

Research article

Cytochrome P450 2E1 polymorphism and nasopharyngeal carcinoma development in Thailand: a correlative studyNarisorn Kongruttanachok¹, Sairoong Sukdikul¹, Surachai Setavarin², Verachai Kerehjanarong³, Pakpoom Supiyaphun³, Narin Voravud⁴, Yong Poovorawan⁵ and Apiwat Mutirangura*¹Address: ¹Genetics unit; Department of Anatomy,, ²National Cancer Institute, Bangkok, Thailand, ³Department of Otolaryngology,, ⁴Medical Oncology unit, and ⁵Viral Hepatitis Research Unit; Department of Medicine, Faculty of Medicine, Chulalongkorn University,E-mail: Narisorn Kongruttanachok - ruttiwan@hotmail.com; Sairoong Sukdikul - sakdikul_s@hotmail.com; Surachai Setavarin - bink506@hotmail.com; Verachai Kerehjanarong - Virachaik@hotmail.com; Pakpoom Supiyaphun - fmedpsp@md2.md.chula.ac.th; Narin Voravud - cu_medonco@hotmail.com; Yong Poovorawan - pyong@chula.ac.th; Apiwat Mutirangura* - mapiwat@chula.ac.th

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world but occurs at relatively high frequency among people of Chinese descent. The cytochrome P450 2E1 enzyme (CYP2E1) is responsible for the metabolic activation of nitrosamines, and has been shown to be a susceptibility gene for NPC development in Taiwan [RR = 2.6; 95%CI = 1.2-5.7]. Since there has been only one report of this link, it was decided to investigate the susceptibility of CYP2E1 to NPC development in other populations. Therefore, the correlation between the RsaI polymorphism of this gene and NPC was studied in-patients including Thai and Chinese in Thailand. The present study comprised 217 cases diagnosed with NPC and 297 healthy controls.

Results: Similar to the result found in Taiwanese, a homozygous uncut genotype demonstrated a higher relative risk both when all cases were analyzed [RR = 2.19; 95%CI = 0.62-8.68] or individual racial groups, Thai [RR = 1.51; 95%CI = 0.08-90.06] or Chinese [RR = 1.99; 95%CI = 0.39-10.87]. The ethnicity-adjusted odds ratio is 2.39 with 95%CI, 0.72-7.89.

Conclusions: Though our finding was not statistically significant due to the moderate sample size of the study, similarity to the study in Taiwan with only a slight loss in precision was demonstrated. The higher RR found for the same genotype in distinct populations confirmed that CYP2E1 is one of several NPC susceptibility genes and that the RsaI minus variant is one mutation that affects phenotype.

Background

Nasopharyngeal carcinoma (NPC) is a rare tumor in most parts of the world, with annual age-standardized incidence rates typically below 1 per 100,000 people/year in both sexes [1]. The tumor occurs most often in

Southern Chinese who reside in Guangdong Province, at an incidence rate 30-50 per 100,000 people/year, in contrast with <1 per 100,000 people/year in white Europeans [2,3,4,5]. The disease also occurs at moderate frequencies (3-10 per 100,000 people/year) in several non-

Chinese ethnic groups such as Malay, Thai and Vietnamese [6]. Numerous factors, both environmental and genetic, have been associated with the risk of developing NPC. The environmental factors include infection with the Epstein-Barr virus (EBV), as well as frequent consumption of high levels of nitrosamine from preserved food such as salted fish [7,8,9]. In addition, host factors also play a major role in NPC development. Unique alleles of the human leukocyte antigen (*HLA*) and cytochrome P450 2E1 (*CYP2E1*) have been shown to be associated with high relative risk in several Asian ethnic groups, including the Chinese in Taiwan [10,11,12].

CYP2E1, an enzyme involved in the metabolic activation of procarcinogens into reactive intermediates capable of forming adducts and damaging DNA, is believed to play an essential role in chemical carcinogenesis [13,14]. Nitrosamine is a substrate of *CYP2E1*. It is believed that nitrosamine, once activated can lead to the development of numerous cancers [15]. Studies have also demonstrated that *CYP2E1* is expressed in the nasal epithelium of human [16]. Evidence from previous epidemiological studies has suggested that salted fish is a food preferred by Chinese people and contains nitrosamines and nitrosamine precursors [9]. Therefore, *CYP2E1* is believed to render the nasopharyngeal epithelium susceptible to NPC development. A previous study in Taiwan employed a PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) assay using the restriction enzyme *RsaI* in order to compare wild-type (+/+) and variant forms (-/-) of the *CYP2E1* gene between NPC patients and the general population [10,11]. The variant form of contains *CYP2E1* polymorphic mutations in the distal 5'-flanking region of the gene, causing a marked difference in its transcriptional activity, as shown by CAT (chloramphenicol acetyltransferase) [17]. The Taiwanese association study showed that individuals homozygous for the variant allele (-/-) were at an increased risk for NPC development (relative risk [RR] = 2.6; 95% confidence interval [CI] = 1.2-5.7) [11]. There are many Chinese people who have immigrated and permanently lived in Thailand for 2 to 3 generations, resulting in a mixed population of Thai and Chinese people. From clinical observation, we had observed at least one-third of NPC patients were Chinese in origin. Since the susceptibility of *CYP2E1* gene to develop NPC had only been reported from Chinese people in Taiwan, it was decided to investigate whether this allele played the same role in other populations. Therefore, we studied the correlation of the polymorphism of the *CYP2E1* gene with NPC in Thai and Chinese populations in Thailand.

Materials and Methods

Sample collection

Blood samples were obtained by venipuncture from 217 NPC patients at Chulalongkorn Hospital and 297 healthy blood donors. All subjects were interviewed and separated into two groups, Thai and Chinese, based on their grandparents' ethnic origin. When their ancestors, including their great grandparents, originated from China, the patients were considered Chinese. When their ancestors originated from Thailand, the patients were considered Thai. There were 99 Thai and 98 Chinese in the control group. The NPC patient group included 132 Thai and 56 Chinese.

PCR-RFLP analysis

Genomic DNA (0.1 µg) extracted from leukocytes was used for each PCR analysis. The amplification was performed with primers as described previously by Hayashi et al. The total reaction volume of 50 µl consisted of 20 µM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 125 µM deoxynucleoside triphosphate, 0.2 µM primers, 4 U Taq DNA polymerase (Gibco), and 0.2 µg of template DNA. The PCR conditions were 40 cycles at 92°C for 1 minute, 60°C for 1 minute and 72 for 2 minutes in a Perkin-Elmer/DNA thermal Cycle 480. Genotypes of *CYP2E1* gene were determined by RFLP analysis. Twenty microliters of PCR products were digested with 10 U *RsaI* restriction enzyme (New England Biolabs) overnight at 37°C. The restricted products were analyzed by electrophoresis on 2% agarose gel. Bands were visualized with an ultraviolet transilluminator after ethidium bromide staining.

Statistical Analysis

The relative risk (RR) was estimated by the odds ratio method, to determine the correlation between genotype of the *CYP2E1* gene and NPC development. In addition, the RR was used to estimate the association of the pattern of genetic inheritance of the *CYP2E1* gene and NPC phenotype. The 95% confidence interval (CI) was computed to determine the statistical significance of the findings. The RR and 95%CI was calculated by using Exact method from Epi info version6 program.

Results

In the present study, we investigated the correlation between the polymorphism of the *CYP2E1* gene and NPC on a total of 217 patients and 297 controls. The diagnosis of NPC was confirmed histologically and by the presence of EBV DNA in the tumor. PCR-RFLP analysis was used to evaluate *RsaI* polymorphism in the *CYP2E1* gene (fig 1). The distribution of alleles in all, Thai and Chinese were found to be in Hardy-Weinberg equilibrium. The calculated frequencies of heterozygous using $2x(+/+)^{1/2} \times (-/-)^{1/2}$ were 0.32, 0.26, 0.42 from all, Thai, and Chi-

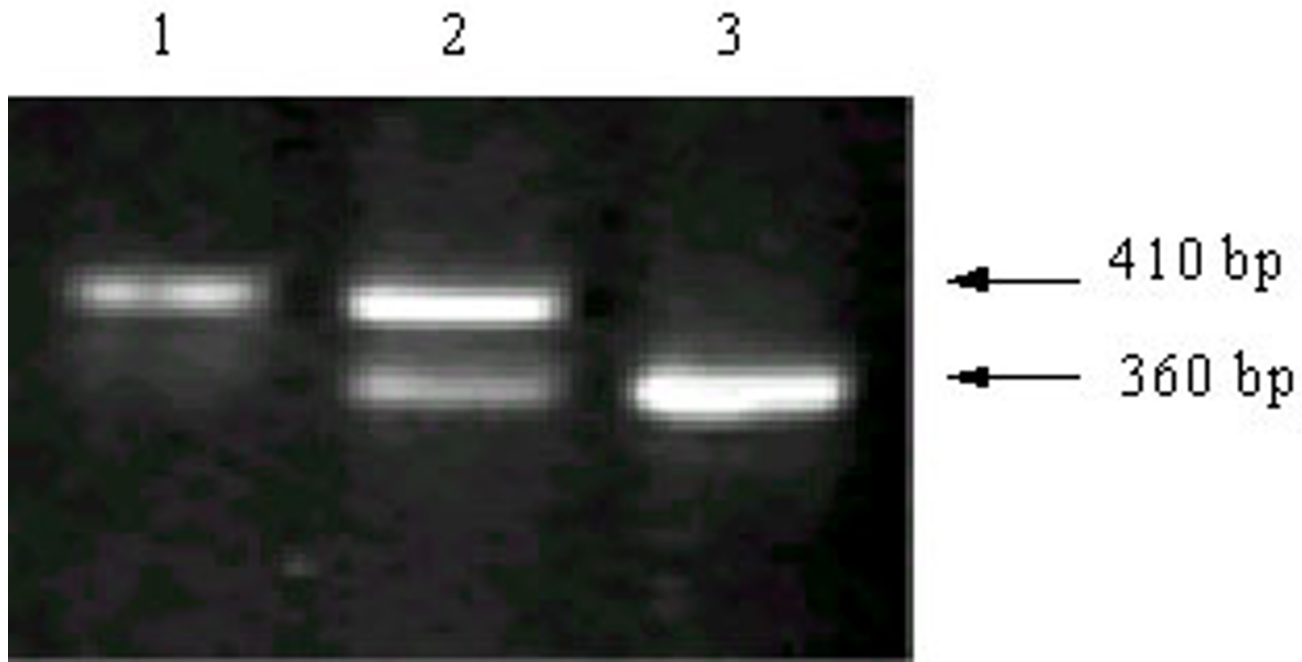


Figure 1
PCR-RFLP assay to detect the polymorphism of the *CYP2E1* gene by *RsaI* enzyme digesting the 410 bp PCR product into 360 bp and 50 bp fragments. Lane 1, homozygous variant (-/-); Lane 2, heterozygous (+/-); Lane 3, homozygous wild type (+/+).

nese NPC patients, and 0.32, 0.26 and 0.42 from all, Thai and Chinese control, respectively. These numbers are similar to actual frequencies of heterozygous from all groups, 0.32, 0.28, 0.42, 0.35, 0.28, 0.52 from all, Thai and Chinese patients and all, Thai and Chinese control, respectively. We found the relative risk of the variant form (-/-) of the *CYP2E1* gene at a high risk [RR = 2.19]. However, this result had no statistical significance [95%CI = 0.62-8.68]. To evaluate whether the lack of significance of the trend was due to a mixed genetic background, both patients and controls were analyzed according to the origins of their ancestors (Thai or Chinese). A slightly increased risk of the variant from (-/-) of the *CYP2E1* gene could be demonstrated in both the Thai and Chinese sample groups [RR = 1.51; 95%CI = 0.08-90.06, RR = 1.99; 95%CI = 0.39-10.87, respectively]. Nevertheless, no statistical significance could be established. The estimated crude odds ratio lies outside of two ethnicity-specific odds ratios for Thai and Chinese indicates ethnicity is a confounder in this genotype-phenotype association. Odds ratio for the combined group of subjects with ethnicity information can be calculated with adjustment for ethnicity. The ethnicity-adjusted odds ratio (-/- vs +/-) is 1.86 (95%CI = 0.55-6.31). A similar adjustment using the whole sample of three groups, Thai, Chinese, and ethnicity unspecified showed an odd ratio of 2.39, with only a slight loss in precision (95%CI = 0.72-7.89). This is clos-

er to the odds ratio of 2.6 from the study in Taiwan. Furthermore, Thai-Taiwanese comparison was done quantitatively by testing $H_0:OR(\text{Thai})=OR(\text{Taiwan})$. Using Mantel-Haenszel test for odds ratio homogeneity, the p-value was 0.81. Thus there is no statistical different for the role of *CYP2E1* on NPC development between these two populations.

In this study, we further analyzed the association of the pattern of genetic inheritance of *CYP2E1* gene and NPC phenotype by calculating the relative risk if the genotype were either autosomal dominant (AD) or autosomal recessive (AR). In autosomal dominance, the contribution of a single variant allele would show a higher RR. Thus the RR for the combination of the heterozygous (+/-) and the variant form (-/-) compared with wild type (+/+) were computed. There was no association between AD heredity and NPC risk in the total, Thai, and Chinese sample groups [RR = 1.00; 95%CI = 0.68-1.47, RR = 1.01; 95%CI = 0.55-1.87, RR = 0.84; 95%CI = 0.41-1.71, respectively] (Table 2). By contrast, AR inheritance requires an abnormality in both alleles of the *CYP2E1* gene. The RR were calculated by comparison between the wild type (+/+), the heterozygous (+/-) or the combination of the wild type and heterozygous (+/+ and +/-) and the variant form (-/-). A higher RR value in all comparisons was shown for AR heredity in all sample groups (Table

3). However, these results showed no statistical significance for either the AD or AR pattern.

Table 1: Frequency distribution and relative risks associated with genotype variants of CYP2E1 detected RFLP using RsaI

CYP2E1	Frequency		RR	95%CI
	Cases	Controls		
Total	217*	297		
+ / +	138	189	1.00	
+ / -	71	103	0.94	0.64 - 1.39
- / -	8	5	2.19	0.62 - 8.68
Thai	132	99		
+ / +	93	70	1.00	
+ / -	37	28	0.99	0.54 - 1.86
- / -	2	1	1.51	0.08 - 90.06
Chinese	56	98		
+ / +	27	43	1.00	
+ / -	24	51	0.75	0.36 - 1.57
- / -	5	4	1.99	0.39 - 10.87

* 29 cases lack precise information regarding ethnicity +, The allele could be digested with RsaI enzyme -, The allele could not be digested with RsaI enzyme

Table 2: Correlation between Autosomal Dominant (AD) pattern of genetic CYP2E1 gene and NPC phenotype

CYP2E1	Frequency		RR (AD)	95%CI
	Cases	Controls		
Total				
+ / +	138	189	1.00	
+ / - and - / -	79	108	1.00	0.68 - 1.47
Thai				
+ / +	93	70	1.00	
+ / - and - / -	39	29	1.01	0.55 - 1.87
Chinese				
+ / +	27	43	1.00	
+ / - and - / -	29	55	0.84	0.41 - 1.71

+, The allele could be digested with RsaI enzyme -, The allele could not be digested with RsaI enzyme

Table 3: Correlation between Autosomal Recessive (AR) pattern of genetic CYP2E1 gene and NPC phenotype

CYP2E1	Frequency		RR (AR)	95%CI
	Cases	Controls		
Total				
+ / +	138	189	1.00	
- / -	8	5	2.19	0.62 - 8.68
+ / -	71	103	1.00	
- / -	8	5	2.32	0.64 - 9.36
+ / + and + / -	209	292	1.00	
- / -	8	5	2.24	0.63 - 8.80
Thai				
+ / +	93	70	1.00	
- / -	2	1	1.51	0.08 - 90.06
+ / -	37	28	1.00	
- / -	2	1	1.51	0.07 - 92.44
+ / + and + / -	130	98	1.00	
- / -	2	1	1.51	0.08 - 89.84
Chinese				
+ / +	27	43	1.00	
- / -	5	4	1.99	0.39 - 10.87
+ / -	24	51	1.00	
- / -	5	4	2.66	0.51 - 14.48
+ / + and + / -	51	94	1.00	
- / -	5	4	2.30	0.47 - 12.08

+, The allele could be digested with RsaI enzyme -, The allele could not be digested with RsaI enzyme

Discussion

We have shown an increased risk of developing NPC associated with the homozygous variant form of the CYP2E1 gene. This higher RR was demonstrated in both the Thai and Chinese populations in Thailand. This finding was similar to the result reported from Taiwan. However, these results were marginal statistical significance, which may well be due to the small sample size employed in the present study. Thus the CYP2E1 gene appears to be a susceptibility gene for NPC development regardless of the patient's genetic background. Patients of both Thai and Chinese ethnic origin revealed a higher relative risk from the same allele, despite their distinct ancestry. Thus it is more likely that the RsaI negative allele affects the phenotype directly rather than being a consequence of linkage disequilibrium from another mutation or gene. This confirms the previous finding that the polymorphic RsaI site was essential for a marked difference in transcriptional activities [17]. A higher level of expression in the variant form would result in larger amounts of pro-carcinogens being changed into carcinogens, that then produce DNA damage. The affect of the distinct expression level of a metabolic gene should be reduced if the

person with abnormal genotype is not exposed to the substrate. For example, Phenylketonuria (PKU) patients would not demonstrate mental retardation if they were prevented completely from exposure to tyrosine [18]. In other words, a mutation can not cause the phenotype without interaction from environmental factors. Regarding NPC development, the role of *CYP2E1* variant may be varied upon the amount of consumed salted fish and/or preserved foods that contain nitrosamine and nitrosamine precursors.

Conclusion

Result of the reported crude odds ratio is 2.19 [95%CI = 0.62-8.68]. If the result is adjusted odds ratio, it will be 2.39 [95%CI = 0.72-7.89], which is closer to the Taiwanese odds ratio of 2.6, with only a slight loss in precision. Thus, this study confirmed a previous study in Taiwan that *CYP2E1* appears to be one of a number of NPC susceptibility genes and the RsaI minus variant is not just a polymorphism but directly influences the development of the phenotype.

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Competing interests

None declared

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