RESEARCH ARTICLE

Association of Cyclin D1 Variants with Head and Neck Cancer Susceptibility: Evidence from a Meta-analysis

Hai Lin[1], Lian Fang[1,2], Dong Lin[3]*

Abstract

Whether cyclin D1 (CCND1) gene variants increase susceptibility to head and neck cancer (HNC) is undetermined. Therefore, we performed the present meta-analysis to systematically assess any possible association between CCND1 variants (G870A and G1722C) and HNC risk. Seventeen studies for CCND1 G870A and three studies for CCND1 G1722C were included. Overall, CCND1 polymorphisms (G870A and G1722C) had no association with increased HNC risk (p>0.05). In the subgroup analysis by smoking status, significantly increased HNC risk was found among smokers under allele contrast, homozygous comparison and recessive models (p<0.05), smoking carriers of A allele and AA genotype appearing at elevated risk. In conclusion, while there was overall a lack of any association between CCND1 polymorphisms (G870A and G1722C) and HNC risk, smokers carrying the A allele and AA genotype of the CCND1 G870A polymorphism may be susceptible to HNC development.

Keywords: Cyclin D1 - variant - polymorphism - meta-analysis - head and neck cancer

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Introduction

Head and neck cancers (HNC) comprising malignant neoplasms of the oral cavity, pharynx, and larynx, are the sixth most common cancers which threatens human life worldwide (Jemal et al., 2010). Although the exact pathogenetic mechanisms of HNC are not yet fully elucidated, there are ample evidences suggesting that HNC are complex multifactorial disorders involving genetic factors, lifestyle and environmental factors (Abusail et al., 2013, Mishra and Meherotra, 2014) and some low-penetrant genes have been identified as potential HNC susceptibility genes (Hopkins et al., 2008, Niu et al., 2012).

Among them, an important one is a cell cycle regulatory gene called cyclin D1 (CCND1), which is located on chromosome 11q13 and encodes CCND1 protein, which plays a pivotal role in regulating the cell cycle at the G1 to S phase transition in the process of cell division and up-regulation of CCND1 has been proved to disrupt normal cell cycle control and participate in the oncogenesis of lung cancer and breast cancer (Cui et al., 2012, Li et al., 2012). The two most commonly studied variants in the CCND1 gene, G870A (rs9344) and G1722C (rs678653), have both been associated with cancer risk. The most important mutation, CCND1 G870A polymorphism, is caused by a G to A transition at codon 241 in exon 4 resulting in the elevated mRNA alternative splicing, which leads to an altered protein having a longer half-life (Qin et al., 2014). Carries of CCND1 A allele may have a longer half-life and bypass the G1/S checkpoint more easily than the G allele, and is crucial in the carcinogenesis and development of brain tumors (Zeybek et al., 2013). A second common variant at nucleotide 1722 within CCND1 3 UTR, CCND1 G1722C, has been proved to be associated with urothelial cancer risk (Lin et al., 2011).

To date, a series of case-control studies have been conducted to clarify the association between CCND1 polymorphisms (G870A and G1722C) and HNC risk. However, the results were inconsistent. One meta-analysis regarding cyclin D1 G870A variant with head and neck cancer susceptibility has been published (Tang et al.,2011). However, the meta-analysis was only based on eleven case–control studies and included relatively modest sample sizes, and did not have detailed subgroup analysis by source of controls, cancer site, gender, smoking status, T stage, histological differentiation and lymph nodes. Additionally, several studies on the issue have been published recently. Therefore, we performed this meta-analysis in order to precisely assess the possible association of CCND1 G870A and G1722C polymorphisms with the susceptibility to develop HNC.

*[1]Department of Otorhinolaryngology, Eye and ENT Hospital of Fudan University, Shanghai, [2]the First Affiliated Hospital of Wenzhou Medical College, Wenzhou, Zhejiang, [3]Department of Biology and Chemical Engineering, Fuqing Branch of Fujian Normal University, Fuqing, Fujian, China *For correspondence: yunfenh@163.com*

Materials and Methods

Literature Search Strategy

The OVID, Medline, Embase, Pubmed, Web of Science databases (up to November 2013) were searched to identify the studies focusing on the association between CCND1 G870A and G1722C variants and susceptibility to HNC. The formats of search terms were used as follows: "cyclin D1", "CCND1", "head and neck cancer", "oral cancer", "pharyngeal cancer", "oropharyngeal cancer", "nasopharyngeal cancer", "laryngeal cancer", "SNP or polymorphism or variant" and the combination of them. The literature retrieval was performed by two authors (H. Lin and D. Lin) independently. Relevant reviews and abstracts of meetings were searched for related studies.

Inclusion and Exclusion Criteria

Eligible studies which satisfied the following inclusion criteria would be included: 1) the study clearly assessed the association between CCND1 polymorphisms (G870A and G1722C) and HNC risk; 2) HNC was diagnosed by histopathological examination; 3)the normal healthy controls had no diagnosis of HNC. On the other hand, the exclusion criteria were used as follows:1) studies without normal healthy controls; 2) studies without essential data and information.

Data Extraction

Two authors (H. Lin and D. Lin) performed the extraction of relevant data respectively from all eligible studies. Disagreement was resolved by discussing between two authors (H. Lin and D. Lin). The relevant data as listed below were extracted: name of first author, publication year, country, ethnicity, source of controls, genotyping method, cancer site, pathologic type, gender, smoking status, alcohol drinking, T stage, histological differentiation and lymph nodes, total number of patients and controls, and distribution of genotypes in these two groups and *p*-value of Hardy-Weinberg equilibrium (HWE) tested in controls.

Statistical Analysis

Pooled Odds ratios (ORs) with 95% confidence intervals (CIs) were used to evaluate the association between CCND1 polymorphisms (G870A and G1722C) and susceptibility to HNC on the basis of the distinct genotype and allele frequencies of CCND1 polymorphisms (G870A and G1722C) in two groups. The five distinct genetic models comprised allele contrast (M v W), homozygous comparison (MM v WW), heterozygous comparison (WM v WW), dominant model (WM+MM v WW) and recessive model (MM v WM+WW) (M mutant allele, W wild-type allele, MM mutant homozygotes, WW wild-type homozygotes, WM heterozygotes). We used I^2 statistic to check heterogeneity. *p*-value of heterogeneity less than 0.1 was confirmed as statistically significant. The summary ORs were calculated under fixed effects model in the case that *p*-value of heterogeneity was more than 0.1. Otherwise, we used random effects model to perform the data calculation. HWE in controls was assessed by the online program. Funnel plots, Begg's test and Egger's linear regression method were used to evaluate publication bias. In case of publication bias, the trim and fill method was applied to assess the influence to the result, $p<0.05$ was confirmed as statistically significant to evaluate the data except heterogeneity test. We conducted subgroup analyses by stratification of ethnicity, source of controls, cancer site, gender, smoking status, T stage, histological differentiation and lymph nodes. In addition, sensitivity analysis was conducted to verify the impact of individual study respectively. All the data statistics and analyses were conducted using Stata version 12.0 (Stata Corporation, College Station, TX).

Results

Study Characteristics

In summary, a total of 578 potentially relevant papers were identified after searching the OVID, Medline, Embase, Pubmed, Web of Science databases. Two authors (H. Lin and D. Lin) excluded ineligible articles independently. Then, 534 papers including duplicates or not related articles were excluded during screening. Then, 45 potentially relevant papers on CCND1 polymorphisms (G870A and G1722C) and susceptibility to HNC were selected. After careful examination of these papers, 26 papers were excluded for the following reasons: five were reviews, six without normal healthy controls, six on cancers other than HNC, two were overlapped studies, five on gene expression other than SNP, two without sufficient genotype data. Then, 19 potentially appropriate papers reported the association of CCND1 polymorphisms (G870A and G1722C) with the risk of HNC. However, 2 papers (Huang et al., 2006; Ye et al., 2008) were ruled out for the studies on premalignant lesion instead of cancer. As a result, 17 papers (Matthias et al., 1998; Zheng et al., 2001; Deng et al., 2002; Wong et al., 2003; Monteiro et al., 2004; Nishimoto et al., 2004; Holley et al., 2005; Catarino et al., 2006; Rydzanicz et al., 2006; Sathyan et al., 2006; Gomes et al., 2008; Marsit et al., 2008; Sui et al., 2009; Jelonek et al., 2010; Tsai et al., 2011; Shih et al., 2012; Sabir et al., 2013) comprising seventeen eligible studies with 3,761 cases and 3,834 controls for CCND1 G870A as well as three eligible studies (Sathyan et al., 2006; Tsai et al., 2011; Shih et al., 2012) with 943 cases and 935 controls for CCND1 G1722C were included. With regard to CCND1 G870A, seven studies (Matthias et al., 1998; Zheng et al., 2001; Monteiro et al., 2004; Holley et al., 2005; Catarino et al., 2006; Rydzanicz et al., 2006; Jelonek et al., 2010) were performed in Caucasians and seven (Deng et al., 2002; Wong et al., 2003; Sathyan et al., 2006; Sui et al., 2009; Tsai et al., 2011; Shih et al., 2012; Sabir et al., 2013) were conducted in Asians and three (Nishimoto et al., 2004; Gomes et al., 2008; Marsit et al., 2008) for mixed population. Fifteen studies (Matthias et al., 1998; Deng et al., 2002; Wong et al., 2003; Monteiro et al., 2004; Nishimoto et al., 2004; Holley et al., 2005; Catarino et al., 2006; Rydzanicz et al., 2006; Sathyan et al., 2006; Gomes et al., 2008; Sui et al., 2009; Jelonek et al., 2010; Tsai et al., 2011; Shih et al., 2012; Sabir et al., 2013) were hospital-based and two studies (Zheng et al., 2001; Marsit et al., 2008) were

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Table 1. Characteristics of all Included Studies

Author	Year	Country	Ethnicity	Source	Genotyping method	Cancer site (PT)	Total Number		Cases			Controls			HWE
							Cases	Controls	WW	WM	MM	WW	WM	MM	of controls
Matthias	1998	Germany	Caucasian	HB	PCR-RFLP	HNC(SCC)	384	191	100	193	91	55	101	35	0.3381
						OC(SCC)	38	191	7	20	11	55	101	35	0.3381
						PC(SCC)	87	191	18	46	23	55	101	35	0.3381
						LC(SCC)	259	191	75	127	57	55	101	35	0.3381
Zheng	2001	America	Caucasian	PB	PCR-SSCP	HNC(SCC)	233	248	62	116	55	78	129	41	0.3135
Deng	2002	China	Asian	HB	PCR-DHPLC	NPC(Mix*)	84	91	19	48	17	14	42	35	0.8115
Wong	2003	Taiwan	Asian	HB	PCR-SSCP	OC(SCC)	70	93	15	36	19	17	49	27	0.5239
Nishimoto	2004	Brazil	Mix	HB	PCR-DHPLC	HNC(SCC)	147	135	53	68	26	40	69	26	0.6985
Monteiro	2004	Portugal	Caucasian	HB	PCR-RFLP	LC(SCC)	66	110	23	30	13	14	49	47	0.8258
Holley	2005	Germany	Caucasian	HB	PCR-RFLP	OC(SCC)	174	155	66	94	14	40	87	28	0.1073
Catarino	2006	Portugal	Caucasian	HB	PCR-RFLP	NPC(Mix§)	94	187	26	42	26	28	105	54	0.0472
Sathyan	2006	India	Asian	HB	PCR-SSCP	OC(SCC)	146	137	36	71	39	40	61	36	0.2031
Rydzanicz	2006	Poland	Caucasian	HB	PCR-RFLP	LC(SCC)	63	102	12	41	10	38	43	21	0.1799
Marsit	2008	America	Mix	PB	ABI3500SDC	HNC(SCC)	698	777	210	314	174	238	396	143	0.3324
Gomes	2008	Brazil	Mix	HB	PCR-RFLP	OC(SCC)	80	80	25	30	25	28	29	23	0.0149
Sui	2009	China	Asian	HB	PCR-RFLP	NPC(SCC)	241	272	60	110	71	115	124	33	0.9617
Jelonek	2010	Poland	Caucasian	HB	PCR-RFLP	HNC(NM)	105	110	23	52	30	32	51	27	0.4577
Tsai	2011	Taiwan	Asian	HB	PCR-RFLP	OC(NM)	620	620	84	323	213	100	365	155	<0.0001
Sabir	2013	Pakistan	Asian	HB	PCR-SSCP	HNC(NM)	380	350	104	147	129	155	103	92	<0.0001
CCND1 G1722C (rs678653)															
Sathyan	2006	India	Asian	HB	PCR-SSCP	OC(SCC)	147	139	44	72	31	44	60	35	0.1167
Tsai	2011	Taiwan	Asian	HB	PCR-RFLP	OC(NM)	620	620	450	127	43	434	136	50	<0.0001
Shih	2012	Taiwan	Asian	HB	PCR-RFLP	NPC(NM)	176	176	127	37	12	124	38	14	0.0001

HB, hospital-based study; PB, population-based study; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism; PCR-DHPLC, polymerase chain reaction-denaturing high performance liquid chromatography; ABI3500SDC, ABI 3500 Sequence Detection System(Applied Biosystems); PT, pathologic type; HNC, head and neck cancer; SCC, squamous cell carcinoma; *82 squamous cell carcinomas; one non differentiated carcinoma and one large round cell carcinoma ;§69 undifferentiated; 11 moderately differentiated; one well differentiated and 13 not stated; LC, laryngeal carcinoma; OC, oral cance; PC, pharyngeal carcinoma; NPC, nasopharyngeal carcinoma; NM, not mentioned; WW, wild-type homozygotes; WM, heterozygotes; MM, mutant homozygotes; HWE, Hardy-Weinberg equilibrium

population-based. In addition, several studies had detailed genotype and allele frequencies under stratification of cancer site, gender, smoking status, alcohol drinking, T stage, histological differentiation and lymph nodes (Table 1 and Table 2). Consequently, we performed subgroup analysis by stratification of ethnicity, source of controls, cancer site, gender, smoking status, T stage, histological differentiation and lymph nodes (only one paper provided detailed and extractable data under stratification of alcohol drinking, so we did not perform subgroup analysis by alcohol drinking). Details of subjects in these studies were outlined in Table 1 and Table 2.

Quantitative synthesis

The main results of our meta-analysis under five distinct genetic models were listed in Table 3. Overall, CCND1 G870A and G1722C polymorphisms had no association with increased HNC risk under all five genetic models (p>0.05).

In addition, after excluding five studies (Catarino et al., 2006; Gomes et al., 2008; Tsai et al., 2011; Shih et al., 2012; Sabir et al., 2013) that significantly deviated from HWE, the results were not substantially altered, indicating statistically obvious robustness in our results (Table 3).

With regard to CCND1 G870A, still no significant association was found in the subgroup analysis by ethnicity, cancer site, gender, T stage, and lymph nodes under five genetic models (p>0.05) (Figure 1, Table 3).

In the subgroup analysis by source of controls, CCND1 G870A polymorphism had statistically significant association with elevated HNC risk in population-based studies performed in America under allele contrast, homozygous comparison and recessive model (p<0.05, Table 3). However, there were only two population-based

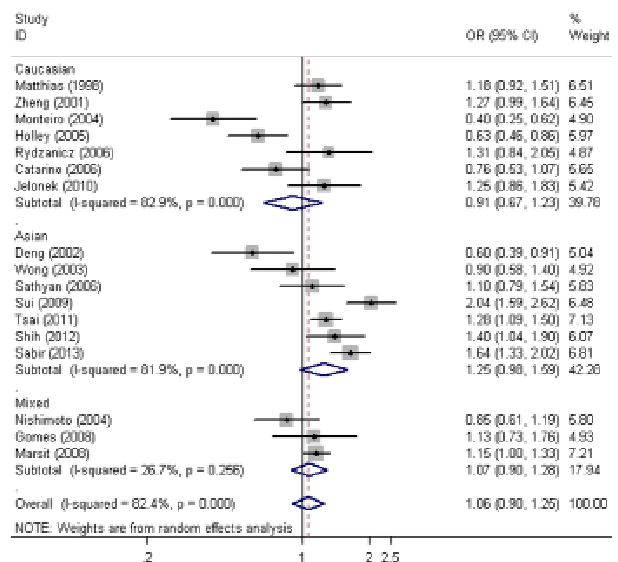

Figure 1. Forest Plot of CCND1 G870A Polymorphism Associated with HNC Risk by Ethnicity Stratification Under Allele Contrast (A versus G, random effects model was used)

studies included in this meta-analysis, so the results should be interpreted with caution.

In the subgroup analysis by smoking status and histological differentiation, significantly increased HNC risk was found among smokers under allele contrast, homozygous comparison and recessive model (p<0.05, Figure 2, Table 3). Carriers of A allele was about 36% more likely (OR=1.36, 95% CI=1.04-1.79, p=0.026) compared to carriers of G allele and carriers of AA genotype, was about 77% more likely (OR=1.77, 95% CI=1.02-3.07, p=0.044) and about 53% more likely (OR=1.53, 95% CI=1.09-2.15, p=0.015), respectively, to

Table 2. Characteristics of Studies for Subgroup Analysis

Author	Subgroup	Total Cases	Number Controls	Cases GG	Cases GA	Cases AA	Controls GG	Controls GA	Controls AA
CCND1 G870A (rs9344)									
	Gender								
Matthias	Male	327	149	86	161	80	45	81	23
Matthias	Female	57	42	14	32	11	10	20	12
Zheng	Male	169	173	35	88	46	30	89	54
Zheng	Female	64	75	20	28	16	11	40	24
Holley	Male	135	125	49	73	13	35	72	18
Holley	Female	38	30	17	21	0	5	15	10
Sabir	Male	224	201	65	88	71	95	56	50
Sabir	Female	156	149	39	59	58	60	47	42
	Smoking status								
Zheng	Smoker	168	163	35	81	52	27	84	52
Zheng	Nonsmoker	65	85	20	35	10	14	45	26
Sui	Smoker	178	87	49		129*	67		20*
Sui	Nonsmoker	63	185	11		52*	48		137*
Tsai	Smoker	458	433	59	240	159	72	261	100
Tsai	Nonsmoker	162	187	25	83	54	28	104	55
Shih	Smoker	77	73	5	37	35	11	44	18
Shih	Nonsmoker	99	103	18	49	32	17	61	25
Sabir	Smoker	191	177	45	78	68	71	57	49
Sabi	Nonsmoker	189	173	59	69	61	84	46	43
Holley	Smoker	144	NM	59	79	6	NM	NM	NM
Holley	Nonsmoker	26	NM	6	14	6	NM	NM	NM
	Alcohol drinking								
Zheng	Drinker	183	162	38	93	52	29	83	50
Zheng	Nondrinker	50	86	17	23	10	12	46	28
Holley	Drinker	141	NM	57	79	5	NM	NM	NM
Holley	Nondrinker	29	NM	8	14	7	NM	NM	NM
	T stage								
Matthias	T1+T2	194	191	53	97	44	55	101	35
Matthias	T3+T4	155	191	32	80	43	55	101	35
Nishimoto	T1+T2	45	135	18		27*	40	69	26
Nishimoto	T3+T4	100	135	34		66*	40	69	26
Holley	T1+T2	124	155	50	67	7	40	87	28
Holley	T3+T4	43	155	15	25	3	40	87	28
Rydzanicz	T1+T2	8	102	2	5	1	38	43	21
Rydzanicz	T3+T4	55	102	10	36	9	38	43	21
	HD								
Matthias	G0/1+G2	160	191	36	82	42	55	101	35
Matthias	G3	54	191	21	27	6	55	101	35
Holley	G0/1+G2	97	155	37	53	7	40	87	28
Holley	G3	19	155	6	12	1	40	87	28
Rydzanicz	G0/1+G2	57	102	11	36	10	38	43	21
Rydzanicz	G3	6	102	1	5	0	38	43	21
	Lymph nodes								
Matthias	Negative	120	191	30	62	28	55	101	35
Matthias	Positive	93	191	27	47	19	55	101	35
Nishimoto	Negative	72	135	27		45*	40	69	26
Nishimoto	Positive	74	135	25		49*	40	69	26
Holley	Negative	27	155	8	16	3	40	87	28
Holley	Positive	134	155	57	71	6	40	87	28
Rydzanicz	Negative	36	102	7	26	3	38	43	21
Rydzanicz	Positive	27	102	5	15	7	38	43	21

HD, Histological differentiation; NM, not mentioned; *GA+AA

have HNC compared to carriers of the GG genotype and GG+GA genotype among smokers. Carriers of A allele and AA genotype may be associated with elevated HNC risk among smokers. Moreover, HNC with histological differentiation G3 had a borderlined association of G870A with decreased HNC risk under homozygous comparison (OR=0.41, 95% CI=0.17-0.99, p=0.048).

Heterogeneity and Publication Bias

There were some significant heterogeneities for the analysis of association between CCND1 G870A polymorphism and susceptibility to HNC under all genetic models (Ph, 0.10, Table 3). Hence, the results were assessed under random effects model. Howerver, no significant heterogeneities were found for CCND1

Table 3. Main Results of Pooled ORs in this Meta-Analysis

Study groups	N	n	M versus W		n	MM versus WW		n	WM versus WW		n	WM +MM versus WW		n	MM versus WW+WM	
			OR (95% CI)	Ph		OR (95% CI)	Ph		OR (95% CI)	Ph		OR (95% CI)	Ph		OR (95% CI)	Ph
CCND1 G870A																
All	17	17	1.06 (0.90-1.25)	<0.001	17	1.10 (0.80-1.52)	<0.001	17	1.05 (0.84-1.30)	<0.001	17	1.05 (0.83-1.33)	<0.001	17	1.12 (0.88-1.41)	<0.001
All in HWE	12	12	1.00 (0.80-1.24)	<0.001	12	0.98 (0.63-1.52)	<0.001	12	1.03 (0.83-1.26)	0.005	12	1.02 (0.77-1.36)	<0.001	12	0.97 (0.69-1.37)	<0.001
Ethnicity																
Caucasian	7	7	0.91 (0.67-1.23)	<0.001	7	0.78 (0.41-1.48)	<0.001	7	0.92 (0.60-1.40)	0.001	7	0.88 (0.55-1.40)	<0.001	7	0.86 (0.55-1.32)	<0.001
Asian	7	7	1.25 (0.98-1.59)	<0.001	7	1.51 (0.96-2.37)	<0.001	7	1.29 (0.97-1.72)	0.037	7	1.30 (0.93-1.80)	0.002	7	1.35 (0.94-1.95)	<0.001
Mixed	3	3	1.07 (0.90-1.28)	0.256	3	1.20 (0.86-1.67)	0.279	3	0.89 (0.72-1.10)	0.628	3	0.99 (0.82-1.20)	0.448	3	1.29 (0.97-1.71)	0.291
Controls source																
HB	15	15	1.04 (0.85-1.27)	<0.001	15	1.03 (0.70-1.52)	<0.001	15	1.05 (0.81-1.36)	<0.001	15	1.03 (0.77-1.37)	<0.001	15	1.05 (0.79-1.39)	<0.001
PB	2	2	1.18 (1.04-1.34)	0.512	2	1.45 (1.12-1.86)	0.508	2	0.95 (0.77-1.17)	0.347	2	1.08 (0.89-1.31)	0.365	2	1.49 (1.20-1.86)	0.826
Cancer site																
OC	6	6	1.05 (0.82-1.34)	0.003	6	1.06 (0.63-1.78)	0.003	6	1.01 (0.82-1.26)	0.400	6	1.05 (0.78-1.42)	0.077	6	1.06 (0.71-1.56)	0.006
LC	3	3	0.83 (0.43-1.59)	<0.001	3	0.68 (0.19-2.43)	0.001	3	1.01 (0.37-2.76)	0.001	3	0.89 (0.31-2.56)	<0.001	3	0.69 (0.30-1.59)	<0.001
PC	5	5	1.10 (0.69-1.75)	<0.001	5	1.31 (0.50-3.41)	<0.001	5	1.00 (0.59-1.70)	0.008	5	1.02 (0.52-1.98)	<0.001	5	1.38 (0.70-2.69)	<0.001
Gender	8	8			8			8			8			8		
Male	4	4	1.10 (0.78-1.57)	0.001	4	1.15 (0.61-2.18)	0.005	4	1.12 (0.67-1.87)	0.005	4	1.13 (0.66-1.91)	0.002	4	1.12 (0.73-1.71)	0.048
Female	4	4	0.72 (0.35-1.48)	<0.001	4	0.49 (0.12-1.97)	<0.001	4	0.82 (0.34-1.96)	0.007	4	0.70 (0.25-1.92)	<0.001	4	0.69 (0.28-1.66)	0.010
Smoking status																
Smoker	5	4	1.36 (1.04-1.79)	0.016	4	1.77 (1.02-3.07)	0.023	4	1.30 (0.80-2.10)	0.042	5	1.18 (0.22-6.17)	<0.001	4	1.53 (1.09-2.15)	0.072
Nonsmoker	5	4	1.05 (0.70-1.58	0.001	4	1.00 (0.48-2.10)	0.006	4	0.99 (0.53-1.85)	0.012	5	1.10 (0.65-1.88)	0.006	4	1.10 (0.70-1.73)	0.054
T stage																
T1+T2	4	3	0.85 (0.49-1.47)	0.010	3	0.59 (0.14-2.60)	0.003	3	0.86 (0.53-1.38)	0.212	4	0.77 (0.48-1.22)	0.117	3	0.61 (0.18-2.11)	0.007
T3+T4	4	3	1.11 (0.70-1.75)	0.028	3	1.13 (0.39-3.29)	0.027	3	1.46 (0.72-2.96)	0.042	4	1.20 (0.68-2.10)	0.023	3	0.87 (0.35-2.14)	0.030
H D																
G0/1+G2	3	3	1.03 (0.62-1.73)	0.003	3	0.96 (0.29-3.14)	0.003	3	1.27 (0.60-2.69)	0.012	3	1.21 (0.55-2.68)	0.004	3	0.81 (0.33-1.98)	0.012
G3	3	3	0.72 (0.50-1.02)	0.843	3	0.41 (0.17-0.99)	0.850	3	0.90 (0.46-1.77)	0.279	3	1.80 (0.31-10.44)	<0.001	3	0.47 (0.21-1.05)	0.741
Lymph nodes																
Negative	4	3	1.09 (0.85-1.41)	0.509	3	1.14 (0.65-1.99)	0.383	3	1.44 (0.72-2.85)	0.105	4	1.39 (0.66-2.91)	0.004	3	0.76 (0.31-1.82)	0.102
Positive	4	3	0.93 (0.50-1.72)	0.001	3	0.73 (0.16-3.42)	<0.001	3	0.98 (0.48-1.99)	0.039	4	0.89 (0.50-1.58)	0.020	3	0.70 (0.23-2.12)	0.005
CCND1 G1722C																
All	3	3	0.90 (0.77-1.06)	0.925	3	0.84 (0.61-1.17)	0.986	3	0.95 (0.77-1.19)	0.650	3	0.92 (0.75-1.12)	0.769	3	0.83 (0.61-1.13)	0.981

N, total number of studies for overall or subgroup analysis; n, number of studies for genotype analysis under five distinct genetic models; M, mutant allele; W, wild-type allele; MM, mutant homozygotes; WW, wild-type homozygotes; WM, heterozygotes; Ph, P-value of Q-test for heterogeneity test; HB, hospital-based study; PB, population-based study; OC, oral cancer; LC, laryngeal carcinoma; PC, pharyngeal carcinoma; HD, histological differentiation

G1722C polymorphism, so the results were assessed under fixed effects model.

In this meta-analysis, no obvious publication bias was found for CCND1 G1722C under all models and CCND1 G870A under allele contrast, heterozygous comparison and dominant model (Figure 3). However, evidence of publication bias was found for CCND1 G870A under homozygous comparison and recessive model ($p<0.05$ for Begg's test and Eggers's linear regression method). Therefore, we performed the trim and fill method. The results showed that there was no need for trimming and filling, the adjusted ORs calculated using the trim and fill technique were identical to the non-adjusted significant ORs (OR=1.10, 95% CI=0.80-1.52, OR=1.12, 95% CI=0.88-1.41), which indicated that the influence to the results could be omitted and our results were stable and statistically robust.

Sensitivity analysis

Sensitivity analysis was performed to reflect the impact of the individual study to the summarized ORs by removing one study each time involved in the meta-analysis.

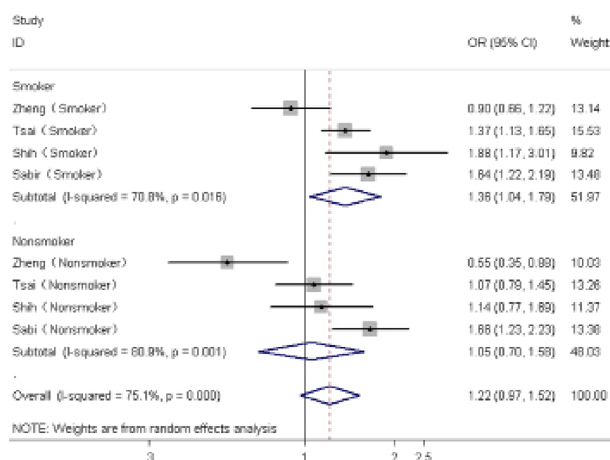

Figure 2. Forest Plot of CCND1 G870A Polymorphism Associated with HNC risk by Smoking Status Stratification Under Allele Contrast (A versus G, random effects model was used)

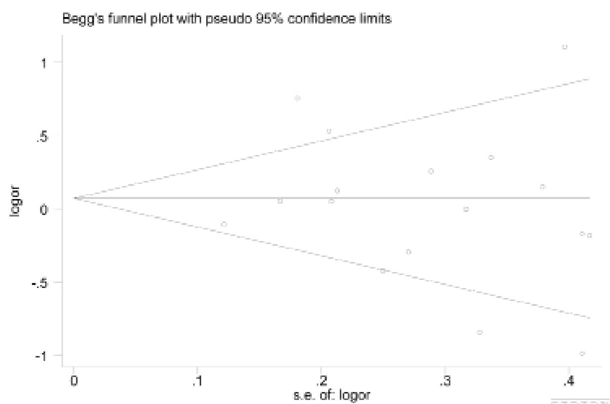

Figure 3. Begg's Funnel Plot of CCND1 G870A Polymorphism Associated with HNC Risk under Heterozygous Comparison (AG versus GG). Each small circle represents a separate study for the indicated association

We found that the summarized ORs with 95% CIs under all genetic models were not significantly altered after sensitivity analysis, indicating that our results were stable and statistically robust.

Discussion

It has been shown that CCND1 acting as a key regulatory protein in the process of cell growth is involved in the pathogenesis of cancer and the A allele or AA genotype of CCND1 G870A polymorphism plays a vital role in the mechanism of carcinogenesis (Zhuo et al., 2012). Up to now, different studies on the association between CCND1 polymorphisms (G870A and G1722C) and HNC risk have showed discrepant results. Thus, our meta-analysis from seventeen papers was performed to precisely assess the possible association of CCND1 polymorphisms (G870A and G1722C) with the susceptibility to develop HNC.

Our meta-analysis indicated the following descriptions: first, CCND1 polymorphisms (G870A and G1722C) had no association with increased HNC risk under all five genetic models by overall analysis; second, still no significant association was found under five genetic models in the subgroup analysis for CCND1 G870A by ethnicity, cancer site, gender, T stage, and lymph nodes; third, significantly increased HNC risk was found among smokers under allele contrast, homozygous comparison and recessive model. Smokers carrying the A allele and AA genotype of CCND1 G870A polymorphism may confer susceptibility to HNC.

Some limitations of our study should be interpreted. First, the included studies were carried out mainly in Caucasians and Asians and only two studies were population-based, which increased the limitation of statistical power. Hence, studies with larger sample sizes and representative population are warranted to verify our findings. Second, with regard to CCND1 G870A, evidence of publication bias was found for the meta-analysis under homozygous comparison and recessive model, although the results of the trim and fill method indicated that the influence to the results could be omitted. Finally, our results were grounded on unadjusted estimates, however, CCND1 polymorphisms (G870A and G1722C) are only two phenotypes of HNC and HNC are intricate disorders, and there are many other factors comprising genes, occupation, lifestyle, obesity and environmental factors that participate in the development of HNC. If the individual data including confounding factors mentioned above were available, a more precise analysis allowing for the adjustment by other covariants should be performed in the future.

In conclusion, our meta-analysis indicated that there was overall lack of association between CCND1 polymorphisms (G870A and G1722C) and HNC risk under all five genetic models and still no significant association was found in the subgroup analysis for CCND1 G870A by ethnicity, cancer site, gender, T stage, and lymph nodes. However, smokers carrying the A allele and AA genotype of CCND1 G870A polymorphism may confer susceptibility to HNC. Studies with large sample

sizes and representative population are warranted to further clarify this finding.

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