Association of the GLB1 rs4678680 genetic variant with risk of HBV-related hepatocellular carcinoma

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ABSTRACT

Accumulated evidences demonstrated that GLB1 is involved in cell senescence and cancer development. The GLB1 rs4678680 single nucleotide polymorphism (SNP) has been identified as a hepatocellular carcinoma (HCC) susceptibility polymorphism by a genome-wide association study in Korean population previously. However, little or nothing was known about its involvement and functional significance in hepatitis B viruses (HBV)-related HCC in Chinese. Therefore, we investigated the association between the GLB1 rs4678680 SNP and HBV-related HCC risk as well as its biological function in vivo. Genotypes were determined in two independent case-control sets from two medical centers of China. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by logistic regression. The potential regulation role the rs4678680 genetic variant on GLB1 expression was examined with HCC and normal liver tissues. We found that The rs4678680 G allele was showed to be risk allele; individuals with the TG genotype had an OR of 1.51 (95% CI = 1.10–2.07, P = 0.010, Shandong set) or 1.49 (95% CI = 1.11–1.99, P = 0.008, Jiangsu set) for developing HBV-related HCC, respectively, compared with individuals with the TT genotype. This association was more pronounced in males, individuals aged older than 57 years and drinkers (all P < 0.05). In the genotype-phenotype correlation analyses of fifty-six human liver tissue samples, rs4678680 TG or GG was associated with a statistically significant increase of GLB1 mRNA expression (P < 0.05). Our data indicated that the GLB1 rs4678680 SNP contributes to susceptibility to develop HBV-related HCC, highlighting the involvement of GLB1 and cell senescence in etiology of HCC.

INTRODUCTION

Although ranking the fifth most common cancer, hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality worldwide [1]. There is a high incidence of HCC in China and other Asia-Pacific region [1]. Notably, China alone accounts for about 50% of all HCC cases in the world [1, 2]. There were several epidemiological features, such as marked variations between geographical regions, racial and ethnic groups, and sex. It has been revealed that men have a higher prevalence of HCC than women, i.e. the male:female ratio of HCC patients is ~2.65:1 in high-risk Chinese populations [2, 3]. Chronic infections with
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As a result, it is still largely unclear if T single nucleotide polymorphism (SNP) in the study.

Shandong and Jiangsu sets are showed in Table 1. The rs4678680 SNP in cases and controls from the GLB1 mRNA expression levels in normal liver tissues.

function of the rs4678680 SNP developing HBV-related HCC. To validate the biological polymorphism plays a part in etiology of HCC in Chinese.

Also, little or nothing has been known about functional significance of GLB1 rs4678680 SNP and HBV-related HCC risk in Shandong and Jiangsu sets (Table 1). The rs4678680 G allele was showed to be risk allele; individuals with the TG genotype had an OR of 1.51 (95% CI = 1.10–2.07, P = 0.010, Shandong set) or 1.49 (95% CI = 1.11–1.99, P = 0.008, Jiangsu set) for developing HBV-related HCC, respectively, compared with individuals with the TT genotype (Table 1). It was also found that carriers of the rs4678680 TG or GG genotype showed significantly and consistently increased risk to develop HBV-related HCC compared with the TT carriers in both case-control sets (Shandong set: OR = 1.53, 95% CI = 1.12–2.10, P = 0.007; Jiangsu set: OR = 1.57, 95% CI = 1.18–2.09, P = 0.002). In the pooled analyses, we observed that the odds of having the rs4678680 TG genotype in cases was 1.56 (95% CI = 1.24–1.97, P = 1.76 × 10⁻⁴) compared with the TT genotype. Similarly, the rs4678680 TG or GG genotype carriers showed a 1.52-fold increased HCC risk compared with the TT genotype carriers (95% CI = 1.19–1.94, P = 0.001).

The risk of HBV-related HCC associated with the GLB1 rs4678680 genotypes was further examined by stratifying for sex (Table 2). A significantly increased risk of HCC associated with the rs4678680 TG or GG genotype compared with the TT genotype was observed in males (Shandong set: OR = 1.60, 95% CI = 1.15–2.23; P = 0.006; Jiangsu set: OR = 1.59, 95% CI = 1.17–2.16; P = 0.003). However, this genetic polymorphism was not significantly associated with HCC risk in females (all P > 0.05). In the stratification analyses with age, elevated risk of HCC associated with the GLB1 rs4678680 TG or GG genotype was only observed among individuals aged older than 57 years (Shandong set: OR = 1.63, 95% CI = 1.01–2.62; P = 0.047), but not among individuals aged 57 years or younger (Shandong set: OR = 1.46, 95% CI = 0.96–2.21; P = 0.076) (Table 3). Similar results were observed among individuals aged older than 57 years in Jiangsu set (rs4678680 TG or GG genotype: OR = 2.10, 95% CI = 1.37–3.21; P = 0.001). Interestingly, the rs4678680 TG or GG genotype was also significantly associated with increased HCC risk in drinkers compared to the TT genotype in both sets (Shandong: OR = 1.90, 95% CI = 1.26–2.86, P = 0.002; Jiangsu: OR = 1.76, allele frequencies for rs4678680 G were 0.087 or 0.094 in cases and 0.059 or 0.058 in controls in Shandong or Jiangsu case-control set. All observed genotype frequencies in both cases and controls conform to Hardy-Weinberg equilibrium. Distributions of the rs4678680 genotypes were compared between HCC cases and controls. Frequencies of rs4678680 TT, TG and GG genotypes among HCC cases differed significantly from those among controls in either Shandong set (χ² = 7.593, P = 0.022, df = 2) or Jiangsu set (χ² = 16.14, P = 3.12 × 10⁻⁴, df = 2).

Unconditional logistic regression analysis was used to examine associations between the GLB1 rs4678680 SNP and HBV-related HCC risk in Shandong and Jiangsu sets (Table 1). The rs4678680 G allele was showed to be risk allele; individuals with the TG genotype had an OR of 1.51 (95% CI = 1.10–2.07, P = 0.010, Shandong set) or 1.49 (95% CI = 1.11–1.99, P = 0.008, Jiangsu set) for developing HBV-related HCC respectively, compared with individuals with the TT genotype (Table 1). It was also found that carriers of the rs4678680 TG or GG genotype showed significantly and consistently increased risk to develop HBV-related HCC compared with the TT carriers in both case-control sets (Shandong set: OR = 1.53, 95% CI = 1.12–2.10, P = 0.007; Jiangsu set: OR = 1.57, 95% CI = 1.18–2.09, P = 0.002). In the pooled analyses, we observed that the odds of having the rs4678680 TG genotype in cases was 1.56 (95% CI = 1.24–1.97, P = 1.76 × 10⁻⁴) compared with the TT genotype. Similarly, the rs4678680 TG or GG genotype carriers showed a 1.52-fold increased HCC risk compared with the TT genotype carriers (95% CI = 1.19–1.94, P = 0.001).

The risk of HBV-related HCC associated with the GLB1 rs4678680 genotypes was further examined by stratifying for sex (Table 2). A significantly increased risk of HCC associated with the rs4678680 TG or GG genotype compared with the TT genotype was observed in males (Shandong set: OR = 1.60, 95% CI = 1.15–2.23; P = 0.006; Jiangsu set: OR = 1.59, 95% CI = 1.17–2.16; P = 0.003). However, this genetic polymorphism was not significantly associated with HCC risk in females (all P > 0.05). In the stratification analyses with age, elevated risk of HCC associated with the GLB1 rs4678680 TG or GG genotype was only observed among individuals aged older than 57 years (Shandong set: OR = 1.63, 95% CI = 1.01–2.62; P = 0.047), but not among individuals aged 57 years or younger (Shandong set: OR = 1.46, 95% CI = 0.96–2.21; P = 0.076) (Table 3). Similar results were observed among individuals aged older than 57 years in Jiangsu set (rs4678680 TG or GG genotype: OR = 2.10, 95% CI = 1.37–3.21; P = 0.001). Interestingly, the rs4678680 TG or GG genotype was also significantly associated with increased HCC risk in drinkers compared to the TT genotype in both sets (Shandong: OR = 1.90, 95% CI = 1.26–2.86, P = 0.002; Jiangsu: OR = 1.76,

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RESULTS

Allele frequencies and genotype distributions of GLB1 rs4678680 SNP in cases and controls from the Shandong and Jiangsu sets are showed in Table 1. The
95% CI = 1.21–2.57, \( P = 0.003 \) (Table 4). However, no such associations was observed among non-drinkers (all \( P > 0.05 \)).

We next examined whether the HCC susceptibility SNP rs4678680 has an allele-specific impact on \( GLB1 \) expression using HCC tissues since it locates in a 18 kb upstream region of \( GLB1 \). As shown in Figure 1, we found that there were significantly higher \( GLB1 \) mRNA levels (mean ± SD) in HCC tissues compared to normal tissues (313.3 ± 33.9 vs. 189.9 ± 21.1; \( P = 0.003 \)). Subjects with the rs4678680 TG or GG genotype had significantly higher \( GLB1 \) mRNA levels than those with the TT genotypes in normal tissues (TG or GG: 367.5 ± 40.2 \[ n = 9 \], TT: 155.9 ± 20.6 \[ n = 47 \]; \( P = 0.001 \)). Similar results were observed when the \( GLB1 \) mRNA levels were compared between rs4678680 TG or GG and TT genotypes in HCC tissues (TG or GG: 590.7 ± 32.2 \[ n = 9 \], TT: 260.1 ± 35.0 \[ n = 47 \]; \( P = 0.001 \)) (Figure 1).

**DISCUSSION**

In the current study, we examined the association between the \( GLB1 \) rs4678680 SNP and risk of developing HBV-related HCC in a case-control design. Although the genetic predisposition to HCC of the \( GLB1 \) rs4678680 polymorphism was firstly identified in Korean populations via GWAS, this is still the first validation study with relative large sample size in different ethnic populations.

We found significantly increased HCC risk among individuals with the \( GLB1 \) rs4678680 TG or GG genotype compared with those with TT genotype in Chinese. In the genotype-phenotype correlation analyses of fifty-six human liver tissue samples, rs4678680 TG or GG was associated with a statistically significant increase of \( GLB1 \) mRNA expression.

The \( GLB1 \) gene provides instructions for producing an enzyme called \( \beta \)-galactosidase. This enzyme is located in lysosomes, which are compartments within cells that break down and recycle different types of molecules. Within lysosomes, GLB1 helps break down certain molecules, including substances called GM1 ganglioside and keratan sulfate. Caldwell et al. found that GLB1 activity is the only biomarker that accurately identifies a small and heterogeneous population of non-proliferating premalignant cells in the pancreas, indicating the utility of GLB1 to predict the senescent state in pancreatic preneoplasia [13]. Additionally, increased GLB1 is a valuable marker in formalin-fixed paraffin-embedded tissues for the senescence-like phenotype and associates with improved prostate cancer outcomes [16]. All these evidences support the involvement of GLB1 in carcinogenesis, possibly through regulating cell senescence.

Several limitations may exist in this case-control study. First, because it was a hospital-based study and the cases were from hospitals, there might be inherent limitations.
Table 2: Risk of HBV-related HCC associated with GLB1 rs4678680 G > T genotypes by sex

<table>
<thead>
<tr>
<th>Studies</th>
<th>Genotypes</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases No. (%)</td>
<td>Controls No. (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 1018</td>
<td>n = 425</td>
</tr>
<tr>
<td>Shandong set</td>
<td>TT</td>
<td>848 (83.3)</td>
<td>373 (87.8)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>164 (16.1)</td>
<td>51 (12.0)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>6 (0.6)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>170 (16.7)</td>
<td>52 (12.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 531</td>
<td>n = 998</td>
</tr>
<tr>
<td>Jiangsu set</td>
<td>TT</td>
<td>437 (82.3)</td>
<td>884 (88.6)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>88 (16.6)</td>
<td>112 (11.2)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>6 (1.1)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>94 (17.7)</td>
<td>114 (11.4)</td>
</tr>
</tbody>
</table>

Note: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; NC, not calculated.
*HCC case vs. chronic HBV carriers, data were calculated by logistic regression with adjustment for age, smoking and drinking.

Figure 1: GLB1 mRNA expression in fifty-six pairs of HCC-normal liver tissues. (A) There were higher GLB1 mRNA expression in HCC tissues than those in normal liver tissues. (B) GLB1 mRNA expression in normal liver tissues grouped by GLB1 rs4678680 genotypes. (C) GLB1 mRNA expression in HCC tissues grouped by GLB1 rs4678680 genotypes.
Table 3: Risk of HBV-related HCC associated with GLB1 rs4678680 G > T genotypes by age

<table>
<thead>
<tr>
<th>Studies</th>
<th>Genotypes</th>
<th>Cases (No. (%))</th>
<th>Controls (No. (%))</th>
<th>OR* (95% CI)</th>
<th>P-value*</th>
<th>Cases (No. (%))</th>
<th>Controls (No. (%))</th>
<th>OR* (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age (≤ 57 years)</td>
<td></td>
<td></td>
<td></td>
<td>Age (&gt; 57 years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 627 516 (82.3)</td>
<td>n = 262 228 (87.0)</td>
<td>Reference</td>
<td>1.46 (0.96–2.23)</td>
<td>0.077</td>
<td>n = 559 471 (84.3)</td>
<td>n = 246 221 (89.8)</td>
<td>Reference</td>
</tr>
<tr>
<td>Shandong set</td>
<td>TG</td>
<td>108 (17.2) 33 (12.6)</td>
<td>226 (17.7) 34 (13.0)</td>
<td>1.46 (0.96–2.21)</td>
<td>0.076</td>
<td>84 (15.0) 25 (10.2)</td>
<td>88 (15.7) 25 (10.2)</td>
<td>1.63 (1.01–2.62)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3 (0.5) 1 (0.4)</td>
<td>4 (0.7) 0 (0)</td>
<td>NC</td>
<td>4 (0.7) 0 (0)</td>
<td>NC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>111 (17.7) 34 (13.0)</td>
<td>115 (17.7) 34 (13.0)</td>
<td>1.46 (0.96–2.21)</td>
<td>0.076</td>
<td>84 (15.0) 25 (10.2)</td>
<td>88 (15.7) 25 (10.2)</td>
<td>1.63 (1.01–2.62)</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 315</td>
<td>n = 633</td>
<td>Reference</td>
<td>265 (84.1) 552 (87.2)</td>
<td>Reference</td>
<td>n = 305</td>
<td>n = 567</td>
<td>Reference</td>
</tr>
<tr>
<td>Jiangsu set</td>
<td>TG</td>
<td>44 (14.0) 79 (12.5)</td>
<td>1.06 (0.70–1.60)</td>
<td>0.791</td>
<td>61 (20.0) 57 (10.1)</td>
<td>2.10 (1.37–3.21)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>6 (1.9) 2 (0.3)</td>
<td>0 (0) 0 (0)</td>
<td>NC</td>
<td>6 (1.9) 2 (0.3)</td>
<td>NC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>50 (15.9) 81 (12.8)</td>
<td>1.19 (0.80–1.78)</td>
<td>0.388</td>
<td>61 (20.0) 57 (10.1)</td>
<td>2.10 (1.37–3.21)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; NC, not calculated. *HCC case vs. chronic HBV carriers, data were calculated by logistic regression with adjustment for sex, smoking and drinking.

Table 4: Risk of HBV-related HCC associated with GLB1 rs4678680 G > T genotypes by alcohol drinking

<table>
<thead>
<tr>
<th>Studies</th>
<th>Genotypes</th>
<th>Nondrinkers (Cases (No. (%)</th>
<th>Controls (No. (%))</th>
<th>OR* (95% CI)</th>
<th>P-value*</th>
<th>Drinkers (Cases (No. (%)</th>
<th>Controls (No. (%))</th>
<th>OR* (95% CI)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n = 410 349 (85.1)</td>
<td>n = 195 168 (86.2)</td>
<td>Reference</td>
<td>1.16 (0.70–1.92)</td>
<td>0.578</td>
<td>n = 776 638 (82.2)</td>
<td>n = 313 281 (89.8)</td>
<td>Reference</td>
</tr>
<tr>
<td>Shandong set</td>
<td>TG</td>
<td>59 (14.4) 26 (13.3)</td>
<td>1.16 (0.70–1.92)</td>
<td>0.578</td>
<td>133 (17.2) 32 (10.2)</td>
<td>1.82 (1.21–2.75)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>2 (0.5) 1 (0.5)</td>
<td>1.25 (0.68–2.29)</td>
<td>0.468</td>
<td>85 (18.3) 60 (12.1)</td>
<td>1.72 (1.17–2.51)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>61 (14.9) 27 (13.8)</td>
<td>1.14 (0.69–1.87)</td>
<td>0.620</td>
<td>138 (17.8) 32 (10.2)</td>
<td>2.10 (1.37–3.21)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n = 516 133 (85.3)</td>
<td>n = 706 629 (89.1)</td>
<td>Reference</td>
<td>133 (17.8) 32 (10.2)</td>
<td>1.90 (1.26–2.86)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Janshu set</td>
<td>TG</td>
<td>20 (12.8) 76 (10.8)</td>
<td>1.25 (0.68–2.29)</td>
<td>0.468</td>
<td>85 (18.3) 60 (12.1)</td>
<td>1.72 (1.17–2.51)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>3 (1.9) 1 (0.1)</td>
<td>1.25 (0.68–2.29)</td>
<td>0.468</td>
<td>85 (18.3) 60 (12.1)</td>
<td>1.72 (1.17–2.51)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG + GG</td>
<td>23 (14.7) 77 (10.9)</td>
<td>1.42 (0.79–2.53)</td>
<td>0.240</td>
<td>88 (18.9) 61 (12.3)</td>
<td>1.76 (1.21–2.57)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; NC, not calculated. *HCC case vs. chronic HBV carriers, data were calculated by logistic regression with adjustment for age, sex and smoking.

selection bias. As a result, it is crucial to confirm these observations in a population-based prospective study. Second, the statistical power for gene-covariate interaction analyses may be limited. Third, since the P value for association between the GLB1 rs4678680 SNP and HCC risk in the current study are not less that 10^{-7}, it is possible that these polymorphisms may not be identified by the aforementioned four large scale HCC GWAS in Chinese [7–10].

In summary, our study elucidated that the GLB1 rs4678680 polymorphism was associated with risk of HBV-related HCC in Chinese populations, highlighting the involvement of GLB1 and cell senescence in etiology of HCC.
MATERIALS AND METHODS

Study subjects

This study consisted of two case-control sets (Supplementary Table S1): (a) Shandong set: 1186 individuals with HBV-related HCC, sex- and age-matched (± 5 years) 508 chronic HBV carriers were recruited at Shandong Cancer Hospital affiliated to Shandong University, Shandong Academy of Medical Sciences (Jinan, Shandong Province, China). (b) Jiangsu set: 620 HBV-related HCC individuals from Huaian No. 2 Hospital (Huaian, Jiangsu Province, China) and sex- and age-matched 1200 chronic HBV carriers as controls. Cases and controls were recruited at Huaian No. 2 Hospital. The case-control sets has been reported previously [17]. A total of 56 pairs of HCC tissue specimens from 56 HCC individuals recruited in this study. All HCC individuals received curative resection in Huaian No. 2 Hospital or Qianfoshan Hospital, Shandong University. Prior to the surgery, no HCC individuals received any local or systemic anticancer treatments. All subjects were ethnic Han Chinese. At recruitment, the written informed consent was obtained from each subject. This study was approved by the institutional Review Boards of Shandong Cancer Hospital, Qianfoshan Hospital and Huaian No. 2 Hospital.

SNP genotyping

The GLB1 rs4678680 polymorphism was analyzed by the MassArray system (Sequenom Inc., San Diego, California, USA). GLB1 rs4678680 PCR primers are 5′-ACGTTGGATGAGTCCAAGCCTGTTCTTC-3′ (Forward) and 5′-ACGTTGGATGTCTGCCGAGTTGTGCAAAG-3′ (Reverse). GLB1 rs4678680 UEP_SEQ primer is 5′-cctcaTGCTTTCTTCCCTTTCTT-3′. GLB1 rs4678680 EXT1_SEQ primer is 5′-cctcaTGCTTTCTTCCCTTTCTT-3′. GLB1 rs4678680 EXT2_SEQ primer is 5′-cctcaTGCTTTCTTCCCTTTCTT-3′. A 15% blind, random sample of study subjects was genotyped in duplicates and the reproducibility was 100%.

Real-time analyses of GLB1 mRNA

SYBR-Green real-time quantity PCR method was used to examine GLB1 mRNA levels in normal liver tissues as described previously [18–20]. Total cellular RNA was isolated and converted to cDNA using the ReverTra Ace qPCR RT Kit (TOYOBO). Relative gene expression quantitation for GLB1 and β-actin as an internal reference gene was carried out using the ABI 7500 real-time PCR system in triplicates. The primers used for GLB1 were 5′-GTCTATTCTTCTCGCTTCT-3′ and 5′-TGTGCTCCATAGTGTTGTA-3′; and for β-actin were 5′-GGCGGCACCACCATGTACCCT-3′ and 5′-AGGGGCCGGACTCGTCTATCATC-3′. The expression of individual GLB1 mRNA measurements was measured relative to expression of β-actin mRNA using the method as described previously [21].

Statistic analyses

The differences in demographic variables and genotype distributions of the GLB1 rs4678680 SNP between cases and controls were examined via Pearson’s χ² test. The associations between genotypes of GLB1 rs4678680 and HBV-related HCC risk were estimated by ORs and their 95% CIs computed by logistic regression models. All ORs were adjusted for age, sex, smoking or drinking status, where it was appropriate. Kruskal-Wallis one-way analysis of variance tests were performed to calculate GLB1 mRNA expression differences between different rs4678680 genotype carriers. A P value of less than 0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. All analyses were performed using SPSS 16.0 (SPSS Inc.).

CONFLICTS OF INTEREST

The authors declare no competing financial interests.

GRANT SUPPORT

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REFERENCES


