RESEARCH COMMUNICATION

MDM2 309T>G Polymorphism and Risk of Squamous Cell Carcinomas of Head and Neck: a Meta-analysis

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Abstract

Purpose: Several studies have reported influence of the murine double minute 2 (MDM2) 309T>G polymorphism on head and neck squamous cell carcinoma (HNSCC) susceptibility. However, the results remain controversial and ambiguous. We therefore carried out a meta-analysis to explore more precisely the association between MDM2 309T>G variants and the risk of HNSCC. Methods: Studies on the association between MDM2 309T>G polymorphism and HNSCC were searched in the PubMed database. All relevant studies that met the inclusion criteria were eligible for the analysis. Four genetic models and generalized odds ratios (ORs) and 95% confidence interval (CIs) were used for the assessment. Results: A total of seven articles with 1,629 cases and 2,472 controls were included in our meta-analysis. Overall, significant associations between the MDM2 SNP309T>G and HNSCC risk for TG vs. TT model and the dominant model (TG+GG vs. TT) were observed (OR=0.82, 95%CI=0.70-0.96 and OR=0.83, 95%CI=0.71-0.96, respectively). On subgroup meta-analysis by ethnicity, a negative association was shown in the Caucasian subgroup (for GG vs. TT: OR=0.661, 95%CI=0.455-0.960; for TG vs. TT: OR=0.653, 95%CI=0.496-0.859; for the dominant model GG+TG vs. TT: OR= 0.657, 95%CI=0.463-0.931). However, in the Asian population no significant association was found. Subgroup analysis by the source of controls also yielded non-significant results. None of the results were materially altered in any genetic model after studies which did not fulfill Hardy-Weinberg equilibrium were excluded. Conclusion: The present meta-analysis suggested that the MDM2 SNP309 G allele probably acts as an important HNSCC protective factor in Caucasians, but no association exists in Asians.

Keywords: MDM2 SNP309T>G - polymorphism - head and neck squamous cell carcinomas - meta-analysis

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Introduction

Head and neck squamous cell carcinomas (HNSCC; including cancers of the oral cavity, oropharynx, hypopharynx, and larynx) constitute the fifth most common cancers worldwide (Jemal et al., 2005). Various risk factors have been proved to be associated with HNSCC including infection with human papillomavirus (HPV)(Fakhry and Gillison 2006; Lescaille et al., 2011), cigarette smoking (Stockwell and Lyman, 1986) and heavy alcohol consumption (Pelucchi et al., 2008). But the pathogenesis of the development and progression of HNSCC is currently far from being clear and is considered as a multistep process with involvement of a series of genetic alterations (Brennan and Boffetta, 2004). Tumor suppressor gene p53, as the "guardian of genome", can be activated by or interact with many other proteins in the network of signaling pathways (Efeyan and Serrano, 2007). And p53 mutations and inactivation play a central role in human cancers including HNSCC (Hollstein et al. ,1991; Polyak et al., 1997; Nylander et al., 2000).

The murine double minute 2 (MDM2) gene encodes a negative regulating protein which promotes rapid degradation of p53 by functioning as an E3 ubiquitin ligase for p53, facilitating polyubiquitination and degradation in proteosomes (Haupt et al. ,1997). Therefore, any change in MDM2 levels may have a great influence on the overall function of the p53 pathway and in vivo, alterations of MDM2 levels have been shown to affect (Wang et al.,. 2010) p53-dependent tumor suppression(Poyurovsky and Prives 2006). It has been reported that the up-regulation of MDM2 can result in the formation of tumors in mice (Jones et al. 1998). More importantly, numerous studies revealed that overexpression of MDM2 in tumor is often associated with poor prognosis (Rasidakis et al., 1998, Freedman and Levine, 1999). Several factors could affect the expression level of MDM2, such as single nucleotide polymorphism. A single nucleotide polymorphism (SNP) was identified on the MDM2 P2 promoter at the 309th nucleotide of intron 1 (a change from T to G, rs2279744) and hence termed SNP309. And the presence of the G allele has been shown to strengthen the binding affinity of the transcriptional activator Sp1 to the MDM2 promoter, contributing to a higher expression of MDM2 mRNA and

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protein and subsequent attenuation of the p53 pathway (Bond et al., 2004). Several reports suggested that the polymorphism is associated with the risk and early onset age of various human cancers (Hu et al., 2007; Wilkening et al., 2007) such as lung cancer (Li et al.. 2006), breast cancer (Economopoulos and Sergentanis 2010), Hepatocellular cancer (Liu et al., 2011), colorectal cancer (Fang et al., 2010), etc. To date, many epidemiological studies have been performed to investigate the relationship between the MDM2 309T>G polymorphism and HNSCC risk (Alhopuro, 2005; Nakashima et al., 2008; Tu et al., 2008; Hamid et al., 2009; Huang et al., 2009; Misra et al., 2009; Chen et al., 2010). However, results remain different or even contradictory partially due to the relatively small sample size of individual studies and sampling effects. Therefore, we conducted a meta-analysis of eligible studies to estimate the effect of MDM2 SNP309T>G on HNSCC risk and provide more information on these controversial results.

Materials and Methods

Literature search

To identify relevant studies eligible for the meta-analysis, we searched PubMed database up to May 22, 2011, using the following search criteria: head and neck cancer/oral cancer/pharyngeal cancer/oropharyngeal cancer/hypopharyngeal cancer/laryngeal cancer, MDM2, SNP/polymorphism/variant. The potentially associated studies were read in their entirety to evaluate their appropriateness for inclusion in the analysis. All references cited in the articles were also scanned to identify relevant publications. The results were limited to papers published in English.

Inclusion and exclusion criteria

The inclusive studies must meet the following criteria: (1) case-control studies; (2) articles about MDM2 309T>G polymorphism and risk of HNSCC; and (3) at least two comparison groups (cancer patient vs. control group); (4) detailed genotyping data.

Data extraction

Two authors extracted the data from each article independently to increase objectivity. Discrepancies were not solved until consensus was reached on every item. From each study, the following items were considered: items of author's last name, year of publication, country of origin, ethnicity, cancer type, source of the control population, genotyping methods, number of cases and controls, genotype frequencies for cases and controls, characteristics of cancer cases and controls.

Statistic analysis

First we evaluated Hardy-Weinberg equilibrium (HWE) for each study using goodness-of-fit test ($\chi 2$ of Fisher's exact test) only in control groups(Zintzaras and Lau, 2008). Crude odds ratios (ORs) with 95% confidence interval (CIs) were calculated to estimate the strength of association between MDM2 309T>G polymorphism and HNSCC risk. In the overall and the subgroup meta-analysis, pooled ORs and 95% CIs for GG vs. TT, TG versus TT, dominant model (TG+GG vs. TT), and recessive model (GG vs. TG+TT) were all calculated. A $\chi 2$–based Q-test was performed to check the heterogeneity of the ORs (Zintzaras and Ioannidis, 2005). If the result of heterogeneity test was $P>0.1$, ORs were pooled according to the fixed-effects model (Mantel-Haenszel model). Otherwise, the random-effects model (DerSimonian and Laird model) was selected (DerSimonian and Laird ,1986). Sensitivity analysis was used to calculate whether one study has great influences on the whole results in the procedure of repeating the meta-analysis by omitting each study one at a time. This analysis was performed for overall and subgroups. The Egger regression test and Begg-Mazumdar test were used to measure the potential publication bias (Macaskillet al., 2001) and the results were considered statistically significant for $P<0.05$. All statistical tests were performed with the software STATA v.10.0 (Stata Corporation, College Station, TX, USA) and Review manager 5.0, using two-side P values.

Results

Studies Characteristics

30 potentially relevant articles from our search of the published literatures, of which 22 articles were excluded and a total 8 articles were identified through literature search and selection according to the inclusion and exclusion criteria. During the extraction of data, one article (Canova et al., 2009) that was not relevant to MDM2 309T>G polymorphism were excluded. Therefore, 7 articles (Alhopuro, 2005, Nakashima et al., 2008; Tu et al., 2008; Hamid et al., 2009; Huang et al., 2009; Misra et al., 2009; Chen et al., 2010) including 1629 cases and 2472 controls were identified and included in the final meta-analysis (Figure 1). All studies were case-control studies with different ethnicities (5 studies of Asians and 2 studies of Caucasians), and sources of controls (4 studies of hospital-based controls and 3 studies of population-based controls). A classic polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was carried out in 4 of the 7. The detailed MDM2 SNP309T>G genotype distributions and allele frequencies for HNSCC cases and controls are listed in Table1. All but one were in agreement with HWE (Misra et al., 2009).

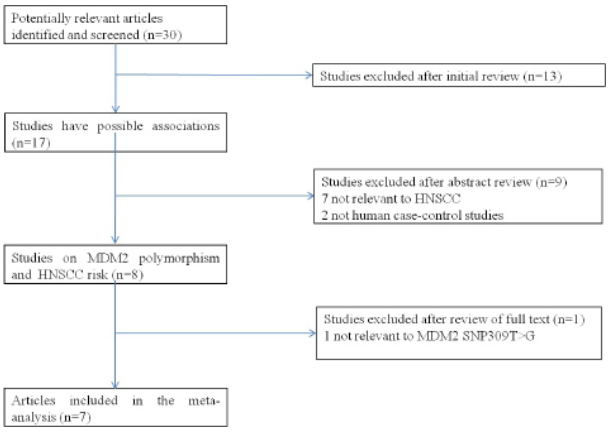

Figure 1. Selection Process

Table 1. MDM2 SNP309T>G Genotype Distribution and Allele Frequency in Cases and Controls

Author-Year	Genotype (N, %)						Allele frequency (N, %)				*P*
	Case			Control			Case		Control		HWE
	TT	TG	GG	TT	TG	GG	T	G	T	G	Controls
Alhopuro (2005)	58 (37)	75 (48)	24 (15)	56 (30)	98 (53)	31 (17)	191 (61)	123 (39)	210 (57)	160 (43)	0.282
Nakashima (2008)	29 (28)	46 (45)	28 (27)	37 (31)	50 (42)	33 (28)	104 (51)	102 (50)	124 (52)	116 (48)	0.07
Tu (2008)	44 (23)	93 (49)	52 (28)	29 (25)	55 (47)	32 (29)	181 (48)	197 (52)	113 (49)	119 (51)	0.582
Hamid (2009)	48 (23)	104 (50)	55 (27)	30 (26)	58 (50)	28 (24)	200 (48)	214 (52)	118 (51)	114 (49)	0.997
Huang (2009)	80 (23)	176 (50)	95 (27)	274 (22)	653 (51)	345 (27)	336 (48)	366 (52)	1201 (47)	1343 (53)	0.286
Misra (2009)	70 (23)	147 (50)	80 (27)	59 (18)	181 (55)	88 (27)	287 (48)	307 (52)	299 (46)	357 (54)	0.042
Chen (2010)	146 (45)	132 (41)	47 (15)	112(33)	165 (49)	58 (17)	424 (65)	226 (35)	389 (58)	281 (42)	0.835

HWE: Hardy-Weinberg equilibrium

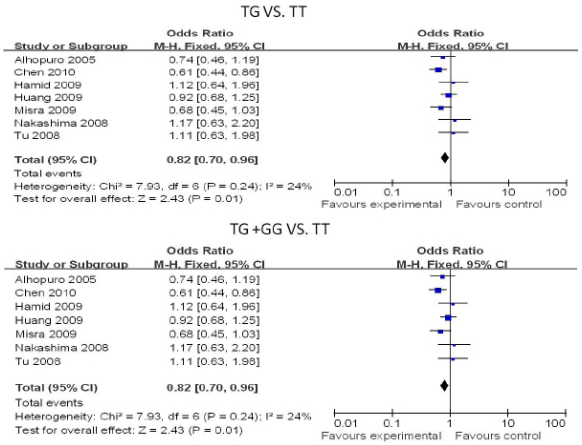

Figure 2. Association between MDM2 SNP309T>G Polymorphisms and HNSCC Risk

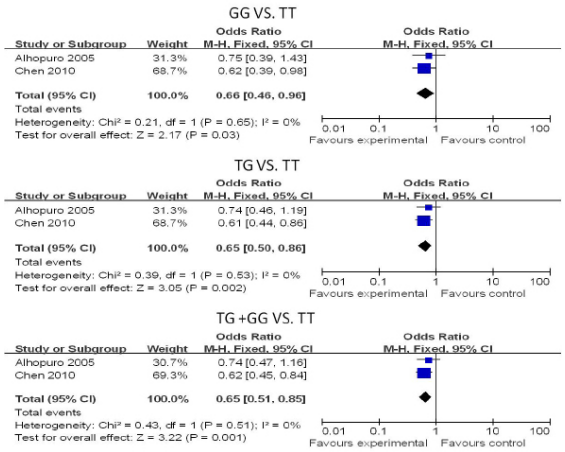

Figure 3. Association between MDM2 309T>G Polymorphisms and HNSCC Risk in Caucasians

Meta-analysis Results

The results of the association between MDM2 309T>G polymorphism and HNSCC risk are summarized in Table 2. Overall, a significant association existed between MDM2 309T>G polymorphism and HNSCC risk (for GG vs. TT: OR=0.82, 95%CI=0.70-0.96, P=0.24 for heterogeneity; for dominant model (TG+GG vs. TT): OR=0.83, 95%CI=0.71-0.96, P=0.20 for heterogeneity, (see Figure 2). In the ethnicity subgroup meta-analysis, in Caucasian group the results suggested obvious significant association between MDM2 309 T>G polymorphism and HNSCC risk (for GG vs. TT: OR=0.66, 95%CI=0.46-0.96, P= 0.65 for heterogeneity; for TG vs. TT: OR=0.65, 95%CI=0.50-0.86, P= 0.53 for heterogeneity; for the dominant model GG+TG vs. TT: OR= 0.65, 95%CI=0.51-0.85, P= 0.51 for heterogeneity (see Figure 3, while in Asians no significant association was found. The results indicated no significant association between MDM2 309 T>G polymorphism and HNSCC susceptibility.

Sensitivity Analysis

A single study involved in the meta-analysis was deleted each time to reflect the influence of individual data-set to the pooled ORs, and the results in any genetic model were not materially altered (data not shown)

Publication Bias

As shown in Figure 4, the hospital-based control' subgroup the shape of Begg's funnel plots seemed

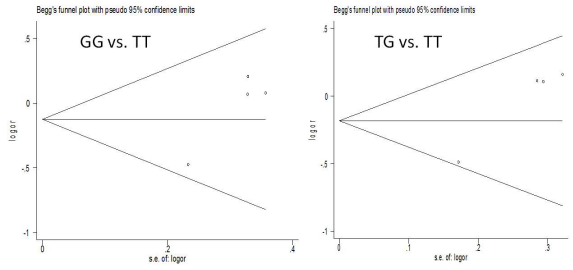

Figure 4. Begg's Funnel Plots

Table 2. Detailed Results of Meta-analysis with Dominant and Recessive Models

Variable	N	GG versus TT		TG versus TT		Dom (TG+GG versus TT)		Rec (GG versus TG+TT)	
		OR (95% CI)	P[a]	OR (95% CI)	P[a]	OR (95% CI)	P[a]	OR (95% CI)	P[a]
Total	7	0.87 (0.72-1.05)	0.59	0.82 (0.70-0.96)[c]	0.24	0.83 (0.71-0.96)[c]	0.20	0.97 (0.83-1.14)	0.97
Ethnicity									
Asian	5	0.95 (0.77-1.19)	0.79	0.92 (0.76-1.12)	0.48	0.94 (0.78-1.13)	0.51	1.01 (0.85-1.21)	1.00
Caucasian	2	0.66 (0.46-0.96)[c]	0.65	0.65 (0.50-0.86)[c]	0.53	0.65 (0.51-0.85)[c]	0.51	0.84 (0.60-1.18)	0.78
Source of control									
Hospital	4	0.88 (0.66-1.18)	0.27	0.92 (0.63-1.33)[b]	0.09	0.88 (0.66-1.18)	0.27	0.95 (0.74-1.22)	0.78
Population	3	0.86 (0.67-1.10)	0.70	0.81 (0.65-1.01)	0.47	0.83 (0.67-1.01)	0.48	0.95 (0.81-1.21)	0.94

[a]P values for heterogeneity test, if P>0.1, ORs were calculated using fix-effects model, otherwise the random-effects model was used; [b]ORs calculated using random-effects model; [c]Results statistically significant; N, Number of studies

obvious asymmetry in both GG vs. TT and TG vs. TT models, and the Egger's test also suggested significant publication bias (P=0.044 and P=0.007, respectively). But the corresponding pooled ORs were not materially altered after deleting any of the four studies (data not shown).

Discussion

We performed a meta-analysis by retrieving eligible studies that investigated the relationship between the widely studied MDM2 polymorphism, SNP309T>G and the risk of HNSCC. Seven independent genetic studies were collected so far, and the aim of the study was to explore accurate estimates of the influence of the variants on HNSCC susceptibility.

In this study, the result of our overall meta-analysis suggests that the MDM2 309 TG genotype and the combined TG/GG genotypes are inversely associated with HNSCC compared with TT genotype. In the subgroup meta-analysis based on ethnicity, compared with TT genotype, a significantly reduced risk of HNSCC was associated with TG genotype, GG genotype and the combined TG/GG genotypes in Caucasian subgroup. While in Asians not any association was found. In addition, in subgroup meta-analysis based on the source of controls, no significant relationship is also observed.

The MDM2 gene is located at small, acentromeric extrachromosomal nuclear bodies and can act as an oncogenes (Mayo and Donner 2002). And there is evidence that decreased MDM2 expression is associated with poor prognosis of HNSCC (Millon et al. 2001). A commonly occurring T-to-G polymorphism at nucleotide 309 (T309G) of MDM2 has been a focus of many case-control association studies of HNSCC in different ethnic populations. However, these studies indicated different or even conflicting results. Nakashima, et al. (Nakashima et al. 2008)found that T309G polymorphism does not affect genetic susceptibility to HNSCC, however, the MDM2 309 G allele was associated with an earlier tumor onset compared with T allele. Chen et al. (Chen et al. 2010) observed that individuals carrying the MDM2 G allele have reduced risk for formation of oral squamous cell carcinoma. In our pooled analysis, we found that the G allele of MDM2 309 might be a protective factor for HNSCC in Caucasians, in contrast no such relationship was found in Asian population. Therefore, it is very possible that MDM2 309T>G polymorphism engenders different risks for ethnic differences in HNSCC. However, this interpretation should be treated with some extent of caution, because the number of studies and participants relative to other meta-analyses on MDM2 309T>G polymorphism is rather small.

Due to the existence of several limitations, all the results in this study should be regarded prudently. First of all, the number of eligible studies and the sample size of individual studies were relatively small. Apparently, these factors may decrease statistical power to reveal the true association. In the second place, although confounding ingredients may influence the association between genetic variants and HNSCC risk, these ingredients (e.g. sex, age, alcohol consumption, cigarette smoking, HPV status and socioeconomic status) were not tested in the current study due to data limitation. In the third place, in the subgroup analysis by ethnicity, only two studies were carried out in Caucasians. Therefore, to perform a more accurate analysis of this polymorphism on HNSCC risk, addition studies with larger sample size and involving different ethnicities (especially non-Asian) are required. More importantly, single polymorphisms analysis may not be reliable markers and haplotypes analysis has been regarded as a more powerful approach in genetic association studies. However, we could not obtain more detailed individual information on genotypes of the other polymorphisms of MDM2, and thus we did not perform linkage disequilibrium and haplotypes analysis.

In conclusion, despite these limitations, the present meta-analysis provided a more precise estimation of the relationship between MDM2 309T>G polymorphism and susceptibility to HNSCC compared with the individual studies. The results indicated that the MDM2 SNP309 G allele probably acts as an important HNSCC protective factor in Caucasians, but in Asians no such association could be shown. The results of our meta-analysis are preliminary and should be considered prudently. Further well-designed and large studies base on MDM2 haplotypes are needed to come to much safer conclusions.

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The authors declare that there is no conflict of interest with this work.

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