Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Catechol-*O*-methyltransferase (*COMT*) gene variants and pain in chronic pancreatitis

A.A.J. van Esch*, E. de Vries, R.H.M. te Morsche, M.G.H. van Oijen, J.B.M.J. Jansen, J.P.H. Drenth

[1]Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24-36 14 760, fax: +31 (0)24-35 40 103, e-mail: A.vanEsch@MDL.umcn.nl

ABSTRACT

Background: Pain is the major symptom of chronic pancreatitis. The role of genetics in pancreatic pain is unclear. Catechol-*O*-methyltransferase (COMT) regulates enkephalin levels and influences pain perception. The *COMT* gene contains functional polymorphisms that have been found to influence human pain perception. The aim of our study was to investigate *COMT* single-nucleotide polymorphisms (SNPs) and diplotypes in chronic pancreatitis patients and healthy controls.

Methods: We genotyped four *COMT* gene SNPs: *c.1-98A>G* (rs6269), *c.186C>T (p.=)* (rs4633), *c.408C>G (p.=)* (rs4818) and *c.472G>A (p.Val158Met)* (rs4680) using a dual-colour discrimination assay in 240 chronic pancreatitis patients and 445 controls. We generated five diplotypes with a frequency >0.5% and compared prevalence between patients and controls.

Results: There was no significant association between the SNPs in the *COMT* gene and chronic pancreatitis. The diplotype ATCA/ACCG was more prevalent in controls compared with patients (OR 0.48, 95% CI 0.24 to 0.93, p=0.03) where the most common diplotype GCGG/ATCA served as reference. However, after correction for multiple testing, this is not a significant difference. The distribution of other diplotypes was not significantly different between patients and controls.

Conclusion: *COMT* SNPs and diplotypes are not associated with chronic pancreatitis. As a consequence, our results do not support a significant role for the *COMT* gene in chronic pancreatitis.

KEYWORDS

Chronic pancreatitis, polymorphism, COMT, pain

INTRODUCTION

Chronic abdominal pain is the major presenting symptom of chronic pancreatitis and the majority of patients will have pain at a given time during the course of their disease. A large majority of patients with chronic pancreatitis presented with pain in a survey of the Asia-Pacific region, varying from 60% in Japan, to 90% in Australia, South Korea and South India and 100% in Singapore.[1] The inter- and intra-individual variation of pain in chronic pancreatitis is high, with pain duration varying from intermittent to persistent and pain intensity ranging from mild to disabling.[2] The inter-individual differences in the response to pain suggest that genetic factors can be involved in its modulation.[3,4]

Recently, several studies have investigated the association between the catechol-*O*-methyltransferase (*COMT*) gene and pain sensitivity.[5-13] In some studies, there was a positive association between *COMT* gene SNPs and pain.[5,10-13] This was not confirmed by other studies.[6-8] Other studies have focused on the association between COMT and the efficacy of pain therapy, such as morphine.[14,15] The COMT enzyme metabolises catecholamines, thereby acting as a key modulator of dopaminergic and adrenergic/noradrenergic neurotransmission.[16,17] Low activity of COMT is associated with activation of dopaminergic neurons, a reduction in the neuronal content of enkephalin and an increase in the regional concentration of μ-opioid system receptors. The μ-opioid system is activated in response to stressors, pain and other salient environmental stimuli, typically reducing pain and stress responses.[9,18] COMT inhibition results in increased pain sensitivity via a $\beta_{2/3}$-adrenergic mechanism.[19]

The *COMT* gene is located on the long arm of chromosome 22, at the gene map locus of 22q11.2. The human *COMT* gene encodes two distinctive proteins: soluble COMT

(S-COMT) and membrane-bound (MB-COMT) through the use of alternative translation initiations sites and promoters.[20] There are different single-nucleotide polymorphisms (SNPs) in the *COMT* gene, which induce important functional alterations of the enzyme. The *COMT* gene contains a common functional polymorphism: *c.472G>A (p.Val158Met)* (rs4680). This substitution is associated with a reduction in thermostability and activity of the enzyme.[21] Individuals with the Val^{158}/Val^{158} genotype have the highest activity of COMT and have been found to be less susceptible to pain compared with other genotypes. Individuals with the Met^{158}/Met^{158} genotype showed diminished regional μ-opioid system responses to pain compared with heterozygotes.[9] The exact mechanism by which diminished COMT activity influences pain perception is not known. However, associations between the low-activity Met^{158} allele are often inconsistent.[22] This suggests that additional SNPs in the *COMT* gene modulate COMT activity. There are three other SNPs in the *COMT* gene that exhibit a strong linkage disequilibrium with the $Val^{158}Met$ variation. One is located in the *S-COMT* promoter region: *c.1-98A>G* (rs6269). The two other SNPs are located in the *MB-COMT* coding region: *c.186C>T (p.=)* (rs4633) and *c.408C>G (p.=)* (rs4818).[23] Furthermore, haplotypes of the *COMT* gene that have functional consequences with respect to COMT enzyme activity have been revealed. Diatchenko identified three different haplotypes formed by the four different SNPs.[23] The use of haplotype reconstruction is preferred because combinations of SNPs might have a synergistic effect on COMT protein function. Since each person has two haplotypes for each gene, one can determine the variation on both haplotypes simultaneously: the diplotype.

The aim of this study was 1) to compare four *COMT* SNPs and the diplotypes between patients with CP and controls and 2) examine the effect of *COMT* gene variants on presence and severity of pain in CP.

MATERIALS AND METHODS

Subjects

We included patients diagnosed with chronic pancreatitis who visited the outpatient clinic at the Department of Gastroenterology and Hepatology of the Radboud University Nijmegen Medical Centre between 1980 and 2009. We sampled patients and performed a cross-sectional study. Therefore, we collected venous blood samples for DNA analysis in these patients at our outpatient clinic. The clinical diagnosis of chronic pancreatitis was based on one or more of the following criteria: presence of typical complaints (recurrent upper abdominal pain, radiating to the back, relieved by leaning forward or sitting upright and increased after eating), suggestive radiological findings, such as pancreatic calcifications or pseudo cysts, and pathological findings (pancreatic ductal irregularities and dilatations) revealed by endoscopic retrograde pancreaticography or magnetic resonance imaging of the pancreas before and after stimulation with secretin. We collected data regarding the cause of pancreatitis. Patients who had an estimated intake of alcohol of more than 60 g (females) or 80 g (males) daily for more than two years were classified as chronic pancreatitis of alcoholic origin. The diagnosis hereditary pancreatitis was established by fulfilling the international diagnostic criteria for hereditary pancreatitis: two first-degree relatives or three or more second-degree relatives, in two or more generations with recurrent acute pancreatitis and/or chronic pancreatitis for which there were no known precipitating factors.[24] Idiopathic pancreatitis was diagnosed if precipitating factors such as alcohol abuse, bile stones, trauma, medication, infection, metabolic disorders, and a positive family history were absent. Patients with other causes of pancreatitis, such as anatomic or tropical, were classified as miscellaneous causes. The controls were unrelated, healthy individuals from the Netherlands who were not suffering from pancreatic disease. We matched cases and controls on gender, while gender is a significant covariate in genetic studies of human pain.

A positive family history for pancreatic diseases was absent in all controls. In addition there was no chronic alcohol abuse (<60 g for females and <80 g for males) in our population. These data were collected through interviews.

Ethics

The study was conducted in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the local medical ethics review committee, the Institutional Review Board from the Radboud University Nijmegen Medical Centre (CWOM no. 0011-0242). All subjects gave their informed consent. The informed consent was obtained verbally in the presence of a witness and documented in the patient's medical file.

Genotyping

All patients donated a venous blood sample. Genomic DNA was extracted from 300 μl whole blood using the Puregene® genomic DNA isolation kit (Gentra Systems, Minneapolis, USA). The 4 *COMT* SNPs (*c.1-98A>G*, *c.186C>T (p.=)*, *c.408C>G (p.=)* and *c.472G>A*) were analysed by a dual-colour discrimination assay, using the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories; Hercules, USA). The PCR amplifications were carried out in a final volume of 25 μl, which contained 200 ng of genomic DNA, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 3 mM $MgCl_2$ 0.25 mM dNTP's, 200 nM of forward and reverse primer, 200 nM of both probes complementary to the two alleles of each SNP labelled at the 5' end with the fluorophore Fam or Hex and at the 3' end with BHQ1 as quencher (primer

sequences available on request) and 3.0 units of Taq-DNA polymerase. Genomic DNA was denatured at 95 °C for five minutes. Forty cycles were carried out, each composing denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 63 °C, and extension for 30 seconds at 72 °C. Genotype assignment was conducted using the iCycler iQ Optical System Software version 3.1 (Bio-Rad Laboratories; Hercules, USA) using the final fluorescent signals.

Statistical methods

After testing for Hardy-Weinberg equilibrium (HWE) among controls, frequency tables were provided for the distribution of the four studied SNPs.[25] Differences between continuous variables were tested using Student's t-test and categorical variables by the χ^2 test. Combination of haplotypes, diplotypes, were generated based on the four studied SNPs; missing SNPs were imputed.

The relative risk associated with minor alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI) with the most common diplotype as a reference. Statistical significance was defined as $p<0.05$. For diplotypes that were only present in either the patient population or healthy controls, no odds ratios could be calculated. Statistical analysis was carried out with SPSS 16.0 for Windows. Pair-wise linkage disequilibrium estimations between polymorphisms and haplotype reconstruction were performed with Haploview version 4.0.[26]

RESULTS

Characteristics of patients and controls

Samples of 685 subjects were included in our study cohort. The characteristics of the patients and controls are shown in *table 1*. The cohort consisted of 240 chronic pancreatitis patients (157 males, 83 females), with a mean age of 48 years (range 17 to 78 years). We included 445 controls (294 male, 150 female) with a mean age of 53 years (range 19 to 90 years). The patients and controls were Caucasians. Of the patients, 44% had alcohol-related chronic pancreatitis. Healthy controls were significantly older (3 years).

The genotyping completion rate was 100%. The observed and expected frequencies of the different SNPs in controls were in Hardy-Weinberg equilibrium. The allele frequencies of the four SNPs in chronic pancreatitis patients and healthy controls are shown in *table 2*. There was no significant association between the SNPs and chronic pancreatitis.

Table 1. *Demographic and clinical characteristics of chronic pancreatitis patients and healthy controls*

	Patients	Controls	*P* value
N	240	445	
Age (mean, range, in years)	48 (17-78)	53 (19-90)	0.001*
Sex (male:female)	157;83	294;150; 1 N/A	0.83
Tobacco use			
• Smoking	158		
• Non-smoking	63		
• Unknown	19	445	
Cause of chronic pancreatitis			
• Alcoholic	106		
• Hereditary	14		
• Idiopathic	103		
• Miscellaneous	17		

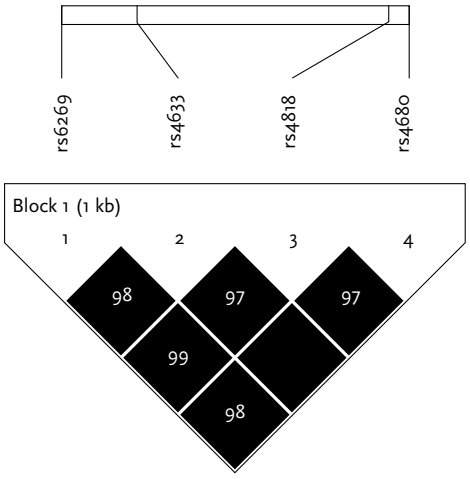
Table 2. *Allele frequencies of the four SNPs in chronic pancreatitis patients and healthy controls*

	Alleles	Patients (n=240)	Controls (n=445)	*P* value
rs6269				0.25
	A/A	84 (35%)	164 (37%)	
	A/G	123 (51%)	202 (45%)	
	G/G	33 (14%)	79 (18%)	
rs4633				0.14
	T/T	70 (29%)	122 (27%)	
	T/C	127 (53%)	214 (48%)	
	C/C	43 (18%)	109 (25%)	
rs4818				0.26
	C/C	82 (34%)	165 (37%)	
	C/G	126 (53%)	206 (46%)	
	G/G	32 (13%)	74 (17%)	
rs4680				0.18
	A/A	70 (29%)	120 (27%)	
	A/G	127 (53%)	218 (49%)	
	G/G	43 (18%)	107 (24%)	

Diplotype analysis

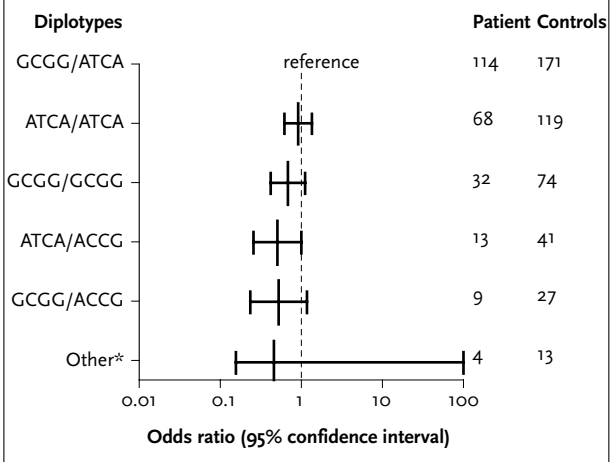
Linkage analysis between the four SNPs showed that they were closely linked (*figure 1*). We then determined haplotypes and combinations of haplotypes (diplotypes). Based on the SNP distribution, five diplotypes with a frequency >0.5% were generated, three of them representing 84% of all diplotypes observed in this study. Diplotype GCGG/ATCA is most prevalent in both groups, but more frequent in patients compared with controls (47.5% *vs* 38.4%). This haplotype served as reference in calculating the odds ratios for the remaining diplotypes (*figure 2*). ATCA/ACCG was more prevalent in controls compared with patients (9.2 *vs* 5.4%, OR 0.48, 95% CI 0.24 to 0.93, p=0.03). After correction for multiple testing, this was no longer a significant difference. The distribution of other diplotypes was not significantly different between patients and controls.

Figure 1. *Linkage disequilibrium plot*



Linkage disequilibrium (LD) plot across the COMT. The box at the top indicates the *COMT* gene with the four investigated SNPs. The LD plot is based on the measure of D'. Each diamond indicates the pair-wise magnitude of LD, with dark grey diamonds indicating strong LD (D' > 0.8).
LD = linkage disequilibrium is the non-random association of alleles at two or more loci, not necessarily on the same chromosome. Linkage disequilibrium describes a situation in which some combinations of alleles or genetic markers occur more or less frequently in a population than would be expected from a random formation of haplotypes from alleles based on their frequencies.

Figure 2. *Diplotype distribution of chronic pancreatitis patients and healthy controls*



Distribution of diplotypes in patients with chronic pancreatitis and healthy controls. The diplotypes are compared with the most prevalent diplotype GCGG/ATCA.

DISCUSSION

This study investigated the association between four SNPs in the *COMT* gene in a large cohort of patients with chronic pancreatitis and healthy controls. We considered the *COMT* gene a candidate in chronic pancreatitis for several reasons. First, COMT has been associated with several chronic pain conditions, such as fibromyalgia syndrome, neuropathic pain and temporomandibular disorder. Second, pain is a major symptom in chronic pancreatitis, which ultimately will be present in nearly all patients and it causes substantial impairments in health-related quality of life in these patients.

Gene association studies in chronic pancreatitis have so far focussed on the presence *vs* absence of the disease.[27] For example, mutations in pancreatic serine protease inhibitor Kazal type 1 (*SPINK 1*) are enriched in patients with idiopathic chronic pancreatitis as well in alcoholic pancreatitis in comparison with background population.[28] Likewise, the G191R variant of anionic trypsinogen gene (*PRSS2*) affords protection against various forms of chronic pancreatitis when compared with healthy controls.[29] We tried to take this further and search for genetic variants that determine an important symptom in chronic pancreatitis: pain.

In our study, we investigated if COMT polymorphisms are associated with chronic pancreatitis, but we were actually interested in the question whether COMT polymorphisms are associated with pain in patients with chronic pancreatitis. COMT itself has no role in the aetiology of CP per se, but its genetic variants have a role in altered pain perception. Our chronic pancreatitis group consisted of patients experiencing pain varying from intermittent to persistent and pain intensity ranging from disabling to no pain or mild pain. We did not directly quantify pain, which makes it difficult to study the exact correlation between COMT and pain due to chronic pancreatitis in this population. It is very complex to investigate pain, due to different levels of pain that patients' experience, the use of analgesic drugs and different pain scales. Furthermore, the difficulty in measuring pain is that there is no validated objective measurement of pain associated with chronic pancreatitis. This is partially due to the unpredictable course of chronic pancreatitis with relapses and remission. Pain in chronic pancreatitis is highly variable and it varies greatly during the lifetime of the disease. But ultimately, the majority of the patients with chronic pancreatitis will experience pain.

Moreover, there are several confounding variables, such as dependence of analgesic drugs and the use of alcohol or other narcotic agents. However, since almost every patient with chronic pancreatitis will experience pain during the course of their disease, we lumped the patients together and investigated COMT in chronic pancreatitis patients from our cohort.

We did not limit ourselves to a single *COMT* SNP, but rather elected to perform haplotype (and diplotype) association studies. Haplotype and diplotype reconstruction, rather than individual SNPs, better predicts variability in pain sensitivity. Diplotype GCGG/ATCA is most prevalent in both groups and more frequent in patients than in controls. ATCA/ACCG was more prevalent in controls compared with patients (OR 0.48,

95% CI 0.24 to 0.93). However, after correction for multiple testing this is not a significant difference.

Furthermore, we demonstrated no association between the SNPs *c.1-98A>G* (rs6269), *c.186C>T (p.=)* (rs4633), *c.408C>G (p.=)* (rs4818) and *c.472G>A (p.Val158Met)* (rs4680) and chronic pancreatitis. As a consequence, our results do not support a significant role for the *COMT* gene in the chronic pancreatitis.

A possible limitation of our study is that we do not have detailed insights into nicotine and alcohol use in our healthy controls. Numerous studies have explored the association of COMT with alcohol dependence. The Met^{158} allele has been associated with late-onset alcoholism in men, but not in the development of early-onset alcoholism with severe antisocial behaviour.[30,31] Second, the Met^{158} allele has also been associated with elevated weekly alcohol consumption in male social drinkers.[32] However, these findings are not consistent, because others failed to find evidence to support an association between alcohol dependence and variation in COMT.[33] In addition, we do not know if the pain pattern is different between patients with idiopathic and alcoholic chronic pancreatitis.

In conclusion, our study shows that the SNPs of the *COMT* gene are not associated with chronic pancreatitis. Because our results do not answer the complete complex of pain, future studies are needed to characterise the joint effect of multiple genes affecting pain.

REFERENCES

1. Garg PK, Tandon RK. Survey on chronic pancreatitis in the Asia-Pacific region. J Gastroenterol Hepatol. 2004;19(9):998-1004.
2. Van Esch AA, Wilder-Smith OH, Jansen JB, et al. Pharmacological management of pain in chronic pancreatitis. Dig Liver Dis. 2006;38(7):518-26.
3. Mogil JS. The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proc Natl Acad Sci U S A. 1999;96(14):7744-51.
4. Reimann F, Cox JJ, Belfer I, et al. Pain perception is altered by a nucleotide polymorphism in SCN9A. Proc Natl Acad Sci USA. 2010;107(11):5148-53.
5. Diatchenko L, Nackley AG, Slade GD, et al. Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. Pain. 2006;125(3):216-24.
6. Hagen K, Pettersen E, Stovner LJ, et al. The association between headache and Val158Met polymorphism in the catechol-O-methyltransferase gene. J Headache Pain. 2006;7(2):70-4.
7. Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA. No association between chronic musculoskeletal complaints and Val158Met polymorphism in the Catechol-O-methyltransferase gene. The HUNT study. BMC Musculoskelet Disord. 2006;7:40.
8. Armero P, Muriel C, Santos J, et al. COMT (Val158Met) polymorphism is not associated to neuropathic pain in a Spanish population. Eur J Pain. 2005;9(3):229-32.
9. Zubieta JK, Heitzeg MM, Smith YR, et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003;299(5610):1240-3.
10. Vargas-Alarcon G, Fragoso JM, Cruz-Robles D, et al. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis Res Ther. 2007;9(5):R110.
11. Jensen KB, Lonsdorf TB, Schalling M, et al. Increased sensitivity to thermal pain following a single opiate dose is influenced by the COMT val(158)met polymorphism. PLoS One. 2009;4(6):e6016.
12. Lee PJ, Delaney P, Keogh J, et al. Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity. Clin J Pain. 2011;27(2):93-101.
13. McLean SA, Diatchenko L, Lee YM, et al. Catechol O-methyltransferase haplotype predicts immediate musculoskeletal neck pain and psychological symptoms after motor vehicle collision. J Pain. 2011;12(1):101-7.
14. Reyes-Gibby CC, Shete S, Rakvag T, et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain. Pain. 2006;17:1-7.
15. Rakvag TT, Klepstad P, Baar C, et al. The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain. 2005;116(1-2):73-8.
16. Cumming P, Brown E, Damsma G, et al. Formation and clearance of interstitial metabolites of dopamine and serotonin in the rat striatum. J Neurochem. 1992;59(5):1905-14.
17. Li PP, Warsh JJ, Godse DD. Formation and clearance of norepinephrine glycol metabolites in mouse brain. J Neurochem. 1984;43(5):1425-33.
18. Zubieta JK, Smith YR, Bueller JA, et al. Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science. 2001;293(5528):311-5.
19. Nackley AG, Tan KS, Fecho K, et al. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both beta2- and beta3-adrenergic receptors. Pain. 2007;128(3):199-208.
20. Mannisto PT, Kaakkola S. COMT: biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev. 1999;51(4):593-628.
21. Lotta T, Vidgren J, Tilgmann C, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase. Biochemistry. 1995;34(13):4202-10.
22. Nackley AG, Shabalina SA, Lambert JE, et al. Low enzymatic activity haplotypes of the human catechol-O-methyltransferase gene: enrichment for marker SNPs. PLoS One. 2009;4(4):e5237.
23. Diatchenko L, Slade GD, Nackley AG, et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet. 2005;14(1):135-43.
24. Howes N, Lerch MM, Greenhalf W, et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. Clin Gastroenterol Hepatol. 2004;2(3):252-61.
25. www.genetik.charite.de. Tests for Hardy-Weinberg equilibrium.
26. www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview.
27. Derikx MH, Drenth JP. Genetic factors in chronic pancreatitis; implications for diagnosis, management and prognosis. Best Pract Res Clin Gastroenterol. 2010;24(3):251-70.
28. Drenth JP, te Morsche R, Jansen JB. Mutations in serine protease inhibitor Kazal type 1 are strongly associated with chronic pancreatitis. Gut. 2002;50(5):687-92.
29. Witt H, Sahin-Toth M, Landt O, et al. A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. Nat Genet. 2006;38(6):668-73.
30. Hallikainen T, Lachman H, Saito T, et al. Lack of association between the functional variant of the COMT gene and early-onset alcoholism associated with severe antisocial behavior. Am J Med Genet. 2000;96(3):348-52.
31. Tiihonen J, Hallikainen T, Lachman H, et al. Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism. Mol Psychiatry. 1999;4(3):286-9.
32. Kauhanen J, Hallikainen T, Tuomainen TP, et al. Association between the functional polymorphism of catechol-O-methyltransferase gene and alcohol consumption among social drinkers. Alcohol Clin Exp Res. 2000;24(2):135-9.
33. Foroud T, Wetherill LF, Dick DM, et al. Lack of association of alcohol dependence and habitual smoking with catechol-O-methyltransferase. Alcohol Clin Exp Res. 2007;31(11):1773-9.