

Glutathione Peroxidase Codon 198 Polymorphism Variant Increases Lung Cancer Risk¹

Duminda Ratnasinghe,² Joseph A. Tangrea, Mark R. Andersen, Michael J. Barrett, Jarmo Virtamo, Philip R. Taylor, and Demetrius Albanes

Division of Clinical Sciences, National Cancer Institute, NIH, Bethesda, Maryland 20892-7058 [D.R., J. A. T., P. R. T., D. A.]; New Chemical Entities, Inc., Thetagen Division, Bothell, Washington 98011 [M. R. A.]; Information Management Services, Inc., Silver Spring, Maryland 20904 [M. J. B.]; and National Public Health Institute, SF-00300 Helsinki, Finland [J. V.]

ABSTRACT

Human cellular glutathione peroxidase 1 (hGPX1) is a selenium-dependent enzyme that participates in the detoxification of hydrogen peroxide and a wide range of organic peroxides. We conducted a case-control study nested within the α -Tocopherol, β -Carotene Cancer Prevention Study cohort to evaluate the association between the proline to leucine polymorphism at codon 198 of hGPX1 and lung cancer risk. Cases ($n = 315$) were matched to controls on age (± 5 years), intervention group, and study clinic using incidence density sampling in a 1:1 ratio. The prevalence of the hGPX1 Pro198leu variant allele was 58% for controls and 71% for cases ($P < 0.001$). Using conditional logistic regression, we found a significant association between hGPX1 genotype and lung cancer risk. The odds ratio for heterozygotes was 1.8 (95% confidence interval, 1.2–2.8) and 2.3 (95% confidence interval, 1.3–3.8) for homozygous variants compared to wild-type individuals. Due to its high prevalence, the hGPX1 variant may contribute significantly to lung cancer risk among Caucasians but not among ethnic Chinese who do not exhibit this polymorphism.

INTRODUCTION

Although environmental and industrial exposures such as radon can increase lung cancer risk, it is well established that tobacco smoke is principally responsible for the incidence of this malignancy. However, not all persons exposed to high concentrations of airborne carcinogens develop lung cancer. Differences in lung cancer risk among individuals with similar carcinogen exposures may be due to genetic predisposition and dietary factors that have been shown to modulate or neutralize carcinogen-derived oxidative radicals.

The risk of developing cancer is determined by both the number and nature of cumulative carcinogenic exposures and by individual genetic variation (1). For example, genetic polymorphisms in detoxification enzymes can substantially alter the magnitude of exposure to carcinogenic substances. hGPX1³ is a selenium-dependent enzyme that participates in the detoxification of hydrogen peroxide and a wide range of organic peroxides with reduced glutathione (2). From a sequence analysis of lung tumor specimens, Moscow *et al.* (3) reported a nucleotide substitution at codon 198 of hGPX1, resulting in the substitution of leucine for proline. They also reported that other polymorphisms cosegregate with the proline to leucine substitution, including six alanines in the polyalanine sequence (instead of five or seven alanines with the wild-type proline), a T for C substitution at +2, and a G for A substitution at –592.

In the present study, we sought to confirm the presence of an

hGPX1 germ-line codon 198 (Pro198leu) polymorphism among members of a cancer prevention trial in Finland and explored the association between the polymorphism and lung cancer risk. We also examined whether age, smoking behavior, serum antioxidant levels, or the trial antioxidant supplementation could modify the hGPX1-lung cancer association.

PATIENTS AND METHODS

Study Cohort. The cases and controls for this study were selected from the cohort of the α -Tocopherol, β -Carotene Cancer Prevention Study, a randomized, placebo-controlled prevention trial designed to determine whether α -tocopherol (50 mg/day), β -carotene (20 mg/day), or both would reduce the incidence of lung, prostate and other cancers. The overall design, rationale, and objectives of this study have been published, as have the main trial findings (4–6). The trial was conducted between 1985 and 1993 in southwestern Finland as a joint project between the National Public Health Institute of Finland and the National Cancer Institute of the United States. Potential participants, all men aged 50–69 years residing in southwestern Finland ($n = 290,406$) were identified from the computer list of the national population registry of Finland. Participants (29,133) had to be current smokers of five or more cigarettes per day, eligible and willing to participate, and give informed consent. Information on variables that possibly could confound the association between the polymorphism of hGPX1 and lung cancer risk were available from α -Tocopherol, β -Carotene Cancer Prevention Study baseline questionnaires. Baseline measurements of serum α -tocopherol and β -carotene levels were also available.

Selection of Cases and Controls. The cases consist of 315 men diagnosed with primary lung cancer (ICD-9, 162) during the years 1985–1994 among those who gave a whole blood sample in 1992 and 1993. Seventy-eight percent of the cases donated blood before cancer diagnosis. Using incidence density sampling, the controls were selected from cohort participants who were alive and free of cancer at the time the matched case was diagnosed. Controls were selected from those who donated blood matched to cases on age (± 5 years), intervention group, and study clinic in a 1:1 ratio.

Genotype Analyses. PCR primers and dual-labeled allele discrimination probes were designed using PrimerExpress version 1.0 (PE Biosystems). Probes were selected that have a predicted T_m near 68°C, with the polymorphic base near the center. Flanking PCR primers were selected based on the calculated penalty score, T_m , length, and amplicon size. Primers and probes were synthesized and purified by PE Biosystems. Oligonucleotide sequences for primers and probes to detect the C to T polymorphism in codon 198 of hGPX1 were: PCR forward, TGTGCCCTACGCAGGTACA; PCR reverse, CCCCCGACACAGCA; C allele probe, ¹²⁵CCTGTCTCAAGGGCCCA-GCTGTG^{TAMRA}; and T allele probe, ¹²⁵CTGTCTCAAGGGCTCAGCTGTGCT^{TAMRA}.

Reactions (10 μ l) contained ~20 ng of genomic DNA isolated from whole blood, 1 \times TaqMan Master Mix, dual-labeled probes (100 nM each), and PCR primers (900 nM each). Reactions were performed in 96-well MicroAmp Optical reaction plates and caps (PE Biosystems). Plates were incubated in PE Biosystems 9600 thermal cyclers at 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 62°C for 1 min. Fluorescence was measured using the ABI Prism 7700 Sequence Detector and analyzed with Sequence Detection System version 1.6.3. Amplified DNA from several individuals exhibiting each genotype was electrophoresed on an agarose gel to confirm amplicon size and sequenced to confirm each genotype. When possible, each plate contained two control DNA samples for each homozygous genotype and

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² To whom requests for reprints should be addressed, at Division of Clinical Sciences, Cancer Prevention Studies Branch, 6006 Executive Boulevard, MSC 7058, Bethesda, MD 20892-7058. E-mail: DR132K@NIH.gov.

³ The abbreviation used is: hGPX1, human glutathione peroxidase 1.

Table 1 Medians of selected subject baseline characteristics by case-control status^a

Characteristic	Cases (n = 315)	Controls (n = 315)	P ^b
Age (yr)	60	59	0.93
Cigarettes per day	20	20	<0.001
Yr smoked	40	39	<0.001
Age started smoking	18	19	<0.001
Body mass index (kg/m ²)	25.3	26.0	<0.04
Alcohol (g/day)	8.9	9.6	0.85
Serum α -tocopherol (mg/l)	11.3	11.5	0.09
Serum β -carotene (μ g/l)	169	166	0.12

^a Based on unmatched data with continuous variables expressed as the median.

^b P determined by Wilcoxon rank-sum tests.

four no template controls. All laboratory personnel were blind to the case-control status of the samples. A random sample of 10% was repeated for quality control and was 100% concordant.

Statistical Analyses. The χ^2 test for heterogeneity was used to test whether the distribution of allele prevalences was the same for cases and controls. Conditional logistic regression was used to examine the association between genotype and lung cancer risk. Potential confounding of the association between the *hGPX1* genotype and cancer risk by other related risk factors was explored using Spearman rank correlation analysis and multivariate logistic regression models, including stepwise regression models. If the potential confounder caused a significant change in the log likelihood estimate ($P < 0.05$) and a $>20\%$ change in the β -coefficient, it was kept in the model for further multivariate analysis. Modification of the effect of genotype by age, tobacco, α -tocopherol, and/or β -carotene supplementation, serum β -carotene and α -tocopherol on lung cancer risk was examined by statistical tests of the first order interaction term in the logistic regression models. Exclusion of cases diagnosed before blood draw did not materially alter any of the risk estimates, therefore, all cases were included in the statistical analyses. All analyses were performed using the statistical software package STATA (STATA Corporation, College Station, TX).

RESULTS

Table 1 shows a case-control comparison of selected baseline subject characteristics. As expected, cigarette smoking and age-started smoking were significantly different in the cases compared with controls. Levels of serum antioxidants, α -tocopherol, and β -carotene were not different in lung cancer cases compared with controls.

Table 2 shows the association between the *hGPX1* genotype and lung cancer risk. The distribution of *hGPX1* alleles was different comparing the cases with controls (χ^2 P for distributions <0.001). In addition, we observed an association between the *hGPX1* genotype and lung cancer risk. Individuals with the heterozygous genotype were at 80% greater risk for lung cancer compared to those with the homozygous wild-type (pro/pro) genotype. Individuals with the homozygous variant genotype (*leu/leu*) were at 130% greater risk for lung cancer compared with homozygous wild-type individuals. Adjustment for baseline covariates such as years smoked or cigarettes smoked per day did not materially alter the risk estimates. However, the risk estimates shown in Table 2 were adjusted for years smoked and cigarettes smoked per day because they were significantly associated with lung cancer risk.

We also evaluated the association between the *hGPX1* genotype and histological subtypes of lung cancer. The relative risk estimates for squamous cell ($n = 140$, odds ratio homozygous wild type, heterozygous variant, and homozygous variant; 1.0, 2.4, and 3.2, respectively) and adenocarcinoma ($n = 56$, odds ratio homozygous wild type, heterozygous variant, and homozygous variant; 1.0, 3.9, and 3.3, respectively) of the lung were statistically significant for both the heterozygous and homozygous variant genotypes compared to those with the homozygous wild-type genotype. By contrast, risk of small cell lung carcinoma ($n = 49$) was not associated with *hGPX1*, possibly due to the relatively small number of cases for this subtype.

Evaluation of age, cigarette smoking, serum α -tocopherol and/or β -carotene at baseline, and trial intervention of α -tocopherol and/or β -carotene as potential modifiers of the association between *hGPX1* and lung cancer risk showed no statistically significant interactions (data not shown).

DISCUSSION

A large proportion of lung cancers have a deletion of the short arm of chromosome 3, in the region of 3p21 (7, 8). *hGPX1* is a selenium-dependent detoxifying enzyme that has been mapped to chromosome 3p21 by *in situ* hybridization of lymphocyte metaphase spreads (3). Glutathione peroxidases protect cells against oxidative damage by reducing hydrogen peroxide and a wide range of organic peroxides with reduced glutathione (2). The cytosolic form of *hGPX1* belongs to a family of selenium-dependent peroxidases that include another cytosolic form, *hGPX2* (9), the plasma-based *hGPX3* (10), and the phospholipid hydroperoxidase *hGPX4* (11).

Many studies have shown that selenium increases *hGPX1* activity and expression (12–14). It is generally assumed that selenium increases the antioxidant capacity of a cell, consequently reducing intracellular oxidative stress. The association between prospectively collected serum selenium and lung cancer has been reported in over a dozen studies (15–19). Most of these studies found that selenium concentrations were slightly lower in the lung cancer cases than in controls.

In the current study, we confirm the presence of a germ-line polymorphism in the coding region of *hGPX1* with notable prevalence of allele variants among middle-aged Finnish smokers. We also show that individuals with one or two variant alleles were at increased risk of lung cancer compared with homozygous wild-type individuals. One possible explanation for the increased lung cancer risk observed among individuals with just one variant allele is that, because different *hGPX1* alleles encode structurally different *hGPX1* subunits, heterozygote individuals may have a less efficient final glutathione peroxidase complex. In addition, serum antioxidant levels or supplements of α -tocopherol (50 mg daily) or β -carotene (20 mg daily) did not seem to modify lung cancer risk associated with the variant *hGPX1* genotypes. This may be due to lack of chemical reactivity of α -tocopherol and/or β -carotene with hydrogen peroxide or organic peroxides. Smoking also did not modify the risk of lung cancer due to *hGPX1* variants.

The association we report here is complicated by the other polymorphisms that cosegregate with the codon 198 polymorphism. These polymorphisms include six alanines in the *hGPX1* polyalanine sequence (instead of five or seven alanines with the wild-type proline), a T for C substitution at +2, and a G for A substitution at –592 with the proline to leucine substitution. Although the exact structural or functional consequences of these *hGPX1* coding region polymorphisms are not known, the substitution of the α -imino acid proline (cyclic amino acid) by leucine is probably the polymorphism that causes the most profound secondary and tertiary conformational change of *hGPX1*, because proline is the only amino acid without a

Table 2 Association between the glutathione peroxidase polymorphism and lung cancer risk^a

<i>hGPX1</i> codon 198	Cases (n = 315)	Controls (n = 313) ^b	Odds ratio
Pro/Pro	91 (29%)	132 (42%)	1.00 (Ref)
Pro/Leu	157 (50%)	135 (43%)	1.8 (1.2–2.8)
Leu/Leu	67 (21%)	46 (15%)	2.3 (1.3–3.8)
P	$P_{\chi^2} < 0.001$		$P_{\text{trend}} < 0.001$

^a Risk estimates from conditional logistic regression after adjusting for total number of cigarettes smoked per day and years of smoking.

^b PCR failed for two control individuals.

free unsubstituted amino group on the α carbon atom and is known to cause a unique kink in the secondary structure of peptides.

One of the strengths of this study is its prospective design. The collection of covariate data (e.g., smoking) before case diagnoses minimized the potential for recall bias. The availability of detailed exposure data also allowed us to explore several relevant gene-environment interactions. The availability of data on prospective serum antioxidant levels and supplements of α -tocopherol or β -carotene given during the intervention trial added another dimension to our study because of the antioxidant functions of hGPX1. The generalizability of these results, however, may be somewhat restricted because the study was conducted among Finnish male smokers. Other case-control studies examining the association of hGPX1 polymorphisms and lung cancer risk are clearly needed. In future studies, our group will attempt to examine the association between the hGPX1 polymorphism and lung cancer risk in other Caucasian populations. Our analysis of ethnic Chinese members of the Yunnan tin corporation cohort (20) revealed that they do not exhibit the codon 198 hGPX1 polymorphism (all 92 noncase samples analyzed were wild type).

In summary, we show that the codon 198 hGPX1 polymorphism is associated with increased risk of lung cancer among male smokers. We also found that neither smoking nor supplementation with either α -tocopherol and/or β -carotene altered the association between the hGPX1 proline to leucine substitution polymorphism and risk of lung cancer. Because of its high prevalence, the codon 198 variant allele of hGPX1 may have substantial public health impact by influencing lung cancer risk among Caucasians but not among ethnic Chinese.

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