Impacts of TCF7L2 gene polymorphisms on the susceptibility of hepatogenous diabetes and hepatocellular carcinoma in cirrhotic patients

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ABSTRACT

Background: Hepatogenous diabetes (HD) occurs as a complication of cirrhosis. Whether genetic factors, rather than only liver damage, play roles in the development of HD is unknown. TCF7L2 gene has been reported to be associated with type 2 diabetes and also cancer risks. We aim to evaluate the impact of TCF7L2 gene on the susceptibility of HD and hepatocellular carcinoma (HCC) in a Chinese Han population.

Patients and methods: A total of 367 adult liver transplant candidates with liver cirrhosis were included. Fifteen tag single nucleotide polymorphisms (SNPs) were selected from HapMap CHB database with a minor allele frequency of >0.2 and r2 of >0.8. Another three SNPs were also chosen because of their close association with type 2 diabetes in East Asian.

Results: Patients with HD presented significantly poorer liver function, higher incidence of cirrhotic complications and higher insulin resistance compared with non-HD patients. Three SNPs were differentially distributed between HD patients and non-HD patients. In multivariate logistic analysis, TCF7L2 rs290487 and rs6585194 polymorphisms were independently associated with HD after adjustment of clinical factors. The TCF7L2 rs290487 C/C variant homozygote showed much higher insulin resistance and significantly increased HD risk compared with T/T and T/C genotypes, while the genetic variant of rs6585194 was protectively against HD. Three SNPs (rs290481, rs290487 and rs290489) located near the 3′ end of TCF7L2 gene were associated with HCC risk with marginal significance. Patients carrying C-C-A haplotype had a significantly higher HCC risk than those with A-T-G.

Conclusions: TCF7L2 polymorphisms were associated with HD and maybe cancer risk as well. Further studies with large samples are needed to verify these results.

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1. Introduction

It is well known that advanced liver diseases are frequently associated with diabetes mellitus. An estimated 60% of cirrhotic patients suffer from impaired glucose metabolism, and around 30% of them may develop into overt diabetes mellitus, which is known as hepatogenous diabetes (HD) (Garcia-Compean et al., 2009; Holstein et al., 2002). HD not only exacerbates the clinical course of cirrhosis (Garcia-Compean et al., 2009). In addition, genetic and environmental factors also have a great influence on the development of diabetes. For instance, TCF7L2 (transcription factor 7-like 2, also known as TCF4) has been uncovered as the most significant type 2 diabetes candidate gene. Genetic variants in this gene are associated with increased risk of type II diabetes in a variety of study populations (Luo et al., 2009). It can directly bind to multiple gluconeogenesis associated genes and involve in the hepatic glucose metabolism (Norton et al., 2011; Oh et al., 2012). Because different types of diabetes usually share genetic similarities (Cervin et al., 2008; Garcia-Compean et al., 2009), TCF7L2 has been reported to be associated with latent autoimmune diabetestes in adult (LADA) (Lukecs et al., 2012) and new-onset diabetes after transplantation (NODAT) (Kurzawski et al., 2011; Ling et al., 2013), it is
hypothesized that there is a potential link between the type 2 diabetes susceptibility genes and HD.

In this study, we aim to evaluate the association between TCF7L2 gene and HD in a Chinese Han population. Furthermore, because TCF7L2 gene is a transcription factor and key component of the Wnt signaling pathway, which is associated with carcinogenesis (Polakis, 2012), we also aim to assess whether TCF7L2 gene polymorphisms increase hepatocellular carcinoma (HCC) risk.

2. Materials and methods

2.1. Patients

A total of 367 adult liver transplant candidates (age > 18 years) with liver cirrhosis were included between January 2005 and December 2009 at the First Affiliated Hospital, Zhejiang University School of Medicine, China. Patients with a family history of diabetes, or diabetes before liver diseases were excluded. There were 315 males and 52 females with a mean age of 46.4 ± 10.3 years. Most of the patients had a history of hepatitis B virus infection. Model for end-stage liver disease (MELD) was used to evaluate disease severity. The mean value of MELD score was 21.7 ± 7.9 at admission to the liver transplant department. The demographic data of patients are summarized in Table 1.

This study was approved by the Institutional Review Board, First Affiliated Hospital, Zhejiang University School of Medicine, the current regulation of the Chinese Government, and the Declaration of Helsinki. Written informed consents were obtained. Data were analyzed anonymously.

2.2. Data collection

The clinical data recorded for analysis included age, gender, primary liver diseases, comorbidities, liver and kidney function, body mass index, blood glucose and insulin levels. HD was defined as diabetes after liver cirrhosis. Diabetes was diagnosed on the basis of 1999 World Health Organization as fasting blood glucose of ≥ 7 mmol/L, or a non-fasting blood glucose of ≥ 11.1 mmol/L, confirmed on at least 2 occasions or a need for anti-diabetic medicines. Impaired fasting glucose (IFG) was defined as 2 fasting blood glucose measurements of ≥ 6.1 and < 7.0 mmol/L without anti-diabetic treatment. The homeostasis model assessment (HOMA) index was calculated to estimate the insulin resistance (IR) (Matthews et al., 1985). All patients could be divided into HD group (n = 100) and non-HD group (n = 267) accordingly.

2.3. Genotyping

Genomic DNA was isolated from EDTA-anticoagulated whole blood of recipients using the QIAamp DNA Blood mini kit (QIAGEN, Hilden, Germany). Single nucleotide polymorphisms (SNPs) in TCF7L2 gene were selected from HapMap CHB database (public data release 21a/phase II, January 2007; http://snp.cshl.org/cgi-perl/gbrowse/hapmap22_B36/) with a minor allele frequency (MAF) of >0.2 and r² of >0.8. Fifteen tag SNPs in TCF7L2 (rs10749127, rs10787475, rs11196224, rs12775879, rs17130188, rs290481, rs290487, rs290489, rs3750804, rs4918792, rs6585194, rs7085532, rs7094463, rs7919409, and rs966227) were chosen for analysis. In addition, another three SNPs (rs7903146, rs11196205 and rs1225372) were also selected because they were reported to be significantly associated with type 2 diabetes in East Asians (Luo et al., 2009). SNPs were successfully genotyped in 97.3% of patients. Applied Biosystems SnP-Shot and TaqMan technology were applied to identify genetic polymorphism. The detailed procedure has been described previously (Ling et al., 2013).

2.4. Statistical analysis

Quantitative variables were expressed as mean ± SD or median. Categorical variables were presented as values. A Student’s t test or Mann–Whitney test was used to compare quantitative variables, and a chi-square test was used to compare categorical variables. Pairwise Linkage disequilibrium methods testing r² and D’ were used to assess linkage between SNPs. Pearson linear regression was used to examine the correlations. Logistic regression analysis was used to evaluate risk factors. Variables with statistical significance in univariate analysis were entered into a stepwise multivariate regression analysis. The predictive models were subsequently established, the risk scores and probability of HD were calculated as described previously (Ling et al., 2013). Hapoview software and SNPStats web tool (http://bioinfo.iconcologia.net/snpstats/start.htm) were used to analyze the Hardy–Weinberg equilibrium, linkage disequilibrium, and haplotype. SPSS version 11.3 (SPSS Inc, Chicago, IL) was used to consider other analyses. A P value of <0.05 was considered to be statistically significant.

3. Results

3.1. Clinical characteristics of HD patients

Among all 367 patients, 100 (27.2%) had HD, 51 (13.9%) had IGF, and 233 (58.9%) showed normal glucose levels. Clinical parameters of HD patients are shown in Table 1. Compared to the non-HD group, the HD group presented significantly higher MELD score (P = 0.022), bilirubin (P = 0.008) and international normalized ratio (P = 0.017), as well as higher incidence of hepatic encephalopathy (P = 0.015) and bleeding esophageal varices (P = 0.029). The HOMA-IR index was significantly higher in the HD group than in the non-HD group (P < 0.001).

Fasting blood glucose levels were significantly correlated with hepatic encephalopathy (r = 0.192, P < 0.001), MELD score (r = 0.161, P = 0.003), international normalized ratio (r = 0.149, P = 0.006) and bilirubin (r = 0.134, P = 0.015).

3.2. Genotype distribution and association with HD

All 18 SNP frequencies were in accordance with the Hardy–Weinberg equilibrium with P values of > 0.05 (Supplemental Table 1). Among the 18 SNPs, TCF7L2 rs290487, rs6585194 and rs7094463 were found to distribute differentially between the two groups (Table 2). The rs290487

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>HD (n = 100)</th>
<th>Non-HD (n = 267)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.9 ± 9.8</td>
<td>47.2 ± 10.5</td>
<td>0.572</td>
</tr>
<tr>
<td>Male/females, n (%)</td>
<td>88 (88)/12 (12)</td>
<td>227 (85)/40 (15)</td>
<td>0.466</td>
</tr>
<tr>
<td>Etiology of cirrhosis, n (%)</td>
<td>87 (87)/13 (13)</td>
<td>234 (88)/33 (12)</td>
<td>0.601</td>
</tr>
<tr>
<td>Comorbidities, n (%)</td>
<td>39 (39)</td>
<td>96 (36)</td>
<td>0.590</td>
</tr>
<tr>
<td>Hepatic encephalopathy</td>
<td>16 (16)</td>
<td>20 (7)</td>
<td>0.015</td>
</tr>
<tr>
<td>Hepatorenal syndrome</td>
<td>11 (11)</td>
<td>21 (8)</td>
<td>0.343</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>20 (20)</td>
<td>30 (11)</td>
<td>0.029</td>
</tr>
<tr>
<td>Ascites</td>
<td>41 (41)</td>
<td>95 (36)</td>
<td>0.339</td>
</tr>
<tr>
<td>Operation history, n (%)</td>
<td>11 (11)/2 (2)</td>
<td>30 (11)/7 (3)</td>
<td>0.949</td>
</tr>
<tr>
<td>MELD score</td>
<td>23.3 ± 8.3</td>
<td>21.1 ± 7.6</td>
<td>0.022</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>15.2 ± 10.0</td>
<td>10.5 ± 8.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.12 ± 0.59</td>
<td>1.02 ± 0.55</td>
<td>0.396</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>2.23 ± 1.04</td>
<td>1.85 ± 0.70</td>
<td>0.019</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>10.4 ± 4.2</td>
<td>4.7 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/L)</td>
<td>21.5 ± 8.2</td>
<td>12.5 ± 6.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>11.1 ± 8.5</td>
<td>2.4 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.5 ± 4.3</td>
<td>21.9 ± 3.7</td>
<td>0.449</td>
</tr>
<tr>
<td>Follow up time (months)</td>
<td>34 ± 20</td>
<td>31 ± 24</td>
<td>0.361</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCC, Hepatocellular carcinoma; MELD, model for end stage liver disease; IR, insulin resistance; BMI, body mass index. * The data were available in 192 patients.
minor C-allele, rs6585194 major C-allele and rs7094463 major A-allele were more frequent in the HD group than in the non-HD group.

In univariate logistic regression analysis, four SNPs were significantly associated with HD (Table 2). The genetic variants in TCF7L2 rs290481 and rs290487 increased the risk of HD, with odds ratio ranging from 1.38 to 1.57. In contrast, genetic variants in TCF7L2 rs6585194 and rs7094463 significantly reduced the risk of HD, with odds ratio ranging from 0.52 to 0.75. Multivariate logistic regression analysis revealed that rs290487 and rs6585194 polymorphisms were independent factors influencing the onset of HD. The effect of genetic variants remained significant after adjustment of MELD score, bilirubin, international normalized ratio, hepatic encephalopathy and bleeding esophageal varices (Table 3).

According to the result of logistic regression analysis, we calculated the risk score for each independent influencing factor. The predicted probabilities of HD are shown in Fig. 1. The TCF7L2 rs290487 C/C variant homozygote presented a high probability of HD (45%). The combination of the two SNPs increased the predictive value compared with rs6585194 alone.

IR was further compared among different genotypes using HOMA-IR index. TCF7L2 rs290487 polymorphism was found to be associated with IR (Fig. 2). Compared to the T/T wild-type, both C/C (6.3 vs. 2.2, \( P = 0.001 \)) and T/C (3.8 vs. 2.2, \( P = 0.004 \)) genotypes showed significantly higher HOMA-IR indexes. The C/C variant homozygote also presented older age (48.1 ± 9.3 years vs. 45.5 ± 10.7 years, \( P = 0.001 \)), higher HOMA-IR than the T/C heterozygote (\( 16.5 \pm 5.1 \) vs. \( 23.2 \pm 8.2 \), \( P = 0.001 \)). Furthermore, the fasting blood glucose levels had significantly lower MELD score, hepatic encephalopathy and bleeding esophageal varices (Table 3).

3.4. Haplotype construction

Linkage disequilibrium analysis showed strong linkages between rs6585194 and rs7094463 (\( D' = 0.98 \)), and between rs290481 and rs290487 (\( D' = 0.89 \)). Haplotypes were constructed with frequency threshold for rare haplotypes of >0.1. Neither the individual haplotypes nor global test was associated with the risk of HD. However, when combined with rs290481, rs290487 and rs290489, patients with G-C-A haplotype had a significantly higher HCC risk than those with A-T-G (odds ratio = 1.74, 95% confidence interval = 1.10–2.76, \( P = 0.018 \)).

4. Discussions

The liver is the central metabolic organ and plays a key role in glucose homeostasis. Glucose intolerance occurs in many cirrhotic patients, and subsequently, diabetes manifests clinically as liver function deteriorates. In this study, we found that HD patients had poorer liver function and higher incidence of cirrhotic complications compared to non-HD patients. Furthermore, the fasting blood glucose levels had significantly positive correlations with the parameters of liver function. These results demonstrated a strong association between the severity of liver disease and glucose intolerance.

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**Table 3**

Multivariate logistic regression analysis of SNPs associated with HD.

<table>
<thead>
<tr>
<th>Genotype OR 95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td></td>
</tr>
<tr>
<td>rs290487 (C/C vs. T/T-T/C)</td>
<td>0.027 2.180 (1.094–4.342)</td>
</tr>
<tr>
<td>rs6585194 (G/G-G/C vs. C/C)</td>
<td>0.011 0.539 (0.334–0.867)</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
</tr>
<tr>
<td>rs290487 (C/C vs. T/T-T/C)</td>
<td>0.016 2.402 (1.178–4.898)</td>
</tr>
<tr>
<td>rs6585194 (G/G-G/C vs. C/C)</td>
<td>0.010 0.523 (0.320–0.853)</td>
</tr>
<tr>
<td>Adjusted</td>
<td></td>
</tr>
<tr>
<td>rs290487 (C/C vs. T/T-T/C)</td>
<td>0.029 2.202 (1.082–4.428)</td>
</tr>
<tr>
<td>rs6585194 (G/G-G/C vs. C/C)</td>
<td>0.004 0.481 (0.293–0.789)</td>
</tr>
</tbody>
</table>

SNP, single-nucleotide polymorphism; HD, hepatogene diabetes; OR, odds ratio; CI, confidence interval.

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HD risk. To some extent, HD can be considered as an indicator of advanced liver disease (Garcia-Compean et al., 2009).

Apart from the clinical implications, genetic factors have also been speculated to involve in the development of HD. Genome-wide association studies have identified several potent diabetes-susceptibility loci (Doria et al., 2008), which have been further proved among various populations around the world including the Han Chinese (Chang et al., 2007; Wen et al., 2010). However, there is a remarkably different genetic background between East Asians and Caucasians (Luo et al., 2007; Wen et al., 2010). The major reason for this inconsistency is that the main cause of cirrhosis is hepatitis B virus, which is different from the Western countries. It has been reported that diabetes is more frequent in patients with hepatitis C virus-related cirrhosis than those with hepatitis B virus-related cirrhosis, because of a direct role of hepatitis C virus in insulin resistance and pancreatic β cell dysfunction (Kwon et al., 2005). Therefore, the results should be verified in some other cohorts with different etiologies of cirrhosis. In addition, patients with family history of diabetes were excluded. This could minimize the possibility of including other types of diabetes but may also exclude risk alleles from study population and underestimate the true risk. One limitation of this study is the small sample size. Another limitation is that the main cause of cirrhosis is hepatitis B virus, which is different from the Western countries. It has been reported that diabetes is more frequent in patients with hepatitis C virus-related cirrhosis than those with hepatitis B virus-related cirrhosis, because of a direct role of hepatitis C virus in insulin resistance and pancreatic β cell dysfunction (Kwon et al., 2005). Therefore, the results should be verified in some other cohorts with different etiologies of cirrhosis. In addition, patients with family history of diabetes were excluded. This could minimize the possibility of including other types of diabetes but may also exclude risk alleles from study population and underestimate the true risk.

In conclusion, HD could be a marker of end-stage liver disease. Besides clinical factors, TCF7L2 gene polymorphisms are associated with HD. The genetic variants near the 3’ end of the TCF7L2 gene may increase insulin resistance and also HD risk. These findings uncover a genetic role in the pathogenesis of HD in cirrhotic patients, and furthermore, provide
first molecular evidence to explain the association between diabetes and HCC. Well designed and large sample studies are required to confirm the results.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2013.03.089.

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