Influence of MDM2 polymorphisms on squamous cell carcinoma susceptibility: a meta-analysis

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Purpose: Controversial associations between single-nucleotide polymorphisms (rs2279744, rs937283, rs3730485) of the MDM2 gene and the etiology of squamous cell carcinomas (SCCs) have been reported. This merits further comprehensive assessment.

Materials and methods: We systematically reviewed the available data and conducted an updated meta-analysis to evaluate the genetic effect of MDM2 polymorphisms in SCC susceptibility, using Stata/SE 12.0 software.

Results: After screening, 7,987 SCC cases and 12,954 controls from 26 eligible case–control studies were enrolled. Overall, compared with the control group, a significantly increased SCC risk was observed for the MDM2 rs2279744 polymorphism in the Asian population (test of association: odds ratio [OR] 1.12, P=0.027 for G vs T; OR 1.26, P=0.016 for GG vs TT; OR 1.25, P<0.001 for GG vs TT + TG; and OR 1.08, P=0.023 for carrier G vs T). In subgroup analysis by SCC type, a similarly increased esophageal SCC risk was detected (OR 1.19, P<0.001 for G vs T; OR 1.46, P<0.001 for GG vs TT; and OR 1.48, P=0.005 for GG vs TT + TG). Furthermore, MDM2–TP53 double mutation was statistically associated with increased SCC susceptibility overall (OR 1.52, P=0.001), especially in the Asian population (OR 1.49, P=0.022). However, no significant difference between the control and case groups was obtained for MDM2 rs937283 or rs3730485 under any genetic model (all P>0.05).

Conclusion: Our results highlight a positive association between the GG genotype of MDM2 rs2279744 polymorphism and an increased risk of esophageal SCC in the Asian population, which needs to be clarified by more large-scale studies.

Keywords: MDM2, SCC, SNP, meta-analysis

Introduction

The MDM2 gene maps to chromosome 12q14.3–q15.1 The MDM2 protein forms a complex with the p53 protein, attenuates the activity of p53, and promotes the subsequent degradation of p53 by acting as a ubiquitin E3 ligase for p53.2,3 The abnormal expression of the MDM2/TP53 genes is linked to carcinogenesis or malignant transformation.4,5 Accumulating evidence supports the link between the alteration of protein structural/functional behavior and single-nucleotide polymorphisms (SNPs) within relative genes.6–11 Multiple prediction or detection techniques, such as structural biology, computational platform, and molecular dynamic simulation, contribute to the investigation of identification and function of disease-associated SNPs.6–11 The SNPs of rs2279744 (T309G or SNP309), rs3730485 (del11518+19) and rs937283 (A2164G), have been identified in the human MDM2 gene.12,13 Previous reports have shown that MDM2 polymorphisms are associated with susceptibility to various clinical diseases, such as bladder cancer,14 hepatocellular carcinoma,15 myelodysplastic syndromes,16 and leukemia.17,18
Keratinization of the epidermal cells often leads to the occurrence of squamous cell carcinoma (SCC), which behaves as the uncontrolled growth of outer abnormal squamous cells of the epidermis.\textsuperscript{19–21} Specific types of SCC, such as head and neck SCC (HNSCC), skin squamous cell carcinoma (SSC), esophageal SCC (ESCC), oral SCC (OSCC), lung SCC (LSCC), and cervical squamous cell carcinoma (CSCC), have been described.\textsuperscript{19–23} The different effects of the genetic mutations within \textit{MDM2} have been reported to be related to the carcinogenesis of specific SCC types. For example, a lower plasma \textit{MDM2} level was observed in laryngeal SCC patients with the GT genotype of \textit{MDM2} rs2279744 than the TT genotype.\textsuperscript{24} The prevalence of \textit{MDM2} rs2279744 might be involved in OSCC onset, rather than increased OSCC risks.\textsuperscript{25} Although several previous meta-analyses on the correlation between \textit{MDM2} rs2279744 polymorphism and the risks of HNSCC, OSCC, or ESCC have been reported,\textsuperscript{26–28} another systematic evaluation with enlarged statistical power is still meaningful. Moreover, the meta-analyses of the association between \textit{MDM2} rs937283 and rs3730485 polymorphisms and SCC risks, or between the \textit{MDM2} rs2279744 polymorphism and other SCC types, such as SSCC and CSCC, have not been reported yet. It was thus worthwhile carrying out an updated systematic review and meta-analysis, in order to reassess the genetic relationship between common \textit{MDM2} polymorphisms (rs2279744, rs937283, and rs3730485) and the overall risks of SCC.

\textbf{Materials and methods}

\textbf{Article search}

We searched for potentially relevant articles (up to May 7, 2016) from seven electronic databases: PubMed, Web of Science, Cochrane, Scopus, Chinese National Knowledge Infrastructure (CNKI), Wanfang, and Weipu. The key terms were as follows: mouse double minute 2 homolog; proto-oncogene proteins c-mdm2; MDM2; MDM2 proto-oncogene, E3 ubiquitin protein ligase; human homolog of mouse double minute 2; murine double minute 2; polymorphism; mutation; SNP; single nucleotide polymorphism; T309G; rs2279744; A2164G; rs937283; del1518; rs3730485; G285C; rs117039649; squamous cell carcinoma; carcinoma, squamous cell; and SCC.

\textbf{Article screening and data extraction}

With the help of EndNote X7 software, potential articles were screened for eligibility according to our strict inclusion/exclusion criteria. Exclusion criteria were duplicated articles, review or conference abstract, not human or clinical data, not relevant to MDM2, not about SCC, meta-analysis, not relevant to mutation, lack of control data, and overlapped data. Eligible case–control studies needed to be linked to SCC risks and contain data on individual genotype numbers of \textit{MDM2} rs2279744, rs937283, and rs3730485 polymorphisms. We independently extracted the following data: first author, year of publication, country, ethnicity, SNPs, sample sizes and genotype frequencies of case/control group, SCC type, source of control, genotyping assay, \textit{P}-values of Hardy–Weinberg equilibrium (HWE), and test of control groups. A detailed discussion was required for the conflicting assessment.

\textbf{Statistical analysis}

Pooled odds ratios (ORs) with 95\% confidence intervals (CIs) and \textit{P}-values of associations based on Mantel–Haenszel statistics were calculated by Stata 12.0 software (StataCorp LP, College Station, TX, USA). \textit{P}>0.05 was considered the exclusion of statistically significant difference between case and control groups. The \textit{P} test (0\%–100\%) and \textit{Q}-statistic were adopted to evaluate the potential heterogeneities across case–control studies. \textit{P}\textgreater{}25\% or \textit{P}-value of \textit{Q}-statistic <0.1 was considered significant heterogeneity, and statistical analysis under a random-effect model and sensitivity analysis were conducted. Six genetic (allele, homozygote, heterozygote, dominant, recessive, and carrier) models were employed. Subgroup analyses by ethnicity, source of controls, HWE or control groups. The \textit{P}25\% or \textit{P}-value of \textit{Q}-statistic <0.1 was considered significant heterogeneity, and statistical analysis under a random-effect model and sensitivity analysis were conducted. Six genetic (allele, homozygote, heterozygote, dominant, recessive, and carrier) models were employed. Subgroup analyses by ethnicity, source of controls, HWE or SCC types were also performed. In addition, potential publication bias was assessed by analysis of Begg’s funnel plots (continuity-corrected) and Egger’s publication-bias plots.

\textbf{Results}

\textbf{Studies selected for meta-analysis}

Figure 1 shows a flow diagram of our article-search strategy. A total of 545 potentially relevant articles were retrieved initially from the databases: PubMed (n=95), Web of Science (n=260), Cochrane (n=0), Scopus (n=73), CNKI (n=54), Wanfang (n=44), and Weipu (n=19). A total of 378 articles were obtained after duplicates had been removed by the EndNote software, and then 336 articles were excluded by screening titles and abstracts according to the exclusion criteria. Specific information is shown in Figure 1. Next, 42 full-text articles were assessed for eligibility; 16 articles were then excluded for lack of control data (n=10) and overlapped data (n=6). Finally, 26 independent articles with 7,987 SCC cases and 12,954 controls were selected for our meta-analysis.\textsuperscript{4,12,13,18,24,25,29–48} We then carefully extracted the data and summarized the characteristics (Table 1).
Polymorphism rs2279744 of MDM2 and SCC susceptibility

A total of 25 case–control studies were enrolled for the meta-analysis of MDM2 rs2279744 and risks of SCC. As shown in Table 2, the results (G vs T, \( F = 70.0\%, P < 0.001 \); GG vs TT, \( F = 59.1\%, P < 0.001 \); TG vs TT, \( F = 72.9\%, P < 0.001 \); GG vs TT + TG, \( F = 36.3\%, P = 0.04 \); carrier G vs T, \( F = 31.5\%, P = 0.068 \)) suggested that between-study heterogeneity existed for MDM2 rs2279744. The random-effect model was thus applied for meta-analysis. The pooled results further showed that an increased SCC risk was observed under the allele model (Table 2, G vs T, OR 1.09, 95% CI 1.0–1.19; GG vs TT, \( F = 1.17 \), 95% CI 1.02–1.36; P=0.03, and recessive model (GG vs TT + TG, \( P = 1.46 \), 95% CI 1.29–1.60; P=0.005), but not other genetic models. Forest plots can be seen for meta-analysis of the allele (Figure 2A), homozygote (Figure 3A), heterozygote (Figure 4A), dominant (Figure 5A), and recessive (Figure 6A) models. These data revealed that the GG genotype of MDM2 rs2279744 was statistically associated with increased SCC susceptibility.

Furthermore, subgroup analyses by ethnicity (Asian/Caucasian), HWE (\( P = 0.05 \)/\( P = 0.05 \)), source of control (population-based/hospital-based), and SCC type (HNSCC/SSCC/ESCC/OSCC/CSCC/LSCC) were performed for all genetic models. As shown in Table 3, a significantly increased SCC risk was observed in the Asian population in four models (G vs T, OR 1.12, \( P = 0.027 \); GG vs TT, OR 1.26, \( P = 0.016 \); GG vs TT + TG, OR 1.25, \( P = 0.001 \); carrier G vs T, OR 1.08, \( P = 0.023 \)). Similar results were obtained in the HWE \( P = 0.05 \) group and the population-based group for the allele, homozygote, recessive, and carrier models (Table 3, all OR > 1, \( P < 0.05 \)). These data further indicated an association between the GG genotype of MDM2 rs2279744 and increased SCC susceptibility in the Asian population. The results of stratified analyses by SCC type showed that a significantly increased ESCC risk was observed for three models (Table 4; G vs T, OR 1.19, \( P = 0.001 \); GG vs TT, OR 1.46, \( P = 0.001 \); GG vs TT + TG, OR 1.48, \( P = 0.005 \)). In addition, an increased SSCC risk was observed in the G vs T model (Table 4, OR 1.19, \( P = 0.016 \)) and the TG + GG vs TT model (OR 1.22, \( P = 0.028 \)), while an increased LSCC risk was only observed in the TG + GG vs TT model (Table 4, OR 1.18, \( P = 0.045 \)). In contrast, no significant difference was observed for OSCC and CSCC group in any genetic models (Table 4, test of association, all \( P > 0.05 \)). These data further suggested that patients with the GG genotype of MDM2 rs2279744 appeared to be at a higher risk of developing ESCC in the Asian population.
Table 1 Characteristics of studies included for meta-analysis

| Study                | Country       | Ethnicity | SNPs      | Case | Control | Source of control | Genotyping assay | HWE P-value |
|---------------------|---------------|-----------|-----------|------|---------|------------------|------------------|-------------|
| Alhopuro et al⁹¹     | Finland       | Caucasian | rs2279744 | 157  | 58/75/24 | PB               | PCR–RFLP and sequencing | 0.28         |
| Almquist et al⁴      | USA           | Caucasian | rs2279744 | 559  | 234/261/164 | PB               | Sequenom platform | 0.69         |
| Cao et al³³          | People’s Republic of China | Asian | rs2279744 | 351  | 50/170/131 | PB               | PIRA–PCR | 0.3          |
| Chen et al¹³         | USA           | Caucasian | rs937283  | 325  | 146/132/47 | HB               | PCR–RFLP | 0.84         |
| Er et al⁹⁰          | People’s Republic of China | Asian | rs2279744 | 121  | 47/31/43 | PB               | PCR–RFLP | 0.16         |
| Hamid et al⁶²        | Mixed         | Asian     | rs2279744 | 207  | 48/104/55 | PB               | PCR–RFLP | 0.1          |
| Hong et al³³         | People’s Republic of China | Asian | rs2279744 | 758  | 203/348/207 | PB               | PCR–RFLP | 0.72         |
| Huang et al⁴⁰        | People’s Republic of China | Asian | rs2279744 | 351  | 80/176/95 | PB               | PCR–RFLP | 0.29         |
| Jiang et al⁷⁹        | People’s Republic of China | Asian | rs2279744 | 96   | 44/35/17 | PB               | PCR–RFLP and sequencing | 0.02         |
| Kohno et al⁴⁰        | Japan         | Asian     | rs2279744 | 377  | 68/138/126 | HB               | Pyrosequencing | 0.22         |
| Li et al³⁶           | People’s Republic of China | Asian | rs2279744 | 132  | 37/70/25 | PB               | PCR–RFLP | 0.09         |
| Liu et al³⁴          | USA           | Caucasian | rs2279744 | 423  | 178/186/59 | HB               | TaqMan assay sequencing | 0.39         |
| Logvinov et al⁹⁰     | Russia        | Caucasian | rs2279744 | 59   | 50/9/0 | PB               | PCR–RFLP | 0.87         |
| Ma et al⁴²           | People’s Republic of China | Asian | rs2279744 | 226  | 49/119/58 | PB               | PCR–RFLP | 0.49         |
| Misra et al³⁸        | India         | Asian     | rs2279744 | 226  | 120/91/15 | PB               | PCR–RFLP | 0.04         |
| Nakashima et al³⁷    | Japan         | Asian     | rs2279744 | 297  | 70/147/80 | PB               | Sequencing and real-time PCR | 0.07         |
| Nak et al³³          | People’s Republic of China | Asian | rs2279744 | 281  | 117/119/45 | PB               | PCR–RFLP | 0.03         |
| Park et al³⁶         | South Korea   | Asian     | rs2279744 | 270  | 57/128/85 | PB               | PCR–RFLP | 0.44         |
| Roszak et al³²       | Poland        | Caucasian | rs2279744 | 379  | 139/169/71 | PB               | PCR–RFLP and sequencing | 0.05         |
| Singh et al³⁵        | India         | Asian     | rs2279744 | 182  | 63/47/45 | PB               | PCR–RFLP and sequencing | 0.00         |
| Tu et al³⁵           | People’s Republic of China | Asian | rs2279744 | 189  | 44/93/52 | PB               | Sequencing | 0.58         |
| Yang et al³⁷         | People’s Republic of China | Asian | rs937283  | 307  | 163/126/18 | PB               | TaqMan assay | 0.92         |
| Yu et al³¹           | USA           | Caucasian | rs937284  | 1,083 | 463/486/134 | PB               | PCR–RFLP | 0.33         |
| Zhang et al¹²        | People’s Republic of China | Asian | rs937284  | 1,078 | 369/52/25 | TaqMan assay | 0.75         |
| Zhang et al³⁶        | People’s Republic of China | Asian | rs2279744 | 146  | 37/58/51 | PB               | Pyrosequencing | 0.43         |

Note: P<0.05 in bold.

Abbreviations: SNPs, single-nucleotide polymorphisms; M, major allele; m, minor allele; HNSCC, head and neck squamous cell carcinoma; SSCC, skin SCC; ESCC, esophageal SCC; OSCC, oral SCC; LSCC, lung SCC; CSCC, cervical SCC; PB, population-based; HB, hospital-based; PCR–RFLP, polymerase chain-reaction restriction fragment-length polymorphism; PIRA–PCR, primer-introduced restriction-analysis PCR; MALDI–TOF, matrix-assisted laser desorption/ionization time of flight; ARMS–PCR, amplification refractory mutation-system (tetra-primer) PCR; HWE, Hardy–Weinberg equilibrium.
Polymorphisms rs937283 and rs3730485 of MDM2 and SCC susceptibility

Next, pooled analysis for the association between rs937283 and rs3730485 polymorphisms of MDM2 and the risks of SCC was conducted (Table 2). A random-effect model was used for the comparison of G vs A, GG vs AA, AG vs AA, AG + GG vs AA, due to the presence of heterogeneity (all 95% CI 1.19–1.95; P=0.001) and the Asian population (test of association, OR 1.49, 95% CI 1.06–2.11; P=0.022). However, no significant difference was observed for other mutations (test of association, all P>0.05). According to our data, the combined effect of the MDM2/TP53 double mutation may contribute to an increased SCC risk, especially in the Asian population.

Table 2 Meta analysis of the association between MDM2 polymorphisms (rs2279744, rs937283, and rs3730485) and SCC susceptibility

| SNP          | Comparison     | No of case–control studies | Total sample size | Test of association | Heterogeneity | Model | Begg\(\alpha\) | Egger\(\beta\) |
|--------------|----------------|-----------------------------|-------------------|---------------------|---------------|-------|----------------|---------------|
| rs2279744 G vs T | 25            | 7,680/12,643                | 1.09 0.041         | 70 <0.001 R         | 0.05 0.963    | 0.43 0.671 |
| rs2279744 GG vs TT | 24           | 7,621/12,483                | 1.17 0.030         | 59.1 <0.001 R       | 0.05 0.96    | -0.33 0.744 |
| rs2279744 TG vs TT | 25           | 7,680/12,643                | 0.87–1.15 0.975   | 72.9 <0.001 R       | 0.79 0.427   | -0.48 0.637 |
| rs2279744 TG + GG vs TT | 25     | 7,680/12,643                | 0.92–1.21 0.422   | 73.7 <0.001 R       | 0.47 0.64   | -0.18 0.861 |
| rs2279744 GG vs TT + TG | 24 | 7,621/12,483                | 1.07–1.13 0.001   | 36.3 0.04 R         | 0.74 0.457   | 0.52 0.607 |
| rs2279744 carrier G vs T | 25 | 7,680/12,643                | 1.06 0.064         | 31.5 0.068 R       | 0.09 0.926   | 0.78 0.446 |
| rs937283 G vs A | 3             | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs937283 GG vs AA | 3             | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs937283 AG vs AA | 3             | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs937283 AG + GG vs AA | 3 | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs937283 GG vs AA + AG | 3 | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs937283 carrier G vs A | 3 | 1,710/1,735                 | 0.99 0.008         | 67 0.048 R          | 0 0.57 0.668 |
| rs3730485 − vs + | 3             | 709/1,000                   | 0.89 0.08          | 0 0.428 F           | 0 0.59 0.659 |
| rs3730485 −/− vs ++ | 3             | 709/1,000                   | 0.82 0.06          | 0 0.428 F           | 0 0.59 0.659 |
| rs3730485 +/− vs ++ | 3             | 709/1,000                   | 0.91 0.10          | 0 0.428 F           | 0 0.59 0.659 |
| rs3730485 −/− vs +/− | 3 | 709/1,000                   | 0.89 0.08          | 0 0.428 F           | 0 0.59 0.659 |
| rs3730485 Carrier – vs + | 3 | 709/1,000                   | 0.92 0.09          | 0 0.428 F           | 0 0.59 0.659 |

Notes: Continuity-corrected; significant P-values in bold.
Abbreviations: SCC, squamous cell carcinoma; OR, odds ratio; CI, confidence interval; F, fixed-effect model; R, random-effect model.

MDM2/TP53 mutations and SCC susceptibility

The MDM2 rs2279744 polymorphism has been reported to suppress the p53 pathway via the modulation of MDM2 expression.\(^3\)\(^4\)\(^9\) We also investigated the genetic relationship between SCC risks and MDM2/TP53 mutations, including MDM2*/TP53*, MDM2/*TP53*, and MDM2*/TP53* in the Caucasian group (Table 4, Egger’s publication-bias plot, P<0.05). According to our data, the combined effect of the MDM2/TP53 double mutation may contribute to an increased SCC risk, especially in the Asian population.

Publication bias and sensitivity analysis

The results of Begg’s funnel plots and Egger’s publication-bias plots demonstrated that the occurrence of large publication bias was excluded under all genetic models (Tables 2 and 4, all P>0.05), apart from the mutations of MDM2*/TP53* and MDM2/*TP53* in the Caucasian group (Table 4, Egger’s publication-bias plot, P<0.05). Egger’s funnel plots of publication bias for the allele (Figure 2B), homozygote (Figure 3B), heterozygote (Figure 4B), dominant (Figure 5B), and recessive (Figure 6B) models of MDM2 rs2279744 polymorphism are shown. With regard to the sensitivity analysis, compared with overall meta-analysis data, no significant difference for the pooled OR value was observed when each study was omitted sequentially (Figure 2C for allele model of MDM2 rs2279744; Figure 3C for homozygote model; Figure 4C for heterozygote model; Figure 5C for dominant model; Figure 6C for recessive model).
model; data not shown for others). Consequently, these data suggested that our statistical results were credible.

**Discussion**

More and more studies on the possible role of the *MDM2* rs2279744 polymorphism in the onset and development of cancer have been reported. Hu et al performed a meta-analysis based on 25 published case–control studies, and reported that *MDM2* rs2279744 seems to be associated with tumor susceptibility.\(^5\) Chen et al reported that the *MDM2* rs2279744 polymorphism may be linked to an increased digestive tract cancer risk in the Asian population.\(^5\)
Here, we further focused on the potential effect of MDM2 rs2279744 in susceptibility to overall SCC and specific SCC types, including HNSCC, SSCC, ESCC, OSCC, CSCC, and LSCC.

Several SCC-related meta-analyses have been carried out previously. A meta-analysis by Liu et al based on seven articles with 1,629 cases and 2,472 controls showed that the G allele of the MDM2 rs2279744 polymorphism seemed to act as an important HNSCC protective factor in the Caucasian population, but not the Asian population. However, in our meta-analysis, we were unable to observe a significant association between HNSCC susceptibility and MDM2 rs2279744.
rs2279744. How to explain this? Seven studies were enrolled in the meta-analysis of Liu et al. Also, data for OSCC in five studies were included as HNSCC. The disease in two studies was defined only as HNSCC. In our subgroup analysis, we tested the relationship between OSCC risk and MDM2 rs2279744. One new study was added in the new meta-analysis for HNSCC. We found that the MDM2 rs2279744 polymorphism did not appear to be associated with OSCC susceptibility, which is partly consistent with the results of Xie et al.27
A meta-analysis by Chen et al based on six case–control studies, including 1,899 cases and 3,016 controls, showed that the MDM2 rs2279744 polymorphism may be associated with increased risks of overall esophageal cancer, including SCC and adenocarcinoma, especially in the Asian population. However, our meta-analysis only targeted the ESCCs. We thus removed one study on esophageal adenocarcinoma and added another new published case–control study. All cases in six case–control studies were Chinese patients, with a mean age of >50 years and male:female ratio of >50%. The
GG genotype of MDM2 rs2279744 was likely to confer an increased susceptibility to ESCC in elderly male patients in People’s Republic of China. The influence of habits and customs, such as drinking or smoking, should be considered. Considering the close association between MDM2 and p53, it is meaningful to investigate the role of gene–gene interaction between MDM2 and TP53 Arg72Pro polymorphism in SCC risks. In our meta-analysis, we observed a

| Study                  | OR (95% CI)   | % weight |
|------------------------|---------------|----------|
| Alhopuro et al         | 0.90 (0.50–1.60) | 2.29     |
| Almqvist et al         | 1.17 (0.82–1.68) | 4.58     |
| Cao et al              | 1.10 (0.84–1.44) | 6.38     |
| Chen et al             | 0.81 (0.53–1.23) | 3.77     |
| Er et al               | 2.85 (1.59–5.10) | 2.29     |
| Hamid et al            | 1.14 (0.67–1.92) | 2.69     |
| Hong et al             | 1.46 (1.19–1.79) | 8.07     |
| Huang et al            | 1.00 (0.76–1.30) | 6.49     |
| Jiang et al            | 0.94 (0.48–1.85) | 1.78     |
| Kohno et al            | 1.22 (0.88–1.68) | 5.31     |
| Li et al               | 1.97 (0.97–3.98) | 1.65     |
| Liu et al              | 0.92 (0.67–1.26) | 5.47     |
| Ma et al               | 1.00 (0.66–1.53) | 3.73     |
| Misra et al            | 1.01 (0.71–1.43) | 4.72     |
| Nakashima et al        | 0.98 (0.55–1.78) | 2.23     |
| Nan et al              | 1.22 (0.84–1.77) | 4.39     |
| Park et al             | 1.20 (0.88–1.65) | 5.43     |
| Roszak et al           | 1.25 (0.87–1.78) | 4.67     |
| Singhal et al          | 2.39 (1.37–4.18) | 2.44     |
| Tu et al               | 1.00 (0.59–1.67) | 2.76     |
| Yu et al               | 1.04 (0.81–1.35) | 6.69     |
| Zhang et al            | 1.97 (0.97–3.98) | 1.65     |
| Zhang et al            | 1.34 (1.05–1.70) | 7.06     |
| Zhou et al             | 1.14 (0.73–1.78) | 3.45     |
| Loginov et al          | (Excluded) 0.00 |          |

Overall ($I^2=36.3\%$, $P=0.040$) 1.18 (1.07–1.30) 100
Table 3 Subgroup analysis by ethnicity, source of controls, and HWE for association between MDM2 rs2279744 and SCC susceptibility

| Comparison | Ethnicity | HWE | Source of control |
|------------|-----------|-----|-------------------|
| G vs T     |           |     |                   |
| No of case-control studies | Asian | 17 | 21 | 22 |
| Case/control total sample size | 4,414/7,507 | 3,266/5,136 | 6,824/11,142 | 856/1,501 |
| OR (95% CI) | 1.12 (1.01–1.24) | 1.03 (0.89–1.19) | 1.09 (1.01–1.17) | 1.09 (0.69–1.71) |
| P-value | 0.027 | 0.669 | 0.026 | 0.711 |
| GG vs TT   |           |     |                   |
| No of case-control studies | Asian | 17 | 20 | 21 |
| Case/control total sample size | 4,414/7,507 | 3,207/4,976 | 6,765/10,982 | 856/1,501 |
| OR (95% CI) | 1.26 (1.04–1.52) | 1.03 (0.84–1.27) | 1.18 (1.03–1.34) | 1.13 (0.55–2.34) |
| P-value | 0.016 | 0.755 | 0.015 | 0.74 |
| TG vs TT   |           |     |                   |
| No of case-control studies | Asian | 17 | 21 | 22 |
| Case/control total sample size | 4,414/7,507 | 3,266/5,136 | 6,824/11,142 | 856/1,501 |
| OR (95% CI) | 0.97 (0.8–1.1) | 0.97 (0.8–1.19) | 1.02 (0.89–1.16) | 0.87 (0.42–1.77) |
| P-value | 0.781 | 0.805 | 0.818 | 0.691 |
| TG + GG vs TT |           |     |                   |
| No of case-control studies | Asian | 17 | 21 | 22 |
| Case/control total sample size | 4,414/7,507 | 3,266/5,136 | 6,824/11,142 | 856/1,501 |
| OR (95% CI) | 1.06 (0.88–1.28) | 1.04 (0.84–1.28) | 1.07 (0.95–1.2) | 0.94 (0.45–1.94) |
| P-value | 0.527 | 0.736 | 0.279 | 0.86 |
| GG vs TT + TG |           |     |                   |
| No of case-control studies | Asian | 17 | 20 | 21 |
| Case/control total sample size | 4,414/7,507 | 3,207/4,976 | 6,765/10,982 | 856/1,501 |
| OR (95% CI) | 1.25 (1.11–1.42) | 1.05 (0.92–1.2) | 1.17 (1.06–1.29) | 1.27 (0.88–1.83) |
| P-value | <0.001 | 0.501 | 0.002 | 0.197 |
| Carrier G vs T |           |     |                   |
| No of case-control studies | Asian | 17 | 21 | 22 |
| Case/control total sample size | 4,414/7,507 | 3,266/5,136 | 6,824/11,142 | 856/1,501 |
| OR (95% CI) | 1.08 (1.01–1.16) | 1.03 (0.91–1.16) | 1.06 (1.01–1.12) | 1.07 (0.77–1.5) |
| P-value | 0.023 | 0.672 | 0.031 | 0.676 |

Note: Significant P-values in bold.

Abbreviations: HWE, Hardy-Weinberg equilibrium; SCC, squamous cell carcinoma; PB, population-based; HB, hospital-based; Y, P-value of HWE >0.05; n, P-value of HWE <0.05; OR, odds ratio; CI, confidence interval.

positive association between MDM2+/TP53+ double mutation and SCC susceptibility in overall or Asian populations. The underlying molecular mechanism on the effect of MDM2 genetic variation in the incidence of ESCC remains unclear. The rs2279744 SNP within the promoter region of MDM2 can lead to a T–G substitution at the 309 nucleotide site, which is closely linked to the high expression of the MDM2 protein via higher binding affinity with the transcriptional activator SP1, and thus enhances the degradation of p53.2 It was possible that MDM2 rs2279744 polymorphism is linked to the increased SCC risks, through influencing the role of p53 pathway in genomic stability and tumor prevention. Chen et al conducted a meta-analysis to investigate the relationship between positive MDM2 expression and clinicopathological characteristics of ESCC, and found that high MDM2 expression was associated with early primary tumor stage and increased risk of regional lymph node metastasis, but not the risk of distant metastasis.53 Vlatković et al reported that loss of MTBP expression seems to be associated with reduced survival in some patients with HNSCC.54 In addition, several reported studies have estimated the role of the interaction between the MDM2/TP53 gene and several environmental factors, including smoking exposure, alcohol consumption, or human papillomavirus infections in SCC susceptibility.13,29,38,40,41 For instance, rs2279744 and rs937283 of MDM2 might be associated
Table 4 Subgroup analysis by disease type for association between MDM2 rs2279744 and SCC susceptibility

| Comparison | HNSCC | SSCC | ESCC | OSCC | CSCC | LSCC |
|------------|-------|------|------|------|------|------|
| G vs T     |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 5   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,605/3,847 |
| OR (95% CI) | 1.16 (0.92–1.15) | 1.16 (1.02–1.32) | 1.19 (1.09–1.3) | 0.92 (0.81–1.05) | 1.19 (0.62–2.25) | 1.16 (0.95–1.42) |
| P-value | 0.659 | 0.022 | <0.001 | 0.223 | 0.603 | 0.153 |
| GG vs TT   |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 4   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,546/3,687 |
| OR (95% CI) | 1.03 (0.82–1.13) | 1.3 (0.98–1.71) | 1.46 (1.23–1.74) | 0.86 (0.7–1.07) | 1.33 (0.5–3.52) | 1.23 (0.92–1.65) |
| P-value | 0.788 | 0.067 | <0.001 | 0.173 | 0.569 | 0.161 |
| TG vs TT   |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 5   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,605/3,847 |
| OR (95% CI) | 1.02 (0.83–0.126) | 1.19 (0.99–1.43) | 0.98 (0.72–1.34) | 0.82 (0.65–1.04) | 0.96 (0.35–0.62) | 1.21 (0.88–1.67) |
| P-value | 0.83 | 0.062 | 0.916 | 0.103 | 0.941 | 0.249 |
| TG + GG vs TT |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 5   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,605/3,847 |
| OR (95% CI) | 1.02 (0.82–0.126) | 1.22 (1.02–1.45) | 1.14 (0.94–1.38) | 0.84 (0.66–1.06) | 1.06 (0.39–2.85) | 1.26 (0.91–1.75) |
| P-value | 0.886 | 0.028 | 0.179 | 0.135 | 0.913 | 0.164 |
| GG vs TT + TG |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 4   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,546/3,687 |
| OR (95% CI) | 1.01 (0.81–0.126) | 1.19 (0.92–1.55) | 1.48 (1.12–1.94) | 0.98 (0.83–1.16) | 1.42 (0.88–2.31) | 1.18 (1–1.38) |
| P-value | 0.91 | 0.181 | 0.005 | 0.801 | 0.154 | 0.045 |
| Carrier G vs T |       |      |      |      |      |      |
| No of case-control studies | 3  | 2   | 6   | 5   | 3   | 5   |
| Case/control total sample size | 1,186/1,210 | 840/1,525 | 1,720/2,694 | 1,369/2,167 | 657/803 | 1,605/3,847 |
| OR (95% CI) | 1.02 (0.9–0.116) | 1.11 (0.96–1.29) | 1.21 (1.01–1.24) | 0.94 (0.84–1.06) | 1.15 (0.71–1.86) | 1.11 (0.92–1.34) |
| P-value | 0.766 | 0.148 | 0.028 | 0.326 | 0.581 | 0.268 |

Note: Significant P-values in bold.

Abbreviations: HNSCC, head and neck squamous cell carcinoma; SSCC, skin SCC; ESCC, esophageal SCC; OSCC, oral SCC; CSCC, cervical SCC; LSCC, lung SCC; OR, odds ratio; CI, confidence interval.

with the occurrence of OSCC patients with HPV16 L1 seropositivity. However, due to the lack of sufficient data, we failed to carry out a subgroup analysis based on these environmental factors.

Our meta-analysis contained several limitations. Very few publications resulted in small sample sizes for the analysis of MDM2 rs937283 and rs3730485. The possible effect of other unpublished studies on our negative conclusion should be taken into consideration. The same limitation of sample size existed in the meta-analysis of MDM2/TP53 double mutation and several subgroup analyses of the MDM2 rs2279744 polymorphism. Heterogeneity and potential publication bias may weaken our conclusion. Demographic features, lifestyle, or clinical characteristics were not considered, due to the lack of data. Larger and independent studies are required to validate the association between MDM2/TP53 mutations and susceptibility to different types of SCC.

Conclusion

Our updated meta-analysis demonstrated that there is a positive association between increased overall SCC risks and the MDM2 rs2279744 polymorphism, rather than rs937283 or rs3730485. We further provided evidence that the GG genotype of MDM2 rs2279744 is more likely to confer an increased genetic susceptibility to ESCC in the Asian population, particularly in Chinese. MDM2 rs2279744 may be a valuable risk factor or diagnostic biomarker for patients with ESCC in People’s Republic of China, and needs more supporting evidence.
Table 5

| Mutation       | Genotype       | Case/control total sample size | Case/control | Test of association |
|----------------|----------------|-------------------------------|--------------|---------------------|
| MDM2/TP53      | Arg/Pro or Pro/Pro | 1,293/2,375                  | 5            |                     |
| Arg72Pro       | T/G or G/G       | 1,293/2,375                  | 5            |                     |
| Arg/Pro        | T/G or G/G       | 1,071/1,532                  | 4            |                     |
| Arg/Pro        | Arg/Pro or Pro/Pro| 2,474/3,139                  | 9            |                     |
| Arg/Pro        | Arg/Pro or Pro/Pro| 4,847/6,473                  | 6            |                     |
| Arg/Pro        | Arg/Pro or Pro/Pro| 1,863/2,473                  | 3            |                     |

Notes: Continuity-corrected significance, 95% CI confidence interval, OR, odds ratio; CI, confidence interval; R, random-effect model.

Abbreviations: SCC, squamous cell carcinoma; MDM2, mouse double minute 2 homolog; TP53, tumour protein 53; Arg72Pro, arginine/proline variant of TP53 at codon 72.

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Disclosure

The authors report no conflicts of interest in this work.

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