Section A -Research paper

Genotyping Analysis of APE1 (Asp148Glu) polymorphism: Triggering Factor For Squamous Cell Head and Neck Cancer

[1]Garima Avasthi, [2]Tridiv Katiyar, [1]Amresh Gupta, [2]Devendra Parmar

[1]Goel Institute of Pharmacy and Sciences, Faizabad Road, Lucknow-226028,U.P.,India.

[2]System Toxicology & Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Vishvigyan Bhawan, Mahatma Gandhi Marg, Lucknow-226001, Uttar Pradesh, India.

DOI:10.48047/ecb/2023.12.si4.724

Abstract

Polymorphisms in DNA repair genes may be associated with differences in DNA repair capacity, thereby influencing the individual susceptibility to smoking-related cancer. We investigated the association of 10 nucleotide-excision repair gene polymorphisms (APE1 Asp148Glu;) with head and neck cancer risk in North Indian poulations. Genotypes were determined by PCR-RFLP and PCR-single base extension assays in 135 head and Neck cancer patients and 150 age- and sex-matched controls, and the results were analyzed using logistic regression adjusted for relevant covariates. A significant association between the APE1 Asp148Glu polymorphism and head and neck cancer risk was found, with adjusted odds ratios (OR) of 3.38 (p=0.001) for the Asp/Glu genotype and 2.39 (p=0.038) for the Glu/Glu genotype.

Introduction

A plethora of genetic events leading to the inactivation of tumour-suppressor genes or activation of proto-oncogenes, or both, govern the development of SCCHN. Molecular techniques can identify genetic and epigenetic alterations in premalignant and invasive lesions, and allow the delineation of a hypothetical progression model for SCCHN carcinogenesis.

The onset of HNC depends on an interplay between genetic factors, such as Fanconi anemia (FA), Bloom syndrome and xeroderma pigmentosum, and environmental factors, such as age, geographic region and various lifestyle contributors. A major risk lifestyle factor is tobacco smoking, while smoking electronic cigarettes, despite being considered less harmful than conventional cigarettes, has also been associated with the development of HNSCC due to their high content of substances that can cause oral mucosal lesions . Another major lifestyle risk factor is alcohol consumption, even in individuals who have never been smokers, while combined use of alcohol and tobacco has been shown to have a synergistic effect on tumor development . Notably, a significant association, albeit to a lesser extent compared to smoking and alcohol consumption, has also been observed between Human papillomavirus (HPV) infection and the development of various HNCs, especially oropharyngeal (OPC), nasopharyngeal (NPC), oral cavity (OC), and larynx cancers. To date, thirteen types of HPV have been characterized as carcinogenic by the International Agency for Research on Cancer, with the HPV16 subtype being regarded as the most potent contributor to the development of HNC.

Detection of APE1 (Asp148Glu) polymorphism

Asp148Glu polymorphic variant of APE1, present in exon-5 region of the gene which was identified by the new restriction fragment length polymorphism (RFLP). The primer sequences used for identifying Asp148Glu (rs3136820) polymorphism is as follows- FP: 5'-TGCATTAGGTACATATGCTGTT-3'and RP: 5'-GCGAGACCCTGTCCCTAA-3'. The reaction mixture in 50µl contained 1X buffer (10 mMTris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 25mM KCl), 200 mM of each nucleotides. 1 unit of Tag polymerase (MBI Fermentas, Gennany), 100 ng of genomic DNA and sterile milliQ water. Amplification was performed on GeneAmp 9700 PCR thermal cycler using the following protocol: 94°C for 5 minutes for initial denaturation followed by 35 cycles of 94°C for 30 seconds, 56°C for 45 seconds and 72°C for 45 seconds and a final elongation step of 72°C for 10 minutes. PCR reaction resulted in a 360bpPCR product. 10µlof the PCR product was digested with 1 U of BtgZI (New England Biolabs, US) in a final volume of 15 µl. This reaction mixture was incubated at 60°C for one hour. Electrophoresis was carried out in a 3.5 % agarose gel containing ethidium bromide and analyzed on VERSA DOC Imaging System (Model 1000, Bio-Rad). Primers designed to amplify BtgZI containing region of the APE1 gene produce a band of 360bp corresponding to the homozygous mutant genotypes, heterozygous genotypes produced 147bp, 213bp & 360bp PCR products, while homozygous wild genotypes gave bands of 147bp, 213bpafter BtgZIdigestion.

Isolation of Genomic DNA: The quantity of the Genomic DNA was estimated by making appropriate dilutions to determine the optical density (OD) at 260nm and 280nm. 5µl of the stock DNA was electrophoresed on a 0.8% agarose gel and stained with ethidium bromide to determine the integrity of the genomic DNA. Isolated DNA from the blood samples of both control and patients were used for identifying the role of DNA repair gene in case-control study.

PCR-RFLP Analysis of APE1 gene forAsp148Glu (rs3136820 or BtgZI) polymorphic site:

Digestion of PCR product with BtgZI to identify Asp148Glu (rs3136820 or BtgZI)polymorphism, produced a fragment size of 147bp and 213bp for homozygous wild allele and an undigested 360bp fragment for homozygous mutant allele. In heterozygous genotype, all the three bands corresponding to 302bp, 201bp, 101bp were visible (Figure 1.1).

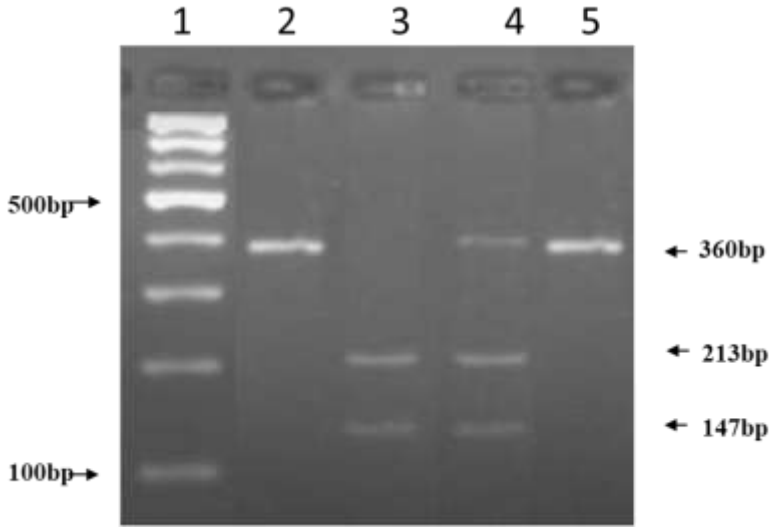

Figure 1.1: Genotyping of Asp148Glu polymorphism of APE1 gene by RFLP (lane 1: 100bp DNA Ladder, lane 2: homozygous mutant genotypes, lane 3: homozygouswild genotype, lane 4: heterozygous mutant genotype, lane 5: PCR product).

SNPs	Genotypes	Controls (n=150)	Cases (n=135)	OR (95%CI)	*P* Value
APE1					
Asp148Glu	Asp/Asp	98 (49.00)	83 (44.87)	1 (Ref.)	
	Asp/Glu	91 (45.50)	85 (45.95)	1.10 (0.72-1.67)	0.64
	Glu/Glu	11 (5.50)	17 (9.18)	1.82 (0.80-4.11)	0.14
	Asp/Glu+Glu/Glu	102 (51.00)	102 (55.13)	1.18(0.79-1.76)	0.41

Among the controls, the genotype frequencies were in Hardy-Weinberg equilibrium. In comparison to the controls (45.50%), the frequency of the Asp148Glu heterozygous genotype was found to be similar in the case (45.95%). In comparison to the controls (5.50%), the frequency of the homozygous mutant genotype was higher in the case (9.18%). The heterozygous genotype's crude OR was 1.10 (95% CI: 0.72-1.67), whereas the crude OR for the homozygous mutant genotype was 1.82 (95% CI: 0.80-4.11), indicating that the Asp148Glu polymorphism was not substantially related with an increased risk of HNSCC in either genotype. However, the incidence of HNSCC was not significantly (OR: 1.18) increased by the combination of both variant (heterozygous plus mutant) Asp148Glu gene types. The risk (7.15-folds) increased in patients with variant genotype of Asp148Glu when compared to non-smokers among controls with wild type genotype. Tobacco smoking also significantly increased the HNSCC risk among the cases.

The present work demonstrated that the presence of genetic variation in DNA repair gene *APE1*, particularly involved in the DNA damage and repair process were significantly associated with

increased risk to HNSCC. Data also explained the cases carrying the variant genotypes ofAsp148Glu, with environmental risk factors (tobacco smoking, chewing & alcohol uses) increases the HNSCC risk to several folds (2-10 folds). 55% patients responded to the treatment while 45% patients were non-responders.

References

1. Bell DA, Badawi AF, Lang NP, Ilett KF, Kadlubar FF, Hirvonen A. Polymorphism in the N-acetyltransferase 1 (NAT1) polyadenylation signal: association of NAT1* 10 allele with higher N-acetylation activity in bladder and colon tissue. Cancer research. 1995 Nov 15;55(22):5226-9.
2. Benhamou S, Sarasin A. ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis. 2002 Nov 1;17(6):463-9.
3. Benhamou S, Tuimala J, Bouchardy C, Dayer P, Sarasin A, Hirvonen A. DNA repair gene XRCC2 and XRCC3 polymorphisms and susceptibility to cancers of the upper aerodigestive tract. International journal of cancer. 2004 Dec 10;112(5):901-4.
4. Berg EL. Phenotypic chemical biology for predicting safety and efficacy. Drug Discovery Today: Technologies. 2017 Mar 1;23:53-60.
5. Black AJ, McLeod HL, Capell HA, Powrie RH, Matowe LK, Pritchard SC, Collie-Duguid ES, Reid DM. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. Annals of internal medicine. 1998 Nov 1;129(9):716-8.
6. Blitzer GC, Smith MA, Harris SL, Kimple RJ. Review of the clinical and biologic aspects of human papillomavirus-positive squamous cell carcinomas of the head and neck. International Journal of Radiation Oncology* Biology* Physics. 2014 Mar 15;88(4):761-70.
7. Boukouvala S, Sim E. Structural analysis of the genes for human arylamine N-acetyltransferases and characterisation of alternative transcripts. Basic & clinical pharmacology & toxicology. 2005 May;96(5):343-51.