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Meta-analysis demonstrates lack of a relationship between *XRCC1*-399 gene polymorphisms and susceptibility to hepatocellular carcinoma

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ABSTRACT. *XRCC1*-399 allele polymorphisms have been reported to be associated with susceptibility to hepatocellular carcinoma (HCC), but the conclusions of the various studies have been inconsistent. We conducted a meta-analysis of available studies to determine whether *XRCC1*-399 alleles influence susceptibility to hepatocellular carcinoma. We searched English-language databases, including PubMed, Medline and Embase, using terms such as "hepatocellular

carcinoma" (or "HCC"), "X-ray repair cross-complementing group 1" (or "*XRCC1*") and "genetic polymorphism" (or "SNP"), among others; we also searched Chinese-language databases, including CNKI, VIP, Wanfang Data, and CBM, using terms such as "ganai", "ganxibaoai", "ganzhongliu", "duotaixing", and "X-xian xiufu jiaocha hubu jiyin 1". Eight independent studies, including 1604 HCC cases and 2185 controls, were included. The pooled odds ratio for *XRCC1*-399 was 0.99 (95% confidence interval = 0.75-1.31). We conclude that *XRCC1*-399 gene polymorphisms are unrelated to risk for HCC.

Key words: *XRCC1*; Gene polymorphism; Hepatocellular carcinoma; Meta-analysis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. Ranking third in the world by incidence, it is a significant public health issue (Zhang et al., 2004). Asian countries account for nearly 78% of the roughly 600,000 cases of HCC reported globally each year [International Agency for Cancer Research: GLOBCAN 2002 (http://www-dep.iarc.fr)]. China alone accounts for more than 50% of the cases worldwide (El-Serag and Rudolph, 2007). In recent years, *XRCC1*-399 allele polymorphisms have been reported to be associated with susceptibility to HCC, but the results of previous studies are inconsistent. We conducted a meta-analysis to ascertain whether specific *XRCC1*-399 alleles are associated with susceptibility to HCC.

MATERIAL AND METHODS

Search strategy

To obtain information regarding case-control studies on *XRCC1* polymorphisms and HCC risk conducted from January 2000 to December 2010, we searched English language databases, including PubMed, Medline, and Embase, using terms such as "hepatocellular carcinoma" or "HCC", "X-ray repair cross-complementing group 1" or "*XRCC1*", and "genetic polymorphism" or "SNP", among others. We also searched Chinese language databases, including CNKI, VIP, Wanfang Data, and CBM, using terms such as "ganai", "ganxibaoai", "ganzhongliu", "duotaixing", and "X-xian xiufu jiaocha hubu jiyin 1". We then contacted the authors of studies containing relevant information to enquire if they did not report results necessary for this analysis. Unpublished data were also included if an abstract was available and if further information was obtained from the authors.

Selection criteria

In the meta-analysis, the following selection criteria were defined and reviewed by 2 independent investigators: A) each trial was an independent case-control study; B) the study purpose and statistical methods were similar; C) enough information was available to calculate the odds ratio (OR); D) *XRCC1*-399 alleles were molecularly typed (high- or low-

resolution); and E) patients were included according to the diagnostic standard defined in 2002, which was based on classical histological characteristics or a serum alpha fetoprotein level higher than 400 ng/mL together with radiological findings (ultrasound or CT) consistent with HCC (Befeler and Di Bisceglie, 2002). The following exclusion criteria were defined as follows: A) incomplete raw data, B) repetitive reports (if more than one version of the same study was retrieved, only the most recent version was used), and C) material and methods used were not well described or reliable. Although assessment of study quality is considered important for systematic reviews and meta-analyses, scoring methods have been considered problematic (Conn and Rantz, 2003) and may not accurately assess the quality measures of interest (Huwiler-Muntener et al., 2002). Therefore, we used reliability of the methods for patient selection, molecular typing, and statistical analysis as quality variables.

Data extraction

The studies were independently evaluated by 2 researchers. Discrepancies in the evaluations of some studies were resolved by discussion between the reviewers. The following data were collected from each study: authors, publication year, study area, publication language, source of cases, numbers of cases and controls, matching criteria of the cases and the controls, the Hardy-Weinberg equilibrium test, *XRCC1* genotyping method and allele frequencies, definitions used for HCC, HCC sample description, and control sample description. Allelic frequency was calculated as the number of cases or controls harboring at least 1 allele type divided by the total number of chromosomes included in each of the corresponding groups.

Statistical analysis

Meta-analysis was performed using Review Manager 5.0 and Stata 10.0. Heterogeneity was calculated with the Cochran Q test ($\alpha = 0.05$) and the Higgins (I^2) test (Petitti, 2000). I^2 values of 25, 50, and 75% were assigned as low, moderate, and high estimates, respectively. A pooled OR was presented as a standard plot with 95% confidence intervals (95%CI) (Woolf, 1955). The meta-analysis was performed using fixed- or random-effect methods, depending on the absence or presence of significant heterogeneity. If the results of the Q test had no significant heterogeneity, the Mantel-Haenszel fixed-effect model (Peto method) was used; if the Q test results had significant heterogeneity, the Dersimonian-Laird random-effect model was used (DerSimonian and Laird, 1986). To reduce heterogeneity and to evaluate whether there was a different genotype effect in predefined subgroups of the studies, we performed a meta-regression analysis and a subgroup analysis according to the source of the cases, number of samples (300 samples as the boundary), publication year (2006 as the boundary), and the matching conditions. Funnel plots and the Egger regression asymmetry test were used to evaluate publication bias (Egger et al., 1997). We performed sensitivity analysis to assess the stability of the results by sequential omission of individual studies. All P values presented are two-tailed.

RESULTS

Characteristics of literature

Following the literature review, we identified 17 studies for potential inclusion in

the meta-analysis; 9 were excluded, comprising 2 review articles (Ye et al., 2006; Huang and Zeng, 2010), 4 repeated reports (Yang et al., 2004; Long et al., 2005; Wang, 2006; Kiran et al., 2009b), 1 study that examined the association between HCC and SNPs of *XRCC1* and p53-249 (Long et al., 2008), 1 study without Hardy-Weinberg equilibrium test (Long et al., 2006), and 1 study that demonstrated an association between HCC related to HBV and an SNP of *XRCC1* and glutathione *S*-transferase (Yu et al., 2003). The remaining 8 studies met our selection criteria and were used for the meta-analysis; 4 were written in Chinese, and 4, in English. A total of 3789 subjects were studied (1604 patients and 2185 controls). The main features of the included studies are shown in Table 1. Six of the 8 studies were conducted in Asia, 1 in Europe, and 1 in Africa. All studies used PCR for genotyping, and logistic regression for statistical analysis. More than three-quarters (77.74%) of the cases were confirmed by biopsy or pathology, and the others were in line with international diagnostic criteria. In 62.5% of the studies, the cases and controls were matched by age and gender.

Table 1. Basic characteristics of the 8 studies.

Author (reference)	Country/region	Source of control	No. of cases GG/GA/AA	No. of controls GG/GA/AA	Match	HWE (P)	G allele frequencies in control group
Long et al., 2004	China	Hospital	72/63/5	362/159/15	Yes	0.62	0.82
Han et al., 2004	China	Hospital	34/7/28	58/15/63	Yes	0.38	0.48
Kirk et al., 2005	Africa	Hospital	120/26/3	248/43/3	Yes	0.46	0.97
Chen et al., 2005	Taiwan China	Crowd	301/223/53	218/143/28	Yes	0.50	0.74
Borentain et al., 2007	Europe	Crowd	27/21/8	27/43/19	No	0.79	0.54
Ren et al., 2008	China	Crowd	32/14/4	46/41/5	No	0.29	0.72
Kiran et al., 2009a	India	Crowd	25/33/5	45/70/27	No	0.99	0.56
Zeng et al., 2010	China	Hospital	286/180/34	304/167/36	Yes	0.05	0.76

HWE = Hardy-Weinberg equilibrium.

Meta-analysis

Heterogeneity test

The heterogeneity test indicates that there were statistically significant differences across the selected studies. The meta-analysis for the *XRCC1*-399 allele did not demonstrate any statistical effect in the pooled population. In the random-effect model, the combined OR for the association of the *XRCC1*-399 allele with the risk for HCC in the pooled population was 0.99 (95%CI = 0.75-1.31; P = 0.00, I^2 = 69%) (Figure 1).

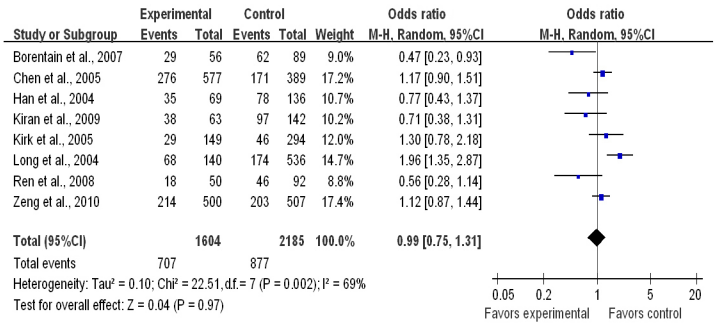

Figure 1. Meta-analysis forest plot. M.-H. = Mantel-Haenzel; d.f. = degrees of freedom.

The meta-regression analysis showed that sample size and matching between the cases and controls by age and gender were sources of heterogeneity. The P values for (GA+AA) *vs* GG in the meta-regression analysis were as follows: sample size, P = 0.00; source of control, P = 0.08; match, P = 0.00; and publication year, P = 0.06.

We also conducted subgroup analyses. Subgroup analyses by sample size (with 300 samples as the boundary) and case-control matching showed that the *XRCC1*-399 allele was not associated with the risk of HCC in the random-effect model (Table 2).

Table 2. Subgroup analysis.

Factor	No. of studies	(GA+AA) *vs* GG	
		Heterogeneity	OR (95%CI)
Sample size			
≤300	4	0.71	0.63 (0.46-0.87)#
>300	4	0.09	1.32 (1.03-1.68)*
Match			
Yes	5	0.06	1.24 (0.97-1.58)*
No	3	0.68	0.58 (0.39-0.85)#

*Fixed effect model; #random effect model. OR = odds ratio; 95%CI = 95% confidence interval.

Publication bias

Figure 2 depicts the funnel plot analysis used to detect the publication bias of each study. The shape of the funnel plot appears symmetrical, suggesting that publication bias did not affect the meta-analysis findings. In addition, we used the Egger linear regression to evaluate the funnel quantitatively, which also indicated no publication bias. The Egger regression asymmetry test showed that the result of (GA+AA) *vs* GG was as follows: $t = 1.64$, P = 0.15; 95%CI = -19.83-3.91.

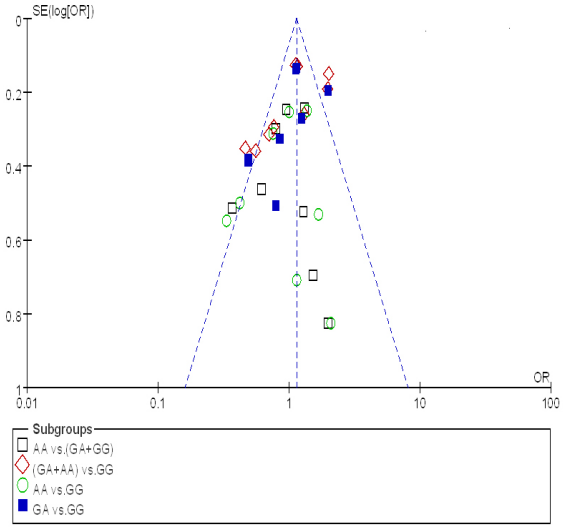

Figure 2. Funnel plots from the meta-analysis. OR = odds ratio; SE = standard error.

Sensitivity analysis

The exclusion of individual studies did not change the result of non-significant association (Table 3).

Table 3. Results of the sensitivity analysis.

Study	OR (95%CI)
Excluded studies (all)	0.99 (0.75-1.31)*
Long et al., 2004	1.01 (0.87-1.18)*
Kirk et al., 2005	1.09 (0.95-1.26)*
Han et al., 2004	1.13 (0.98-1.31)*
Zeng et al., 2010	1.10 (0.93-1.30)*
Chen et al., 2005	1.08 (0.92-1.28)*
Borentain et al., 2007	1.15 (1.00-1.33)*
Ren et al., 2008	1.14 (0.99-1.31)*
Kiran et al., 2009a	1.14 (0.98-1.31)*

*Fixed-effect model. OR = odds ratio; 95%CI = 95% confidence interval.

DISCUSSION

Recently, an increasing number of published studies investigated the associations between *XRCC1*-399 polymorphisms and various tumors, including HCC. However, the results of previous studies are inconclusive when considered as a whole. The same gene polymorphism may have different effects in different populations. Even considering the same tumor in the same population, different sample sizes may lead to different conclusions due to the lack of statistical power to find genes with minor effects. We carried out a meta-analysis to detect the association between SNPs of *XRCC1*-399 and susceptibility to HCC, which increases the statistical power and reveals possible sources of heterogeneity.

The aim of this meta-analysis was to ascertain whether specific *XRCC1*-399 alleles are associated with susceptibility to HCC. We analyzed published studies that investigated the association between *XRCC1*-399 alleles and HCC. Our meta-analysis of 8 studies revealed that *XRCC1*-399 alleles were not significantly associated with the risk of HCC in the pooled population.

The heterogeneity test indicated that there were statistically significant differences across the individual studies. The meta-regression clearly showed that the sample size (with 300 samples as the boundary) and case-control matching were sources of heterogeneity.

Statistically, the results show that *XRCC1*-399 polymorphisms were not significantly associated with susceptibility to HCC. However, there is significant heterogeneity in the literature. In addition to sample size and case-control matching, various factors may have contributed to the inconsistent results observed. HCC is a complex genetic disease involving multiple genes. The first source of heterogeneity is the study population. Different populations usually have different genetic backgrounds, and different genetic backgrounds may have a different genetic structure. Differences in the genetic structures of dissimilar populations may result in diverse disease outcomes. Different populations also have different allele frequencies, such as Asian, European, and African populations. Even allele frequencies can differ in different areas of the same country. As shown in Table 1, the allele frequencies of *XRCC1*-399 across China

differed significantly. However, due to the limited data in the literature, we could not determine further whether there are different effects caused by *XRCC1*-399 polymorphisms in different populations. Second, the observed heterogeneity may be clinical. Clinical heterogeneity can arise due to differences in age, gender, diet, time of onset, choice of treatment, treatment duration, severity of disease, and so on. Different populations usually have different dietary habits, which may lead to differences in the intake of nutrients in specific populations. Certain nutrients may play a role in the process of tumor formation and development. Third, environmental and genetic heterogeneity may be significant. Differences in the external environment and other susceptible genes may cause heterogeneity across studies. In addition, HCC is a multi-gene disease. Gene interactions may also have affected the heterogeneity of the studies. Lastly, the heterogeneity may have been due to the random effect. A correlation analysis must be based on a large sample size. The number of studies selected for this meta-analysis was relatively small, with a small overall sample size and a low incidence of HCC.

A meta-analysis can increase the statistical power and reveal sources of heterogeneity, but some limitations remain. Even if the statistical tests found no theoretical statistical significance, differences may actually have existed. Due to the lack of original, individual data, we used uncorrected ORs for the combined analysis. This approach may have reduced the accuracy of the results. In the subgroup analysis, some groups also had limited sample sizes, resulting in the limited statistical power of our meta-analysis to detect weak effects. Moreover, because the data were not complete, it was difficult to carry out subgroup analysis of more factors.

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