Review

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Signature of genetic associations in oral cancer

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Abstract

Oral cancer etiology is complex and controlled by multi-factorial events including genetic events. Candidate gene studies, genome-wide association studies, and next-generation sequencing identified various chromosomal loci to be associated with oral cancer. There is no available review that could give us the comprehensive picture of genetic loci identified to be associated with oral cancer by candidate gene studies–based, genome-wide association studies–based, and next-generation sequencing–based approaches. A systematic literature search was performed in the PubMed database to identify the loci associated with oral cancer by exclusive candidate gene studies–based, genome-wide association studies–based, and next-generation sequencing–based study approaches. The information of loci associated with oral cancer is made online through the resource "ORNATE." Next, screening of the loci validated by candidate gene studies and next-generation sequencing approach or by two independent studies within candidate gene studies or next-generation sequencing approaches were performed. A total of 264 loci were identified to be associated with oral cancer by candidate gene studies, genome-wide association studies, and next-generation sequencing approaches. In total, 28 loci, that is, 14q32.33 *(AKT1)*, 5q22.2 *(APC)*, 11q22.3 *(ATM)*, 2q33.1 *(CASP8)*, 11q13.3 *(CCND1)*, 16q22.1 *(CDH1)*, 9p21.3 *(CDKN2A)*, 1q31.1 *(COX-2)*, 7p11.2 *(EGFR)*, 22q13.2 *(EP300)*, 4q35.2 *(FAT1)*, 4q31.3 *(FBXW7)*, 4p16.3 *(FGFR3)*, 1p13.3 *(GSTM1-GSTT1)*, 11q13.2 *(GSTP1)*, 11p15.5 *(H-RAS)*, 3p25.3 *(hOGG1)*, 1q32.1 *(IL-10)*, 4q13.3 *(IL-8)*, 12p12.1 *(KRAS)*, 12q15 *(MDM2)*, 12q13.12 *(MLL2)*, 9q34.3 *(NOTCH1)*, 17p13.1 *(p53)*, 3q26.32 *(PIK3CA)*, 10q23.31 *(PTEN)*, 13q14.2 *(RB1)*, and 5q14.2 *(XRCC4)*, were validated to be associated with oral cancer. "ORNATE" gives a snapshot of genetic loci associated with oral cancer. All 28 loci were validated to be linked to oral cancer for which further fine-mapping followed by gene-by-gene and gene–environment interaction studies is needed to confirm their involvement in modifying oral cancer.

Keywords

Association studies, candidate gene studies, genome-wide association studies, next-generation sequencing, oral cancer

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Introduction

Every year, around 274,300 new cases of oral cancer (OC) are diagnosed in Asia associated with a high mortality rate due to probable delayed diagnosis of the disease.[1] The risk of OC development depends on multiple factors, such as exposure to environmental factors, human papillomavirus (HPV), alcohol, tobacco, areca nut, and genetic factors, such as an alteration in DNA.[2,3]

Several decades of intensive research have expanded our knowledge of cancer pathogenesis at the molecular

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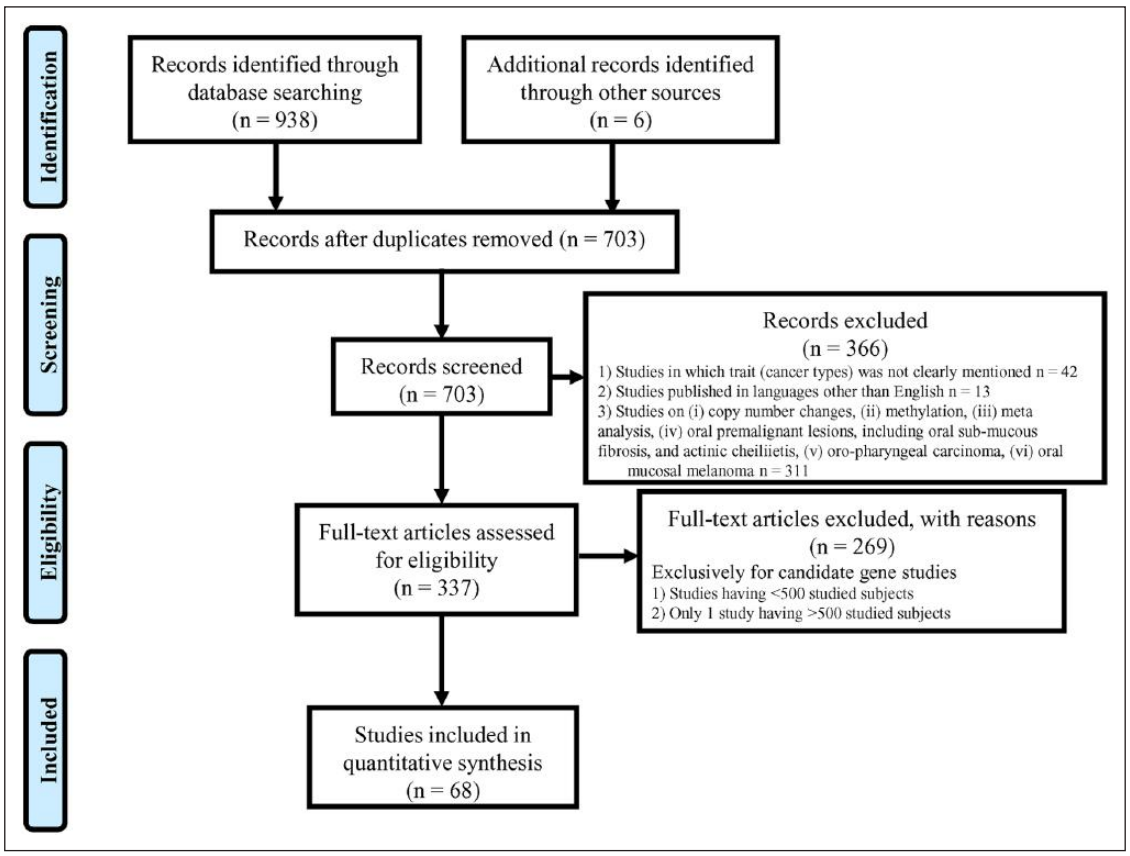

Figure 1. Flow chart describing the included/excluded literature.

level, thus providing new targets for early diagnosis, disease characterization, and therapy selection. Genetic profiling of individual patient is being expected to play a key role in efficient disease diagnosis, adverse drug responses, and drug treatment. Moreover, mutations within genetic loci are used for very early cancer diagnosis, prognosis, and predicting therapeutic responses.[4–6] Advances in methods and technology in the field of genomics are now expected to enable construction of a comprehensive panel of genes that may be targeted for defining treatment strategies for OC.

Genetics of OC is studied at the population level following three approaches, that is, (a) candidate gene studies (CGS) which target associations between genetic variations within pre-specified genes of interest,[7,8] (b) genome-wide association studies (GWAS) that, unlikely to CGS, scan the whole genome through the single-nucleotide polymorphism (SNP) array and target sequence variants with small effects,[9,10] and (c) next-generation sequencing (NGS)-based studies where entire genome is deep sequenced and variants with large effects are studied.[11,12] Each of these approaches identified various genetic loci to be associated with OC. However, there is no available review of the literature (Supplement S1) that could give us the comprehensive knowledge of genetic loci identified to be associated with OC by CGS, GWAS, and NGS. Absence of any relevant literature inspired us to perform a comprehensive review with the following aims: (a) to give an overview of genetic loci reported to be associated with OC by CGS-, GWAS-, and NGS-based studies and (b) to screen/identify loci validated by at least two independent CGS or NGS studies or validated by both CGS and NGS study approaches.

Materials and methods

Comprehensive search was performed (based on reported odds ratios) to know the genetic association studies of polymorphism/s associated with OC using exclusive CGS, GWAS, and NGS approaches (Figure 1 and Supplement S2; flow charts 1–4). Since the polymorphism/s in tight linkage disequilibrium (LD; $r^2 \geq 0.8$) with the reported OC-associated polymorphism/s could be the causal one (if two or more than two polymorphisms are in tight LD, it is difficult to dissect which among them is causal), we considered and discussed the whole genetic locus rather than individual polymorphism. (a) Studies screening by CGS approach: only those studies in which the same genetic locus was identified by at least 2 independent studies and having >500 studied subjects were picked up (single study

having >500 studied subjects was not excluded); (b) studies screening by GWAS approach: studies in which same locus was identified by at least two independent studies, one from GWAS and other one/s validated by at least one CGS- or NGS-based approach were selected; (c) studies screening by NGS approach: first criterion was to screen the studies in which same locus was identified by at least two independent studies. Second criterion was to screen studies where same locus was identified by at least two independent studies, one from NGS and other one/s validated by at least one CGS or GWAS approaches. Due to limited GWAS studies on OC,[10,13,14] the validation of OC-associated genetic loci was performed for CGS and NGS studies.

The studies were screened using the following criteria.

Inclusion criteria

Original studies on human OC, squamous cell carcinoma of the oral cavity, lip, and buccal mucosa, showing genetic associations were considered. The genetic locus was screened if polymorphism/s within it was/were found to be associated with OC.

Exclusion criteria

1. The studies in which trait (cancer types) was not clearly mentioned.
2. The studies published in languages other than English.
3. Studies on (a) copy number changes, (b) methylation, (c) meta-analysis, (d) oral premalignant lesions, including oral sub-mucous fibrosis, and actinic cheilitis, (e) oro-pharyngeal carcinoma, and (f) oral mucosal melanoma.

Open source link development and genetic loci mapping on chromosome

For an overview of the loci screened in this study, a resource "ORNATE" was developed, which comprises the information of loci (encompassing genes) associated with OC and their underlying references (http://bmi.icmr.org.in/ornate/). ORNATE was designed using front-end technologies HTML, CSS, jQuery (JavaScript library) and back end with PHP (version 5.6) and MySql (version 5.4).

To locate the OC-associated loci on human chromosomes, mapping was done using a Genome Decoration tool (http://www.ncbi.nlm.nih.gov/genome/tools/gdp/).

Results

A total of 264 loci were found to be linked to OC by CGS (155 loci), GWAS (20 loci), and NGS (89 loci) approaches (Table S1 and "ORNATE"). Of these 264 loci, 28 loci, that is, 14q32.33 (*AKT1*), 5q22.2 (*APC*), 11q22.3 (*ATM*), 2q33.1 (*CASP8*), 11q13.3 (*CCND1*), 16q22.1 (*CDH1*), 9p21.3 (*CDKN2A*), 1q31.1 (*COX-2*), 7p11.2 (*EGFR*), 22q13.2 (*EP300*), 4q35.2 (*FAT1*), 4q31.3 (*FBXW7*), 4p16.3 (*FGFR3*), 1p13.3 (*GSTM1-GSTT1*), 11q13.2 (*GSTP1*), 11p15.5 (*H-RAS*), 3p25.3 (*hOGG1*), 1q32.1 (*IL-10*), 4q13.3 (*IL-8*), 12p12.1 (*KRAS*), 12q15 (*MDM2*), 12q13.12 (*MLL2*), 9q34.3 (*NOTCH1*), 17p13.1 (*p53*), 3q26.32 (*PIK3CA*), 10q23.31 (*PTEN*), 13q14.2 (*RB1*), and 5q14.2 (*XRCC4*), were validated to be associated with OC by CGS and NGS approaches (Figure 2 and Table 1). For better understanding of loci distribution, the validated loci were mapped on human chromosomes (Figure 3).

A total of 172 OC-associated loci were identified by CGS approach (Table S1 and Supplement S2; flow chart 2); of which 14q32.33 (*AKT1*), 5q22.2 (*APC*), 11q22.3 (*ATM*), 2q33.1 (*CASP8*), 16q22.1 (*CDH1*), 7p11.2 (*EGFR*), 4p16.3 (*FGFR3*), 11p15.5 (*H-RAS*), 12p12.1 (*KRAS*), 9q34.3 (*NOTCH1*), 17p13.1 (*p53*), and 13q14.2 (*RB1*) were validated by NGS (Table 1) and 18q21.2 (*DCC*), 3p14.2 (*FHIT*), 6q16.3 (*GRIK2*), 16q12.2 (*MMP-2*), and 2p22.3 (*RASGRP3*) by GWAS (Table S1). Hence, overall, 155 loci were identified only in CGS.

A total of 20 loci were found to be linked to OC via GWAS approach (Table S1); of which 18q21.2 (*DCC*), 3p14.2 (*FHIT*), 6q16.3 (*GRIK2)*, 16q12.2 (*MMP-2*), and 2p22.3 (*RASGRP3*) were further validated in studies by CGS approach.

Through NGS-based approach, 89 OC-associated loci were identified (Table 1); of which 9p21.3 (*CDKN2A*), 22q13.2 (*EP300*), 4q35.2 (*FAT1*), 4q31.3 (*FBXW7*), 12q13.12 (*MLL2*), 3q26.32 (*PIK3CA*), and 10q23.31 (*PTEN*) were found in at least two independent studies and 14q32.33 (*AKT*), 5q22.2 (*APC*), 11q22.3 (*ATM*), 2q33.1 (*CASP8*), 16q22.1 (*CDH1*), 7p11.2 (*EGFR*), 4p16.3 (*FGFR3*), 11p15.5 (*H-RAS*), 12p12.1 (*KRAS*), 9q34.3 (*NOTCH1*), 917p13.1 (*p53*), and 13q14.2 (*RB1*) were also confirmed in studies following CGS approach.

Conclusion

The "ORNATE" gives the status of studies from literature in which genetic variants (within loci) were found to be associated with OC. With the non-availability of any resource giving systematic information about genetic loci associated with OC, "ORNATE" may be a useful tool for comparing/deciding experiments on OC genetic association studies.

In this study, 28 loci were validated to be linked to OC via CGS and NGS approach. Genetic alterations are considered to be important in the development and progression of OC, leading to dysregulation of essential cellular signaling pathways such as the Ras-MAPK-ERK (Ras–mitogen-activated protein kinase–extracellular signal–regulated protein kinase), the PI3K/Akt/mTOR (phosphoinositide 3-kinase/Akt/

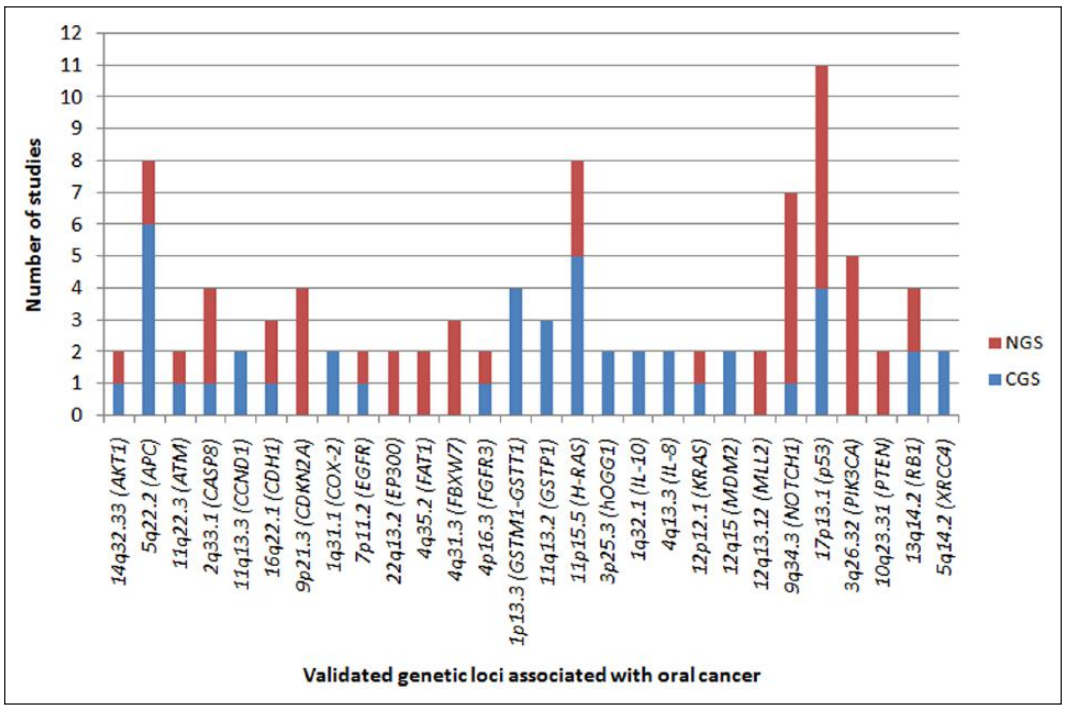

Figure 2. Validated 28 oral cancer–associated genetic loci. On x-axis is the associated genetic loci and their representative gene, whereas on y-axis is the number of identified associated studies.

mechanistic target of rapamycin), the JAK/STAT3 (Janus kinase/signal transducer and activator of transcription 3), and the PLCγ/PKC (phospholipase C-γ/protein kinase C).[67,68] In addition to mutation correlates of OC, several studies identifying differential gene expression patterns in OC patient samples are available.[69,70] Integrating mutation and gene expression data set to identify patterns of molecular events relevant to initiation and progression of tumor may be of importance for OC diagnosis, prognosis, and therapy.[71]

The tumor suppressor gene *p53* is commonly mutated in OC.[72,73] Alterations in the *p53* gene make it functionally inactive in oral tumors. Moreover, functional restoration of the *p53* gene in OC cell lines and in animal models of OC has shown to relapse oral carcinogenesis.[74] It is suggested that *p53* polymorphism together with HPV infection increases the susceptibility of OC.[75] Another important tumor suppressor gene *NOTCH1* is reported to be mutated in 54% for oral squamous cell carcinoma (OSCC) and 60% for preneoplastic lesions in Chinese patients.[57] *NOTCH1* mutations and altered expression are proposed to be the drivers of OSCC progression.[57,76] The *MDM2* gene, a proto-oncogene promoting tumor formation by targeting tumor suppressor proteins, such as p53, is amplified in 25%–40% of all human cancers.[77] The co-expression of p53/MDM2 proteins is suggested to be an indicator of aggressive tumor behavior in OSCC.[78] The proto-oncogene *Akt1* promotes cell proliferation, survival, and metastasis of cancer cells and its overexpression relates to the progression of OC.[79]

In the *RAS* gene family, involved in the molecular pathogenesis of OSCC, mutations in the *HRAS* gene are reported to be the highest in OC. Importantly, *HRAS* mutations are common in Asian patients who chew betel quid.[80]

Expression of the oncogene *PIK3CA* increases in OSCC.[81] However, the association of SNPs with the expression level has not been observed.[82] This may be due to the occurrence of somatic mutations of the *PIK3CA* late in the development of OSCC and tumor progression occurring through the PI3K-AKT signaling pathway.[83] The frequency of *PIK3CA* and *HRAS* mutation in Asian OC patients is similar to the Caucasian OC patients, despite differences in their risk habit exposure as betel quid chewing versus smoking and alcohol drinking, respectively.[84]

The epidermal growth factor receptor (EGFR) is involved in the regulation of cell proliferation, metastasis, migration, invasion, angiogenesis, and inhibition of apoptosis. Overexpression of EGFR is associated with aggressive phenotype and poor prognosis in OC.[85] Cetuximab, a monoclonal antibody targeting EGFR, is a Food and Drug Administration (FDA)-approved drug in OSCC, with a significant effect in combination therapy.[86]

Overexpression of cyclooxygenase-2 (COX-2), enzyme involved in the production of prostanoids from arachidonic acid, results in enhanced synthesis of prostaglandins promoting angiogenesis, apoptosis, and cancer progression.[87] Polymorphisms and expression of the *COX-2* gene are associated with the pathogenesis of OSCC and may be of prognostic significance.[87–89]

Table 1. List of chromosome locus and their corresponding oral cancer–associated and validated gene.

S. no.	Chromosome locus	Genes associated with oral cancer	CGS	NGS
1	14q32.33	*AKT1*	Studies: 1[15] Subjects: 389	Studies: 1[16] Subjects: 345
2	5q22.2	*APC*	Studies: 6[17–22] Subjects: 285	Studies: 2[12,16] Subjects: 395
3	11q22.3	*ATM*	Studies: 1[23] Subjects: 1240	Studies: 1[16] Subjects: 345
4	2q33.1	*CASP8*	Studies: 1[24] Subjects: 1012	Studies: 3[25–27] Subjects: 208
5	11q13.3	*CCND1*	Studies: 2[28–29] Subjects: 1866	
6	16q22.1	*CDH1*	Studies: 1[30] Subjects: 598	Studies: 2[12,16] Subjects: 395
7	9p21.3	*CDKN2A*		Studies: 4[16,25,26,31] Subjects: 663
8	1q31.1	*COX-2*	Studies: 2[32,33] Subjects: 1396	
9	7p11.2	*EGFR*	Studies: 1[34] Subjects: 306	Studies: 1[16] Subjects: 345
10	22q13.2	*EP300*		Studies: 2[26,27] Subjects: 188
11	4q35.2	*FAT1*		Studies: 2[26,27] Subjects: 188
12	4q31.3	*FBXW7*		Studies: 3[12,16,31] Subjects: 624
13	4p16.3	*FGFR3*	Studies: 1[35] Subjects: 20	Studies: 1[16] Subjects: 345
14	1p13.3	*GSTM1-GSTT1*	Studies: 4[36–39] Subjects: 4041	
15	11q13.2	*GSTP1*	Studies: 3[40–42] Subjects: 1774	
16	11p15.5	*H-RAS*	Studies: 5[43–47] Subjects: 623	Studies: 3[16,27,31] Subjects: 624
17	3p25.3	*hOGG1*	Studies: 2[48,49] Subjects: 2480	
18	1q32.1	*IL-10*	Studies: 2[8,50] Subjects: 2324	
19	4q13.3	*IL-8*	Studies: 2[51,52] Subjects: 1220	
20	12p12.1	*KRAS*	Studies: 1[53] Subjects: 131	Studies: 1[16] Subjects: 345
21	12q15	*MDM2*	Studies: 2[54,55] Subjects: 1308	
22	12q13.12	*MLL2*		Studies: 2[26,27] Subjects: 188
23	9q34.3	*NOTCH1*	Studies: 1[56] Subjects: 84	Studies: 6[16,26,27,31,57,58] Subjects: 960
24	17p13.1	*p53*	Studies: 4[55,59–61] Subjects: 2624	Studies: 7[12,16,25–27,31,62] Subjects: 948
25	3q26.32	*PIK3CA*		Studies: 5[12,16,25,26,31] Subjects: 713
26	10q23.31	*PTEN*		Studies: 2[16,31] Subjects: 565
27	13q14.2	*RB1*	Studies: 2[63,64] Subjects: 1205	Studies: 2[16,31] Subjects: 565
28	5q14.2	*XRCC4*	Studies: 2[65,66] Subjects: 1272	

CGS: candidate gene studies; NGS: next-generation sequencing.
The studies represent the total number of articles screened where the corresponding genetic locus (encompassing gene) was found to be associated with oral cancer. Subjects are dealt with the sum of the number of participants from the screened studies.

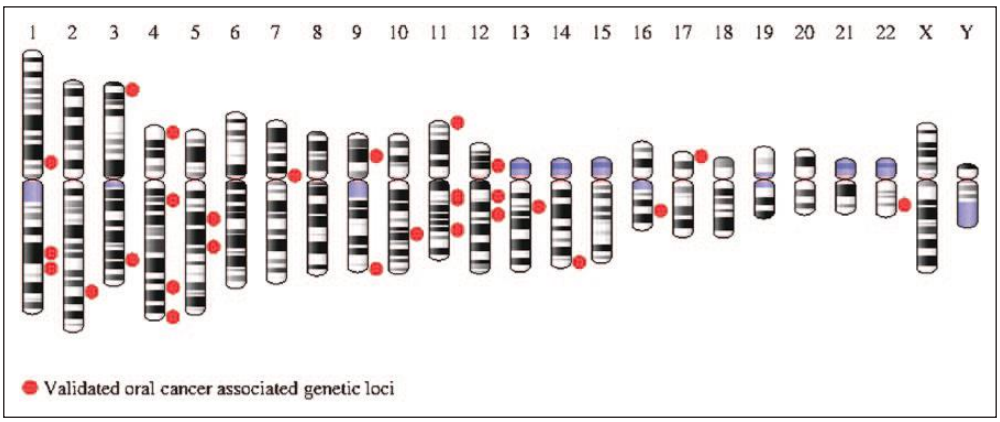

Figure 3. Validated 28 oral cancer–associated genetic loci mapped on the human chromosomes. Information of genetic loci and their encompassing gene mapped on the figure can be found in Table 1. References underlying the loci are available on "ORNATE."

Deregulation of *CASP8*, a key controller of apoptosis, is crucially involved in carcinogenesis. Importantly, polymorphisms in the *CASP8* are suggested to play a protective role in the development of tumor in OSCC patients.[24]

Interleukin (IL)-8 and IL-10 cytokines play an essential role in OC. IL-10 is an immunosuppressive cytokine, while IL-8 is a pro-inflammatory cytokine both involved in the process of angiogenesis. These cytokines, studied at both mutation and expression level, may serve as biomarkers of prognostic value.[90–93] The heterogeneity in cancer cells between and/or within patients makes it a complex disease to cure.[94] Identifying germ line mutations which may serve as biomarkers for predicting OC-susceptible individuals and/or who may respond differently to a particular cancer therapy is very important.[4] In addition to this, studying genetic mutations and aberrations in tumor cells, all together provide a window of opportunity for defining treatment strategies for OC. Recent data demonstrate that OC is a mutationally heterogeneous class of cancer. Efforts to improve disease prognosis by combining genetic information from NGS with traditional clinicopathological prognostic parameters are ongoing.[5] Precision oncology goals to define treatment strategies based on patient-specific somatic mutations and abnormal molecular pathways. However, discrepancies in data sets may be accepted due to differences in disease stage, exposed carcinogen, ethnicity, and genetic background of individual patient.[5] Recent research focuses on OC subtype stratification, and molecular characterization may help in prognosis and defining personalized treatment strategies. In addition, retrospective study design with formalin-fixed paraffin-embedded samples paved the path to study the prognostic significance of common genetic alterations. Such study has aided in identifying a prognostic gene signature, *HRAS, BRAF, FGFR3, SMAD4, KIT, PTEN, NOTCH1, AKT1, CTNNB1*, and *PTPN11*, that predicts both disease-free survival and overall survival.[16]

On 28 validated hotspots, further fine-mapping on larger cohorts, based on the 1000 Genomes Project data sets,[95] is needed to confirm already known associations and find novel genetic variants associated with OC. Moreover, gene-by-gene interaction analysis[96] using complex statistical models[97] may also help to comprehend the genomic network regulating oral carcinogenesis. Polygenic disease, such as OC, is also influenced by environmental factors, including alcohol, betel quid, and smoking, which along with genetic variations plays a modulating role in determining an individual's susceptibility to disease.[3] Statistical methods using multifaceted gene–environment analysis are also required to unravel the complex biological mechanism/s modulating OC.[96]

The holistic approach used for screening studies on OC-associated loci could be utilized for studying other cancer subtypes, but for now, well-defined studies on larger cohorts of different ethnicity needs to be performed. In order to improve patient care with targeted therapies leading to more accuracy and specificity, the combined analysis of the so far available genetic data sets is required. This review is a step toward this direction with an aim to filter down the genetic loci from the available data set in the public domain which may be of clinical relevance in defining strategies pertaining to OC treatment.

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Declaration of conflicting interests

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References

1. Krishna Rao SV, Mejia G, Roberts-Thomson K, et al. Epidemiology of oral cancer in Asia in the past decade—an update (2000–2012). *Asian Pac J Cancer Prev* 2013; 14: 5567–5577.
2. http://www.cancercenter.com/oral-cancer/risk-factors/
3. Hung HC, Chuang J, Chien YC, et al. Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 901–905.
4. Santosh AB, Jones T and Harvey J. A review on oral cancer biomarkers: understanding the past and learning from the present. *J Cancer Res Ther* 2016; 12: 486–492.
5. Wang H-M, Liao C-T, Yen T-C, et al. Clues toward precision medicine in oral squamous cell carcinoma: utility of next-generation sequencing for the prognostic stratification of high-risk patients harboring neck lymph node extracapsular extension. *Oncotarget* 2016; 7: 63082–63092.
6. Fruscella M, Ponzetto A, Crema A, et al. The extraordinary progress in very early cancer diagnosis and personalized therapy: the role of oncomarkers and nanotechnology. *J Nanotechnol* 2016; 2016: 1–18.
7. Tabor HK, Risch NJ and Myers RM. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 2002; 3: 391–397.
8. Tsai C-W, Chang W-S, Lin K-C, et al. Significant association of Interleukin-10 genotypes and oral cancer susceptibility in Taiwan. *Anticancer Res* 2014; 34: 3731–3737.
9. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005; 308: 385–389.
10. Bhatnagar R, Dabholkar J and Saranath D. Genome-wide disease association study in chewing tobacco associated oral cancers. *Oral Oncol* 2012; 48: 831–835.
11. Luo L, Boerwinkle E and Xiong M. Association studies for next-generation sequencing. *Genome Res* 2011; 21: 1099–1108.
12. Er T-K, Wang Y-Y, Chen C-C, et al. Molecular characterization of oral squamous cell carcinoma using targeted next-generation sequencing. *Oral Dis* 2015; 21: 872–878.
13. Johansson M, Roberts A, Chen D, et al. Using prior information from the medical literature in GWAS of oral cancer identifies novel susceptibility variant on chromosome 4—the AdAPT method. *PLoS ONE* 2012; 7: e36888.
14. Lunde ML, Roman E, Warnakulasuriya S, et al. Profiling of chromosomal changes in potentially malignant and malignant oral mucosal lesions from South and South-East Asia using array-comparative genomic hybridization. *Cancer Genomics Proteomics* 2014; 11: 127–140.
15. Wang Y, Lin L, Xu H, et al. Genetic variants in AKT1 gene were associated with risk and survival of OSCC in Chinese Han Population. *J Oral Pathol Med* 2015; 44: 45–50.
16. Chen S-J, Liu H, Liao C-T, et al. Ultra-deep targeted sequencing of advanced oral squamous cell carcinoma identifies a mutation-based prognostic gene signature. *Oncotarget* 2015; 6: 18066–18080.
17. Rivero ER, Horta MC, Silva Guerra EN, et al. Loss of heterozygosity of the APC gene in oral squamous cell carcinoma. *Pathol Res Pract* 2008; 204: 793–797.
18. Kok S-H, Lee J-J, Hsu H-C, et al. Mutations of the adenomatous polyposis coli gene in areca quid and tobacco-associated oral squamous cell carcinomas in Taiwan. *J Oral Pathol Med* 2002; 31: 395–401.
19. Chang KW, Lin SC, Mangold KA, et al. Alterations of adenomatous polyposis Coli (APC) gene in oral squamous cell carcinoma. *Int J Oral Maxillofac Surg* 2000; 29: 223–226.
20. Tandle A, Sanghavi V and Saranath D. Infrequent loss of heterozygosity at adenomatous polyposis coli gene locus in Indian oral cancers. *Cancer Lett* 2000; 157: 155–160.
21. Imai FL, Uzawa K, Shiiba M, et al. Evidence of multi-step oncogenesis of oral squamous cell carcinoma. *Oncol Rep* 1998; 5: 1489–1491.
22. Uzawa K, Yoshida H, Suzuki H, et al. Abnormalities of the adenomatous polyposis coli gene in human oral squamous-cell carcinoma. *Int J Cancer* 1994; 58: 814–817.
23. Bau D-T, Chang C-H, Tsai M-H, et al. Association between DNA repair gene ATM polymorphisms and oral cancer susceptibility. *Laryngoscope* 2010; 120: 2417–2422.
24. Tang YI, Liu Y, Zhao W, et al. Caspase-8 polymorphisms and risk of oral squamous cell carcinoma. *Exp Ther Med* 2015; 10: 2267–2276.
25. Al-Hebshi NN, Li S, Nasher AT, et al. Exome sequencing of oral squamous cell carcinoma in users of Arabian snuff reveals novel candidates for driver genes. *Int J Cancer* 2016; 139: 363–372.
26. Vettore AL, Ramnarayanan K, Poore G, et al. Mutational landscapes of tongue carcinoma reveal recurrent mutations in genes of therapeutic and prognostic relevance. *Genome Med* 2015; 7: 98.
27. India Project Team of the International Cancer Genome Consortium. Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. *Nat Commun* 2013; 4: 2873.
28. Tsai M-H, Tsai C-W, Tsou Y-A, et al. Significant association of cyclin D1 single nucleotide polymorphisms with oral cancer in Taiwan. *Anticancer Res* 2011; 31: 227–231.
29. Lin Y-C, Yeh C-J, Wang L-H, et al. The effect of CCND1 +870A>G and VEGF +936C>T polymorphisms on oral cancer development and disease-free survival in a Taiwan population. *Oral Oncol* 2012; 48: 535–540.
30. Chien M-H, Chou LS-S, Chung T-T, et al. Effects of E-cadherin (CDH1) gene promoter polymorphisms on the risk and clinicopathologic development of oral cancer. *Head Neck* 2012; 34: 405–411.
31. Oikawa Y, Morita K-I, Kayamori K, et al. Receptor tyrosine kinase amplification is predictive of distant metastasis

in patients with oral squamous cell carcinoma. *Cancer Sci* 2017; 108: 256–266.
32. Chiang S-L, Chen P-H, Lee C-H, et al. Up-regulation of inflammatory signalings by areca nut extract and role of cyclooxygenase-2–1195G>a polymorphism reveal risk of oral cancer. *Cancer Res* 2008; 68: 8489–8498.
33. Lin Y-C, Huang H-I, Wang L-H, et al. Polymorphisms of COX-2–765G>C and p53 codon 72 and risks of oral squamous cell carcinoma in a Taiwan population. *Oral Oncol* 2008; 44: 798–804.
34. Kang D, Gridley G, Huang W-Y, et al. Microsatellite polymorphisms in the epidermal growth factor receptor (EGFR) gene and the transforming growth factor-alpha (TGFA) gene and risk of oral cancer in Puerto Rico. *Pharmacogenet Genomics* 2005; 15: 343–347.
35. Chou A, Dekker N and Jordan RC. Identification of novel fibroblast growth factor receptor 3 gene mutations in actinic cheilitis and squamous cell carcinoma of the lip. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; 107: 535–541.
36. Anantharaman D, Samant TA, Sen S, et al. Polymorphisms in tobacco metabolism and DNA repair genes modulate oral precancer and cancer risk. *Oral Oncol* 2011; 47: 866–872.
37. Anantharaman D, Chaubal PM, Kannan S, et al. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. *Carcinogenesis* 2007; 28: 1455–1462.
38. Buch SC, Notani PN and Bhisey RA. Polymorphism at GSTM1, GSTM3 and GSTT1 gene loci and susceptibility to oral cancer in an Indian population. *Carcinogenesis* 2002; 23: 803–807.
39. Yadav DS, Devi TR, Ihsan R, et al. Polymorphisms of glutathione-S-transferase genes and the risk of aerodigestive tract cancers in the Northeast Indian population. *Genet Test Mol Biomarkers* 2010; 14: 715–723.
40. Datta S, Majumder M, Biswas NK, et al. Increased risk of oral cancer in relation to common Indian mitochondrial polymorphisms and Autosomal GSTP1 locus. *Cancer* 2007; 110: 1991–1999.
41. Sikdar N, Paul RR and 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.
42. Matthias C, Bockmühl U, Jahnke V, et al. The glutathione S-transferase GSTP1 polymorphism: effects on susceptibility to oral/pharyngeal and laryngeal carcinomas. *Pharmacogenetics* 1998; 8: 1–6.
43. Murugan AK, Hong NT, Cuc TT, et al. Detection of two novel mutations and relatively high incidence of H-RAS mutations in Vietnamese oral cancer. *Oral Oncol* 2009; 45: e161–e166.
44. Sathyan KM, Nalinakumari KR and Kannan S. H-Ras mutation modulates the expression of major cell cycle regulatory proteins and disease prognosis in oral carcinoma. *Mod Pathol* 2007; 20: 1141–1148.
45. Sathyan KM, Nalinakumari KR, Abraham T, et al. Influence of single nucleotide polymorphisms in H-Ras and cyclin D1 genes on oral cancer susceptibility. *Oral Oncol* 2006; 42: 607–613.
46. Anderson JA, Irish JC, McLachlin CM, et al. H-ras oncogene mutation and human papillomavirus infection in oral carcinomas. *Arch Otolaryngol Head Neck Surg* 1994; 120: 755–760.
47. Saranath D, Bhoite LT, Mehta AR, et al. Loss of allelic heterozygosity at the Harvey ras locus in human oral carcinomas. *J Cancer Res Clin Oncol* 1991; 117: 484–488.
48. Tsai C-W, Tsai M-H, Tsou Y-A, et al. The joint effect of smoking and hOGG1 genotype on oral cancer in Taiwan. *Anticancer Res* 2012; 32: 3799–3803.
49. Tsou Y-A, Hua C-H, Tseng H-C, et al. The joint effect of hOGG1 single nucleotide polymorphism and betel quid chewing on oral cancer in Taiwan. *Anticancer Res* 2010; 30: 4205–4208.
50. Yao J-G, Gao L-B, Liu Y-G, et al. Genetic variation in interleukin-10 gene and risk of oral cancer. *Clin Chim Acta* 2008; 388: 84–88.
51. Singh PK, Chandra G, Bogra J, et al. Association of genetic polymorphism in the interleukin-8 gene with risk of oral cancer and its correlation with pain. *Biochem Genet* 2016; 54: 95–106.
52. Liu C-M, Yeh C-J, Yu C-C, et al. Impact of interleukin-8 gene polymorphisms and environmental factors on oral cancer susceptibility in Taiwan. *Oral Dis* 2012; 18: 307–314.
53. Wang W-Y, Chien Y-C, Wong Y-K, et al. Effects of KRAS mutation and polymorphism on the risk and prognosis of oral squamous cell carcinoma. *Head Neck* 2012; 34: 663–666.
54. Chen X, Sturgis EM, Lei D, et al. Human papillomavirus seropositivity synergizes with MDM2 variants to increase the risk of oral squamous cell carcinoma. *Cancer Res* 2010; 70: 7199–7208.
55. Misra C, Majumder M, Bajaj S, et al. Polymorphisms at p53, p73, and MDM2 loci modulate the risk of tobacco associated leukoplakia and oral cancer. *Mol Carcinog* 2009; 48: 790–800.
56. Aoyama K, Ota Y, Kajiwara K, et al. Frequent mutations in NOTCH1 ligand-binding regions in Japanese oral squamous cell carcinoma. *Biochem Biophys Res Commun* 2014; 452: 980–985.
57. Izumchenko E, Sun K, Jones S, et al. Notch1 mutations are drivers of oral tumorigenesis. *Cancer Prev Res* 2015; 8: 277–286.
58. Song X, Xia R, Li J, et al. Common and complex Notch1 mutations in Chinese oral squamous cell carcinoma. *Clin Cancer Res* 2014; 20: 701–710.
59. Saleem S, Abbasi ZA, Hameed A, et al. Novel p53 codon 240 Ser > Thr coding region mutation in the patients of oral squamous cell carcinoma (OSCC). *Tumour Biol* 2014; 35: 7945–7950.
60. Wang Z, Sturgis EM, Zhang Y, et al. Combined p53-related genetic variants together with HPV infection increase oral cancer risk. *Int J Cancer* 2012; 131: E251–E258.
61. Chen X, Sturgis EM, El-Naggar AK, et al. Combined effects of the p53 codon 72 and p73 G4C14-to-A4T14 polymorphisms on the risk of HPV16-associated oral cancer in never-smokers. *Carcinogenesis* 2008; 29: 2120–2125.
62. Pickering CR, Zhang J, Neskey DM, et al. Squamous cell carcinoma of the oral tongue in young non-smokers

is genomically similar to tumors in older smokers. *Clin Cancer Res* 2014; 20: 3842–3848.
63. Murali A, Varghese BT, Kumar RR, et al. Combination of genetic variants in cyclin D1 and retinoblastoma genes predict clinical outcome in oral cancer patients. *Tumour Biol* 2016; 37: 3609–3617.
64. Murali A, Nalinakumari KR, Thomas S, et al. Association of single nucleotide polymorphisms in cell cycle regulatory genes with oral cancer susceptibility. *Br J Oral Maxillofac Surg* 2014; 52: 652–658.
65. Tseng H-C, Tsai M-H, Chiu C-F, et al. Association of XRCC4 codon 247 polymorphism with oral cancer susceptibility in Taiwan. *Anticancer Res*; 28: 1687–1691.
66. Chiu C-F, Tsai M-H, Tseng H-C, et al. A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. *Oral Oncol* 2008; 44: 898–902.
67. Gong W, Xiao Y, Wei Z, et al. Toward the use of precision medicine for the treatment of head and neck squamous cell carcinoma. *Oncotarget* 2017; 8: 2141–2152.
68. Murugan AK, Munirajan AK and Tsuchida N. Genetic deregulation of the PIK3CA oncogene in oral cancer. *Cancer Lett* 2013; 338: 193–203.
69. Chen C, Méndez E, Houck J, et al. Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 2008; 17: 2152–2162.
70. Oliveira-Costa JP, de Carvalho AF, da Silveira da GG, et al. Gene expression patterns through oral squamous cell carcinoma development: PD-L1 expression in primary tumor and circulating tumor cells. *Oncotarget* 2015; 6: 20902–20920.
71. Fleck JL, Pavel AB and Cassandras CG. Integrating mutation and gene expression cross-sectional data to infer cancer progression. *BMC Syst Biol* 2016; 10: 12.
72. Hsieh LL, Wang PF, Chen IH, et al. Characteristics of mutations in the p53 gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. *Carcinogenesis* 2001; 22: 1497–1503.
73. Greenblatt MS, Bennett WP, Hollstein M, et al. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 1994; 54: 4855–4878.
74. Jurel SK, Gupta DS, Singh RD, et al. Genes and oral cancer. *Indian J Hum Genet* 2014; 20: 4–9.
75. Hou J, Gu Y, Hou W, et al. P53 codon 72 polymorphism, human papillomavirus infection, and their interaction to oral carcinoma susceptibility. *BMC Genet* 2015; 16: 72.
76. Yoshida R, Nagata M, Nakayama H, et al. The pathological significance of Notch1 in oral squamous cell carcinoma. *Lab Invest* 2013; 93: 1068–1081.
77. Denaro N, Lo Nigro C, Natoli G, et al. The role of p53 and MDM2 in head and neck cancer. *ISRN Otolaryngol* 2011; 2011: 931813.
78. Shwe M, Chiguchi G, Yamada S, et al. P53 and MDM2 coexpression in tobacco and betel chewing-associated oral squamous cell carcinomas. *J Med Dent Sci* 2001; 48: 113–119.
79. Nakashiro K-I, Tanaka H, Goda H, et al. Identification of Akt1 as a potent therapeutic target for oral squamous cell carcinoma. *Int J Oncol* 2015; 47: 1273–1281.
80. Murugan AK, Munirajan AK and Tsuchida N. Ras oncogenes in oral cancer: the past 20 years. *Oral Oncol* 2012; 48: 383–392.
81. Wan X, Li X, Yang J, et al. Genetic association between PIK3CA gene and oral squamous cell carcinoma: a case control study conducted in Chongqing, China. *Int J Clin Exp Pathol* 2015; 8: 13360–13366.
82. Cohen Y, Goldenberg-Cohen N, Shalmon B, et al. Mutational analysis of PTEN/PIK3CA/AKT pathway in oral squamous cell carcinoma. *Oral Oncol* 2011; 47: 946–950.
83. Kozaki K, Imoto I, Pimkhaokham A, et al. PIK3CA mutation is an oncogenic aberration at advanced stages of oral squamous cell carcinoma. *Cancer Sci* 2006; 97: 1351–1358.
84. Zanaruddin SNS, Yee PS, Hor SY, et al. Common oncogenic mutations are infrequent in oral squamous cell carcinoma of Asian origin. *PLoS ONE* 2013; 8: e80229.
85. Ribeiro FAP, Noguti J, Oshima CTF, et al. Effective targeting of the epidermal growth factor receptor (EGFR) for treating oral cancer: a promising approach. *Anticancer Res* 2014; 34: 1547–1552.
86. Scully C. Cancers of the oral mucosa treatment & management, http://emedicine.medscape.com/article/1075729-treatment
87. Mohammad S, Ram H, Gupta PN, et al. Overexpression of COX-2 in oral squamous cell carcinoma patients undergoing chemoradiotherapy. *Natl J Maxillofac Surg* 2011; 2: 17–21.
88. Li D, Hao S-H, Sun Y, et al. Functional polymorphisms in COX-2 gene are correlated with the risk of oral cancer. *Biomed Res Int* 2015; 2015: 580652.
89. Pandey M, Prakash O, Santhi WS, et al. Overexpression of COX-2 gene in oral cancer is independent of stage of disease and degree of differentiation. *Int J Oral Maxillofac Surg* 2008; 37: 379–383.
90. Hussain SR, Ahmad MK, Mahdi AA, et al. Association of interleukin-10 (A1082G) gene polymorphism with oral squamous cell carcinoma in north Indian population. *J Genet* 2016; 95: 249–255.
91. Singh PK, Ahmad MK, Kumar V, et al. Genetic polymorphism of interleukin-10 (-A592C) among oral cancer with squamous cell carcinoma. *Arch Oral Biol* 2017; 77: 18–22.
92. Arduino PG, Menegatti E, Cappello N, et al. Possible role for interleukins as biomarkers for mortality and recurrence in oral cancer. *Int J Biol Markers* 2015; 30: e262–e266.
93. Fujita Y, Okamoto M, Goda H, et al. Prognostic significance of interleukin-8 and CD163-positive cell-infiltration in tumor tissues in patients with oral squamous cell carcinoma. *PLoS ONE* 2014; 9: e110378.
94. Alizadeh AA, Aranda V, Bardelli A, et al. Toward understanding and exploiting tumor heterogeneity. *Nat Med* 2015; 21: 846–853.
95. Sharma V, Michel S, Gaertner V, et al. Fine-mapping of IgE-associated loci 1q23, 5q31, and 12q13 using 1000 Genomes Project data. *Allergy* 2014; 69: 1077–1084.
96. Yang C-H, Lin Y-D, Yen C-Y, et al. A systematic gene-gene and gene-environment interaction analysis of DNA repair genes XRCC1, XRCC2, XRCC3, XRCC4, and oral cancer risk. *OMICS* 2015; 19: 238–247.
97. Mahachie John JM, Van Lishout F and Van Steen K. Model-based multifactor dimensionality reduction to detect epistasis for quantitative traits in the presence of error-free and noisy data. *Eur J Hum Genet* 2011; 19: 696–703.