Short Communication

Association of the hOGG1 Ser326Cys Polymorphism with Lung Cancer Risk

Loïc Le Marchand,1 Timothy Donlon, Annette Lum-Jones, Ann Seifried, and Lynne R. Wilkens
Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, Hawaii 96813

Abstract
Oxidative stress may be one mechanism by which tobacco smoke causes lung cancer. A common oxidative damage to DNA is the highly mutagenic 7,8-dihydro-8-oxoguanine adduct, which can be repaired by 8-oxoguanine glycosylase 1 (OGG1). A Ser326Cys substitution polymorphism in the hOGG1 gene has been suggested, based on in vitro data, to reduce the activity of the enzyme. We tested the association of this polymorphism with lung cancer in a population-based, case control study of 298 cases and 405 controls of Caucasian, Japanese, or Native Hawaiian ancestry in Hawaii. Subjects were genotyped with a PCR-RFLP assay, and odds ratios were estimated by logistic regression after adjustment for other observed risk factors, including smoking and vegetable intake. We found marked differences in the frequencies of the hOGG1 Cys variant allele among ethnic groups (45% in Hawaiians, 42% in Japanese, and 22% in Caucasians). The homozygous Cys/Cys genotype was also found to be more common in cases than controls (P = 0.008), with an odds ratio of 2.1 (95% confidence interval: 1.2–3.7) for this genotype compared with the Ser/Ser genotype. Heterozygous individuals were not at increased risk. This association with the Cys/Cys genotype was observed for each sex, ethnic group, and lung cancer cell type. There was also the suggestion that vegetable intake may not be protective against lung cancer among subjects with the Cys/Cys genotype. These data suggest that the presence of two hOGG1 326Cys alleles confers a 2-fold increased risk of lung cancer. Additional studies need to be conducted to confirm this association.

Introduction
DNA damage generated by ROS has been implicated in carcinogenesis, particularly in lung cancer (1–4). Tobacco smoke, a major cause of this disease, contains free radicals, as well as various compounds, including catechol and hydroquinone, that can generate ROS (5).

One abundant type of DNA damage resulting from ROS exposure produces 8-OHdG, which has been shown to be highly mutagenic, yielding G:C to T:A transversions (6, 7). The relevance of this specific DNA alteration to lung cancer is suggested by the elevated 8-OHdG content of peripheral leucocyte and lung tissue DNA observed in smokers and lung cancer patients (8–10) and by the high frequency of G:C to T:A transversions in TP53 in lung tumors (11).

The gene coding for 8-oxoguanine DNA glycosylase 1, a major excision repair enzyme for 8-OHdG, has been identified recently as a homologue to the yeast MMH/OGG1 gene (12–14). The hOGG1 gene maps to 3p25, a chromosomal region that is commonly lost in lung tumors (5). A few polymorphisms have been described in the hOGG1 gene, including one resulting in a Ser-Cys substitution at codon 326, which has been associated with a reduced enzyme activity in a bacterial complementation assay system (15). In two small hospital-based, case control studies of lung cancer in Okinawa (16) and Germany (17), a 2-fold increased risk was found, respectively, for squamous cell carcinoma and all lung cancer for the Cys/Cys genotype, compared with the Ser/Ser genotype. However, these associations were not statistically significant.

We sought to investigate the associations of this polymorphisms in the hOGG1 gene with lung cancer in a relatively large population-based, case control study conducted in Hawaii.

Materials and Methods
The methods and subjects for this study have been described previously (18). Lung cancer patients were identified by the rapid-reporting system of the Hawaii Tumor Registry, a member of the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute. Eligible cases were all patients with histologically confirmed primary lung cancer who were newly diagnosed between January 1, 1992 and March 31, 1997 in all main medical centers on the island of Oahu, Hawaii. Other eligibility criteria included age between 18 and 79 years, Oahu residency, no previous history of lung cancer, and ethnicity (any Hawaiian/part-Hawaiian heritage or no part-Hawaiian but with three of four grandparents being Japanese or Caucasian). Controls were selected randomly from a list of Oahu residents interviewed by the State of Hawaii Department of Health as part of a health survey of a 2% random sample of households in the state. This source was supplemented with controls over age 65 from Health Care Financing Administration participants on Oahu. One control was matched to each case on sex, ethnicity, and age (±2 years). The participation rate for the interview among cases and controls was 64 and 62%, respectively. A total of 341 cases (76% of interviewed cases) and 456 population controls (80% of interviewed controls) additionally donated a blood specimen for the study. The

Received 6/8/01; revised 1/2/02; accepted 1/14/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by Grants RO1 CA55874 and CA85997 and Contract NO1-PC-67001 from the U.S. National Cancer Institute and by Grant EDT-78 from the American Cancer Society.

2 To whom requests for reprints should be addressed, at Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Suite 407, Honolulu, HI 96813. Phone: (808) 586-2988; Fax: (808) 586-2982.

3 The abbreviations used are: ROS, reactive oxygen species; 8-OHdG, 7,8-dihydro-8-oxoguanine; OR, odds ratio; CI, confidence interval; LOH, loss of heterozygosity.
analysis presented here was conducted with the 298 cases and 405 controls with available DNA.

In-person interviews were conducted at the subjects’ homes by trained interviewers. On average, cases were interviewed in ≤4 months of diagnosis. Information was collected on the types (nonfiltered cigarettes, filtered cigarettes, cigars, and pipes) of tobacco product ever smoked daily for at least 6 months and, for each tobacco product, the usual amount smoked per day, age when started, the overall duration of use, and for ex-smokers, age when smoking stopped. We also inquired about any periods of smoking cessation for each tobacco product during the subject’s life. The questionnaire included detailed demographic information, including ethnic origin of each grandparent, a quantitative food frequency questionnaire, a history of various relevant medical conditions and occupational exposures, and a family history of lung diseases.

Laboratory personnel were blinded to the case control status of the subjects. The hOGG1 Ser326Cys polymorphism in exon 7 described by Sugimura et al. (16) was assessed by PCR-RFLP using primers HOOGG1F: 5′-GGAGAAGGTGCTTG-TGGGGAAT-3′ and HOOGG1R: 5′-ACTGTCACTAGTCTT-MHETC-3′. Amplification consisted of a 5-min denaturation at 95°C followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 1 min. An incubation step at 72°C was added at the end of the reaction for 7 min. Instead of the single-strand conformational polymorphism method used by Sugimura et al. (16), we used a simple RFLP method to identify the Ser326Cys variant, because the C-to-G transversion creates a new Fnu4HI restriction site. The PCR product is 200 bp in length and is digested by the Fnu4HI restriction enzyme to two 100-bp fragments for the 326Cys allele and is undigested for the 326Ser allele. Fragments were separated on a 2% NuSieve GTG agarose gel (FMC Bioproducts, Rockland, ME) and compared with genotype standards (confirmed by direct sequencing). A representative gel is shown in Fig. 1.

Unconditional logistic regression (19) was used to compute ORs and 95% CIs, with adjustment for several covariates found to be associated with risk (sex and race, using indicator variables; age, smoking duration and amount, and saturated fat and total vegetable intakes, as continuous variables). Various ways of modeling the smoking effect were explored, and the best fitting model was one that included an indicator variable for smoking status (ever versus never smoked) and separate continuous terms for duration and amount (18). The likelihood ratio test was used to test the statistical significance of modeled effects. We also used this test to determine the significance of interactions (additive on the logistic scale) among certain variables with respect to lung cancer risk. The test compared main effects, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest. Gene dosage effects were modeled by assigning the value 1, 2, or 3 to a genotype trend variable according to the subject’s number of variant alleles (zero, one and two variant alleles, respectively).

Results
The characteristics of the study population were published previously (18). A group (40%) of subjects was Caucasian, 38% were Japanese, and 24% were Hawaiian. Table 1 includes the distribution of the subjects by case control status, ethnicity, and hOGG1 genotype. On the basis of the controls, the frequency for the hOGG1 Cys variant allele was 44.8% in Hawaiians, 42% in Japanese, and 21.7% in Caucasians. The ethnic-specific allele distributions were all consistent with the Hardy-Weinberg equilibrium.

The homozygous variant hOGG1 genotype (Cys/Cys) was more common in cases than controls (P = 0.008; Table 1). The lung cancer OR for this genotype was 2.1 (95% CI: 1.2–3.7) compared with the homozygous wild type (Ser/Ser). The corresponding OR and 95% CI for men and women were 1.9 (0.9–4) and 2.2 (0.9–5.1), respectively. The OR for the Cys/Cys genotype compared with the combined Ser/Ser and Ser/Cys genotypes was 2.5 (1.5–4.2), 1.9 (0.5–6.8), 2.2 (1.1–4.7), and 3.4 (1.3–8.9) for all subjects, Caucasians, Japanese, and Hawaiians, respectively. The direct association with the Cys/Cys genotype was observed for each ethnic group (Table 1) and each cell type (Table 2), although the association appeared somewhat stronger for squamous cell carcinoma than for the other cell types.

Because vegetables were strongly associated with lung cancer risk in our study and because they are a major source of dietary antioxidants, we examined the modifying effect of the Cys/Cys genotype on the association between vegetable intake and lung cancer (Table 3). Although among subjects with the Ser/Ser or Ser/Cys genotype, risk was halved for vegetable consumption > median, compared with ≤ median, there was no apparent protection from vegetables among subjects with the Cys/Cys genotype. However, the P for interaction did not reach statistical significance (P = 0.24). Finally, the interactions of the hOGG1 polymorphism with, successively, pack-years of cigarette smoking (>median versus ≤median) and the GSTMI deletion polymorphism were investigated. No interactions were suggested. We were unable to examine the modifying effect of smoking status because very few cases were lifetime non-smokers.

Discussion
In this population-based, case control study, lung cancer risk was found to be increased 2-fold in individuals with the homozygous hOGG1 Cys/Cys variant genotype (95% CI: 1.2–3.7). This association was observed for each sex, ethnic group, and cell type. The data also suggested that vegetable intake may not protect against lung cancer among subjects with the homozygous hOGG1 Cys/Cys variant genotype.

The frequencies found among our controls for the hOGG1
were 405 in each model.

Sugimura et al. 326Cys

* OR and 95% CI adjusted by unconditional logistic regression for age, sex, ethnicity (when appropriate), smoking status, years of smoking, (years of smoking)², number of cigarettes per day, and saturated fat and vegetable intakes.

b Number of cases/number of controls.

c P for the genotype variable assigned the values 1, 2, or 3 according to the subject’s number of variant Cys alleles (0, 1, and 2, respectively).

Table 2 OR* (95% CI) for the hOGG1 Ser326Cys genotype by lung cancer cell type

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Squamous cell carcinoma (n = 66)</th>
<th>Adenocarcinoma (n = 141)</th>
<th>Small cell (n = 43)</th>
<th>Other (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>3.7 (1.7–8.3)</td>
<td>2.1 (1.1–3.9)</td>
<td>3.4 (1.1–10.4)</td>
<td>2.4 (1.4–4.1)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, ethnicity (when appropriate), smoking status, years of smoking, (years of smoking)², number of cigarettes per day, and saturated fat and vegetable intakes by unconditional logistic regression.

b Number of cases. Five cases were of unspecified cell type. Number of controls was 405 in each model.

326Cys variant allele were very similar to those reported by Sugimura et al. (Ref. 16; 41.2% for Micronesians compared with 44.8% in Hawaiians; 43.6 versus 42% for Japanese; and 19.5 versus 21.7% for Caucasians). Additionally in agreement with our findings, the hOGG1 Cys/Cys genotype has been associated with a 2.3-fold increased risk of squamous cell carcinoma of the lung (95% CI: 0.9–5.6) in a hospital-based, case control study conducted in Okinawa (16). No association was observed with adenocarcinoma in that study. Although the OR was strongest for squamous cell carcinoma in our data, an association was also found for adenocarcinoma, as well as for small cell carcinoma. A 2.2-fold increased risk of lung cancer (95% CI: 0.4–11.8) was also found for the Cys/Cys genotype compared with the Ser/Ser genotype in a small, hospital-based case control study conducted in Germany among heavy smokers (17). The allele frequency for the Cys allele was also 22% in that study of Caucasians.

Another polymorphism in hOGG1 (a G to T transition at position –18) has also been associated with a 3-fold increased risk of adenocarcinoma of the lung (95% CI: 1.3–7.8) in a recent Japanese study (20). However, this polymorphism was found to be rare (frequency of 2.8% in Japanese and <1% in Caucasians), and its functional significance is unknown (17, 20). Overall, the limited epidemiological data available to date suggest that one or two polymorphisms in the hOGG1 gene may be associated with lung cancer.

The oxidatively damaged guanine, 8-OHdG, is produced abundantly in DNA as a result of endogenous and exogenous oxidative stress or exposure to ionizing radiation or chemical carcinogens (21–23). Levels of this adduct are known to be elevated in the peripheral leukocytes and lung tissue of smokers, as well as in lung tumors (5). 8-OHdG does not impede DNA chain elongation but preferentially mispairs with adenine during DNA replication, inducing GC to TA transversions (24, 25). The DNA excision repair enzyme 8-OHdG glycosylase is thought to play a key role in repairing 8-OHdG lesions. Inactivation of the gene coding for this enzyme (MutM) has been shown to create a mutator phenotype with accumulation of GC to TA transversions in Escherichia coli and yeast (26, 27). The human homologue of this gene, hOGG1, is located on chromosome 3p, which is subject to frequent and early LOH during lung cancer development. Indeed, a recent study has shown that lung tumor exhibiting 3p LOH contained higher levels of 8-OHdG adducts, compared with tumors without LOH at this site (28). However, in this study, which included only three subjects with the Cys/Cys genotype, no association was found between the Cys allele and 8-OHdG levels in normal lung DNA (28). If it can be confirmed that the hOGG1 Cys/Cys genotype conferred a reduced 8-OHdG repair activity (15), smokers with this genotype would be expected to be at increased risk for lung cancer. Although the epidemiological evidence to date suggests that this may be the case, additional studies in other populations are needed to confirm this association.

Although not statistically significant, our data suggest that a high vegetable intake may not be helpful in preventing lung cancer among individuals predicted to have a poor 8-OHdG repair capacity (i.e., those with the hOGG1 Cys/Cys genotype). This finding was unanticipated because dietary antioxidants from vegetables would be expected to prevent oxidative DNA damage and not to interfere with 8-OHdG repair. This result may be because of chance but may be worth considering in future studies.

In summary, consistent with available data, this study suggests that the hOGG1 Cys/Cys genotype may confer a 2-fold increased risk of lung cancer.

Acknowledgments

We thank the Hawaii Tumor Registry, Castle Medical Center, Kaiser-Permanente Medical Center, Kuakini Medical Center, Queen’s Medical Center, Straub Clinic and Hospital, St. Francis Medical Center, Tripler Medical Center, and Wahsawa...
Short Communication: Association of the hOGG1 Ser326Cys Polymorphism

General Hospital for their collaboration. We also thank Ronette Hunt, Barbara Burden, Geraldine Kaneshiro, and Yun Oh Jung for technical assistance.

References

Association of the hOGG1 Ser326Cys Polymorphism with Lung Cancer Risk

Loïc Le Marchand, Timothy Donlon, Annette Lum-Jones, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/11/4/409

Cited articles
This article cites 26 articles, 11 of which you can access for free at:
http://cebp.aacrjournals.org/content/11/4/409.full#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/11/4/409.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.