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Short Communication

Systematic evaluation of genetic polymorphisms in carcinogen metabolizing enzymes associated with oral potentially malignant disorders progression to oral cancer risk

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Abstract *Background/purpose:* Increasing studies investigate the association of polymorphisms in carcinogen-metabolizing enzymes with oral potentially malignant disorder (OPMD) progression to oral squamous cell carcinoma (OSCC) risk. However, these results remain inconsistent and conflicting. This pooled analysis aimed to systematically evaluate the 5 enzymes (CYP1A1, GSTM1, GSTM3, GSTT1 and GSTP1) polymorphisms in OPMD versus OSCC.
Materials and methods: A systematic literature search was conducted to identify all eligible case—control studies on the association between SNPs in the 5 enzymes in OPMD versus OSCC. Pooled odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated to access association strength.
Results: No significant association of CYP1A1 MspI polymorphism (OR, 1.38; 95%CI, 0.80—2.38) in OPMD with OSCC risk was observed based on 5 eligible studies involving 624 cases of OPMD

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polymorphisms

and 1529 OSCC. As for *GST* genes, an increased risk of GSTM1 null genotype (OR, 1.72; 95%CI, 1.24–2.37) with OPMD, especially oral leukoplakia, progression to OSCC was found based on 11 eligible studies involving 1195 OPMD patients and 2219 OSCC controls. However, there was no significant association of GSTT1, GSTM3 or GSTP1 polymorphisms in OPMD with OSCC risk.
Conclusion: This is the first pooled analysis on polymorphisms in carcinogen-metabolizing enzymes in OPMD versus OSCC. The results suggested that the GSTM1 null genotype was a significant genetic risk factor for OPMD progression to OSCC, highlighting its potential for improving risk stratification and early detection, particularly for oral leukoplakia.

Introduction

Oral squamous cell carcinoma (OSCC) accounts for over 90 % of oral cancer, which is one of the most common cancers worldwide. OSCC is frequently preceded by oral potentially malignant disorders (OPMD) such as oral leukoplakia (OLK) and oral submucous fibrosis (OSF).[1] The occurrence and malignant progression of OPMD correlate with environmental risk factors including tobacco, alcohol, and betel quid consumption.[2] The interaction between environmental exposures and genetic factors is mediated by Phase I and Phase II carcinogen metabolizing enzymes, which regulate the bioactivation and detoxification of carcinogens within the oral mucosa. Cytochrome P450 (CYP) enzymes, notably CYP1A1, represent crucial Phase I enzymes that catalyze the bioactivation of lipophilic carcinogens into highly reactive intermediates.[3] Glutathione S-transferases (GSTs), particularly the four isoenzymes GSTM1, GSTM3, GSTT1 and GSTP1, constitute important Phase II enzymes that metabolize carcinogens for detoxification.[4]

Single-nucleotide polymorphisms (SNPs) in carcinogen metabolizing enzymes may alter metabolic efficiency, leading to increased DNA damage and subsequent gene mutations.[5] The polymorphism of *CYP1A1* MspI can cause change in the enzyme activity of CYP1A1. The null genotype or variation of *GST* genes could cause the inactivation of GSTM1, GSTM3, GSTT1 and GSTP1 enzymes.[5] Recently, we have systematically evaluated the associations of SNPs in carcinogen-metabolizing *CYP* and *GST* genes with risk of OPMD occurrence.[6] Although SNPs in these enzymes in OPMD versus OSCC had been investigated by some previous studies,[7–18] these conclusions were conflicting and inconsistent. Therefore, to gain more precise evidence for the association, we further systematically evaluate the associations of SNPs in carcinogen metabolizing enzymes with OPMD progression to OSCC risk by pooling data from all eligible case–control studies.

Materials and methods

Search strategy and data extraction

A comprehensive literature search was conducted on PubMed, Medline, and Web of Science databases for all relevant publications on SNPs within OPMD versus OSCC, without any restriction on September 30, 2025. Herein, alteration of SNPs in OPMD compared with in OSCC may have the risk of malignant progression. According to the search strategy described in Supplementary Table S1, we used medical subject terms (SNP OR polymorphism OR genotype*) AND (cytochrome P450 OR glutathione OR CYP1* OR GSTM* OR GSTT* OR GSTP*) AND the synonyms and subtypes of OPMD in all fields. The asterisk indicates a wildcard used to search for all endings including fifth or more root words. Additionally, the reference lists given in relevant articles and reviews were also considered for eligible studies. The inclusion criteria for eligible articles were as follows: (i) human case–control studies; (ii) studies on associations of polymorphisms in carcinogen metabolizing genes with OPMD versus OSCC; (iii) sufficient genotyping data for the computation of odds ratio (OR) and 95 % confidence interval (CI); and (iv) histologically confirmed diagnosis of both OPMD and OSCC. On the contrary, the exclusion criteria were as follows: (i) not a human case–control study; (ii) overlapping or duplicate publications; (iii) no genotype data reported; and (iv) the number of the studies on a single gene was <3.

Base on the flow diagram of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (Supplementary Fig. S1), 12 eligible case–control studies were retrieved for detailed evaluation from the literature databases (Table S2).[7–18] The following information were extracted from each study: first author' name, publication year, country of origin, ethnicity, genotyping methods, number of cases and controls, age, sex, tobacco and alcohol use, betel quid chewing, source of controls, genotype distributions of cases and controls (Supplementary Table S2). Ethical approval and informed consent were not applicable for a meta-analysis.

Statistical analysis

As per the statistical method described previously,[22] the strength of association of SNPs with OPMD susceptibility was determined by OR with 95 % CI. The statistical significance of the pooled OR was evaluated using the Z-test, and the heterogeneity of the ORs was tested by χ^2–based Q-test and I^2 statistics. ORs were pooled according to the fixed-effects model (Mantel-Haenszel model) and random-effects model (DerSimonian-Laird model). Additionally, the potential publication bias was visually examined by the Begg's funnel plot and Egger's test. All statistical analyses were performed with the software Review manager 5.4 (The Cochrane Collaboration, Oxford, UK) using two-sided *P* value, and the value of <0.05 was considered as statistically significant.

Results

CYP genotypes in OPMD versus OSCC

Among SNPs in *CYP* genes, there were 5 eligible studies on CYP1A1 polymorphism in OPMD versus OSCC (Table S2). A total of 624 OPMD patients and 1529 OSCC ones were identified from India, China, and Pakistan for CYP1A1 MspI variant. Overall, no significant association between CYP1A1 MspI polymorphism and OPMD versus OSCC was found with the random-effects model (pooled OR, 1.38; 95%CI, 0.80–2.38; $P_{heterogeneity} = 0.0003$; Fig. 1A). In the stratified analysis, there was also lack of significant associations in OLK subgroup and non-specified OPMD subgroup. Begg's funnel plot showed that there was evidence for publication bias of CYP1A1 MspI polymorphisms in OPMD versus OSCC ($P < 0.05$, Egger's test; Fig. S2A).

GST genotypes in OPMD versus OSCC

Among SNPs in *GST* genes, there were 11 eligible studies on GSTM1 polymorphism in OPMD versus OSCC (Table S2). A total of 1195 OPMD patients and 2219 OSCC ones were identified from India. Overall, a significant increased risk of OPMD progression to OSCC with GSTM1 null genotype was found with the random-effects model (pooled OR, 1.48; 95%CI, 1.08–2.03; $P_{heterogeneity} < 0.00001$; Fig. 1B). Stratified analysis revealed a significantly increased risk in the OLK subgroup (OR, 1.67; 95%CI, 1.03–2.71), but not in the OSF subgroup (OR, 2.13; 95%CI, 0.75–6.01) and the non-specified OPMD subgroup (OR, 1.12; 95%CI, 0.79–1.59). Begg's funnel plot showed that there was evidence for publication bias of GSTM1 polymorphism in OPMD versus OSCC ($P < 0.05$, Egger's test; Fig. S2B).

There were 8 eligible studies evaluating GSTT1 polymorphism OPMD versus OSCC. A total of 905 OPMD patients and 1909 OSCC cases were identified from India and Pakistan (Fig. 1C). There were 3 eligible studies on GSTM3 polymorphism in OPMD versus OSCC. A total of 321 OPMD patients and 581 OSCC cases were identified from India (Fig. 1D). Besides, there were 3 eligible studies on GSTP1 polymorphism in OPMD versus OSCC. A total of 525 OPMD patients and 1231 OSCC ones were identified from India (Fig. 1E). No significant association of SNPs in GSTT1, GSTM3 and GSTP1 with the risk of OPMD progression to OSCC was found in overall and subgroup analysis. Begg's funnel plot showed that there was no evidence for publication bias of GSTT1, GSTM3 and GSTP1 polymorphisms in OPMD versus OSCC ($P > 0.05$, Egger's test; Fig. S2C–E).

Discussion

It is recognized that tobacco, alcohol, and betel quid consumption as environmental risk factors and environment–gene interaction contribute to the occurrence and malignant progression of OPMD. This interaction is exemplified by the metabolism of the procarcinogens and carcinogens, which undergo biotransformation and detoxification mediated by Phase I (CYP1A1, and CYP2E1) and Phase II (GSTM1, GSTM3, GSTT1 and GSTP1) carcinogen metabolism enzymes.[2–4] The evaluation of polymorphisms in CYP1A1 MspI, GSTM1, and GSTT1 in OSCC versus health controls[19–22] and polymorphisms in GSTM1, GSTT1, and GSTP1 in OPMD (OLK and OSF) versus health controls[23,24] have reported by the previous meta-analyses, respectively. Moreover, we recently evaluated polymorphisms in CYP1A1, CYP2E1, GSTM1, GSTM3, GSTT1 and GSTP1 in OPMD (OLK and OSF) versus health controls.[6] However, there is a lack of the evaluation of polymorphisms in these enzymes in OSCC versus OPMD. Hence, this study for the first time attempts to identify all qualified studies to draw a more precise estimation of the genetic associations between SNPs in these enzymes and OPMD progression in a single meta-analysis.

Among Phase I carcinogen metabolism enzymes, CYP1A1 MspI polymorphism is a common variant, characterized by a T to C transition at the 3081 MspI restriction site. The previous meta-analyses established that the CYP1A1 MspI polymorphism was significantly associated with OSCC risk, and may be a genetic risk factor for oral cancer, particularly among the Asian population.[19,20] However, we recently observed that the CYP1A1 MspI polymorphism was not significantly associated with OPMD occurrence.[6] We further observed that this polymorphism was not significantly associated with OPMD progression to OSCC in this analysis. Among Phase II enzymes, we recently observed that the GSTM1 null genotype was significantly associated with OPMD occurrence, especially OLK,[6] in agreement with the results of the previous meta-analysis.[23] We further observed that this genotype was also significantly associated with OPMD, particularly OLK, progression to OSCC in this analysis. For the other *GST* genes, we observed no significant associations of GSTT1, GSTM3 or GSTP1 polymorphisms with OPMD progression to OSCC. The previous meta-analyses found that GSTM1 and GSTT1 null genotypes significantly enhances the risk of oral cancer, compared with health controls.[21,22] Overall, GSTM1 null genotype as a significant genetic risk factor for both OPMD occurrence and malignant progression was substantially established.

Although the efforts in conducting a systematic evaluation, some limitations need to be addressed in this analysis. The number of the eligible studies was limited, and the pooled sample size for most analyses was still small. The effects of confounding factors, such as tobacco use, alcohol consumption, betel nut chewing, and differences in tumor site, could not be estimated due to unavailability of data in most studies. Moreover, the evidence of heterogeneity was observed in some genetic models possibly owing to methodological variations in different studies. The majority of the patients were enrolled from India, and the analysis of these polymorphisms did not involve populations other than Asians, which may have introduced potential biases. Therefore, more well-designed studies with larger sample sizes and multiple ethnic groups are required to confirm this finding and to strengthen the genetic associations of polymorphisms in carcinogen metabolizing enzymes with OPMD progression to oral cancer risk.

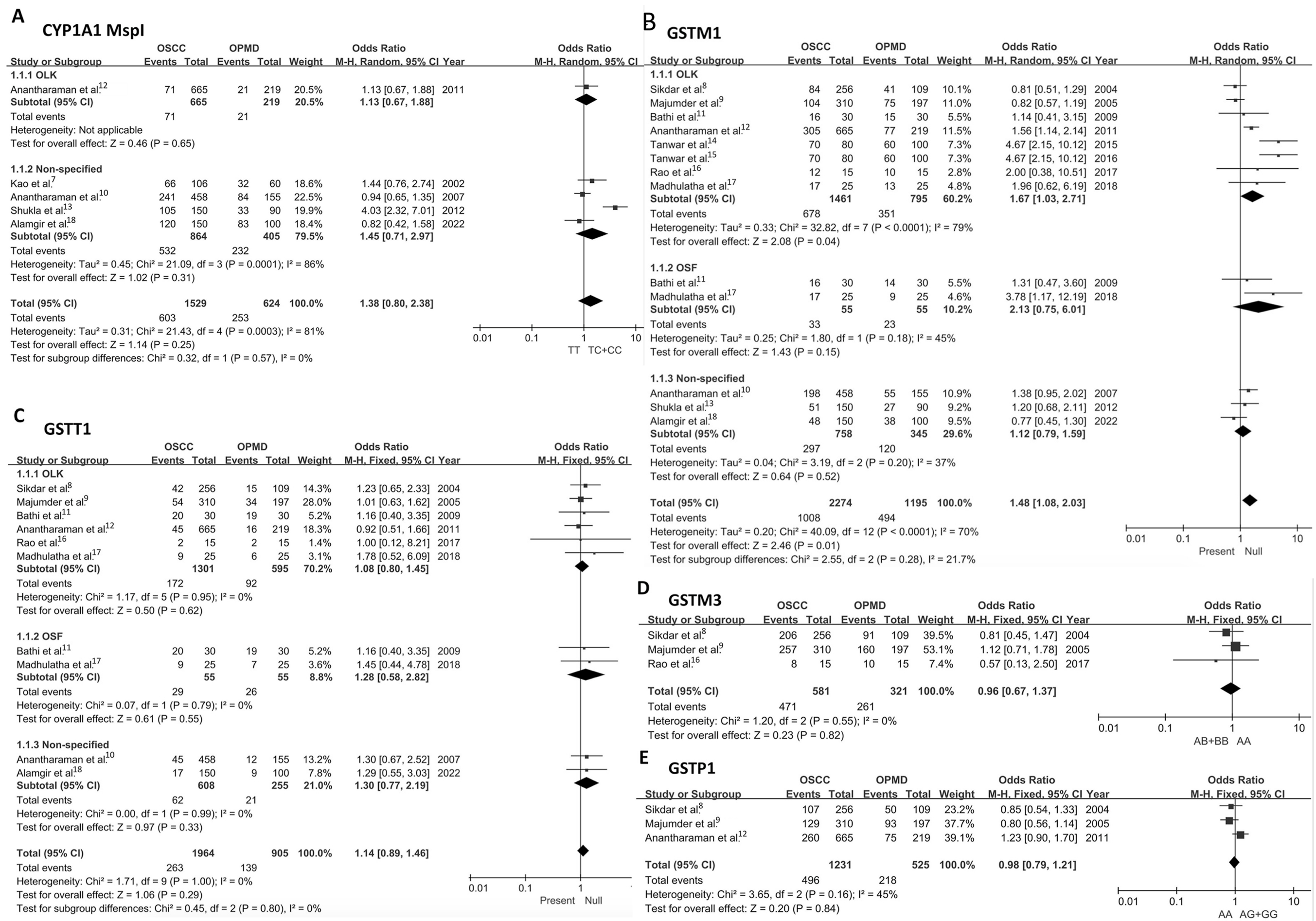

Figure 1 Forest plots of meta-analysis of genetic polymorphisms in carcinogen metabolizing enzymes and oral squamous cell carcinoma (OSCC) compared to oral potentially malignant disorders (OPMD). **(A)** CYP2A1 MspI, **(B)** GSTM1, **(C)** GSTT1, (**D**) GSTM3, (**E**) GSTP1. CI, confidence interval. OLK, oral leukoplakia. OSF, oral submucous fibrosis.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2025.12.021.

References

1. Saldivia-Siracusa C, Carlos de Souza ES, Barros da Silva AV, et al. Automated classification of oral potentially malignant disorders and oral squamous cell carcinoma using a convolutional neural network framework: a cross-sectional study. *Lancet Reg Health Am* 2025;47:101138.
2. Yang CS. Influences of dietary and other factors on xenobiotic metabolism and carcinogenesis-a review article in memory of Dr. Allan H. Conney (1930-2013). *Nutr Cancer* 2015;67:1207—13.
3. Singh RD, Avadhesh A, Sharma G, et al. Potential of cytochrome P450, a family of xenobiotic metabolizing enzymes, in cancer therapy. *Antioxidants Redox Signal* 2023;38:853—76.
4. Guedes Pinto T, Cury PR, Renno ACM, Dagli MLZ, Ribeiro DA. Is placental glutathione S-Transferase (GST-P) a suitable biomarker for oral carcinogenesis: a scoping review. *Pathol Res Pract* 2023;249:154762.
5. Shridhar K, Aggarwal A, Walia GK, et al. Single nucleotide polymorphisms as markers of genetic susceptibility for oral potentially malignant disorders risk: review of evidence to date. *Oral Oncol* 2016;61:146—51.
6. Lou C, Zhu Y, Shi L, Liu W. Systematic evaluation of genetic polymorphisms in carcinogen metabolizing enzymes associated with the risk of oral potentially malignant disorders occurrence. *J Dent Sci* 2026;21:604—9.
7. Kao SY, Wu CH, Lin SC, et al. Genetic polymorphism of cytochrome P4501A1 and susceptibility to oral squamous cell carcinoma and oral precancer lesions associated with smoking/betel use. *J Oral Pathol Med* 2002;31:505—11.
8. Sikdar N, Paul RR, Roy B. Glutathione S-transferase M3 (A/A) genotype as a risk factor for oral cancer and leukoplakia among Indian tobacco smokers. *Int J Cancer* 2004;109:95—101.
9. Majumder M, Sikdar N, Paul RR, Roy B. Increased risk of oral leukoplakia and cancer among mixed tobacco users carrying XRCC1 variant haplotypes and cancer among smokers carrying two risk genotypes: one on each of two loci, GSTM3 and XRCC1 (Codon 280). *Cancer Epidemiol Biomarkers Prev* 2005;14:2106—12.
10. Anantharaman D, Chaubal PM, Kannan S, Bhisey RA, Mahimkar MB. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. *Carcinogenesis* 2007;28:1455—62.
11. Bathi RJ, Rao R, Mutalik S. GST null genotype and antioxidants: risk indicators for oral pre-cancer and cancer. *Indian J Dent Res* 2009;20:298—303.
12. Anantharaman D, Samant TA, Sen S, Mahimkar MB. Polymorphisms in tobacco metabolism and DNA repair genes modulate oral precancer and cancer risk. *Oral Oncol* 2011;47:866—72.
13. Shukla D, Dinesh Kale A, Hallikerimath S, Vivekanandhan S, Venkatakanthaiah Y. Genetic polymorphism of drug metabolizing enzymes (GSTM1 and CYP1A1) as risk factors for oral premalignant lesions and oral cancer. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2012;156:253—9.
14. Tanwar R, Iyengar AR, Nagesh KS, Patil S, Subhash BV. Prevalence of glutathione S-transferase M1 null polymorphism in tobacco users, oral leukoplakia and oral squamous cell carcinoma patients in South Indian population: a polymerase chain reaction study. *Contemp Clin Dent* 2015;6:S59—64.
15. Tanwar R, Iyengar AR, Nagesh KS, Patil S, Subhash BV. GSTM1 null polymorphism prevalence in tobacco users, oral leukoplakia and oral squamous cell carcinoma patients in South Indian population: a polymerase chain reaction study. *Indian J Dent Res* 2016;27:353—8.
16. Rao AK, Parameswar P, Majumdar S, Uppala D, Kotina S, Vennamaneni NH. Evaluation of genetic polymorphisms in glutathione s-transferase theta1, glutathione s-transferase mu1, and glutathione s-transferase mu3 in oral leukoplakia and oral squamous cell carcinoma with deleterious habits using polymerase chain reaction. *Int J Appl Basic Med Res* 2017;7:181—5.
17. Madhulatha G, Das S, Venkateswarlu N, Pujar A, Jyothy A, Munshi A. GSTM1 and GSTT1 null polymorphism and antioxidant levels in oral submucous fibrosis, leukoplakia and oral cancer patients among a South Indian population. *J Oral Maxillofac Surg Med Pathol* 2018;30:169—74.
18. Alamgir MM, Jamal Q, Mirza T. Genetic profiles of different ethnicities living in Karachi as regards to tobacco-metabolising enzyme systems and the risk of oral cancer. *J Pakistan Med Assoc* 2022;72:1092—6.
19. Xu JL, Xia R, Sun L, et al. Association of CYP1A1 MspI polymorphism with oral cancer risk in Asian populations: a meta-analysis. *Genet Mol Res* 2016;15. gmr.15027688.
20. Xie S, Luo C, Shan X, Zhao S, He J, Cai Z. CYP1A1 MspI polymorphism and the risk of oral squamous cell carcinoma: evidence from a meta-analysis. *Mol Clin Oncol* 2016;4:660—6.
21. Kumar KV, Goturi A, Nagaraj M, Goud EVSS. Null genotypes of glutathione S-transferase M1 and T1 and risk of oral cancer: a meta-analysis. *J Oral Maxillofac Pathol* 2022;26:592.
22. Li JY, Huang LN, Xue HL, Zhu QQ, Li CH. Glutathione S-transferase mu-1, glutathione S-transferase theta-1 null genotypes, and oral cancer risk: a meta-analysis in the Chinese population. *J Cancer Res Therapeut* 2018;14:S1052—6.
23. He P, Wei M, Wang Y, Liu Q. Associations among glutathione s-transferase T1, M1, and P1 polymorphisms and the risk of oral leukoplakia. *Genet Test Mol Biomark* 2016;20:312—21.
24. Motahari P, Neshanifard N. Glutathione S-transferase M1 and T1 null polymorphisms and risk of oral submucous fibrosis: a meta-analysis of South Asian populations. *BMC Oral Health* 2025;25:1108.