Short Research Communication

Association of the TP53 rs1042522 C>G polymorphism and hepatoblastoma risk in Chinese children

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Abstract

The TP53 gene encodes an important class of cell cycle and tumor-suppressing factors that play critical roles in maintaining genomic stability. The TP53 Arg72Pro (rs1042522 C>G) polymorphism has been reported to be associated with the risk of several types of adult cancers; however, its risk for pediatric cancers remains unclear. Here, we analyzed the association of the TP53 gene rs1042522 C>G polymorphism with hepatoblastoma (HB) susceptibility in a hospital-based study among Chinese children. A total of 213 HB patients and 958 healthy controls were enrolled in the study. Genotypes were determined by a TaqMan assay, and the strength of the association was assessed by the odds ratios and 95% confidence intervals generated from logistic regression models, adjusted for age, gender, and clinical stage. No significant association between the TP53 rs1042522 C>G polymorphism and HB susceptibility was detected in the main analysis or in stratification analyses of age, gender, and clinical stages. Overall, the TP53 gene rs1042522 C>G polymorphism is not associated with HB susceptibility in the Chinese population, other polymorphisms alone or in combination should be investigated to further clarify HB susceptibility.

Key words: TP53; rs1042522 C>G; polymorphism; genetic association; hepatoblastoma

Introduction

Hepatoblastoma (HB) is an embryonic tumor derived from hepatic precursor cells, and is the most common hepatic malignancy in children, accounting for approximately 80% of all childhood liver tumors [1, 2]. HB predominantly occurs in children under the age of 5 years, although those aged between 6 months and 3 years have the highest incidence, and the median age is 17 months, with a greater incidence in boys [3]. An enlarged hepatic mass and increased alpha fetoprotein level are the main clinical features of HB [4]. Complete surgical resection remains the mainstay treatment, and neoadjuvant and postoperative chemotherapy help to substantially increase overall survival [5].

Although most HB cases are sporadic, some patients also have a family history of cancers and certain cases are associated with genetic aberrations. HB is also associated with Edwards syndrome, Beckwith-Wiedemann syndrome, familial adenomatous polyposis, and other genetic syndromes [6], which can significantly increase the risk of developing HB [7, 8]. HB development has been linked to abnormal methylation of certain imprinted differentially methylated regions in tumor-specific genes, such as 11p15.5 and 20q13.3, which have high rates of genetic and epigenetic changes along with
abnormally high expression [9]. In addition, HB development has been associated with abnormal Wnt signaling pathway activation due to somatic or germ-line mutations of various genes [10]. Besides these specific factors, HB has been linked to chromosomal abnormalities, genetic factors, low birth weight, and various adverse factors during pregnancy, either acting alone or in combination [11].

The treatment and prognosis of HB is currently based on a risk stratification system. Complete surgical resection is generally curative for well-differentiated fetal HB, while chemotherapy is needed for other subtypes, and a cisplatin-based chemotherapy regimen is particularly effective [12]. With state-of-the-art treatment, the 5-year overall survival rate of HB is over 70% [11]. However, treatment of cases associated with high risk factors remains challenging. Therefore, further investigation is warranted to unveil the detailed pathogenesis and enable better prognosis.

TP53 is an important tumor suppressor gene, located on chromosome 17p13.1 comprising 11 exons and 10 introns. TP53 encodes a protein of 393 amino acids, which has three major domains: an N-terminal transactivation site, intermediate DNA-binding region, and C-terminal oligomerization site [13]. TP53 exerts important biological functions by regulating the cell cycle, promoting cell apoptosis, participating in DNA recombination and repair, and stabilizing genome function. Moreover, TP53 protein can stimulate the expression of genes involved in the inhibition of angiogenesis, which may further prevent tumor formation [14, 15].

Given these important functions, a variety of malignancies may occur when the TP53 gene is inactivated [15]. Among those genetic variants that impact gene function, single nucleotide polymorphism (SNP) plays an important role [16, 17]. Potentially functional SNPs located within cell death genes may influence cancer risk in various ways [18-21]. To date, more than 200 polymorphic loci have been detected in TP53 [22], and the three major single nucleotide polymorphisms are associated with tumorigenesis [23]. The first is a single nucleotide polymorphism (rs1042522 C>G; CGC-CCC) located at codon 72 of exon 4 of TP53, which results in substitution of an arginine (Arg) to proline (Pro) residue in the protein [24]. The second polymorphism is a 16-bp insertion repeat in the third intron region of TP53 [25, 26]. The third polymorphism occurs at the MSP I restriction site of TP53 in the sixth intron [27]. Among these three polymorphisms, rs1042522 C>G is the most important and extensively investigated one [28]. Several studies have shown that these three polymorphisms are associated with the genetic susceptibility of many tumors [26, 29, 30]. In particular, the TP53 rs1042522 C>G polymorphism is associated with susceptibility to multiple malignancies such as breast cancer, lung cancer, and cervical cancer [28, 31-33], suggesting that this part of the gene plays an important role in the development of adult cancers. However, the influence of the TP53 rs1042522 C>G polymorphism has not been investigated in HB.

Therefore, in this study, we analyzed the relationship between the TP53 gene rs1042522 C>G polymorphism and HB susceptibility in a cohort of children with HB and healthy controls in the Han Chinese population.

Materials and Methods

Study subjects

A total of 213 HB patients and 958 healthy control subjects from Chinese genetically unrelated Han people were enrolled in the study, including subjects from four hospitals in Guangdong province, Henan province, Shaanxi province, and Shannxi province (Supplemental Table 1). All patients were younger than 18 years and were diagnosed as having HB based on a histopathological examination. None of the patients had a history of any other tumors. The control subjects were randomly chosen among children living in the same area as the HB patients, with match age and sex.

This study was approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center (Guangzhou, China). Informed consent for participation in the study was obtained from the legal guardians of all subjects. All patient records were anonymized and de-identified prior to analysis.

Genotyping

The TP53 gene rs1042522 C>G polymorphism was genotyped on a TaqMan platform (Applied Biosystems, Foster City, CA, USA) as reported previously [34-36]. Quality control was performed with eight negative control and positive control samples in each 384-well plate. In addition, 10% of the samples were randomly selected for a second genotyping for validation of the assay, and the concordance rate was 100%.

Statistical analysis

The χ² test was used to detect differences in demographic variables, risk factors distribution, and TP53 genotype distribution between the case and control groups. The χ² was also used to test whether the distribution of TP53 genotypes was consistent with Hardy-Weinberg equilibrium (HWE)
expectations. Univariate and multivariate unconditional logistic regression analyses were used to test the association of single nucleotide polymorphism genotypes with HB risk based on the generated odds ratios (ORs) and 95% confidence intervals (CIs). Adjusted ORs were calculated using multivariate analysis adjusting for age, and gender. The χ² test and logistic regression analysis were used to analyze the statistical differences in age, gender, and clinical stage of HB patients with different genotypes. Polymorphic loci were evaluated by dominant and recessive models, according to the P values, ORs, and 95% CIs. All statistical analyses were performed using SAS software (version 9.1; SAS Institute, Cary, NC). P < 0.05 was considered to indicate a statistically significant difference or association.

**Results**

**Characteristics of the study population**

Overall, the frequency distribution of selected variables did not differ between HB patients and controls. The distributions of age, gender, and clinical stages of the study subjects are summarized in Table 1. There was no significant difference between HB patients and controls regarding the distribution of age (P = 0.105) and gender (P = 0.973). Males were predominant in both the HB and control groups. Most of the patients had stage II disease, followed by stage I, stage III, and stage IV. There were also no significant differences in the age and gender distribution between HB patients and healthy control recruited from each province (P > 0.05).

**TP53 rs1042522 C>G polymorphism and HB susceptibility**

The genotype distributions of the TP53 rs1042522 C>G polymorphism in HB patients and controls are summarized in Table 2. Three genotypes, CC, CG, and GG, were detected at the rs1042522 locus of TP53 gene, with similar frequencies of each genotype in the two groups, although the frequencies of the CC and GG genotypes were slightly higher in HB cases, whereas the CG frequency was slightly higher in controls. No significant deviation from HWE was detected in the control group (P = 0.485). Moreover, there was no significant association observed between the TP53 rs1042522 C>G polymorphism and HB susceptibility in any comparison (Table 2).

**Stratification analysis of the TP53 rs1042522 C>G polymorphism and HB risk**

We further explored the association between the TP53 rs1042522 C>G polymorphism and HB risk in analyses stratified by age, gender, and clinical stages (Table 3). No significant associations were observed in children older than 17 months or in those 17 months or younger. In addition, the CG/GG genotypes were not significantly associated with HB risk in either females or males. Finally, CG/GG genotypes were not associated with HB risk in patients at stage I+II or stage III+IV.

![Table 1. Frequency distribution of selected variables in hepatoblastoma patients and controls](http://www.jcancer.org)

**Table 1. Frequency distribution of selected variables in hepatoblastoma patients and controls**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n = 213)</th>
<th>Controls (n = 958)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age range, months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>23.62 ± 24.36</td>
<td>23.75 ± 18.30</td>
<td>0.105</td>
</tr>
<tr>
<td>&lt;17</td>
<td>114</td>
<td>454</td>
<td>0.105</td>
</tr>
<tr>
<td>≥17</td>
<td>99</td>
<td>504</td>
<td>0.5261</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.973</td>
</tr>
<tr>
<td>Female</td>
<td>84</td>
<td>379</td>
<td>0.5936</td>
</tr>
<tr>
<td>Male</td>
<td>129</td>
<td>579</td>
<td>0.6044</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>42</td>
<td>19.72</td>
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</tr>
<tr>
<td>II</td>
<td>55</td>
<td>25.82</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>40</td>
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<tr>
<td>NA</td>
<td>61</td>
<td>28.64</td>
<td></td>
</tr>
</tbody>
</table>

* Based on a two-sided χ² test for distributions between hepatoblastoma patients and cancer-free controls.

**Discussion**

Overall, this first hospital-based case-control study of its kind indicates that the TP53 gene rs1042522 C>G polymorphism may not be associated with HB susceptibility in Chinese children.

HB is a malignant embryonic tumor and is the most common hepatic malignancy in children [37]. Although the overall incidence is relatively low, with an annual incidence rate of confirmed cases of 1 in 1,000,000 children up to 18 months old [38], recent studies indicate an increasing trend. In particular, the number of HB cases doubled from 1975 to 2009, and has increased by about 4% per year from 1992 to 2004, representing an emerging threat to children's health [39].

Inactivation of the TP53 tumor suppressor gene is associated with an increased risk of a variety of malignant tumors [40]. Specifically, TP53 gene polymorphisms have been associated with the susceptibility to many cancers [32]. Moreover, rare variants in TP53 gene also contribute to childhood neuroblastoma susceptibility [41]. The rs1042522 C>G polymorphism of TP53 is the most extensively studied and of paramount importance for cancer risk [33]. Although the polymorphism results in the same basic structure of the TP53 protein as the wild-type, the molecular biological behavior and functions are different [42].
The polymorphic TP53 Pro-type protein shows stronger transcriptional activity than the wild-type TP53 Arg protein and up-regulates downstream gene expression [43]. By contrast, the Arg-type TP53 protein not only has a stronger inhibition function on transformed cell growth than TP53 Pro but also exhibits a stronger function of promoting cell apoptosis and repairing cell damage [44]. In addition, the TP53 Arg-type protein is easily degraded by the high-risk HPV E6 protein, whereas the TP53 Pro-type protein is not degraded by E6 [45].

With this increased understanding of the different biological functions of TP53 Pro and TP53 Arg proteins, many studies have demonstrated that the rs1042522 C>G polymorphism was associated with increased susceptibility to various cancers, including breast cancer, lung cancer, thyroid cancer, and cervical cancer [29, 46-48]. The first TP53 Arg/Pro polymorphism with cancer susceptibility were discovered in 1998, in which Rosenthal et al. [49] found that British women that were homozygous for TP53 Arg tended to have a 7-fold increased risk of cervical cancer compared with those harboring the heterozygous TP53 Pro/Arg and homozygous TP53 Pro forms.

In the present study, we genotyped 213 HB patients and 958 cancer-free controls from four different hospitals across China to evaluate the association between the TP53 gene rs1042522 C>G polymorphism and HB susceptibility. Although our overall results suggest no association, it is important to consider that HB is a multi-factorial disease resulting from multiplicative interactions between environmental factors and genetic backgrounds. Thus, a main limitation of this study is the lack of available information on some valuable parameters such as parental exposure, dietary intake, and living environment. Selection bias is another obvious potentially confounding factor, as the study population certainly is not representative of the whole Chinese population.

Moreover, the analysis of this study is limited by at least the three following points. First, the number of HB patients included in the study is relatively small, which could have weakened the statistical power. Second, we only focused on one polymorphism, and thus the potential associations of other known polymorphisms of the TP53 gene with HB risk, alone or in combination, should be investigated. Third, as mentioned above, the interaction of environmental factors with the polymorphism was not addressed. Thus, to better elucidate the role of the TP53 polymorphism with HB susceptibility, future study designs should try to avoid these shortcomings as much as possible.

Despite these limitations, this study represents the largest case-control study conducted to date to explore the correlation between the TP53 rs1042522 C>G polymorphism and HB risk in the Chinese population. We found no such risk, pointing to a need for further validation of this association in other populations, as well as in other forms of pediatric cancers. Moreover, further investigations of polymorphisms that might mediate the risk of HB would help gain a better understanding of the pathogenesis and improve prognosis in the face of the increasing incidence of this otherwise rare malignancy in children.

**Abbreviations**

HB, hepatoblastoma; SNP, single nucleotide polymorphism; Arg, arginine; Pro, proline; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval.
Supplementary Material


Acknowledgements

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Competing Interests

The authors have declared that no competing interest exists.

References