Genetic polymorphism of *CCND1* G870A and esophageal cancer susceptibility: A meta-analysis

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Abstract. The association between the polymorphism of cyclin D1 (*CCND1*) and esophageal carcinogenicity has been widely examined, however, it remains controversial. To evaluate the importance of *CCND1* G870A polymorphism with regard to the risk of esophageal cancer, a meta-analysis was carried out that reviewed the available literature in order to clarify the controversies. This meta-analysis included 1,154 cases and 1,678 controls for *CCND1* G870A polymorphism from seven published case-control studies. The odds ratio (OR) and 95% confidence interval (CI) were calculated using the Stata software version 11.1. The results were pooled using a dominant model to appropriately reflect a biological model of the genetic effect. No significant association was observed in the Caucasian (OR=1.64; 95% CI, 0.84-3.20) or the Asian populations (OR=1.30; 95% CI, 0.65-2.62), while no significant association was found in esophageal squamous cell carcinoma (ESCC) (OR=1.74; 95% CI, 0.79-3.81) or esophageal adenocarcinoma (EADC) (OR=1.18; 95% CI, 0.77-1.81). However, the comparison of A vs. G in *CCND1* G870A showed significant differential susceptibility to esophageal cancer (OR=1.26; 95% CI, 1.00-1.59). These findings suggested that the *CCND1* G870A polymorphism has no association with esophageal cancer risk in ethnicity and histology, respectively. Further studies are required to assess these associations in greater detail.

Introduction

Esophageal cancer is a global health problem that ranked eighth in terms of incidence and sixth in terms of mortality in 2002 (1). Esophageal tumors usually lead to dysphagia, pain and other symptoms and are diagnosed via a biopsy. Esophageal cancer is divided into squamous cell carcinoma and adenocarcinoma. Squamous cell carcinoma arises from the cells that line the upper part of the esophagus. Adenocarcinoma arises from glandular cells that are present at the junction of the esophagus and stomach (2). Genetic as well as environmental factors play a role in the carcinogenesis of esophageal cancer (3,4). Individual variations in cancer risk have been associated with specific polymorphisms of various genes that are present in a significant proportion of the normal population.

Cyclin D1 (*CCND1*) is a mitogenic sensor for the cell cycle mechanism and cellular oncogene (5). High activity of cyclin D1 can lead to chemoresistance due to the dual roles of cyclin D1 in promoting cell proliferation and inhibiting drug-induced apoptosis (6). Overexpression of cyclin D1 contributes to immature cell passage through the G1-S transition, induces propagation of unrepaired DNA damage and the accumulation of genetic errors and a selective growth advantage for the altered cells (7). Cyclin D1 are important regulators of the cell cycle and apoptosis and each contains functional single nucleotide polymorphisms (SNPs) (*CCND1* G870A) that are involved in the susceptibility and outcome of various human malignancies (8).

Poor statistical power has been controversial for data from individually published studies. The aim of this study was to evaluate the association between *CCND1* G870A and esophageal cancer risk in a large-scale case-control study. However, we analyzed two subgroups based on ethnicity and histology, in which the variant alleles potentially exert a stronger biologic effect.

Materials and methods

Search strategy. Relevant studies were selected by searching PubMed, EMBASE and HuGENet databases prior to July 1, 2012. A highly sensitive search strategy was used to identify relevant articles exploring the following terms or their combination: '*CCND1*', 'cyclin D1', '*PRAD1*', 'polymorphism', 'G870A', 'rs603965' and 'esophageal cancer'. Reference lists of identified studies and related reviews and proceedings of international meetings were reviewed to ensure identification of all potentially eligible studies.

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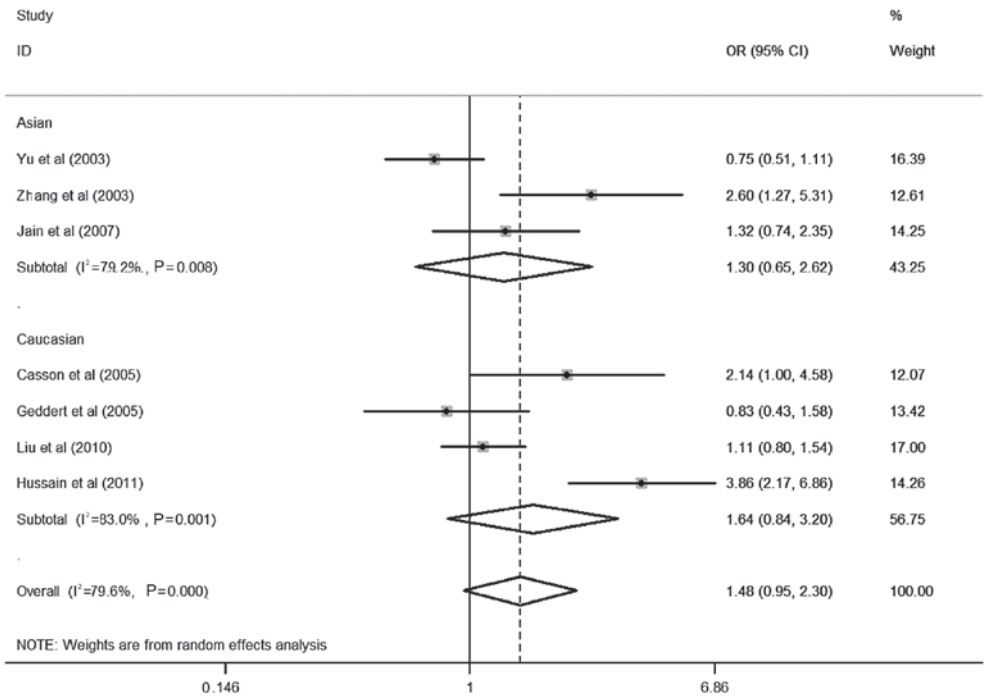

Figure 1. Forest plot (random-effects analysis) of esophageal cancer risk associated with *CCND1* G870A polymorphism based on ethnicity using a dominant genetic model.

Eligibility criteria. Identified studies included in our meta-analysis had to be: i) originally on *CCND1* G870A polymorphism and esophageal cancer risk; ii) a study to obtain G870A genotypes and the genotype distributions being reported separately; iii) case-control or cohort studies; iv) on a control population without severe respiratory disease and malignant tumor patients and v) a clear information source of the population.

Data extraction. From each eligible study, we extracted the following information: first author, year of publication, country of origin, ethnicity, sum of cases and controls with genotypes, diagnostic standards and genotype methods.

Statistical analysis. Funnel plots, together with the Begg's rank correlation test, were used to assess publication bias. Additionally, a subgroup analysis of the Caucasian and Asian populations was performed to account for the subpopulation structure. The effect of these polymorphisms on esophageal cancer risk was estimated using a logistic regression analysis to obtain pooled estimates of the odds ratio (OR) and a 95% confidence interval (CI). The most plausible gene effect model was determined without assuming a priority genetic model to avoid multiple comparisons.

First, the Pearson's Chi-square test (P≥0.05) (9) was used to check for deviations from the Hardy-Weinberg equilibrium (HWE) in the controls in each study. The measurement of the overall inconsistency index (I^2) (10) was then carried out to describe the proportion of total variation caused by heterogeneity (11). Meta-regression was performed to explore potential heterogeneity in various types of study designs. The I^2 test detected mainly moderate heterogeneity for the SNPs, thus a logistic regression was performed to evaluate gene effects via the random-effects model.

The parameters θ_2 and θ_3 were calculated using the formula: log it $(\pi_{ij}) = \alpha_i + \theta_2\ z_{i2} + \theta_3\ z_{i3}$, and $OR_{AB/AA} = \exp(\theta_2)$, $OR_{BB/AA} = \exp(\theta_2)$, αi are indicators of the study-specific fixed-effects, and θ_2 and θ_3 are dummy variables of genotypes AB and BB. The appropriate genetic model was identified using the criteria (12): i) no association: $\theta_2=\theta_3$ ($OR_{AB/AA}=OR_{BB/AA}=1$); ii) dominant model: $\theta_2\neq0, \theta_3\neq0$ and $\theta_2=\theta_3$ ($OR_{AB/AA}=OR_{BB/AA}\neq1$); iii) recessive model: $\theta_2=0$ ($OR_{AB/AA}=1$) and $\theta_3\neq0$ ($OR_{BB/AA}\neq1$); iv) co-dominant model: $\theta_2\neq0$, $\theta_3\neq0$ and $2\theta_2=\theta_3$ ($OR^2_{AB/AA}=OR_{BB/AA}$).

Metagen (http://bioinformatics.biol.uoa.gr/~pbagos/metagen/) was used by selecting the genetic model. Statistical analyses were carried out using the Stata software version 11.1 (Stata Corporation, College Station, TX, USA). The statistical evaluations were carried out using a two-sided test with a significance level of 0.05.

Results

Study inclusion and characteristics. Based on the inclusion criteria, we searched the PubMed, EMBASE and HuGENet databases by reading titles and abstracts. Ten original study articles concerning the association between *CCND1* G870A polymorphism and esophageal cancer risk were retrieved. The full text of these articles was reviewed intensively and three articles without the control group were excluded. Thus, seven case-control studies (1,154 esophageal cancer cases and 1,678 controls) on the association between *CCND1* G870 polymorphism and esophageal cancer risk were included in this meta-analysis (13-19). Of these included articles, four studies were on Caucasians (15,16,18,19) and three studies were on Asians (13,14,17).

The eligible studies were consistent with HWE. Their characteristics are shown in Table I, and frequency of *CCND1*

Table I. Characteristics of all eligible studies in this meta-analysis.

Authors	Year	Country	Ethnicity	Eligible subjects		Selective characteristics of cases and controls		Source of controls	Method	Ref.
				Cases	Controls	Cases	Controls			
Yu, *et al*	2003	China	Asian	321	345	Most of the cases and controls have been characterized in a molecular epidemiological study	Population controls were accrued from a database of the nutritional survey conducted in the same regions	Population	PCR-RFLP	(13)
Zhang, *et al*	2003	China	Asian	120	183	Histological tumor typing was carried out on the basis of the resection specimens in the Department of Pathology of the same hospital	The healthy controls were recruited from blood donors	Population	PCR	(14)
Casson, *et al*	2005	Canada	Caucasian	56	95	Cases that underwent diagnostic esophagogastroscopy were asked to provide three additional biopsy samples, in addition to the usual number of biopsies, which were placed in formalin for immunohistochemistry and routine histopathologic diagnosis	Patients who attended for consultation regarding unrelated, benign conditions were screened for GERD-related symptoms, a history of 'hiatus hernia', 'dyspepsia', antacid use; if negative, patients were asked to participate as controls	Hospital-based	PCR	(15)
Geddert, *et al*	2005	Germany	Caucasian	56	253	Tumors with their epicenter in the esophagus were regarded as esophageal in origin	The healthy controls were recruited from blood donors	Population	PCR	(16)
Jain, *et al*	2007	India	Asian	151	201	Histologically confirmed esophageal cancer patients of squamous cell carcinoma or adenocarcinoma were inducted into this study.	During the same period, blood samples of 201 unrelated controls were randomly selected from the outpatient clinics	Hospital-based	PCR-RFLP	(17)
Liu, *et al*	2010	USA	Caucasian	299	450	Patients with histologically confirmed EADC	Healthy unrelated age-, gender-, and gender-matched visitor controls with no history of cancer or gastroesophageal reflux disease were recruited from the same institutions	Hospital-based	TaqMan assays	(18)
Hussain, *et al*	2011	India	Caucasian	151	151	This study included a total of 151 histologically confirmed, untreated squamous cell carcinoma patients	The controls were recruited from a healthy Indian population	Population	PCR-RFLP	(19)

PCR-RFLP, polymerase chain reaction-restriction fragment length; PCR, polymerase chain reaction; GERD, gastroesophageal reflux disease; EADC, esophageal adenocarcinoma.

Table II. Heterogeneity test showing frequency of *CCND1* G870A polymorphism in various populations.

Authors	Year	Ethnicity	Cases (%)			Controls (%)			P-value for HWE	Refs.
			GG	GA	AA	GG	GA	AA		
Yu, *et al*	2003	Asian	21.2	48.9	29.9	16.8	51.3	31.9	0.354	(13)
Zhang, *et al*	2003	Asian	9.2	61.7	29.2	20.8	55.7	23.5	0.118	(14)
Casson, *et al*	2005	Caucasian	21.4	48.2	30.4	36.8	54.7	8.4	0.063	(15)
Geddert, *et al*	2005	Caucasian	28.6	46.4	25.0	24.9	53.8	21.3	0.224	(16)
Jain, *et al*	2007	Asian	14.6	50.3	35.1	18.4	55.2	26.4	0.114	(17)
Liu, *et al*	2010	Caucasian	26.4	51.5	22.1	28.4	47.8	23.8	0.369	(18)
Hussain, *et al*	2011	Caucasian	13.2	65.6	21.2	37.1	47.7	15.2	0.986	(19)

CCND1, cyclin D1; HWE, Hardy-Weinberg equilibrium.

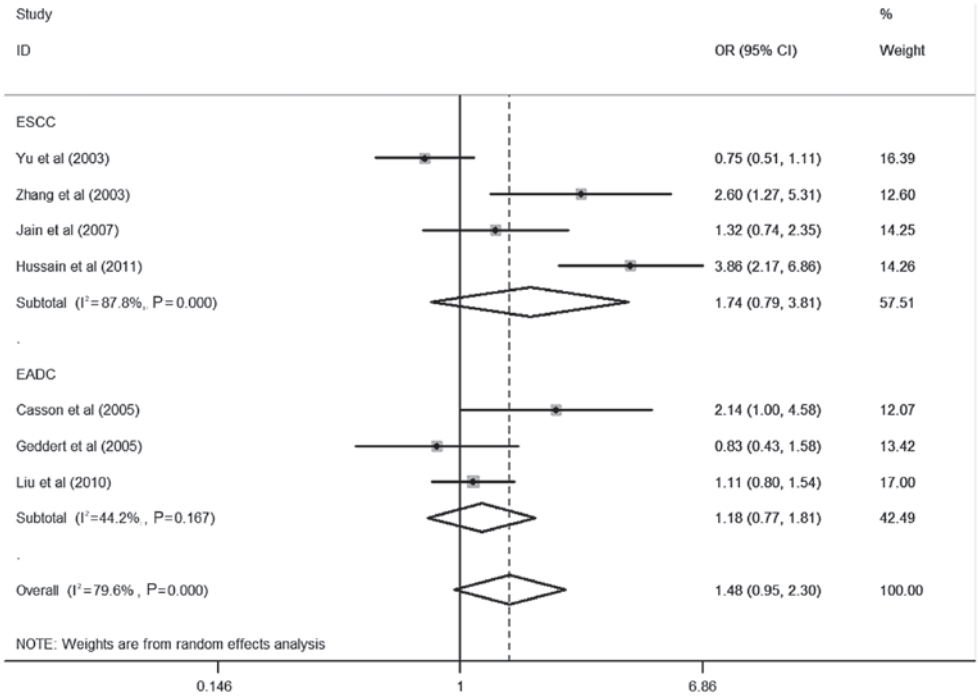

Figure 2. Forest plot (random-effects analysis) of esophageal cancer risk associated with *CCND1* G870A polymorphism based on histology using a dominant genetic model.

G870A polymorphism in various populations are listed in Table II. Casson *et al* (15), Jain *et al* (17) and Hussain *et al* (19) showed a significant association between this polymorphism and esophageal cancer. However, four other studies demonstrated no significant association between this polymorphism and esophageal cancer (13,14,16,18).

Meta-analysis results. Table II shows the detailed results of the heterogeneity test, the most appropriate genetic model and the association between *CCND1* G870A polymorphism and esophageal cancer risk evaluated using OR with 95% CI. In the overall analysis, using the random-effects model, significant associations were detected in the comparison of A vs. G in *CCND1* G870A polymorphism and esophageal cancer risk were observed in dominant models (OR=1.26; 95% CI, 1.00-1.59). Moreover, in stratified studies based on ethnicity, no significant association was found in Caucasian (OR=1.64; 95% CI, 0.84-3.20) or Asian populations (OR=1.30; 95% CI, 0.65-2.62) (Fig. 1), and no significant association was found in esophageal squamous cell carcinoma (ESCC) (OR=1.74; 95% CI, 0.79-3.81) and esophageal adenocarcinoma (EADC) (OR=1.18; 95% CI, 0.77-1.81) (Fig. 2).

Publication bias. The Begg's rank correlation test and funnel plots were performed to access the publication bias. No evident asymmetry was observed in the shapes of the funnel plots, indicating no evidence of publication bias in this meta-analysis (t=1.55, P=0.182).

Discussion

The aim of this study was to elucidate the association between the *CCND1* G870A polymorphism and esophageal cancer risk. A meta-analysis published in 2011, showed that the

CCND1 G870A genotype exhibited a statistically significant risk for cancers of the digestive tract (20). In the present study we explored the association between *CCND1* G870A polymorphism and the risk of esophageal cancer based on various ethnicities and histology in the most appropriate genetic model and detected no significant associations in Caucasians and Asians. Additionally, no significant association was found in ESCC and EADC, whereas the comparison of A vs. G in *CCND1* G870A showed a significant differential susceptibility to esophageal cancer. However, the reason for the results of the *CCND1* polymorphism is unclear. Environmental differences and genetic backgrounds play a role in the association between the *CCND1* G870A polymorphism and the risk for esophageal cancer. Notably, the results of our meta-analysis were inconsistent with the findings of Chen *et al* (20), likely due to the different genetic model selected. We identified the most appropriate genetic model using the method described by Bagos *et al* (11), which allows for the assessment of heterogeneity, thus our result is likely to be more accurate. Additionally, we considered the association between *CCND1* G870A polymorphism and the risk of esophageal cancer rather than cancers of the digestive tract.

Heterogeneity is inevitable in a meta-analysis (21,22), and was therefore also evident in our meta-analysis. Sources of heterogeneity may be derived from various channels: studies included in this meta-analysis cover various ethnicities and environments. Moreover, various methodologies including source of the controls, diagnostic criteria and genotypic methods may lead to heterogeneity.

Additional possible limitations should be taken into account and contributed to the poor statistical power of this meta-analysis. For example, we only selected articles published electronically from databases in English and excluded articles published in other languages, in print or not published at all. Although the Begg's funnel plot and the Egger's test did not detect it, publish bias may exist. Moreover, since not all the included articles contain complete data, a subgroup analysis should be conducted.

In conclusion, the present meta-analysis has demonstrated that the comparison of A vs. G in the *CCND1* G870A polymorphism may increase the risk of esophageal cancer. Considering the limitations of this meta-analysis, additional large-scale investigations, as well as well-designed and more accurate methods of genotypic are required to confirm the association between *CCND1* G870A and esophageal cancer risk under the complex landscape of the cell cycle and cancer risk.

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