RESEARCH ARTICLE

Hepatitis B Virus Gene C1653T Polymorphism Mutation and Hepatocellular Carcinoma Risk: an Updated Meta-analysis

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Abstract

Although there have been many studies investigating possible associations between the C1653T mutation and risk of HCC, the results have been inconsistent. We conducted searches of the published literature in Pubmed and Embase databases up to January 2013. Seventeen studies with a total of 1,085 HCC cases and 1,365 healthy controls were retrieved.We found a significant association between the C1653T mutation and HCC risk (OR = 2.01, 95% CI= 1.49–2.70). In the subgroup analysis by ethnicity, a significant association was also found in Asians (OR = 2.07, 95% CI= 1.71–2.51). In subgroup analysis by HBV genotype, B and C were linked with development of HCC (B:OR = 2.21, 95% CI= 1.13–4.34; C:OR = 2.26, 95% CI= 1.61–3.16). However, no significant association was found between the C1653T mutation and HCC risk in HBeAg positive cases. In conclusion, this meta-analysis suggests that the C1653T mutation may be associated with susceptibility to HCC.

Keywords: HBV - C1653T mutation - HCC - meta-analysis

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Introduction

Hepatocellular carcinoma(HCC) is a malignant tumor composed of cells resembling hepatocytes which ranks the sixth most common neoplasm and the third most frequent cause of cancer death (Ferlay et al., 2010). It had an estimated global incidence of more than 500,000 cases in the year 2000, and its incidence is rising in many countries.Most cases of HCC (80%) arise in eastern Asia and sub-Saharan Africa, where the main risk factors for HCC are alcoholism, hepatitis B, hepatitis C, aflatoxin, cirrhosis of the liver, hemochromatosis, wilson's disease and type 2 Diabetes (Parkin et al., 2000). However, The most frequent risk factor for HCC is chronic HBV infection, which accounts for more than 50% of all cases (Sherman et al., 2010).

Although a considerable amount of research has been conducted, the molecular basis of HBV-related hepatocarcinogenesis remains unknown (Brechot et al., 2004). Several studies have examined mutations within the HBV genome that may be associated with HCC. HBV contains an incomplete double-stranded DNA genome consisting of four main regions: the PreS/S region (nucleotides or nt 2854–155), the enhancer II (EnhII; nt 1636–1744) region, basal core promoter (BCP, nucleotides 1751–1769) region and the precore region. It has been demonstrated that mutations in the HBV genome which is pertinent to HCC invariably occur at the PreS region, EnhII, BCP and precore region.

The C1653T mutation in the EnhII region of the core promoter that converts histidine at amino acid 94 of the HBx protein to tyrosine (Shinkai et al., 2007; Yuen et al., 2008). Since then, numerous studies have confirmed the association between C1653T mutation and HCC. However, the results have been inconsistent. Up to now, there has been only one published meta-analysis article focusing on the C1653T mutation,and the conclusion is that C1653T is associated with an increased risk of HCC (Liu et al., 2009). With much more studies focusing on the correlation of C1653T mutation with HCC,we update the meta-analysis and product a subgroup analysis to give a more comprehensive understanding on C1653T mutation with HCC risk.

Materials and Methods

Literature search strategy

We searched Pubmed and Embase databases by two reviewers (Huaping Shi and Xinyou Xie) to retrieve papers linking C1653T polymorphism and HCC risk available by January 2013 without language restrictions, using the following key words: "HBV", "C1653T", "liver cancer", "polymorphism", "single nucleotide polymorphism", "genetic polymorphism", "HCC" and "hepatocellular carcinoma". The reference lists of major textbooks, reviews, and included articles were identified through manual searches to find other potentially eligible studies. Inclusion and exclusion criteria

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Table 1. Characteristics of the Included Studies for Meta-analysis

Study included	Year	Area	Race	Detection method	Mutations /Cases		Mutations /Controls		HBV genotype	HBeAg
Cho EY1	2011	Korea	Asian	sequence	28	69	28	125	-	-
Choi CS 2	2009	Korea	Asian	sequence	21	42	15	46	C	-
Fang ZL3	2008	China	Asian	sequence	04	30	08	30	-	positive
Guo XY4	2008	China	Asian	nested-PCR	22	58	20	71	-	positive
Ja KK5	2009	Korea	Asian	sequence	12	135	03	135	C	-
Jang JW6	2012	Korea	Asian	sequence	46	75	19	75	C	-
Qu LS7	2011	China	Asian	sequence	42	134	19	114	B, C	-
Sakamoto T8	2006	Philippine	Asian	sequence	08	31	05	69	A, B, C	positive
Shinkai N9	2007	Japan	Asian	sequence	45	80	24	80	C	-
Tanaka Y10	2006	Japan	Asian	sequence	58	148	44	180	C	-
Tangkijvanich P11	2010	Thailand	Asian	sequence	16	60	07	60	B, C	-
Tatsukawa M12	2011	Japan	Asian	sequence	20	40	06	52	C	-
Wang ZH13	2007	China	Asian	PCC	09	47	03	164	B, C	positive
Welschinger R14	2010	South Africa	African	PCC	09	84	10	50	A, B, C, D, F	positive
Yuan J15	2007	China	Asian	PCC	02	08	09	59	B, C	positive
Zhang KY16	2007	Japan	Asian	PCC	12	24	11	20	B, C	-
Zhu Y17	2010	China	Asian	PCC	11	20	01	35	B, C	positive

Studies were included in this meta-analysis if they met the following criteria: i) case-control studies that addressed HCC cases and healthy controls; ii) studies that evaluated the association between C1653T polymorphism and HCC risk; and iii) studies that included sufficient data for extraction.Studies were excluded when: i) not case-control studies that evaluated the association between C1653T polymorphism and HCC risk; ii) case reports, letters, reviews, meta-analysis, and editorial articles; iii) studies that were based on incomplete data and those with no usable data reported and duplicate data.

Data extraction

Using a standardized form, data from published studies were extracted independently by two reviewers (Huaping Shi and Xinyou Xie) to populate files with the necessary information. The following information was extracted from each of the included articles: first author, year of publication, country, ethnicity,detection method, number of cases and controls, HBV genotype and HBeAg status.For conflicting evaluations, agreement was reached following discussion among the authors.

Statistical analysis

Meta-analysis was performed using the STATA package version 12.0 (Stata Corporation, College Station, TX, USA). The strength of the associations between C1653T polymorphism and HCC risk was estimated by odds ratio (OR) and 95% confidence interval (95%CI). Between study heterogeneities were estimated using the I^2 test. I^2 values of 25, 50 and 75% were defined as low, moderate and high estimates, respectively. When $I^2 > 50\%$ indicated heterogeneity across studies, the random effects model was used for meta-analysis, or else the fixed effects model was used. Subgroup analysis based on ethnicity, genotype and HBeAg were used to explore and explain the diversity among the results of different studies.Sensitivity analysis was mainly performed by sequential omission of individual studies. Publication bias was investigated by Begg's funnel plot ($P<0.05$ was considered statistically significant).

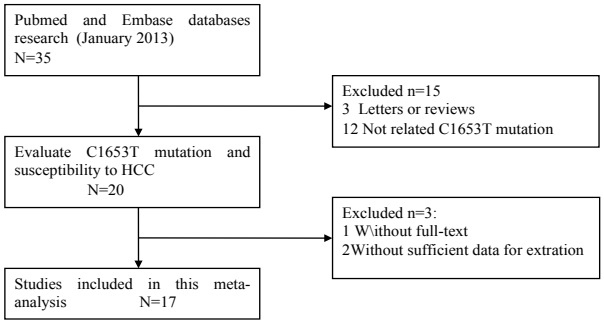

Figure 1. Flow Diagram of Study Searching and Selection Process

Results

Studies included in the meta-analysis

The search strategy retrieved 35 potentially relevant studies. Base on the inclusion criteria, only 17 case-control studies (Sakamoto et al., 2006; Tanaka et al., 2006; Shinkai et al., 2007; Wang et al., 2007; Yuan et al., 2007; Zhang et al., 2007; Fang et al., 2008; Guo et al., 2008; Jang et al., 2008; Choi et al., 2009; Ja et al., 2009; Tangkijvanich et al., 2010; Welschinger et al., 2010; Zhu et al., 2010; Cho et al., 2011; Qu et al., 2011; Tatsukawa et al., 2011) with full-text were included in this meta-analysis and 18 studies were excluded. The flow chart of study selection is summarized in Figure 1. These seventeen selected case-control studies included 1085 HCC cases and 1365 healthy controls. All studies were case-control studies that evaluated the association between C1653T mutation and HCC risk. All the articles were written in English. The baseline characteristics of all included studies are summarized in Table 1. Of these studies, sixteen reported on Asians, and one reported on Africans.

Main results, subgroup and sensitivity analysis

A summary of the meta-analysis findings of the association between C1653T mutation and HCC risk is shown in Table 2. Results showed that C1653T correlated with an increased risk of HCC (Figure 2,OR = 2.01, 95%CI= 1.49–2.70). In subgroup analysis by ethnicity,

Table 2. Summary of the Odds Ratio and its 95% Confidence Interval in the Meta-analysis

Subgroup	No. of	Sample size		Type of	Test of heterogeneity		Test of association		Test of publication bias	
	study	Case	Control	model	I^2	*P*	OR	95% CI	z	*P*
Overall	17	1085	1365	Random	50.9%	0.01	2.01	1.49-2.70	0.30	0.76
Subgroup analysis by ethnicity										
Africans	1	84	50	/	/		0.54	0.20-1.41	/	
Asians	16	1081	1315	Fixed	41.3%	0.04	2.07	1.71-2.51	1.58	0.12
Subgroup analysis by genotype										
Genotype A	2	115	119	Random	82.9%	0.02	1.34	0.21-8.54	0.00	1.00
Genotype B	8	408	571	Random	66.2%	0.01	2.21	1.13-4.34	1.11	0.27
Genotype C	14	928	1139	Random	51.7%	0.01	2.26	1.61-3.16	1.97	0.05
Subgroup analysis by HBeAg										
HBeAg positive	7	278	478	Random	73.8%	0.00	2.05	0.83-5.02	1.20	0.23
HBeAg negative	0	/	/	/	/	/				

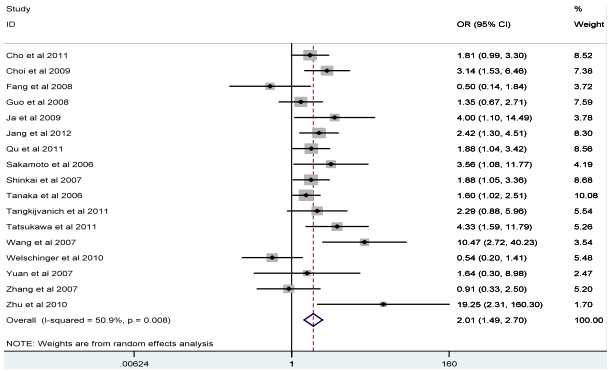

Figure 2. Meta-analysis of the Relationship Between the C1653T Mutation and HCC Risk

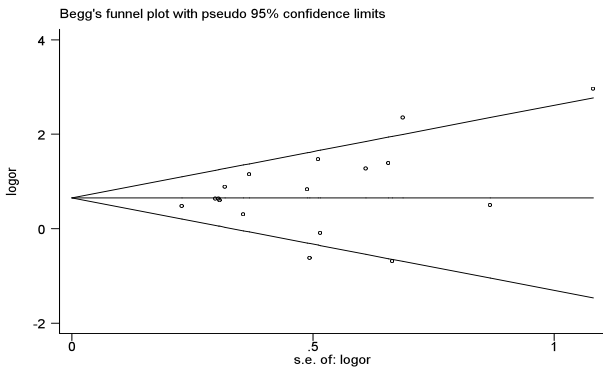

Figure 3. Begg's Funnel Plot Test of Publication Bias for the Association the C1653T Mutation and HCC

the studies included were divided into Asians and Africans populations, a significant association was found between C1653T mutation and HCC risk in Asians (OR = 2.07, 95%CI= 1.71–2.51). While only one study (Welschinger et al., 2010) focused on the Africans and no correlation was found. In subgroup analysis by HBV genotype, HBV genotype B and C are with development of HCC (Genotype B:OR = 2.21, 95%CI= 1.13–4.34; Genotype C:OR = 2.26, 95%CI= 1.61–3.16). And in subgroup analysis by HBV genotype, no significant association was found between C1653T mutation and HCC risk in HBeAg positive.

Sensitivity analysis was performed by sequential omission of individual studies. The significance of pooled ORs in all individual and subgroup analyses was not influenced excessively by omitting any single study. The publication bias of the meta-analysis of the association between C1653T mutation and HCC risk was detected by Begg's funnel plot, all graphical funnel plots of the included studies appeared to be symmetrical. There was no evidence of publication bias visually from the funnel plot which implied that the publication bias was low in the present meta-analysis (Figure 3).

Discussion

Although several studies have evaluated the association between C1653T mutation and HCC, the association remains poorly understood. Our meta-analysis quantitatively assessed the association between C1653T mutation and susceptibility to HCC.Finally, 17 case-control studies were included and assessed, involving a total of 1085 HCC cases and 1365 healthy controls. The main meta-analysis results showed that significant associations between C1653T mutation and HCC risk. In the subgroup analysis by ethnicity, a significant association was found between C1653T mutation and HCC risk in Asians and no correlation was found in Africans, suggesting a possible role of ethnic differences in genetic backgrounds and the environment in which they lived. Genotypes B and C are found commonly in Asia,and genotype C causes more-serious liver disease than genotype B(Sakurai et al., 2004). In subgroup analysis by HBV genotype, HBV genotype B and C are with development of HCC (Genotype B:OR = 2.21, 95%CI= 1.13–4.34; Genotype C:OR = 2.26, 95%CI= 1.61–3.16), this studied results was consistent with preiously published studies that Genotype C is associated with more severe and rapid progress of liver disease compared to genotype B (Chu et al., 2002; Chan et al., 2004). HBeAg positivity may be one of independent predictive factor for HCC And in the subgroup analysis by HBeAg status, our results suggest that HBeAg-positive C1653T mutation may be not involved with the susceptibility of HCC.

The mechanism of how C1653T mutation relates to HCC risk is still unclear. HBx, the nonstructural regulatory protein of HBV, has been strongly associated with the development of liver cancer in some HBx-transgenic mouse strains or with increased progression to liver cancer in other toxin-exposed HBx-transgenic mouse strains. The T1653 mutation resulted in a histidine-totyrosine amino acid substitution at codon 94 of the X protein,which is the center of the immunodominant antigenic domain of amino acids (aa) 85 to 110 (Stemler

et al., 1990). Indeed, codon 94 (nt 1653 to 1655) is within the function domain of the X protein, which has been reported to play a central role in transactivation (Kumar et al., 1996). And the C1653T mutation might enhance the binding affinity of related factors and enhancer II/core promoter activity, cause activation of an indolent immune response, as well as abrogates both the antiproliferative and transactivation effects of wildtype HBx (Lee et al., 1998; Sirma et al., 1999).These changes may be involved in hepatocarcinogenesis.

There were also some limitations in our meta-analysis. First, because of incomplete raw data or publication limitations, some relevant studies could not be included in our analysis. Secondly, the small sample size available was not ideal for detecting small genetic effects. Thirdly, we were not able to address all the sources of heterogeneity that existed among studies, although we could have made subgroup stratifications analysis for the limited number of published studies. Finally, the genotype information stratified for the main confounding variables was not available in the original papers and the confounding factors addressed across the different studies were variable.

In conclusion, the previously reported controversial conclusions on the correlation of C1653T mutation with HCC susceptibility may arise from statistical bias, such as limited sample size or some methodological errors. As suggested by our meta-analysis with a total of 17 eligible studies, C1653T mutation appear to act as an independent risk factor of developing HCC, and further studies are needed to determine its contribution to the genesis of hepatocarcinogenesis.

Acknowledgements

The author(s) declare that they have no competing interests.

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