

Original Article

CYP2E1 RsaI/PstI polymorphisms contributed to oral cancer susceptibility: a meta-analysis

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Abstract: Several studies have investigated the associations between CYP2E1 RsaI/PstI polymorphisms and oral cancer risk, but results have been inconclusive. In order to derive a more precise estimation of the relationship, a meta-analysis was performed. PubMed and China National Knowledge Infrastructure (CNKI) searches were carried out for relevant studies published before September 2014. Meta-analysis was performed with the Stata, version 11.0. A total of 14 case control studies, including 1,962 cases and 3,271 controls, were selected. Overall, significant association was found between the CYP2E1 RsaI/PstI polymorphisms and oral cancer risk (for c1c1 vs. c1c2, OR=0.72, 95% CI=0.56-0.91; for c1c1 vs. c2c2, OR=0.45, 95% CI=0.25-0.82), while not for the dominant model (c1c1 vs. c1c2+c2c2, OR=0.84, 95% CI=0.69-1.01). In the subgroup analysis by ethnicity, statistically significant association was found in Caucasian, East Asian and South Asian. This meta-analysis suggests that the CYP2E1 RsaI/PstI polymorphisms are a risk factor for developing oral cancer.

Keywords: CYP2E1, polymorphism, oral cancer, meta-analysis

Introduction

Oral cancer is the sixth most common cancer worldwide and a serious and growing health problem in many parts of the world [1]. The etiology of oral cancer is multifactorial. The most important etiological factors are tobacco, alcohol and betel quid consumption, these factors act separately or synergistically, so that the attributable risk of oral cancer due to both tobacco and alcohol is estimated to be more than 80% [1]. Nowadays, the mechanism by which alcohol participates in oral carcinogenesis is unclear [2]. The isoenzymatic differences of alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH) and cytochrome P450 E1 (CYP2E1) might be a risk factor in oral squamous cell carcinoma (OSCC) [3-6].

Cytochrome P450 2E1 (CYP2E1) belonging to the cytochrome P450 (CYP) family are the predominant enzymes of the phase I oxidative xenobiotic metabolism [6]. The human CYP2E1 gene is located on chromosome 10 (10q24.3-pter) and has a total of 9 exons with several polymorphisms, some affecting the expression

of the protein [7]. CYP2E1 expression has been recognized in oral epithelial cell lines cultures, in human oral mucosa and tongue of rats [8]. This enzyme activates low molecular weight molecules such as ethanol, carcinogens, certain toxins and drugs [9]. Among the most studied polymorphisms of the CYP2E1 gene is the wild allele CYP2E1*5A (dbSNP rs3813867) with a RsaI restriction site at position -1259 and CYP2E1*5B (dbSNP rs2031920) with a PstI restriction site at position -1019. As both sites are located in the in the 5'-flanking region and are segregated completely, they could play an important role in the CYP2E1 gene transcription [10]. These two polymorphisms are in close linkage disequilibrium and are known to generate the CYP2E1_1 (c1) allele and the less common CYP2E1_2 (c2) allele. So there are three genotypes: wild-type homozygous genotype (c1/c1), heterozygous genotype (c1/c2) and variant homozygous genotype (c2/c2).

Hung et al. first reported the association between the risk of oral cancer and CYP2E1 genotype [11]. They found that the dominant genotype was associated with a significantly

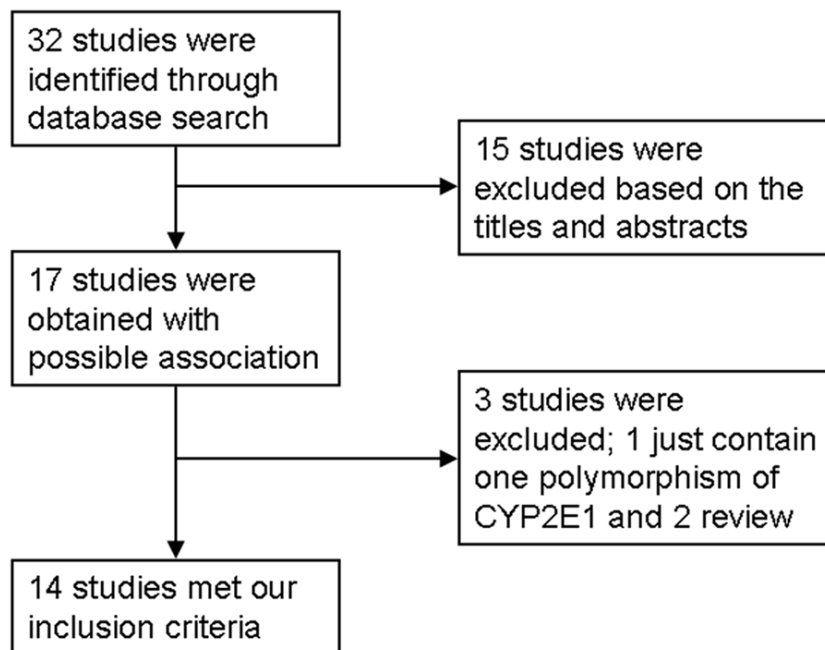


Figure 1. Flow chart of the study selection procedure.

increased risk association in Chinese. Since then, some studies on this topic had been done. But the results were different or even contradictory, and most studies included only small numbers of cases and controls. To determine the effects of this genotype on the risk of oral cancer, we undertook a meta-analysis based on the present published data.

Materials and methods

Studies identification

To identify all studies that examined the association of CYP2E1 genotype with oral cancer risk, we conducted a literature search of PubMed and Chinese National Knowledge Infrastructure (CNKI) databases, without a language limitation, covering all papers published up to September 2014, using the following keywords and subject terms: CYP2E1, oral cancer, head and neck cancer and polymorphism. We evaluated potentially relevant publications by checking their titles and abstracts and then obtained the most relevant publications for a detailed examination. Moreover, the reference lists of the selected papers were also screened for other potential articles that may have been missed in the initial search. Only published studies with full text articles were included. When more than one of the same patient po-

pulation was included in several publications, only the most recent or complete study was used in this meta-analysis.

Selection criteria

After searching, we reviewed all papers in accordance with the criteria defined below for further analysis: (a) case-control studies which evaluate the association between CYP2E1 RsaI/PstI polymorphisms and oral cancer risk, (b) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI), (c) genotype distribution of control population must be in Hardy-Weinberg equilibrium (HWE). Accordingly, the following exclusion criteria were also used: (a) the design and the definition of the experiments were obviously different from those of the selected papers; (b) the source of cases and controls and other essential information were not provided; (c) reviews and duplicated publications.

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Data extraction

Data were carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria mentioned above. Disagreement was resolved by discussion between the two authors (YG and SZ). If these two authors could not reach a consensus, another author was consulted to resolve the dispute and a final decision was made by the majority of the votes. The following data were collected from each study: first author's name, publication date, ethnicity, genotype frequency, and design of experiment (population or hospital based controls). Different ethnicities were categorized as Caucasian, East Asian, South Asian South American and African. Design of experiments was stratified into population based studies and hospital based studies. We did not define any minimum number of patients to include in our meta-analysis.

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Table 1. Main characteristics of all studies included in the meta-analysis

Author	Year	Ethnicity	Design	Method	Case	Control	Case			Control			HWE (P)
							c1/c1	c1/c2	c2/c2	c1/c1	c1/c2	c2/c2	
Hung	1997	East Asia	PB	PCR-RFLP	41	122	20	19	2	76	42	4	0.53
Balaji	2011	South Asia	PB	TaqMan	157	132	151	6	0	125	7	0	0.75
Brocic	2011	Caucasian	Mixed	PCR-RFLP	123	177	105	13	5	160	16	1	0.4
Gattás	2006	South American	HB	PCR-RFLP	38	102	31	7	0	96	6	0	0.76
Sugimura	2006	East Asia	HB	PCR-RFLP	122	241	72	39	11	164	70	7	0.89
Zavras	2002	Caucasian	HB	PCR-RFLP	93	99	92	1	0	98	1	0	0.96
Bouchardy	2000	Caucasian	HB	PCR-RFLP	67	172	59	7	1	164	8	0	0.75
Morita	1999	East Asia	PB	PCR-RFLP	31	164	18	13	0	105	52	7	0.86
Katoh	1999	East Asia	PB	PCR-RFLP	92	147	53	36	3	95	45	7	0.58
Liu	2001	Caucasian	HB	PCR-RFLP	112	224	105	7	0	210	14	0	0.63
Liu	2001	African	HB	PCR-RFLP	55	156	55	0	0	155	1	0	0.97
Soya	2008	South Asia	HB	PCR-RFLP	187	220	179	8		212	8		NA
Marques	2006	South American	HB	PCR-RFLP	231	212	200	31		187	25		NA
Anantharaman	2011	South Asia	HB	PCR-RFLP	423	700	414	9		665	35		NA
Buch	2008	Caucasian	PB	PCR-RFLP	190	403	176	14		364	39		NA

PB: population-based study; HB: hospital-based study; NA: not available.

Statistical analysis

Crude ORs with 95% CIs were used to assess the strength of association between the CYP2E1 RsaI/PstI polymorphisms with oral cancer risk. The pooled ORs were performed for an additive model (c1c1 versus c2c2 and c1c1 versus c1c2) and a dominant model (c1c1 versus c1c2+c2c2). Stratification analysis was performed by ethnicity and study design (hospital-based studies and population-based studies).

A Chi-square test was used to determine if the distribution of genotypes among controls was departure from Hardy-Weinberg equilibrium (HWE), $P < 0.05$ means a departure. The Q-test and I^2 statistics were used to investigate the degree of heterogeneity among studies [12]. A P value greater than 0.05 for the Q-test indicates a lack of heterogeneity among studies, so the pooled OR estimate of the each study was calculated by the fixed-effects model (the Mantel-Haenszel method [13]). Otherwise, the random-effects model (the DerSimonian and Laird method [14]) was used. Sensitivity analysis was performed to assess the stability of the results. A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs. An estimate of potential publication bias was assessed by visual inspection of funnel plots [15], in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot indicates a possible publication bias. The symmetry of the fun-

nel plot was further evaluated by Egger's linear regression test ($P < 0.05$ was considered indicative of significant publication bias) [16]. Statistical analysis was performed using STATA version 11 (Stata Corporation, College Station, TX).

Results

Study characteristics

Through literature search, we found 32 articles. Based on the inclusion criteria, 17 studies were found, but only 14 studies met our inclusion criteria [4, 8, 11, 17-27]. The three studies were excluded for the following reasons: one study just contain one polymorphism of CYP2E1 [28]; two studies were review [2, 29] (**Figure 1**). Among the 14 studies, two populations (Caucasians and African) were included in one study [8], so we divided the relevant data into two studies; four studies were just included in the dominant model for they provided the genotype of c1c2+c2c2 as a whole [24-27]. The data for this analysis included 1,962 cases and 3,271 controls from 14 studies. **Table 1** lists the identified studies and their main characteristics.

Meta-analysis results

To summarize the published data, we did a comprehensive meta-analysis. The data was extracted from 14 case control studies. The meta-analysis included 1,962 cases and 3,271 controls.

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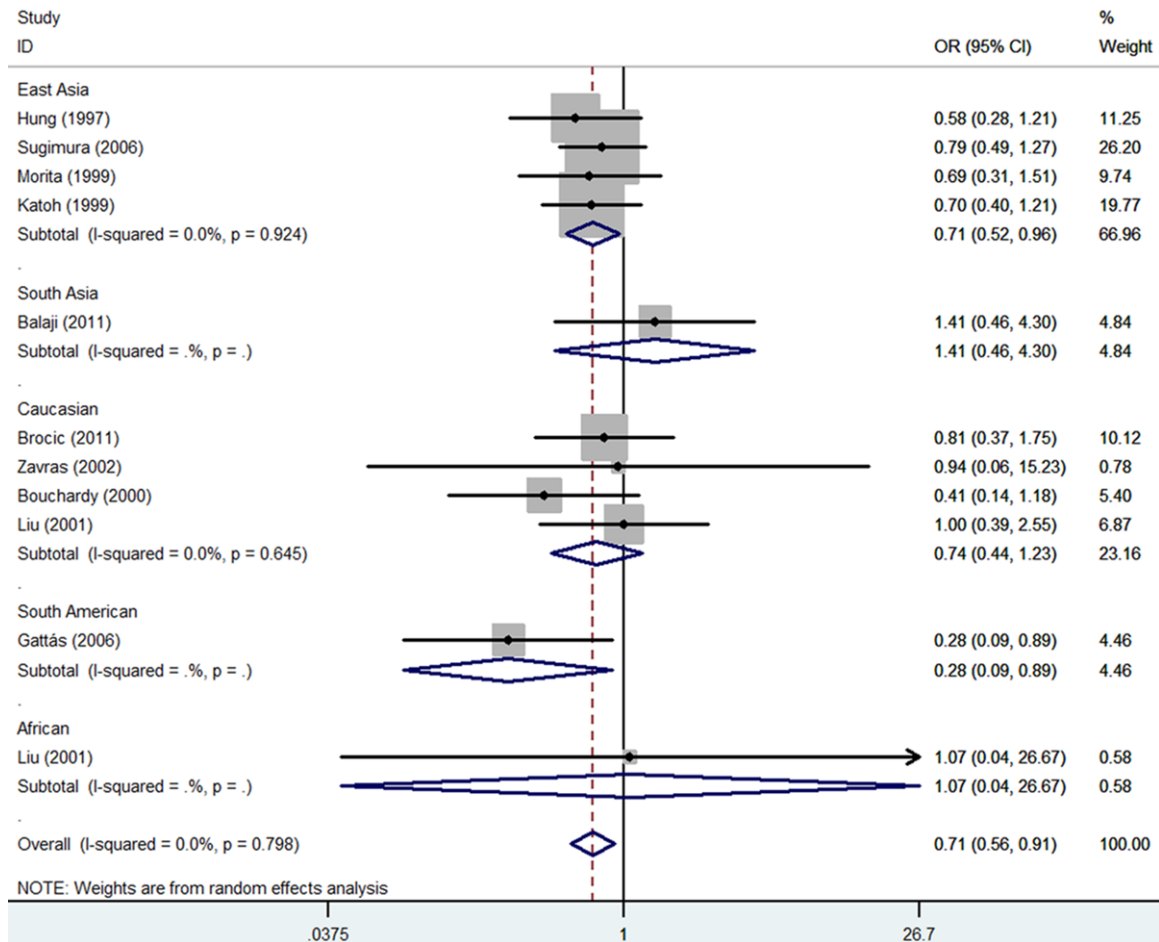


Figure 2. Forest plot of OR of oral cancer risk associated with CYP2E1 RsaI/PstI polymorphism for c1c1 vs. c1c2. Horizontal lines represent 95% confidence intervals. Each square represents the OR point estimate and its size is proportional to the weight of the study. The diamond (and broken line) represents the overall summary estimate, with confidence interval given by its width. The unbroken vertical line is at the null value (OR=1.0).

The overall data shown that significant association was found between the CYP2E1 RsaI/PstI polymorphisms and oral cancer risk (for c1c1 vs. c1c2, OR=0.72, 95% CI=0.56-0.91; for c1c1 vs. c2c2, OR=0.45, 95% CI=0.25-0.82), while not for the dominant model (c1c1 vs. c1c2+c2c2, OR=0.84, 95% CI= 0.69-1.01). Then, the 14 studies were analyzed by stratified based on ethnicity and study design. In the stratified analysis of study design, the association was found in hospital based study for the additive model (for c1c1 vs. c1c2, OR=0.69, 95% CI=0.48-1.00; for c1c1 vs. c2c2, OR=0.26, 95% CI=0.10-0.66). In the subgroup analysis by ethnicity, statistically significant association was found in Caucasian, East Asian and South Asian (**Figure 2**). The details were listed in **Table 2**.

Sensitive analysis

Sensitivity analyses were conducted to determine whether modification of the inclusion criteria of the meta-analysis affected the final results. A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data-set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown), indicating that our results were statistically robust.

Publication bias

Begg's funnel plots and Egger's tests were performed to assess publication bias. The shapes of the funnel plots revealed no obvious asymmetry (**Figure 3**). The Egger's test was then

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Table 2. Summary of ORs for CYP2E1 RsaI/PstI polymorphism and oral cancer risk

Subgroup	N	c1c1 vs. c1c2		N	c1c1 vs. c2c2		N	c1c1 vs. c1c2+c2c2	
		OR (95% CI)	Ph		OR (95% CI)	Ph		OR (95% CI)	Ph
Ethnicity									
Caucasian	4	0.75 (0.45-1.24)	0.65	2	0.13 (0.02-0.78)	0.97	5	0.85 (0.58-1.24)	0.23
East Asian	4	0.71 (0.53-0.96)	0.92	4	0.57 (0.29-1.11)	0.22	4	0.69 (0.52-0.92)	0.94
South Asian	1	1.41 (0.46-4.30)	NA	0	NA	NA	3	1.66 (1.00-2.77)	0.24
Design									
HB	6	0.69 (0.48-1.00)	0.51	2	0.26 (0.10-0.66)	0.62	9	0.85 (0.65-1.10)	0.06
PB	4	0.72 (0.49-1.03)	0.63	3	1.13 (0.42-3.08)	0.58	5	0.87 (0.64-1.18)	0.38
Total	10	0.72 (0.56-0.91)	0.79	6	0.45 (0.25-0.82)	0.26	15	0.84 (0.69-1.01)	0.13

N: Number of comparisons.

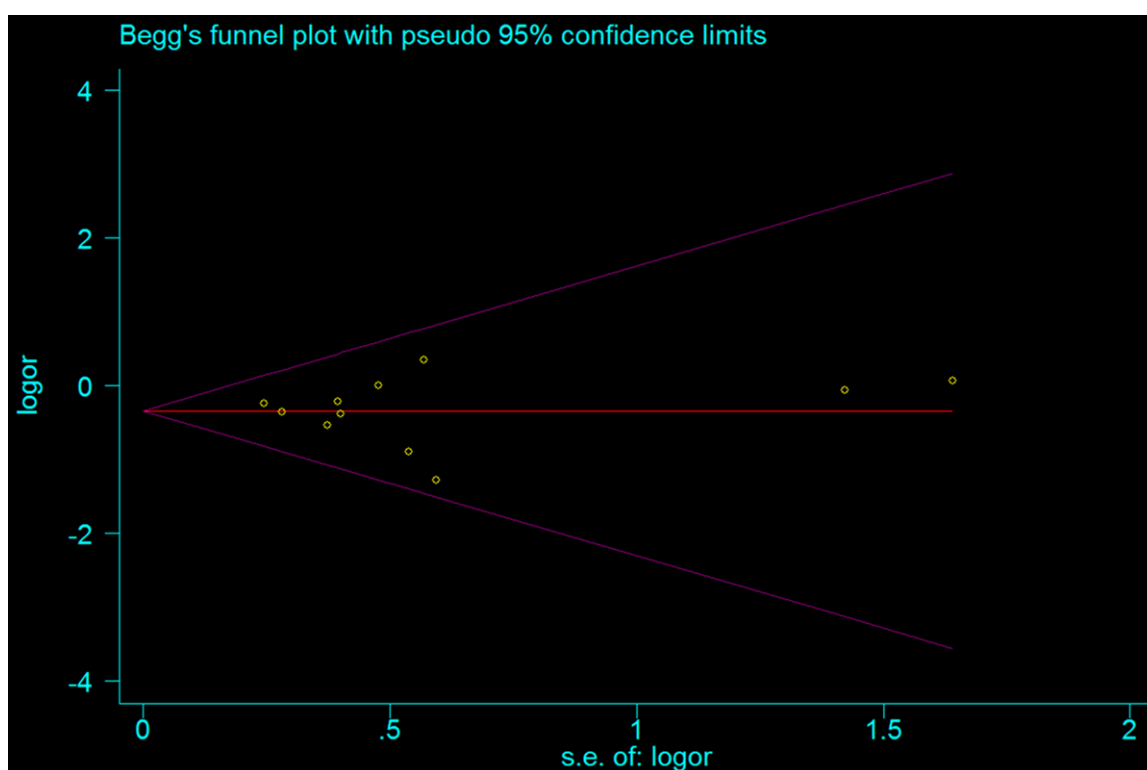


Figure 3. Begg's funnel plot of CYP2E1 RsaI/PstI polymorphism and oral cancer risk.

used to statistically assess funnel plot symmetry. The results suggested no evidence of publication bias (data not shown). The results indicated that the results of these meta-analyses are relatively stable and that publication bias is unlikely to affect the results of the meta-analyses.

Discussion

Oral cancer is a major cause of morbidity and mortality worldwide. Use of tobacco and alcohol are established causal factors in the progress of oral carcinogenesis [30]. A growing body

of evidence implicates human oral bacteria in the etiology of oral and gastrointestinal cancers. More than 700 bacterial species inhabit the oral cavity, including at least 11 bacterial phyla and 70 genera. Oral bacteria may activate alcohol and smoking-related carcinogens locally or act systemically, through chronic inflammation [31].

Although development of oral cancer is associated with exposure to tobacco and alcohol, only a small proportion of exposed individuals will develop cancer, suggesting the involvement of genetic factors. Biological evidence indicated

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that CYP2E1 are the predominant enzymes of the phase I oxidative xenobiotic metabolism [6]. This enzyme activates low molecular weight molecules such as ethanol, carcinogens, certain toxins and drugs and maybe associated with the risk of oral cancer [8, 9]. Among the most studies polymorphism of CYP2E1 gene is the RsaI and PstI polymorphisms, they were both located on the un-transcription region and control the transcription of CYP2E1.

Some studies had focused on the association between CYP2E1 RsaI/PstI polymorphisms and oral cancer risk, but the results were different. As we know that individual study in small sample size may have not enough statistical power to detect a small risk factor. So, in this meta-analysis, we involved a total of 1,962 cases and 3,271 controls and investigated the associations of the CYP2E1 RsaI/PstI polymorphisms with oral cancer risk.

The results indicated that the significant reduced oral cancer risk was found in CYP2E1 c1c1 carriers, which means that CYP2E1 c2 is the risk factor of oral cancer. In the subgroup analysis of study design, the individuals carrying the c1c1 genotype showed a lower oral cancer risk compared with those with the c1c2 or c2c2 genotype for hospital based study, while not for the population based studies. This may be due to the fact that the hospital based studies have some biases because such controls may just represent a sample of ill defined reference population, and may not be a true representative of the general population, particularly when the genotypes under investigation were associated with the disease conditions that the hospital based controls may have.

In the subgroup analysis by ethnicity, different risk was found in different populations. Most interesting is that the individuals with c1c1 had a significant increased oral cancer risk comparing with individual c1c2+c2c2 in South Asian, while the association is opposite in East Asian. The results were consistent with the results of Tang et al. [32]. It might be common for the same polymorphism playing different roles in cancer susceptibility among different ethnic populations, because cancer is a complicated multi-genetic disease, and different genetic backgrounds may contribute to the discrepancy [33]. From this result, we could know that South Asians and East Asians had different

genetics backgrounds and they could not be taken as a whole population in the analysis.

There are some limitations to this meta-analysis. First, only published studies were included in the meta-analysis. It is possible that some related unpublished studies that might meet the inclusion criteria were missed; therefore, publication bias may have been present, even though statistical analysis indicated this not to be the case. Second, our results were based on unadjusted estimates and a more precise analysis could be conducted if individual data were available; this would allow for adjustment by other covariates such as age, ethnicity, environmental factors and lifestyle; Third, in the subgroup analyses, the number of some groups was relatively small, not having enough statistical power to explore the association of the polymorphism with oral cancer susceptibility. However, our meta-analysis also had some advantages. First, substantial number of cases and controls were pooled from different studies, which significantly increased the statistical power of the analysis. Second, no publication bias was detected; indicating that the pooled result should be reliable.

In summary, our meta-analysis indicates that CYP2E1 RsaI/PstI polymorphism is associated with the risk of oral cancer and c2 allele is a risk factor of oral cancer. However, it is necessary to conduct large sample studies using standardized unbiased genotyping methods and well-matched controls.

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Disclosure of conflict of interest

None.

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