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Influence of *CYP1A1*, *CYP2E1*, *GSTM3* and *NAT2* genetic polymorphisms in oral cancer susceptibility: Results from a case-control study in Rio de Janeiro

Christiane F.S. Marques [a], Sérgio Koifman [b], Rosalina J. Koifman [b], Paolo Boffetta [c], Paul Brennan [c], Ana Hatagima [a,*]

[a] *Genetics Department, Laboratório de Genética Humana—IOC—FIOCRUZ, Pavilhão Leônidas Deane—6° Andar —Sala 615, Avenida Brazil, 4365 Manguinhos, Rio de Janeiro, RJ 21045-900, Brazil*
[b] *Department of Epidemiology and Quantitative Methods in Health, Escola Nacional de Saúde Pública, Rio de Janeiro, 21041-210, Brazil*
[c] *International Agency for Research on Cancer, Lyon 69372, France*

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Summary Xenobiotic metabolizing enzymes are involved in the detoxification of many carcinogens and may be important in modulating cancer susceptibility. CYP1A1, CYP2E1, GSTM3, and NAT2 polymorphisms were determined in peripheral blood DNA of 231 oral cancer patients and 212 hospital controls in Rio de Janeiro, Brazil, using the PCR–RFLP technique. NAT2 polymorphism distribution was different between cases and controls ($P = 0.035$), with an overrepresentation of *NAT2*11* mutant allele in controls. Risk analysis showed that *NAT2 4/4* individuals (OR = 1.95, 95% CI = 1.05–3.60) and combined GSTM3 and NAT2 heterozygotes (OR = 1.94, 95% CI = 1.04–3.66) were at increased oral cancer risk. No statistically significant association was observed for CYP1A1 and CYP2E1 polymorphisms. Our results suggest that NAT2 polymorphism, alone or combined with GSTM3, may modulate susceptibility to oral cancer in Rio de Janeiro.

* Corresponding author. Tel.: +55 21 3865 8213; fax: +55 21 2260 4282.
E-mail addresses: chrismarques@oi.com.br (C.F.S. Marques), anah@ioc.fiocruz.br (A. Hatagima).

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Introduction

Oral cancer is a serious public health problem, with over 200,000 new cases reported annually worldwide, two-thirds of which occur in developing countries, and with an overall 5-year mortality rate of approximately 50%.[1] In Brazil, it is the seventh most incident cancer type, with over 11,000 new cases in 2002.[2] Molecular epidemiology has shown strong evidence for the contribution of both environmental and genetic risk factors in human cancers.[3] Tobacco and alcohol consumption are major environmental risk factors for oral cancer, and a dose–effect relationship has been observed.[2,4] Several common genetic polymorphisms may modify the multi-step carcinogenic process, including the genes involved in xenobiotic biotransformation, DNA repair, hormone metabolism, immune system regulation and development, apoptosis, and cell cycle control.[5,6] Biotransformation processes include detoxification pathways, but sometimes the reactive intermediates can bind to DNA or other macromolecules,[7] leading to cellular and genetic damage, initiating carcinogenesis.[8] Genetic polymorphisms in xenobiotic metabolizing enzymes (XMEs) may contribute to the individual variability in the metabolism of chemical substances which have been associated with cancer.[9] The higher activity of phase I enzymes, such as the ones coded by the cytochrome P450 (CYP) family, and/or lower activity of phase II enzymes, coded by glutathione *S*-transferase (GST) and *N*-acetyltransferase (NAT), may have implications for the metabolism of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons (PAHs), alcohol, and other carcinogenic substances.[5] Despite the biological plausibility, inconsistent associations have been described for polymorphic XMEs and cancer, with controversial results.[10]

In the last decade, considerable progress has been made in understanding the genetic basis of the development of oral cancer as it results from an accumulation of genetic alterations.[6] However, little is known about which genes are involved and how important they are, or which can be used as predictive or informative risk factors for early detection, prognosis, and treatment management. Few studies have been conducted in this area, especially in developing countries.

The identification of genes modulating cancer risk may have several implications, including the possibility of developing chemoprevention programs for highly sensitive individuals, allowing early intervention and the implementation of efficient prevention and treatment strategies. The objectives of this study were to determine the allelic and genotypic frequencies of *CYP1A1*, *CYP2E1*, *GSTM3*, and *NAT2* polymorphisms in a case-control sample from Rio de Janeiro, Brazil, and investigate their role as molecular markers for oral cancer risk.

Subjects and methods

Cases were 15–79 year-old patients with histopathological confirmation, mainly of squamous cell carcinoma, with no previous treatment, diagnosed at the Hospital of the National Cancer Institute (INCA), Rio de Janeiro, between 1999 and 2002. Controls were selected from two public hospitals near the INCA, with non-tumor pathologies (excluding those related to alcohol and tobacco consumption), and were frequency-matched to cases in relation to age, gender, and skin color. Individuals were classified by skin color as Whites (European descendants), Mulattoes (mixed European and African), or Blacks (African-descendants). Peripheral blood samples collected in EDTA Vacutainer® tubes were used for genomic DNA extraction following a standard protocol.[11] All proceedings were approved by the Institute's research ethics committee and followed informed consent by participating individuals.

Genetic polymorphisms were assessed by previously described PCR–RFLP protocols, with minor modifications (Table 1).[12–15] In brief, the amplification of target DNA was achieved by PCR optimized conditions as follows: a final reaction volume of 30 μL was composed of 50–100 ng of DNA, 200 μM of each dTNP, 1 U of Taq DNA polymerase and 1× PCR buffer (Pharmacia™), and 30 pmol of each primer. Negative controls were included in every run, and the success of amplification was confirmed in agarose 1.5% gels stained with ethidium bromide and visualized under ultraviolet (UV) light. Endonuclease digestions were performed similarly, using overnight 37 °C incubation conditions (New England Biolabs™, according to manufacturer's

Table 1 Primer sequences for each studied polymorphism and their respective annealing temperature and genotyping conditions

Gene and target region	Primer sequence (5′–3′)	Annealing temperature (°C)	Amplified product (bp)	Endonuclease	Reference
CYP1A1 Exon 7	F-GTCTCCCTCTGGTTACAGGA R-GAAAGACCTCCCAGCGGTCA	55	171	*Hinc*II	[12]
CYP2E1 5′ upstream non-coding region	F-CCAGTCGAGTCTACATTGTCA R-TTCATTCTGTCTTCTAATGG	53	410	*Pst*I	[13]
GSTM3 Intron 6	F-CCTCAGTACTTGGAAGAGCT R-CACATGAAAGCCTTCAGGTT	58	273/270	*Mnl*I	[14]
NAT2 Coding region	F-CCTCAGTACTTGGAAGAGCT R-CACATGAAAGCCTTCAGGTT	57	1093	*Kpn*I	[15]

F: forward sequence.
R: reverse sequence.