Meta Gene 1 (2013) 126–137

Contents lists available at ScienceDirect

Meta Gene

The association between polymorphism of *P53* codon 72 Arg/Pro and hepatocellular carcinoma susceptibility: evidence from a meta-analysis of 15 studies with 3704 cases☆,☆☆

Surong Hu [a], Lianying Zhao [b], Jingting Yang [a], Miao Hu [a,*]

[a] *Department of Geriatrics, Changzhou NO 2 People's Hospital, Affiliated Hospital of Nanjing Medical University, Changzhou, China*
[b] *Kunshan Agency for Public Health Inspection, Soochow, China*

ARTICLE INFO

Article history:
Received 9 September 2013
Revised 25 September 2013
Accepted 25 September 2013

Keywords:
p53
codon 72
rs1042522
Hepatocellular carcinoma

ABSTRACT

Background: Emerging evidence has shown that *p53*gene participates in human carcinogenesis as tumor suppressors. Polymorphism of *p53* gene codon 72 Arg/Pro (rs1042522) may influence the function of p53 protein and then affect the processing of carcinogenesis. It has been suggested that *p53* codon 72 Arg/Pro polymorphism is associated with susceptibility to hepatocellular carcinoma (HCC). However, published results are inconsistent and inconclusive. To examine the validity of the association between the polymorphism and HCC risk, we performed this meta-analysis.

Methodology/principal findings: We have conducted a search of case–control studies on the associations of *p53* codon 72 polymorphism with susceptibility to HCC in PubMed, ScienceDirect, Bio-Med central, Springer-link, EBSCO, Wanfang databases and Chinese National Knowledge Infrastructure (CNKI) databases. A total of 15 studies were identified with 3704 cases and 4559 controls for codon 72 Arg/Pro polymorphism. The result did support a significant genetic association between Pro allele and susceptibility to HCC in all the genetic models. Similarly, subgroup analysis showed significant associations between the Arg/Pro polymorphism and susceptibility to HCC when stratifying by race, gender, source of controls and hepatitis virus infection status.

Abbreviations: HCC, hepatocellular carcinoma; CNKI, Chinese National Knowledge Infrastructure; HBV, hepatitis B virus; HCV, hepatitis C virus; AFB1, aflatoxin B1; HWE, Hardy–Weinberg equilibrium; CIs, confidence intervals; PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism; PCR–ASP, polymerase chain reaction–allele specific polymerase chain reaction; PCR–SSCP, polymerase chain reaction–Single strand conformation polymorphism analysis; P_H, between-study heterogeneity

☆☆ Grant support: We have no support or funding to report.

* Corresponding author at: Changzhou NO 2 People's Hospital, Affiliated Hospital of Nanjing Medical University, 29 Xinglong Road, Changzhou 213003, Jiangsu Province, China. Tel.: +86 051988132379; fax: +86 051988132769.

2214-5400/$ – see front matter
http://dx.doi.org/10.1016/j.mgene.2013.09.010

Conclusions/significance: This meta-analysis suggests that *p53* codon 72 Arg/Pro polymorphism may be associated with the risk of HCC, especially in subgroup analysis of Asian and Caucasian population, hospital-based population, the female, and the individuals infected with hepatitis virus. However, well-designed studies based on different ethnic groups with larger sample size and more detailed data are needed to confirm these conclusions.

1. Introduction

Hepatocellular carcinoma (HCC), as the most frequent primary cancer of the liver, is the sixth most common cancer and the third most common cause of cancer-related deaths worldwide with about 600,000 deaths every year (Jemal et al., 2011). The major etiologies of hepatocellular carcinoma (HCC) include infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), cigarette smoking, alcohol drinking and aflatoxin B1 (AFB1) exposure (Pogribny and Rusyn, in press). However, not all individuals with HCC have been exposed to the carcinogenic factors mentioned above; although HBV and HCV infections are the major causes of HCC, only a fraction of infected patients develop HCC during their lifetime, which suggests that the etiology of HCC is still not yet clarified (Pogribny and Rusyn, in press). Current studies indicated that genetic factors could also contribute to the etiology of HCC (Pogribny and Rusyn, in press; Dokianakis et al., 2000; Pandith et al., 2012)

The tumor protein p53 (encoded by *P53* gene), as described by many studies, suppresses the cell cycle and induces apoptosis on activation after DNA damage. *P53* gene mutations and polymorphisms have been widely associated with cancer (El-Serag, 2011). A common SNP at codon 72 of *p53* gene (rs1042522), encoding either a proline (Pro) or an arginine (Arg) residue by a transversion of G to C, has been demonstrated to be associated with host's susceptibility to malignant tumors, including HCC (Dokianakis et al., 2000; Pandith et al., 2012; Staib et al., 2003). The mechanism is probably that this polymorphism occurs in the proline-rich domain of p53 protein, which is necessary for the protein to fully induce apoptosis, and it is found that in cell lines containing inducible versions of alleles encoding the Pro and Arg variants, the Arg variant induces apoptosis more markedly than the Pro variant (Dumont et al., 2003).

In recent years, a lot of studies were conducted to investigate the association between *p53* codon 72 polymorphism and HCC susceptibility in humans, but these studies reported conflicting results (Son et al., 2013; Mohana et al., 2013; Ezzikouri et al., 2007; Sumbul et al., 2012; Xu et al., 2011; Yoon et al., 2008; Zhu et al., 2005; Anzola et al., 2003; Yu et al., 1999; Leveri et al., 2004; Di Vuolo et al., 2011; Mah et al., 2011; Yang et al., 2013; Peng et al., 2004; Zhang et al., 2012). Meta-analysis has the advantage of reducing errors by pooling large amount of available data and providing a more precise estimate on cancer susceptibility. The purpose of this meta-analysis is to quantitatively derive more comprehensive and precise estimation of the associations between the *p53* codon 72 polymorphism and susceptibility to HCC.

2. Methods

2.1. Searching

We carried out a publication search in PubMed, ScienceDirect, Bio-Med central, Springer-link, EBSCO, Wanfang databases and Chinese National Knowledge Infrastructure (CNKI) databases with the following search terms: (*p53* Arg72Pro OR rs1042522 OR codon 72) AND ("liver cancer" OR "hepatocellular carcinoma" OR HCC) AND (polymorphism OR mutation OR variation) by two independent investigators (Miao Hu and Lianying Zhao, last search update: May 1th, 2013). Publication country and publication language were not restricted in our research. We examined reference lists manually to further identify potentially relevant studies, and contacted the corresponding authors of conference abstracts without sufficient data to get additional information by e-mail. If the author had

refused to provide the data required in this meta-analysis or we had acquired no reply, the item would be excluded.

All the items matching the inclusion criteria were retrieved for further examination and data extraction. Investigators include experts in biologists, epidemiologist and qualified graduate researchers. All of the investigators have received training in literature search, statistics and evidence-based medicine.

2.2. Selection

We set the following criteria for studies recruited in our meta-analysis: (a) evaluated the associations between *p53* codon 72 polymorphism and susceptibility to HCC, (b) studied on human beings, (c) study designed as case–control, (d) there was sufficient data for the computation of odds ratios and corresponding 95% confidence intervals (ORs, 95% CIs) and (e) if more than one article was published by the same author using the same case series, we selected the study with the largest series. We assessed the methodological qualities of included studies by the description of title, author, year, country, ethnicity, source of sample, the set of controls and cases, value of Hardy–Weinberg equilibrium (HWE), and factors of risk.

2.3. Data extraction

Two investigators (Miao Hu and Lianying Zhao) screened titles, abstracts and full texts independently using a standardized screening guide. Data extraction was carried out independently after the concealment of titles, authors, journals, supporting organizations and funds to avoid investigators' bias. After the data abstraction, discrepancies and differences were resolved by consultation and discussion. Characteristics of the enrolled studies were assigned in the structured form (Table 1), including first author's name, publication time, ethnicity, study country origin, genotyping method, total numbers of cases and controls and genotype frequencies of cases and controls. The two investigators (Miao Hu and Lianying Zhao) checked the data extraction results and reached consensus on all of the data extracted. If different results were generated, they would check the data and have a discussion to come to an agreement. Two senior investigators (Surong Hu and Jingting Yang) would be invited to the discussion if disagreement had still existed.

2.4. Quantitative data synthesis

For each study, the departure of frequencies of *p53* codon 72 polymorphism from expectation under Hardy–Weinberg equilibrium (HWE) was assessed by $\chi 2$ test in controls. The strength of the association between *p53* codon 72 polymorphism and HCC risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The pooled OR with the corresponding 95% CI was used to estimate the strength of the association between *p53* codon 72 Arg/Pro polymorphism and HCC risk by the contrasts of Arg vs Pro, additive model (ArgArg vs ProPro, ArgPro vs. ProPro), dominant model (ArgArg/ArgPro vs. ProPro), and recessive model (ArgArg vs ArgPro/ProPro) respectively. The significance of pooled OR was determined by Z-test ($P < 0.05$ was considered statistically significant). Statistical heterogeneity among the studies was checked by chi-square-based Q-test. A P value greater than 0.10 for Q-test indicates no significant heterogeneity existing among studies (Lau et al., 1997), then the pooled OR was estimated by the fixed-effects model; otherwise, if the heterogeneity was significant, the random-effects model would be employed. Sensitivity analysis was carried out by deleting one single study each time to examine the influence of individual data set on the pooled ORs. Publication bias of literatures was assessed using funnel plots and Egger's test. An asymmetric plot suggests a possible publication bias and the P value of Egger's test less than 0.05 was considered representative of statistically significant publication bias (Egger et al., 1997). All of the statistical tests were performed with STATA software version 10.0 (STATA Corporation, College Station, TX, USA).

Table 1
Characteristics of studies included in the meta-analysis.

Author	Year	Source of controls	Ethnicity	Country	Genotyping method	Cases	Controls	HWE	Cases			Controls		
									Arg/Arg	Arg/Pro	Pro/Pro	Arg/Arg	Arg/Pro	Pro/Pro
Myung Su Son	2013	PBC	Asian	South Korea	PCR–RFLP	157	201	0.09	52	88	17	61	110	30
Subramania	2013	PBC	Asian	India	PCR–RFLP	93	93	0.007	67	21	5	75	14	4
Sayeh Ezzikouri	2007	PBC	Caucasian	Morocca	PCR–RFLP	96	222	0.68	52	31	13	129	79	14
Ahmet Taner	2012	HBC	Caucasian	Turkey	PCR–RFLP	119	119	0.92	46	52	21	49	63	7
Yan Xu	2011	PBC	Asian	China	PCR–RFLP	501	548	0.4	152	245	104	162	262	124
Young Joon Yoon	2008	HBC	Asian	Korea	PCR–RFLP	287	296	0.98	110	111	66	124	135	37
Zhong-Zheng Zhu	2005	HBC	Asian	China	PCR–RFLP	507	541	0.39	145	273	89	188	270	83
Monica Anzola	2003	PBC	Caucasian	Spain	PCR–ASP,PCR–SSCP	97	111	0.38	46	47	4	65	42	4
MING-WHEI YU	1999	HBC	Asian	Taiwan	PCR–RFLP	80	328	0.02	28	35	17	112	141	75
Michela Leveri	2004	PBC	Caucasian	Italy	PCR–RFLP	86	254	0.3	46	33	7	122	113	19
Valeria Di Vuolo	2011	PBC	Caucasian	Italy	PCR–ASP	61	122	0.43	38	20	3	71	42	9
Yone-Han Mah	2011	HBC	Asian	Taiwan	PCR–RFLP	93	214	0.24	29	26	38	65	98	51
Yun Yang	2013	HBC	Asian	China	Taqman RT–PCR	350	326	0.64	103	174	73	117	160	49
Peng T	2004	PBC	Asian	China	PCR–RFLP	192	192	0.48	81	69	42	54	91	47
Zhang YY	2012	HBC	Asian	China	Taqman RT–PCR	985	992	0.9	221	501	263	244	498	250

Hardy–Weinberg equilibrium (HWE) was evaluated using the goodness-of-fit chi-square test. P values were presented. $P < 0.05$ was considered representative of a departure from HWE. HBC: Hospital-based case–control study, PBC: Population-based case–control study, PCR–RFLP: polymerase chain reaction–restriction fragment length polymorphism; PCR–ASP: PCR–allele specific polymerase chain reaction; PCR–SSCP: PCR–Single strand conformation polymorphism analysis.

3. Results

3.1. Study characteristics

A total of 126 articles were retrieved after the first search in PubMed, ScienceDirect, Bio-Med central, Springer-link, EBSCO, Wanfang databases and Chinese National Knowledge Infrastructure (CNKI) databases. As shown in Fig. 1, after selection, 15 case–control studies with 3704 cases and 4559 controls fulfilled the inclusion criteria, among them, 10 studies were carried out on Asian population, 5 were on Caucasian population. Characteristics of included studies are summarized in Table 1. Most of the studies

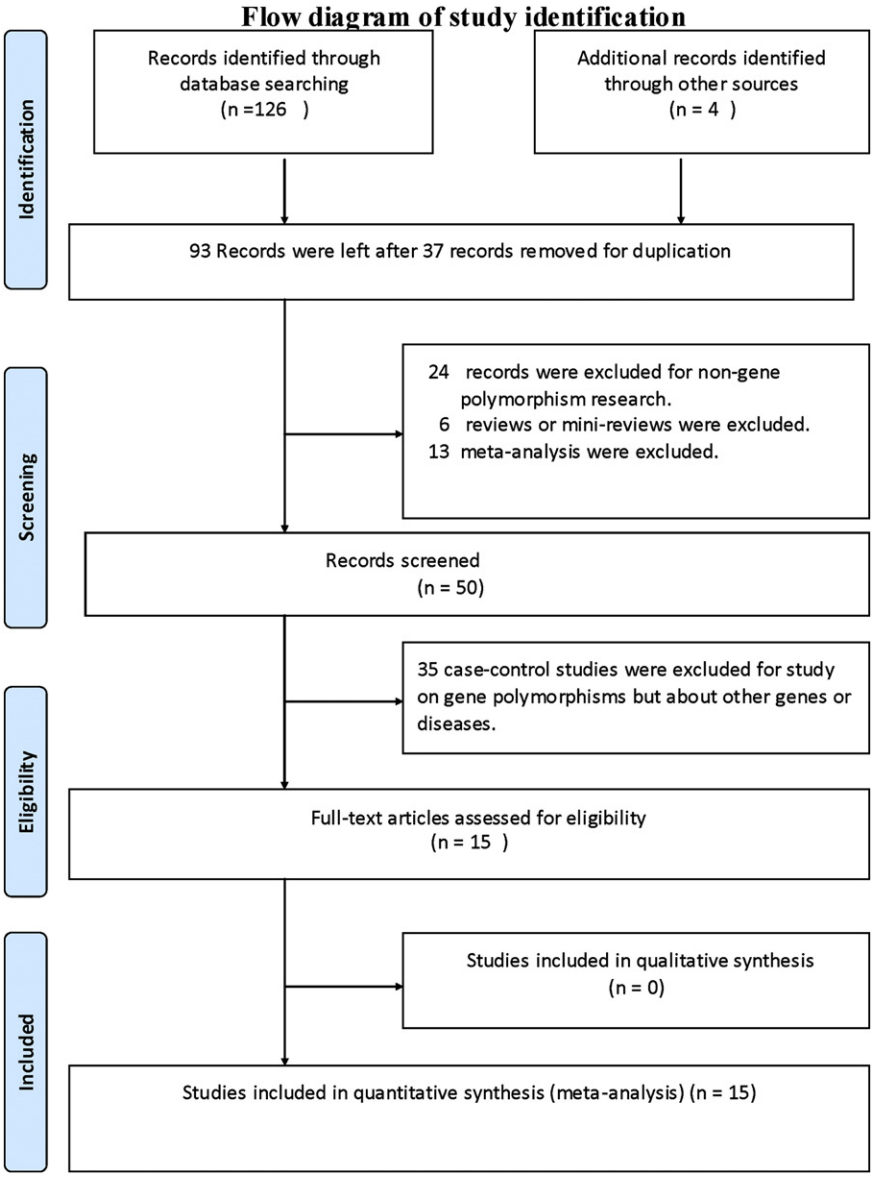

Fig. 1. Flow diagram of study identification.

included in our meta-analysis employed the same genotyping method: PCR–RFLP (polymerase chain reaction–restriction fragment length polymorphism), 2 studies employed the genotyping methods: PCR–ASP (polymerase chain reaction–allele specific polymerase chain reaction) and PCR–SSCP (polymerase chain reaction–Single strand conformation polymorphism analysis). The genotype distribution among controls of all studies agreed with HWE, except the two studies by Myung Su Son (Son et al., 2013) and Yu (Yu et al., 1999). MOOSE checklist was generated to provide detailed description of this meta-analysis (data available when asked).

3.2. Meta-analysis results

3.2.1. The P53 codon 72 Arg/Pro polymorphism and HCC susceptibility in total studies

Overall, there is significant association between *p53* codon 72 Arg/Pro polymorphism and susceptibility to HCC, which could be identified in the following genetic models (Arg vs Pro: OR = 0.37, 95%CI 0.35–0.39, P = 0.0001; ArgArg vs ProPro: OR = 0.83, 95% CI 0.73–0.94, P = 0.005; ArgPro vs ProPro: OR = 0.85, 95% CI 0.75–0.96, P = 0.009; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.85, 95% CI 0.76–0.95, P = 0.004); but there is no significant correlation in the recessive model: (ArgArg vs ArgPro/ProPro: OR = 0.93, 95% CI 0.84–1.02, P = 0.113).

3.2.2. Ethnicity

In the ethnicity subgroup analysis, the pooled ORs indicated that the *p53* codon 72 Arg/Pro polymorphism was significantly associated with an elevated risk of HCC both in Asians and Caucasians under the following genetic models (for Asians, Arg vs Pro: OR = 0.39, 95% CI 0.36–0.41, P = 0.0001; ArgArg vs ProPro: OR = 0.85, 95% CI 0.74–0.98, P = 0.0024; ArgPro vs ProPro: OR = 0.88, 95% CI 0.78–1.00, P = 0.044; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.87, 95% CI 0.78–0.98, P = 0.023; for Caucasian population, Arg vs Pro: OR = 0.27, 95% CI 0.24–0.32, P = 0.0001; ArgArg vs ProPro: OR = 0.61, 95% CI 0.39–0.94, P = 0.025; ArgPro vs ProPro: OR = 0.55, 95% CI 0.35–0.87, P = 0.01; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.57, 95% CI 0.38–0.88, P = 0.01).

3.2.3. Source of controls

In the subgroup analysis by source of controls, among population-based studies, the significant relationship of the *p53* codon 72 Arg/Pro polymorphism with HCC susceptibility was only found in Arg vs Pro genetic models (Arg vs Pro OR = 0.37,95%CI 0.33–0.40,P = 0.0001) (Table 2). Conversely, among the hospital-based studies, the significant relationship was found in all of the genetic models, which suggested that Pro carriers were more susceptible to HCC in hospital-based studies. (Arg vs Pro: OR = 0.37, 95% CI 0.34–0.40, P = 0.0001; ArgArg vs ProPro: OR = 0.71, 95% CI 0.61–0.84, P = 0.0001; ArgPro vs ProPro: OR = 0.79, 95% CI 0.68–0.91, P = 0.001; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.77, 95% CI 0.67–0.88, P = 0.0001, recessive model: ArgArg vs ArgPro/ProPro OR = 0.85,95%CI 0.75–0.96,p = 0.007) (Table 2).

3.2.4. Gender

As shown in Table 2, female carriers of the Pro allele were more susceptible to HCC than males (for females, Arg vs Pro: OR = 0.6, 95% CI 0.37–0.97, P = 0.036; ArgArg vs ProPro: OR = 0.23, 95% CI 0.07–0.77, P = 0.02; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.27, 95% CI 0.08–0.86, P = 0.03) (Table 2).

3.2.5. Hepatitis virus infection status (HVS)

When stratifying by hepatitis virus infection status, we found that individuals with positive hepatitis virus infection was significantly associated with an elevated risk of HCC in most of the genetic contrast models (Arg vs Pro: OR = 0.86, 95% CI 0.76–0.98, P = 0.022; ArgArg vs ProPro: OR = 0.70, 95% CI 0.54–0.90, P = 0.006; ArgPro vs ProPro: OR = 0.7, 95% CI 0.55–0.91, P = 0.006; dominant model: ArgArg/ArgPro vs ProPro: OR = 0.71, 95% CI 0.56–0.89, P = 0.003, recessive model ArgArg vs ArgPro/ProPro OR = 0.91,95%CI 0.76–1.1,p = 0.331) (Table 2). However, the combined ORs for individuals with negative HVS infection in all genetic models did not suggest an association with the risk of HCC (Table 2).

3.2.6. Heterogeneity analysis

The results for heterogeneity analysis are presented in Table 2 in detail. The between-study heterogeneity (P_H) was significant among the total population in all genetic models (Arg vs Pro, $P_H = 0.0001$; ArgArg vs. ArgPro, $P_H = 0.01$; ArgPro vs. ProPro, $P_H = 0.005$; dominant model: ArgArg/ArgPro vs. ProPro $P_H = 0.005$) except the recessive model: (ProPro vs. ArgArg/ArgPro, $P_H = 0.078$) (Table 2). Subgroup analysis by ethnicity suggested that all of the heterogeneity was from the Asian population (Table 2). The heterogeneity was also significant in subgroups of hospital-based studies (Table 2), however, the heterogeneity was not so obvious in other subgroup analyses including gender and hepatitis virus infection status (Table 2). Therefore, the

Table 2
Meta-analysis results for the polymorphism of p53 codon 72 Arg/Pro and HCC risk.

Group	Population	Cases/controls	Genetic model	Pooled OR	[95%CI]	P	P(h-t)
Total	Total	3704/4559	Arg/Pro	0.37	0.35,0.39	0.0001	0.0001
			ArgArg vs ProPro	0.83	0.73,0.94	0.005	0.01
			ArgPro vs ProPro	0.85	0.75,0.96	0.009	0.005
			ArgArg + ArgPro vs ProPro	0.85	0.76,0.95	0.004	0.005
			ArgArg vs ArgPro + ProPro	0.93	0.84,1.02	0.113	0.078
Ethnicity	Asian	3245/3731	Arg/Pro	0.39	0.36,0.41	0.0001	0.0002
			ArgArg vsProPro	0.85	0.74,0.98	0.024	0.012
			ArgPro vs ProPro	0.88	0.78,1.00	0.044	0.009
			ArgArg + ArgPro vs ProPro	0.87	0.78,0.98	0.023	0.008
			ArgArg vs ArgPro + ProPro	0.92	0.83,1.03	0.138	0.034
	Caucasian	459/828	Arg/Pro	0.27	0.24,0.32	0.0001	0.383
			ArgArg vsProPro	0.61	0.39,0.94	0.025	0.22
			ArgPro vs ProPro	0.55	0.35,0.87	0.01	0.22
			ArgArg + ArgPro vs ProPro	0.57	0.38,0.88	0.01	0.191
			ArgArg vs ArgPro + ProPro	0.93	0.74,1.18	0.571	0.423
Source of controls	PBC	1283/1743	Arg/Pro	0.37	0.33,0.40	0.0001	0.0001
			ArgArg vsProPro	1.14	0.90,1.43	0.282	0.248
			ArgPro vs ProPro	1.01	0.81,1.27	0.905	0.506
			ArgArg + ArgPro vs ProPro	1.07	0.87,1.32	0.527	0.455
			ArgArg vs ArgPro + ProPro	1.07	0.92,1.25	0.396	0.054
	HBC	2421/2816	Arg/Pro	0.37	0.34,0.40	0.0001	0.021
			ArgArg vsProPro	0.71	0.61,0.84	0.0001	0.129
			ArgPro vs ProPro	0.79	0.68,0.91	0.001	0.001
			ArgArg + ArgPro vs ProPro	0.77	0.67,0.88	0.0001	0.005
			ArgArg vs ArgPro + ProPro	0.85	0.75,0.96	0.007	0.829
Gender	Male	236/553	Arg/Pro	0.91	0.72,1.16	0.456	0.31
			ArgArg vsProPro	0.72	0.44,1.15	0.17	0.31
			ArgPro vs ProPro	0.66	0.41,1.06	0.083	0.066
			ArgArg + ArgPro vs ProPro	0.71	0.46,1.09	0.117	0.075
			ArgArg vs ArgPro + ProPro	1.02	0.74,1.42	0.9	0.65
	Female	59/116	Arg/Pro	0.6	0.37,0.97	0.036	0.294
			ArgArg vsProPro	0.23	0.07,0.77	0.02	0.99
			ArgPro vs ProPro	0.34	0.1,1.12	0.08	0.58
			ArgArg + ArgPro vs ProPro	0.27	0.08,0.86	0.03	0.77
			ArgArg vs ArgPro + ProPro	0.62	0.32,1.18	0.14	0.2
HVS infection	HVS(+)	951/1334	Arg/Pro	0.86	0.76,0.98	0.022	0.271
			ArgArg vsProPro	0.7	0.54,0.90	0.006	0.314
			ArgPro vs ProPro	0.7	0.55,0.91	0.006	0.241
			ArgArg + ArgPro vs ProPro	0.71	0.56,0.89	0.003	0.22
			ArgArg vs ArgPro + ProPro	0.91	0.76,1.1	0.331	0.557
	HVS(−)	229/603	Arg/Pro	0.8	0.6,1.07	0.139	0.593
			ArgArg vsProPro	0.55	0.27,1.13	0.104	0.641
			ArgPro vs ProPro	0.61	0.29,1.29	0.193	0.479
			ArgArg + ArgPro vs ProPro	0.55	0.28,1.12	0.098	0.604
			ArgArg vs ArgPro + ProPro	0.83	0.58,1.20	0.322	0.621

PBC: Population-based case–control study, HBC: hospital-based case–control study; HVS: hepatitis virus infection, P(h-t): P-value for heterogeneity test. Random-effects model was used when the p-value for heterogeneity test ≤ 0.10, otherwise the fixed-effect model was used. OR, odds ratio; CI, confidence interval.

random-effect model was adopted when the heterogeneity was obvious: $P_H <= 0.1$, otherwise, the fixed-effect model was employed.

3.2.7. Publication bias

Funnel plot and Egger's test were performed to assess the publication bias of the literature (Fig. 2). Symmetrical funnel plots were obtained in the codon 72 Arg/Pro polymorphism tested in all of the models. Egger's test further confirmed the absence of publication bias in this meta-analysis ($P > 0.05$). No evidence of publication bias was observed in any comparison model.

3.2.8. Sensitivity analysis

We deleted one single study from the overall pooled analysis each time to check the influence of the removed data set to the pooled ORs. No study was observed to change the homogeneity in heterozygote comparison (Fig. 2).

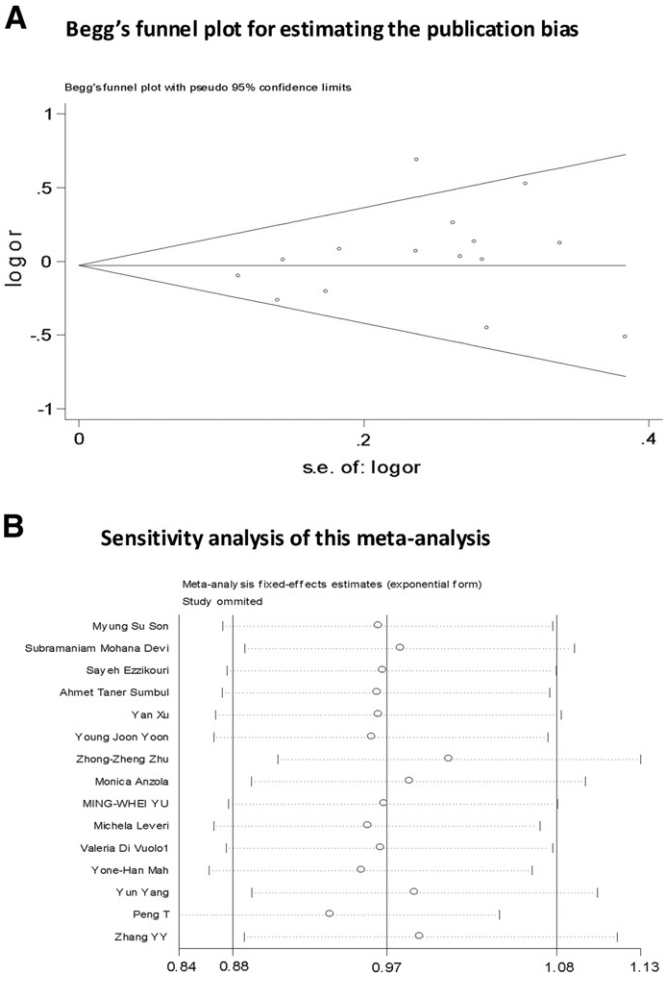

Fig. 2. A. Begg's funnel plot for estimating the publication bias risk in this meta-analysis. Log OR is plotted versus standard error of Log OR for each included study. ($P > 0.05$) Every circle dot represents a separate study for the indicated association by allele contrast. B. Sensitivity analysis of this meta-analysis. This figure shows the influence of individual studies on the summary OR. The middle vertical axis indicates the overall OR and the two vertical axes indicate its 95% CI. Every hollow round indicates the pooled OR when the left study is omitted in this meta-analysis. The two ends of every broken line represent the 95% CI.

4. Discussion

The incidence rates are increasing over all HCC age-adjusted incidence rates tripled between 1975 and 2005, rising from 1.6 per 100,000 to 4.9 per 100,000 (Altekruse et al., 2009). Mutations in the *p53* gene are the most frequently reported somatic gene alteration in human cancer, and *p53* is frequently inactivated in various types of malignant tumors, including HCC (Bressac et al., 1991). Loss of p53 function due to genomic alteration or interaction with viral agents has postulated as a critical step in the development of HCC (Sheen et al., 2003). A common polymorphism located in exon 4 of *P53* gene (rs1042522), resulting in a non-conservative arginine to a proline change at codon 72, has been studied by a lot of researches to figure out the association between the polymorphism and risk for HCC, but the conclusions are inconsistent. The SNP (rs1042522) were investigated using the HapMap Database (http://hapmap.ncbi.nlm.nih.gov/) and HaploView software.

Until recently, a number of studies have been conducted to find the relationship between *p53* codon 72 polymorphism and HCC risk, however, the conclusions are controversial and dubious because of the limited sample size, potential selection bias, and other unsuspected reasons. As a powerful statistical method, meta-analysis can provide a quantitative approach for pooling the results of different researches on the same topic, and for estimating and explaining their diversity. In this study, we made a meta-analysis including 15 case–control studies with 3704 cases and 4559 controls. It is the most comprehensive and accurate research to study the relationship between *p53* codon 72 polymorphism and HCC risk by now.

Overall, our results did support a significant genetic association between Pro allele and susceptibility to HCC in all the genetic models. The underlying mechanisms by which the *P53* codon 72 Arg/Pro polymorphisms influence cancer risk are not fully understood, but the results of our study were consistent with the previous experimental findings: it is likely that the different functions of Arg allele and Pro allele affect cell cycle regulation (Pim et al., 2004), DNA repair capacity (Siddique et al., 2006), apoptosis (Dumont et al., 2003), tumor development (Whibley et al., 2009), tumor progression (Whibley et al., 2009) and consequently influence susceptibility of HCC.

Considering the potential relationship between the well-known risks and HCC, we further made the subgroup analysis stratified by ethnicity, source of controls, gender and hepatitis virus infection status, and found several interesting results:

(1) When stratifying by ethnicity, the meta-analysis suggested that a significant association between the homozygous and dominant genetic models of *p53* codon 72 Arg/Pro and susceptibility of HCC, particularly in Asians and Caucasians. This is not similar to the findings of Chen et al., 2011 and Ding et al. (2012), but supports the conclusion of Lv et al., 2013, as the samples Chen and Ding collected were much less than ours, we consider our results more reliable. Although Arg and Pro alleles occur at different frequencies in various races now (Puente et al., 2006); our research indicated that the Pro allele may have such a strong positive effect on the acceleration of hepatocarcinogenesis in both Asians and Caucasians that it could even conceal the differences between the races.

(2) When stratifying by source of controls, in the hospital-based study, the pro allele could still increase the risk of HCC in all the genetic models, however, in the population-based study, we could not reach the same conclusion. These findings support the conclusion of the previous studies (Puente et al., 2006; Yeh et al., 2010; Xu et al., 2012). Our explanation for the findings is that although the Pro allele of codon 72 is important in the carcinogenesis of HCC, it has to be combined with other potential risk factors that only exist in the hospital-based populations to cause the hepatocarcinogenesis, instead of leading to HCC independently. Nonetheless, the findings should be interpreted with caution for 2 reasons: the hospital-based studies could not represent the whole population; and the random-effects model was employed in the hospital-based studies because the heterogeneity in these studies was significant, which would reduce the accuracy of our conclusions.

(3) When stratifying by gender, for the women, the pro carriers have higher risk of HCC than non-pro carriers, but for the male, there is no significant association between the p53 codon 72 polymorphism and HCC risk, although it is well known that men have a higher incidence of hepatocellular carcinoma (HCC) than women (Yeh et al., 2010), and the recent article suggested that estrogen had an effect on attenuates tumor progression in hepatocellular carcinoma too (Xu et al., 2012). Our explanation for the finding is that Pro allele might play a role with estrogen

synergistically, instead of androgen in the carcinogenesis of HCC, however, the mechanism of the Synergistic effect of estrogen and Pro allele in the carcinogenesis of HCC has not been clarified, it needs to be further investigated in future studies; and this finding may be occasional due to the limited sample sizes in subgroup analysis by gender.

(4) When stratifying by hepatitis virus infection status, we found that in the HVS positive group, patients with Pro allele had a significantly higher risk of HCC than the controls, in contrast, in the HVS negative group, there are no significant differences on the susceptibility of HCC between different genotypes. Our explanation for this phenomenon is that hepatitis virus was implicated in the etiology of P53 mutation and promoted the tumorigenesis of HCC (Hussain et al., 2007; Bréchot, 2004).

4.1. 3 comparisons with other meta-analyses

There are several meta-analyses which have already studied the associations between p53 codon 72 polymorphism and susceptibility to HCC (Puente et al., 2006; Yeh et al., 2010; Xu et al., 2012). However, our updated research is the most comprehensive and accurate study until now as we collected 15 case–control studies with 3704 cases and 4559 controls, more than any other meta-analyses of the same type, and we have reached several conclusions different from others in the subgroup analysis. (1) In the subgroup analysis by ethnicity, we found that the Pro allele would increase the risk of HCC in both Asian and Caucasian subgroup, which is not similar to the findings of Chen et al., 2011 and Ding et al. (2012),as the samples we collected are much more than any other research, we consider our conclusion the most reliable; (2) in the subgroup analysis by hepatitis virus infection, we found that patients with Pro allele had a significantly higher risk of HCC than the controls in HVS positive group, but the same conclusion couldn't be reached in the HVS negative group, which is not similar to the conclusions of previous meta-analyses (Chen et al., 2011; Lv et al., 2013),the probable reason is that the previous meta-analyses and ours have different standards of sample recruitment in the HVS subgroup, for example: we believe that Zhu et al. studied the same population in the series of his researches, so we selected his research with the largest samples (Zhu et al., 2005), however, in the meta-analysis of Lv et al., 2013, he recruited 2 of Zhu's articles, and it would change the conclusion of the meta-analysis, as Zhu's research samples were relatively larger in the meta-analysis. In a word, our results suggest that Arg72Pro genotyping test in the HVS carriers may be of great importance for screening out the patients of HCC.

5. Limitation

However, there are still some limitations in this meta-analysis: (1) there is no research about the sub-Saharan population, which would reduce the comprehensiveness of the meta-analysis in the total population research. (2) Only published studies were included in the meta-analysis, therefore, publication bias might have occurred, even though the use of a statistical test did not show it. (3) Meta-analysis is a retrospective research that is subject to the methodological limitations. (4) Available data for subgroup factors including alcohol consumption, smoking, and exposure to aflatoxin were lacking. These factors may become potential determinants to influence the evaluation of the associations between codon 72 polymorphism and risk of HCC, but we could not explore these potential relationships due to the lack of sufficient data.

In conclusion, this meta-analysis suggests that the *p53* codon 72 Arg/Pro polymorphism may be associated with hepatocellular carcinoma, especially when stratifying by race, gender, source of controls and hepatitis virus infection status. Due to limited number of cases and the insufficient data about the related risks of HCC, larger and well-designed multicenter studies based on different ethnic groups are needed to confirm our results and explore the potential synergistic effects of gene–environment on HCC risk.

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