

Original Article

ASSOCIATION BETWEEN GENETIC POLYMORPHISMS OF HUMAN CYTOCHROME CYP2E1 AND RISK OF NASOPHARYNGEAL CARCINOMA IN ALGERIA POPULATION

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ABSTRACT

Objective: The present study was carried out to analyze the relationship between CYP2E1 genetic polymorphism and the risk of developing nasopharyngeal cancer (NPC).

Methods: The study involved 100 patients with nasopharyngeal carcinoma, compared with 180 healthy controls. Genomic DNA samples were assayed for restriction fragment length polymorphisms in the CYP2E1 by PCR amplification followed by digestion with RsaI. The frequency comparison between the two groups was determined by the χ^2 test.

Results: Our results showed that the distribution of CYP2E1-/-allele frequency was found to be significantly different between controls (22 %) and cancer cases (26%) along with twice increase of the NPC risk in Algeria (OR=2.2, CI 95% [1.02-2.54]).

Conclusion: Our results are in line with those found for other populations and subsequent they emphasize the importance of the involvement of this gene in the development of the NPC detoxification process, which exhibited more and more strong association with environmental factors.

Keywords: Genetic polymorphism, Nasopharyngeal carcinoma, Cytochrome P450 2E1, RsaI, Algeria Population

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INTRODUCTION

Cancers are among the most important non-communicable diseases that impose a major disease burden to the society [1], there are over 100 different types of cancer [2]. Nasopharyngeal cancer (NPC) is a rare cancer in most parts of the world [3] with annual age-standardized incidence rates typically below 1 per 100,000 people/year, in both sexes [4], but occurs at relatively high rates in some geographic regions and among certain ethnic groups with the highest incidence [5]. Intermediate incidence (8-12 cases/100,000/y) was reported in the North African population [6].

The distinct geographical and ethnic distribution of NPC seem to be associated with certain environmental and hereditary factors [7]. Numerous environmental factors have been associated with risk of developing nasopharyngeal carcinoma, including infection with the Epstein-Barr virus, occupational exposure to cigarette smoking, and various dietary factors [8]. Host factors, including human leukocyte antigens and cytochrome P450 2E1 (CYP2E1), have also been postulated as an important molecule in nasopharyngeal carcinoma development [8, 9].

The cytochrome P450 enzymes (CYP) represent a large family of proteins involved in the metabolism of drugs and other xenobiotics, as well as some endogenous substrates. CYP450 consists of the superfamily of hem proteins that catalyze the oxidative metabolism of a wide variety of exogenous chemicals including drugs [10]. CYP2E1, a member of the cytochrome P-450 superfamily, is involved in the metabolic activation of many low molecular weight compounds such as N-nitrosamines, aniline, vinyl chloride, and urethane [11]. This enzyme is also believed to participate in the oxidation of other compounds; like ethanol, to produce reactive free radicals that may initiate lipid peroxidation and consequently carcinogenesis [12]. N-Nitrosamines presented in tobacco and diet are well-recognized as carcinogens, involved in cancer development in many body parts, including the esophagus and stomach [13].

Functional CYP2E1 gene polymorphisms might, therefore, affect susceptibility cancers, for which a role is suspected for etiological

agents such as N-nitrosamines [14]. Among the known genetic polymorphisms in the CYP2E1 detectable by Dra I and Taq I digestion are not thought to affect the transcription or the function of the enzyme encoded by the gene. In contrast, the RsaI variant corresponding to a C-1054T substitution (rs2031920) in the 5'-flanking region appears to be associated with decreased enzyme activity or inactivity [15]. The CYP2E1 gene is present in the population in various polymorphic forms. The variants detectable by RsaI digestion (called the variant form and wild-type form). The form of variant contains polymorphic base substitution sites in a region of the gene that is not transcribed but that appears to be involved in the transcriptional regulation of CYP2E1 expression [16, 17]. However, there has been considered as one *in vitro* study, showing that the variant allele (-/-) increased expression of a reporter gene constructs [18].

In the present study, we examined the relationship between CYP2E1 genotypes and the risk of developing NPC pathogenesis within 100 NPC cases and 180 matched healthy controls from Algeria. PCR and RFLP methods were used to identify polymorphisms in the gene of interest. RsaI restriction enzyme digestions were utilized to detect genotypic polymorphisms. The use of this restriction enzyme was dictated by previous findings suggesting that the two forms of the gene detected by RsaI digestion exhibit widely different levels of expression.

MATERIALS AND METHODS

Study of patient populations and genomic DNA preparation

The present study was conducted on 100 patients with NPC and 180 controls. All subjects were residents of East region of Algeria. The cases were recruited over a period from January; 2012 to December; 2015 at the Cancer Center of Batna, and were histologically confirmed as undifferentiated NPC. Controls were frequency-matched to the cases depending to age (± 2 y), gender, and residence. In addition, controls of the present study were required to have no previous history of NPC identification. At recruitment, each participant was personally interviewed, in order to obtain detailed

information on ethnicity and family disease history sociodemographic characteristics, recent and prior tobacco use. Subjects were afterwards asked to consent to the collection of approximately 5 ml blood; the 280 samples were taken with their consent and permission. All DNA samples were extracted from whole blood collected in K3E (K3EDTA) anticoagulant tubes according to the protocol stand by the manufacturer, using the wizard genomic DNA kit part ® 050 (PROMEGA, United States of America).

PCR-RFLP analysis

Genomic DNA (0.1 µg) extracted from whole blood was used for each PCR analysis. The amplification was performed with primers as described previously by Hayashi et al.1991. The total reaction volume of 50 µl consisted of 20µM Tris-HCl (pH 8.4), 50 mmol KCl, 1.5 mmol MgCl₂, 125 µM deoxynucleoside triphosphate, 0.2 µM primers, 4 U Taq DNA polymerase (Gibco), and 0.2 µg of template DNA. The method of Hayashi et al. [19] was used to identify genotypes of CYP2E1 using RsaI digestion. In brief, two primers used were: (a)-5' CAGTCGAG-TCTACATTGTCA-3', and (b) 5'-TTCATTCTGTCTTCTAAC-TGG-3', which produce 410 bp fragment. The PCR conditions were 40 cycles at 92 °C for 1 minute, 60 °C for 1 minute, 72 °C for 2 min and final elongation step of 5 min at 72 °C in a Perkin-Elmer/DNA thermal Cycle 480. Twenty µl of each sample was digested with 10 units RsaI at 37 °C overnight. The restricted products were analyzed by electrophoresis on 2% agarose gel. Bands were visualized with an ultraviolet trans illuminator after ethidium bromide staining.

Statistical analysis

Pearson's χ^2 test was used to examine differences in distributions of genotypes studied between cases and controls. ORs with 95% CIs

calculated using unconditional logistic regression and adjusted for age, gender, smoking status, were used to determine the association between genotype of the CYP2E1 gene and NPC development. The 95% confidence interval (CI) was computed to determine the statistical significance of the findings. Statistical significance was declared if two-sided $P < 0.05$ or if the 95% CI did not include unity. All statistical analysis was performed by SPSS (8.0) software.

RESULTS

The selected demographic variables of study subjects are summarized in table 1. The mean age of the case subjects was 47 y (range, 20–more than 60 y); 63% of the case subjects were male. Comparable statistics for control subjects were 49 y (range, 20–more than 60) and 55.5% were male. The majority of our case subjects and our control subjects aged between 40 and 60 y. Although an effort was made to obtain a frequency match on smoking status between cases and controls, more smokers were present in the case group compared to controls ($p = 0.012$). No family history of esophageal cancer was observed among controls and cases.

This study is based on the analyses of 100 cases with NPC and 180 normal controls. The DNA fragment of the CYP2E1 gene was 410bp after PCR amplification with primer RsaI. The expected sizes of the products after digestion with RsaI were as follow: homozygous wild-type (+/+) containing restriction site in each DNA chain, resulting in electrophoresis bands of 360 bp and 50 bp; variant homozygote (-/-) without a restriction site and an electrophoresis band of 410 bp, and heterozygote (+/-) with restriction sites in one of the DNA chains, become into 3 fragments of 410 bp, 360 bp, and 50 bp.

Table 1: Demographic variable of the study subjects

Variable	Control subjects (n = 180)	Cancer cases (n = 100)
Gender, n (%)		
Male	100 (55.5)	63 (63.0)
Female	80 (44.4)	37 (37.0)
Mean age year (range)	49 (20.+60)	47 (20.+60)
20–40 %	35 (19.44)	20 (20.0)
40–60 %	97 (53.88)	54 (54.0)
+60 %	48 (26.66)	26 (26.0)
Smoking status, n (%)		
Never	115 (63.9)	38 (38.0)
Current	65 (36.1)	62 (62.0)
Histologically (%)		
differentiated		25 (25.0)
undifferentiated		85 (85.0)

The distributions of three CYP2E1 genotypes were then compared within carcinoma cases, and controls. As shown in table 2, 11.0% of cancer cases were homozygous for the (-/-) allele of CYP2E1; these were significantly higher ($\chi^2 = 30.6$; $P = 0.0001$) than that seen in controls (5.0%). Subjects with the homozygous variant (-/-) genotype had a 2.2-fold increased risk of developing nasopharynx

carcinoma (adjusted OR= 2.2; 95% CI= 1.02–2.54) compared with subjects with the wild-type and heterozygous (+/+and+/-) genotypes. The allele frequencies for the wild-type and variant form (+/and-/-) of the CYP2E1 gene were found to be 0.77 and 0.22 among the control population as; compared to 0.73 and 0.26 within cancer cases, respectively.

Table 2: Frequency distribution among case and control subjects and relative risk associated with genotypic variants of CYP2E1 detected by restriction enzyme digestion with Rsa I

Frequency	Case subjects (n = 100)	Control subjects (n = 180)	p	OR (95% CI)
CYP2E1 genotype				
Rsa I				
+/+	58 (58.0)	108 (60.0)		[0.72-1.2] 0.96
+/-	31 (31.0)	63 (35.0)		[0.66-1.09] 0.88
-/-	11 (11.0)	9 (5.0)	<0.05	[1.02-2.54] 2.2
Allele frequency				
+/+	0.73	0.77		
-/-	0.26	0.22		

+/+homozygote for the common allele; +/-, heterozygote; -/-homozygote for the variant allele.

Results of the analysis of the interaction between smoking and CYP2E1 polymorphisms on the risk of the cancer were also examined. Table 3

shows the ORs of nasopharyngeal carcinoma related to CYP2E1 genotypes by exposure to tobacco smoking. An excessive risk with

borderline significance (OR= 1.78; 95% CI= 0.98-2.9) was found to be related to smoking only among those with at least one copy variant allele (-/-) of the CYP2E1 compared with nonsmokers. We observed a strong and statistically significant modification of the CYP2E1 effect by smoking status (table 3). Among smokers, individuals homozygous for the variant allele (-/-) of the CYP2E1 gene were at a 3.3-fold increased risk of disease relative to nonsmokers who were

homozygous for the variant allele (-/-) of the CYP2E1 gene (OR= 0.3; 95% CI =0.08-1.01). We also examined the effect of CYP2E1 genotype separately for age (table 4). A stronger association between homozygosity for variant allele (-/-) of the CYP2E1 and nasopharyngeal carcinoma was observed among patients whose age is between 40 and 60 y (OR= 3.7; 95% CI= 1.78-5.96) compared with patients whose age is more than 60 y (OR= 1.92; 95% CI = 1.03-2.33).

Table 3: Joint effect of CYP2E1 genotypic variants detected by Rsa I digestion and cigarette smoking on nasopharyngeal carcinoma risk

Smoking	+/+		+/-		-/-	
	No. of case/No. of control subjects	OR (95% CI)	No. of case/No. of control subjects	OR (95% CI)	No. of case/No. of control subjects	OR (95% CI)
Smokers	30/36	[0.87-2.07] 1.14	24/25	[0.98-2.9] 1.78	8/4	[1.22-4.58]3.33
Nonsmokers	28/72	[0.18-1.23] 0.46	7/38	0.09-1.13] 0.22	3/5	[0.08-1.01] 0.3

+/+homozygote for the common allele; +/-, heterozygote; -/-homozygote for the variant allele.

Table 4: Joint effect of CYP2E1 genotypic variants detected by Rsa I digestion and age on nasopharyngeal carcinoma risk

Age/Year	+/+		+/-		-/-	
	No. of case/No. of control subjects	Or (95% CI)	No. of case/No. of control subjects	Or (95% CI)	No. of case/No. of control subjects	Or (95% CI)
20-40	11/15	[0.42-2.42] 1.03	5/16	[0.09-1.56]0.53	4/4	[1.12-3.69]1.89
40-60	28/57	[0.32-2.09] 0.83	22/38	[0.39-2.34]1.05	4/2	[1.78-5.96] 3.7
+60	19/36	[1.23-2.35] 0.93	4/9	[0.21-1.97] 0.79	3/3	[1.03-2.33] 1.92

+/+homozygote for the common allele; +/-, heterozygote; -/-homozygote for the variant allele.

DISCUSSION

Epidemiological studies have shown that up to 90% of all cancers are related to environmental factors. Most of the environmental carcinogens need to be metabolically activated to exert their carcinogenic effects [20, 21]. Genetic polymorphisms in enzymes involved in carcinogen metabolism have shown to influence the susceptibility to cancer [22, 23]. Cytochrome P4502E1 (CYP2E1) plays an important role in this process. It participates in the metabolic activation of carcinogenic nitrosamines. Several recent studies show that the genetic polymorphisms of metabolizing enzymes are associated with some cancers such as lung cancer [24, 25], nasopharyngeal cancer [25, 26] and colorectal cancer [27]. But the results of those studies of the relation between CYP2E1 and cancer susceptibility are inconsistent [28].

The possibility of making N-nitrosated compounds involved in NPC cancer has been an issue for many years. In this study, cigarette smoke was positively associated with NPC cancer [29, 30]. Tobacco smoke contains many potential carcinogens, also including nitroso compounds [31]. The results obtained from the current study confirm our previous data, suggesting an association between CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma. On the basis of this study of 100 patients diagnosed with nasopharyngeal carcinoma and 180 control subjects, the distribution of a homozygous variant form of the CYP2E1 gene much in different races. Frequencies of the variant allele (-/-) are: 4% in European-Americans, 29.5% in Thailand, 18% in Taiwanese [19], 8% in China, and 1.94% in Turkish population.

Our study finds that frequencies of the variant allele (-/-) are 26% in Algeria population, alike to the allele frequencies of Thailand, but not comparable to the North African population that could not be performed. In regards of a combination of these genotypes and risk of NPC, the overall risk of NPC was found to be 2.2 times higher in case of homozygous variant (-/-) state. Since CYP2E1 is involved in the metabolic activation of numerous procarcinogens [32], this observation is in agreement with recent studies showing that the homozygous variant (-/-) genotype of CYP2E1 was associated with increased risk of nasopharyngeal [33] in Tunisia, Caucasian, Asian [5] and Taiwan populations [34].

In; contrary the results also exist reporting no association between CYP2E1 (-/-) genotype and cancers or reporting that excessive risk of certain types of cancer is associated with (+/+) variant genotype [21]. The reason for this discrepancy is not clear, but several

possibilities, such as ethnic differences in allele frequencies and specific exposures associated with the polymorphism, should be considered [25].

Several studies reported that homozygous variant form was associated with enhanced enzyme activity. Hayashi *et al.* [35] reported that enhanced activity of variant form DNA was about 10 times than that of wild-type DNA. The variant allele (-/-) of the CYP2E1 affects the phenotype directly rather than being a consequence of linkage disequilibrium from another mutation or gene [35]. This difference in the transcriptional activities might associate with the susceptibility in human carcinogenesis.

A higher level of expression in the variant form would result in larger amounts of procarcinogens being changed into carcinogens that then produce DNA damage.

This result of genotype-phenotype relationship further supports our and others findings showing that the variant allele (-/-) of the CYP2E1 is a genetic susceptibility factor for certain types of exposures. We also found a significant association between the homozygous variant (-/-) genotype and age in our study, individuals in the 40-60 y age group showed a strong association in the CNP in the presence of the homozygous variant (-/-) genotype of the CYP2E1 (OR=3.7; 95% IC =1.78-5.96). This shows that an early exposure to tobacco would cause the expression of a number more important of cancer mutations. Such patients present factor genetic regulation of oncogenic expression.

CONCLUSION

In conclusion, our results demonstrate a significant association between CYP2E1 genetic Polymorphism and nasopharyngeal carcinoma risk. The association was restricted to smokers, genetic susceptibility factors for nasopharyngeal carcinoma identified in our study could serve as useful biomarkers for targeting prevention of cancer.

ABBREVIATION

CYP2E1-cytochrome P450 2E1, PCR-polymerase chain reaction, RFLP-restriction fragment length polymorphism, CI-confidence interval, NPC-nasopharyngeal cancer, χ^2 -pearson's χ^2 test, OR-odds ratio.

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LIMITATIONS

The short duration of the study, limited sample size, and patients lost to follow-up due to certain conditions.

AUTHORS CONTRIBUTIONS

I have participated sufficiently in the conception and design of this work and the analysis of the data, as well as the writing of the manuscript, to take public responsibility for it. Pr. SATTA Dalila has supervised and finalisation of the manuscript. Pr. BENDJEMANA Katia has supervised the different techniques involved in the analysis and identification of results. ABDENNEBI Monia, DOUIK Hayat and GUÉMIRA Fethi have selected and collected data of the study, in addition to the revision of the manuscript together with Prof. SATTA Dalila.

CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest with respect to the authorship, research, or publication of this article.

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