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BRIEF COMMUNICATION

# N-Methylpurine DNA Glycosylase and OGG1 DNA Repair Activities: Opposite Associations With Lung Cancer Risk

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**Only a minority of smokers develop lung cancer, possibly due to genetic predisposition, including DNA repair deficiencies. To examine whether inter-individual variations in DNA repair activity of N-methylpurine DNA glycosylase (MPG) are associated with lung cancer, we conducted a blinded, population-based, case–control study with 100 lung cancer case patients and 100 matched control subjects and analyzed the data with conditional logistic regression. All statistical tests were two-sided. MPG enzyme activity in peripheral blood mononuclear cells from case patients was higher than in control subjects, results opposite that of 8-oxoguanine DNA glycosylase (OGG1) DNA repair enzyme activity. For lung cancer associated with one standard deviation increase in MPG activity, the adjusted odds ratio was 1.8 (95% confidence interval [CI] = 1.2 to 2.6; *P* = .006). A combined MPG and OGG1 activities score was more strongly associated with lung cancer risk than either activity alone, with an odds ratio of 2.3 (95% CI = 1.4 to 3.6; *P* < .001). These results form a basis for a future panel of risk biomarkers for lung cancer risk assessment and prevention.**

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Lung cancer, the leading cause of cancer death, is caused primarily by tobacco smoke, as manifested by the fact that 80% to 90% of lung cancer patients are smokers. However, "only" 10% to 15% of heavy smokers develop lung cancer, suggesting the existence of personal risk factors of genetic origin, which predispose a fraction of smokers to the disease (1–4). A major development in the lung cancer field was the recent conclusion of the National Lung Screening Trial study, which showed that early detection of lung cancer by low-dose computed tomography screening followed by treatment reduced mortality from lung cancer by 20% (5) [see also (6)]. The implementation of these impressive findings is complicated by several factors, among them the very high fraction of false positives and the high cost of mass screening. Clearly, risk and diagnostic lung cancer biomarkers might be useful for avoiding unnecessary computed tomography scanning, reducing the false-positive rate, and making computed tomography screening more effective.

Because DNA repair is a major mechanism for preventing mutations and therefore cancer (7,8), we and others have sought to harness the power of DNA repair to develop risk biomarkers for cancer in general and lung cancer in particular (9–14). Using a functional approach, we have addressed the repair of oxidative DNA damage, which is caused by tobacco smoke, radiation, and metabolism and is associated with inflammation and carcinogenesis (15–17). Because base excision repair is a major mechanism to repair oxidative DNA damage (7,18–20), we have previously developed an epidemiology-competent assay to measure the enzymatic activity of the base excision repair enzyme 8-oxoguanine DNA glycosylase,1 (OGG1) in protein extracts prepared from peripheral blood mononuclear cells (PBMCs) (21). Using this assay, we found that reduced OGG1 activity is associated with increased risk of lung cancer (13) and head and neck cancer (14). In this study, we investigated the role in lung cancer risk of the DNA glycosylase N-methylpurine DNA glycosylase (MPG; also termed AAG and ANPG), which has a different and broader DNA damage specificity than OGG1. MPG repairs hypoxanthine, which is formed by spontaneous or oxidative deamination of adenine; secondary oxidative lesions such as 1,N6-ethenoadenine, which are generated by endogenous processes through the reaction of lipid peroxidation-derived aldehydes and hydroxyalkenals with DNA; and alkylated bases such as 3-methyladenine and 7-methylguanine (22,23).

We developed a robust and reproducible assay for measuring the enzymatic activity of MPG in protein extracts prepared from PBMCs. We used as a substrate a short synthetic DNA carrying a site-specific hypoxanthine. The removal of the hypoxanthine by MPG in the extract created in the substrate an abasic site, whose cleavage by alkali treatment was used as a measure for MPG activity. This assay is commonly used in the DNA repair field [eg, (22,24)] and is similar to the previously developed OGG assay (21). The development of the assay will be described elsewhere; however, the final assay conditions are presented in the Supplementary Materials and Methods (available online). The coefficient of variation of the MPG assay was 11.1%, calculated based on the average of coefficient of variation values obtained in two different ways:

1. The coefficient of variation of 34 repeats, each performed on a different day with two different extracts, was 9.6%. These extracts were run as references in each of the 34 experiments in which the study specimens were assayed.
2. The coefficient of variation obtained by twice running 18 different extracts on different days was 12.6%. The average of the two coefficients of variation was 11.1%.

To examine whether there is an association between MPG activity and lung cancer,

we performed a blinded, population-based, case—control study with 100 lung cancer patients and 100 control subjects matched for sex, age (±1 year), area of residence, and ethnicity. The mean age was 67 in both the case patients and control subjects groups; the distribution by sex was 60% males and 40% females, and 77% were Jews and 23% were non-Jews. The smoking status was 22% current smokers, 28% former smokers, and 50% never smokers in the control subjects group, and 37% current smokers, 36% former smokers, and 24% never smokers in the case patients group. We did not match for smoking status, but we adjusted for smoking in the statistical analysis.

The odds ratio (OR) of lung cancer was estimated for MPG and OGG tests, and for the combination of the two tests, using conditional logistic regression models with smoking status as an adjusting variable. A combined OGG–MPG score was calculated from the regression coefficients obtained from the model combining MPG and OGG, and was used to estimate the odds ratio per one standard deviation (SD). *P* values were based on the two-sided Wald test. All statistical tests were two-sided. Statistical analyses were performed using S-Plus 2000, Professional Release 1 (1988–99 Mathsoft, Inc., Boston, MA) and/or SAS software (version 9.2; SAS Institute Inc., Cary, NC). Additional details of the statistical analysis are in the Supplementary Materials and Methods (available online).

Unexpectedly, the distribution of MPG activity in case patients was shifted to higher MPG activity values than in control subjects, spanning a range of 77 to 271 and 41 to 249 units/μg protein, respectively (Supplementary Figure 1, A, available online). Consistently, the mean MPG activity in case patients was 174 units/μg protein (95% confidence interval [CI] = 167 to 181), statistically significantly higher than in control subjects, in whom the mean MPG activity was 161 units/μg protein (95% CI = 154 to 168; *P* = .004) (Table 1) (25). This suggested that high MPG activity might be associated with risk of lung cancer, which is counterintuitive given the function of DNA repair in genome protection. There was no statistically significant

**Table 1.** Distribution of selected characteristics and N-methylpurine DNA glycosylase (MPG) activity value in lung cancer patients and control subjects*

| | Control subjects (n = 100)† | | | Case patients (n = 100)† | | |
|---|---|---|---|---|---|---|
| **Variable** | **No.** | **MPG mean (95% CI)** | ***P*** | **No.** | **MPG mean (95% CI)** | ***P*‡** |
| All§ | 97 | 161 (154 to 168) | .36‖ | 97 | 174 (167 to 181) | .004 |
| SQCC | | | | 29 | 174 (162 to 186) | |
| Adenocarcinoma | | | | 45 | 177 (168 to 187) | .30¶ |
| Age, y | | | .23# | | | .56¶ |
| ≤65 | 40 | 163 (153 to 172) | | 40 | 180 (169 to 190) | |
| >65 | 57 | 159 (150 to 169) | | 57 | 170 (161 to 179) | |
| Sex | | | .06# | | | .55¶ |
| Male | 58 | 156 (150 to 163) | | 58 | 171 (163 to 180) | |
| Female | 39 | 167 (154 to 181) | | 39 | 178 (168 to 187) | |
| Smoking status | | | .74# | | | .80¶ |
| Never smoked | 49 | 161 (151 to 172) | | 23 | 172 (160 to 184) | |
| Former smoker | 27 | 156 (146 to 165) | | 34 | 174 (164 to 183) | |
| Current smoker | 21 | 166 (151 to 181) | | 37 | 176 (165 to 187) | |

* One hundred case patients were recruited from the Rambam Medical Center in Haifa, located in northern Israel. Control subjects were enrollees of Clalit Health Services (CHS) identified from the same geographical area. CHS is the largest health care provider in Israel and covered, during the study years, approximately 60% of all persons aged older than 50 in Israel. Because health-care coverage in Israel is mandatory and is provided by four groups akin to not-for-profit health maintenance organizations, all study participants (case patients and control subjects) had similar basic health insurance plans and access to health services. One hundred control subjects were individually matched to the case patients by sex, year of birth, area of residence (defined by primary clinic location), and ethnic group (Jews vs non-Jews). Control subjects were selected from the enrollee list of Clalit, from which multiple matched candidates were available and one was randomly assigned as a control subject. Case patients and control subjects were excluded only if they had a former diagnosis of lung cancer. Specimens were collected between April 2008 and December 2009. The general response rate of case patients in our study is about 90% and of control subjects about 50%. Participants provided written informed consent at time of recruitment, and were interviewed in-person to obtain information about their personal and family history of cancer and smoking history. Diagnoses of lung cancer, all of primary origin, were made independently by the diagnosing hospitals and included information on histological type, tumor, nodes and metastasis (TNM) staging, and tumor grade. The institutional review board at Carmel Medical Center, Haifa, approved all procedures. Blood samples were drawn from case patients prior to the operative procedure or any treatment intervention. CI = confidence interval.

† MPG activity was measured as described in the Supplementary Materials and Methods (available online). Three participants did not have a known MPG value and were excluded from the analysis along with their matched subjects. Three case patients did not have a known smoking status.

‡ Analysis of covariance comparing case patients with control subjects, with matched pairs and smoking status as a covariate. The *P* value is based on the *F* test that yields a two-sided comparison.

§ Of the 100 lung cancer case patients, 30 had squamous cell carcinoma (SQCC), 46 had adenocarcinoma, 14 had bronchioloalveolar cell carcinoma, 4 had adenosquamous carcinoma, 4 had adenobronchioloalveolar cell carcinoma, 1 had small cell carcinoma, and 1 had unknown histology.

‖ Analysis of covariance comparing histological type within case patients, with smoking status, age (continuous), and sex as covariates. The *P* value is based on the *F* test that yields a two-sided comparison.

¶ *F* test for interaction between case–control status and the variable of interest. This estimates whether the difference in mean MPG between case patients and control subjects differs between the subgroups of the variable in question (eg, between men and women). None of the differences were statistically significant, indicating no interaction.

# *F* test from analysis of covariance comparing subsets defined by the variable of interest and stratified by case patients and control subjects, with smoking status, age (continuous), or sex as covariates, as appropriate. This estimates whether there are real differences in MPG between the subgroups of the variable in question (eg, between those aged ≤65 years and those aged >65 years) after adjustment for disease status, and other appropriate covariates from among age, sex, and smoking [see reference (25)].

difference in MPG activity between males and females, between those aged less than or equal to 65 years and those aged greater than 65 years, and between adenocarcinoma and squamous cell carcinoma (Table 1). To determine whether smoking status is associated with MPG activity level, we calculated the mean MPG activity level for each smoking status in case patients and control subjects. As can be seen in Table 1, the mean levels of MPG activity level among current, former, and never smokers were similar, indicating that smoking did not affect the level of MPG enzyme activity in PBMCs.

We used conditional logistic regression to examine the association between the level of MPG enzyme activity and the probability of having the disease, adjusted for smoking status. Using MPG activity as a continuous variable, the adjusted odds ratio for lung cancer associated with one standard deviation (1 SD = 34 units; variables were expressed in standard deviation units to allow meaningful comparisons with the OGG test and with a combination of OGG and MPG) increase in MPG activity was statistically significantly greater than 1.0 (OR = 1.8; 95% CI = 1.2 to 2.6; $P$ = .006) (Table 2) (26,27), indicating an association with increased lung cancer risk. To substantiate the MPG results obtained with the hypoxanthine substrate, we assayed the same specimens using a similar MPG activity assay, except that a 1,N6-ethenoadenine–containing substrate was used instead of the hypoxanthine substrate, and we obtained similar results (data not shown).

We also wanted to examine whether using a combination of MPG activity and the previously developed OGG activity provides a stronger association with lung cancer than each assay alone. As part of our aim to facilitate the utility of the assays, we developed a fluorescence-based OGG assay. The assay is similar to the $^{32}$P-labeled OGG test that we have used before (13,14,21), except that the substrate used was the 3' end labeled with Yakima Yellow, repair reactions were performed using a robot, and analysis of reaction products was automated, using capillary gel electrophoresis (the ABI3130XL genetic analyzer). The development of the assay will be described elsewhere; however, the final assay conditions are presented in the Supplementary Materials and Methods (available online). Supplementary Figure 1, B (available online) shows the distribution of OGG activity in the same specimens of case patients and control subjects in which MPG activity was measured, spanning a range of 3.5 to 9.1 and 3.1 to 9.4 units/μg protein, respectively. A clear shift to lower OGG values was observed in case patients compared with control subjects (Supplementary Figure 1, B, available online), consistent with our previous results. The mean OGG value in case patients was 6.3 (95% CI = 6.1 to 6.6) units/μg protein, statistically significantly lower than in control subjects, in whom the mean OGG activity was 6.7 (95% CI = 6.6 to 7.0; $P$ = .009). A comparison of OGG activity to MPG activity in the same specimens showed a very weak correlation (Pearson coefficient = 0.12). When OGG was used as a continuous variable, the adjusted odds ratio for lung cancer associated with a one standard deviation (1.1 unit/μg protein) decrease in OGG activity was statistically significantly greater than 1.0 (OR = 1.5; 95% CI = 1.1 to 2.1; $P$ = .01) (Table 2).

When both continuous OGG and MPG were included in the logistic regression model, both were statistically significant (for OGG, OR = 1.6; 95% CI = 1.1 to 2.3; Wald test $P$ = .007; and for MPG, OR = 2.0; 95%CI = 1.3 to 3.1; Wald test $P$ = .002) (Table 2). Thus, not only did each test remain statistically significantly associated with lung cancer risk when combined, but also their respective odds ratios and levels of statistical significance were strengthened. Forming a combined MPG–OGG score from this model, the estimated odds ratio per one

**Table 2.** Conditional logistic regression analyses of N-methylpurine DNA glycosylase (MPG) and 8-oxoguanine DNA glycosylase (OGG) activity in lung cancer patients and control subjects

| Variable* | SD of variable (units/μg protein) | Adjusted for | No. of case patients | No. of control subjects | Adjusted odds ratio (95% CI)† | *P* |
|---|---|---|---|---|---|---|
| MPG‡§ (per 1 SD increase) | 34.0 | Smoking | 94 | 94 | 1.8 (1.2 to 2.6) | .006 |
| OGG‡ (per 1 SD decrease) | 1.08 | Smoking | 96 | 96 | 1.5 (1.1 to 2.1) | .01 |
| Combined model‖ | | | | | | |
| MPG (per 1 SD increase) | 34.0 | Smoking and OGG | 94 | 94 | 2.0 (1.3 to 3.1) | .002 |
| OGG (per 1 SD decrease) | 1.08 | Smoking and MPG | 94 | 94 | 1.6 (1.1 to 2.3) | .007 |
| Combined score¶(per 1 SD decrease) | 0.82 | Smoking | 94 | 94 | 2.3 (1.4 to 3.6) | <.001 |

* Variables were expressed in standard deviation (SD) units (standardized coefficients) to allow meaningful comparisons with the OGG test and with a combination of OGG and MPG [eg, see (26)].

† Conditional logistic regression for matched sets including smoking status (current smoker, former smoker, never smoker) as a confounding variable together with continuous MPG activity or OGG activity or both. *P* values are from two-sided Wald tests. CI = confidence interval

‡ MPG and OGG activities were measured as described in the Supplementary Materials and Methods (available online).

§ The odds ratio for smoking that was obtained with this model was: former smoker vs never smoker: 3.6 (95% CI = 1.4 to 8.9); current smoker vs never smoker: 4.0 (95% CI = 1.7 to 9.5). These odds ratios are consistent with the lung cancer risk associated with smoking (by duration or amount) in Israel, which is lower than in many other countries (27).

‖ We examined the interaction between the OGG and MPG in the model with continuous OGG and MPG levels and found that they did not reach conventional statistical significance ($P$ = .06).

¶ The score was defined as 0.4584 × OGG – 0.0204 × MPG and was fitted in the conditional logistic regression model as a continuous variable. OGG and MPG represent enzyme activities in units per microgram of protein.

standard deviation was 2.3 (95% CI = 1.4 to 3.6; Wald test $P < .001$) (Table 2), higher than the odds ratio for each single assay.

The two main outcomes of this study are 1) high MPG enzyme activity is associated with lung cancer risk, which is opposite to the effect of OGG, and 2) the combination of MPG and OGG activities provides a stronger association with lung cancer than each activity alone.

The association of increased MPG enzyme activity with lung cancer risk was unexpected because DNA repair functions to preserve the genome and protects against cancer. Previous studies on the activity of MPG on 1,N6-ethenoadenine in PBMCs from lung cancer patients reported conflicting results, with activity in lung cancer patients compared with activity in healthy individuals being lower in one study (28) and higher in another study (29). The current case–control study was performed under strict epidemiological criteria: it was blinded, population based, and matched for sex, age (±1 year), area of residence, and ethnicity. In addition, two different assays of MPG were performed, which were conducted under different conditions and directed to different substrate lesions, suggesting that the association observed is real. Several mechanisms can explain this unexpected result. Unlike OGG1, which has a relatively narrow range of substrate specificity, MPG repairs a much broader range of DNA lesions (18,19,30). Moreover, MPG binds several lesions without being able to repair them (eg, 3-methylcytosine; 1-methyladenine; 3,N4-ethenocytosine) (22). This nonproductive binding may inhibit the repair of these lesions by their designated repair enzyme on the one hand and, on the other hand, prevent MPG from acting on its substrate DNA lesions. Indeed, it was found that the tight binding of MPG to 3,N4-ethenocytosine inhibits its repair by ALKBH2 (31) and, at the same time, prevents MPG from acting on its own substrates (32).

High activity of MPG may cause the accumulation of toxic base excision repair intermediates (eg, abasic sites), as was suggested to explain the observation that overproduction of MPG sensitized mammalian cells to DNA-damaging agents, which is opposite to the expected protective effect (33,34). Elevated levels of MPG were reported to cause frameshift mutagenesis and microsatellite instability, most likely via binding to one and two base-pair loops and inhibiting their repair by mismatch repair (35).

When further considering the effect of MPG, it is quite common that enzymes operate in organisms within a defined activity range, a result of the need to optimize simultaneously over many activities, such that values that are too low or too high are disadvantageous. Indeed, the notion of the deleterious effects of imbalance in DNA repair has been previously raised (36,37,38). Yet, it is surprising that, within the normal population, increased MPG DNA repair activity is associated with increased risk of lung cancer. Of course, MPG is a DNA repair enzyme that fulfills a protective role, and therefore we propose that MPG activity that is too low will also be associated with increased cancer risk. It is possible that in larger studies MPG will display a bimodal behavior, whereby both low and high activities will be associated with increased lung cancer risk.

Like OGG, MPG activity showed no interaction with smoking. This suggests that MPG is a smoking-independent risk factor for lung cancer, implying that the same odds ratios due to DNA repair test levels apply to smokers as to nonsmokers. A limitation of this study was that the sample size in this study was too small to perform a detailed analysis of subgroups, such as smokers and nonsmokers; for that, a larger study will be necessary. It should be pointed out that measuring enzymatic DNA repair activity in PBMCs measures baseline DNA repair capabilities, prior to any challenge, and those reflect the inherent baseline repair ability of an individual, as we have suggested for OGG enzymatic activity (13,14). Being a functional DNA repair enzyme assay, such an assay is likely to integrate genetic, environmental, and lifestyle factors, which makes it a potential effective measure of DNA repair (15,39).

The validation of high MPG activity and low OGG activity as risk biomarkers for lung cancer with a predictive value will require a prospective study. If successful, it might provide, in combination with additional risk biomarkers, a useful tool for risk assessment of lung cancer and for selecting high-risk individuals for smoking cessation programs and for computed tomography screening for early detection of lung cancer.

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