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Cyclin D1 polymorphism and oral cancer: a meta-analysis

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Abstract Cyclin D1 (CCND1) plays a critical role in the G1 to S-phase cell cycle transition. Data on the association between the CCND1 A870G polymorphism and oral cancer are conflicting. To assess the relationship between the CCND1 A870G genotype and the risk of developing oral cancer, we performed a meta-analysis. We searched PubMed to December 1, 2011, for studies on this topic that had been published in the English. For each study, we calculated odds ratios (ORs) and 95 % confidence intervals (CIs), assuming the frequency of allele comparison, homozygote comparison, recessive and dominant genetic models. We then calculated pooled ORs and 95 % CIs. Seven studies were included in the meta-analysis. The CCND1 G allele was not associated with oral cancer in the frequency of allele comparison (G vs. A: OR = 0.882; 95 % CI = 0.684–1.137; $p = 0.001$ for heterogeneity). In the subgroup analysis, the CCND1 G allele was associated with a borderline significantly decreased risk of developing oral cancer in Asians in the frequency of allele comparison (G vs. A: OR = 0.800; 95 % CI = 0.636–1.006; $p = 0.089$ for heterogeneity), and the association between the GG genotype and oral cancer was significant in Asians with respect to both the homozygote comparison (GG vs. AA: OR = 0.644; 95 % CI = 0.491–0.843; $p = 0.186$ for heterogeneity) and the dominant genetic model (GG + AG vs. AA: OR = 0.713; 95 % CI = 0.584–0.870; $p = 0.293$ for heterogeneity). Our analysis provides evidence that genotypes for the CCND1 A870G polymorphism may be associated with an increased risk of developing oral cancer in the Asian population.

Keywords CCND1 · Genetic polymorphisms · Oral cancer · Meta-analysis

Abbreviations

CI	Confidence interval
CCND1	Cyclin D1
OR	Odds ratio
MAF	Minor allele frequency

Introduction

Oral cancer is one of the most frequent cancers worldwide [1], and is associated with abnormalities of cell cycle regulation [2].

Cyclin D1 (CCND1) plays a critical role in the G1 to S-phase cell cycle transition [2, 3], and may be involved in the development of some carcinomas in a cyclin dependant kinase independent pattern [4, 5]. Dysregulation of CCND1 is a commonly observed characteristic of human carcinomas, and an overexpression of CCND1 has been reported as a potential biomarker for cancers in humans, for example oral cancers [6–8]. The CCND1 gene, *CCND1*, is located on chromosome 11q13. The gene is polymorphic with a common A/G substitution at nucleotide 870 (A870G, rs9344) in the conserved splice donor region of exon 4 [9]. The A870G single nucleotide polymorphism has been shown to increase

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Table 1 Characteristics of published studies used in the meta-analysis

Reference	Years	Country of origin	Ethnicity	Sample size (case/control)	Cases			Controls			MAF in controls	HWE
					GG	AG	AA	GG	AG	AA		
Liu et al. [12].	2011	China	Asian	102/101	23	43	36	45	29	27	0.411	<0.001
Tsai et al. [13].	2011	China	Asian	620/620	84	323	213	100	365	155	0.544	<0.001
Gomes et al. [14].	2008	Brazil	Mixed	80/80	25	30	25	28	29	23	0.469	0.015
Sathyan et al. [15].	2006	India	Asian	176/142	36	71	39	40	61	36	0.485	0.203
Holley et al. [16].	2005	Germany	Caucasian	174/155	66	94	14	40	87	28	0.461	0.107
Wong et al. [17].	2003	China	Asian	70/93	15	36	19	17	49	27	0.554	0.524
Matthias et al. [18].	1998	Germany	Caucasian	38/191	7	20	11	55	101	35	0.448	0.338

MAF Minor Allele Frequency, *HWE* Hardy–Weinberg equilibrium

the frequency of alternative splicing and can lead to an increase in the half-life of the protein [9, 10]. The variant *CCND1* corresponding to the A allele may have a longer half-life than the G allele, which may bypass the G1/S-checkpoint [11].

Over the past two decades, a number of case–control studies have been conducted to investigate the association between the *CCND1* A870G polymorphism and the risk of developing oral cancer in humans. However, these studies have reported conflicting results. Data in the literature on the association between the *CCND1* A870G polymorphism and the risk of oral cancer, together with the designation of the *CCND1* A870G risk allele, are contradictory and inconclusive, possibly due to the relatively small included populations, which compromised the power of the studies. Meta-analysis is a powerful tool for analyzing cumulative data from studies where individual sample sizes are small and the statistical power is therefore low. Thus, we have undertaken this meta-analysis of the association between *CCND1* A870G and oral cancer.

Materials and methods

Identification and eligibility of relevant studies

PubMed MEDLINE searches were undertaken using the search terms: '*CCND1*'or '*Cyclin D1*', 'polymorphism', and 'oral cancer' or 'oral tumor' or 'oral carcinoma' (last updated on December 1, 2011). The searches were complemented by a review of the bibliographies of the retrieved papers and review articles. All articles were published in English. In order to minimize heterogeneity and facilitate the interpretation of our results, we used the following inclusion criteria were: studies were case–control in design and included genotyping of oral cancer. For studies that did not provide raw data of allele frequencies in the initial publication, we attempted to obtain this information by correspondence with the authors. When such information could not be obtained, the studies were excluded. When study populations overlapped, we generally retained only studies with the most extensive data for the meta-analysis, in order to avoid duplication.

Eligible studies

We identified 10 published reports of potentially eligible studies [12–21]. Of these, we excluded one study as genotype counts could not be obtained despite attempts to contact the authors [19]. We also excluded two further

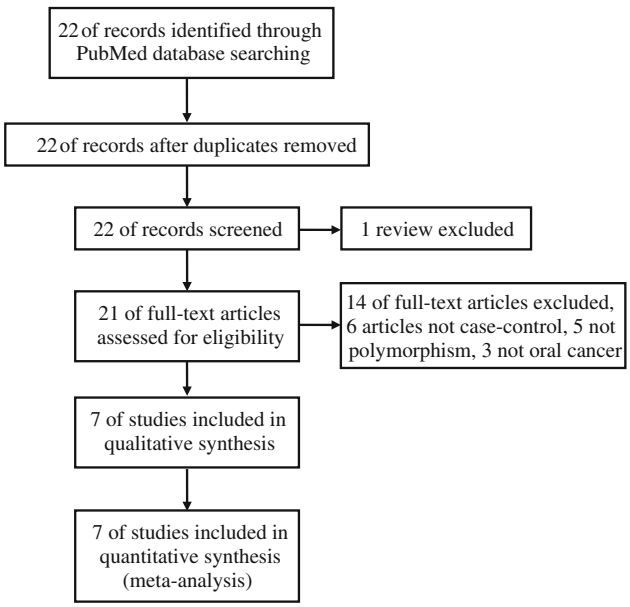

Fig. 1 Flow diagram of articles selection process for *CCND1* A870G gene polymorphism and oral cancer risk meta-analysis

Table 2 Results of the meta-analysis of the association between the *CCND1* A870G polymorphism and oral cancer in seven studies [the random-effects model (if $p_{\text{Heterogeneity}} < 0.10$) or the fixed-effects model (if $p_{\text{Heterogeneity}} \geq 0.10$) was used to summarize the combined OR]

Genetic comparison	Population	Random- or fixed-effects model OR (95 % CI); *p*	Heterogeneity (*p* value, I^2) (%)
G vs. A	All	0.882(0.684–1.137);0.334	0.001,74.8
	Caucasian	1.042(0.439–2.474);0.925	0.003,88.5
	Asian	0.800(0.636–1.006);0.056	0.089,53.9
GG vs. AA	All	0.826(0.500–1.365);0.456	0.001,73.2
	Caucasian	1.193(0.153–9.319);0.866	0.001,90.3
	Asian	**0.644(0.491-0.843);0.001**	0.186,37.3
GG vs. (AG + AA)	All	0.833(0.574–1.207);0.334	0.005,67.5
	Caucasian	1.054(0.344–3.225);0.927	0.024,80.4
	Asian	0.723(0.479–1.092);0.123	0.062,59.0
(GG + AG) vs. AA	All	0.897(0.631–1.275);0.543	0.010,64.1
	Caucasian	1.194(0.268–5.312);0.816	0.004,87.8
	Asian	**0.713(0.584–0.870);0.001**	0.293,19.4

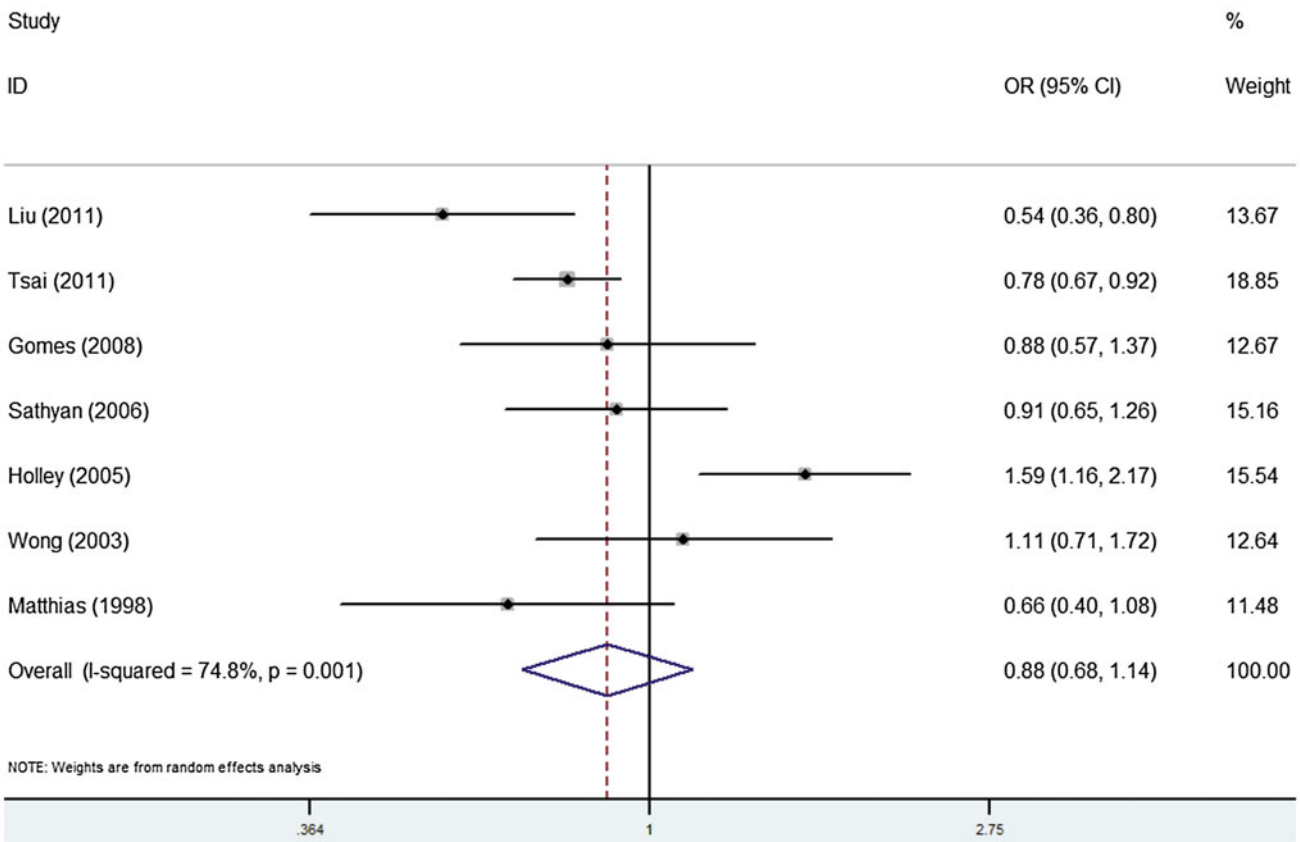

Fig. 2 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the G versus A comparison model

studies, as the cases included oral premalignant lesions [20, 21] (Table 1).

CCND1 genotyping methods

In five studies, genomic DNA was extracted from peripheral blood samples. Among these, three used polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) analysis for genotyping [13, 16, 18], and two used the PCR-single strand conformation polymorphism (PCR-SSCP) assay [15, 17].

In one of the remaining studies [14], genomic DNA was extracted from oral mucosa swabs, and was used for genotyping with PCR–RFLP assays.

In a further study [12], DNA was extracted from a buccal swab sample, and was used for genotyping with the PCR–RFLP method.

Data extraction

Data for the analyses, which included the first author's surname, year of publication, country where the study was conducted, ethnicity of the study population, genotype frequencies and minor allele frequency (MAF) in the controls, were extracted from the published articles and summarized in a consistent manner to aid comparison.

Statistical analysis

We calculated the OR that corresponded to the 95 % CI, in accordance with the method described by Woolf [22], to evaluate the association between the *CCND1* polymorphism and oral cancer. Four comparisons were performed: the frequency of the allele (G vs. A), a comparison of homozygotes (GG vs. AA), a dominant genetic model (GG + AG vs. AA), and a recessive genetic model (GG vs. AG + AA). We applied two models of meta-analysis for dichotomous outcomes, according to the results of heterogeneity tests among individual studies, using the software Stata 11.0 (Stata Corp., College Station, Texas) or Review Manager (RevMan) 5.0 (Cochrane Collaboration, 2008; www.cc-ims.net/RevMan), a fixed-effects model (Mantel–Haenszel) [23] and a random-effects model (DerSimonian and Laird) [24]. Heterogeneity between studies was assessed using the Chi-square-based Q statistic test [25]. The Q statistic test was considered significant at $p < 0.10$. The random-effects model (if $p < 0.10$) or the fixed-effects model (if $p \geq 0.10$) was used to summarize the combined OR. The significance of the pooled OR was determined by the *Z*-test. A p value < 0.05 was considered significant. Publication bias was investigated with the funnel plot, in which the standard error (SE) of log (OR) for each study was plotted against the respective log (OR). An asymmetric plot suggested a possible publication bias. Funnel plot asymmetry was assessed further using Egger's linear regression method [26]. The significance of the intercept was determined by the *t* test, and a p value < 0.05 was considered significant.

The χ^2 goodness-of-fit test was used to evaluate whether genotypes within the control subjects conformed to the Hardy–Weinberg equilibrium (HWE). Analysis was performed using the software Stata version 11.0 and Review Manager 5.0. All p-values were two-sided.

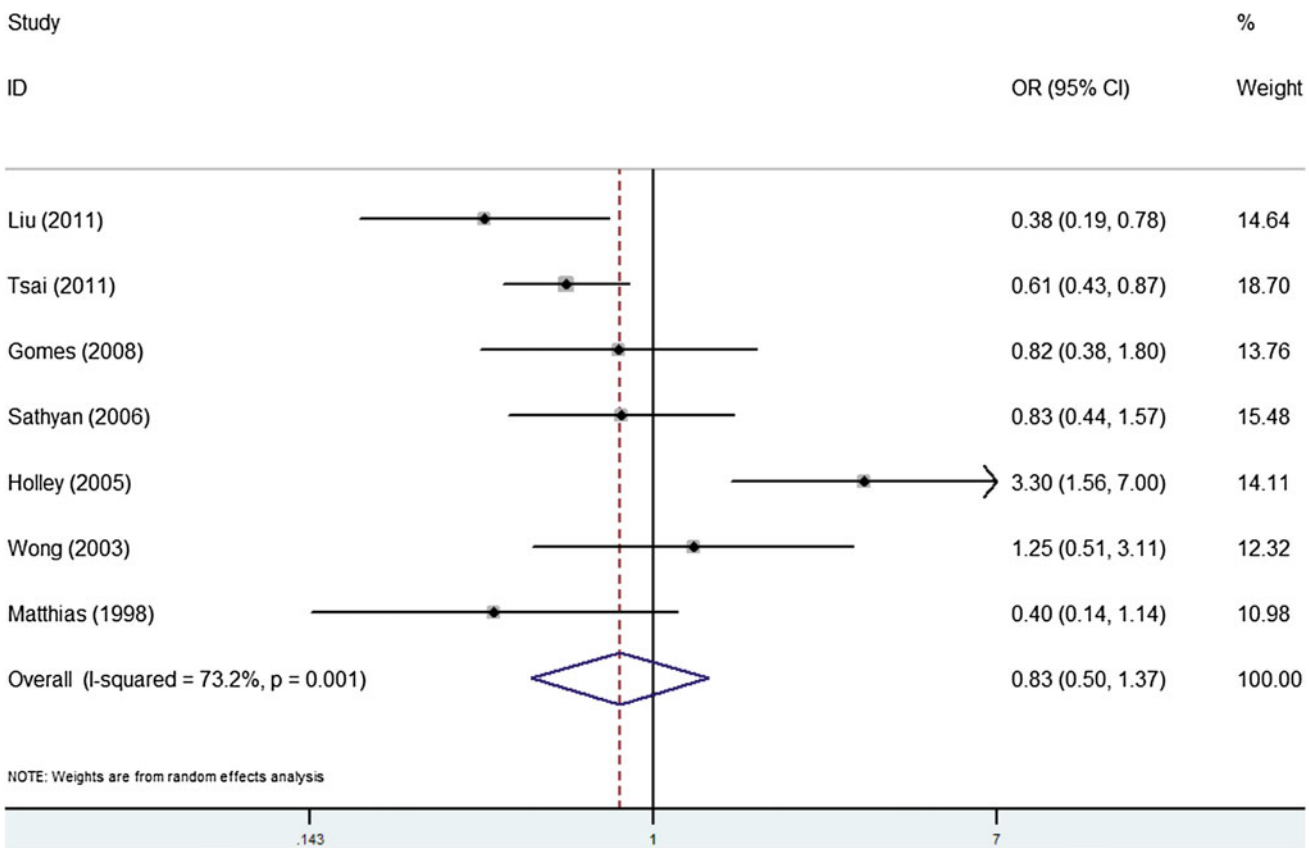

Fig. 3 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the homozygote comparison model

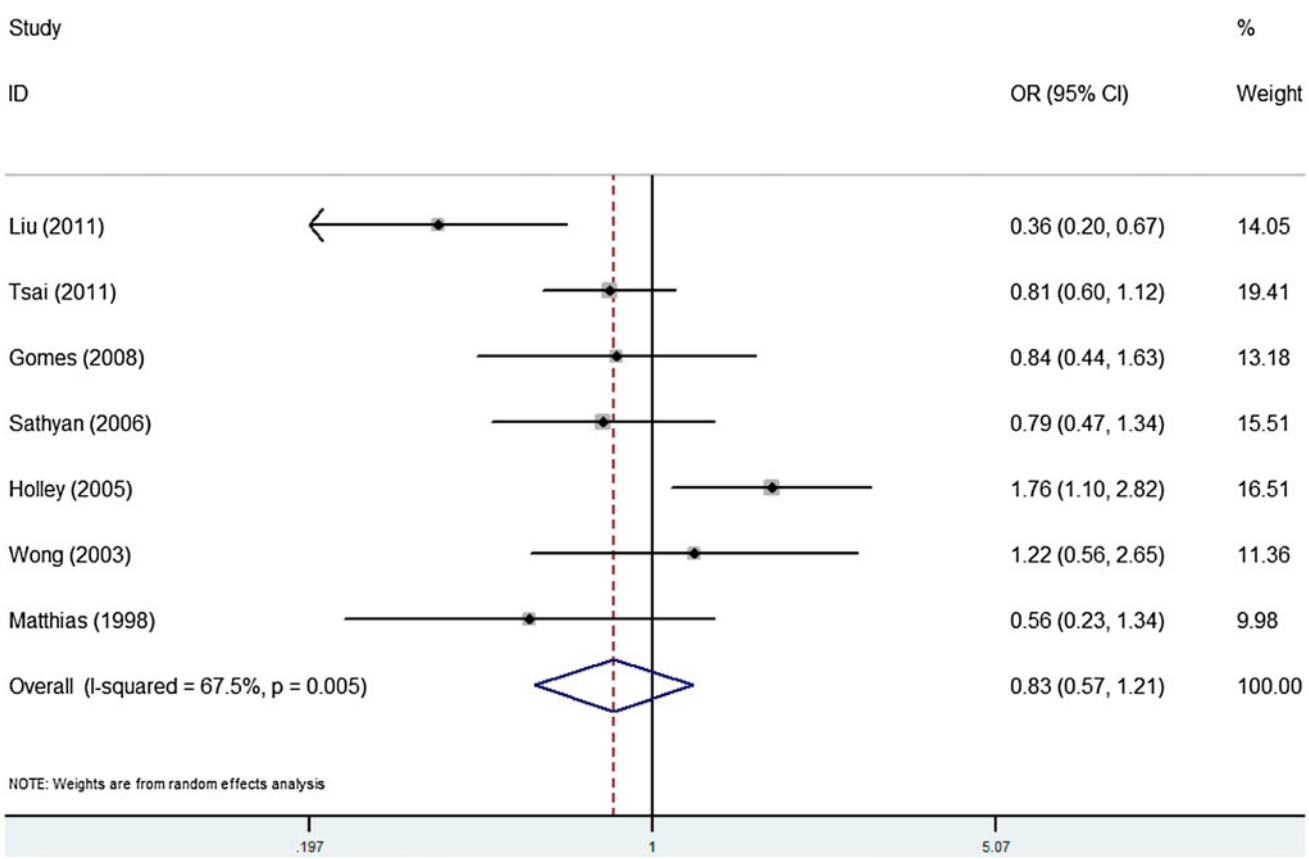

Fig. 4 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the recessive genetic model

Results

Characteristics of published studies

In total, seven articles published in English met the inclusion criteria. The seven studies (1,260 cases and 1,382 controls) were published between 1998 and December 1, 2011 (Table 1, Fig. 1). Among them, three articles originated from China, two from Germany, one from India and one from Brazil. A further four articles were from Asia and two were Caucasian in origin. Genotype distributions in the control groups in three studies [12–14] did not conform to the HWE equilibrium ($p = 0.00004$, 0.000003, and 0.0149, respectively), which indicated the presence of genotyping errors and/or population stratification.

Meta-analysis

Main results

As can be seen in Table 2, the *CCND1* G allele was not associated with an increased risk of developing oral cancer in terms of the frequency of allele comparison (G vs. A: OR = 0.882; 95 % CI = 0.684–1.137; $p = 0.001$ for heterogeneity) (Fig. 2). The *CCND1* GG genotype was not associated with oral cancer when compared the AA genotype, as revealed by both the homozygote comparison (GG vs. AA: OR = 0.826; 95 % CI = 0.500–1.365; $p = 0.001$ for heterogeneity) (Fig. 3) and the recessive genetic model (GG vs. AG + AA: OR = 0.833; 95 % CI = 0.574–1.207; $p = 0.005$ for heterogeneity) (Fig. 4). In addition, analysis of the dominant model did not indicate a significant association between the *CCND1* A870G polymorphism and oral cancer (GG + AG vs. AA: OR = 0.897; 95 % CI = 0.631–1.275; $p = 0.010$ for heterogeneity) (Fig. 5).

When we removed the three studies that deviated from the HWE equilibrium, the association between *CCND1* A870G and oral cancer was also not significant with respect to allele comparison (G vs. A: OR = 1.036; 95 % CI = 0.719–1.492; $p = 0.013$ for heterogeneity), the homozygote comparison (GG vs. AA: OR = 1.124; 95 % CI = 0.489–2.583; $p = 0.006$ for heterogeneity), the recessive genetic model (GG vs. AG + AA: OR = 1.042; 95 % CI = 0.628–1.727; $p = 0.055$ for heterogeneity) and the dominant genetic model (GG + AG vs. AA: OR = 1.120; 95 % CI = 0.634–1.979; $p = 0.032$ for heterogeneity).

In subgroup analysis, the *CCND1* G allele was associated with a borderline significantly decreased risk of

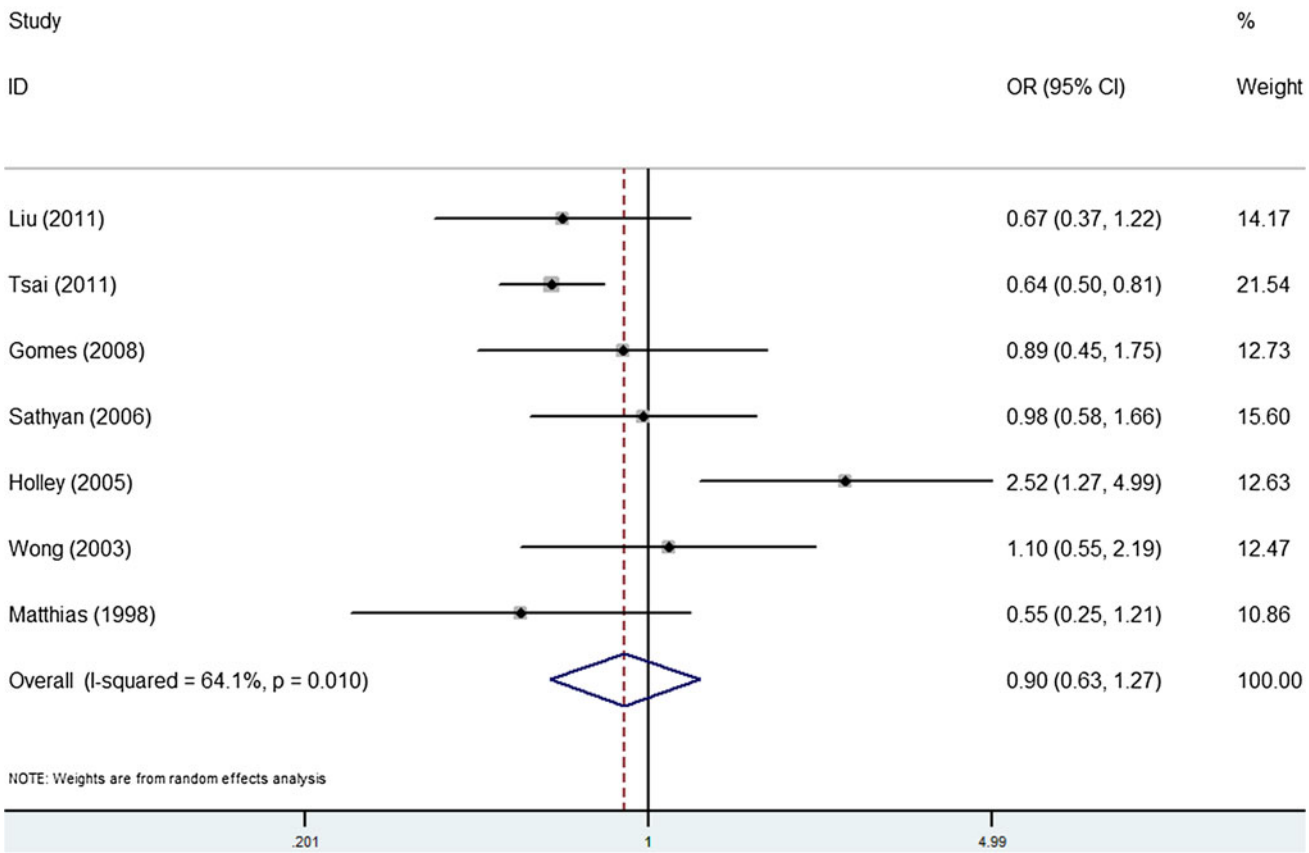

Fig. 5 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the dominant genetic model

developing oral cancer in Asians, in the frequency of allele comparison (G vs. A: OR = 0.800; 95 % CI = 0.636–1.006; $p = 0.089$ for heterogeneity). The association between the GG genotype and oral cancer was also significant in Asians with respect to both the homozygote comparison (GG vs. AA: OR = 0.644; 95 % CI = 0.491–0.843; $p = 0.186$ for heterogeneity) (Fig. 6) and the dominant genetic model (GG + AG vs. AA: OR = 0.713; 95 % CI = 0.584–0.870; $p = 0.293$ for heterogeneity) (Fig. 7). On the other hand, significant associations were not identified between the *CCND1* A870G polymorphism and oral cancer in Caucasians, either through allele comparison, homozygote comparison, or analysis of the recessive and dominant models (2).

Publication bias

Begg's funnel plot and Egger's test were performed to determine whether a publication bias existed in the literature. Firstly, the possibility of a publication bias was evaluated using a funnel plot of the estimate of log OR for the genotype G vs. A against the reciprocal of its SE (Table 3). The results of the frequency of allele comparison indicated that there was no publication bias, both in Begg's test ($z = 0.15$, $p > |z| = 0.881$) and Egger's test ($t = -0.73$, $p > |t| = 0.497$). Secondly, publication bias was evaluated using a funnel plot of the estimate of log OR for the genotype GG vs. AA against the reciprocal of its SE (Table 3). The results for the homozygote comparison GG vs. AA indicated no publication bias in Begg's test ($z = 0.45$, $p > |z| = 0.652$) and Egger's ($t = -1.01$, $p > |t| = 0.359$). The results of Begg's and Egger's tests for the recessive genetic model and the dominant model also indicated a low probability of publication bias (Table 3). Thus, we considered that no publication bias was present.

Discussion

CCND1 promotes cell migration, regulates cellular metabolism and conveys transcriptional functions [5]. Our meta-analysis was based on seven studies that provided data on the *CCND1* A870G polymorphism and the risk of developing oral cancer, and included over 1,260 cases and 1,382 controls. The results of our analysis provide evidence that genotypes for the *CCND1* A870G polymorphism might be associated with oral cancer in the Asian population. The *CCND1* A870G A allele may have a longer half-

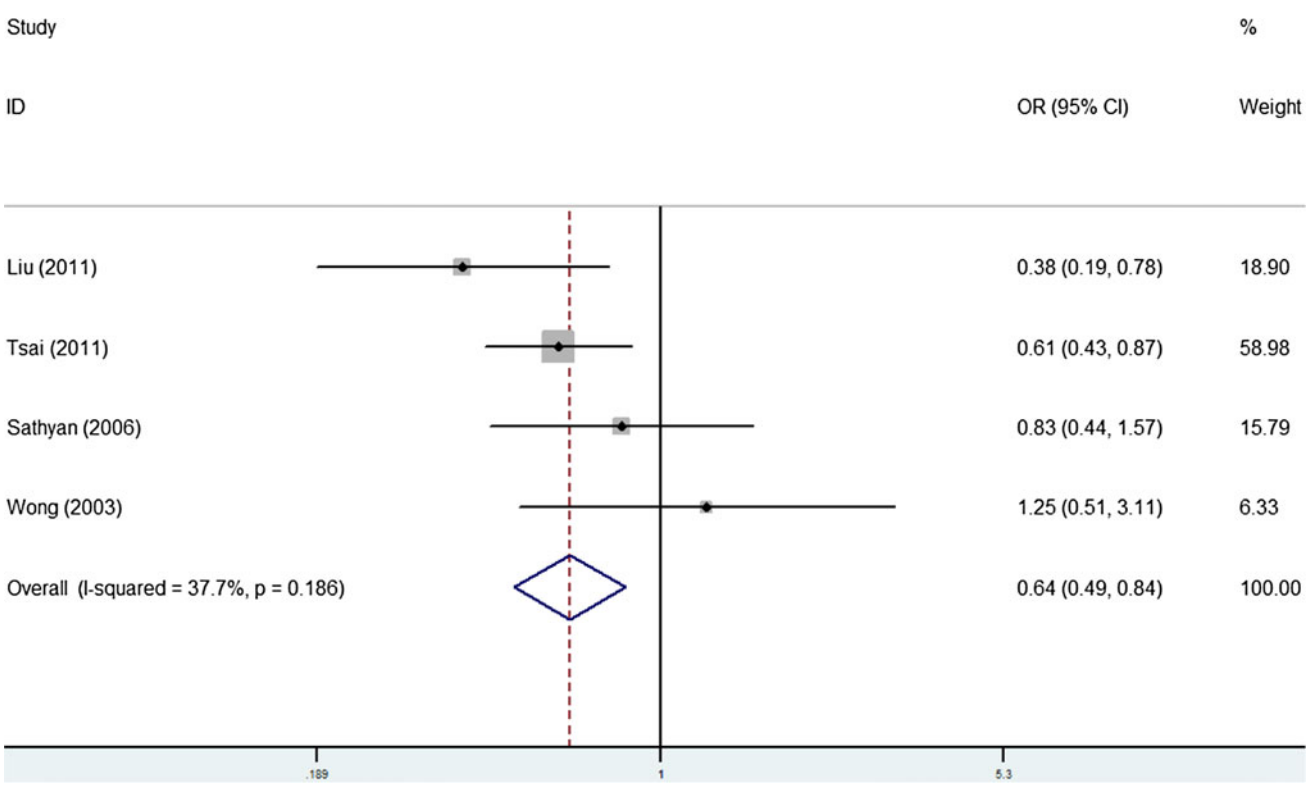

Fig. 6 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the homozygote comparison model in the Asian population

life than the G allele and may bypass the G1/S-checkpoint [11], which is in accordance with our results that revealed that the *CCND1* G allele is protective and decreases the risk of developing oral cancer.

Matthias et al. [18] were the first to investigate the association between the incidence of oral cancer and the *CCND1* A870G polymorphism. Subsequent studies revealed controversial findings, with some studies failing to find evidence of an association between the *CCND1* A870G polymorphism and oral cancer [14, 15, 17]. The purpose of this meta-analysis was to assess whether an association exists between the *CCND1* A870G polymorphism and the risk of developing oral cancer. Most of the studies included in our meta-analysis involved less than two hundred cases, and the statistical power was therefore too low to allow convincing conclusions to be drawn from individual studies. Consequently, the CIs around the ORs were wide. Our meta-analysis suggests that genotypes for the *CCND1* A870G polymorphism might be associated with the risk of developing oral cancer in the Asian population.

There was significant heterogeneity for the *CCND1* A870G polymorphism among the seven studies. Many factors might contribute to this heterogeneity, with ethnicity one such factor, as allele and genotype distributions for the *CCND1* A870G locus varied between different ethnic groups. We categorized the seven studies into different subgroups on the basis of ethnicity. In the Asian group, the results indicated a significant association between the *CCND1* A870G polymorphism and oral cancer. However, heterogeneity was observed in the Caucasian group, and no significant associations were found between the *CCND1* A870G polymorphism and oral cancer in Caucasians.

Considering CCND1 A870G mutant alleles in the control group, OR, case samples and control samples, the power of our meta-analysis ($\alpha = 0.05$) was 0.359 in 1,230 cases and 1,377 controls with OR = 0.882. In subgroup analysis, the power of our meta-analysis ($\alpha = 0.05$) was 0.674 in 938 cases and 951 controls with OR = 0.800 in Asians.

This study has limitations. The number of studies included in the meta-analysis was small. Given that both positive and negative studies had been published, publication bias concerning the association between the *CCND1* A870G polymorphism and oral cancer appears to have been low. The funnel plots were symmetrical for the *CCND1* A870G polymorphism, which indicates a lack of publication bias. However, studies with nonsignificant findings could reduce the chance of publication bias. The seven included studies were undertaken in different countries.

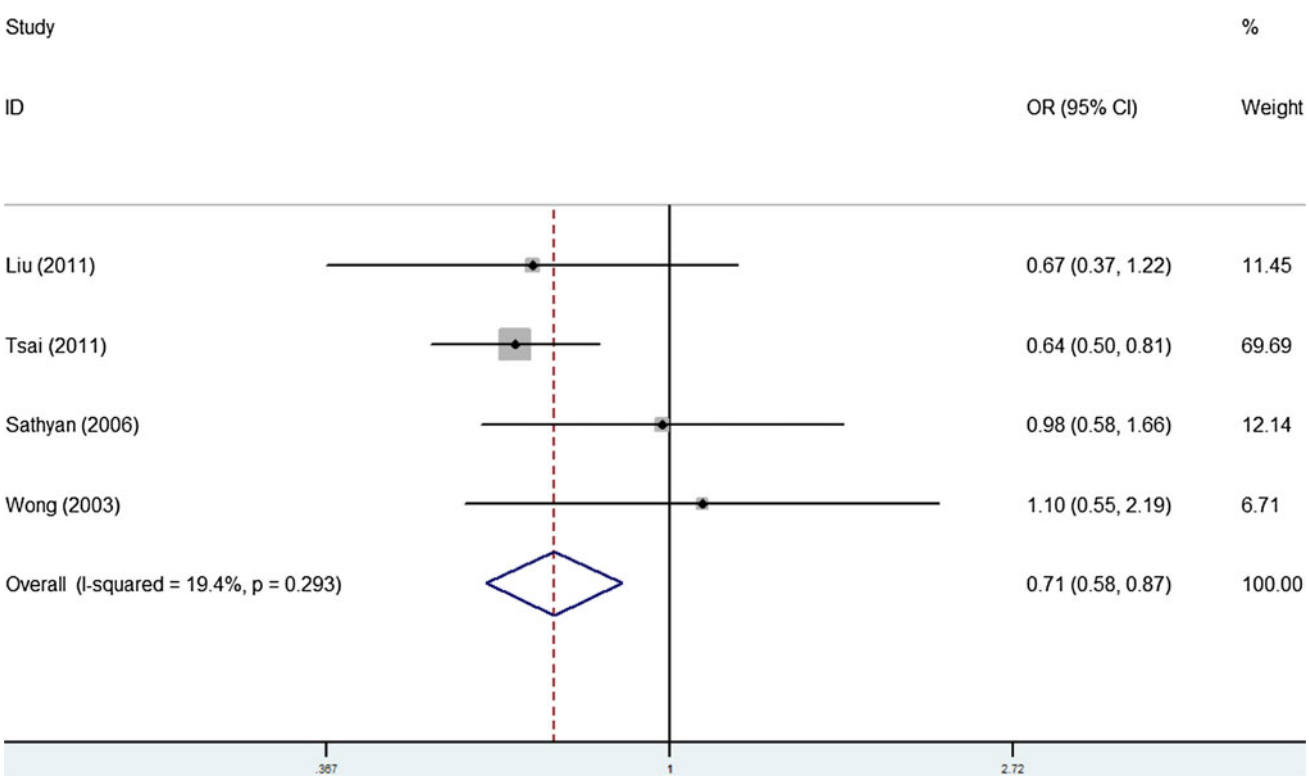

Fig. 7 Forest plot of the *CCND1* A870G polymorphism and the risk of developing oral cancer in the dominant genetic model in the Asian population

Table 3 Tests for publication bias (Egger's test and Begger's test) in population

Genetic comparison	Population	Egger's test (*t*, *p*)	Begger's test (*z*, *p*)
G vs. A	All	−0.73, 0.497	−0.15, 0.881
	Caucasian	–	−1.00, 0.317
	Asian	−0.95, 0.443	0.68, 0.497
GG vs. AA	All	−1.01, 0.359	0.45, 0.652
	Caucasian	–	−1.00, 0.317
	Asian	−1.23, 0.345	0.68, 0.497
GG vs. (AG + AA)	All	0.08, 0.938	−0.15, 0.881
	Caucasian	–	−1.00, 0.317
	Asian	−0.22, 0.845	0.00, 1.000
(GG + AG) vs. AA	All	−2.11, 0.089	0.75, 0.453
	Caucasian	–	−1.00, 0.317
	Asian	−3.39, 0.077	1.36, 0.174

In conclusion, the results of this meta-analysis support a substantial association between the *CCND1* A870G polymorphism and the risk of developing oral cancer in the Asian population. The existence of genetic structures in this population might lead to the identification of false positive genetic associations due to an unbalanced distribution between cases and controls. Oral cancer appears to be the result of complex interactions between genetic factors and the environment. Large-scale, population-based association studies are now required to investigate potential gene–gene and gene–environment interactions that involve the *CCND1* A870G polymorphism and that could affect the risk of developing oral cancer. Such studies might eventually lead to a better and more comprehensive understanding of the association between the *CCND1* A870G polymorphism and oral cancer.

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Disclosures All authors have no disclosures or conflict of interest.

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