RESEARCH ARTICLE

CYP2E1 RsaI/PstI Polymorphism and Liver Cancer Risk among East Asians: a Huge Review and Meta-analysis

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Abstract

Published data on any association between the CYP2E1 RsaI/PstI (c1/c2) polymorphism and liver cancer risk among east Asians are inconclusive. The aim of this Human Genome Epidemiology (HuGE) review and meta-analysis was to derive a more precise estimation of the relationship. A literature search of Pubmed, Embase, Web of science and CBM databases from inception through July 2012 was conducted. Twelve case-control studies were included with a total of 1,552 liver cancer cases and 1,763 healthy controls. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association under five genetic models. When all the eligible studies were pooled into the meta-analysis, the results showed that the c2 allele and the c2 carrier (c2/c2 + c2/c1) of RsaI/PstI polymorphism were associated with decreased risk of liver cancer among east Asians (c2 vs. c1: OR = 0.75, 95%CI: 0.59-0.95, P = 0.016; c2/c2 + c2/c1 vs. c1/c1: OR = 0.76, 95%CI: 0.58-1.00, P = 0.050). In the stratified analysis by country, significant associations were observed between RsaI/PstI polymorphism and decreased risk of liver cancer among the Chinese population (c2 vs. c1: OR = 0.70, 95%CI: 0.54-0.91, P = 0.007; c2/c2 + c2/c1 vs. c1/c1: OR = 0.72, 95%CI: 0.54-0.95, P = 0.020), but not among Japanese and Korean populations. Results from the current meta-analysis indicates that the c2 allele of CYP2E1 RsaI/PstI (c1/c2) polymorphism may be a protective factor for HCC among east Asians, especially among China populations.

Keywords: Liver cancer - CYP2E1 - polymorphism - East Asian - susceptibility - meta-analysis

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Introduction

Among primary human liver cancer, hepatocellular carcinoma (HCC) is by far the predominant histological subtype, accounting for 70% to 85% of the total liver cancer incidents (Perz et al., 2006). It has been estimated that 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 and half of these cases and deaths were present in China, which indicated that the liver cancer outbreak was largely attributed to Asians (Jemal et al., 2010). Generally, liver cancer complicates several chronic liver diseases, mainly those induced by hepatitis B virus (HBV) and hepatitis C virus (HCV) (Ruiz et al., 1992). HBV infection has been evaluated to contribute to about 60% of the total liver cancer in developing countries and to about 23% of cancer in developed countries; the corresponding percentages for HCV infection are 33% in developing countries and 20% in developed countries (Parkin, 2006). But chronic HBV or HCV infection is not the only cause for HCC, other environmental risk factors, such as tobacco smoking, alcohol drinking, aflatoxin exposure, physical inactivity and westernized diets, are also related to the susceptibility to liver cancer (McGlynn and London, 2012). Furthermore, despite of environmental factors, a wide range of studies have identified various genetic factors to be implicated in the liver cancer risks, such as LAPTM4B (Wang et al., 2012), COX-2 (He et al., 2011), ERCC1 (Hu et al., 2010) and CYP2E1 (Kato et al., 2003). The pathways of liver cancer development are heterogeneous and influenced by various environmental and genetic factors mentioned above, but the mechanisms of these factors, both individually and in conjunction with viral infection are not well understood. Recently, it has been reported there exists a possible molecular mechanism linking individual genetic differences in cigarette smoking, alcohol consumption and aflatoxin metabolism with the pathogenesis of liver cancer and involving activation and detoxification of chemical carcinogens to their active and carcinogenic metabolites, which may repair the induced DNA damage (Kirk et al., 2006).

The P450 2E1 (CYP2E1) gene, located on chromosome 10q26.3, spanning approximately 11.8 kb in length and consisting of 9 exons, is responsible for encoding a member enzyme of the cytochrome P450 superfamily involved in drug metabolism and is suggested to be associated with the risk of liver cancer (Yu et al., 2002). As one of the key alcohol-metabolizing enzymes induced by ethanol, CYP2E1 accounts for the metabolic activation of carcinogenic N-nitrosamines, polycyclic aromatic hydrocarbons and other low-molecular weight compounds (Lai and Shields, 1999). In addition, various halogenated anesthetics and drugs were identified as

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substrates for CYP2E1 (Tanaka et al., 2000). Thus, the CYP2E1 gene could play an important role of human susceptibility to liver cancer under various exogenous factors. The mechanism of action is hypothesized that some pro-carcinogens and ectogenic compounds can be activated and catalyzed in the first step of metabolism by CYP2E1 and then create reactive metabolic intermediates, which can constitute DNA adducts and lead to genetic mutations (Wang et al., 2008). Several important single nucleotide polymorphisms (SNPs) have been identified in the CYP2E1 gene. The RsaI/PstI polymorphism in the promoter region of CYP2E1 gene has been reported to affect the transcriptional activity of CYP2E1 (Hayashi et al., 1991). To date, the etiology studies have showed that the rare allele possibly performed different effect in liver cancer, in term of the CYP2E1 RsaI/PstI (c1/c2) polymorphism and the risk for developing liver cancer among east Asians, the conclusions of previous studies were not consentaneous. Munaka et al suggested that CYP2E1 RsaI/PstI polymorphism combined with habitual alcohol drinking was likely to lead to an increased risk of liver cancer (Munaka et al., 2003). Similar conclusions were also obtained in several other studies (Zhang et al., 2000; Kato et al., 2003; Sheng et al., 2009). However, there existed some inconsistent results indicating that there was a lack of association between the polymorphisms of CYP2E1 and liver cancer susceptibility among Asian populations (Lee et al., 1997; Yu et al., 2002; Jiang et al., 2004; Wu et al., 2007; Ye et al., 2008). The contradictory findings may due to the inadequate sample size, the neglect of considering interactions between CYP2E1 genotype and different tumor type, and the unadjusted estimates (Chuang et al., 2010). Therefore, we attempt to perform a meta-analysis of all eligible case-control studies to evaluate the relationships between CYP2E1 gene polymorphisms and liver cancer susceptibility, which may shed light on a comprehensive profiling of CYP2E1 gene function and benefit a better understanding of the biological mechanisms associated with liver cancer formation and progression. Such knowledge is promisingly to be further utilized as a diagnostic tool for accurate determination of therapeutic strategies in liver cancer treatment.

Materials and Methods

Literature search

Relevant papers published before July 1, 2012 were identified through a search of Pubmed, Embase, Web of science and CBM databases using the following terms: ("Genetic polymorphism" or "polymorphism" or "SNP" or "gene mutation" or "genetic variants") and ("liver neoplasms" or "hepatic neoplasms" or "liver cancer" or "hepatic cancer" or "liver cancer" or "hepatocellular cancer") and ("cytochrome P-450 CYP2E1" or "CY 2E1" or "CYP IIE1" or "cytochrome P450 2E1" or CYP2E1"). The references of the eligible articles or textbooks were also reviewed to check through manual searches to find other potentially studies. Any disagreement was resolved by discussion between the authors.

Inclusion and Exclusion Criteria

Studies included in our meta-analysis have to meet the following criteria: (1) case-control study or cohort study focused on associations between CYP2E1 RsaI/PstI polymorphism and liver cancer susceptibility; (2) all patients with the diagnosis of liver cancer confirmed by pathological or histological examination; (3) sufficient published data about the size of the sample, odds ratio (OR), and their 95% confidence interval (CI); (4) published in English or Chinese language. Studies were excluded when they were: (1) not case-control study or cohort study; (2) duplicate of previous publication; (3) based on incomplete data; (4) meta-analyses, letters, reviews or editorial articles.

Data Extraction

Using a standardized form, data from published studies were extracted independently by two authors to populate the necessary information. For each study, the following characteristics were collected: the first author, year of publication, country, language, ethnicity, study design, numbers of subjects, source of cases and controls, pathological type, detecting sample, genotype method, allele and genotype frequencies, and evidence of Hardy-Weinberg equilibrium (HWE) in controls. In case of conflicting evaluations, an agreement was reached following a discussion between the authors.

Quality assessment of included studies

Two authors independently assessed the quality of papers according to modified STROBE quality score systems (da Costa et al., 2011; Zhang et al., 2011). Forty assessment items related with the quality appraisal were used in this meta-analysis, scores ranging from 0 to 40. Scores of 0-20, 20-30 and 30-40 were defined as low, moderate and high quality, respectively. Disagreement was resolved by discussion between the authors.

Statistical Analysis

The strength of the association between CYP2E1 RsaI/PstI polymorphism and liver cancer susceptibility was measured by ORs with 95%CIs in allele model (c2 vs. c2), dominant model (c2/c2 + c1/c2 vs. c1/c1), recessive model (c2/c2 vs. c1/c1 + c1/c2), homozygous model (c2/c2 vs. c1/c1), and heterozygous model (c2/c2 vs. c1/c2). The statistical significance of the pooled OR was examined by Z test. Between-study variations and heterogeneities were estimated using Cochran's Q-statistic, and $P < 0.05$ was considered to be manifestation of statistically significant heterogeneity (Higgins and Thompson, 2002). We also quantified the effect of heterogeneity by using I^2 test, which ranges from 0 to 100% and represents the proportion of inter-study variability that can be contributed to heterogeneity rather than by chance (Zintzaras and Ioannidis, 2005). When a significant Q-test ($P < 0.05$) or $I^2 > 50\%$ indicated that heterogeneity among studies existed, the random effects model (DerSimonian Laird method) was conducted for meta-analysis. Otherwise, the fixed effects model (Mantel-Haenszel method) was used. To establish the effect of heterogeneity on the conclusions

Table 1. Characteristics of Included Studies in This Meta-analysis

First author	Year	Country	Language	Number		Source		Gene	Chromosome	SNP	Alias name	Quality scores
				Case	Control	Case	Control					
Kato et al	1995	Japan	English	150	203	HB	HB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	21
Yu et al	1995	China	English	30	150	HB	HB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	26
Lee et al	1997	Korea	English	171	31	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	25
Liu et al	2000	China	Chinese	84	144	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	27
Zhang et al	2000	China	Chinese	30	26	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	31
Yu et al	2002	China	English	248	248	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	23
Kato et al	2003	Japan	English	99	135	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	28
Munaka et al	2003	Japan	English	78	138	HB	HB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	27
Jiang et al	2004	China	Chinese	208	208	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	22
Sheng et al	2007	China	Chinese	91	102	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	26
Wu et al	2007	China	Chinese	63	86	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	29
Ye et al	2008	China	Chinese	300	292	HB	PB	CYP2E1	10	Rsa I (c1/c2)	Pst I/Rsa I (c1/c2)	32

HB, hospital-based; PB, population-based; SNP, single nucleotide polymorphism

Table 2. The Genotype Distribution of CYP2E1 RsaI/PstI (c1/c2) Polymorphism

First author	Year	SNP	Case								Control								HWE test	
			Total	c1	c2	c1/c1	c1/c2	c2/c2	TA	MAF	Total	c1	c2	c1/c1	c1/c2	c2/c2	TA	MAF	P	Test
Kato et al	1995	Rsa I (c1/c2)	15	24	6	10	4	1	30	0.20	203	309	97	120	69	14	406	0.24	0.352	HWE
Yu et al	1995	Rsa I (c1/c2)	30	55	5	25	5	0	60	0.08	150	239	61	95	49	6	300	0.20	0.919	HWE
Lee et al	1997	Rsa I (c1/c2)	171	268	74	104	60	7	342	0.22	31	52	10	23	6	2	62	0.16	0.113	HWE
Liu et al	2000	Rsa I (c1/c2)	84	142	26	60	22	2	168	0.15	144	217	71	80	57	7	288	0.25	0.432	HWE
Zhang et al	2000	Rsa I (c1/c2)	30	52	8	23	6	1	60	0.13	26	34	18	17	9	0	52	0.35	0.009	non-HWE
Yu et al	2002	Rsa I (c1/c2)	131	207	55	83	41	7	262	0.21	134	201	67	77	47	10	268	0.25	0.454	HWE
Kato et al	2003	Rsa I (c1/c2)	93	0	0	57	0	0	186	-	115	0	0	68	0	0	230	-	-	HWE
Munaka et al	2003	Rsa I (c1/c2)	77	0	0	45	0	0	154	-	138	0	0	89	0	0	276	-	-	HWE
Jiang et al	2004	Rsa I (c1/c2)	207	320	94	122	76	9	414	0.23	208	329	87	131	67	10	416	0.21	0.705	HWE
Sheng et al	2007	Rsa I (c1/c2)	91	149	33	58	33	0	182	0.18	102	151	53	49	53	0	204	0.26	0.000	non-HWE
Wu et al	2007	Rsa I (c1/c2)	63	103	23	43	17	3	126	0.18	86	125	47	47	31	8	172	0.27	0.391	HWE
Ye et al	2008	Rsa I (c1/c2)	300	493	107	203	87	10	600	0.18	292	473	111	196	81	15	584	0.19	0.091	HWE

SNP , single nucleotide polymorphism; TA, total alleles; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium

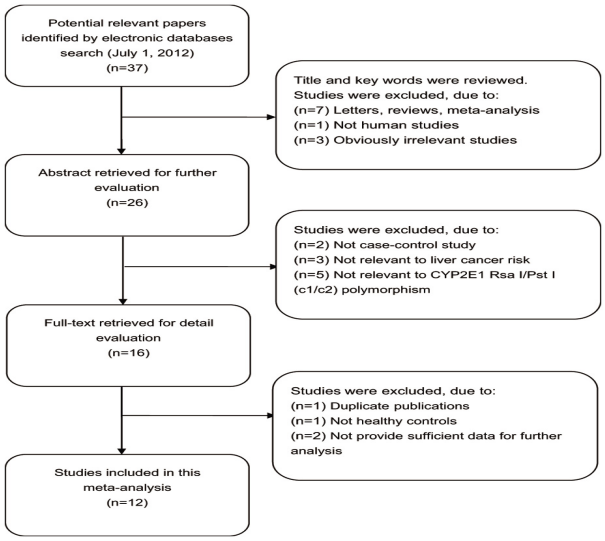

Figure 1. Flow Chart Shows Study Selection Process

of meta-analyses, we also performed subgroup analysis by cancer type, ethnicity, source of controls, HWE test, and genotype method. We tested whether genotype frequencies of controls were in HWE using the χ^2 test. Sensitivity was performed by omitting each study in turn to assess the stability of results. Begger's funnel plots were used to detect publication bias. In addition, Egger's linear regression test which measures funnel plot asymmetry using a natural logarithm scale of OR was used to evaluate the publication bias (Peters et al., 2006). All the P values were two-sided. All analyses were calculated using STATA Version 12.0 software (Stata Corp, College Station, TX).

Results

The characteristics of included studies

According to the inclusion criteria, 12 case-control studies (Kato et al., 1995; Yu et al., 1995; Lee et al., 1997; Liu et al., 2000; Zhang et al., 2000; Yu et al., 2002; Kato et al., 2003; Munaka et al., 2003; Jiang et al., 2004; Wu et al., 2007; Ye et al., 2008; Sheng et al., 2009) were included and 25 articles were excluded. The publication year of involved studies ranged from 1995 to 2008. The flow chart of study selection is shown in Figure 1. In total, 1,552 cases and 1,763 healthy controls were involved in this meta-analysis, which evaluated the relationship between CYP2E1 RsaI/PstI (c1/c2) polymorphism and liver cancer risk among east Asians. All patients fulfilled the diagnosis criteria of liver cancer confirmed by pathological examination of the surgical specimen. Studies had been carried out in Japan, China and Korea. The source of controls was mainly based on a healthy population, of which three (Kato et al., 1995; Yu et al., 1995; Munaka et al., 2003) were hospital-based and nine were population-based. All studies extracted DNA from peripheral blood and PCR-RFLP was used to validate genotype in all studies. All quality scores of included studies were higher than 20 (moderate-high quality). HWE test was conducted on genotype distribution of the controls in all included studies, all of studies performed HWE except two studies (Zhang et al., 2000; Sheng et al., 2009). The characteristics and methodological quality of the included studies are summarized in Table 1. The genotype distribution of CYP2E1 RsaI/PstI (c1/

Table 3. Meta-analysis of the Association Between CYP2E1 Rsa I/Pst I (c1/c2) Polymorphism and Liver Cancer Risk among Asians

Subgroups	c2 allele vs. c1 allele (allele model)				c2/c2 + c1/c2 vs. c1/c1 (dominant model)				c2/c2 vs. c1/c1 + c1/c2 (recessive model)				c2/c2 vs. c1/c1 (homozygous model)				c2/c2 vs. c1/c2 (heterozygous model)			
	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph	OR	95%CI	P	Ph
Overall	0.75	0.59-0.95	0.016†	0.031	0.76	0.58-1.00	0.050†	0.044	0.68	0.45-1.04	0.076	0.987	0.66	0.43-1.01	0.053	0.962	0.74	0.47-1.15	0.18	0.975
Country																				
China	0.7	0.54-0.91	0.007†	0.026	0.72	0.54-0.95	0.020	0.058	0.67	0.43-1.06	0.085	0.947	0.64	0.41-1.01	0.053	0.880	0.75	0.47-1.20	0.232	0.972
Japan	0.8	0.32-2.01	0.629	-	0.72	0.24-2.19	0.566	-	0.96	0.12-7.88	0.973		0.86	0.10-7.20	0.887	-	1.23	0.13-11.87	0.857	-
Korea	1.44	0.70-2.96	0.327	-	1.85	0.78-4.38	0.161	-	0.62	0.12-3.13	0.562	-	0.77	0.15-3.97	0.759	-	0.35	0.06-2.08	0.248	-
Source of control																				
Population-based	0.77	0.60-0.99	0.044†	0.030	0.8	0.60-1.07	0.128†	0.045	0.68	0.44-1.06	0.091	0.959	0.67	0.43-1.04	0.072	0.909	0.72	0.45-1.15	0.167	0.924
Hospital-based	0.54	0.25-1.18	0.123	0.236	0.48	0.23-1.02	0.058	0.336	0.64	0.12-3.45	0.600	0.589	0.53	0.10-2.91	0.466	0.546	0.98	0.16-5.89	0.978	0.763
Language																				
English	0.8	0.50-1.28	0.356	0.155	0.81	0.44-1.47	0.480†	0.098	0.67	0.31-1.43	0.301	0.960	0.64	0.30-1.37	0.252	0.937	0.73	0.32-1.66	0.447	0.828
Chinese	0.71	0.53-0.97	0.030†	0.022	0.74	0.53-1.03	0.072†	0.052	0.69	0.41-1.15	0.149	0.829	0.66	0.40-1.11	0.118	0.721	0.74	0.43-1.26	0.269	0.866
HWE test																				
HWE	0.82	0.64-1.03	0.090	0.087	0.81	0.60-1.10	0.172†	0.045	0.66	0.43-1.02	0.06	0.993	0.64	0.42-0.99	0.043	0.962	0.71	0.45-1.11	0.135	0.994
non-HWE	0.48	0.23-0.99	0.047†	0.151	0.54	0.32-0.90	0.018	0.894	2.7	0.11-69.04	0.549	-	2.23	0.09-58.19	0.629	-	4.39	0.15-125.29	0.388	-

OR, odds ratios; 95%CI, 95% confidence interval; Ph, P value of heterogeneity test; †, estimates for random effects model

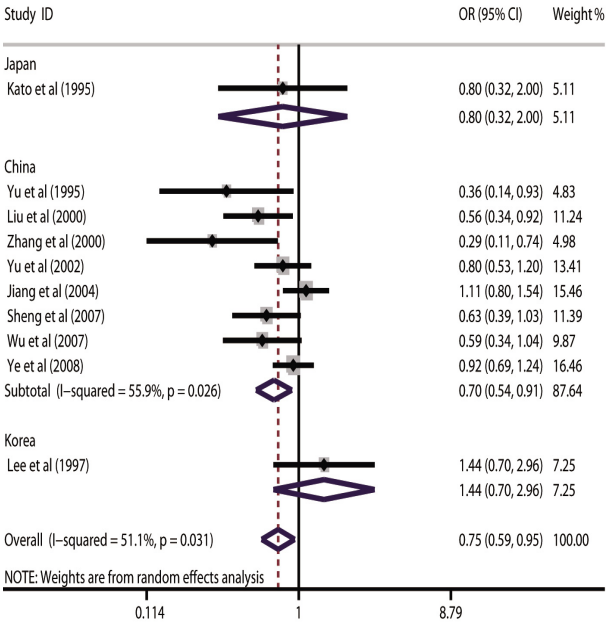

Figure 2. Forest Plot Showed the Association Between CYP2E1 RsaI/PstI (c1/c2) Polymorphism and liver Cancer Risk among East Asians under Allele Model (c2 vs. c1)

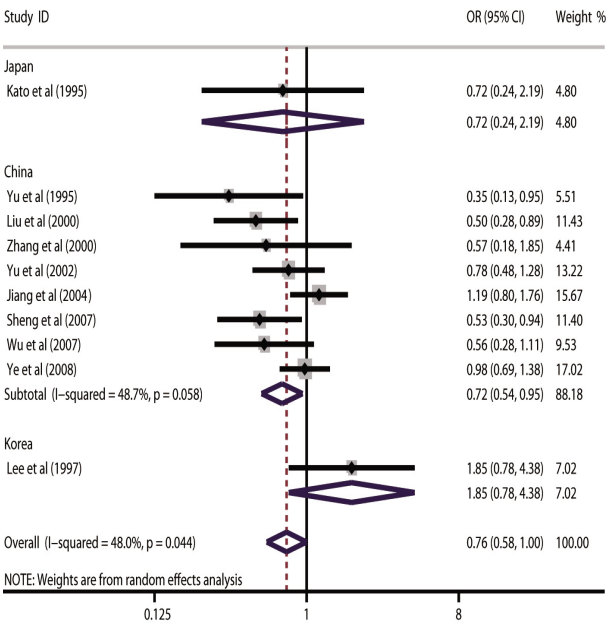

Figure 3. Forest Plot Showed the Association Between CYP2E1 RsaI/PstI (c1/c2) Polymorphism and the Risk of Liver Cancer among East Asians under Dominant Model (c2/c2 + c2/c1 vs. c1/c1)

c2) polymorphism were presented in Table 2.

Association between CYP2E1 RsaI/PstI polymorphism and liver cancer risk among east Asians

A summary of the meta-analysis findings of the association between CYP2E1 RsaI/PstI (c1/c2) polymorphism and liver cancer risk among east Asians is provided in Table 3. The heterogeneity is obvious under allele model and dominant model (all $P < 0.05$), which might result from difference of country, source of controls, language and HWE test, so random effects model was used. The meta-analysis result showed that the c2 allele and the c2 carrier (c2/c2 + c2/c1) of RsaI/PstI polymorphism were associated with decreased risk of liver cancer among east Asians (c2 vs. c1: OR = 0.75, 95%CI: 0.59-0.95, P = 0.016; c2/c2 + c2/c1 vs. c1/c1: OR = 0.76, 95%CI: 0.58-1.00, P = 0.050).

In the stratified analysis by country, significant associations were observed between CYP2E1 RsaI/PstI polymorphism and decreased risk of liver cancer among Chinese (c2 vs. c1: OR = 0.70, 95%CI: 0.54-0.91, P = 0.007; c2/c2 + c2/c1 vs. c1/c1: OR = 0.72, 95%CI: 0.54-0.95, P = 0.020) (Figure 2-3). While we found no statistically significant associations for liver cancer among Japan and Korea populations. According to the different source of controls, we also performed further subgroup analysis. The result indicated that the c2 allele of RsaI/PstI polymorphism might be a protective factor for liver cancer in population-based subgroup under allele model (OR = 0.77, 95%CI: 0.60-0.99, P = 0.044). However, no significant association was found between RsaI/PstI polymorphism and liver cancer risk in hospital-based group. We also performed subgroup analyses based on language and HWE test, results suggested that RsaI/PstI polymorphism might increase the risk of liver cancer in Chinese and non-HWE subgroups, but the result was lack of credibility due to potential bias (Table 3).

Sensitivity analysis

Sensitivity analysis is regarded as an indispensable step for the analysis of various multiple-criteria decision-making problems. It was performed to assess the influence of each individual study on the pooled ORs by omission of individual studies. The analysis results suggested that

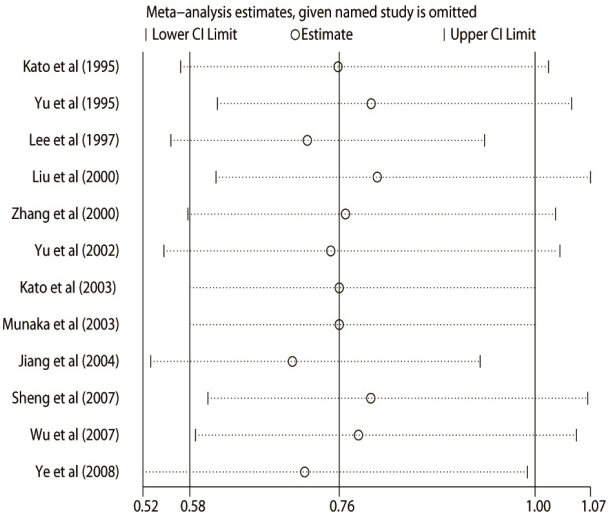

Figure 4. Sensitivity Analysis of the Summary Odds Ratio Coefficients on the Association Between CYP2E1 RsaI/Pst I Polymorphism and Liver Cancer Risk among East Asians. A: allele model (c2 vs. c1); B: dominant model (c2/c2 + c1/c2 vs. c1/c1)

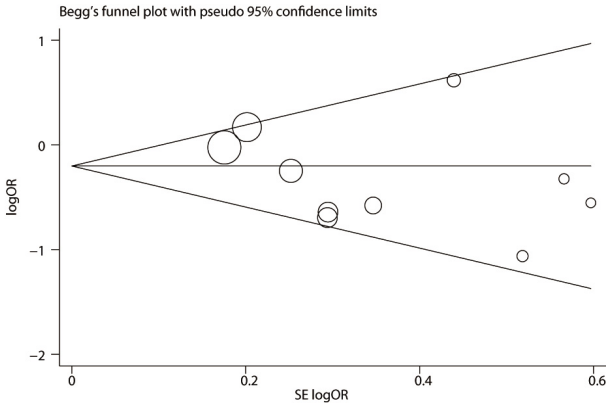

Figure 5. Begger's Funnel Plot of the Meta-analysis of Between CYP2E1 RsaI/Pst I Polymorphism and Liver Cancer Risk among East Asians. A: allele model (c2 vs. c1); B: dominant model (c2/c2 + c1/c2 vs. c1/c1)

no individual study significantly affected the pooled ORs under allele and dominant models of CYP2E1 RsaI/PstI polymorphism (Figure 4), indicating that our results are statistically robust.

Publication bias

Begger's funnel plot and Egger's linear regression test were performed to assess the publication bias of included studies. The shapes of the funnel plots in all genetic models did not reveal any evidence of obvious asymmetry under allele and dominant models of CYP2E1 RsaI/PstI polymorphism (Figure 5). Egger's test also showed that there was no significantly statistical evidence of publication bias for any of the genetic models (P = 0.074 for allele model; P = 0.205 for dominant model; P = 0.866 for recessive model; P = 0.821 for homozygous model; P = 0.307 for heterozygous model).

Discussion

Due to the complex functional mechanism of CYP2E1 in tumorigenesis of liver cancer, growing number of studies suggested that CYP2E1 RsaI/PstI polymorphism played an important role in the development of liver cancer. Unfortunately, the results of these studies have appeared in the literature either supporting or negating the significant association. Previous clinical and genetic study suggested that mutations of CYP2E1 gene may play an important role in cigarette smoking-related liver carcinogenesis (Yu et al., 1995). Further studies revealed that the variations of CYP2E1 RsaI/PstI polymorphism also carried weight in the development of alcohol-related liver cancer (Ye et al., 2008). However, several later studies indicated the lack of evidence for the association between CYP2E1 RsaI/PstI polymorphism and liver cancer susceptibility (Jiang et al., 2004; Wu et al., 2007). To provide a comprehensive and reliable conclusion, we conducted the present meta-analysis of 12 independent case-control studies, including 1,552 liver cancer patients and 1,763 healthy controls. The main results of this meta-analysis confirmed that the c2 allele of CYP2E1 RsaI/PstI polymorphism was a protector for liver cancer risk among east Asians. These results were supported by a previous Japanese study, which indicated that the expression of CYP2E1 in tumor cells tended to decrease as the cells were less differentiated (Hirose et al. 2002). Although the exact function of RsaI/PstI polymorphism in tumorigenesis of liver was not clear yet, a possible reason is that the mutations of RsaI/PstI polymorphism may influence the CYP2E1 enzyme metabolizes and activates toxicological substrates (Cheung et al., 2005). CYP2E1 metabolically activates a large number of toxicants and carcinogens and thus is of great toxicological importance (Gonzalez, 2007). Recent study revealed that inhibition of CYP2E1 would led to significant decreases in high glucose mediated oxidative stress and toxicity (Chandrasekaran et al., 2012). The c2 allele enhanced the ability of CYP2E1 as a principal P-450 responsible for the metabolism of ethanol and a major component of the microsomal ethanol-oxidizing system because ethanol exerts its carcinogenic effect in the liver among others via the induction of CYP2E1 and the generation of carcinogenic etheno-DNA adducts (Lee et al., 1996; Millonig et al., 2011). CYP2E1 potentiates Fas-mediated HepG2 cells toxicity via the induction of oxidative stress to promote apoptosis, too (Yan et al., 2008). Human CYP2E1 also involved in the formation of glycidamide from acrylamide for glycidamide is suspected of being the ultimate carcinogenic metabolite of acrylamide (Settels et al., 2008). In addition, CYP2E1 plays an important role in the metabolize of acetaminophen (APAP), which is a common cause of drug-induced hepatotoxicity if overdosed (Gonzalez, 2007). In the subgroup analysis by country, we observed significant associations between CYP2E1 RsaI/PstI polymorphism and decreased risk of liver cancer among Chinese. However, no association between RsaI/PstI polymorphism and Japanese or Koreans was found. This result was lack of reliability due to the estimation of effect size from a single study. Another influencing factor for this result might be the high incidence of HBV in Chinese population. For chronic hepatitis B virus carriers with high-titer viremia (>10(5) virions/ml) are at increased risk for liver cancer, hepatitis B virus may also impact the function of cytochrome P450 (P450) CYP2E1 enzyme (Harris et al., 2003). What's more, alteration in the

level of CYP2E1 might influence the health effects of the environmental pollutants (Lucas et al. 2001). Therefore the differences of environment in Asian countries might contribute to the distinction of CYP2E1 impact. Different living habits in different ethnicity might be contributed, too. The result of subgroup analysis based on the source of controls showed that the c2 allele of CYP2E1 RsaI/PstI (c1/c2) polymorphism was a protective factor for liver cancer susceptibility in population-based studies. It might be cause by the similarity between the etiopathogenesis of liver cancer patients and other chronic liver disease patients recruited as controls.

In interpreting our results of the current meta-analysis, some limitations need to be addressed. Firstly, the sample size is still relatively small and might not provide sufficient power to estimate the association between CYP2E1 RsaI/PstI polymorphism and liver cancer risk. In addition, the selection bias may exist because of the differences in the source of controls or detection samples. Besides, our meta-analysis was based on unadjusted ORs estimates because not all published presented adjusted ORs or when they did, the ORs were not adjusted by the same potential confounders, such as ethnicity, age, gender, geographic distribution, etc. Even tiny differences of those aspects could be the main cause for the heterogeneity, thereby they would influence the results of the study much further. Nevertheless, it is well acknowledged that many other factors, such as gene-gene or gene-environment interaction may affect the risk of liver cancer. Finally, although all cases and controls of each study were well defined with similar inclusion criteria, there may be potential factors that were not taken into account that may have influenced our results. In spite of these limitations, our meta-analysis still had some advantages. To the best of our knowledge, this is the first meta-analysis of the relationship of CYP2E1 gene polymorphisms and liver cancer risk among east Asians. It is worthwhile to mention that we established perfectly searching strategy based on computer-assisted and manual search, which allowed the eligible studies included as possible as it can. By this means, the quality of studies included in current meta-analysis was satisfactory according to our selection criteria. Besides, explicit methods for study selection, data extraction, and data analysis were well designed before initiating. Last but not the least, there was no evidence of publication bias in this meta-analysis and the sensitivity analysis indicated that the results are statistically robust.

In conclusion, this meta-analysis provides reliable evidence that the c2 allele of CYP2E1 RsaI/PstI (c1/c2) polymorphism may be a protective factor for HCC among east Asians, especially among Chinese populations. Because of the importance of CYP2E1 gene in metabolism of liver, it may display as a prerequisite for marker based molecular therapies. Due to the limitations showed above in this analysis, it is critical that further studies focused on the relationship between the CYP2E1 Rsa I/Pst I (c1/c2) polymorphism and HBV-related HCC are needed.=

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