

MD Consult information may not be reproduced, retransmitted, stored, distributed, disseminated, sold, published, broadcast or circulated in any medium to anyone, including but not limited to others in the same company or organization, without the express prior written permission of MD Consult, except as otherwise expressly permitted under fair use provisions of U.S. Copyright Law. [Subscriber Agreement](#)

**Journal of Occupational and Environmental Medicine**

Volume 43 • Number 3 • March 1, 2001

Copyright © 2001 American College of Occupational and Environmental Medicine

## ORIGINAL ARTICLES

---

### **p53 Gene Expression in Relation to Indoor Exposure to Unvented Coal Smoke in Xuan Wei, China**

Qing Lan, MD, MS

Zumei Feng, MD

Defa Tian, MD

Xingzhou He, MD

Nathaniel Rothman, MD, MPH, MHS

Linwei Tian, MD

Xubang Lu, MD

Mary Beth Terry, PhD

Judy L. Mumford, PhD

From the University of North Carolina, Chapel Hill (Dr Lan, Dr Feng, Dr Tian); the Chinese Academy of Preventive Medicine, Beijing (Dr He); the Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD (Dr Lan, Dr Rothman); the University of California, Berkeley (Dr Tian); Xuan Wei Hospital, Yunnan, China (Dr Lu); the Division of Epidemiology, Joseph L. Mailman School of Public Health, Columbia University (Dr Terry); and the US Environmental Protection Agency, Research Triangle Park (Dr Mumford)

Address correspondence to: Dr Qing Lan, Occupational Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, MSC 7240, 6120 Executive Boulevard, EPS 8109, Bethesda, MD 20892-7240; e-mail [qingl@mail.nih.gov](mailto:qingl@mail.nih.gov).

Lung cancer mortality rates in Xuan Wei County, which are among the highest in China, have previously been associated with exposure to indoor emissions from burning smoky coal. To determine if this association is stronger among lung cancer patients with abnormal expression of p53, we performed a population-based case-control study. Ninety-seven newly diagnosed lung cancer patients and 97 controls, individually matched by age, sex, and home fuel type, were enrolled. We used immunocytochemical methods to assess p53 protein accumulation in exfoliated tumor cells isolated from sputum samples. As expected, the amount of lifetime smoky coal use was associated with an overall increase in lung cancer risk. Compared with subjects who used less than 130 tons of smoky coal during their lifetime, the odds ratios (OR) for lung cancer were 1.48 (95% confidence interval [CI], 0.73 to 3.02) for subjects exposed to 130 to 240 tons, and 3.21 (95% CI, 1.23 to 9.03) for subjects who used more than 240 tons of smoky coal (*P* for trend 0.01). The effect was due almost exclusively to the pattern in women, almost all of whom were nonsmokers. Further, among highly exposed women, the association was substantially larger and achieved statistical significance only among patients with sputum samples that were positive for p53 overexpression (OR, 18.72; 95% CI, 1.77 to 383.38 vs OR, 4.80; 95% CI, 0.66 to 43.87 for p53-negative cases). This study suggests that exposure to the

<http://home.mdconsult.com/das/article/body/1/jorg=journal&source=MI&sp=11910054&s...> 4/11/2003

combustion products of smoky coal in Xuan Wei is more strongly associated with women who have lung cancer accompanied by p53 protein overexpression in exfoliated tumor cells.

## Introduction

In Xuan Wei, a rural county of Yunnan Province, China, tobacco smoking is very rare in the female population (smoking rate <0.1%)<sup>[1]</sup> but common in the male population (smoking rate >70%).<sup>[2]</sup> Lung cancer mortality in Xuan Wei is among the highest in China: eight times the national average for women, and four times the national average for men. The high mortality rate has been linked to the heavy indoor air pollution resulting from burning smoky coal in unventilated homes.<sup>[1][3]</sup>

Mutations in oncogenes and tumor suppressor **genes** are considered critical events in the pathogenesis of cancer.<sup>[4]</sup> Mutations in the p53 **gene** are the most common genetic changes associated with human tumors, including lung cancer.<sup>[5]</sup> Further, the pattern of p53 mutations may provide some insight into causative exposures for a variety of tumors,<sup>[6][7][8]</sup> and the mutation frequency may be able to function as a molecular dosimeter of such exposures.<sup>[6]</sup> Studies show that the specific mutation patterns of G:C to T:A transversions are the most commonly observed mutations following experimental exposure to polycyclic aromatic hydrocarbons, such as B(a)P.<sup>[9][10]</sup> This mutation pattern in the p53 **gene** has been associated with smoking in lung cancer cases, and the frequency of p53 mutation has been correlated with lifetime cigarette consumption.<sup>[11][12][13]</sup>

Mutations in p53 can result in an altered p53 protein that has a prolonged half-life and accumulates in nuclei, whereas wild-type p53 eludes detection because of its short half-life; this provides a rationale for using p53 protein overexpression (ie, p53 positivity) as a measure of certain classes of p53 mutations.<sup>[14]</sup><sup>[15]</sup> Our previous pilot studies examined the feasibility of detecting p53 protein accumulation in sputum samples and developed methods for detecting p53 overexpression in sputum cells using a color immunocytochemistry staining method and an immunofluorescence assay.<sup>[2][16]</sup> Further, the proportion of p53-positive sputum samples from a series of lung cancer cases in Xuan Wei County was similar to the proportion detected in tumor tissue collected from lung cancer patients in the same geographic area.<sup>[13]</sup> Residents in this area are generally reluctant to undergo invasive diagnostic procedures, and autopsy rates are very low, so it is difficult to perform molecular epidemiological studies using immunocytochemical or molecular analysis of tumor samples in a substantial number of cases.

The previous pilot study also examined the role of p53 in lung pathogenesis by comparing the p53 positivity of lung cancer cases from Xuan Wei with that of another area having relatively lower air pollution levels.<sup>[2]</sup> The p53-positivity rate was found to be higher in the former group. In the current study, we extended these findings by performing a population-based case-control study, in which all study subjects were from the same county. We estimated lifetime smoky coal exposure for each subject and were able to control for such potential confounding factors as tobacco smoking. Because tumor tissue was collected from very few subjects in the present study, the evaluation of p53 overexpression was done in sputum samples only. By requiring the presence of alveolar macrophages to ensure that sputum samples came from deep within the lung, by collecting samples from controls to assess the proportion of p53 overexpression in healthy subjects, and by using strict criteria for determining if a sputum sample was p53-positive, we were able to study the determinants of p53 overexpression in lung cancer cases identified from this unique population.

## Materials and Methods

The methods used for this population-based case-control study are described in detail elsewhere.<sup>[12]</sup> Briefly, all residents living in Xuan Wei were considered the target population. During the period from March 1995 to March 1996, a total of 135 new lung cancer cases, as diagnosed by a minimum of clinical symptoms and radiography, were identified. Among the 135 patients, 133 agreed to participate in this study (98%). The criteria for inclusion of lung cancer cases in the study were (1) confirmation of the clinical diagnosis of lung cancer based on the examination of tissue or tracheal lavage, or (2) detection of tumor cells in a tracheal brushing or sputum sample. A total of 105 cases met the above criteria. Among these, 97 had detectable tumor cells with Papanicolaou's staining and were included in the p53 staining study.

One control was selected for each lung cancer case, matching for sex, age ( $\pm 2$  years), village, and type of fuel currently used for cooking and heating at home. Within 2 weeks after the diagnosis and recruitment of each lung cancer case, a control was selected randomly from the list of household registrations (which included age and sex) from the same villages in which the cancer patient lived. A standardized closed-question form of questionnaire was used to obtain demographic information, smoking history, family and personal medical history, and information on other variables from the patients and controls. Trained investigators interviewed all patients at the hospital; the controls were interviewed in their homes. For the protection of the subjects, this international research study was conducted according to the guidelines of the World Medical Association Declaration of Helsinki. All subjects in this study signed a consent form. The research protocol was approved by an Environmental Protection Agency Human Subjects Research Review Official.

A series of five sputum samples were collected on 5 consecutive mornings from the lung cancer patients and the controls. The first-morning specimen resulting from the overnight accumulation of secretions contained more exfoliated cells for assay.<sup>[18]</sup> All sputum samples were collected by spontaneous expectoration and, from the patients, before surgery or any other type of treatment. All subjects rinsed their mouth with water to remove extraneous material. They were then instructed to take a deep breath, cough deeply, and expectorate into a plastic cup. Sputum was stored in 40 mL of Saccomanno's fluid (39% ethanol, 3% polyoxyethylene, and 2% isopropanol; Lerner Laboratories, Pittsburgh, PA) to fix and preserve the cells. The sputum samples were stored at 4°C and were transported to the United States by air.

The Saccomanno blending technique<sup>[18]</sup> was used to prepare the sputum samples prior to Papanicolaou's and p53 protein immunohistochemical staining. Each sample was blended for 8 to 15 seconds in a blender to break the mucus and free the cells, after which it was centrifuged at 600 g for 10 minutes. The supernant was discarded, and the cell pellet was resuspended in fresh Saccomanno fluid by vortexing to a cell concentration of approximately 1 million/mL. An aliquot (6 to 8 drops, or 200  $\mu$ L) of the samples was spun in a cytospin at 700 rpm for 7 minutes to form a monolayer of cells on glass microscope slides, which was then allowed to air dry at room temperature before staining. To determine if the sputum samples were derived from the lower respiratory tract, and to confirm the presence of tumor and atypical cells in sputum samples from the lung cancer patients, we used the cytological examination method described by Saccomanno to determine the quality of the samples.<sup>[18]</sup> Cytospin preparations were assayed for Papanicolaou's and p53 staining. Eight slides were made for each participant.

All of the patients were evaluated for the evidence of p53 protein expression (eight slides per subject) for p53 staining. The detection method used was the ABC ELITE mouse immunoglobulin G detection kit (Vector Laboratories, Burlingame, CA). Briefly, the immunocytochemistry method used a three-step indirect process based on the streptavidin-biotin complex, with peroxides-conjugated streptavidin

molecules, and diaminobenzidine as a chromogen. The slides were then counterstained with hematoxylin. Details of this protocol have been previously published. <sup>[2]</sup>

The criteria for inclusion of the sputum samples to be assayed for p53 immunoreactivity were as follows: (1) the presence of at least five alveolar macrophages in sputum on eight slides to ensure that the samples were from deep in the lung for both patients and controls, and (2) the presence of at least three tumor cells on eight slides by Papanicolaou's staining for the lung cancer patients. p53 immunostaining in sputum was evaluated by a scoring system for quantity and intensity. A positive state was considered if (1) the cell nuclear immunocytochemical staining had an intensity score of moderate or strong, and (2) at least three tumor cells showed brown staining in nuclear immunocytochemical staining in the eight cytospin slides assayed. No tumor cells were detected in the controls by Papanicolaou's staining. The results of the staining (Papanicolaou's and p53) were reviewed independently and blindly by two investigators (including D.T., a trained pathologist) to identify the tumor cells and the p53 staining in the exfoliated sputum cells. The results of p53 immunostaining were examined and scored by a trained investigator (Q.L.) and then independently confirmed by a pathologist (D.T.). The few discrepancies were resolved by joint review.

The relationship between risk factors (smoky coal and smoking) and lung cancer risk was analyzed by logistic regression. The variables considered in this analysis were age, sex, smoking pack-years, and the amount of smoky coal use without ventilation. Smoky coal use was defined as lifetime-accumulated smoky coal use without ventilation. The average tons of smoky coal use per year in this study were similar to that of our former study, in which subjects had used an average of 6 to 8 tons per year over several decades. <sup>[20]</sup> Two levels according to the approximate median distribution in the control group defined the variables age and pack-years. Because the smoky coal use distribution was skewed, the cut point for smoky coal use was chosen at 60% and 90% among controls.

Conditional logistic regression was used for calculating the adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) in all lung cancer cases and controls. Unconditional logistic regression was used for calculating the adjusted ORs and 95% CI in p53+ and p53- lung cancer cases and controls. This analysis was also done separately according to gender. Because of the small sample size, the analysis was performed using exact statistical methods (StatXact 4 software). <sup>[20]</sup> Tests for trends were conducted using a likelihood ratio statistic in a logistic regression model. Crude and adjusted ORs were similar in all analyses; only the adjusted ORs are presented.

## Results

This study included 97 patients and 97 controls; 36 pairs were female and 61 pairs were male. None of the women were smokers, and only eight of the men were nonsmokers. Of the 97 patients in this study, 44 (45%) showed evidence of p53 protein accumulation detected by immunocytochemistry. Among the patients, 31% of the women and 54% of the men were p53-positive. Patients who were p53-positive were older (mean age at diagnosis, 56.5 years) than those who were p53-negative (mean age at diagnosis, 53.5 years), although this difference was not statistically significant ( $P = 0.22$ ). All of the controls examined for p53 by immunocytochemistry staining showed negative results.

Table 1 shows the distribution of age, pack-years of tobacco smoking, and smoky coal use. Table 2 shows the relationship between smoky coal use, tobacco use, and lung cancer for all cases and controls. The positive and statistically significant relationship between smoky coal use and lung cancer risk was substantially stronger among women. The effect of high levels of exposure to smoky coal was

particularly pronounced and was significant only among female patients who were p53-positive. This effect was 3.43 times higher (95% CI, 0.39 to 40.77) than the risk among p53-negative patients, but the difference was not statistically significant. The male heavy smokers had a slightly higher risk of lung cancer than the lighter smokers. The effect was almost entirely restricted to p53-positive patients.

**Table 1. Distribution of Characteristics in Control Subjects and Lung Cancer Cases**

Factors	Controls		Cases			
	n	%	p53-Positive (n = 44)		p53-Negative (n = 53)	
			n	%	n	%
Age						
<55	42	43	16	36	25	47
≥55	55	57	28	64	28	53
Pack-years <sup>‡</sup>						
<20	27	44	8	24	13	46
≥20	34	56	25	76	15	54
Smoky coal <sup>†</sup> (tons)						
Total						
<130	57	59	20	45	23	43
130–240	31	32	12	27	21	40
>240	9	9	12	27	9	9
Male						
<130	36	59	17	52	14	50
130–240	19	31	10	30	10	36
>240	6	10	6	18	4	14
Female						
<130	21	58	3	27	9	36
130–240	12	33	2	18	11	44
>240	3	8	6	55	5	20

\* Male participants only.

† Smoky coal use without ventilation.

**Table 2. Multivariate Adjusted ORs and 95% CIs for p53+ and p53– Lung Cancer in Xuan Wei, China<sup>\*</sup>**

Smoky coal <sup>‡</sup> (tons)	Combined p53+ and p53–		p53+		p53–	
	OR	95% CI	OR	95% CI	OR	95% CI

<http://home.mdconsult.com/das/article/body/1/jorg=journal&source=MI&sp=11910054&s...> 4/11/2003

Total						
<130	1.0		1.0		1.0	
130-240	1.48	0.73-3.20	1.08	0.40-2.85	1.63	0.73-0.64
>240	3.21	1.23-9.03	3.98	1.23-13.65	2.39	0.74-7.80
<i>P</i> for trend	0.01		0.03		0.07	
Female						
<130	1.0		1.0		1.0	
130-240	2.21	0.64-8.14	1.49	0.10-17.22	2.47	0.66-10.19
>240	7.94	1.46-60.44	18.72	1.77-383.38	4.80	0.66-43.87
<i>P</i> for trend	0.008		0.007		0.07	
Male						
<130	1.0		1.0		1.0	
130-240	1.30	0.50-3.15	1.01	0.32-3.13	1.53	0.47-43.98
>240	1.88	0.54-7.09	2.01	0.44-9.27	1.81	0.32-9.37
<i>P</i> for trend	0.32		0.47		0.41	
Pack-years †						
<20	1.0		1.0		1.0	
≥20	1.57	0.66-3.81	2.51	0.82-8.39	0.99	0.33-3.01

\* OR, odds ratio; CI, confidence interval.

† Adjusted for age and gender and pack-years of smoke.

‡ Adjusted for age and gender and smoky coal use without ventilation.

## Discussion

We performed a population-based case-control study of smoky coal use and p53 overexpression in sputum samples from lung cancer patients and matched controls in Xuan Wei County in Yunan Province, China. Smoky coal use was associated with an increased risk of lung cancer, particularly among women (all of whom were nonsmokers). Moreover, exposure to high levels of smoky coal among women was strongly and significantly associated only with lung tumors with overexpressed p53 protein detected in exfoliated tumor cells from sputum samples.

Our study has several strengths. It was conducted in a unique rural population with heavy indoor air pollution and high lung cancer mortality. It was shown previously that indoor benzo(a)pyrene concentrations during cooking in this area are comparable with occupational exposure levels reported in some coke oven plants.<sup>(1)</sup> Further, this study was population-based and had very high participation rates among both cases and controls. In addition, we were able to quantitatively estimate smoky coal use for each subject.

The primary limitations of our study are that we used sputum samples rather than tumor tissue, and immunohistochemical detection of p53 protein expression rather than direct sequencing of p53 mutations. Residents in this region of China are generally reluctant to undergo invasive diagnostic procedures, and autopsy rates are very low, so it is difficult to perform studies using immunocytochemical or molecular analysis of tumor samples in a substantial number of cases in a timely manner. To ensure that sputum cells derived at least in part from the lung in both cases and controls, we required the presence of alveolar macrophages to consider a sputum sample acceptable. To ensure that sputum samples from the patients contained tumor cells, we used Papanicolaou's staining to confirm the presence of tumor and atypical cells. Finally, our sample size was relatively small, so our study had low power to detect the association between smoky coal use and p53 status among lung cancer patients. Nevertheless, we found some evidence that, in women, high exposure to the combustion products of smoky coal is more strongly associated with lung cancer accompanied by p53 protein overexpression in exfoliated tumor cells.

If there is truly a stronger relationship between smoky coal exposure and lung cancer risk for p53-positive compared with p53-negative cases, then the overall effect of the error with our method would have been to weaken the association.<sup>[21]</sup> This assumes that the probability of incorrectly classifying p53 status was independent of exposure to smoky coal. Therefore, it is possible that an even greater difference between these risks exists in p53-positive versus p53-negative subjects than what we observed in our study.

There is some evidence that lung tumors in populations exposed to polycyclic aromatic hydrocarbons may have altered p53 expression or mutation patterns. For example, Rusin et al reported that nonsmokers with lung cancers from Silesia, Poland, had a higher frequency of G:C to T:A transversions than that previously reported for nonsmokers.<sup>[22]</sup> People in that highly industrialized area are exposed to polluted air that contains polycyclic aromatic hydrocarbons, such as benzo(a)pyrene.<sup>[22]</sup> One study reported an association between p53-positive immunostaining and a history of heavy smoking<sup>[23]</sup>; another found that duration of smoking was linked with the presence of p53 mutation.<sup>[23][24]</sup> In addition, a recent case-series analysis of lung cancer showed that smoking was associated with a 4.4-fold higher risk of having p53 mutations in tumor samples compared with never-smokers who had no history of environmental tobacco smoke exposure. Also, the risk was higher for heavier smokers and those with a longer duration of smoking.<sup>[24]</sup>

In summary, our study suggests that exposure to the combustion products of smoky coal in Xuan Wei County, China, is strongly associated with the risk of developing lung tumors that overexpress p53. Future research is needed to clarify the relationship between smoky coal and lung cancer tumor mutation patterns in larger studies within Xuan Wei County and in other populations with substantial environmental exposure to polycyclic aromatic hydrocarbons.

#### **Acknowledgment**

The authors are grateful to Chaofu Huang for his assistance with our data collection in Xuan Wei. We thank Dr Robert Chapman at USEPA and Dr Aaron Blair at the NCI for reviewing the article. The research described here has been reviewed by the National Environmental and Health Effects Research Laboratory of the USEPA and NCI/NIH. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

## References

1. Mumford JL, He XZ, Chapman RS, et al. Lung cancer and indoor air pollution in Xuan Wei, China. *Science*. 1987; 235: 217–220. [Abstract](#)
2. Mumford JL, Tian D, Younes M, et al. Detection of p53 protein accumulation in sputum and lung adenocarcinoma associated with indoor exposure to unvented coal smoke in China. *Anticancer Res*. 1999; 19: 951–958. [Abstract](#)
3. Lan Q, Chen W, Chen H, He XZ. Risk factors for lung cancer in non-smokers in Xuanwei County of China. *Biomed Environ Sci*. 1993; 6: 112–118. [Abstract](#)
4. Bishop JM. The molecular genetics of cancer. *Science*. 1987; 235: 305–311. [Abstract](#)
5. Harris CC. p53: at the crossroads of molecular carcinogenesis and risk assessment. *Science*. 1993; 262: 1980–1981. [Citation](#)
6. Harris CC. Molecular basis of multistage carcinogenesis. *Princess Takamatsu Symp*. 1991; 22: 3–19. [Abstract](#)
7. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science*. 1991; 253: 49–53. [Abstract](#)
8. Vogelstein B, Kinzler KW. p53 function and dysfunction. *Cell*. 1992; 70: 523–526. [Citation](#)
9. Cherpillod P, Amstad PA. Benzo[a]pyrene-induced mutagenesis of p53 hot-spot codons 248 and 249 in human hepatocytes. *Mol Carcinog*. 1995; 13: 15–20. [Abstract](#)
10. Ruggeri B, DiRado M, Zhang SY, Bauer B, Goodrow T, Klein-Szanto AJ. Benzo[a]pyrene-induced murine skin tumors exhibit frequent and characteristic G to T mutations in the p53 gene. *Proc Natl Acad Sci USA*. 1993; 90: 1013–1017. [Abstract](#)
11. Lee LN, Shew JY, Sheu JC, et al. Exon 8 mutation of p53 gene associated with nodal metastasis in non-small-cell lung cancer. *Am J Respir Crit Care Med*. 1994; 150: 1667–1671. [Abstract](#)
12. Takeshima Y, Seyama T, Bennett WP, et al. p53 mutations in lung cancers from non-smoking atomic-bomb survivors. *Lancet*. 1993;342:1520–1521. (Erratum in *Lancet*. 1994;343:1302.)
13. Wang X, Christiani DC, Wiencke JK, et al. Mutations in the p53 gene in lung cancer are associated with cigarette smoking and asbestos exposure. *Cancer Epidemiol Biomarkers Prev*. 1995; 4: 543–548. [Abstract](#)
14. Hall PA, Lane DP. p53 in tumour pathology: can we trust immunohistochemistry?—Revisited [editorial; see comments]. *J Pathol*. 1994; 172: 1–4. [Citation](#)
15. Korobowicz E, Zdunek M. Immunohistochemical study of p53 in non-small cell lung cancer before and after preoperative chemotherapy. *Pol J Pathol* 2000; 51: 71–76. [Abstract](#)
16. Feng Z, Tian D, Lan Q, Mumford JL. A sensitive immunofluorescence assay for detection of p53 protein accumulation in sputum. *Anticancer Res*. 1999; 19: 3847–3852. [Abstract](#)
17. Lan Q, He X, Costa DJ, et al. Indoor coal combustion emissions, GSTM1 and GSTT1 genotypes, and lung cancer risk: a case-control study in Xuan Wei, China. *Cancer Epidemiol Biomarkers Prev*. 2000; 9: 605–608. [Abstract](#)
18. Saccomanno G. *Diagnostic Pulmonary Cytology: Procedure in Sputum Cytology*. Chicago, IL: *American Society of Clinical Pathologists Press*; 1986: 3–5.
19. He XZ, Yang RD. Lung Cancer and Indoor Air Pollution From Coal Burning. *Yuan Nan Science and Technology*

<http://home.mdconsult.com/das/article/body/1/jorg=journal&source=MI&sp=11910054&s...> 4/11/2003

*Publishing House*; 1994: 1–61.

20. Cytel Software Corporation. StatXact 4 for Windows. Cambridge, MA: *Cytel*; 1999.

21. Terry MB, Gammon MD, Ng-Mak D, Thompson WD. p53 protein overexpression in relation to risk factors for breast cancer [letter; comment]. *Am J Epidemiol*. 1998; 147: 511–512. [Citation](#)

22. Rusin M, Butkiewicz D, Malusecka E, et al. Molecular epidemiological study of non-small-cell lung cancer from an environmentally polluted region of Poland. *Br J Cancer*. 1999; 80: 1445–1452. [Abstract](#)

23. Liloglou T, Ross H, Prime W, et al. p53 gene aberrations in non-small-cell lung carcinomas from a smoking population. *Br J Cancer*. 1997; 75: 1119–1124. [Abstract](#)

24. Husgafvel-Pursiainen K, Boffetta P, Kannio A, et al. p53 mutations and exposure to environmental tobacco smoke in a multicenter study on lung cancer. *Cancer Res*. 2000; 60: 2906–2911. [Abstract](#)

MD Consult L.L.C. <http://www.mdconsult.com>

**Bookmark URL:** [/das/journal/view/27614874/N/11910054?ja=214802&PAGE=1.html&ANCHOR=top&source=MI](http://home.mdconsult.com/das/journal/view/27614874/N/11910054?ja=214802&PAGE=1.html&ANCHOR=top&source=MI)