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ORIGINAL RESEARCH ARTICLE

Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism

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Catechol-O-methyltransferase (COMT) is an enzyme which has a crucial role in the metabolism of dopamine. It has been suggested that a common functional genetic polymorphism in the COMT gene, which results in 3 to 4-fold difference in COMT enzyme activity,[1,2] may contribute to the etiology of mental disorders such as bipolar disorder and alcoholism.[1] Since ethanol-induced euphoria is associated with the rapid release of dopamine in limbic areas, it is conceivable that subjects who inherit the allele encoding the low activity COMT variant would have a relatively low dopamine inactivation rate, and therefore would be more vulnerable to the development of ethanol dependence. The aim of this study was to test this hypothesis among type 1 (late-onset) alcoholics. The COMT polymorphism was determined in two independent male late onset (type 1) alcoholic populations in Turku (*n* = 67) and Kuopio (*n* = 56). The high (H) and low (L) activity COMT genotype and allele frequencies were compared with previously published data from 3140 Finnish blood donors (general population) and 267 race- and gender-matched controls. The frequency of low activity allele (L) was markedly higher among the patients both in Turku (*P* = 0.023) and in Kuopio (*P* = 0.005) when compared with the general population. When all patients were compared with the general population (blood donors), the difference was even more significant (*P* = 0.0004). When genotypes of all alcoholics (*n* = 123) were compared with genotypes of matched controls, the odds ratio (OR) for alcoholism for those subjects having the LL genotype *vs* those with HH genotype was 2.51, 95% CI 1.22–5.19, *P* = 0.006. Also, L allele frequency was significantly higher among alcoholics when compared with controls (*P* = 0.009). The estimate for population etiological (attributable) fraction for the LL genotype in alcoholism was 13.3% (95% CI 2.3–25.7%). The results indicate that the COMT polymorphism contributes significantly to the development of late-onset alcoholism.

Catechol-O-methyltransferase (COMT) is an enzyme which has a crucial role in the metabolism of dopamine. A common functional polymorphism in the COMT gene is responsible for enzyme activity variability found in the general population. It has been suggested that this genetic polymorphism may contribute to the etiology of mental disorders such as schizophrenia, obsessive compulsive disorder (OCD) and alcoholism.[1] There is solid evidence that a G → A transition at COMT codon 158 is associated with three- to four-fold variation in COMT enzyme activity in human hepatic tissue and red blood cells.[1,2] Homozygosity for the high activity allele (HH genotype) is found in approximately 25% of Caucasians. Homozygosity for the low activity allele (LL genotype) is also found in approximately 25% of Caucasians. Heterozygotes (LH genotype) have intermediate levels of COMT activity.[1,2] Thus far empirical evidence for an association between the COMT L allele and mental disorders has been reported in patients with velo-cardio-facial syndrome (VCFS),[3] rapid cycling bipolar disorder,[4,5] schizophrenia and schizoaffective disorder with increased violent behavior,[6,7] and in patients with OCD.[8] On the other hand, some studies have failed to show any association between COMT polymorphism and schizophrenia or affective disorders.[9–12] In addition, Li *et al*[13] reported an association between COMT H allele and schizophrenia rather than transmission of the L allele.

Alcoholism has been classified into two subgroups, type 1 and type 2.[14] Type 2 alcoholism is associated with early onset, high novelty seeking and impulsive antisocial behavior. The majority of alcoholics can be classified as type 1, which is characterized by late onset (over 25 years) and no prominent antisocial behavior.[14] *In vivo* brain imaging studies in humans have indicated that a dysfunction in dopaminergic neurotransmission occurs in type 1 alcoholics[15–18] but not in type 2 alcoholics.[17] Since ethanol-induced euphoria is associated with the rapid release of dopamine in limbic areas,[19] it is logical to assume that those subjects having the low activity COMT allele—resulting in low dopamine inactivation rate—would be more vulnerable to the development of ethanol dependence than those having the high activity COMT allele.

The COMT genotype and allele frequencies are shown in Table 1. The COMT genotypic distributions were in Hardy–Weinberg equilibrium in both patients and controls. The L allele frequency was higher among the patients both in Turku (χ^2 = 5.21, P = 0.023) and in Kuopio (χ^2 = 8.03, P = 0.005) when compared with

Table 1 COMT genotype and allele frequencies

	Genotype			*Allele*	
	LL	*LH*	*HH*	*L*	*H*
Cases (*n* = 123)					
Turku (*n* = 67)	22 (0.33)	35 (0.52)	10 (0.15)	0.59	0.41
Kuopio (*n* = 56)	20 (0.36)	30 (0.54)	6 (0.11)	0.63	0.38
Controls					
General population[a] (*n* = 3140)	0.24	0.50	0.26	0.49	0.51
Matched controls (*n* = 267)	67 (0.25)	136 (0.51)	64 (0.24)	0.51	0.49

[a]Data obtained from the study by Syvänen *et al*.[2]

the general population. When the pooled data from all patients were compared with the data from the general population (blood donors), the difference was highly statistically significant (χ^2 = 12.7, P = 0.0004). When genotypes of all alcoholics (n = 123) were compared with genotypes of matched controls, the odds ratio (OR) for alcoholism for those subjects having the LL genotype *vs* those with the HH genotype was 2.51, 95% CI 1.22–5.19, P = 0.006. The age-adjustment in the logistic regression analysis had only a small effect on the odds ratio (OR for LL *vs* HH genotype 2.79, 95% CI 1.23–6.35, P = 0.015). Also, the L allele frequency was significantly higher among alcoholics when compared with controls (χ^2 = 6.78, P = 0.009). The odds ratio (OR) for alcoholism for LL genotype *vs* LH or HH genotype combined was 1.55 (95% CI 0.95–2.53, χ^2 = 3.43, P = 0.064). Since the controls were from the same area as alcoholics from the Kuopio sample, the allele and genotype frequencies were also compared between these two populations (Kuopio alcoholics *vs* controls) The L allele frequency was higher among Kuopio alcoholics (χ^2 = 5.29, P = 0.021) and OR for LL *vs* HH genotype was 3.18 (95% CI 1.11–9.53, χ^2 = 5.83, P = 0.016). The population etiological (attributable) fraction for the LL genotype in alcoholism was 13.3% (95% CI 2.3–25.7%). This was calculated by using OR obtained for LL *vs* LH plus HH genotypes in the comparison of alcoholics and the general population (OR 1.64). The respective estimate based on OR from the comparison with the matched controls from the Kuopio area was 12%.

The results suggest that the low activity COMT allele plays a role in the development of type 1 alcoholism, and that the LL genotype is associated with an increased risk for alcoholism. The results are consistent with data obtained from animal studies which show that reward processes are mediated by dopaminergic pathways that project from the ventral tegmental area to the nucleus accumbens and frontal cortex,[20] and that addictive drugs, such as cocaine, amphetamine, opioids and ethanol increase dopaminergic transmission in the brain.[19,21–23] The results are consistent with the hypothesis that ethanol induces longer-lasting (and more effective) dopamine release in the brains of those subjects with low activity (LL) COMT genotype, which makes them more vulnerable to the development of alcoholism.

The observed allele frequency in the general Finnish population (blood donors) is similar to previous studies which have reported equal or even slightly higher H allele (than L allele) frequency among Caucasian populations.[3,8–10] This suggests that the observed differences between our index populations and our general population are not caused by biased (too low) frequency of the H allele among the blood donors. However, since no exact data were available about the gender and age distribution of the blood donors, we also compared the genotype frequencies with race- and gender-matched controls and also adjusted the effect of age with logistic regression analysis. The differences remained almost identical. Because this study was based on a case-control design, there may still remain some hidden population stratification which we could not exclude with the adjustment of race, age and gender.

It is quite probable that low activity COMT genotype is not a specific risk factor for type 1 alcoholism, but, rather, contributes to the risk to a number of mental disorders including OCD,[8] violence in schizophrenics,[6,7] and ultra rapid cycling bipolar disorder.[4,5] This is quite logical, since, in addition to the reward process, the dopaminergic neurotransmission has a role in the regulation of aggressive behavior[24] and motor impulse control.[25] Since type 1 alcoholism is associated with dopaminergic deficits, but type 2 with serotonergic deficits,[26] it is also quite possible that the COMT polymorphism does not have any significant role in the development of type 2 alcoholism. In conclusion, the results indicate that the COMT low activity allele significantly contributes to the development of late-onset alcoholism, though we cannot totally exclude the possibility that the examined polymorphism is in linkage disequilibrium with some unknown polymorphism, which contributes to the vulnerability to type 1 alcoholism.

Methods

Study subjects

The study protocol was approved by the Ethical Committees of Kuopio University/Kuopio University Hos-

pital and Turku University/Turku University Hospital. The index populations consisted of two independent Finnish samples of male late-onset (type 1) alcoholics from the region of Turku in south-western Finland (n = 67) and the region of Kuopio in eastern Finland (n = 56). The ages (mean ± SD) of alcoholic subjects were 42.1 ± 8.5 years in Turku and 47.3 ± 8.5 years in Kuopio. Inclusion criteria were serious alcohol-related problems (severe abuse or dependence) starting after the age of 25 years. All patients underwent a clinical examination and self-administered Michigan Alcoholism Screening Test (MAST).[27] Exclusion criteria were major mental disorders (screened with Hopkins Symptom Checklist 90 and clinical interview by medical doctor), history of violent or severe antisocial behavior or severe somatic disorder. The COMT polymorphism was studied among alcoholic populations in Turku and Kuopio by comparing the genotype and allele frequencies with previously published data from 3140 blood donors[2] (general population) and 267 race- and gender-matched unrelated controls (mean age ± SD 54.6 ± 6.92) (from the Kuopio Ischemic Heart Disease Risk Factor study[28]) reporting low or moderate alcohol use (self-report average alcohol intake 1–7 drinks/weeks and MCV $<$ 101 FI, GGT $<$ 80 u L^{-1}). The Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) is a population-based study undertaken to investigate previously unestablished risk factors for acute myocardial infarction, extracoronary atherosclerosis and related outcomes among men in eastern Finland. The study population is a random sample of men living in the town of Kuopio or neighboring rural communities, stratified and balanced according to age, who were 42, 48, 54, or 60 years old at the base-line examination. The base-line study was carried out between March 1984 and December 1989. Of 3235 eligible men, 2682 (83%) participated.[28,29] All index and control subjects were white Caucasian subjects of Finnish origin.

Statistical analysis

Genotype and allele distributions were analysed by Chi-square test. Odds ratios and 95% confidence intervals were calculated as described[30] as well as the etiological (attributable) fraction.[31] The association was adjusted for age by using a logistic regression analysis.

Genotype analysis

COMT genotypes were determined by restriction fragment length polymorphism (RFLP) analysis from DNA extracted from the subjects' peripheral blood by an investigator unaware of phenotype, as described in Reference 1. Briefly, the polymorphism is generated by the presence of a G or A encoding a valine or methionine at codon 158. A 210-base pair ^{32}P-radiolabelled PCR product was generated using the primers 5′-CTCATCACCATCGAGATCAA and 5′-GATGACCCTGGTGATAGTGG (nucleotides 1881–1900 and 2071–2090 in GenBank accession number z26491).[32] The PCR product (10 μl) was treated with 5 units of *Nla* III for 3 h at 37°C and the separated by electrophoresis uing 8% non-denaturing polyacrylamide gels.[1]

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