Research article

**Cytochrome P450 2E1 polymorphism and nasopharyngeal carcinoma development in Thailand: a correlative study**

Narisorn Kongruttanachok¹, Sairoong Sukdikul¹, Surachai Setavarin², Verachai Kerekhjanarong³, Pakpoom Supiyaphun³, Narin Voravud⁴, Yong Poovorawan⁵ and Apiwat Mutirangura*¹

Address: 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 Kerekhjanarong - 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

*Corresponding author

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 Epit info version 6 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 x (-/-)1/2 were 0.32, 0.26, 0.42 from all, Thai, and Chi-
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 closer to the odds ratio of 2.6 from the study in Taiwan. Furthermore, Thai-Taiwanese comparison was done quantitatively by testing Ho:OR(Thai)=OR(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 2).
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      |
| + / +    | 79        | 108     | 1.00      | 0.68 - 1.47 |
| Thai     | 93        | 70      | 1.00      |
| + / +    | 39        | 29      | 1.01      | 0.55 - 1.87 |
| Chinese  | 27        | 43      | 1.00      |
| + / +    | 24        | 51      | 1.00      |
| + / -    | 5         | 4       | 2.66      | 0.51 - 14.48 |

+, 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      | 0.64 - 9.36 |
| + / -    | 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 procarcinogens being changed into carcinogens, that then produce DNA damage. The effect 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 nitrosoamine 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.

**Acknowledgments**

We deeply indebted to the staff of the Department of Otolaryngology and the Radiotherapy Section, Department of Radiology, Chulalongkorn Hospital and National Blood Center for the recruitment of patients and collection of materials. This work was supported by Molecular Biology Project, Faculty of Medicine, Chulalongkorn University and the Thailand research Fund.

**Competing interests**

None declared

**References**

1. Parkin DM, Muir CS: Cancer Incidence in Five Continents: Comparability and quality of data. IARC Sci Publ 1992, 120:45-173
2. Simons MJ, Wee GB, Singh D, Dharmalingham S, Yong NK, Chau JC, Ho JH, Day NE, De The G: Immunogenetic aspects of nasopharyngeal carcinoma. V. Confirmation of a Chinese-related HLA profile (A2, Singapore 2) associated with an increased risk in Chinese for nasopharyngeal carcinoma. Natl Cancer Inst Monogr 1977, 47:147-51
3. Cui HY: Apparent correlation between nasopharyngeal carcinoma and HLA phenotype. Chung Hwa Chung Liu Tsa Chih 1982, 4(4):249-53
4. Chan SH: Aetiology of nasopharyngeal carcinoma. Ann Acad Med Singapore 1990, 19(2):201-7
5. Zhu XN, Chen R, Kong FH, Liu W: Human leukocyte antigens - A, -B, -C, and -DR and nasopharyngeal carcinoma in northern China. Ann Otol Rhinol Laryngol 1990, 99(4 Pt 1):286-7
6. Muir CS: Nasopharyngeal carcinoma in non-Chinese populations with special reference to south-east Asia and Africa. Int J Cancer 1971, 15:351-63
7. Choi PH, Suen MW, Huang DP, Lo KW, Lee JC: Nasopharyngeal carcinoma: genetic changes, Epstein-Barr virus infection, or both. A clinical and molecular study of 36 patients. Cancer 1993, 15:2873-8
8. Hildesheim A, Levine PH: Etiology of nasopharyngeal carcinoma: a review. Epidemiol 1993, 15(2):466-85
9. Zheng YM, Tuppen P, Hubert A, Jeanne D, Pan YJ, Zeng Y, de The G: Environmental and dietary risk factors for nasopharyngeal carcinoma: a case-control study in Zangwu County, Guangxi, China. Br J Cancer 1994, 69(3):508-14
10. Hildesheim A, Chen CJ, Caporaso NE, Cheng YJ, Hoover RN, Hsu MM, Levine PH, Chen IH, Chen JY, Yang CS, et al: Cytochrome P4502E1 genetic polymorphisms and risk of nasopharyngeal carcinoma: results from a case-control study conducted in Taiwan. Cancer Epidemiol Biomarkers Prev 1995, 4(6):607-10
11. Hildesheim A, Anderson LM, Chen CJ, Cheng YJ, Brinton LA, Daly AK, Reed CD, Chen IH, Caporaso NE, Hsu MM, et al: CYP2E1 genetic polymorphisms and risk of nasopharyngeal carcinoma in Taiwan. J Natl Cancer Inst 1997, 20(89(16)):1207-12
12. Nebert DW: Role of genetics and drug metabolism in human cancer risk. Mutat Res 1991, 247(2):267-81
13. Poulsen HE, Loft S, Wassermann K: Cancer risk related to genetic polymorphisms in carcinogen metabolism and DNA repair. Pharmacol Toxicol 1993, 72 Suppl 1:93-103
14. Bartsch H, Montesano R: Relevance of nitrosamines to human cancer. Carcinogenesis 1984, 5(11):1381-93
15. Gervasi PG, Longo V, Naldi F, Panattoni G, Ursino FX: Enobiotic-metabolizing enzymes in human respiratory nasal mucosa. Biochem Pharmacol 1991, 41(41):177-84
16. Poirier S, Bouvier G, Malavelle C, Oshshima H, Shao YM, Hubert A, Zeng Y, de The G, Bartsch H: Volatile nitrosamine levels and genotoxicity of food samples from high-risk areas for nasopharyngeal carcinoma before and after nitrosation. Int J Cancer 1989, 5(44(6)):1088-94
17. Hayashi S, Watanabe J, Kawaiji K: Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIIE1 gene. J Biochem (Tokyo) 1991, 110(4):559-65
18. Bruce RK: Human Genetics: A problem-Based Approach. Massachusetts: Blackwell Science 1999

**Pre-publication history**

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/content/backmatter/1471-2407-1-4-b1.pdf