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An unlikely role for the NAT2 genotypes and haplotypes in the oral cancer of south Indians

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ABSTRACT

The arylamine N-acetyltransferase 2 (NAT2) enzyme detoxifies a wide spectrum of naturally occurring xenobiotics including carcinogens and drugs. Acetylation catalysed by the *NAT2* is an important process in metabolic activation of arylamines to electrophilic intermediates that initiate carcinogenesis. Polymorphism in N-acetyltransferase 2 gene was reported to be associated with the susceptibility of various cancers.

Objective: The aim of our study was to determine whether there is any association between the susceptibility to oral cancer amongst the variations of NAT2 genotypes.

Design: This study was carried out in 157 patients with oral cancer. The control group consisted of 132 healthy volunteers. The most common polymorphisms rs1799929, rs1799930 and rs1799931 on the *NAT2* gene were screened for the genotypes using TaqMan allelic discrimination.

Results: All the three SNPs were polymorphic with minor allele frequency of 0.339, 0.372 and 0.061 for rs1799929, rs1799930 and rs1799931, respectively. None of the polymorphic site deviated from HWE in controls. There were no significant differences in genotype or allele frequencies of three SNPs between controls and cases with oral cancer. Risk of oral cancer development for the carriers of the individual deduced phenotypes was also not statistically significant. Of the 3 studied polymorphisms, 2 were in strong LD and form one haplotype block. None of the haplotype had shown significant association with the oral cancer.

Conclusions: Our study concludes that the NAT2 genotypes, phenotypes and haplotypes are not involved in the susceptibility to oral cancer in South Indian subjects.

1. Introduction

Oropharyngeal cancer is more common in developing countries than in developed countries [1]. Oral Cancer is the third most common cancer in India after Cervical and Breast Cancer amongst women [2]. In India, the age standardised incidence rate of oral cancer is reported at 12.6 per 100,000 people. The accessibility and visibility of the oral cavity to the patient and clinician makes the diagnosis of oral squamous cell carcinoma (OSCC) relatively straight forward [3]. The use of tobacco and alcohol are the most common risk factors for the development of oral cavity cancer. The development of cancer is a multistep process which involves accumulation of DNA alterations, resulting in neoplastic transformation and uncontrolled growth. Several genetic polymorphisms of the genes that involved in xenobiotic metabolism, DNA repair, hormone metabolism, immune system regulation and development, apoptosis and cell cycle control may play an important role in carcinogenesis process [4–6].

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The N-acetyltransferases (NAT; E.C.2.3.1.5) are xenobiotic-metabolising enzymes (XME) that involved in the metabolism of drugs, environmental toxins and aromatic amine carcinogens present in cigarette smoke. N-Acetyltransferases catalyse the transfer of an acetyl group from acetylCoA (Ac-CoA) to the nitrogen or oxygen atom of arylamines, hydrazines, and their N-hydroxylated metabolites [7]. *NAT2* gene (MIM # 243400) codes for the NAT2 proteins that have variable enzymatic activity or stability, leading to slow or rapid acetylation [8,9]. The human *NAT2* gene spans 9.9 kb and is located on chromosome 8p22. *NAT2* consists of a non-coding exon at the 5′ end separated by a 9 kb intron from an uninterrupted coding region of 873 bp that encodes a 290 amino acid protein. The *NAT2* gene is polymorphic and 36 alleles have been described till date (http://louisville.edu/medschool/pharmacology/NAT.html). Several of the *NAT2** alleles share sequence variations, and not all sequence variations would lead to change in the enzyme activity of the encoding protein. Early genotyping studies screened for the presence of the $C^{481}T$, the $G^{590}A$, the $G^{857}A$ and sometimes the $G^{191}A$ nucleotide changes, all of which were shown to cause a slow acetylation phenotype [10]. A threefold decrease in clearance was reported between fast acetylators and slow acetylators [11]. The frequency of the slow acetylator phenotype varies considerably amongst ethnic groups [12], and this is due to the different frequencies of the polymorphisms that correspond to the slow acetylator alleles. In Caucasian and African populations, the frequency of the slow acetylation phenotype varies between 40 and 70%, whilst in Asian populations, such as Japanese, Chinese, Korean, and Thai, it ranges from 10 to 30% [13]. The present study was aimed to investigate association between oral cancer and three sequence variations, which were reported to result in impaired acetylation.

2. Materials and methods

2.1. Subjects

The study group consisted of 157 oral cancer patients (all were confirmed by histopathology to be squamous cell carcinoma) and 132 controls. For cases and controls, the information regarding age, gender, occupation and details about duration, frequency, nature of tobacco habit (smoking or smokeless) and alcohol consumption were noted through a detailed questionnaire. The diagnosis of oral cancer patients was confirmed histopathologically in the Kanchipuram cancer hospital between the years 2006 and 2009. Genetically unrelated healthy individuals, who had no personal history of cancer of any organ, were recruited from the Sri Ramachandra hospital as the control subjects. Sample sizes for this study were calculated using power and sample size calculation program software (version 2.1.31). Previous studies indicated that the probability of slow acetylators amongst controls is 0.52. Based on a power analysis, 133 oral cancer and 133 controls are large enough to detect a significant odds ratio of 0.5, with a power of 80% and an alpha of 5%. All the patients participated in the study had given informed written consent prior to the study. This study was approved by Ethics Committee of Sri Ramachandra University, Chennai, and Department of Health and Family welfare, Government of Tamil Nadu state, India.

2.2. Genotyping

Three millilitres of blood sample were collected from all the participants. Genomic DNA from blood samples was extracted using the published protocol [14]. Three SNPs of the *NAT2* gene [c.481C>T (p.L161L, dbSNP rs1799929), c.590G>A (p.R197Q, dbSNP rs1799930) and c.857G>A (p.G286E, dbSNP rs1799931), were genotyped. The primers and probes for all the SNPs (Table 1) used in this study were purchased from Applied Biosystems, Foster City, CA, USA. Each reaction contained 2.5 μL TaqMan Universal PCR Master Mix, 0.125 μL TaqMan SNP Genotyping Assay, 1.375 μL distilled water and 1 μL DNA (10 ng/μL), with a final reaction volume of 5 μL. For each SNP, a positive control for wild type, heterozygote and variant genotype was provided. The plate also contained at least two no template controls without any DNA. Before analysing the DNA, a pilot test was conducted to confirm the accuracy of the assay. After a successful pilot test, sample analysis was carried out in 384-well optical reaction microplates (Applied

Table 1 – Primers and probes used for genotyping *NAT2* gene polymorphisms.

Gene/polymorphism	Primers/probe	Sequence
NAT2/rs1799929	Forward	CTGCTTGACAGAAGAGAGAGGAATC
	Reverse	AGAAATTCTTTGTTTGTAATATACTGCTCTCTCC
	Probe 1 (VIC)[a]	TGATTTGGTCCA**G**GTACCA[b]
	Probe 2 (FAM)	TGATTTGGTCCA**A**GTACCA
NAT2/rs1799930	Forward	CCTGCCAAAGAAGAAACACCAAAA
	Reverse	GAGACGTCTGCAGGTATGTATTCAT
	Probe 1 (VIC)	CTTGAACCTC**A**AACAAT
	Probe 2 (FAM)	TTGAACCTC**G**AACAAT
NAT2/rs1799931	Forward	GGAGAAATCTCGTGCCCAAAC
	Reverse	GGGTGATACATACACAAGGGTTTATTTTG
	Probe 1 (VIC)	CTGGTGATG**A**ATCCCTT
	Probe 2 (FAM)	TGGTGATG**G**ATCCCTT

[a] Probes corresponding to different alleles were labelled with VIC and FAM fluorescent dyes (Applied Biosystems).
[b] Polymorphic bases are underlined.