

# DNA Repair Activity for Oxidative Damage and Risk of Lung Cancer

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**Background:** Although smoking is a major cause of lung cancer, only a proportion of smokers develop lung cancer, suggesting a genetic predisposition in some individuals. Because tobacco smoking is associated with the increased formation of DNA lesions, including those induced from oxidative damage, we investigated whether the activity of the DNA repair enzyme 8-oxoguanine DNA *N*-glycosylase (OGG), which repairs the oxidative DNA lesion 8-oxoguanine, is associated with lung cancer. **Methods:** We conducted a molecular epidemiologic case-control study that included 68 case patients with non-small-cell lung cancer and 68 healthy control subjects, frequency matched for age and sex. Enzymatic OGG activity was determined in protein extracts prepared from peripheral blood mononuclear cells or lung tissue by assaying the cleavage product of a radiolabeled synthetic DNA oligonucleotide containing an 8-oxoguanine residue. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined by conditional logistic regression. All statistical tests were two-sided. **Results:** OGG activity was lower in peripheral blood mononuclear cells from case patients than in those from control subjects. After adjustment for age and smoking status, individuals in the lowest tertile of OGG activity had an increased risk of non-small-cell lung cancer compared with individuals in the highest tertile (OR = 4.8, 95% CI = 1.5 to 15.9). The adjusted OR associated with a unit decrease in OGG activity was statistically significantly increased (OR = 1.9, 95% CI = 1.3 to 2.8). There was no interaction between OGG activity and smoking status. The estimated relative risk of lung cancer for smokers with low OGG activity was 34- or 124-fold higher for smokers with a low OGG activity of 6.0 or 4.0 U/ $\mu$ g protein, respectively, than for nonsmokers with a normal OGG activity of 7.0 U/ $\mu$ g protein, illustrating the cumulative effect of low OGG activity and smoking. **Conclusions:** Low OGG activity is associated with an increased risk of lung cancer. Although prospective studies are needed to validate the results, they suggest that smoking cessation in individuals with reduced OGG activity might be an effective strategy in lung cancer prevention. [J Natl Cancer Inst 2003;95:1312-19]

The high incidence and poor prognosis of lung cancer make it a major health problem worldwide (1-3). A major cause of lung cancer is tobacco smoking, reflected by the fact that 80%-90% of lung cancer patients smoke (1). Although approximately 10% of heavy smokers (with more than 20 pack-years of smoking) develop lung cancer, the percentage of all smokers who develop lung cancer is substantially lower (4). This indicates that the carcinogenic effect of tobacco smoke affects only a fraction of those who smoke, most likely a result of differences in genetic predisposition to lung cancer (5-7). The identification of genetic risk factors might be useful in lung cancer prevention.

Tobacco smoke contains multiple carcinogens that are known to chemically modify DNA (8) and lead to the formation of mutations (9). The accumulation of mutations in critical oncogenes and tumor suppressor genes promotes cancer (10). A critical cellular response that counteracts the carcinogenic effects of DNA damage is DNA repair (11). There are several known pathways of DNA repair, including base excision, nucleotide excision, and mismatch repair, all of which act to remove DNA lesions and prevent mutations, thereby restoring genetic integrity. The importance of DNA repair pathways is illustrated by a number of hereditary diseases, in which individuals with defects in DNA repair genes are highly susceptible to cancer. For example, defects in the nucleotide excision-repair pathway are associated with a high predisposition to skin cancer in patients with xeroderma pigmentosum (12), and defects in mismatch repair genes are associated with hereditary non-polyposis colorectal cancer (13).

Several studies have investigated whether reduced DNA repair is associated with lung cancer [reviewed in (14,15)]. Reduced repair of benzo[*a*]pyrene DNA adducts, as measured by the host-cell reactivation assay, is associated with lung cancer (16). This method measures the expression of a reporter gene from a plasmid that has been treated with a DNA-damaging agent and introduced into cultured cells by transfection. The plasmids are repaired inside the cells, leading to increased gene expression. Because benzo[*a*]pyrene-DNA lesions are repaired via nucleotide excision repair, the degree of reporter gene expression is believed to reflect the activity of this repair pathway (17). In addition, reduced DNA repair of bleomycin-induced damage was found to be associated with lung cancer, using the comet assay (18). This method measures single-strand breaks in genomic DNA as a generalized measure of DNA repair activity, although it is difficult to relate that activity to specific DNA repair pathways. Nevertheless, these studies suggested that the reduced activity of various DNA repair mechanisms predisposes individuals to lung cancer and prompted us to search for defined DNA repair activities that may be risk factors for lung cancer. In particular, we sought to identify DNA repair mechanisms whose reduced activity predisposes smokers to lung cancer.

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Because tobacco smoke induces the formation of oxidative DNA damage, we investigated whether reduced repair of oxidative DNA damage is associated with lung cancer. We focused on the repair of DNA containing 8-oxoguanine, a mutagenic lesion that is formed by oxidation (19), ionizing radiation (20), and tobacco smoke (21). 8-Oxoguanine is removed from DNA, primarily by base excision repair, in a process that is initiated by the enzyme 8-oxoguanine DNA *N*-glycosylase encoded by the OGG1 gene. This enzyme cleaves the bond linking the oxidized guanine to the deoxyribose and then breaks the DNA strand via its apurinic lyase activity [reviewed in (22)]. An additional DNA glycosylase with a similar activity is the recently discovered NEIL1; however, its activity is minor compared with that of the OGG1 gene product (23). In this study, we examined whether OGG activity is associated with non-small-cell lung cancer (NSCLC).

## SUBJECTS AND METHODS

### Study Subjects

Lung cancer patients were enrolled from the Lung Institute in the Sheba Medical Center during the period from April 1999 through January 2002. All patients had histopathologically confirmed operable NSCLC. The healthy control subjects were volunteers from the Sheba Medical Center and the Weizmann Institute of Science, including employees, retired employees, and relatives of employees. Each subject was interviewed by the team physician (M. Krupsky). A standard questionnaire was used to collect information on demographic data and lung cancer risk factors, including smoking status and family history of first-degree relatives with cancer. Exclusion criteria were prior chemotherapy or radiation therapy (for the case patients), prior cancer (for control subjects), and previous smoking (for case patients and control subjects). The study involved only people who never smoked (nonsmokers) or who currently smoked (current smokers). A total of 110 lung cancer patients were enrolled, of whom eight were excluded. One hundred two patients were included in the analysis. Of the 129 healthy subjects who were enrolled, six were excluded. The groups were frequency-matched for sex and age by dividing the case subjects and control subjects by sex and 15-year age groups, calculating the percentages in each group, and randomly sampling sets with comparable frequencies for each sex and age group. This finally led to matched groups of 68 lung cancer patients and 68 healthy control subjects, for which the statistical analysis was performed. The age and sex distribution of case patients and control subjects is shown in Table 1. All subjects provided written informed consent. The study was approved by the institutional ethics committees of the Sheba Medical Center and the Weizmann Institute of Science.

### Isolation of Peripheral Blood Mononuclear Cells and Preparation of Protein Extracts

Each study participant provided a sample (10 mL) of peripheral blood collected in a 50-mL tube containing 1.4 mL of an anticoagulant citrate-phosphate-dextrose-adenine solution (CPDA-1; Teva Medical, Ashdod, Israel). Blood samples were taken from case patients at time points ranging from 4 months before surgery to more than 1 year after surgery. Blood samples were taken from control subjects during the same time period when samples were collected from case patients. The blood

**Table 1.** Age and sex distribution of control subjects and case patients

Variable	Control subjects		Case patients		<i>P</i> †
	No. (%)	Mean age (95% CI)*	No. (%)	Mean age (95% CI)	
All subjects	68 (100)	65 (63 to 67)	68 (100)	66 (64 to 69)	.472
Male					
All	37 (54)	64 (61 to 68)	38 (56)	65 (61 to 69)	.854
Age, y					
40–55	11 (16)	51 (49 to 53)	9 (13)	49 (44 to 53)	.185
56–70	13 (19)	66 (64 to 68)	15 (22)	64 (62 to 66)	.240
71–85	13 (19)	74 (72 to 77)	14 (21)	76 (74 to 78)	.178
Female					
All	31 (46)	66 (63 to 69)	30 (44)	68 (65 to 72)	.332
Age, y					
40–55	4 (6)	50 (44 to 57)	4 (6)	51 (44 to 58)	.804
56–70	18 (26)	65 (62 to 67)	15 (22)	66 (64 to 68)	.354
71–85	9 (13)	76 (73 to 78)	11 (16)	78 (75 to 80)	.210

\*CI = confidence interval.

†The results of two-sided Student's *t* tests (with equal variance) between the case and control subjects.

samples were processed within 18–24 hours of collection. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation on a polysucrose–sodium metrizoate medium in UNI-SEP tubes (NOVamed, Jerusalem, Israel). After centrifugation, the PBMCs were washed with phosphate-buffered saline and were treated with 5 mL of a buffer containing 155 mM NH<sub>4</sub>Cl, 0.01 M KHCO<sub>3</sub>, and 0.1 mM EDTA for 4 minutes at room temperature to lyse any red blood cells. The PBMCs were pelleted by centrifugation and, after discarding the supernatant, were resuspended at a concentration of 20 000 cells/μL in a buffer containing 50 mM Tris–HCl (pH 7.1), 1 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM spermidine, 0.1 mM spermine, and a protease inhibitor cocktail (Sigma, St. Louis, MO). The PBMCs were incubated on ice for 30 minutes, after which they were frozen in liquid nitrogen and stored at –80 °C.

To generate protein extracts, the frozen PBMCs were thawed at 30 °C and incubated in a potassium chloride solution (220 mM) for 30 minutes on ice. Cell debris was removed by centrifugation at 11 000g for 15 minutes at 4 °C. Glycerol was then added to the protein extract to a final concentration of 10%, and the extract was frozen in liquid nitrogen. Protein concentration in the extract was determined with the bicinchoninic acid assay kit (Pierce, Rockford, IL), using bovine γ-globulin as a standard.

Non-tumor lung samples were obtained from NSCLC patients who underwent lobectomy or pneumonectomy as the primary treatment. One big piece, distal to the tumor (10–15 cm), was removed from the resected tissue and immediately frozen in liquid nitrogen. Small slices (each weighing approximately 40–90 mg) were taken from the frozen sample, pulverized with a pestle and mortar, and used for preparing protein extracts as described above for the PBMCs. DNA content in lung tissue homogenates was determined by using a fluorescence-enhanced assay with bisbenzimidazole (Hoechst 33258; Sigma) and calf thymus DNA as a standard, as described (24).

### OGG Activity Assay

Because OGG removes 8-oxoguanine residues from DNA, we developed an assay to quantify this activity. The assay monitors the ability of OGG to remove an 8-oxoguanine residue from

a radiolabeled synthetic DNA oligonucleotide, generating two DNA products that can be distinguished on the basis of size. Enzymatic activity of OGG was determined by incubating a 32-base-pair-long oligonucleotide containing a site-specific 8-oxoguanine residue with the PBMC protein extract. The oligonucleotides were synthesized by the Synthesis Unit of the Department of Biological Services at the Weizmann Institute, using the Expedite 8909 DNA Synthesizer (Applied Biosystems, Foster City, CA). The 8-oxoguanine phosphoramidite building block was purchased from Glen Research (Sterling, VA) and used to generate an oligonucleotide with the sequence 5'-CCG GTGCATGACACTGTGTOACCTATCCTCAGCG-3' (O = 8-oxoguanine). The oligonucleotide was <sup>32</sup>P-labeled using T4 polynucleotide kinase, and annealed to a complementary oligonucleotide 5'-CGCTGAGGATAGGTCACAGTGTCATG CACCGG-3' in the presence of 150 mM NaCl for 10 minutes at 70°C, followed by cooling in a water bath kept at room temperature. The radiolabeled duplex DNA was separated from unannealed oligonucleotides by polyacrylamide gel electrophoresis (PAGE) on a native 10% gel. The duplex DNA was purified from the gel to be used as a substrate in the OGG activity assay.

For the OGG activity assay, the standard reaction mixture (20 μL) contained 50 mM Tris-HCl (pH 7.1), 1 mM EDTA, 115 mM KCl, 20 μg of bovine γ-globulin, 2 pmol of polyA polydT, 0.5 pmol of radiolabeled duplex DNA, and 8–12 μg of protein extract. The reaction mixture was incubated at 37°C for 30 minutes, and the reaction was stopped by the addition of 15 mM EDTA and 0.2% sodium dodecyl sulfate. The proteins in the reaction mixture were then degraded by proteinase K (20 μg) during an incubation for 1 hour at 37°C. The reaction mixture was then incubated with 80 mM NaOH for 30 minutes at 37°C, and the denatured DNA products were subjected to electrophoresis on a 15% polyacrylamide gel containing 8 M urea (urea-PAGE) in 89 mM Tris-borate and 2.5 mM EDTA (pH 8.0) at 1500 V for 2 hours at 45–50°C. The distribution of the radiolabeled DNA products was visualized and quantified using a Fuji BAS 2500 PhosphorImager. One unit of OGG activity is defined as the amount of protein that cleaves 1 fmol of DNA substrate in 1 hour at 37°C, under the standard reaction conditions described here. Specific OGG activity was calculated by dividing the activity (in units) by the amount of protein (in micrograms) in the reaction mixture. For a tissue such as the lung, where a substantial fraction of the proteins originates from the extracellular matrix rather than from cellular contents, OGG activity cannot be normalized to the total protein amount and was therefore normalized to the total DNA amount, better representing the intracellular contents. To normalize the OGG activity to DNA content, an additional 0.5 mg of lung tissue was used to determine the amount of DNA. Specific activity was then calculated by dividing OGG activity (in units) by the amount of DNA (in nanograms) in the reaction mixture. A single assay required 200 000 purified cells, which can be obtained from 0.5–2 mL of whole blood or 2.5 mg of lung tissue. The activity in each sample was measured in duplicate, and each assay was performed twice.

### Statistical Analysis

Student's *t* test (with equal variance) was performed to compare mean age between control subjects and case patients, mean OGG activity for younger (aged ≤60 years) versus older (aged >60 years) participants within the case patients and within con-

trol subjects, and mean OGG activity between case subjects with adenocarcinoma and squamous cell carcinoma. Other mean OGG activities were compared using analysis of covariance, with age (treated as a continuous variable) as a covariate for adjustment.

Adjusted odds ratios (ORs), and confidence intervals (CIs) were calculated by conditional logistic regression models, with adjustment for age (years), and smoking status (smoker or nonsmoker). In addition, we calculated odds ratios and confidence intervals using nonconditional logistic regression, with adjustment for age, smoking status, and sex. The two methods yielded similar results. OGG activity values were analyzed as a continuous variable or partitioned in two ways. First, the median OGG activity of control subjects was used as the cutoff value (OGG activity cut point at 7.2 U/μg protein). Values above this median were considered to be normal (i.e., sufficient) OGG activity, whereas values equal to or below the median were considered to be low (i.e., suboptimal) OGG activity. Second, a partition by tertiles was performed, where the upper tertile of the control subjects (OGG activity >7.5 U/μg protein) was chosen as the referent group. To perform the linear trend test, the tertiles were recoded as 1, 2, and 3 and treated as one continuous variable in the multiple logistic regression.

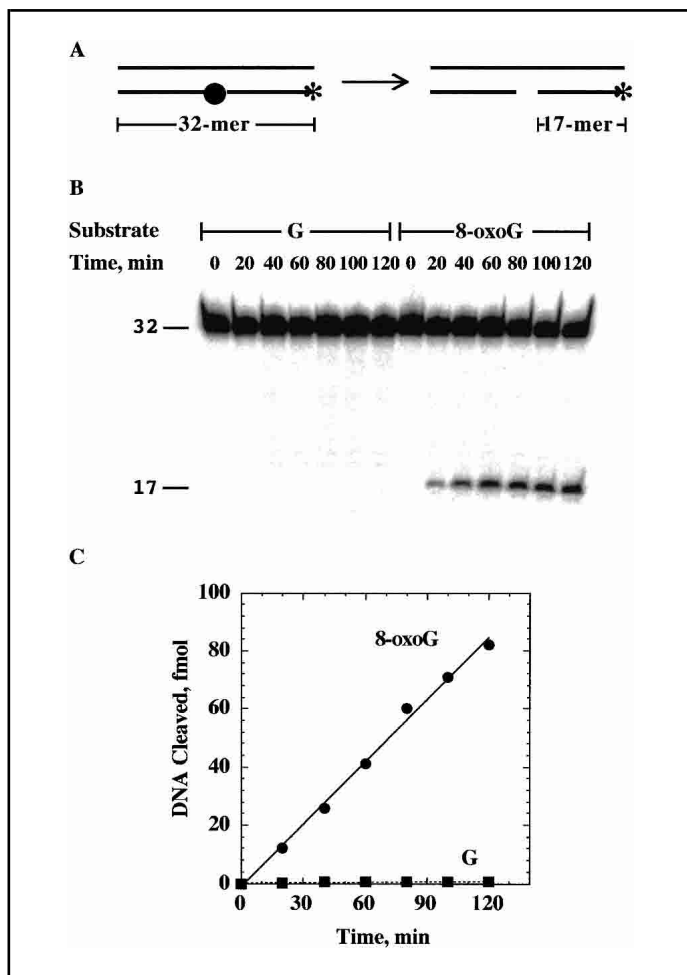
To integrate the effects of OGG activity and smoking at a given age we used the formula (25):  $OR_i = e^{-0.64969(OGGA_i - OGGA_0) + 2.86785(S_i - S_0)}$ , where  $OGGA_i$  and  $OGGA_0$  are the OGG values of individual *i* and of the reference, respectively, and  $S_i$  (smoking status of individual *i*) and  $S_0$  (the reference smoking status) are either 1 or 0 for a smoker or a nonsmoker, respectively. In this equation, the estimate of the change in the log OR that would result from a one-unit increase in each of the variables when the other variable is fixed were derived from the conditional logistic regression model where OGG activity values were analyzed as a continuous variable.

A linear regression analysis was performed for the relationship between OGG activity in the lung (the dependent variable) and OGG activity in PBMC (the independent variable). All statistical tests were two-sided and were performed using SAS (Statistical Analysis System) software (version 8e for Windows; SAS Institute, Cary, NC).

## RESULTS

### OGG Activity Assay

To examine whether reduced OGG activity is associated with NSCLC, we first developed a reproducible assay to measure OGG activity in PBMCs. Because OGG activity removes 8-oxoguanine residues from DNA, we generated a synthetic DNA substrate containing a single 8-oxoguanine residue. The DNA oligonucleotide was radiolabeled with <sup>32</sup>P at the 5' end of the DNA strand containing the lesion (Fig. 1, A). OGG activity removes the modified guanine, leading to the formation of an abasic site. This site is then cleaved by the AP lyase activity of the enzyme or by the action of AP endonucleases present in the protein extract. The cleaved DNA fragments can then be separated by urea-PAGE, and the radiolabeled fragments can be distinguished on the basis of size (fragments of 32 or 17 nucleotides in length). The extent of the cleaved DNA can be quantified by phosphorimaging. We first determined a time course of OGG activity in PBMC protein extracts (Fig. 1, B). DNA cleav-



**Fig. 1.** Assay for the activity of the DNA repair enzyme 8-oxoguanine DNA *N*-glycosylase (OGG). **A**) Outline of the assay. A radiolabeled synthetic short double-stranded DNA carrying a site-specific 8-oxoguanine is incubated with a protein extract. OGG activity cleaves the 8-oxoguanine site (filled circle). After denaturation, this leads to the conversion of the radiolabeled 32 bases to a 17-base oligonucleotide. Cleavage products are separated by polyacrylamide gel electrophoresis in the presence of urea. Asterisks represent the radiolabeled DNA terminus. **B**) Time course of OGG activity in a protein extract prepared from peripheral blood mononuclear cells. The substrate containing 8-oxoguanine (8-oxoG) is cleaved to yield the 17-mer, whereas the control DNA (G) without the oxidized guanine residue is not cleaved. **C**) The amount of radioactivity in the 17- and 32-base fragments was quantified using phosphorimaging and is expressed as a function of the amount of cleaved DNA (in femtomoles) versus time (in minutes). Filled squares = control DNA (G); filled circles = DNA containing 8-oxoguanine. The results from a representative experiment are shown.

age occurred only in the substrate containing 8-oxoguanine and not in the control substrate containing an unmodified guanine residue instead of 8-oxoguanine. We observed a linear increase in DNA cleavage (Fig. 1, B) with an increasing time of incubation (Fig. 1, C). The amount of DNA cleavage was also linear in relation to the protein concentration within the reaction mixture (data not shown). The OGG activity assay was accurate and reproducible, with a coefficient of variation of 10%. We tested OGG activity from the same eight individuals over a period of 3 years and found that the coefficient of variation ranged from 2% to 12%, with an average of 7% (95% CI = 4% to 10%) (Table 2). Thus, we concluded that OGG activity values from the same individual are stable over a period of at least 3 years.

**Table 2.** Stability over time of 8-oxoguanine DNA *N*-glycosylase (OGG) activity in peripheral blood mononuclear cells isolated from the same individual\*

Subject	Age	Sex	OGGA value			Average OGGA	CV, %
			Time of assay†				
			0	6 mo	3 y		
S1	27	F	6.4	6.7	6.4	6.5	3
S4	38	F	5.6	6.5	5.4	5.8	10
S6	47	M	6.3	7.3	5.8	6.5	12
S7	34	M	7.9	7.0	7.8	7.6	7
S8	47	M	7.8	7.4	8.3	7.8	6
S9	37	F	8.5	7.4	7.0	7.6	10
S11	29	F	8.1	7.5	8.8	8.1	8
S13	48	F	6.6	6.4	6.3	6.4	2
Average CV, %							7 (4 to 10)
(95% CI)							

\*OGGA = 8-oxoguanine DNA *N*-glycosylase activity; CV = coefficient of variation; CI = confidence interval; M = male; F = female.

†Time of assay refers to the time the blood samples were drawn and analyzed. OGG activity was assayed in peripheral blood mononuclear cells from the same individual in April 1999 (t = 0), October 1999 (t = 6 mo), and May 2002 (t = 3 y), as described in the "Subjects and Methods" section. The average CV of all individuals combined over 3 years was 7% (95% CI = 4% to 10%).

### Reduced OGG Activity in NSCLC Patients

To examine whether there is an association between OGG activity and lung cancer, we performed a case-control study with 68 lung cancer patients and 68 healthy control subjects, frequency matched for age and sex (Table 1). The mean OGG activity in the control subjects was 7.1 U/ $\mu$ g protein (95% CI = 6.8 to 7.3 U/ $\mu$ g protein) (Table 3). There was no statistically significant difference in mean OGG activity between men (7.0 U/ $\mu$ g protein, 95% CI = 6.7 to 7.4 U/ $\mu$ g protein) and women (7.1 U/ $\mu$ g protein, 95% CI = 6.7 to 7.5 U/ $\mu$ g protein) ( $P = .589$ ), or between smokers (7.3 U/ $\mu$ g protein, 95% CI = 6.9 to 7.6 U/ $\mu$ g protein) and nonsmokers (7.0 U/ $\mu$ g protein, 95% CI = 6.7 to 7.3 U/ $\mu$ g protein) ( $P = .955$ ; Table 3). By contrast, there was a small but statistically significant 9.2% decrease in mean OGG activity values between individuals aged 60 years or younger (mean OGG activity value = 7.6 U/ $\mu$ g protein, 95% CI = 7.1 to 8.0 U/ $\mu$ g protein) and individuals aged older than 60 years (mean OGG activity value = 6.9 U/ $\mu$ g protein, 95% CI = 6.6 to 7.1 U/ $\mu$ g protein) ( $P = .012$ ; Table 3).

The mean OGG activity value of case patients (5.8 U/ $\mu$ g protein, 95% CI = 5.5 to 6.2 U/ $\mu$ g protein) was statistically significantly lower than the mean value of controls (7.1 U/ $\mu$ g protein, 95% CI = 6.8 to 7.3 U/ $\mu$ g protein) ( $P < .001$ ). The mean OGG activity value of case patients with adenocarcinoma was similar to that of case patients with squamous cell carcinoma (Table 3). Analyses of the case patients showed no statistically significant differences in the mean OGG activity values between men and women, between smokers and nonsmokers, or between individuals aged 60 years or younger and those aged older than 60 years (Table 3).

We next compared the distributions of OGG activity values in case patients with those in control subjects. Overall, the distribution of OGG activity values in case patients was shifted to lower values than those in control subjects (Fig. 2), with 41% of case patients but only 4% of control subjects having OGG activity values less than or equal to 5.5.

**Table 3.** Distribution of selected characteristics and DNA repair 8-oxoguanine DNA *N*-glycosylase (OGG) activity values between non-small-cell lung cancer case patients and control subjects\*

Variable	Control subjects (n = 68)		Case patients (n = 68)		P†
	No.	OGGA value (95% CI)	No.	OGGA value (95% CI)	
All	68	7.1 (6.8 to 7.3)	68‡	5.8 (5.5 to 6.2)	<.001
SQCC			17	5.5 (4.6 to 6.4)	
Adenocarcinoma			27	5.9 (5.3 to 6.5)	
				P = .411§	
Age, y					
≤60	20	7.6 (7.1 to 8.0)	19	6.2 (5.5 to 6.9)	.003
>60	48	6.9 (6.6 to 7.1)	49	5.7 (5.3 to 6.2)	<.001
		P = .012§		P = .256§	
Sex					
Male	37	7.0 (6.7 to 7.4)	38	5.9 (5.4 to 6.5)	.001
Female	31	7.1 (6.7 to 7.5)	30	5.7 (5.3 to 6.2)	<.001
		P = .589		P = .637	
Smoking status					
Nonsmoker	51	7.0 (6.7 to 7.3)	16	6.1 (5.4 to 6.8)	.012
Smoker	17	7.3 (6.9 to 7.6)	52	5.8 (5.3 to 6.2)	.002
		P = .955		P = .457	

\*OGG activity (OGGA) was measured as described in the "Subjects and Methods" section. OGGA value: mean (95% confidence interval [CI]), in enzyme units per microgram protein extract. SQCC = squamous cell carcinoma.

†Results of analysis of covariance between case patients and control subjects, with age (treated as a continuous variable) as a covariate.

‡Of the 68 case patients, 17 had squamous cell carcinoma, 27 had adenocarcinoma, and 24 were either other subtypes or unclassified non-small-cell lung cancer.

§Result of two-sided Student's *t* tests with equal variance.

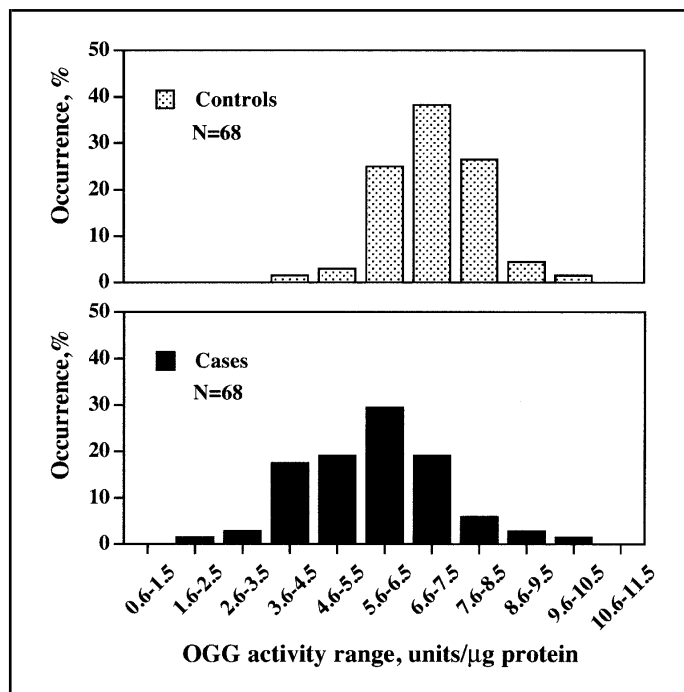
||Results of analysis of covariance among case patients or among control subjects, with age (treated as a continuous variable) as a covariate.

### OGG Activity in PBMCs Reflects OGG Activity in the Lungs

We next determined whether OGG activity values in PBMC reflect OGG activity values in the lung. Because surgery was the primary therapy for the case patients, we obtained lung and blood samples from some of the patients and compared their levels of OGG activity. An initial analysis of OGG activity in samples from seven case patients, using linear regression with OGG activity in PBMC as the dependent variable and OGG activity in lungs as the independent variable, revealed a good fit, with  $R^2 = 0.858$  and  $P = .003$  (Fig. 3). This result indicates that OGG activity in PBMCs can serve as a surrogate for OGG activity in the lungs.

### OGG Activity in PBMCs From NSCLC Patients is not Related to the Presence of a Tumor

In case-control studies, there is a possibility that the examined variable is a consequence of the disease rather than a risk factor. For example, in our study, the OGG activity value may be affected by factors secreted into the blood stream by the tumor. Because the primary treatment for NSCLC is surgery, if blood samples are taken from patients after the tumor has been removed, the effect (if any) of putative secreted factors might decay. We therefore examined whether there was a correlation between OGG activity and the time between surgery and taking the blood sample, a time period that ranged from 4 months before surgery to more than 1 year after surgery. For 62 of the original group of 102 patients, blood samples were taken within

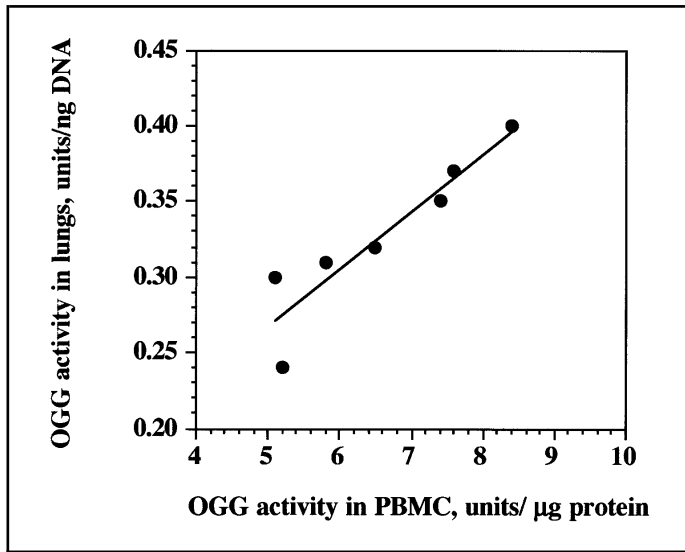


**Fig. 2.** Distribution of 8-oxoguanine DNA *N*-glycosylase (OGG) activity, an enzyme involved in DNA repair, from peripheral blood mononuclear cells from non-small-cell lung cancer patients and control subjects. OGG activity is measured in cell extracts as the ability to cleave a radiolabeled synthetic DNA oligonucleotide that contains a single site-specific 8-oxoguanine residue. The activity is quantified as enzyme units per microgram protein. OGG activity values of 68 control subjects (upper panel) and 68 case patients (lower panel) are shown.

4 months after the date of surgery. We found no correlation between OGG activity and time between surgery and taking the blood sample, indicating that this variable had no effect on the level of OGG activity in PBMCs. Although this result is not a definitive proof, it is consistent with the notion that reduced OGG activity in PBMCs is not directly related to the presence of the lung tumor.

### Reduced OGG Activity in PBMCs is Associated With NSCLC

To determine whether there was an association between reduced OGG activity and NSCLC, we used conditional logistic regression, in which the presence or absence of lung cancer was the binary dependent variable, age was a continuous variable, smoking status (smokers versus nonsmokers) was a dichotomous variable, and OGG activity was categorized by three different methods. Reduced OGG activity was found to be associated with NSCLC, as indicated by the statistically significant odds ratios obtained using three conditional logistic regression models. When OGG activity was used as a continuous variable, with adjustment for age and smoking status, the adjusted odds ratio for lung cancer associated with a one-unit decrease in OGG activity was statistically significantly increased (OR = 1.9, 95% CI = 1.3 to 2.8;  $P < .001$ ) (Table 4). When OGG activity values were dichotomized by the median OGG activity of the control subjects (with OGG activity above the median defined as normal and that equal to or below the median as low), the adjusted odds ratio for lung cancer associated with low OGG activity was 5.2 (95% CI = 1.9 to 14.1;  $P = .001$ ) (Table 4). In the third con-



**Fig. 3.** Relationship between DNA repair 8-oxoguanine DNA *N*-glycosylase (OGG) activities in protein extracts from peripheral blood mononuclear cells (PBMCs) and lung tissue in the same seven lung cancer patients. OGG activity is measured in cell extracts as the ability to cleave a radiolabeled synthetic DNA oligonucleotide that contains a single site-specific 8-oxoguanine residue. The activity in PBMCs is quantified as enzyme units per microgram protein. OGG activity from lung tissue is quantified as enzyme units per nanogram DNA. A linear regression analysis was performed with OGG activity in the lung as the dependent variable and OGG activity in PBMCs as the independent variable. The goodness of fit was  $R^2 = .858$ , and  $P = .003$ .

**Table 4.** Conditional logistic regression analysis of 8-oxoguanine DNA *N*-glycosylase (OGG) activity value in lung cancer patients and control subjects\*

Variable	No. of case patients (%)	No. of control subjects (%)	Adjusted <sup>†</sup> OR (95% CI)
OGGA <sup>‡</sup>	68 (100.0)	68 (100.0)	1.9 (1.3 to 2.8) $P < .001$
OGGA (by median) <sup>§</sup>			
>7.2 U/ $\mu$ g protein	12 (17.6)	33 (48.5)	1.0 (referent)
$\leq$ 7.2 U/ $\mu$ g protein	56 (82.4)	35 (51.5)	5.2 (1.9 to 14.1) $P = .001$
OGGA (by tertiles) <sup>  </sup>			
>7.5 U/ $\mu$ g protein	7 (10.3)	22 (32.4)	1.0 (referent)
6.7–7.5 U/ $\mu$ g protein	13 (19.1)	24 (35.3)	1.4 (0.4 to 4.7) $P = .62$
<6.7 U/ $\mu$ g protein	48 (70.6)	22 (32.4)	4.8 (1.5 to 15.9) $P = .01$
Trend test <sup>¶</sup>			$P = .001$

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

<sup>†</sup>Conditional logistic regression for matched sets adjusted for age and smoking status (smoker, nonsmoker).

<sup>‡</sup>OGG activity (OGGA) was measured as described in the “Subjects and Methods” section and was fitted in the conditional logistic regression model as a continuous variable. The odds ratio for smoking, obtained with this model, was 18 (95% CI = 6 to 53;  $P < .001$ ).

<sup>§</sup>Median of the control subjects’ values.

<sup>||</sup>Tertiles of the control subjects’ values. The upper tertile was chosen as the referent.

<sup>¶</sup>Adjusted for the same variable as described above.

ditional logistic regression model, OGG activity values were further divided by tertiles of OGG activity in the control subjects. By using the tertile with the highest OGG activity as the referent group, the adjusted odds ratio for lung cancer for the lowest tertile was 4.8 (95% CI = 1.5 to 15.9;  $P = .01$ ) (Table

4). The odds ratios of the middle tertile were not statistically significant (Table 4).

#### Combined Effect of Reduced OGG Activity and Smoking on the Estimated Relative Risk for NSCLC

For diseases that do not occur frequently, such as lung cancer, and assuming that the groups of case patients and control subjects are reasonably representative of the population, the odds ratio can be used as the estimated relative risk (26). Thus, the adjusted odds ratios in Table 4 provide the estimated relative risk for lung cancer that is associated with low OGG activity. A more useful relative risk estimate is obtained by combining the effects of the known risk factor (i.e., smoking) and the new suggested risk factor (reduced OGG activity). The absence of a statistical interaction between smoking and reduced OGG activity implies that the two are independent risk factors for lung cancer. This suggests that for individuals affected by both risk factors—namely, smokers with reduced OGG activity—the combined estimated relative risk might be a multiplication of the estimated relative risk of each of the risk factors alone. We used the conditional logistic regression model with OGG activity as a continuous variable to determine the association of smoking with NSCLC. We found that smoking was strongly associated with NSCLC, with OR = 18 (95% CI = 6.0 to 53;  $P < .001$ ). This value is similar to the currently accepted measures of the relative risk of smokers to develop lung cancer (1,27). To illustrate the combined effect of low OGG activity and smoking, and using the conditional logistic regression equation presented in the “Subjects and Methods” section, the estimated relative risk of lung cancer for smokers with OGG activity of 6.0 or 4.0 was 34- or 124-fold higher than that for nonsmokers with an OGG activity of 7.0. By contrast, nonsmokers with the same respective low OGG activity value had estimated relative risks only 1.9- or 7.0-fold higher than those for nonsmokers with an OGG activity of 7.0.

#### DISCUSSION

The OGG activity test offers a new functional DNA repair assay suitable for molecular epidemiologic studies of oxidative DNA damage and cancer risk. The test measures the removal of 8-oxoguanine from DNA and is based on assays that are used to measure the activity of various DNA *N*-glycosylases (28). The OGG activity test has the advantages of being simple, accurate, and reproducible, making it applicable for population screening. A disadvantage of the assay is that the PBMCs have to be isolated from the blood sample within 24 hours. However, after being purified, PBMCs can be stored frozen for at least a year before being assayed without losing OGG activity.

Our data indicate that reduced OGG activity is associated with NSCLC and that reduced activity is a statistically significant risk factor for lung cancer. This conclusion is supported by the following considerations: 1) The mean OGG activity values for case patients were statistically significantly lower than those for control subjects when either the entire groups were compared or when any subgroups were compared (i.e., male case patients versus male control subjects, case patients who were smokers versus control subjects who were smokers; Table 2). 2) The odds ratios of lung cancer associated with low OGG activity were large and highly statistically significant. In addition to indicating a strong association, this result also argues against the possibility of a selection bias in the control group. 3) The odds ratios for

OGG activity being a risk factor for lung cancer were statistically significant after adjustments were made for possible confounding effects of sex, age, and smoking status. 4) Smoking, which is a known risk factor for lung cancer, yielded a highly statistically significant adjusted odds ratio of 18, which is similar to the currently accepted relative risk of smokers to develop lung cancer (1,27). This result indicates that the case and control groups were properly selected. 5) OGG activity in PBMCs was linearly correlated with OGG activity in lung tissue, indicating that OGG activity in the blood is indicative of OGG activity in the lung. 6) The measured OGG activity values were not affected by the time the blood samples were taken, relative to the time of surgical removal of the lung tumor. Thus reduced OGG activity was probably not associated with the presence of the tumor. 7) The association between OGG activity and lung cancer has a biologic explanation in that individuals with low OGG activity have more lesions in their DNA; therefore, they will accumulate mutations faster.

Our findings illustrate the cumulative effects of genes and environment or, more precisely, internal and external factors in cancer risk, and specifically suggest a role of reduced repair of oxidative DNA damage in lung cancer. Lower activity of OGG (the internal factor), possibly determined by genetic factors, leads to a reduced ability to repair oxidative DNA damage and, as a result, the rate of mutation increases in association with a higher estimated relative risk of cancer. In smokers, tobacco smoke induces in the lungs not only 8-oxoguanine DNA damage but also many other lesions, such as polycyclic aromatic hydrocarbon DNA adducts (8). Consequently, the estimated relative risk of cancer in smokers increases with tobacco use (the external factor). In smokers who have reduced OGG activity, mutations accumulate faster than in smokers with normal OGG activity or in nonsmokers with reduced OGG activity, leading to an overall higher estimated relative risk.

A possible connection between OGG1 polymorphisms and lung cancer was previously explored by analyzing six polymorphic sites in the OGG1 gene, including the common Ser326Cys polymorphism (29). None of the polymorphic sites in OGG1 was associated with lung cancer susceptibility in Caucasians (30). By contrast, the Ser326Cys polymorphism might be a risk factor for lung cancer in the Japanese population (31). Although identification of a polymorphism by DNA sequencing is easier than activity assays, the critical parameter is, of course, the repair activity. OGG1 gene polymorphisms are only one of many parameters that might affect OGG1 activity. Other parameters include differences in levels of expression, stability, post-translational modifications, and the presence of natural inhibitors and activators. For example, AP endonuclease is a natural stimulator of OGG1 activity (32). Therefore, we believe that, in general, because activity measurements are directly associated with protein function, they should be more effective than gene-specific polymorphisms as predictors of cancer risk.

The result that the combined estimated relative risk of lung cancer due to smoking and reduced OGG activity is a multiplication of each of the estimated relative risks of these risk factors alone suggests that a substantial fraction of lung cancer cases might result from a combination of smoking and reduced OGG activity. If so, then screening for smokers with low OGG activity, followed by smoking cessation in these individuals, may lead to a decrease in the incidence of lung cancer. Smoking cessation in such susceptible individuals may be more successful

than that in the general population, because it would be based on a personal genetic susceptibility (low OGG activity). Such an approach of targeted smoking cessation, on the basis of personal genetic susceptibility, may provide a successful and cost-effective strategy to prevent lung cancer and may be extended to include additional DNA repair assays. However, to fully explore the predictive potential of the OGG activity test, prospective epidemiologic studies are required.

Low OGG activity is also associated with lung cancer in nonsmokers, although the estimated relative risk is much lower than in smokers. It seems possible that such individuals may reduce their risk of lung cancer by avoiding exposure to external sources of DNA damage, such as secondary smoke or ionizing radiation, and by modifying dietary habits to include known antioxidants that may reduce oxidative DNA damage. Although large population studies found that antioxidants did not reduce the risk of cancer (15,33), whether these food additives might reduce the risk of cancer when used by individuals with low ability to repair oxidative DNA damage remains to be examined by future studies.

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## NOTES

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