# Cytochrome P4502E1 Genetic Polymorphisms and Risk of Nasopharyngeal Carcinoma: Results from a Case-Control Study Conducted in Taiwan[1]

**Allan Hildesheim,[2] Chien-Jen Chen, Neil E. Caporaso, Yu-Juen Cheng, Robert N. Hoover, Mow-Ming Hsu, Paul H. Levine, I-How Chen, Jen-Yang Chen, Czau-Siung Yang, Ann K. Daly, and Jeffrey R. Idle**

Epidemiology and Biostatistics Program [A. H., R. N. H.], Genetic Epidemiology Branch [N. E. C.], Viral Epidemiology Branch [P. H. L.], National Cancer Institute, Bethesda, Maryland; Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan [C-J. C., Y-J. C.]; Department of Otolaryngology, National Taiwan University Hospital, Taipei, Taiwan [M-M. H.]; Department of Otolaryngology, MacKay Hospital, Taipei, Taiwan [I-H. C.]; Institute of Microbiology, College of Medicine, National Taiwan University, Taipei, Taiwan [J-Y. C., C-S. Y.]; GenoType Limited, Newcastle Upon Tyne, United Kingdom [A. K. D., J. R. I.]

## Abstract

**CYP2E1 is responsible for the metabolic activation of nitrosamines believed to be involved in the pathogenesis of various tumors. Nasopharyngeal carcinoma (NPC) is a tumor thought to be linked to nitrosamine exposure. To investigate the possible role of *CYP2E1* genetic polymorphisms in the etiology of this tumor, we investigated 50 histologically confirmed NPC cases from Taiwan and 50 controls matched to cases on age, sex, and residence. Samples were examined for RFLPs in the *CYP2E1* gene by PCR amplification followed by digestion with *Dra*I and *Rsa*I. Among healthy controls, the allelic frequency of wild-type and variant forms of *CYP2E1* were 79 and 21%, respectively, using *Dra*I enzyme digestion and 82 and 18%, respectively, using *Rsa*I enzyme digestion. As compared with individuals who were homozygous for the wild-type *CYP2E1* gene, those found to be homozygous for the variant form of the gene by *Dra*I digestion were at a 5-fold excess risk of disease (95% confidence interval = 0.95–16). Similarly, subjects homozygous for the variant form of the *CYP2E1* gene by *Rsa*I digestion were at 7.7-fold excess risk of developing NPC (95% confidence interval = 0.87–68). Individuals found to be heterozygous for the gene were at similar risk of disease compared to those homozygous for the wild-type gene. A strong association was observed between the RFLPs detected by *Dra*I and *Rsa*I digestion of *CYP2E1*; a correlation coefficient of 0.86 for controls and 0.91 for cases was observed. Interestingly, all individuals with the variant form of *CYP2E1* detected by *Rsa*I enzyme digestion also exhibited the variant form of the gene using *Dra*I enzyme digestion, although the reverse was not always true. Our results confirm previous findings suggesting that the distribution of CYP genotypes among Oriental populations varies from that observed among Caucasians and demonstrate for the first time a possible association between *CYP2E1* genetic polymorphisms and the risk of developing NPC.**

## Introduction

The CYP[3] family of enzymes is responsible for the metabolism of numerous xenobiotics (1). In addition, some of the CYP enzymes are involved in the activation of procarcinogens into reactive intermediates capable of forming adducts and damaging DNA, a step believed to be essential in chemical carcinogenesis (1–3). Nitrosamines are among the substrates of CYP enzymes believed, once activated, to be linked to the development of numerous cancers (4). Of the over 250 CYP enzymes cloned to date, CYP2E1 is believed to play an important role in the activation of these potentially carcinogenic nitrosamines (5, 6).

NPC has been associated with nitrosamine exposure (7). Evidence from previous epidemiological studies suggests that salted fish (7, 8) and cigarette smoke (7, 9–12), both of which contain nitrosamines and nitrosamine precursors (13–15), are associated with NPC risk. Animal studies have demonstrated that rats fed nitrosamine-containing salted fish develop nasal cavity tumors (16, 17).

Furthermore, a recent study conducted in two regions of China having different rates of NPC showed that individuals residing in the community with high NPC rates had an increased ability to endogenously form nitrosamines compared to individuals residing in the community with low NPC rates (18). Because the carcinogenic action of nitrosamines requires that they first be activated and because CYP enzymes have been shown to be involved in the activation process and to be expressed in the nasal epithelium of numerous animal species and humans (7, 19–23), we hypothesized that genotypic and/or phenotypic differences in CYP enzymes responsible for the activation of nitrosamines might partially account for differences in risk of developing NPC. In the present report, we investigate the possible role of *CYP2E1* genotypes in NPC pathogenesis among 50 NPC cases and 50 matched healthy controls from Taiwan. PCR and RFLP methods were used to identify polymorphisms in the gene of interest. *Dra*I and *Rsa*I restriction enzyme digestions were utilized to detect genotypic

Received 2/24/95; revised 5/5/95; accepted 5/11/95.

[1] This study was supported by funding from the United States National Cancer Institute and the Department of Health, Executive Yuan in Taiwan (Project DOH-834M1).

[2] To whom requests for reprints should be addressed, at Environmental Epidemiology Branch, National Cancer Institute, Executive Plaza North Room 443, Bethesda, MD 20892-7374.

[3] The abbreviations used are: CYP, cytochrome P-450; NPC, nasopharyngeal carcinoma; CI, confidence interval.

polymorphisms. The use of these restriction enzymes was dictated by previous findings suggesting that the variant detected by *Dra*I digestion might be associated with lung cancer risk and that the two forms of the gene detected by *Rsa*I digestion exhibit widely different levels of expression (24–27).

## Materials and Methods

As part of a larger epidemiological investigation of NPC currently underway in Taipei, Taiwan, 50 incident cases of NPC were selected for the present study. These subjects were randomly chosen from among the participants in the larger investigation for whom adequate amounts of lymphocytes were available. Cases were diagnosed in one of two large referral hospitals in Taipei. All cases were histologically confirmed as having NPC by an ear, nose, and throat specialist (M-M. H.). Subjects were required to be <75 years old, to have no prior diagnosis or treatment for NPC, and to have resided in Taipei city or county for at least 6 months.

For each eligible NPC case, an individually matched healthy control was selected for study. Controls were matched to the NPC cases on age (5-year groupings), sex, and residence. In addition, controls were required to have no history of NPC before identification for the present study.

All subjects were asked to donate 30 ml of blood for study. Samples were collected and kept at room temperature until processing began. Peripheral blood lymphocytes obtained by Ficoll-Hypaque separation were diluted in a freezing medium containing RPMI 1640 (70%), DMSO (15%), and FCS (15%) and stored in a liquid nitrogen tank. Shipments to the United States were made in dry ice on a monthly basis. Once frozen, samples were never thawed until testing.

DNA extraction was performed by using standard methods (28). All PCR reactions were carried out in a final volume of 50 μl. The reaction buffer was 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% (v/v) Triton X-100 with each primer at 0.25 μM, nucleotides at 0.25 mM, and Tbr polymerase (1 unit/50 μl reaction; NBL Gene Sciences, Cramlington, Northumberland, United Kingdom). The assays for *CYP2E1* (*Rsa*I) also contained 3% DMSO.

The method of Hayashi *et al.* (26) was used to identify genotypes of *CYP2E1* using *Rsa*I digestion. In brief, two primers used were: (a) CCAGTCGAGTCTACATTGTCA; and (b) TTCATTCTGTCTTCTAACTGG. The PCR conditions were 35 cycles of 1 min at 95°C, 2 min at 45°C and 2 min at 70°C. Twenty μl of each sample was digested with 10 units *Rsa*I at 37°C for 3 h and the products analyzed by electrophoresis on a 4% Nusieve 3:1 agarose gel. Bands were visualized on a UV transilluminator (Fig. 1*A*). A sample of DNA from a subject known to be heterozygous was included in each set of 10 samples. All samples were analyzed twice, and concordant results were obtained on both occasions.

The method described previously by Kim *et al.* (29) was used to determine *CYP2E1* genotypes using *Dra*I digestion. The primers were: (*a*) TCGTCAGTTCCTGAAAGCAGG; and (*b*) GAGCTCTGATGCAAGTATCGCA. The PCR conditions were 35 cycles of 1 min at 95°C, 2 min at 55°C, and 2 min at 70°C. Twenty μl of each sample was digested with 10 units *Dra*I at 37°C for 3 h and the products analyzed by electrophoresis on a 1.8% agarose gel. Bands were visualized on a UV transilluminator (Fig. 1*B*). Samples of DNA from subjects known to be homozygous wild type, heterozygous, and homozygous mutant were included in each set of 20 samples. All samples were analyzed on two separate occasions and yielded concordant results.

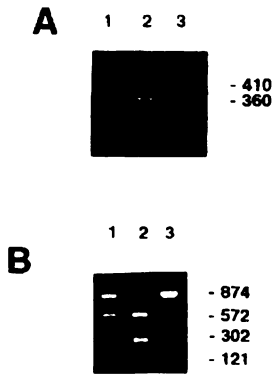


*Fig. 1.* Detection of the *Rsa*I and *Dra*I polymorphisms. *A*, analysis with *Rsa*I. *Lane 1*, heterozygous; *Lane 2*, homozygous wild type; *Lane 3*, homozygous variant. *B*, analysis with *Dra*I. *Lane 1*, heterozygous; *Lane 2*, homozygous wild type; *Lane 3*, homozygous variant. The fragment sizes are indicated.

*Table 1* Demographic characteristics of study subjects

| | Cases | Controls |
|---|---|---|
| Sample size | 50 | 50 |
| Age | | |
| Mean (yrs) | 45.1 | 45.0 |
| Range (yrs) | 17–63 | 19–64 |
| Sex | | |
| % Male | 82% | 82% |
| % Female | 18% | 18% |

A 10% random sample ($n$ = 10) of subjects were blindly tested twice for each of the assays described above. The results were concordant for all 10 blind duplicate sets on all assays.

Allele and genotype frequencies were computed. The relative risk, as estimated by the odds ratio, was the estimate used to determine the association between CYP genotype and disease (30). 95% CIs were calculated to determine the statistical significance of findings (30). Correlation coefficients were computed by using the method proposed by Pearson (31).

## Results

The age and sex distributions of the 50 cases and 50 controls selected for study are presented in Table 1. Eighty two % of cases were male and the median age of cases was 45.1 years (range = 17–63 years). The similar age and sex distribution observed among controls, relative to the cases, was dictated by the matching criteria utilized in selecting controls for the present study.

*CYP2E1* RFLP data were available on 48 NPC cases and 50 healthy controls; 2 cases had insufficient DNA available for analysis. The distribution of alleles among our healthy controls is presented in Table 2. As shown in the table, the wild-type and variant forms of *CYP2E1* detected by *Dra*I digestion were present in 79 and 21% of control subjects, respectively. Similarly, the wild-type and variant forms of *CYP2E1* detected by *Rsa*I digestion were present in 82 and 18% of control subjects, respectively.

Table 3 presents results of our analysis that investigated the association of *CYP2E1* genotypes and NPC risk. Subjects who were homozygous for the variant form of *CYP2E1* by *Dra*I digestion were at a 5-fold excess risk of disease compared to

*Table 2* Allele frequencies of CYP2E1 genotype among healthy control subjects

| | Wild type | Variant |
|---|---|---|
| CYP2E1-*DraI* | 79.0% | 21.0% |
| CYP2E1-*RsaI* | 82.0% | 18.0% |

*Table 3* Frequency distribution among cases and controls and relative risks associated with genotypic variants of CYP2E1

| CYP2E1 | Frequency | | RR[a] | 95% CI |
|---|---|---|---|---|
| | Cases | Controls | | |
| *DraI* | | | | |
| Homozygous wild type | 25 | 31 | 1.0 | |
| Heterozygous | 15 | 17 | 1.1 | 0.45–2.7 |
| Homozygous variant | 8 | 2 | 5.0 | 0.95–16 |
| *RsaI* | | | | |
| Homozygous wild type | 30 | 33 | 1.0 | |
| Heterozygous | 11 | 16 | 0.76 | 0.30–1.9 |
| Homozygous variant | 7 | 1 | 7.7 | 0.87–68 |

[a] RR, relative risk.

*Table 4* Correlation and joint distribution of the *DraI* and *RsaI* genotypes

| *DraI/RsaI* | Homozygous wild type | Heterozygous | Homozygous variant |
|---|---|---|---|
| Among NPC cases | | | |
| Homozygous wild type | 25 | 0 | 0 |
| Heterozygous | 4 | 11 | 0 |
| Homozygous variant | 1 | 0 | 7 |
| Correlation coefficient | | 0.91 ($P < 0.001$) | |
| Among healthy controls | | | |
| Homozygous wild type | 31 | 0 | 0 |
| Heterozygous | 2 | 15 | 0 |
| Homozygous variant | 0 | 1 | 1 |
| Correlation coefficient | | 0.86 ($P < 0.001$) | |

subjects who were homozygous for the wild-type gene (95% CI = 0.95–16). Similarly, subjects who were homozygous for the variant form of *CYP2E1* by *Rsa*I digestion were at a 7.7-fold excess risk of disease compared to subjects who were homozygous for the wild-type gene (95% CI = 0.87–68). No excess in risk was observed for individuals heterozygous for the wild-type and variant forms of the gene using either *Dra*I or *Rsa*I digestions, relative to those who were homozygous for the wild-type gene.

Previous studies have reported a high degree of association between RFLPs observed after *Dra*I and *Rsa*I digestion. We examined this issue among our Taiwanese study population (Table 4). Our results suggest a strong association between the RFLPs observed by *Dra*I and *Rsa*I digestion. The two RFLPs were associated in 47 (94%) of 50 healthy controls and in 43 (86%) of 48 NPC cases examined. Interestingly, all individuals with the variant form of *CYP2E1* detected using *Rsa*I enzyme digestion also exhibited the variant form of the gene using *Dra*I enzyme digestion, although the reverse was not always true.

## Discussion

Results from the present study suggest that genotypic variants of the *CYP2E1* gene might be associated with risk of NPC. Subjects found to be homozygous for the variant form of *CYP2E1* using *Dra*I digestion were at a 5-fold excess risk of disease and those homozygous for the variant form of the gene using *Rsa*I digestion were at a near 8-fold excess risk of disease. These results were of marginal statistical significance due to the relative small size of the present study. Given the strong association observed between the variants of *CYP2E1* detected by *Dra*I and *Rsa*I digestion, we were unable to disentangle the independent effects of these polymorphisms on disease risk. However, it is interesting to note that a previous study reported a 10-fold increase in gene expression of the variant form of the *CYP2E1* gene (detected by *Rsa*I digestion) relative to the wild-type form of the gene, when a chloramphenicol acetyltransferase-containing construct was transfected into HepG2 cells and analyzed for chloramphenicol acetyltransferase activity (26). The polymorphism detected by *Dra*I digestion of *CYP2E1* is located in intron 6, and no functional significance of this polymorphism is currently known to exist.

NPC is a tumor thought to occur, in part, as a result of exposure to nitrosamines (7). Studies have consistently observed that individuals who consume salted fish and other processed/preserved foods known to contain nitrosamines and nitrosamine precursors are at excess risk of developing NPC (13, 14). Risk of disease appears to be particularly accentuated when exposure occurs at young ages (8). Also, several studies to date have reported that cigarette smoking is related to risk of NPC (7, 9–12), although some studies have failed to observe this association (7, 32, 33). As with salted fish, the nitrosamines present in cigarettes are believed to be the chemical carcinogens linked to NPC development.

Because nitrosamines require activation to impart their carcinogenicity and CYP2E1 is known to be important in the activation of numerous nitrosamines, the association between *CYP2E1* genotype and NPC risk observed in this study is biologically plausible (5, 6). Furthermore, evidence suggesting that CYP enzymes are expressed in the nasal cavity of numerous animal species and humans further strengthens the biological plausibility of the observed association (19–21, 23). The fact that an increased risk of disease was apparent only among individuals who were homozygous for the variant form of the gene suggests that *CYP2E1* could function in a recessive manner.

Results from our study are consistent with those from previous investigations suggesting that the allelic frequency distribution of the different forms of *CYP2E1* varies between Caucasian and Oriental populations (27). The allelic frequency distribution of the variant form of the *CYP2E1* gene observed in two studies conducted among Japanese individuals was very similar to that noted in this report (~20%; Refs. 24, 27). In contrast, the allelic frequency distribution of the variant form of the *CYP2E1* gene is reported to be much lower among Caucasians (≤10%; Refs. 25, 34).

In summary, results from the present study suggest an association between *CYP2E1* genotype and risk of developing NPC. Larger studies that are capable of simultaneously assessing *CYP2E1* polymorphisms and nitrosamine exposure are needed to confirm and extend the present findings.

## Acknowledgments

The authors would like to acknowledge the team involved in the field work in Taiwan (P-L. Chan, H-Y. Chang, Y-T. Chang, S-I. Chao, C-F. Chen, H-C. Chen, H-L. Chen, K-S. Chiang, Y-C. Chien, H-M. Cho, C-C. Chu, T-T. Dan, F-Y. Hsu, H-R. Hsu, Y-P. Huang, J-H. Lin, P-H. Lin, Y-S. Lin, D-H. Liu, W-L. Liu, S-M. Peng, H-C. Teng, C-T. Wu, and P-C. Yen) and the individuals from WESTAT and Microbiological Associates, Rockville, MD, involved in this project. Their assistance was instrumental to the success of this study. We would also like to thank Dr. Hong-Hsin Lee for his support throughout the project.

## References


1. Nebert, D. W. Role of genetics and drug metabolism in human cancer risk. Mut. Res., *247:* 267–281, 1991.

2. Poulsen, H. E., Loft, S., and Wassermann, K. Cancer risk related to genetic polymorphisms in carcinogen metabolism and DNA repair. Pharmacol. Toxicol., *72:* 93–103, 1993.

3. Ingelman-Sundberg, M., Johansson, I., Persson, I., Oscarson, M., Hu, Y., Bertilsson, L., Dahl, M. L., and Sjoqvist, F. Genetic polymorphism of cytochrome P450. Functional consequences and possible relationship to disease and alcohol toxicity. *In:* B. Jansson, H. Jornvall, U. Rydberg, L. Terenius, and B. L. Vallee (eds.), Toward a molecular basis of alcohol use and abuse, pp. 197–207. Basel, Switzerland: Birkhauser Verlag, 1994.

4. Bartsch, H., and Montesano, R. Revelance of nitrosamines to human cancer. Carcinogenesis (Lond.), *5:* 1381–1393, 1984.

5. Nouso, K., Thorgeirsson, S. S., and Battula, N. Stable expression of human cytochrome P450IIE1 in mammalian cells: metabolic activation of nitrosodimethylamine and formation of adducts with cellular DNA. Cancer Res., *52:* 1796–1800, 1992.

6. Koop, D. R. Oxidative and reductive metabolism by cytochrome P450 2E1. FASEB J., *6:* 724–730, 1992.

7. Hildesheim, A., and Levine, P. H. Etiology of nasopharyngeal carcinoma: a review. Epidemiol. Rev., *15:* 466–485, 1993.

8. Yu, M. C. Diet and nasopharyngeal carcinoma. FEMS Microbiol. Immunol., *64:* 235–242, 1990.

9. Lin, T. M., Chen K. P., Lin, C. C., Hsu, M. M., Tu, S. M., Chiang, T. C., Jung, P. F., and Hirayama, T. Retrospective study on nasopharyngeal carcinoma. J. Natl. Cancer Inst., *51:* 1403–1408, 1973.

10. Mabuchi, K., Bross, D. S., and Kessler, I. I. Cigarette smoking and nasopharyngeal carcinoma. Cancer (Phila.), *55:* 2874–2876, 1985.

11. Chen, C. J., Liang, K. Y., Chang, Y. S., Wang, Y. F., Hsieh, T., Hsu, M. M., Chen, J. Y., and Liu, M. Y. Multiple risk factors of nasopharyngeal carcinoma: Epstein-Barr virus, malarial infection, cigarette smoking and familial tendency. Anticancer Res., *10:* 547–554, 1990.

12. Nam, J. M., McLaughlin, J. K., and Blot, W. J. Cigarette smoking, alcohol, and nasopharyngeal carcinoma: a case-control study among U. S. whites. J. Natl. Cancer Inst., *84:* 619–622, 1992.

13. Poirier, S., Bouvier, G., Malaveille, C., Ohshima, H., Shao, Y. M., Hubert, A., Zeng, Y., de The, G., and Bartsch, H. Volatile nitrosamine levels and genotoxicity of food samples from high-risk areas for nasopharyngeal carcinoma before and after nitrosation. Int. J. Cancer, *44:* 1088–1094, 1989.

14. Poirier, S., Ohshima, H., de The, G., Hubert, A., Bourgade, M. C., and Bartsch, H. Volatile nitrosamine levels in common food from Tunisia. South China and Greenland, high-risk areas for nasopharyngeal carcinoma (NPC). Int. J. Cancer, *39:* 293–296, 1987.

15. Hecht, S. S., and Hoffmann, D. Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis (Lond.), *9:* 875–884, 1988.

16. Yu, M. C., Nichols, P. W., Zou, X. N., Estes, J., and Henderson, B. E. Induction of malignant nasal cavity tumors in Wistar rats fed Chinese salted fish. Br. J. Cancer, *60:* 198–201, 1989.

17. Huang, D. P., Ho, J. H. C., Saw, D., and Teoh, T. B. Carcinoma of the nasal and paranasal regions in rats fed Cantonese salted marine fish, pp. 315–328, IARC Sci. Pub. No. 20. Lyon, France: IARC, 1978.

18. Zeng, Y., Ohshima, H., Bouvier, G., Roy, P., Jianming, Z., Li, B., Brouet, I., de The, G., and Bartsch, H. Urinary excretion of nitrosamino acids and nitrate by inhabitants of high- and low-risk areas for nasopharyngeal carcinoma in southern China. Cancer Epidemiol., Biomarkers & Prev., *2:* 195–200, 1993.

19. Longo, V., Pacifici, G. M., Panattoni, G., Ursino, F., and Gervasi, P. G. Metabolism of diethylnitrosamine by microsomes of human respiratory nasal mucosa and liver. Biochem. Pharmacol., *38:* 1867–1869, 1989.

20. Gervasi, P. G., Longo, V., Naldi, F., Panattoni, G., and Ursino, F. Xenobiotic-metabolizing enzymes in human respiratory nasal mucosa. Biochem. Pharmacol., *41:* 177–184, 1991.

21. Smith, T. J., Guo, Z., Hong, J. Y., Ning, S. M., Thomas, P. E., and Yang, C. S. Kinetics and enzyme involvement in the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in microsomes of rat lung and nasal mucosa. Carcinogenesis (Lond.), *13:* 1409–1414, 1992.

22. Ding, X., and Coon, M. J. Induction of cytochrome P-450 isozyme 3a (P-450IIE1) in rabbit olfactory mucosa by ethanol and acetone. Drug Metabol. Dispos., *18:* 742–745, 1990.

23. Porter, T. D., Khani, S. C., and Coon, M. J. Induction and tissue-specific expression of rabbit cytochrome P450IIE1 and IIE2 genes. Mol. Pharmacol., *36:* 61–65, 1992.

24. Uematsu, F., Kikuchi, H., Motomiya, M., Abe, T., Sagami, I., Ohmachi, T., Wakui, A., Kanamaru, R., and Watanabe, M. Association between restriction fragment length polymorphism of the human cytochrome P450IIE1 gene and susceptibility to lung cancer. Jpn. J. Cancer Res., *82:* 254–256, 1991.

25. Hirvonen, A., Husgafvel-Pursiainen, K., Anttila, S., Karjalainen, A., and Vainio, H. The human *CYP2E1* gene and lung cancer: *Dra*I and *Rsa*I restriction fragment length polymorphisms in a Finnish study population. Carcinogenesis (Lond.), *14:* 85–88, 1993.

26. Hayashi, S. I., Watanabe, J., and Kawajiri, K. Genetic polymorphism in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J. Biochem., *110:* 559–565, 1991.

27. Kato, S., Shields, P. G., Caporaso, N. E., Hoover, R. N., Trump, B. F., Sugimura, H., Weston, A., and Harris, C. C. Cytochrome P450IIE1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer Res., *52:* 6712–6715, 1992.

28. Buffone, G. J., and Darlington, G. J. Isolation of DNA from biological specimens without extraction with phenol. Clin. Chem., *31:* 164–165, 1985.

29. Kim, R. B., O'Shea, D., and Wilkinson, G. R. Relationship in healthy subjects between *CYP2E1* genetic polymorphisms and the 6-hydroxylation of chlorzoxazone: a putative measure of *CYP2E1* activity. Pharmacogenetics, *4:* 162–165, 1994.

30. Breslow, N. E., and Day, N. E. Statistical methods in cancer research, Vol. 1. The analysis of case-control studies. Lyon, France: IARC, 1980.

31. Dixon, W. J., and Massey, F. J. Introduction to Statistical Analysis. New York: McGraw-Hill, Inc., 1983.

32. Ning, J. P., Yu, M. C., Wang, Q. S., and Henderson, B. E. Consumption of salted fish and other risk factors for nasopharyngeal carcinoma (NPC) in Tianjin, a low-risk region for NPC in the People's Republic of China. J. Natl. Cancer Inst., *82:* 291–296, 1990.

33. Henderson, B. E., Louie, E., R. N.,, Jing, J. S. H., Buell, P., and Gardner, M. B. Risk factors associated with nasopharyngeal carcinoma. N. Engl. J. Med., *295:* 1101–1106, 1976.

34. Persson, I., Johansson, I., Bergling, H., Dahl, M. L., Seidegard, J., Rylander, R., Rannug, A., Hogberg, J., and Sundberg, M. I. Genetic polymorphism of cytochrome P4502E1 in a Swedish population: relationship to incidence of lung cancer. FEBS Lett., *3:* 207–211, 1993.

# Cytochrome P4502E1 genetic polymorphisms and risk of nasopharyngeal carcinoma: results from a case-control study conducted in Taiwan.

A Hildesheim, C J Chen, N E Caporaso, et al.

*Cancer Epidemiol Biomarkers Prev* 1995;4:607-610.