RESEARCH ARTICLE

Association Between TP53 Arg72Pro Polymorphism and Hepatocellular Carcinoma Risk: A Meta-analysis

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Abstract

<u>Background</u>: Previous studies on the association between the TP53 Arg72Pro polymorphism and hepatocellular carcinoma (HCC) risk obtained controversial findings. This study aimed to quantify the strength of the association by meta-analysis. <u>Methods</u>: We searched PubMed and Wangfang databases for published studies on the association between the TP53 Arg72Pro polymorphism and HCC risk, using the pooled odds ratio (OR) with its 95% confidence intervals (95% CI) for assessment. <u>Results</u>: 10 studies with a total of 2,026 cases and 2,733 controls were finally included into this meta-analysis. Overall, the TP53 Arg72Pro polymorphism was not associated with HCC risk (all P values greaterth HCC risk in Caucasians in three genetic models (For Pro versus Arg, OR = 1.20, 95%CI 1.03-1.41; For ProPro versus ArgArg, OR = 1.74, 95%CI 1.23-2.47; For ProPro versus ArgPro/ArgArg, OR = 1.85, 95%CI 1.33-2.57). However, there was no significant association between the TP53 Arg72Pro polymorphism and HCC risk in East Asians (all P values greater than 0.10). No evidence of publication bias was observed. <u>Conclusion</u>: Meta-analyses of available data suggest an obvious association between the TP53 Arg72Pro and HCC risk in Caucasians. However, the TP53 Arg72Pro polymorphism may have a race-specific effect on HCC risk and further studies are needed to elucidate this possible effect.

Keywords: Hepatocellular carcinoma - TP53 Arg72Pro - polymorphism - meta-analysis - ethnicity

Introduction

Liver cancer, which consists predominantly of hepatocellular carcinoma (HCC), is the sixth most common cancer worldwide and the third most common cause of cancer mortality (Jemal et al., 2011, Forner et al., 2012). Besides, HCC is the fastest growing cause of cancer-related deaths in Asians and most HCC cases come from East Asians (Bridges et al., 2011, Kimman et al., 2012). As a complex and multi-factorial process, hepatocellular carcinogenesis is still not fully understood and urgently need further studies (El-Serag, 2011). Currently, hepatitis B virus (HBV), hepatitis C virus (HCV), liver cirrhosis, and exposure to aflatoxin B1 (El-Serag, 2011, Forner et al., 2012) are proven to be major risk factors of HCC (Feng, 2012, Forner et al., 2012). However, most individuals exposing to these risk factors never develop HCC, while many HCC cases are from individuals without those major risk factors, suggesting that hosts' genetic factors also play an important role in hepatocarcinogenesis (Sia and Villanueva, 2011, Feng, 2012). The tumor suppressor p53 is a key player in stress responses and preserves genomic stability by responding to various insults including DNA damage, metabolic stress and oncogene activation (Riley et al., 2008, Reinhardt and Schumacher, 2012). TP53 gene

is a tumor suppressor gene encoding p53 and frequently mutates in various cancers, and those mutations can cause dramatic defects in p53 function and are often hallmarks of most human cancers (Riley et al., 2008, Whibley et al., 2009). A polymorphism at codon 72 of the TP53 gene (Arg72Pro; rs1042522) has been intensively investigated and reported to affect the function of p53 network which is central to the development of cancer (Dumont et al., 2003, Whibley et al., 2009). Many studies investigated the association between TP53 Arg72Pro polymorphism and HCC risk, but reported controversial findings (Yu et al., 1999, Anzola et al., 2003, Leveri et al., 2004, Peng et al., 2004, Yoon et al., 2008, Di Vuolo et al., 2011, Ezzikouri et al., 2011, Sumbul et al., 2012). Meta-analysis was a statistical procedure for combining data from published studies to acquire a precise estimation of the major effect (Stroup et al., 2000). Thus, to quantify the strength of the association between the TP53 Arg72Pro polymorphism and HCC risk, we conducted a comprehensive meta-analysis of published studies.

Materials and Methods

Search strategy and Inclusion criteria

We searched PubMed and Wangfang databases using

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the following search strategy: ('liver tumor' or 'liver cancer' or 'hepatocellular carcinoma') and ('p53' or 'codon 72' or 'Arg72Pro' or 'rs1042522') and ('polymorphism' or 'polymorphisms' or 'mutation' or 'mutations' or 'SNP') for published studies. There was no language limitation. All references cited in the studies were also reviewed to identify additional published articles not indexed in the common database. The inclusion criteria were: (1) case-control studies which evaluated the association between TP53 Arg72Pro polymorphism and HCC risk; (2) used an unrelated case-control design; (3) had available genotype frequency for estimating an odds ratio (OR) with its 95% confidence interval (95%CI). Overlapping study or studies comparing different laboratory methods were all excluded. In studies with overlapping cases or controls, the most recent and/or the largest study with extractable data was included in the meta-analysis. In addition, family-based association studies were excluded because they use different study designs.

Data extraction

Two investigators independently extracted data, and disagreements were resolved through consensus. The following information was extracted from included studies: publishing year, ethnicity, inclusion criteria for HCC patients and controls, demographics, genotyping method, and the genotype distribution of TP53 Arg72Pro polymorphism. The frequencies of the alleles were extracted or calculated for cases and controls. Difference was settled by reaching an agreement between all investigators.

Statistical analysis

We performed a meta-analysis of the association between the TP53 Arg72Pro polymorphism and HCC risk under the allele contrast (Pro versus Arg), homozygote (ProPro versus ArgArg), heterozygote (ArgPro versus ArgArg), recessive (ProPro versus ArgPro/ArgArg), and dominant (ProPro/ArgPro versus ArgArg) models. We calculated the pooled OR with its corresponding 95%CI to assess this possible association. The significance of the pooled OR was determined by the Z test and a P value of less than 0.05 was considered significant. In our study, two models of meta-analysis for dichotomous outcomes were conducted: the random-effects model and the fixed-effects model. The random-effects model was conducted using the DerSimonian and Laird's method (DerSimonian and Laird, 1986), while the fixed-effects model was conducted using the Mantel-Haenszel's method (Mantel and Haenszel, 1959). To assess the between-study heterogeneity, the I^2 statistic were calculated, and I^2 values of 25%, 50%, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively (Higgins et al., 2003). The random-effects model was used to pool the data when obvious heterogeneity existed (I^2 value > 50%). Otherwise, the fixed-effects model was used to pool the data when low heterogeneity existed (I^2 value < 50%). For additional analyses, the cases and controls were subgrouped by ethnicity, and the ethnicity was categorized into Caucasians, East Asians, Africans and others. To validate the credibility of outcomes in this meta-analysis,

sensitivity analysis was performed by sequential omission of individual studies or by omitting studies without high quality. Potential publication bias was assessed by visual inspection of the funnel plot, and an asymmetric plot suggested possible publication bias (Stuck et al., 1998). In addition, we also performed Egger linear regression test at the P < 0.10 level of significance to assess the publication bias (Egger et al., 1997). All analyses were performed using STATA version 12.0 (StataCorp LP, College Station, Texas). A P value < 0.05 was considered statistically significant, except where specified.

Results

Characteristics of included studies

With our search criterion, a total of 39 abstracts were identified from Pubmed and Wanfang databases. After discarding those overlapping records and those records which did not meet the criteria clearly, 14 publications were preliminarily identified for further detailed evaluation (Yu et al., 1999, Anzola et al., 2003, Leveri et al., 2004, Peng et al., 2004, Zhu et al., 2005, Zhu et al., 2005, Zhu et al., 2005, Zhu et al., 2005, Ezzikouri et al., 2007, Yoon et al., 2008, Di Vuolo et al., 2011, Ezzikouri et al., 2011, Xu et al., 2011, Sumbul et al., 2012). After extracting data and reviewing each original paper, 4 publications were further excluded for overlapping data (Zhu et al., 2005, Zhu et al., 2005, Zhu et al., 2005, Ezzikouri et al., 2007). Finally, 10 studies with a total of 2,026 HCC cases and 2,733 controls were finally included into this meta-analysis (Yu et al., 1999, Anzola et al., 2003, Leveri et al., 2004, Peng et al., 2004, Zhu et al., 2005, Yoon et al., 2008, Di Vuolo et al., 2011, Ezzikouri et al., 2011, Xu et al., 2011, Sumbul et al., 2012). Ethnic groups among these studies were as following: 5 from Caucasians (Anzola et al., 2003, Leveri et al., 2004, Di Vuolo et al., 2011, Ezzikouri et al., 2011, Sumbul et al., 2012), and 5 from East Asians (Yu et al., 1999, Peng et al., 2004, Zhu et al., 2005, Yoon et al., 2008, Xu et al., 2011). The number of cases varied from 61 to 507 (mean, 203), and the number of controls varied from 111 to 548 (mean, 273).

Meta-analysis results

Table 1 listed the main results of this meta-analysis. Overall, meta-analyses of total 10 studies showed the TP53 Arg72Pro polymorphism was not associated with HCC risk (For Pro versus Arg, OR = 1.07, 95%CI 0.92-1.23; For ProPro versus ArgArg, OR = 1.00, 95%CI 0.83-1.21; For ArgPro versus ArgArg, OR = 0.99, 95%CI 0.87-1.13; For ProPro versus ArgPro/ArgArg, OR = 1.04, 95%CI 0.72-1.18; For ProPro/ArgPro versus ArgArg, OR = 1.25, 95%CI 0.94-1.67) (Table 1).

Subgroup analyses by ethnicity showed that the TP53 Arg72Pro polymorphism was associated with HCC risk in Caucasians under three genetic models (For Pro versus Arg, OR = 1.20, 95%CI 1.03-1.41; For ProPro versus ArgArg, OR = 1.74, 95%CI 1.23-2.47; For ProPro versus ArgPro/ArgArg, OR = 1.85, 95%CI 1.33-2.57) (Table 1, Figure 1). However, there was no significant association between the TP53 Arg72Pro polymorphism and HCC risk in East Asians (All P values were more than 0.10).

Table 1. Summary of Odds ratios (OR) with 95% Confidence Interval (95C%CI) in this Meta-analysis

| Contrasts	Odds Ratio		Model	Heterogeneity	
	OR[95%CI]	P value		I² (%)	P value
Analyses of total studies					
Pro versus Arg	1.07(0.92-1.23)	0.376	Random	56.90%	0.013
ProPro versus ArgArg	1.00(0.83-1.21)	0.224	Random	60.40%	0.007
ArgPro versus ArgArg	0.99(0.87-1.13)	0.885	Fixed	43.80%	0.067
ProPro and ArgPro versus ArgArg	1.04(0.72-1.18)	0.12	Random	48.40%	0.042
ProPro versus ArgArg and ArgPro	1.25(0.94-1.67)	0.12	Random	57.60%	0.012
Analyses in East Asians					
Pro versus Arg	1.00 (0.83-1.21)	0.995	Random	65.80%	0.02
ProPro versus ArgArg	1.04(0.72-1.52)	0.822	Random	63.60%	0.027
ArgPro versus ArgArg	0.96(0.77-1.20)	0.688	Random	66.70%	0.017
ProPro and ArgPro versus ArgArg	0.97(0.72-1.30)	0.82	Fixed	69.90%	0.01
ProPro versus ArgArg and ArgPro	1.02(0.85-1.22)	0.837	Fixed	35.40%	0.185
Analyses in Caucasians					
Pro versus Arg	1.20(1.03-1.41)	0.019	Fixed	32.10%	0.208
ProPro versus ArgArg	1.74(1.23-2.47)	0.002	Fixed	29.50%	0.225
ArgPro versus ArgArg	0.96(0.77-1.20)	0.729	Fixed	0.00%	0.422
ProPro and ArgPro versus ArgArg	1.09(0.88-1.34)	0.438	Fixed	0.00%	0.414
ProPro versus ArgArg and ArgPro	1.85(1.33-2.57)	<0.001	Fixed	35.10%	0.187
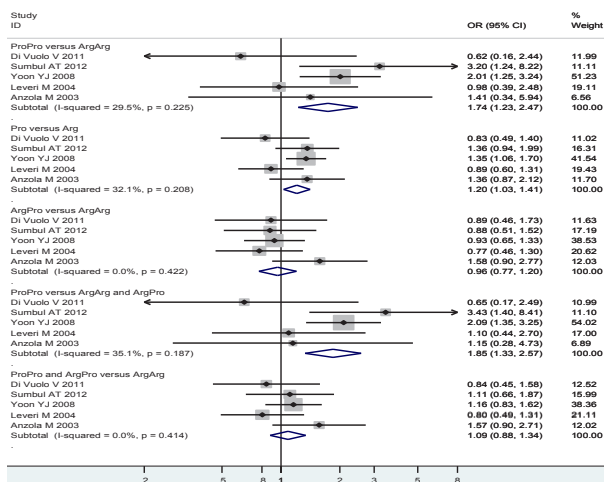
Figure 1. Forest Plot of Pooled OR with 95% CI for TP53 Arg72Pro Polymorphism with HCC Risk in Caucasians
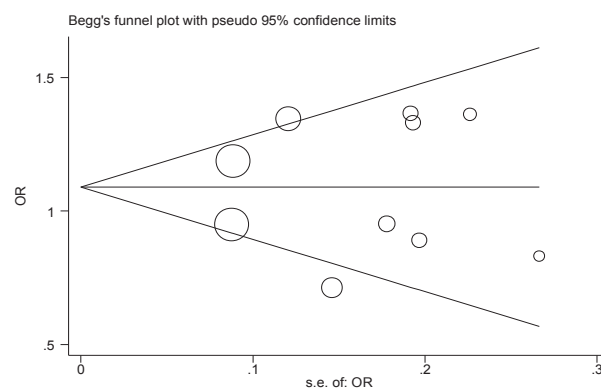

Figure 2. Funnel plot for Publication Bias Test in the Meta-analysis Investigating the Association Between TP53 Arg72Pro and HCC Risk (Pro vs. Arg, Each point represents a separate study for the indicated association, P = 0.944

Publication bias

Funnel plot and Egger's test were performed to assess the publication bias of this meta-analysis. The shape of the funnel plots for most genetic contrast models seemed symmetrical, and all the P values of Egger's tests were more than 0.05, providing statistical evidence of funnel plot symmetry. As was shown in Figure 2, in the meta-analysis investigating the association between the TP53 Arg72Pro and HCC risk under the allele genetic comparison model (Pro vs. Arg), the funnel plot's shape seemed symmetrical, suggesting no presence of publication bias, and the P value of the Egger's test for the allele genetic comparison model was 0.944, providing statistical evidence for funnel plot's symmetry (Figure 2). The results above suggested that publication bias was not evident in our meta-analyses.

Discussion

Although there were many genetic association studies investigating the effect of the TP53 Arg72Pro

polymorphism on HCC cancer risk, the results from those studies were controversial (Yu et al., 1999, Anzola et al., 2003, Leveri et al., 2004, Peng et al., 2004, Yoon et al., 2008, Di Vuolo et al., 2011, Ezzikouri et al., 2011, Sumbul et al., 2012). Those conflicting results may partially due to the relatively small sample size of individual studies and sampling effects, because each of these studies typically involved a few cases and controls. Meta-analysis is a statistical procedure for combining results from published studies to acquire a precise estimation of the major effect (Stroup et al., 2000). One meta-analysis was published to assess the association between the TP53 Arg72Pro polymorphism and HCC risk, but it only included 6 studies and failed to prove an obvious association between the TP53 Arg72Pro polymorphism and HCC risk in Caucasians and Asians (Chen et al., 2011). Besides, several large scale case-control studies have been published to further assess the association between the TP53 Arg72Pro and HCC risk since 2011 (Di Vuolo et al., 2011, Ezzikouri et al., 2011, Xu et al., 2011, Sumbul et al., 2012). Thus, to provide the evidence regarding the association the TP53 Arg72Pro and HCC risk, we conducted a comprehensive meta-analysis of

epidemiological studies investigating this association.

TP53 gene is one of the most widely investigated genes because of its role as a tumor suppressor gene, which plays a key role in the development and progression of cancers (Whibley et al., 2009). It has been proven that TP53 genetic polymorphisms can affect the functions of p53 and TP53 gene variants have drawn increasing attention in the etiology of several cancers (Whibley et al., 2009). Somatic mutation of TP53 that results in the absence or dysfunction of p53 is one of the most common mechanisms by which the p53 pathway is damaged during carcinogenesis (Dumont et al., 2003).TP53 Arg72Pro is a G-to-C polymorphism at the second position of codon 72 in exon 4 resulting in amino acid substitution from Arg to Pro (Dumont et al., 2003). The p53-Arg72 protein is more effective in inducing apoptosis and protecting cells from cancerization than the p53-Pro72 protein, suggesting that the TP53 Pro variant might be a weaker tumor suppressor than its Arg counterpart (Dumont et al., 2003). Thus, there is obvious biological evidence for the different effects on cancer development between the two different variants and the TP53 Pro variant might be a weaker tumor suppressor compared with Arg allele (Dumont et al., 2003).

The present meta-analysis included ten case-control studies with a total of 2026 cases and 2733 controls, providing the most comprehensive assessment of the association between the TP53 Arg72Pro polymorphism and HCC risk up to now. Overall, meta-analyses of total 10 studies showed the TP53 Arg72Pro polymorphism was not associated with HCC risk (All P values were more than 0.10). Subgroup analyses by ethnicity showed that the TP53 Arg72Pro polymorphism was associated with HCC risk in Caucasians under three genetic models (For Pro versus Arg, OR = 1.20, 95CI 1.03-1.41; For ProPro versus ArgArg, OR = 1.74, 95CI 1.23-2.47; For ProPro versus ArgPro/ArgArg, OR = 1.85, 95CI 1.33-2.57). However, there was no significant association in East Asians (All P values were more than 0.10). Thus, meta-analyses of available data suggest an obvious association between the TP53 Arg72Pro and HCC risk in Caucasians, and the TP53 Arg72Pro polymorphism may have a race-specific effect on HCC risk and further studies are needed to elucidate this possible effect. Our meta-analysis suggests an obvious association between TP53 Arg72Pro and HCC risk. Thus, both biological evidence and epidemiological evidence confirm the association between TP53 Arg72Pro polymorphism and HCC risk.

Our analysis had several limitations that should be considered. Firstly, there were only five studies in Asians. More studies with large sample size and careful design are needed to further identify this association in Asians more comprehensively. Secondly, this meta-analysis was based on unadjusted estimates owing to the lack of adjusted estimates. However, a more precise analysis could be performed if adjusted estimates were available in all studies. Besides, serious variability in the study design and the selection of controls was revealed in this meta-analysis. The controls in some studies were selected from non-cancer patients, while the controls in other several studies were just selected from healthy individuals. Finally, misclassification bias was also possible in this meta-analysis, and most studies could not exclude latent cancer cases in the controls.

In conclusion, meta-analyses of available data suggest an obvious association between the TP53 Arg72Pro and HCC risk in Caucasians. TP53 Arg72Pro polymorphism may have a race-specific effect on HCC risk and further studies are needed to elucidate this possible effect.

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