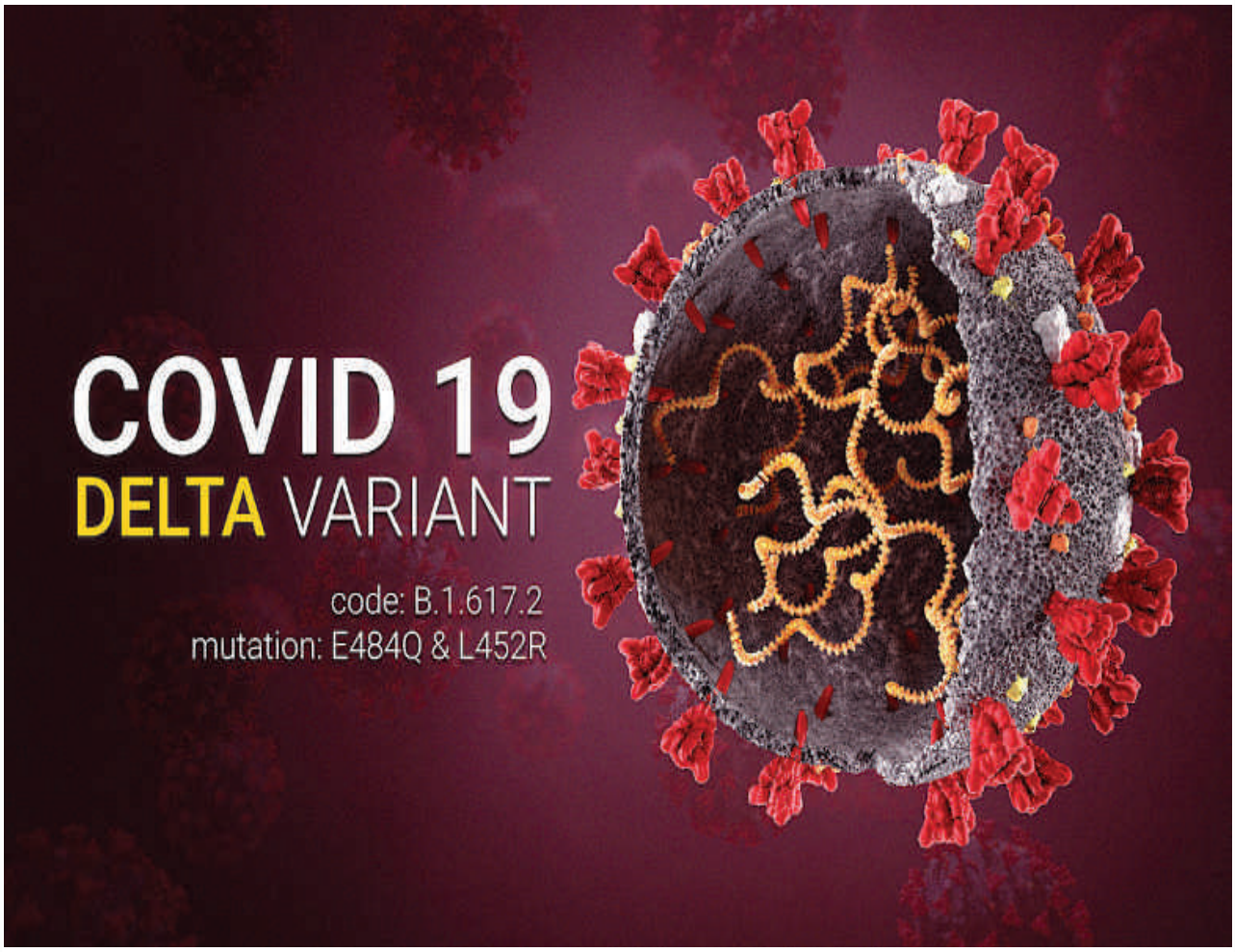
The Gulf Journal of Oncology

Indexed By PubMed and Medline Database

Issue 37, Sep 2021
ISSN No. 2078-2101



The Official Journal of the Gulf Federation For Cancer Control

Table of Contents

Original Articles

Review Article

Case Reports

Conference Highlights/Scientific Contributions

Original Article

Decoding The Genetic Alterations In Cytochrome P450 Family 3 Genes And Its Association With HNSCC

S.Kamala Devi[1], A.Paramasivam[2], A.S.Smiline Girija[1], J. Vijayashree Priyadharsini[2]

[1]Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 600077, India.
[2]Cellular and Molecular Research Centre, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai 600077, India.

Abstract

Introduction: Cytochrome P450 (CYPs) are enzymes belonging to the family of heme–containing proteins, most commonly found in the endoplasmic reticulum and mitochondria. These enzymes catalyze a variety of functions including metabolism of steroids, fatty acids, natural compounds, drugs and carcinogenic chemicals. The inherent association of CYPs with disease conditions have turned the focus into the genetic alterations or variations associated with phenotypes such as drug responsiveness, chemical toxicity and bioconversion of procarcinogens to active carcinogens.

Materials and methods: The present observation study utilizes several computational tools to identify and predict the possible outcomes of gene alterations in *CYP3* family of genes with head and neck squamous cell carcinoma (HNSCC). cBioportal hosts an exhaustive collection of datasets of various cancers which was the primary source of analysis. Oncoprint data obtained was further analysed using tools such as PROVEAN, I–Mutant and gnomAD.

Results: A total of 8 genes of the *CYP3* family were analyzed, among which 4 genes were found to harbour gross abnormalities and variations. The genes *CYP3A4, CYP3A5, CYP3A7, CYP3A43* showed a common pattern of gene amplification in a group of patients. Truncating and missense variants were also identified of which *rs199908125* of *CYP3A4* and *rs768530577* of *CYP3A5* were reported in different populations.

Discussion: The gnomAD analysis revealed a few polymorphic rare variants with minor allele frequency less than 0.01, which could have a putative association with HNSCC. Five out of eight variants identified were found to be deleterious exhibiting decreased protein stability.

Conclusion: Further screening of the genetic abnormalities through experimental validation in different populations are warranted to derive an association between the gene identifiers and disease phenotype.

Keywords: Cytochrome P450; HNSCC; *In silico*, mutations, amplification, deletions

Introduction

Malignant tumors of the upper aerodigestive tract which includes the oral cavity, nasopharynx, oropharynx, hypopharynx and larynx are collectively known as head and neck squamous cell carcinoma (HNSCC)[1]. The GLOBOCAN 2018 statistics on incidence of various cancer types world–wide reported a steep rise in the incidence of head and neck cancer in developing countries including India, Pakistan, Srilanka, Bangladesh and Taiwan. Despite the fact that infection due to human papilloma virus is related to HNC in the United states, a different set of risk factors such as habitual use of tobacco products such as pan, gutka etc., have been attributed to the increasing numbers of cases in the Asian subcontinent[2–4]. The chemicals present in smokeless and smoking tobacco undergoes biotransformation into carcinogenic chemicals by xenobiotic metabolizing enzymes, cytochrome P450s[5]. CYP3A4 enzyme is abundantly placed in liver and intestine and are known to metabolize drugs and xenobiotics. Several CYP3A4 inhibitors such as antibiotics, anti–HIV agents, anti–depressants, calcium channel blockers, steroids, phytocompounds and dietary compounds have been identified[6]. Numerous evolutionary

Corresponding author: Dr. J. Vijayashree Priyadharsini, Research Scientist, Cellular and Molecular Research Centre, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai – 77, India.
E–Mail: vijayashreej.sdc@saveetha.com.
Phone: 9941125984

changes involving gene duplications, deletions, and gene conversions were observed in CYP3A family.. Interestingly, a few of the alterations in CYP3 family have been positively selected in some populations owing to environmental, dietary and other physiological conditions[7]. Human genetic variations have always aided in understanding the inter–individual difference in the susceptibility or resistance to diseases, metabolism of chemicals and xenobiotics etc., In line with the above fact, it is imperative to understand the role of genes involved in the biotransformation of pro–carcinogenic chemicals. Any abnormality in the genes might give rise to alternated gene expression profile of the proteins encoded. Alternatively, variations in the genes might interfere with the functional consequences of cells thereby exerting a pathogenic effect. Computational approach has aided in screening of a large number of samples from different ethnic groups and populations, thereby opening new avenues into the search for biomarkers which can be associated with disease phenotype. The present study is one such attempt to identify potential or putative drivers involved in the pathogenesis of HNSCC by employing an *in silico* approach.

Materials and methods

Sample data set: The cBioPortal for Cancer Genomics (http://cbioportal.org) hosts several datasets [8,9]. The sample data set includes 528 HNSCC cases (530 samples) of which 504 samples harboured information about copy number variations and sequence data. The demographic details of cases in the head and neck squamous cell carcinoma (TCGA, Firehose Legacy) dataset were recorded (Table 1).

Oncoprint data analysis: Submission of user defined query of 8 genes belonging to the cytochrome P450 family 3 returned a window with OncoPrint data which demonstrated the presence of alterations in 4 genes *CYP3A4, CYP3A5, CYP3A7, CYP3A43* and no alterations in other genes *viz., CYP3A51P, CYP3A52P, CYP3A54P, CYP3A137P.* The somatic mutation frequency and the site of mutation in the candidate genes were documented [8,9] (Table 2).

gnomAD analysis: The genome aggregation database (gnomAD) hosts a large collection of data spanning 125,748 exome sequences and 15,708 whole genome sequences from unrelated individuals sequenced, deposited as part of various disease–specific or population genetic studies. This data source was used to verify whether the variants identified in the present study are reported elsewhere in the other populations [10] (Table 2).

Protein stability analysis: I–Mutant server was used for prediction of protein stability changes upon single nucleotide mutations leading to change in the amino acid being encoded by the triplet codon. The prediction was based on running the query with protein sequence downloaded in the FASTA format from the public domain (https://www.ncbi.nlm.nih.gov/protein/). The stability changes were further assessed using the free energy stability change ($\Delta\Delta G$) value. A value < 0 and > 0 implies decrease and increase in protein stability respectively [11] (Table 3).

Pathogenicity analysis: PROVEAN (Protein Variation Effect Analyzer) predicts the impact on the biological function of a protein upon substitution with an amino acid. The results returned scores based on amino acid substitutions and classified them as either neutral or deleterious depending on the PROVEAN scores[12]. A score less than –2.5 or greater than –2.5 was considered to be deleterious and neutral respectively (Table 3).

Results

Demographic data: The dataset (TCGA, Firehose Legacy) included in the present study had information on 528 HNSCC patients (530 samples). The male:female ratio was found to be 2.7:1, with age group ranging from 19 – 90 years. The number of individuals with the history of smoking and alcohol was roughly around 98% and 67%. The dataset had samples from patients of American (85.6%), African (9.1%), Asian (2.1%) and American

Gender	Male (n = 386) Female (n = 142)
Mutation count	6–3181
Diagnosis age	19–90 years
Smoking status	Smokers: 515 Data not available: 12 Unknown: 1
Alcohol history	Yes – 352 No – 165 Data not available: 11
Neoplasm Histologic grade	Grade 1: 63 Grade 2: 311 Grade 3: 125 Grade 4: 7 Grade GX: 18 Data not available: 4
Race category	White: 452 African: 48 Asian: 11 American Indian or Alaska native: 2 Data not available: 15

Table 1: Demographic details of patients analyzed in the present study (as obtained from the cBioportal site)

Gene	Protein	Alteration	Loci	% of alteration	Variant allele frequency in tumor sample	gnomAD frequency data
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	Amplification E262K L449I M89I Q352H	7q22.1	6	 0.33 0.30 0.03 0.23	 rs199908125 Novel Novel Novel
CYP3A5	Cytochrome P450 family 3 subfamily A member 5	Amplification R162W	7q22.1	6	 0.05	 rs768530577
CYP3A7	Cytochrome P450 family 3 subfamily A member 7	Amplification E417K	7q22.1	6	 0.17	 Novel
CYP3A43	Cytochrome P450 family 3 subfamily A member 43	Amplification G391R G153A X418_splice	7q22.1	6	 0.19 0.15 0.33	 Novel Novel Novel

Table 2: Genetic alterations in Cytochrome P450 family 3 genes in HNSCC patients

Gene	Variation	I–Mutant	Score	PROVEAN Score	Prediction
CYP3A4	E262K L449I M89I Q352H	Decrease Decrease Decrease Decrease	–1.80 –1.06 –0.01 –1.50	1.355 –1.425 –0.893 –3.747	Neutral Neutral Neutral Deleterious
CYP3A5	R162W	Decrease	–0.41	–4.005	Deleterious
CYP3A7	E417K	Decrease	–1.68	–3.651	Deleterious
CYP3A43	G391R G153A	Decrease Increase	–1.23 0.10	–6.934 –3.978	Deleterious Deleterious

Table 3: Protein stability analysis and pathogenicity of predicted missense mutations in Cytochrome P450 family 3 genes in HNSCC patients

Indian (0.4%) decent. The distribution of patients based on the histologic grade of neoplasm is given in Table 1, of which 59% of patients had grade 2 tumor.

Oncoprint data analysis: The oncoprint data analysis revealed gene amplification in 4 genes, with the frequency of about 6% (Table 2). An interesting finding of the present study is that gene amplification (6%) of *CYP3A4, CYP3A5, CYP3A7, CYP3A43* were observed in the same set of 27 patients. Apart from this observation several truncating and mis–sense variants of unknown significance have also been documented (Figure 1;Table 2). The genetic loci of the genes identified with alterations falls in the chromosome 7q22.1. A splice site mutation in the *CYP3A43* gene has also been identified (Figure 2).

gnomAD analysis: The analysis revealed 2 reported variants *rs199908125 (CYP3A4)* and *rs768530577 (CYP3A5)* genes with a minor allele frequency of less than 0.01, which implies the fact that they are rare variants which could have a putative association with disease phenotype. Several novel non–synonymous and truncating mutations were also identified (Table 2).

Protein stability and pathogenicity analysis: The protein stability analysis showed the missense variant G153A of *CYP3A43* exhibiting increased stability of protein, whereas the other variants showed decrease in stability. Pathogenicity analysis employing PROVEAN demonstrated three neutral variants and five other variants producing deleterious consequences *viz.,* Q352H of *CYP3A4*, R162W of CYP3A5, E417K of *CYP3A7*, G391R and G153A of *CYP3A43* (Table 3).

Discussion

CYP3 family of proteins represent a large group of drug metabolising enzymes which is abundantly expressed in liver

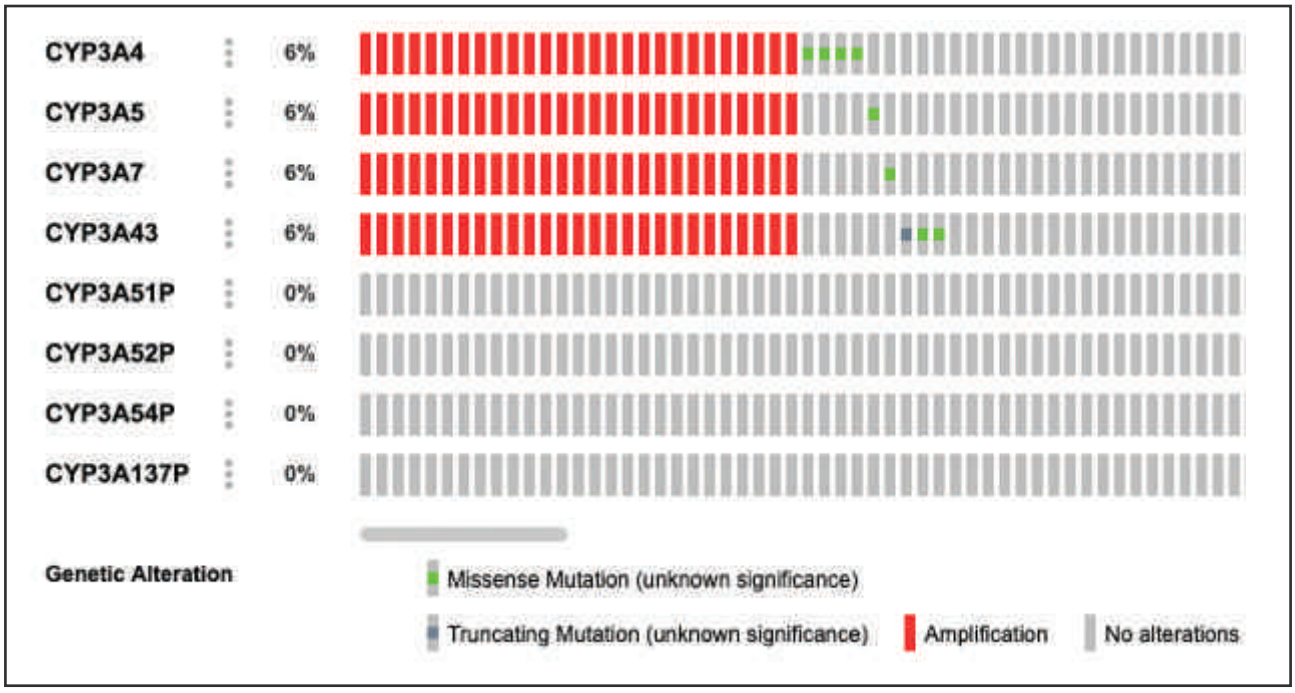


Figure 1: The Oncoprint data depicts the gene alterations in CYP3 family of genes. Each of the grey bar represents HNSC patients.

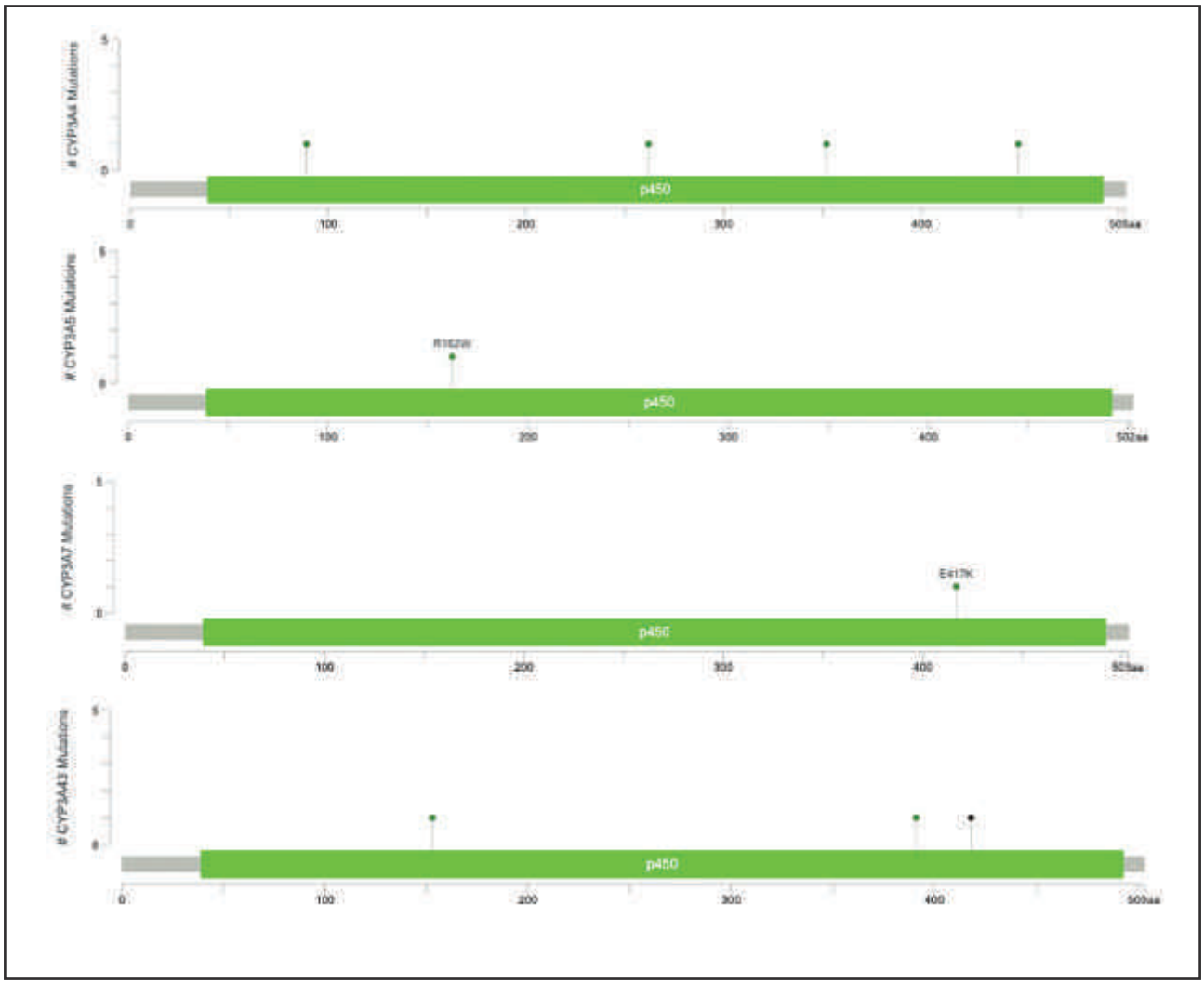


Figure 2: Variants identified in four genes viz., CYP3A4, CYP3A5, CYP3A7 and CYP3A43, of the CYP3 gene family.

and other extra–hepatic tissues such as prostate, breast, colon, small intestine, brain and gut, with the expression of *CYP3A4* being predominately higher than the other members [13]. *CYP3A7* is the main form of the enzyme expressed in foetal liver[14] and *CYP3A43* is the least expressed[15]. Although the role of gene alteration and expression is widely studied in different types of cancer, its role and association with HNSCC or oral cancer remains less understood. The expression of *CYP3A4* was found to be higher, which was also related to metastasis in Ewing's sarcoma[16]. Different variants of *CYP3A4* increased the expression to a higher level which was found to be associated with neuroblastoma mortality. Zhou et al., presented an extensive Human Genome Epidemiology (HuGE) review and meta–analysis about the *CYP3A4*1B* polymorphism with cancer risk. The frequency of alleles related to the polymorphism showed dramatic variation in different populations grabbing attention of researchers to assess the functional role of the variant in association with several types of cancer. The meta–analysis revealed the G allele and the GG genotype to be the risk allele and genotype respectively, which could increase the risk of cancer in African population[17]. A recent study also assessed the consequences of variants in *CYP3A4* gene which returned interesting results related to variable allele frequencies in different populations and their clinical relevance. The present study also identified four variants in *CYP3A4* gene, of which E262K *(rs199908125)* was a reported variant. This variant showed the highest frequency in Latino groups followed by European and African population. The identification of causal variants and their functional association with disease phenotype could aid in developing mutation panels for a specific disease condition in designated population [18].

Genetic variations in *CYP3A5* have been known to induce changes in the catalytic property of enzymes which leads to inter–individual variations in drug metabolism and susceptibility or resistance to debilitating diseases such as cancer. The most common polymorphism identified in the gene CYP3A5*3; rs776746 A>G was shown to play a vital role in the cancer development. A pooled meta–analysis by Wang et al., revealed that the risk of colorectal, acute and chronic leukemia increases in the presence of the variant allele [19]. The homozygous mutant form of *CYP3A5 *3/*3* was found to increase the mortality rate of neuroblastoma by about 4.3 folds (20). Inter–individual variations identified in *CYP3A7* gene has been widely studied in association with neuroblastoma and other cancers types. The genetic variation in *CYP3A7*1C* and up–regulation of *CYP3A7* was associated with adverse reactions in chronic lymphocytic leukemia (CLL), breast and lung cancer [21]. Although, not many reports confined to the functionality of *CYP3A43* gene is available, the present study identified genetic alterations in the form of amplifications, mis–sense and truncating variants, with the most interesting finding being a common type of amplification present in all four major genes of the CYP3 family identified in a group of patients (Figure 1). Siemes et al., reported that heterozygous carriers with G–allele of *CYP3A43* gene exhibited a five–fold increase in mortality with early onset prostate cancer [22]. Since, the risk of oral cancer is amplified by the habitual use of tobacco and related products, the study gains importance through identification of potential variations and genetic aberration in gene encoding xenobiotic metabolising enzymes which could have a crucial role in the disease pathogenesis. *In silico* analysis is considered to be a boon for the researchers wherein an exhaustive collection of data can be analyzed to identify possible associations with a disease phenotype [23–28].

Despite the fact that computational approach provides a comprehensive view of genetic alterations in the selected gene panel, there exists certain limitations which has to be addressed. Some of the limitations were (a) the dataset selected had individuals from different ethnic groups or populations, which made data analysis and interpretation to be more generic. Each of the individuals might have been exposed to different group of chemical carcinogens or pollutants based on the geographical locations hence a precise association could not be derived in such situations, (b) since both genetic and epigenetic factors act simultaneously to exhibit a disease phenotype, investigations on epigenetic factors have to be considered so as to provide a clear picture on trans–generational effects of the persistent chemicals on human tissues. However, with all the limitations addressed, the present study has taken a step forward to address the genetic abnormalities and variations in the "CYP3 gene family" which could be associated with malignant transformation of cells. The present study identified a panel of altered genes of the cytochrome P450 family 3 exhibiting putative association with HNSCC. Further experimental validation is warranted to provide substantial evidences on the functional consequences of the alterations observed.

Acknowledgements

The authors thank the patients who were included in the TCGA study and the developers of cBioportal and UALCAN databases without whom such kind of analysis would have been out of reach for budding researchers.

Funding

None

Conflicts of interest

The authors declare that they have no conflict of interest.

References

1. Gregoire V, Lefebvre JL, Licitra L, Felip E; On behalf of the EHNS–ESMO–ESTRO Guidelines Working Group. Squamous cell carcinoma of the head and neck: EHNS–ESMO–ESTRO Clinical Practice Guidelines for diagnosis, treatment and follow–up. Ann Oncol 2010;21:v184–6.
2. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26:123–41.
3. Krishna Rao SV, Mejia G, Roberts–Thomson K, Logan R. Epidemiology of oral cancer in Asia in the past decade–An update (2000–2012). Asian Pac J Cancer Prev 2013;14:5567–77.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortalityworldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68(6):394–424.
5. Nebert, D., Dalton, T. The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. Nat Rev Cancer 6, 947–960 (2006).
6. Zhou SF. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. Curr Drug Metab 2008;9(4):310-322.
7. Qiu H, Taudien S, Herlyn H, et al. CYP3 phylogenomics: evidence for positive selection of CYP3A4 and CYP3A7. Pharmacogenet Genomics 2008;18(1):53-66.
8. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data (published correction appears in Cancer Discov.2012;2(5):401-404.
9. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):pl1.
10. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL et al., The mutational constraint spectrum quantified from variation in 141,456 humans. 2020; bioRxiv 531210; doi: https://doi.org/10.1101/531210
11. Capriotti E, Fariselli P, Casadio R. I–Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res 2005;33(Web Server issue):W306–10.
12. Choi Y, Chan AP (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 31(16): 2745–2747.
13. Basheer L, Kerem Z. Interactions between CYP3A4 and Dietary Polyphenols. Oxid Med Cell Longev. 2015;2015:854015.
14. Neunzig I, Drăgan CA, Widjaja M, et al. Whole–cell biotransformation assay for investigation of the human drug metabolizing enzyme CYP3A7. Biochim Biophys Acta. 2011;1814(1):161–167.
15. Daly AK. Significance of the minor cytochrome P450 3A isoforms. Clin Pharmacokinet. 2006;45(1):13–31.
16. Zia H, Murray GI, Vyhlidal CA, et al. CYP3A isoforms in Ewing's sarcoma tumours: an immunohistochemical study with clinical correlation. Int J Exp Pathol. 2015;96(2):81–86.
17. Zhou LP, Yao F, Luan H, et al. CYP3A4*1B polymorphism and cancer risk: a HuGE review and meta–analysis. Tumour Biol. 2013;34(2):649-660.
18. Guttman Y, Nudel A, Kerem Z. Polymorphism in Cytochrome P450 3A4 Is Ethnicity Related. Frontiers in Genetics. 2019 ;10:224.
19. Wang BS, Liu Z, Xu WX, Sun SL. CYP3A5*3 polymorphism and cancer risk: a meta–analysis and meta–regression. Tumour Biol. 2013;34(4):2357-2366.
20. Darwish MH, Farah RA, Farhat GN, et al. Association of CYP3A4/5 genotypes and expression with the survival of patients with neuroblastoma. Mol Med Rep. 2015;11(2):1462–1468.
21. Johnson N, De Ieso P, Migliorini G, et al. Cytochrome P450 allele CYP3A7*1C associates with adverse outcomes in chronic lymphocytic leukemia, breast, and lung cancer. Cancer Res. 2016;76(6):1485–1493.
22. Siemes C, Visser LE, de Jong FH, et al. Cytochrome P450 3A gene variation, steroid hormone serum levels and prostate cancer––The Rotterdam Study. Steroids. 2010;75(12):1024–1032.
23. Vijayashree P, Paramasivam A. Virtual screening of mutations in antioxidant genes and its putative association with HNSCC: An in silico approach. Mutat Res Fund Res Fund Mol M. 2020: 111710 (Epub ahead of print).
24. Anita R, Paramasivam A, Vijayashree Priyadharsini J, et al. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. Am J Cancer Res. (2020)10: 2546–2554.
25. Aparna J, Smiline–Girija AS, Paramasivam A, Vijayashree–Priyadharsini J. Deciphering the genetic alterations in matrix metallo–proteinase gene family and its putative association with head and neck squamous cell carcinoma. Mol Biol Res Commun. 2021;10(1):13–22.
26. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. Environ Sci Pollut Res Int. 2021;28(21):27162–27173.
27. Jaikumarr Ram A, Girija As S, Jayaseelan VP, Arumugam P. Overexpression of BASP1 Indicates a Poor Prognosis in Head and Neck Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2020;21(11):3435–3439.
28. Fathima T, Arumugam P, Girija As S, Priyadharsini JV. Decoding the Genetic Alterations in Genes of DNMT Family (DNA Methyl–Transferase) and their Association with Head and Neck Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2020 Dec 1;21(12):3605–3612.