat (VNTR) polymorphism of unknown function is found in an intron and a variable 44-base insertion/deletion polymorphism is found in the promoter region (termed 5-HTTLPR).[4–6] The VNTR polymorphism has been reported to be associated with affective illness,[5,7,8] though other studies have failed to replicate the findings.[9–11] The 5-HTTLPR polymorphism appears to influence gene function by affecting transcription efficiency. Transfection assays show that the long (L) promoter polymorphism is associated with a two-fold increase in activity when compared with the short (S) polymorphism.[1,4] The S allele has been reported to be associated with affective illness[12] and anxiety-related traits[1,13] though, again, other studies have failed to find an association.[8,10,14,15]

Alcoholism has been classified into two subgroups, type 1 and 2.[16] The majority of alcoholics can be classified as type 1 which is characterized by late onset (over 25 years) and no prominent antisocial behavior. Type 2 alcoholism is associated with early onset (under 25 years), high novelty seeking and impulsive,—also often violent—antisocial behavior.[16] Type 2 alcoholism has been postulated to share the same genetic etiology with antisocial personality (ASP) disorder.[17] *In vivo* brain imaging studies in humans have indicated disturbed dopaminergic neurotransmission in type 1 alcoholics but not in type 2 alcoholics.[18,19] Instead, type 2 alcoholism has been suggested to be associated with serotonergic deficits on the basis of brain imaging studies.[20,21] Low concentrations of cerebrospinal fluid 5-hydroxyindolacetic acid (CSF 5-HIAA) in impulsive violent offenders have also been described in several studies.[22–27] Results by Coccaro *et al* imply that low platelet 5-HTT site density, which may reflect reduction in central 5-HT neurotransmission, correlates strongly with aggressive behavior in subjects with personality disorder.[28]

It can be concluded from these studies that: (1) the 5-HTTLPR polymorphism alters 5-HTT expression and 5-HT uptake contributing to variance in brain serotonergic neurotransmission (the 'S' allele being associated with lower uptake activity); and (2) there is evidence that brain serotonergic transmission is disturbed in antisocial, impulsive, and violent type 2 alcoholic subjects. Consequently, it is possible that the 5-HTTLPR polymorphism may contribute to the type 2 alcoholism phenotype. To test this hypothesis, we genotyped the 5-HTTLPR in habitually violent type 2 alcoholics and compared it with race and gender-matched healthy controls and non-violent late-onset (type 1) alcoholics.

The 5-HTTLPR genotype and allele frequencies are shown in Table 1. The genotype distributions were in Hardy–Weinberg equilibrium among type 2 alcoholics

Table 1 5-HTTLPR genotypes and allele frequencies

	Genotype			*Allele*	
	LL	*LS*	*SS*	*L*	*S*
Type 1 ($n = 114$)	50 (0.44)	37 (0.32)	27 (0.24)	0.60	0.40
Type 2 ($n = 51$)	15 (0.30)	18 (0.35)	18 (0.35)	0.47	0.53
Healthy controls ($n = 54$)	26 (0.48)	20 (0.37)	8 (0.15)	0.67	0.33

The table shows the serotonin transporter promoter genotype and allele frequencies among type 1 and type 2 alcoholic subjects and healthy controls (L = Long, S = short allele of 44-bp insertion-deletion polymorphism).

and controls, but not among type 1 alcoholics ($\chi^2 = 11.9$, 1 d.f., $P < 0.001$). The S allele frequency was higher among type 2 alcoholics when compared with healthy controls ($\chi^2 = 8.24$, $P = 0.004$) and type 1 alcoholics ($\chi^2 = 4.86$, $P = 0.028$). Since genotype distribution among type 1 alcoholics was not in Hardy–Weinberg equilibrium, the difference in allele frequency between type 2 and type 1 alcoholics was also studied with the more conservative Cochran–Armitage trend test ($P = 0.055$). The OR for being a type 2 alcoholic compared with controls, and having the SS genotype, as opposed to the LL genotype, was 3.90, 95% Cl 1.37–11.11, $P = 0.011$. Adjustment for age in the logistic regression analysis only slightly attenuated the relationship (OR 3.59). For homozygous SS genotype *vs* LS and LL genotypes, OR was 3.14, 95% CI 1.12–9.02, $P = 0.015$. There were no statistically significant differences in genotype or allele distributions when type 1 alcoholics were compared with healthy controls.

These results confirm our hypothesis that markedly increased risk for type 2 alcoholism, comorbid with ASP disorder and impulsive, habitually violent behavior, is associated with the 5-HTTLPR 'S' allele. The homogyzous SS subjects seemed to be most vulnerable to this type of behavioral pattern (OR 3.90 when compared with race and gender-matched healthy controls). The results are congruent with previous findings which show a reduction in central 5-HT transmission in impulsive-aggressive patients.[25,29,30] The results are also similar to the findings of Sander *et al*, who found an association between the 5-HTTLPR and severe alcoholism.[31] However, Jorm *et al*, did not find any association between 5-HTTLPR and personality traits and alcohol abuse.[32]

All offenders in our sample had early onset alcoholism and, therefore, it is not possible to conclude explicitly if the S allele is associated with impulsive violent behavior *per se* or with ASP disorder and type 2 alcoholism. However, the results indicate that the S allele is not associated with late onset (type 1) alcoholism. Our sample consisted of males, and therefore, the findings may not be generalizable to females.

Exactly how the 5-HTT 'SS' genotype alters 5-HT transmission in type 2 alcoholics is unclear. A reduction in 5-HT uptake could increase synaptic 5-HT and induce desensitization of various postsynaptic 5-HT receptors.[28,33,34] Alternatively an increase in synaptic 5-HT could stimulate presynaptic 5-HT autoreceptor activity which would lead to a decrease in subsequent 5-HT release. Work on 5-HTT knockout mice would be helpful in elucidating the effects of reduced 5-HTT expression on 5-HT transmission.

On the basis of previous studies,[35,36] it can be estimated that about 70% of homicide offences in Finland (and probably other severe habitual violence) is committed by subjects with ASP disorder and alcoholism. Our results suggest that the 5-HTTLPR polymorphism contributes significantly to severe habitual violent offending in Finland. Our findings also further strengthen the hypothesis of serotonergic dysfunction in type 2 alcoholism.

Patients and methods

Study subjects

The study protocol was approved by the Ethical Committees of University of Kuopio/University Hospital and University of Turku/University Hospital. The type 1 alcoholic population (total $n = 114$) consisted of two independent Finnish samples of male alcoholics from the regions of Turku ($n = 65$) and Kuopio ($n = 49$). The sample in Turku consisted of alcoholics entering a detoxification program in that area, and patients in Kuopio were recruited with the help of the local rehabilitation center for alcoholics, where they had obtained treatment for their alcoholism. The ages (mean ± SD) of these subjects were 43.8 ± 8.8 years. Inclusion criteria were serious alcohol-related problems (alcohol abuse or dependence resulting in a failure to fulfill obligations at work or in recurrent social problems) starting after the age of 25 years (the onset of alcohol-related problems was determined within one-year accuracy on the basis of the subjective anamnestic data). All type 1 patients fulfilled the DSM-III-R criteria for alcohol abuse or dependence, and underwent a clinical examination and self-administered Michigan Alcoholism Screening Test (MAST[37]). Exclusion criteria were major mental disorders such as schizophrenia, schizophreniform and schizoaffective disorders, mood disorders with psychotic features, organic mental syndromes and disorders, and paranoid and other psychoses (screened with Hopkins Symptoms Checklist 90 and a clinical interview by a medical doctor), history of violent or severe antisocial behavior or severe somatic disorder.

The type 2 alcoholics ($n = 51$) had been committed for forensic psychiatric examination in a state mental hospital (Niuvanniemi Hospital, Kuopio, Finland) after committing an impulsive violent offence (homicide, attempted homicide, aggravated violent assault, assault, sexual offence or arson). In Finland, most of

those persons with serious violent offences are committed for forensic psychiatric evaluation (eg 70% of all homicide offenders are evaluated[38]), and most of those offenders considered as very violent or dangerous are evaluated in Niuvanniemi Hospital regardless their home-site. Therefore, the offenders included in this study are representative of habitually violent offenders in the Finnish male population. On the basis of anamnestic data, all of them were recidivistic offenders, although four of them had not been convicted in court before. Thirteen (25%) of them had committed at least two homicides or attempted homicides, and 21 (41%) had committed at least one attempted homicide and other aggravated violent assaults. The age (mean ± SD) of these subjects is 30.1 ± 8.4 years. All type 2 alcoholics were subjected to an extensive forensic psychiatric examination including a psychiatric evaluation, standardized psychological tests, evaluation of physical condition with laboratory tests, electroencephalography (EEG), magnetic resonance imaging (MRI), and staff observation in a security ward for 4–8 weeks. Inclusion criteria were serious alcohol-related problems (abuse or dependence had resulted in recurrent social problems and recurrent substance-related legal problems fulfilling DSM-III-R criteria for alcohol abuse or dependence) before the age of 25 years (the onset of alcohol-related problems was determined within one-year accuracy on the basis of objective anamnestic data gathered during the forensic psychiatric evaluation), and the comorbid diagnosis of ASP disorder fulfilling DSM-IV criteria. Exclusion criteria were major mental disorders such as schizophrenia, schizophreniform and schizoaffective disorders, mood disorders with psychotic features, organic mental syndromes and disorders, and paranoid and other psychoses, severe somatic disorder, or non-Finnish ethnic origin. The 5-HTTLPR polymorphism was studied among type 1 and type 2 alcoholic populations by comparing the genotype and allele frequencies between both groups and 54 unrelated healthy controls from Turku and Kuopio regions (males aged mean ± SD 44.1 ± 7.9 years).

The controls were recruited from two industrial corporations in two cities in Southern Finland via occupational health services. These volunteers had no major mental disorders according to interviews and medical records. The controls were carefully screened for alcohol abuse/dependence with clinical interview, MAST (cut-off score five points) and laboratory tests (serum ASAT, ALAT, gamma-GT). Ten subjects were excluded because of their excessive alcohol use. All index and healthy control subjects were white Caucasian males of Finnish origin. The subjects in each group were considered to be representative of their phenotype, and no further matching or adjusting was done in addition to race, gender and age.

Genotype analysis

The 5-HTT promoter polymorphism was detected by PCR using primers TGAATGCCAGCAGCAGCACC TAACCC and TTCTGGTGCCACCTAGACGC. The PCR reaction was carried out in a 20-μl volume containing approximately 100 ng of genomic DNA and Deep Vent polymerase (New England Biolabs, Beverly, MA, USA). After an initial denaturation step of 96°C for 2 min, the cycling parameters were 40 cycles consisting of 96°C for 30 s, 61°C for 1 min, and 71°C for 1 min. The PCR fragment was radiolabelled by including 5 μCi^{32} P-dCTP in the reaction mix. In order to facilitate complete melting of this GC-rich region of the genome, 7-deazaguaine (New England Biolabs) to a final concentration of 0.5 mM was added. The PCR product is a 406/450 base pair fragment that was resolved by electrophoresis through a 4% non-denaturing acrylamide gel and visualized by autoradiography.

Statistical analysis

Allele and genotype distributions were analyzed by 2×2 contingency tables χ^2 test. Odds ratios (OR) and 95% confidence intervals (Cl) were calculated as described.[39] The effect of age was studied by using logistic regression analysis.

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