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Association between the catechol-*O*-methyltransferase (rs4680: Val158Met) polymorphism and serum alanine aminotransferase activity

Mineyoshi Hiyoshi [a,*], Hirokazu Uemura [a], Kokichi Arisawa [a], Mariko Nakamoto [b], Asahi Hishida [c], Rieko Okada [c], Keitaro Matsuo [d], Yoshikuni Kita [e], Hideshi Niimura [f], Nagato Kuriyama [g], Hinako Nanri [h], Keizo Ohnaka [i], Sadao Suzuki [j], Haruo Mikami [k], Michiaki Kubo [l], Hideo Tanaka [d], Nobuyuki Hamajima [c] and for the J-MICC Study Group

[a] *Department of Preventive Medicine, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan*
[b] *Department of Public Health and Applied Nutrition, Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima, Japan*
[c] *Department of Preventive Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[d] *Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan*
[e] *Department of Health Science, Shiga University of Medical Science, Otsu, Japan*
[f] *Department of International Island and Community Medicine, Kagoshima University Graduate School of Medical and Dental Science, Kagoshima, Japan*
[g] *Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan*
[h] *Department of Preventive Medicine, Faculty of Medicine, Saga University, Saga, Japan*
[i] *Department of Geriatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan*
[j] *Department of Public Health, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan*
[k] *Division of Cancer Prevention and Epidemiology, Chiba Cancer Center, Chiba, Japan*
[l] *Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Yokohama, Japan*

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ABSTRACT

In our previous proteomic study in rat liver damaged by carbon tetrachloride, soluble catechol-*O*-methyltransferase (COMT) increased as a phosphorylated form and decreased as a dephosphorylated form. This finding raised the possibility that the COMT protein is associated with liver function. Thus, we hypothesized that (1) the COMT gene contributes to liver homeostasis and (2) a COMT polymorphism (rs4680: Val158Met) causing thermolability of enzymatic activity affects liver enzymes (e.g., aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (γ-GT)) in serum. To investigate (2), we statistically analyzed the association between COMT genotypes and serum ALT activity in a cross-sectional study using data from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study. We conducted a multiple logistic regression analysis for males (n = 838) and females (n = 970). Those participants having missing values or a past history of liver cirrhosis or liver cancer were excluded. ALT values were divided into two; elevated (30 IU/L ≤; males n = 239, females n = 90) and normal (<30 IU/L; males n = 599, females n = 880). In females, non-adjusted and adjusted odds ratios for ALT values in the rs4680 A/A homozygote (n = 126) compared with the wild-type G/G homozygote (n = 397) were 0.37 (95% CI 0.14–0.96) and 0.34 (95% CI 0.13–0.93), respectively. In males, an analysis of the population aged 35–69 did not reveal any significant difference, but the population aged 45–54 had a significant difference in the non-adjusted and adjusted odds ratio in the G/A heterozygote (n = 89) (0.50 (95% CI 0.27–0.92) and 0.35 (95% CI 0.18–0.71)) and in the A/A homozygote (n = 22) (0.34 (95% CI 0.11–0.99) and 0.22 (95% CI 0.07–0.72)), compared with the G/G homozygote (n = 88). These data suggest that the COMT polymorphism affects serum ALT activity to maintain liver function.

1. Introduction

In the progression of liver damage, the liver proteome is predicted to alter the expression and post-translational modification of many proteins. Regeneration-, apoptosis-, and protection-related proteins should be affected according to the level of damage. For example, hepatocyte growth factor, a potent liver regenerating factor, increases according to the damage (Costantini et al., 2010; Hamanoue et al.,

Abbreviations: J-MICC, Japan Multi-Institutional Collaborative Cohort; COMT, catechol-*O*-methyltransferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, gamma-glutamyl transpeptidase; 95% CI, 95% confidence interval; HCC, hepatocellular carcinoma; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; DDT, D-dopachrome tautomerase; Cdk5, cyclin-dependent kinase 5; DARPP-32, dopamine- and cAMP-regulated phosphoprotein of molecular weight 32000.

* Corresponding author at: Department of Preventive Medicine, Institute of Health Biosciences, the University of Tokushima Graduate School, 3-18-15, Kuramoto-cho, Tokushima 770–8503, Japan. Tel.: +81 88 633 7073; fax: +81 88 633 7074.
E-mail address: hiyoshi@basic.med.tokushima-u.ac.jp (M. Hiyoshi).

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1992; Shiota et al., 1995). To obtain a better understanding of liver damage, we have performed a proteomic analysis of rat liver damaged by carbon tetrachloride (Hiyoshi et al., 2009). Previously, we found a post-translational modification of the soluble catechol-*O*-methyltransferase (COMT; EC 2.1.1.6) protein (up: phosphorylated form, down: dephosphoryrated form) (data not shown). We did not discuss this finding at the time, because it was not a focus of the study.

However, Øverbye and Seglen (2009) found that COMT existed in 7 different forms including membrane-bound and soluble forms in rat liver. These results suggest that the COMT protein was modified after translation, which affected the enzymatic activity or selection of substrates. The modified COMT proteins may affect favorable flow pass in metabolic pathways and contribute to liver homeostasis. Although our (de)phosphorylated data corresponded to their results, the paper did not focus on the association between phosphorylation and liver damage.

Against this background, we hypothesized that the COMT protein is associated with liver function, and COMT polymorphisms that impact on enzymatic activity affect liver homeostasis.

COMT catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholestrogen (2-hydroxyestrone and 2-hydroxyestradiol-17beta), catecholamine (dopamine, L-noradrenaline, and L-adrenaline), and their metabolites (3,4-dihydroxyphenylacetate, 3,4-dihydroxyphenylethyleneglycol, and 3,4-dihydroxymandelate). Catecholestrogen is involved in estrogen metabolism, and the catecholamine and their metabolites are involved in tyrosine metabolism.

The COMT polymorphism (rs4680: Val158Met (G/A)) has been investigated experimentally, and thermolability has been demonstrated (Li et al., 2005; Rutherford et al., 2008; Weinshilboum and Dunnette, 1981). Furthermore, clinical and epidemiological studies have revealed this polymorphism to be associated with neurological and psychiatric disorders (Gothelf et al., 2005; Ohnishi et al., 2006; Sweet et al., 2005). Although a few studies examined the association between rs4680 and hepatocellular carcinoma (HCC), the results were not significant (Rossi et al., 2003; Yin et al., 2004; Yuan et al., 2008). However there might have been difficulties detecting significant differences (e.g., small sample size or the combined analysis of males and females). Because COMT is abundant in liver, it is important to understand the association between its polymorphism and liver function.

To evaluate the association between COMT genotypes and liver function,we used data from the Japan Multi-Institutional Collaborative Cohort (J-MICC) Study.

The J-MICC Study was organized by 10 research teams in 2005 (Hamajima et al., 2007). Its aim was to elucidate the associations between various common diseases, especially cancer, and lifestyle and genetic factors in the general Japanese population. At the beginning, we gathered 4,519 participants (male: 2,124, female: 2,395 from 10 research teams) and genotyped them for important polymorphisms clinically for cross-sectional studies (Wakai et al., 2011). Various associations between phenotypes and genotypes were analyzed by the 10 research teams.

In this study, we used serum alanine aminotransferase (ALT) activity as an index of liver function, because it has excellent specificity compared with other liver enzymes (e.g., aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (γ-GT)).

Generally, serum ALT activity increases according to the amount of damage to a liver, and 30 IU/L is used as a cutoff value. Ishiguro et al. revealed an association between elevated ALT activity (30 IU/L≤) and the risk of HCC in a Japan Public Health Center-based prospective Study (Ishiguro et al., 2009). They described that hepatitis virus (B or C) -negative or -positive subjects with elevated ALT activity (30–69 IU/L) have a 6.5 or 12-fold higher risk for HCC, than populations with normal ALT activity (<30 IU/L), respectively. Because, in our cross-sectional study, participants having elevated ALT activity (30 IU/L≤) should be considered to be at higher risk for liver disease, we discuss the impact of the COMT polymorphism on liver function through serum ALT activity.

2. Materials and methods

2.1. Cross-sectional study (liver function) in the J-MICC study

Hamajima et al. (2007) have already outlined the overall J-MICC study, and Wakai et al. (2011) have reported the details of this cross-sectional study. Participants aged 35–69 were collected by 10 research teams from among the general Japanese population. A large number of participants provided venous blood, health examination data, and questionnaire data on lifestyle and medical factors.

In 2009, various single nucleotide polymorphisms were selected by 10 research teams to evaluate the various associations between phenotypes and genotypes.

In this study, on the basis of our previous proteomic observation, we focused on COMT polymorphisms to evaluate the association with serum ALT activity as an index of liver damage. All participants in the J-MICC study provided written informed consent. The ethics committees of all the research teams' institutions reviewed and approved the protocol for the J-MICC Study.

2.2. COMT polymorphism

In this cross-sectional study, rs4680 (Val158Met (G/A)) among COMT polymorphisms was selected to investigate the association with liver function because it is a well-characterized polymorphism. Genotyping was performed using the invader assay (Third Wave Technologies, Madison, WI, USA) (Ohnishi et al., 2001) in RIKEN (Laboratory for Genotyping Development, Center for Genomic Medicine).

2.3. Definition of elevated ALT Level

Because Ishiguro et al. (2009) reported that individuals with elevated levels of ALT activity (30 IU/L≤) had higher risk for HCC than those with normal ALT activity (<30 IU/L), 30 IU/L≤is defined as elevated, and <30 IU/L as normal. Furthermore, because serum ALT activity has the highest specificity as an index of liver damage, to minimize the influences of other damaged tissues, we did not use other indexes of liver damage (e.g., aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (γ-GT) activities in serum).

2.4. Statistical analysis

The participants having missing values (e.g., serum ALT activity, genotype, and other confounding factors in Table 1) and past histories of liver cirrhosis or liver cancer were excluded. Furthermore, to minimize the influences of differences of area-specific ALT activity (e.g., assay system), 2 areas' populations with higher or lower ALT activities than other areas from the results of multiple comparison (Scheffe test after Kruskal Wallis test) were also excluded. Finally, the rest of the participants were evaluated as males and females, separately. The differences in age, AST, ALT, γ-GT activities, and BMI between males and females were tested by unpaired *t*-test. In addition, the differences in alcohol consumption, smoking, use of medical drugs, hepatitis B virus (HBV) positivity, and hepatitis C virus (HCV) positivity between males and females were tested by χ^2-test. In females, because only 6 participants were HCV carriers, a Yates 2×2 χ^2 test was done.

They were divided into two groups based on serum ALT activities (30 IU/L≤or <30 IU/L), and the association between COMT genotypes and serum ALT activities was analyzed by multiple logistic regression for males (n = 838) and females (n = 970), separately.