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Meta-analysis of epidemiological studies of association of P53 codon 72 polymorphism with bladder cancer

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ABSTRACT. Although there have been many studies investigating a possible association between p53 codon 72 polymorphism and risk of bladder cancer, the results have been inconsistent. We conducted a meta-analysis of six epidemiological studies, which included 597 bladder cancer cases and 731 controls. Patients with bladder cancer had a significantly lower frequency of Pro/Arg [odds ratio (OR) = 0.80, 95% confidence interval (CI) = 0.64-0.99], when compared to controls. Stratifying for race, we found that among Caucasians, patients with bladder cancer had a significantly higher frequency of Arg/Arg (OR = 1.64, 95%CI = 1.18-2.28) and a lower frequency of Pro/Arg (OR = 0.62, 95%CI = 0.44-0.86), compared to controls. Stratifying various studies by the stage of bladder cancer, we found that invasive bladder cancers had a significantly lower frequency of Arg/Arg (OR = 0.58, 95%CI = 0.36-0.93) and a higher frequency of Pro/Arg (OR = 0.62, 95%CI = 0.44-0.86) than did non-invasive bladder cancers. No significant association

was found between this genotype and human papilloma virus. Based on our meta-analysis, we suggest that p53 codon 72 polymorphism is associated with bladder cancer and that genotypic distribution of this polymorphism varies with the stage of bladder cancer.

Key words: Bladder cancer; p53 codon 72; Gene polymorphism; Meta-analysis

INTRODUCTION

Bladder cancer is the second most common genitourinary malignant disease in the USA, with an expected 69,000 newly diagnosed cases in 2008, and 14,000 deaths (Jemal et al., 2008). Risk factors for the development of bladder cancer can be classified into three subsets: genetic and molecular abnormalities, chemical or environmental exposures, and chronic irritation. Genetic and molecular factors include oncogenes, tumor suppressor genes, and those newly implicated such as the fragile histidine triad gene (Kaufman et al., 2009). Of all newly diagnosed cases of bladder cancer, about 70% present as superficial tumors, but as many as 50-70% of those superficial tumors will recur, and roughly 10-20% will progress to muscularis propria invasive disease (Rubben et al., 1988). To predict which patients will progress from superficial to muscularis propria invasive disease remains a challenge (Kaufman et al., 2009)

Over the past decade, polymorphism at codon 72 of the TP53 gene (Pro72Arg; rs1042522) has been investigated. Three genotypes occur: arginine homozygotes (Arg/Arg), proline homozygotes (Pro/Pro), and heterozygotes (Pro/Arg) (Klug et al., 2009). A guanine/cytosine variant at the second position of codon 72 on exon 4 leads to Arg72 or Pro72 protein variants with markedly altered primary structures and different biochemical functions. The current view is that the P53-Arg72 protein is more effective than the P53-Pro72 protein at inducing apoptosis and protecting cells from tumor development. The P53 tumor-suppressor protein has been called the guardian of human cells against cancer (Hollstein et al., 1991). P53 is a nuclear protein that induces cell cycle arrest, apoptosis, inhibition of angiogenesis, metastasis, and DNA repair (Levine, 1997). P53 codon 72 polymorphisms have been reported to be associated with cancers of the stomach (Zhou et al., 2007), esophagus (Lee et al., 2006), colorectum (Koushik et al., 2006), lung (Matakidou et al., 2003), breast (Tommiska et al., 2005), and cervix (Klug et al., 2009).

Although there have been many studies investigating a possible association between p53 codon 72 polymorphism and risk of bladder cancer, the results have been inconsistent. We conducted a meta-analysis to assess whether a relationship exists between p53 codon 72 polymorphism and risk of bladder cancer.

METHODS

Literature search

We searched various databases including PubMed, Embase, and the Cochrane Library to identify studies on p53 polymorphism and bladder cancer published before 2010. The following key words were used: ‘p53’ or ‘codon 72’, ‘bladder’, ‘carcinoma’ or ‘cancer’ or ‘tumor’. The reference lists of reviews and retrieved articles were handsearched at the same time.

We did not consider abstracts or unpublished reports. All studies on p53 polymorphism and bladder cancer published before 2010 were included. No language restrictions were applied; all non-English articles were translated if necessary.

Selection criteria

Titles and abstracts of all citations and retrieved studies were reviewed by two independent researchers. To be eligible for inclusion, studies had to be case-control that reported genotypic frequencies for both case and control populations. Interim analyses, overlapping study populations, and comparisons of laboratory methods were excluded.

Statistical analysis

We imported data into STATA, version 9.2 (Stata Corporation, College Station, TX, USA). To determine whether to use the fixed- or random-effects models, we measured statistical heterogeneity between and within groups using the Q-statistic, where $P < 0.05$ was considered to be statistically significant. Heterogeneity was also assessed through visual examination of L'Abbe plots. We used fixed-effects methods if the result of the Q-test was not significant. Otherwise, we calculated pooled estimates and confidence intervals, assuming a random-effects model. While publication bias was not expected, we assessed this possibility using Begg funnel plots and Egger's bias test (Begg and Mazumdar, 1994; Egger et al., 1997). We calculated separate pooled estimates for different ethnic groups and geographic regions. Subgroup analysis was conducted on the basis of race, human papilloma virus (HPV) and the stage of bladder cancer.

RESULTS

Six studies were included in the meta-analysis (Chen et al., 2000; Toruner et al., 2001; Soulitzis et al., 2002; Mabrouk et al., 2003; Horikawa et al., 2008; Murgel de Castro Santos et al., 2009), for a total of 1328 subjects (597 bladder cancer cases and 731 controls). Table 1 provides the general characteristics of the studies. Of these studies, three reported on Caucasians, two reported on Asians, and one reported on Africans.

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

Study (author, year)	Design	Study period	Population (country)	Genotyping method	No. of cases	No. of controls	Arg/Arg cases	Pro/Arg cases	Pro/Pro cases	Arg/Arg controls	Pro/Arg controls	Pro/Pro controls
Chen et al., 2000	HCC	1998-1999	Asians (China)	PCR	58	59	26	25	7	25	26	8
Toruner et al., 2001	HCC	nr	Caucasians (Turkey)	PCR	121	114	43	57	21	42	55	17
Soulitzis et al., 2002	PCC	nr	Caucasians (Greece)	PCR	50	99	30	18	2	24	64	11
Mabrouk et al., 2003	PCC	nr	Africans (Tunisia)	PCR-RFLP	47	34	21	23	3	13	19	2
Horikawa et al., 2008	HCC	1990-2004	Asians (Japan)	PCR	227	266	73	118	36	93	136	38
Murgel et al., 2009	PCC	nr	Caucasians (Brazil)	PCR-RFLP	94	159	64	24	6	90	60	9

HCC = hospital-based case-control; PCC = population-based case-control; nr = not reported; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism.

The combined results based on all studies showed that patients with bladder cancer had a significantly lower frequency of Pro/Arg [odds ratio (OR) = 0.80, 95% confidence interval (CI) = 0.64-0.99] than did controls. When stratifying for race, we found that among Cau-

casians, patients with bladder cancer had a significantly higher frequency of Arg/Arg (OR = 1.64, 95%CI = 1.18-2.28) and a lower frequency of Pro/Arg (OR = 0.62, 95%CI = 0.44-0.86) compared to controls (Figures 1-3).

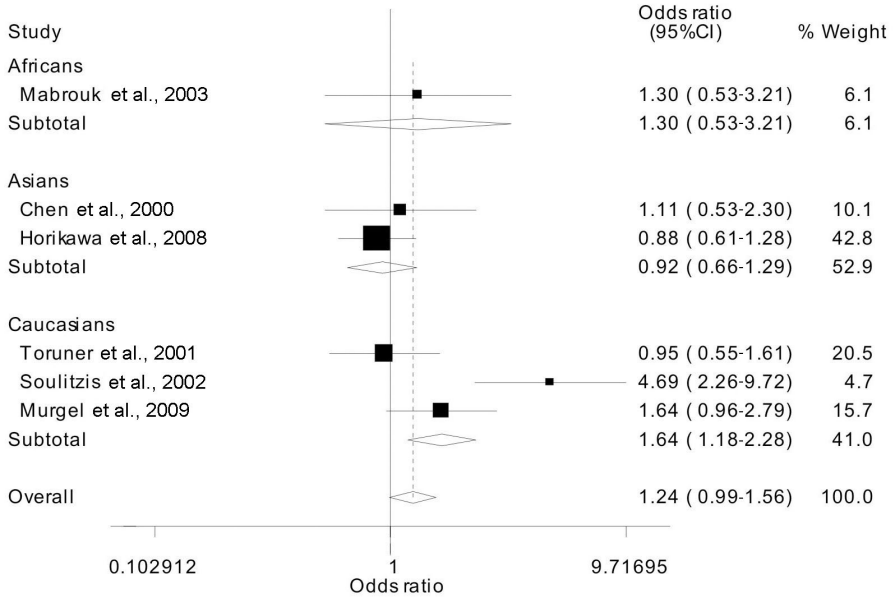

Figure 1. Meta-analysis of p53 codon 72 Arg/Arg and bladder cancer risk.

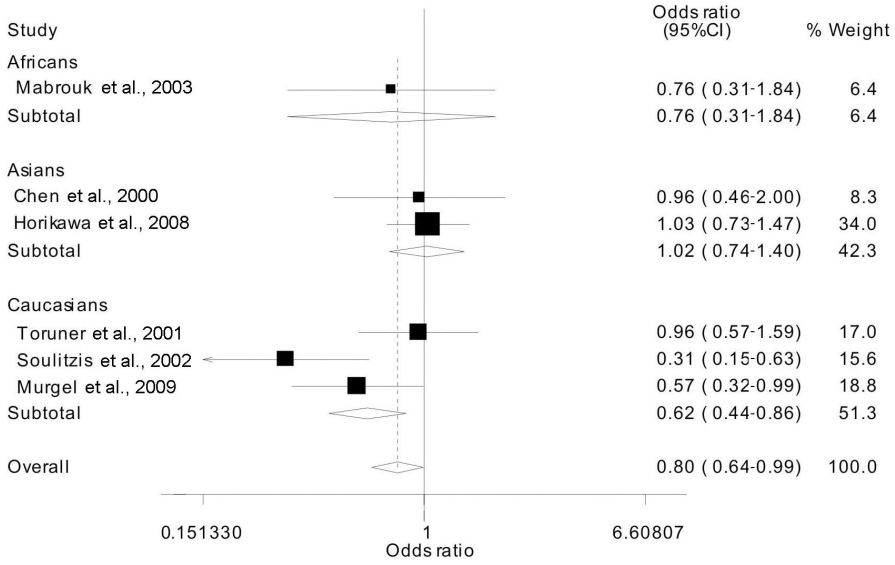

Figure 2. Meta-analysis of p53 codon 72 Pro/Arg and bladder cancer risk.

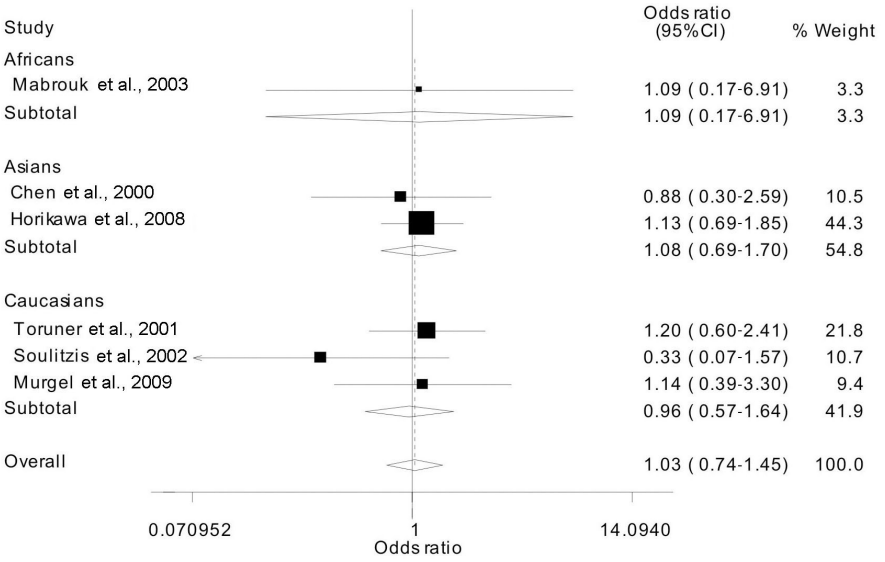

Figure 3. Meta-analysis of p53 codon 72 Pro/Pro and bladder cancer risk.

Stratifying the various studies by the stage of bladder cancer, we found that the invasive bladder cancers had a significantly lower frequency of Arg/Arg (OR = 0.58, 95%CI = 0.36-0.93) and a higher frequency of Pro/Arg (OR = 1.36, 95%CI = 0.45-4.16) than did the non-invasive bladder cancers. No statistical association was found between this genotype and HPV (Table 2).

Table 2. Meta-analysis of p53 codon 72 polymorphism and bladder cancer.

Stratification of bladder cancer	No. of studies	OR (95%CI) of Arg/Arg	P for heterogeneity	OR (95%CI) of Pro/Arg	P for heterogeneity	OR (95%CI) of Pro/Pro	P for heterogeneity
Stage: Invasive versus non-invasive	3	0.58 (0.36-0.93)	0.06	1.36 (0.45-4.16)	0.04	1.95 (1.10-3.46)	1.00
Asians	2	0.40 (0.22-0.73)	0.001	2.25 (0.44-11.44)	0.01	2.00 (0.99-4.06)	0.08
Caucasians	1	1.26 (0.55-2.87)	na	0.55 (0.24-1.24)	na	1.85 (0.69-4.97)	na
HPV: Positive versus negative	1	3.80 (0.41-35.28)	na	0.32 (0.03-2.96)	na	na	na
Asians	0						
Caucasians	1	3.80 (0.41-35.28)	na	0.32 (0.03-2.96)	na	na	na

OR = odds ratio; CI = confidence interval; na = not applicable; HPV = human papilloma virus.

DISCUSSION

Although several studies have investigated the association between p53 codon 72 polymorphism and risk of bladder cancer in the last ten years, small studies of genetic associations often have insufficient power, increasing the risk that chance could be responsible for their conclusions. Combining data from many studies has the advantage of reducing random error (Ioannidis et al., 2008). Meta-analysis enabled us to apply the same kind of criteria to all the study datasets and to obtain precise estimates for subgroups. Our meta-analysis of 1328 subjects from 6 studies provides evidence that p53 codon 72 polymorphism may be associated with bladder cancer, and that differences in genotype distribution may be associated with the stage of bladder cancer, described for the first time.

Many studies have examined the association between p53 codon 72 polymorphisms and other cancers. To explore the true association between p53 codon 72 polymorphism and lung cancer risk, Dai et al. (2009) conducted a pooled analysis of 32 case-control studies involving 19,255 subjects. Their results suggested that the Pro allele at p53 codon 72 was emerging as a low-penetrance susceptibility allele for lung cancer development. The meta-analysis by Zhou et al. (2007) suggested that p53 codon 72 polymorphism may be associated with gastric cancer among Asians, and that differences in genotype distribution may be associated with the location, stage, and histological differentiation of gastric cancer. The meta-analysis by Sousa et al. (2007) revealed that the p53 Arg/Arg genotype did not seem to represent a risk marker for the development of cervical lesions in the majority of the European countries studied. However, in countries with low incidence rates of cervical cancer, this polymorphism could represent a significant genetic marker.

No statistical association was found between this genotype and HPV in our meta-analysis. Soulitzis et al. (2002) examined tumor specimens from all bladder cancer patients for the presence of HPV. HPV was detected in 12% of tumor specimens, which is consistent with previous studies performed in bladder cancer, confirming that HPV is a significant factor in the development of a small percent of tumors (Maloney et al., 1994; Lopez-Beltran et al., 1996; Simoneau et al., 1999). The lack of a statistical association between HPV infection and the homozygous p53Arg genotype in bladder cancer supports the synergistic action of the virus, especially in an environment with exposure to carcinogens, rather than its causative role in the development of the disease.

Our study has a number of possible limitations. First, the database for the meta-analysis included limited numbers of studies on ethnic groups; only two studies reported on Asians and only one study reported on Africans, reflecting the current lack of epidemiologic studies in these populations. Second, only published studies were included in the meta-analysis; therefore, publication bias may have occurred. Third, this meta-analysis is based on unadjusted estimates, while a more precise analysis could be performed if individual data were available. Another potential limitation was the small sample size in the analyses. Therefore, the power in the analyses was not sufficient to detect small increased risks. Finally, meta-analysis remains a retrospective research that is subject to the methodological deficiencies of the studies included.

To our knowledge, this is the first meta-analysis as a quantitative summary of the evidence for p53 codon 72 polymorphism and bladder cancer risk. This analysis supports conclusions that p53 codon 72 polymorphism may be associated with bladder cancer, and that differences in genotype distribution may be associated with the stage of bladder cancer. These findings warrant larger studies to clarify the role of p53 codon 72 polymorphism and to evaluate gene-environment interactions for p53.

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