Catechol-O-methyltransferase promoter hypomethylation is associated with the risk of coronary heart disease

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Abstract. Catechol-O-methyltransferase (COMT) gene variation is known to be associated with the risk of acute coronary events. The purpose of the present study was to investigate the contribution of COMT promoter methylation towards the risk of coronary heart disease (CHD). COMT methylation was evaluated in 48 CHD cases and 48 well-matched non-CHD controls using bisulfite pyrosequencing technology. The results demonstrated that CHD cases had a significantly lower level of methylation at COMT CpG3 sites compared with the controls (33.77±5.71 vs. 36.42±5.00%; P=0.018). Further analysis, according to gender, showed that CpG3 methylation was associated with CHD in males (P=0.038) but not in females (P=0.253), suggesting that there is a gender disparity in the association between COMT methylation and CHD. In conclusion, it was determined that COMT CpG3 hypomethylation is associated with an increased risk of CHD in males.

Introduction

Coronary heart disease (CHD) is typically caused by arteriosclerosis, which is characterized by the narrowing or blockage of coronary arteries (1). CHD is a complex disease, and a variety of genetic and epigenetic factors have been associated with an increased risk of CHD (2-4). Aberrant methylation of cytosine residues in gene CpG islands is an important molecular mechanism involved in the regulation of gene expression in response to the environment (5). Global DNA hypomethylation has been described in the atherosclerotic human aorta (6). DNA methylation studies on CHD have primarily focused on genes related to estrogen receptors (ESR1 and ESR2), the immune system (FOXP3, PLA2G7 and MMP-9), lipid metabolism (ABCA1, KLF2 and LRP1), oxidative stress (GSTP1, BNIP3 and EC-COD), blood coagulation (TM and P2Y12) and genes on chromosome 9p21 (BAX, BCL-2 and TIMP3) (7). In addition, low promoter methylation was found to account for increased expression of the 15-lipoxygenase (ALOX15) gene (8). Furthermore, coagulation factor VII (F7) promoter hypomethylation has been correlated with higher plasma expression levels of activated coagulation factor VII (FVIIa), and has been shown to contribute towards an increased risk of CHD (9).

The catechol-O-methyltransferase (COMT) gene, located on chromosome 22q 11.21, encodes a regulatory enzyme, catecholamine and other catechols (10,11). COMT is able to transfer a methyl group to degrade dopamine, catecholamine, epinephrine and norepinephrine (12). In addition, COMT is associated with numerous human disorders, such as schizophrenia and bipolar disorder (13,14). Furthermore, a number of studies have revealed an association between COMT and metabolic syndromes, such as hypertension and diabetes, that are closely associated with CHD (15,16). COMT gene variation serves an important role in the increased risk of acute coronary events (17).

Hypomethylation of the COMT promoter has been detected in patients with schizophrenia and bipolar disorder (13,18). However, it is unknown whether methylation of the COMT promoter contributes towards the risk of CHD. In light of previous findings, the aim of the current study was to assess the association between COMT gene promoter methylation and the risk of CHD.

Materials and methods

Sample selection. In total, 48 cases of CHD (24 males and 24 females) and 48 gender-matched non-CHD controls from a Han Chinese population were selected from Ningbo

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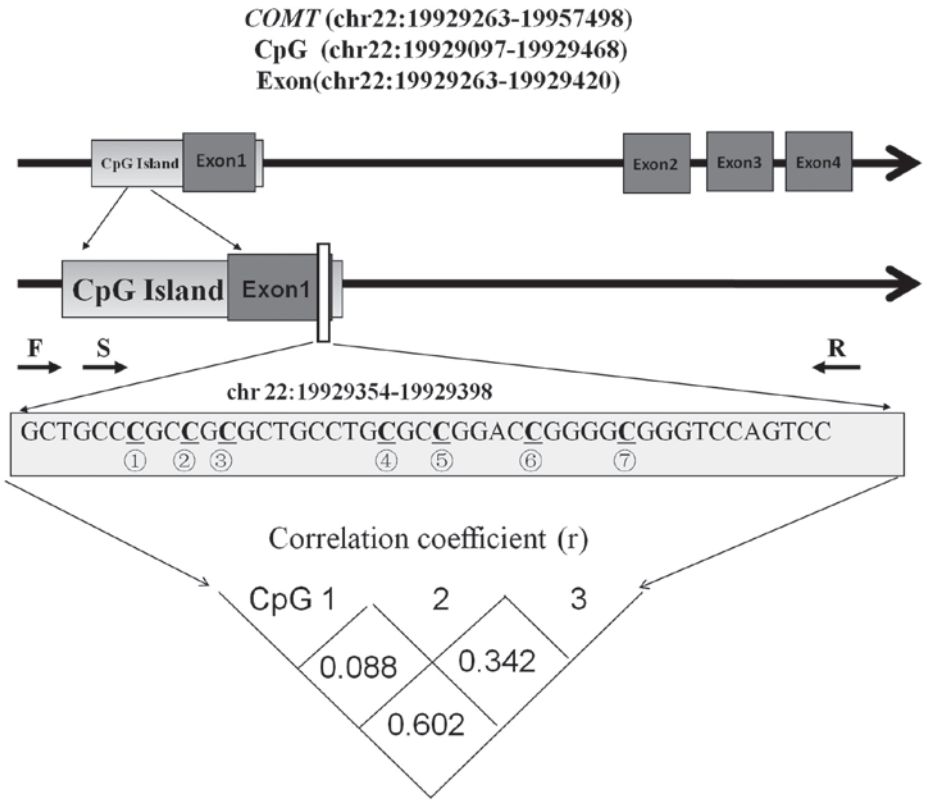

Figure 1. Three CpG sites of the COMT gene promoter region. COMT, catechol-O-methyltransferase; F, forward primer; S, sequencing primer; R, reverse primer.

First Hospital of Ningbo University (Ningbo, China). The mean ages of the CHD cases and non-CHD controls were 64.0 years (range, 50.0-85.0 years) and 62.2 years (range, 52.0-71.0 years), respectively. Unrelated individuals were examined by coronary angiography and diagnosed by experienced cardiologists. The classification details and inclusion criteria were as previously described (19-21). Peripheral blood samples were collected between June 2013 and June 2014. The current case-control study was approved by the Ethics Committee of Ningbo First Hospital. Written informed consent was obtained from each of the participants.

DNA methylation assay. Genomic DNA was collected from peripheral blood samples using a nucleic acid extraction automatic analyzer (Lab-Aid 820; Zeesan Biotech Co., Ltd., Xiamen, China). DNA was quantified using a PicoGreen double-stranded DNA Quantification kit (Molecular Probes; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. Pyrosequencing using PyroMark Gold Q24 Reagent (Qiagen, Hilden, Germany) was conducted to identify the methylation level of the promoter region of the COMT gene. Sodium bisulfite was used as a chemical modifier of genomic DNA to convert unmethylated cytosine to uracil. Polymerase chain reaction (Pyromark PCR kit; Qiagen) was performed using a mixture of primers designed using PyroMark Assay Design software, version 2.0 (Qiagen) yielding a fragment of COMT gene promoter (103 bp). The PCR reaction mixtures contained 10 μl ZymoTaq™ Premix (Zymo Research Corporation, Irvine, CA, USA), 2 μl DNA template, 1.5 μl each primer and 5 μl DNase/RNase-free water. PCR amplification was conducted under the following conditions: 95˚C for 10 min, followed by 45 cycles of 95˚C for 20 sec, 57.5˚C for 20 sec and 72˚C for 30 sec The primer sequences used were: Forward, 5'-GGGTTTTTGGGGTAGTTAG-3'; reverse, 5'-biotin-TAACCAACCCTCTCACCT-3'; and 5'-TTTGGG GTAGTTAGG-3' for the sequencing primer. The methylation rate of each CG site was quantified and analyzed using the PyroMark Assay Design software.

Statistical analysis. Statistical tests were analyzed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). An independent sample t-test was applied to identify COMT methylation differences between CHD cases and controls. A non-parametric approach was used for data that could not be normalized. $P<0.05$ was considered to indicate a statistically significant difference. The figures were created using GraphPad Prism version 6 software (GraphPad Software, Inc., La Jolla, CA, USA) or R software (version 3.1; https://www.r-project.org/).

Results

Correlation between the DNA methylation levels of the three CpG sites. As shown in Fig. 1, seven CpG sites were identified in the selected fragment (chr22:19929354-19929398, Human GRCh37/hg19 Assembly) of the promoter region of the COMT gene. However, due to sequencing limitations, reliable methylation results were only available for the three CpG sites preceding the fragment and, thus, these three sites

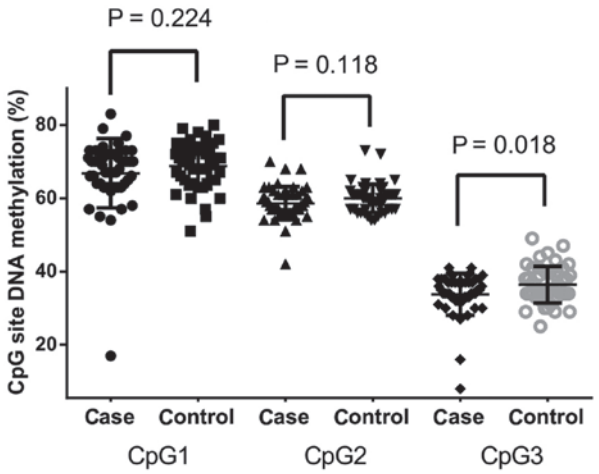

Figure 2. Comparison of the methylation levels of three CpG sites in coronary heart disease cases and controls.

were chosen to represent promoter methylation of the COMT gene. The results demonstrated that there was a moderate correlation between the DNA methylation levels of the three CpG sites (Fig. 1). Therefore, the three CpG sites were tested separately in the subsequent association analyses.

Comparison of the methylation levels of the three CpG sites in CHD. The follow-up case-control comparison showed that there was a significantly lower level of CpG3 methylation in CHD cases compared with non-CHD controls (Fig. 2; 33.77±5.71 vs. 36.42±5.00%; P=0.018). However, no significant difference in the methylation level was found between the cases and controls in CpG1 (Fig. 2; P=0.224) and CpG2 (Fig. 2, P=0.118). Further subgroup analysis by gender (Fig. 3) suggested that the significant association between CpG3 methylation was retained in males (Fig. 3C; 33.71±7.49 vs. 37.79±5.61%; P=0.038) but not in females (Fig. 3C; P=0.253) at the CpG3 site.

Correlation between COMT and the three CpG sites. Further correlation tests (Fig. 4) demonstrated that COMT CpG3 methylation was associated with age in controls (Fig. 4C; r=-0.297, P=0.040) but not in CHD cases. However, gender-stratified analysis in the control group did not identify any significant association between males (P=0.072, data not shown) or females (P=0.300, data not shown). Therefore, additional studies are required to confirm this inverse correlation in the non-CHD controls.

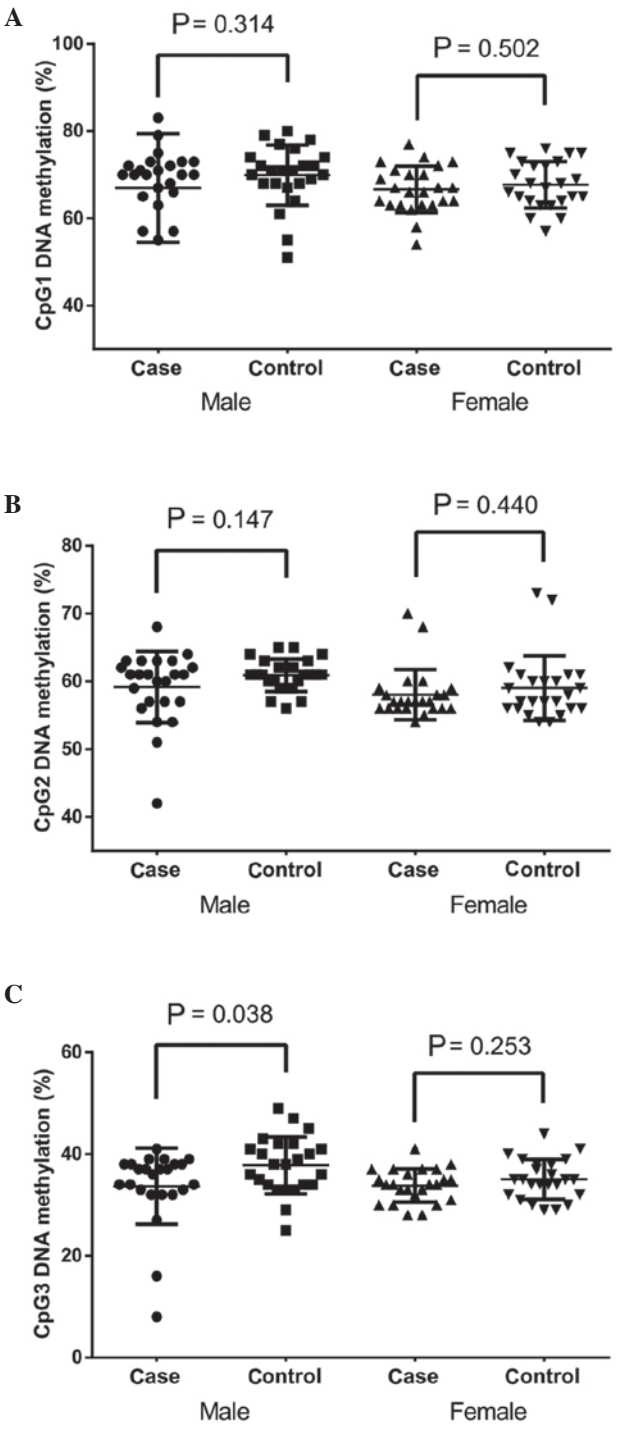

Figure 3. Comparison of the methylation levels of (A) CpG1, (B) CpG2 and (C) CpG3 in coronary heart disease cases and controls by gender.

Discussion

Findings of the current study demonstrated that CHD cases have lower levels of methylated COMT CpG3 compared with the controls. Further breakdown analysis by gender showed that CpG3 methylation was significantly associated with CHD in males (P=0.038) but not in females (P=0.253), suggesting a gender disparity in the association between COMT methylation and CHD. Additionally, the COMT CpG3 methylation level was inversely associated with age in the controls but not in cases with CHD.

COMT encodes a methyltransferase that demethylates s-adenosylmethionine to S-adenosyl-L-homocysteine, the immediate precursor of homocysteine (22). Therefore, COMT, as a homocysteine metabolism-mediated gene, has been hypothesized to serve a vital role in the increased risk of CHD (17). A low activity COMT genotype has a protective effect against cardiovascular diseases (23). The results in the present study show significant promoter hypomethylation of COMT among CHD cases, indicating a potential biomarker for predicting the occurrence and development of CHD.

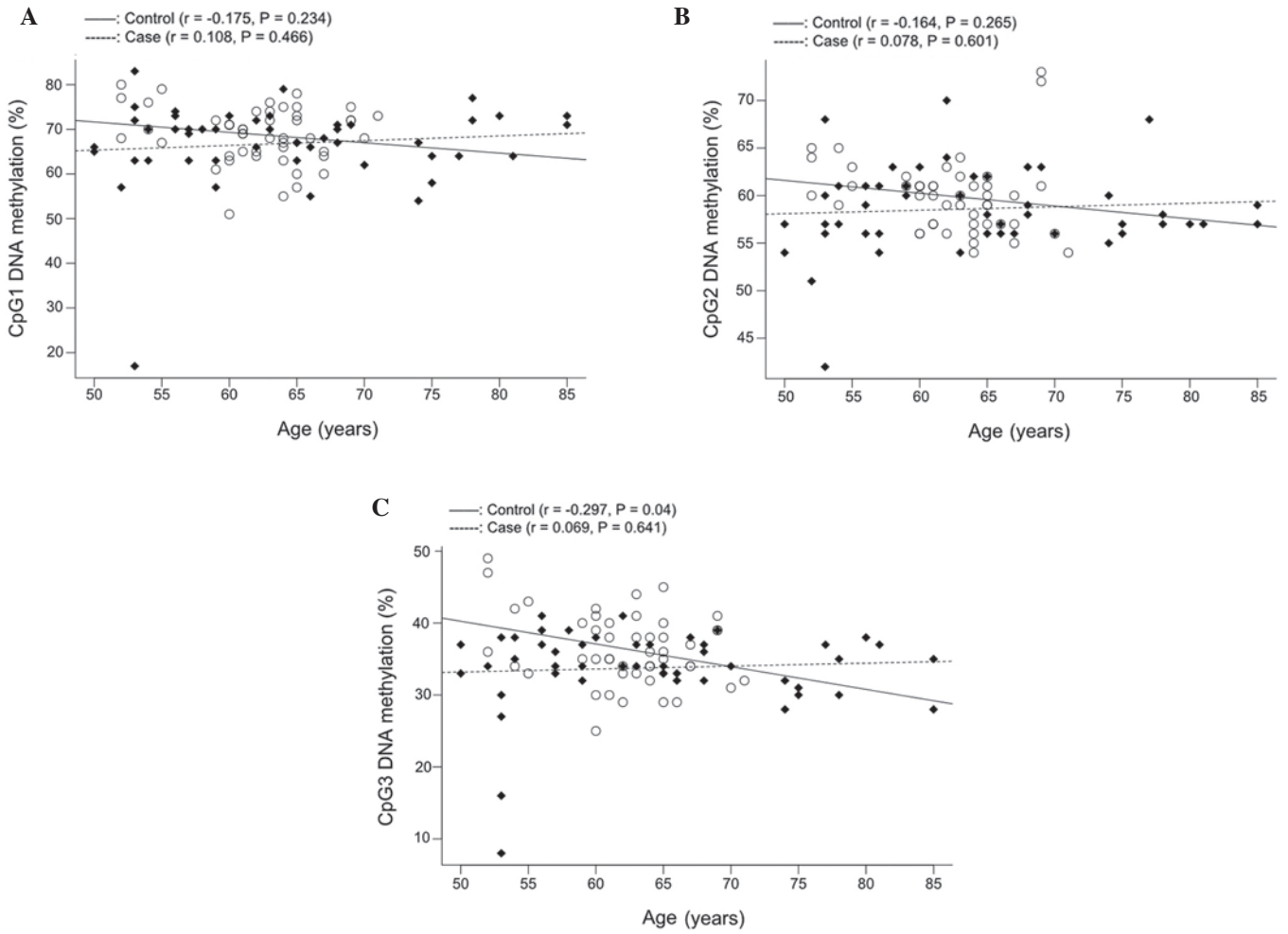

Figure 4. Pearson correlation between catechol-O-methyltransferase (A) CpG1, (B) CpG2 and (C) CpG3 site methylation and age.

Gender disparity exists in the pathogenesis of CHD (24). The current study identified a significant difference in the association between CpG3 methylation and CHD with regards to males and females. However, as there is no evidence suggesting that COMT serves an important role in the metabolism of estrogen, further studies are required to determine whether COMT interacts with other proteins associated with the metabolism of estrogen (25).

Altered DNA methylation patterns are one of the molecular mechanisms that underlie the phenotypic changes associated with human aging (26). In the present study, it was observed that COMT CpG3 methylation levels was reduced with increasing age in controls. It can, therefore, be hypothesized that COMT hypermethylation serves an important protective role against cardiovascular diseases, although the exact mechanisms underlying this role need to be investigated in future studies.

The current study had a number of limitations. Firstly, the sample size in the present study was relatively small. Larger sample sizes and the inclusion of other ethnic populations are required in order to confirm the findings. Secondly, COMT methylation was measured in the DNA of the peripheral blood, which included numerous types of cells, such as granulocytes and lymphocytes, which potentially affect the specificity of the methylation assay, as COMT methylation levels may vary between cell types. Thirdly, only three CpG sites from a fragment of the gene promoter region were selected to represent the entire COMT promoter. Although the cases with CHD were gender matched with the controls, the potential effects of other unknown environmental factors on the findings regarding COMT methylation cannot be excluded.

In conclusion, it was observed that COMT promoter hypomethylation is associated with CHD in males. The results contribute towards a better understanding of the role of COMT methylation in the pathophysiology of CHD.

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