hOGG1 Ser326Cys Polymorphism and Lung Cancer Susceptibility[1]

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Abstract

The human homologue of the yeast *OGG1* gene, *hOGG1*, has been cloned, and its genetic structure has been determined. Several polymorphisms in the *hOGG1* gene were detected in the Japanese populations, and among them, the Ser-Cys polymorphism at codon 326 has been shown to have a functional difference in complementation of mutant *Escherichia coli* that is defective in the repair of 8-hydroxyguanine. Activity in the repair of 8-hydroxyguanine is greater in hOGG1-Ser^{326} protein than in hOGG1^{326} protein. Because many environmental carcinogens produce 8-hydroxyguanine residue and mismatching to this modified base potentially causes oncogenic mutations, the capacity to repair these lesions can be involved in cancer susceptibility in human beings. We, therefore, examined allele distributions of the Ser326Cys polymorphism in a case-control study of male lung cancer in Okinawa. The analyses based on 241 cases and 197 hospital controls disclosed the following findings. (*a*) Those with the *Cys/Cys* genotype were at an increased risk of squamous cell carcinoma and nonadenocarcinoma compared to those with the *Ser/Cys* and those with the *Ser/Ser* genotypes combined. The odds ratios adjusted for age and smoking history were 3.01 (95% confidence interval, 1.33–6.83) and 2.18 (95% confidence interval, 1.05–4.54), respectively. (*b*) The odds ratios for other histological subtypes of lung cancer or those in total were not significant. Those for *Cys/Cys* or *Ser/Cys* genotype against *Ser/Ser* did not reach statistical significance in any cell type. (*c*) The distributions of this polymorphism varied for different populations (Chinese, Japanese, Micronesians, Melanesians, Hungarians, and Australian Caucasians), with much less prevalence of *Cys* allele in the latter three populations. Although our sample size was limited, these results indicate that the Ser326Cys variant may be related to squamous cell lung cancer susceptibility. The *Cys/Cys* genotype appears to be more susceptible to squamous cell carcinoma, although the risk is less than that previously reported to be associated with the *CYPIA1* gene. Further studies are needed to assess the importance of the interpopulation variation to cancer susceptibility.

Introduction

Molecular cloning of a human counterpart of the yeast *OGG1* (*hOGG1*; Refs. 1–6) paved the way for possible application of hOGG1 variants as genetic markers for individual susceptibility to various cancers. This is because 8-hydroxyguanine, assumed to be repaired by *hOGG1*, is highly mutagenic, causing a major oxidative DNA damage induced by reactive oxygen species (7, 8). Moreover, the content of 8-hydroxyguanine in DNA of nontumor lung tissue has been shown to be higher in lung cancer patients than in controls (9). Our previous structural studies as well as those by others revealed the presence of several polymorphisms inside *hOGG1* in the Japanese population (10–12). Among them, the polymorphism Ser326Cys is associated with complementation activity for *Escherichia coli* mutants that are defective in the repair of 8-hydroxyguanine (10). On the basis of our previous studies, the capacity to complement is ~7 times higher in the 326Ser protein than in the 326Cys protein, suggesting that *326Cys* allele may give an individual more susceptibility to the formation of 8-hydroxyguanine in DNA (10, 11). Because previous studies suggest a role of oxidative DNA damage in lung carcinogenesis (3), we evaluated this presumably functional polymorphism of *hOGG1* in a case-control framework of human lung cancer.

Subjects and Methods

Case-Control DNA Set. Samples of DNA from lung cancer patients and hospital controls used here were the same as those

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used in our previous case-control study for *CYPIA1* (13). Briefly, all lung cancer patients and hospital controls were males, recruited from the National Okinawa Hospital, whose diagnoses of lung cancer were verified histopathologically and cytologically. Histological categorization was performed based on the WHO criteria (14, 15). DNA was available from 241 cases and 197 hospital controls. The cases constituted a sequential series of newly diagnosed lung cancer patients at the National Okinawa Hospital from 1993 to 1996. In our previous study (16), we showed that lung cancer patients in this hospital could represent lung cancer in Okinawa. Controls were randomly selected from in-patients at the hospital during the same study period. The participation rate was nearly 100% in both cases and controls, and only six cases were excluded from the final analysis because their diagnoses were not pathologically confirmed. The diagnoses of the controls included 19 tuberculoses, 8 cancers outside the lung, 8 benign tumors, 15 bronchopneumonia, 8 pyothorax, 7 chronic obstructive lung diseases, 29 other respiratory diseases, 13 benign liver diseases, 15 other gastrointestinal diseases, 13 diabetes, 9 fractures, 15 other orthopedic diseases, 25 neuromuscular diseases, 11 others, and 2 healthy individuals. Smoking history was obtained via interviews by doctors and nurses and was reviewed by the researchers. Smoking variables such as starting and quitting ages of smoking and numbers of cigarettes per day were also collected.

PCR-SSCP[3] Analysis. The genomic structure of *hOGG1* is shown in Fig. 1. The primers used for identification of the Ser326Cys polymorphism in exon 7 were 5′-TGAATTCGGAAGGTGCTTGGGGAAT-3′ and 5′-ACTGTCACTAGTCTCACCAG-3′. A forward primer, 5′-GGAAGGTGCTTGGGGAAT-3′, without an *Eco*RI restriction site was also used for the same DNA in combination with the same backward primer, 5′-ACTGTCACTAGTCTCACCAG-3′. The PCR product was denatured with formamide at 95°C for 15 min, quenched on ice, and loaded to polyacrylamide gels under several conditions. Visualization was performed with a silver stain kit (Wako, Osaka, Japan) as described previously (17). Unambiguous migration differences between the two allelic bands were obtained by electrophoresis performed at room temperature and 4°C with a glycerol concentration of 12%. In addition, each allelic PCR product was cloned and sequenced to confirm the genotypes inferred from SSCP analysis. Sequencing involved a dye-primer cycle sequence kit (Amersham, Cleveland, OH) and an ABI 301 automated sequencer (Applied Biosystems, Foster City, CA).

Statistical Analysis. The strengths of associations between lung cancer and the *hOGG1* polymorphism were measured as ORs. The ORs were obtained using unconditional logistic regression analysis (18). Crude ORs and those adjusted for age and smoking habits were calculated. Because the Ser/Ser genotype was thought to have the highest effective enzymatic activity, we used this genotype as our reference for lung cancer risk. Thus, the ORs for the *Ser/Cys* and *Cys/Cys* genotypes relative to the *Ser/Ser* genotype were computed. The ORs for the *Cys/Cys* genotype *versus* other genotypes combined were also computed because only the *Cys/Cys* genotype might influence the individual repair activity. We calculated crude and age-adjusted ORs by smoking dosage, that is, cigarette-years [(cigarettes per day) × (smoking years)] of ≥800 or <800. The χ^2 test was used to test for deviations of genotype distributions from Hardy-Weinberg equilibrium (19). We evaluated how likely our study was to detect a significant ORs for lung cancer at 5% level, when the real ORs ranged from 1.5 to 4.0 (20), and we also calculated the required number of cases of total lung cancer, squamous cell carcinoma, adenocarcinoma, and non-adenocarcinoma (lung carcinoma other than adenocarcinoma, that is, squamous cell, small cell, large cell, and other carcinoma), based on fixed 80% power, 5% type I error, and the ORs from this study.

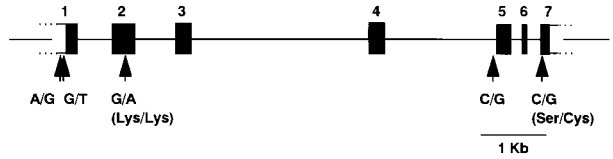

Fig. 1. Genomic structure of *hOGG1* showing previously identified polymorphic sites (10, 33, 38). The Ser-Cys polymorphism is in exon 7 (C/G) and in linkage disequilibrium with the polymorphism in intron 4 (C/G; Ref. 10).

Population Study. Six populations that were free from cancer were studied to detect a difference in the distributions of the polymorphism among populations: Chinese in Jiangsu Province, Australian Caucasians, Hungarians (21), Japanese in Tokyo, Micronesians from the Republic of Palau and Yap State, and Melanesians from three islands of Vanuatu (22, 23). All were randomly sampled, unrelated individuals. Differences in the distributions among the populations were tested by the χ^2 test. Deviation from the Hardy-Weinberg equilibrium was also tested (19).

Results

Identification of the Ser326Cys Polymorphism by Non-Radioisotope SSCP. Electrophoresis at room temperature with 12% glycerol discriminated three genotypes, as shown in Fig. 2*a*. Migration patterns were different when the primers without restriction enzyme site tags were used, but the genotypes assigned were consistent (Fig. 2*a*). Confirmatory sequence results are shown in Fig. 2*b*.

Association of the Ser326Cys Polymorphism with Lung Cancer. The age distribution and smoking habits among the cases and controls for which DNA was available are shown in Table 1. The mean ages ± SDs in cases and controls were 67.6 ± 9.2 and 62.0 ± 11.3 years, respectively. As expected, cases tended to be heavier smokers than controls. Histological subtypes of the cases were: squamous cell carcinoma, 49.0% (n = 118); adenocarcinoma, 32.4% (n = 78); small cell carcinoma, 11.6% (n = 28); and others, 7.0% (n = 17). The distribution of histological subtypes of lung cancer in Okinawa is known to be different from that in other parts of Japan (24, 25), and the distribution here described is consistent with that of lung cancer in Okinawa (25).

The distribution of the Ser326Cys polymorphism and the ORs for *Ser/Cys* or *Cys/Cys* genotype *versus Ser/Ser* are summarized in Table 2. The distribution among the controls was consistent with Hardy-Weinberg equilibrium; it did not deviate from the expected distribution (P = 0.22). For all lung cancer cases, the OR adjusted for age and smoking habits was not significantly greater than unity. The contribution of the Ser326Cys polymorphism in each histological subcategory is also shown in Table 2. The OR was not statistically significant for any lung cancer subtype. Table 3 shows the ORs for *Cys/Cys* genotype *versus* the combined *Ser/Ser* and *Ser/Cys* geno-

[3] The abbreviations used are: SSCP, single-strand conformation polymorphism; OR, odds ratio; CI, confidence interval.

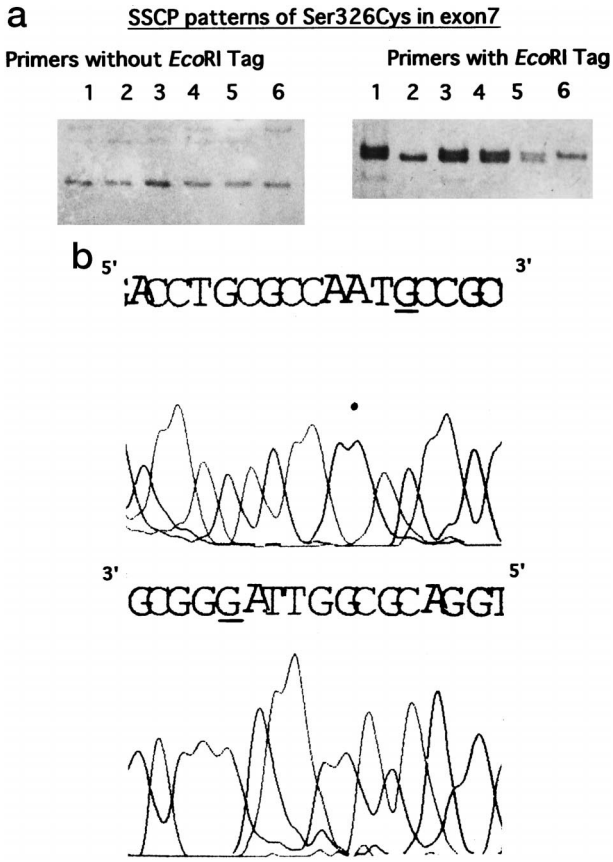

Fig. 2. *a*, three genotypes revealed by SSCP analysis in 12% polyacrylamide gels. *Lanes 1*, *3*, *4*, and *5*, *Ser/Cys*; *Lane 2*, *Cys/Cys*; and *Lane 6*, *Ser/Ser*. *Right*, PCR products with *Eco*RI tag primers. *b*, confirmatory base substitution identification by sequencing. Differences (*underlined*) in a base G (*top*, *Cys* allele) and C (*bottom*, showing complimentary sequence, *Ser* allele) at codon 326 are indicated.

types. The ORs were not significant for all cases, but interestingly, in squamous cell carcinoma, homozygous *Cys/Cys* individuals showed a significantly higher risk than the other genotypes after adjustment for age and smoking history (OR = 3.01, 95% CI, 1.33–6.83; $P = 0.008$). Similarly, for nonadenocarcinoma, a significantly higher risk was also observed (OR = 2.18; 95% CI, 1.05–4.54; $P = 0.037$).

We did analyses including the other smoking variables, such as numbers of cigarettes smoked per day, the results were not materially changed (data not shown).

ORs by Smoking Dosage. The ORs by smoking dosage are shown in Tables 2 and 3. The OR for *Cys/Cys* genotype *versus* either the *Ser/Ser* homozygote or the two other genotypes combined was greater in heavy smokers (cigarette-years ≥800) but was not statistically significant. Further adjustment for smoking-years in each strata did not modify the ORs substantially (data not shown).

Statistical Power. Our power calculations indicated that the analyses had the power to detect an OR of 2.5 at 5% significance level in ~80% or more likelihood for all lung cancer cases, squamous cell carcinoma, and nonadenocarcinoma (data not shown). For other cell types and analysis by smoking dosage, however, the statistical power was found to be lower (data not shown). The required numbers of cases, based on 80% power and 5% type I error, were 387, 80, 545, and 172 cases for total lung cancer, squamous cell carcinoma, adenocarcinoma, and nonadenocarcinoma, respectively, when the same number of cases and controls are recruited and the risk of *Cys/Cys* genotype *versus* other genotypes combined is evaluated.

Table 1 Age distribution and smoking habits among cases and controls

	Cases		Controls	
	No.	%	No.	%
Age (yr)				
30–39	1	0.4	7	3.6
40–49	10	4.1	19	9.6
50–59	31	12.9	52	26.4
60–69	89	36.9	70	35.5
70–79	90	37.3	40	20.3
≥80	20	8.3	9	4.6
Mean ± SD	67.6 ± 9.2		62.0 ± 11.3	
Smoking habits				
Never smoked				
Ex-smokers (cigarette-years)				
0–399	13	5.4	52	26.4
400–799	12	5.0	12	6.1
800–1199	11	4.6	11	5.6
1200+	23	9.5	7	3.6
Current smokers (cigarette-years)	31	12.9	10	5.1
0–399	13	5.4	16	8.1
400–799	28	11.6	29	14.7
800–1199	57	23.7	20	10.2
1200–1599	24	10.0	7	3.6
1600–1999	10	4.2	5	2.5
2000+	15	6.2	4	2.0
Unknown	4	1.7	24	12.2

Distribution in Different Populations. As shown in Table 4 all the distributions of this genotype in Japanese (Tokyo), Chinese (Jiangsu), Hungarian Caucasians, and Micronesians were consistent with Hardy-Weinberg equilibrium, but those of Melanesians ($P = 2.8 \times 10^{-5}$) and Australian Caucasians ($P = 0.002$) were not. The *Ser/Ser* genotype was less frequent in Chinese, whereas the prevalence of the *Cys/Cys* genotype was relatively low in Australian Caucasians, Melanesians, and Hungarians. A significant difference in genotype distributions was detected in these populations ($P = 6.4 \times 10^{-31}$).

Discussion

8-Hydroxyguanine is one of the most common form of DNA damage caused by oxidative DNA injury (26). The increase of 8-hydroxyguanine content in DNA has been shown to elevate cancer risk (27). The repair system for this damaged DNA in *E. coli* has been well characterized, and a human homologue of yeast *OGG1* was recently cloned (1–6). Genetic polymorphisms of *hOGG1* have been documented (10), and it is expected that they play a role in human cancer susceptibility.

We took advantage of a lung cancer case-control DNA set in Okinawa, which previously disclosed the *CypIA1* Ile-Val polymorphism as a possible high-risk marker for lung cancer (13). There are several pieces of evidence implicating oxyradical and subsequent 8-hydroxyguanine formation in human lung carcinogenesis (9, 28, 29). Therefore, the interindividual differences to repair these lesions may be associated with lung cancer susceptibility. Because the content of 8-hydroxyguanine is increased in smokers' leukocytes (26), this association is expected to be most noticeable with tobacco-related subtypes. In a bacterial complementation assay system, the *Ser* allele was

Table 2 ORs according to the hOGG1 Ser326Cys polymorphism *versus* Ser/Ser genotype as a reference and those stratified by smoking dosage

Genotype	No. of cases/controls	Crude			Adjusted for age and smoking habits		
		OR	95% CI	*P*	OR	95% CI	*P*
Total							
Ser/Ser	85/63	1.00			1.00		
Ser/Cys	115/107	0.80	0.52–1.21	0.287	0.64	0.39–1.06	0.081
Cys/Cys	41/27	1.13	0.63–2.02	0.692	1.31	0.65–2.62	0.446
Squamous cell carcinoma							
Ser/Ser	40/63	1.00			1.00		
Ser/Cys	54/107	0.80	0.48–1.33	0.381	0.63	0.33–1.18	0.150
Cys/Cys	24/27	1.40	0.71–2.76	0.331	2.27	0.92–5.60	0.075
Small cell carcinoma							
Ser/Ser	11/63	1.00			1.00		
Ser/Cys	15/107	0.80	0.35–1.86	0.608	0.58	0.23–1.47	0.252
Cys/Cys	2/27	0.42	0.09–2.05	0.285	0.51	0.09–2.87	0.447
Adenocarcinoma							
Ser/Ser	25/63	1.00			1.00		
Ser/Cys	40/107	0.94	0.52–1.70	0.843	0.77	0.40–1.47	0.424
Cys/Cys	13/27	1.21	0.54–2.72	0.639	1.34	0.53–3.39	0.537
Nonadenocarcinoma							
Ser/Ser	57/63	1.00			1.00		
Ser/Cys	73/107	0.75	0.47–1.20	0.235	0.59	0.33–1.05	0.071
Cys/Cys	28/27	1.15	0.61–2.17	0.675	1.59	0.71–3.57	0.262
					Adjusted for age		
					OR	95% CI	*P*
Cigarette-years <800							
Ser/Ser	28/38	1.00			1.00		
Ser/Cys	34/62	0.74	0.39–1.42	0.368	0.74	0.38–1.43	0.362
Cys/Cys	16/20	1.09	0.48–2.46	0.844	0.97	0.42–2.25	0.943
Cigarette-years ≥800							
Ser/Ser	57/15	1.00			1.00		
Ser/Cys	81/34	0.63	0.31–1.26	0.188	0.63	0.31–1.26	0.190
Cys/Cys	25/4	1.65	0.50–5.46	0.416	1.73	0.52–5.77	0.376

~7 times more capable of complementing a repair-deficient strain than the *Cys* allele (11). Thus, we assigned the *Ser/Ser* genotype or *Ser/Ser* and *Ser/Cys* genotypes combined as reference groups (10), but we should be aware that there is no solid biological rationale for this assignment of *Ser/Ser* plus *Ser/Cys* as a reference at this moment.

Compared to the *Ser/Ser* genotype, the *Ser-Cys* polymorphism was not associated with an increased risk of lung cancer in any subtype (Table 2). However, when homozygous *Cys/Cys* were compared against the other genotypes combined, the increased risk was observed in squamous cell carcinoma and nonadenocarcinoma after adjustment for age and smoking habits (Table 3). We still do not have a convincing explanation for our finding that only *Cys/Cys* was a high-risk genotype. These observations can be interpreted in several ways. Although we do not yet have *in vitro* data, these results are consistent with a recessive, reduced repair activity of the *Cys* allele. The hypothesis that only homozygous status of *Cys* (alternatively, the *Ser* allele is dominant) influences individual repair activity would explain the observation. Considering the assumed strong relationship of oxyradicals in tobacco smoke to squamous cell carcinoma, our observation that the significant association was limited to squamous cell carcinoma may reflect hOGG1 commitment in at least some of tobacco-related carcinogenesis. Consistent with this interpretation, the ORs for heavy smokers were higher (although not statistically significant) for all comparisons (Tables 2 and 3). Currently, other subtypes such as adenocarcinoma of the lung are also considered to be associated with smoking, although the relative risk was much lower than in squamous cell and small cell carcinoma (30). To the best of our knowledge, the involvement of oxyradicals in carcinogenesis of different subtype of lung cancer has never been addressed, and this issue remains to be investigated.

Our data may imply that the association of this genotype and lung cancer should not, however, be overemphasized. The lack of association with small cell carcinoma may be due to the low statistical power concomitant with the small number of these cancers. Our power calculation showed that our study seems to have a sufficient power to detect the risk only in squamous cell and nonadenocarcinoma. *hOGG1* polymorphisms other than Ser326Cys may play a role, or the contribution of this gene to repair 8-hydroxyguanine may be small relative to other repair systems, such as the nucleotide excision repair system (31).

As far as we know, this is the first case-control study of *hOGG1* Ser326Cys polymorphism as it relates to human lung cancer, and the findings may be a local phenomenon restricted to Okinawan lung cancer. Further studies will be necessary to clarify the involvement of this gene in human lung carcinogenesis in general. It is possible that the Ser326Cys polymorphism may be involved greater to oxidative damage repair in the other organs, and thus, to carcinogenesis or other pathological conditions, such as carcinoma of the stomach (32, 33).

It is well known that candidate genetic markers responsible for cancer susceptibility sometimes vary in frequencies among populations. Thus, it is very important to know the basic prevalence in multiple populations to evaluate the significance of the genetic markers in cancer risk assessment (34). The

Table 3 ORs for the Cys/Cys genotype of *hOGG1* Ser326Cys polymorphism *versus* the other genotypes combined and those stratified by smoking dosage

Genotype	No. of cases/controls	Crude			Adjusted for age and smoking habits		
		OR	95% CI	*P*	OR	95% CI	*P*
Total							
Ser/Ser + Ser/Cys	200/170	1.00			1.00		
Cys/Cys	41/27	1.29	0.76–2.19	0.343	1.71	0.92–3.19	0.091
Squamous cell carcinoma							
Ser/Ser + Ser/Cys	94/170	1.00			1.00		
Cys/Cys	24/27	1.61	0.88–2.94	0.124	3.01	1.33–6.83	0.008[a]
Small cell carcinoma							
Ser/Ser + Ser/Cys	26/170	1.00			1.00		
Cys/Cys	2/27	0.48	0.11–2.16	0.342	0.72	0.14–3.66	0.691
Adenocarcinoma							
Ser/Ser + Ser/Cys	65/170	1.00			1.00		
Cys/Cys	13/27	1.26	0.61–2.59	0.531	1.58	0.68–3.65	0.290
Nonadenocarcinoma							
Ser/Ser + Ser/Cys	130/170	1.00			1.00		
Cys/Cys	28/27	1.36	0.76–2.41	0.300	2.18	1.05–4.54	0.037[a]
					Adjusted for age		
					OR	95% CI	*P*
Cigarette-years <800							
Ser/Ser + Ser/Cys	62/100	1.00			1.00		
Cys/Cys	16/20	1.29	0.62–2.68	0.494	1.16	0.55–2.46	0.697
Cigarette-years ≥800							
Ser/Ser + Ser/Cys	138/49	1.00			1.00		
Cys/Cys	25/4	2.22	0.74–6.70	0.157	2.33	0.77–7.09	0.137

[a] $P < 0.05$.

Table 4 Ser326Cys polymorphism of *hOGG1* gene in six different ethnic groups[a]

Genotype	Chinese (Jiangsu)	Hungarian	Australian Caucasian	Japanese (Tokyo)	Melanesian (Vanuatu)	Micronesian (Yap)
Ser/Ser	12 (12.0)	95 (63.7)	55 (39.9)	26 (27.7)	41 (74.5)	41 (25.8)
Ser/Cys	53 (54.1)	50 (33.6)	78 (56.5)	54 (57.4)	12 (21.8)	105 (66.0)
Cys/Cys	33 (33.9)	4 (2.7)	5 (3.6)	14 (14.9)	2 (3.7)	13 (8.2)

[a] Numbers in parenthesis are percentages.

distribution of the Ser326Cys polymorphism was significantly different among several ethnic groups. Further research on the importance of *hOGG1* gene in oxidative damage repair is needed to determine the applicability of the Ser326Cys polymorphism as a marker for increased cancer risk in light of the highly variable allele frequencies we have observed among populations. The differences of this polymorphism among populations will influence the interpretations and strategies for its use as a potential tool for estimation of a particular individual risk, as has been done for *CYPIA1* (34–37). In the populations that have low *Cys* allele frequencies, like the Hungarians, our power analysis indicated that required sample sizes needed to obtain significant results would be so large that such studies would not be economically feasible. Previously, we reported an association of *CYPIA1* Ile-Val polymorphism with lung cancer in this area and observed a significantly increased risk of *Val/Val* genotype for some of lung cancer (13). As far as the ORs are comparable (they would be comparable if the sample size was sufficiently large and CIs of the ORs were not so wide), the lung cancer risk associated with the Ser326Cys polymorphisms appears to be smaller than that associated with the Ile-Val polymorphism of *CYPIA1* (13).

In conclusion, the Ser-Cys *hOGG1* polymorphisms, especially the *Cys/Cys* genotype, has a potential role as a high risk marker for at least a subset of tobacco-related lung cancer. We found increased ORs among Cys/Cys individuals with higher smoking dosage, consistent with the hypothesis that smoking induced 8-hydroxyguanine is repaired less in heavy smokers with this susceptible genotype, although the association was not statistically significant.

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