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Replication the association of 2q32.2–q32.3 and 14q32.11 with hepatocellular carcinoma

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ABSTRACT

Hepatocellular carcinoma (HCC) is a malignant tumor. The morbidity and mortality of HCC tend to ascend and become a serious threat to the population health. Genetic studies of HCC have identified several susceptibility loci of HCC. In this study, we aim to replicate the association of these loci in our samples from Chinese population and further investigate the genetic interaction. We selected 16 SNPs within 1p36.22, 2q32.2–q32.3, 3p21.33, 8p12, 14q32.11 and 21q21.3 and genotyped in 507 HCC patients and 3014 controls by using Sequenom MassARRAY system. Association analyses were performed by using PLINK 1.07. We observed that the *STAT4* (2q32.2–q32.3) at rs7574865 ($P = 1.17 \times 10^{-3}$, OR = 0.79) and *EFCAB11* (14q32.11) at rs8013403 ($P = 1.54 \times 10^{-3}$, OR = 0.80) were significantly associated with HCC in this study. In 3p21.33, genetic variant rs6795737 within *GLB1* was also observed with suggestive evidence ($P = 9.98 \times 10^{-3}$, OR = 0.84). In the interaction analysis, the pair of associated SNPs (rs7574865 within *STAT4*, rs8013403 within *EFCAB11*) generated evidence for interaction ($P = 4.10 \times 10^{-3}$). In summary, our work first reported the association of 14q32.11 (*EFCAB11*) with HCC in Chinese Han population and revealed the genetic interaction between *STAT4* (2q32.2–q32.3) and *EFCAB11* (14q32.11) in HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor with a high incidence, which ranks as fifth most common malignant tumors and the second leading cause of cancer mortality (Yang and Roberts, 2010). There are more than 600,000 new cases worldwide each year according to the latest statistic, especially in the Asia-Pacific region (Parkin et al., 2005). HCC is a disease with high morbidity in China, which develops mainly from hepatitis B cirrhosis. Meanwhile the gradual increase of hepatitis C virus infection is also the predisposing factor (El-Serag and Rudolph, 2007; Tanaka et al., 2011). In China, the HCC patients account for 55% of the whole number worldwide (Parkin et al., 2005). Nearly 230,000 patients died from HCC per year, which is 18.8% within the cancer death, just behind gastric carcinoma. The morbidity and mortality of HCC tend to ascend and HCC has become a serious health threat (Yang and Roberts, 2010). With the development of molecular biology and genetic engineering, the genetic study of HCC is becoming a research hotspot to explore the pathogenesis and improve the clinical efficiency as well as survival rate of HCC.

Recently, several GWASs in Asian populations have identified at least 8 susceptibility loci for HCC, including 1p36.22 (*KIF1B*, *UBE4B*, *PGD*) (Zhang et al., 2010), 2q32.2–q32.3 (*STAT4*) (Jiang et al., 2013), 3p21.33 (*GLB1*) (Clifford et al., 2010), 8p12 (Chan et al., 2011), 14q32.11 (*EFCAB11*) (Clifford et al., 2010), 21q21.3 (*GRIK1*) (Sun et al., 2012), 22q12.2 (*DEPDC5*) (Miki et al., 2011) and MHC region (Jiang et al., 2013; Clifford et al., 2010; Sun et al., 2012; Kumar et al., 2011).

Abbreviations: A, adenine; G, guanine; C, cytosine; T, thymine; HCC, hepatocellular carcinoma; *EFCAB11*, EF-hand calcium binding domain 11; *STAT4*, signal transducer and activator of transcription 4; *GLB1*, galactosidase, beta 1; GWAS, genome wide association study; *KIF1B*, kinesin family member 1B; *UBE4B*, ubiquitination factor E4B; *PGD*, phosphogluconate dehydrogenase; *GRIK1*, glutamate receptor, ionotropic, kainate 1; *DEPDC5*, DEP domain containing 5; MHC, major histocompatibility complex; SNP, single-nucleotide polymorphism; HBV, hepatitis B virus; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; OR, odds ratio; IL, interleukin; IFN, interferon; TBA, testing balanced accuracy; CVC, cross-validation consistency; MDR, multifactor dimensionality reduction.

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In this study, we selected tag-SNPs within the above loci (non-MHC region) to validate in Chinese population and further investigate the interactions associated with disease between these genetic variants.

2. Materials and methods

2.1. Subjects

A total of 507 HCC patients and 3014 health controls were enrolled in this study (Table 1). All samples were unrelated and Chinese Han in origin from the same region of Anhui Province. Patients with HCC were recruited from No. 1 Hospital of Anhui Medical University in China. Diagnosis with HCC was based on (i) positive findings on cytological or pathological examination and/or (ii) positive images on angiogram, ultrasonography, computed tomography and/or magnetic resonance imaging, combined with an α-fetoprotein concentration of ≥400 ng/ml. Clinical information was collected from the affected individuals through a full clinical checkup by physician specialists. We assessed the severity of these 507 HCC patients according to Barcelona Clinic Liver Cancer (BCLC) staging (Bruix and Sherman, 2005), 52.27% of patients were with BCLC-0 and BCLC-A, and only 9.27% of patients were with BCLC-D (Table 1). All controls were clinically assessed to be without HCC and no family history of HCC (including first-, second- and third-degree relatives) or systemic disorders. Chronic HBV carriers were defined as individuals that were positive for both HBV surface antigen (HBsAg) and immunoglobulin G antibody to HBV core antigen for at least 6 months. 414 (81.66%) and 286 (9.49%) individuals carried HBV in HCC cases and controls, respectively (Table 1). The study was approved by the Institutional Ethics Committee of Anhui Medical University and was conducted according to Declaration of Helsinki principles.

Collection of blood samples and clinical information from cases and controls was undertaken after written informed consent was obtained from all participants. DNA was extracted from peripheral blood lymphocytes using QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNAs were analyzed by agarose gel electrophoresis, quantified by spectrophotometer, and stored at −80 °C until use.

2.2. SNP selection and genotyping

SNP selection was according to, (1) the associated SNPs within 8 susceptibility loci of HCC in previous study, (2) the tag-SNPs ($r^2 < 0.8$) with MAF > 5% within the above 7 susceptibility loci. Totally, 16 SNPs within 1p36.22, 2q32.2–q32.3, 3p21.33, 8p12, 14q32.11 and 21q21.3 were successfully genotyped in 507 HCC patients and 3014 controls. SNPs were genotyped using the Sequenom MassARRAY system at the State Key Laboratory Incubation Base of Dermatology, Ministry of National Science and Technology, Hefei, Anhui, China. Approximately 15 ng of genomic DNA was used to genotype each sample. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design 3.0 software (Sequenom). The DNA samples were amplified by multiplex PCR reactions, and the PCR products were then used for locus-specific single-base extension reactions. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was performed using MALDI-TOF MS. The mass spectrograms were analyzed by the MassARRAY Typer software (Sequenom).

Table 1
Summary of samples in this study.

	Cases	Controls
Male/female	403/104	2189/825
Mean ages	54.96	44.19
Mean onset ages	45.58	N/A
With hepatitis B virus affected	414 (81.66%)	286 (9.49%)
BCLC-0	38 (7.50%)	N/A
BCLC-A	227 (44.77%)	N/A
BCLC-B	126 (24.85%)	N/A
BCLC-C	69 (13.61%)	N/A
BCLC-D	47 (9.27%)	N/A

BCLC: Barcelona Clinic Liver Cancer staging.

2.3. Statistical analysis

The SNPs were analyzed for association with the disease by comparing the minor allele frequency in the cases and the controls using PLINK 1.07 software (Purcell et al., 2007). The level of associated significance was assigned at *P* values of less than 0.003 after Bonferroni Multiple Testing correction. The *P* values were adjusted by age and gender in the case–control association analysis. All the SNPs passed the quality control in terms of a call rate of >95%, Hardy–Weinberg equilibrium (HWE) ($P > 0.01$) in the controls. The genetic statistical power for all genotyped SNPs was estimated using CaTS-Power Calculator software (Skol et al., 2006). We considered the pairs of associated SNPs to look for interactions between the susceptibility loci. We evaluated two-SNP interaction using multifactor dimensionality reduction (MDR, V2.0 Beta 2) (Ritchie et al., 2001), as a genetically model-free and nonparametric alternative to logistic regression (Moore et al., 2006; Hahn et al., 2003). Models are evaluated on the testing balanced accuracy (TBA) statistic (Velez et al., 2007), the cross-validation consistency (CVC) (Cherkassky and Ma, 2009) and the statistical significance of the model. The satisfactory TBA score is above 0.55 and $P < 0.05$ was the statistical significance of the best models.

3. Results

Totally, 16 SNPs were genotyped in 507 HCC cases and 3014 controls (Table 2). We replicated the association of *STAT4* (2q32.2–q32.3) with HCC at rs7574865 ($P = 1.17 \times 10^{-3}$, OR = 0.79) and observed that the variant rs8013403 within 14q32.11 (*EFCAB11*) was significantly associated with HCC in this study ($P = 1.54 \times 10^{-3}$, OR = 0.80) (Table 2). In 3p21.33, genetic variant rs6795737 within *GLB1* was also observed with suggestive evidence ($P = 9.98 \times 10^{-3}$, OR = 0.84) (Table 2). Those SNPs located within loci 1p36.22, 8p12 and 21q21.3 did not reach the threshold significant association for HCC in this study ($P < 0.003$, after Bonferroni Multiple Testing correction) (Table 2). In the cases of stratified analysis, we compared the MAF of rs8013403 and rs7574865 and no significant difference of MAF was observed between HCC cases with HBV affected and HBV unaffected cases (Table 3).

We made efforts to find the best-fit association model among the recessive, dominant and additive models for the 2 significant SNPs in this study. For rs8013403, the most significant association evidence was observed under the recessive model ($P = 3.20 \times 10^{-3}$, OR = 0.68); the homozygous and heterozygous odds ratio (OR_{hom}/OR_{het}) estimates of this SNP suggested that heterozygote AG had approximate effect with homozygote GG (0.93 vs. 1), while AA had an OR of 0.66, much smaller than effect of AG or GG, which supported this recessive model. The additive model was the best fit for the association of rs7574865_T ($P = 1.36 \times 10^{-3}$, OR = 78); the OR_{hom}/OR_{het} estimates further suggested the additive effects of this SNP (0.59/0.80/ref) (Table 4).

In the interaction analysis, the pair of associated SNPs (rs7574865 within *STAT4*, rs8013403 within *EFCAB11*) generated evidence for interaction associated with disease ($P = 4.10 \times 10^{-3}$) by logistic regression analysis based on the TBA statistic (Table 5). Odds ratio estimates for *EFCAB11* gene genotypes at rs8013403 stratified by *STAT4* gene genotypes at rs7574865 showed that individuals carrying combined genotype (TT/AA, TT/AG, TG/AA) of protective (minor) allele T (rs7574865) and A (rs8013403) had stronger effect (OR = 0.55, 0.6 and 0.5) than single SNP effect (OR = 0.79 and 0.80), which suggested the combined effect of *STAT4* and *EFCAB11* on HCC (Fig. 1).

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