

Short Communication

Exon 5 Polymorphisms in the *O*⁶-Alkylguanine DNA Alkyltransferase Gene and Lung Cancer Risk in Non-Smokers Exposed to Second-Hand Smoke

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Abstract

Purpose: The objective of the study was to examine the association of three exon 5 variants in the *O*⁶-alkylguanine DNA alkyltransferase (AGT) gene involved in the repair of the mutagenic DNA lesion *O*⁶-alkylguanine formed by nitrosamines, with lung cancer risk in never-smokers.

Experimental Design: Exon 5 of the AGT gene was sequenced in genomic DNA from 136 cases and 133 hospital- or population-based controls for whom questionnaire information on second-hand smoke and diet was available to determine the frequencies of the Gly¹⁶⁰Arg, Ile¹⁴³Val, and Lys¹⁷⁸Arg variant alleles.

Results: No codon ¹⁶⁰Arg variant alleles were found in the study population. The codon ¹⁴³Val and ¹⁷⁸Arg variant alleles, present at allele frequencies of 0.07, showed 100% linkage. The odds ratio (OR) of lung cancer for these variant carriers was 2.05 [95% confidence interval (CI) 1.03–4.07]. The risk varied between the different lung cancer pathologies with an increased risk for adenocarcinoma (OR 2.67, 95% CI 1.21–5.87) or small cell carcinoma (OR 4.83, 95% CI 0.91–25.7) but not for squamous cell carcinoma (OR 1.07, 95% CI 0.27–4.18). Compared with individuals carrying the mutant alleles unexposed to second-hand smoke, the OR for exposed variant carriers was 1.95 (95% CI 0.53–1.15); a similar interaction, although not significant, was

observed for low consumption of cruciferous vegetables and for green vegetables and tomatoes.

Conclusions: These results point toward a role of AGT polymorphisms in lung cancer susceptibility among never-smokers, in particular among subjects exposed to environmental carcinogens.

Introduction

Second-hand smoke, a widely studied lung cancer risk factor among non-smokers, is a complex mixture containing several groups of carcinogens, including carcinogenic *N*-nitroso compounds (1), the metabolites of which form the mutagenic DNA lesion *O*⁶-alkylguanine. This adduct is predominantly repaired via the activity of *O*⁶-alkylguanine DNA alkyltransferase (AGT), which irreversibly transfers the alkyl group to an internal cysteine acceptor site, leading to its inactivation and the restoration of the guanine moiety in the DNA. Animals lacking AGT are more sensitive to carcinogenesis and show increased cytotoxicity when exposed to alkylating agents (2).

Several polymorphisms of the human AGT have been described. A Gly¹⁶⁰Arg variant in exon 5, 15 amino acids away from the cysteine in the active site, was originally reported at an allele frequency of ~15% (3); however, lower frequencies, in the order of 0–1.5%, were found in subsequent studies (4–7). The exon 5 variants Ile¹⁴³Val and Lys¹⁷⁸Arg have been reported at allele frequencies of up to 21% with a strong linkage disequilibrium being noted between them (4, 7, 8). In addition, five polymorphisms in the promoter, one in exon 1 and two in exon 3, have been reported (9). In one study, presence of the codon 143 valine allele was shown to be associated with an increased lung cancer risk among smokers [odds ratio (OR) 2.1, 95% confidence interval (CI) 1.01–4.7; Ref. 7].

The goal of this present study was to examine the role of the three exon 5 variants as potential susceptibility factors for lung cancer in never-smokers. As alterations of the active site cysteine by oxidative stress may change the active site's status, the potential interaction of the polymorphisms with other environmental factors such as dietary constituents containing antioxidants was also addressed.

Materials and Methods

Subjects. The DNA used was isolated from peripheral blood samples collected from participants in a multicenter case-control study of lung cancer in never-smokers conducted in eight countries between 1992 and 1994 (10). All participants provided an informed consent and relevant review boards approved the study. Study subjects (136 incident cases and 133 controls, Table 1) were all Caucasian and had smoked <400 cigarettes or an equivalent amount of cigars, cigarillos, or pipe tobacco during their life; they had completed a questionnaire assessing exposure to second-hand tobacco smoke and fre-

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Note: V. Gaborieau participated in the management of the project.

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Table 1 Selected characteristics of cases and controls^a

	Cases (n = 136)	Hospital controls (n = 80)	Population controls (n = 53)
Gender			
Female	119 (87.5)	58 (72.5)	44 (83.0)
Male	17 (12.5)	22 (27.5)	9 (17.0)
		$\chi^2 P = 0.02$	
Age (mean, yrs)	62.9 (13.4)	61.9 (14.0)	61.2 (12.6)
		$\chi^2 P = 1.00$	
Country			
Brazil	3 (2.2)	18 (22.5)	—
France	8 (5.9)	10 (12.5)	—
Germany	19 (14.0)	—	16 (30.2)
Italy	7 (5.2)	22 (27.5)	5 (9.4)
Poland	9 (6.6)	10 (12.5)	—
Romania	12 (8.8)	2 (2.5)	9 (17.0)
Russia	21 (15.4)	18 (22.5)	—
Sweden	57 (41.9)	—	23 (43.4)
		$\chi^2 P < 0.01$	
Educational level			
Up to secondary	63 (46.3)	57 (71.3)	21 (39.6)
More than secondary	73 (53.7)	23 (28.7)	32 (60.4)
		$\chi^2 P < 0.01$	

^a Controls are either healthy individuals randomly selected from population registries (Sweden and Germany) or hospital patients admitted for diseases not related to tobacco smoking (other countries). The figures in parentheses are either percentages (gender, country, educational level) or SD (age).

quency of consumption of selected foods and food groups (11). Cases were recruited immediately after diagnosis among patients with histologically or cytologically confirmed primary lung cancer and were enrolled in the study before any therapy was started. Controls were selected either among patients from the same hospital as the cases or, in two countries, from healthy individuals identified from population registries. All hospital-based controls suffered from non tobacco-related diseases, *i.e.*, injuries; diseases of the musculoskeletal system; diseases of the genitourinary system; diseases of the digestive system such as hernia, diverticula, and fistula; chronic sinusitis; and benign neoplasms and malignant melanoma of the skin.

Genotyping. Exon 5 of the AGT gene was sequenced using PCR primers designed on the basis of the published human sequence (GenBank accession no. NT_008818). The amplification was carried out using 300 ng of sense (5'-TTGTCCA-GATCCCTGACTGA-3') and 300 ng of antisense (5'-ATC-CGATGCAGTGTTACACG-3') primers to generate a 310-bp fragment. Sequencing reactions were performed on the ABI 310 genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol (Big dye Terminator cycle sequencing kit; Applied Biosystems). Analysis of all samples was carried out blindly for case/control status. For quality control purposes, a 10% subset of samples were sequenced bi-directionally or reanalyzed with the results being 100% concordant (data not presented).

Statistical Methods. Multivariate analysis was performed using unconditional logistic regression. The ORs and 95% CIs presented were adjusted for study center. Additional adjustment for age, gender, and education did not modify the results. Exposure to second-hand smoke was defined as ever exposure from the spouse or at the workplace; dietary exposure was defined as intake below the median level in the study population. Statistical analysis was conducted using STATA 7.0 software (12), and all tests were two-sided.

Results

The allele frequency of the codon ¹⁴³Val and the codon ¹⁷⁸Arg variants among controls was 0.07, although some variation was noted between different countries: from 0% in Poland and France to 13% in Sweden. All individuals carrying the codon ¹⁴³Val variant were found to also carry the codon ¹⁷⁸Arg variant. It was therefore assumed that complete linkage disequilibrium existed between these two variants. The genotype distributions were consistent with Hardy-Weinberg equilibrium in both cases and controls. No codon ¹⁶⁰Arg variant alleles were found in the 269 samples sequenced. A statistically significant elevation in the risk of lung cancer was associated with the codon ¹⁴³Val/¹⁷⁸Arg variant alleles (OR 2.05, 95% CI 1.03–4.07): the OR was increased for adenocarcinoma and small cell carcinoma but not for squamous cell carcinoma (Table 2). Testing for heterogeneity showed that the histology-specific ORs were different ($P = 0.001$). As compared with wild-type individuals unexposed to second-hand smoke, the OR for exposed wild-type individuals was 1.25 (95% CI 0.65–2.38), whereas for exposed individuals carrying the mutant alleles, the OR was 1.95 (95% CI 0.53–1.15) compared with unexposed subjects (Table 3). Although not significant, an interaction similar to the one observed for second-hand smoke was suggested between low consumption of cruciferous vegetables (OR = 2.45, 95% CI 0.47–13.00) or green vegetables and tomatoes (OR = 1.92, 95% CI 0.27–13.58) and presence of these variants (Table 3).

Discussion

This study confirms previous results of an increased risk of lung cancer among carriers of AGT exon 5 variants and suggests that the risk might be strongest in the presence of low-level exposure to environmental pollutants, as in the case of non-smokers exposed to second-hand smoke. This study suffers from several potential limitations; firstly, the stratified analyses were underpowered, although the main analysis was not affected by limited power and resulted in statistically significant ORs. Secondly, controls were hospital or community based. However, the fact that the genotype distribution of cases and controls was in Hardy Weinberg equilibrium argues against bias in the selection of controls.

Although a mechanistic interpretation of these results is not straightforward, codons 143 and 178 are both close to the

Table 2 Odds ratio of lung cancer for codon 143 and 178 polymorphisms^a

	Cases	Controls	Odds ratio	95% confidence interval
All subjects				
Wild-type	102	115	1.00	
Mutant ^b	34	18	2.05	1.03–4.07
Squamous cell carcinoma				
Wild-type	17	115	1.00	
Mutant ^b	3	18	1.07	0.27–4.18
Small cell carcinoma				
Wild-type	4	115	1.00	
Mutant ^b	3	18	4.83	0.91–25.7
Adenocarcinoma				
Wild-type	47	115	1.00	
Mutant ^b	24	18	2.67	1.21–5.87

^a Odds ratios are adjusted for country. Reference category: wild-type.

^b Heterozygous or homozygous mutant; one case of adenocarcinoma was homozygous mutant, all other cases and controls were either homozygous wild-type or heterozygous.

Table 3 Interaction between codon 143 and 178 polymorphisms and exposure to second-hand smoke and dietary factors^a

Exposure	Polymorphism	Unexposed		Exposed	
		Ca/Co	Odds ratio (95% confidence interval)	Ca/Co	Odds ratio (95% confidence interval)
Second-hand smoke	Wild-type	26/41	1.00 ^b	76/74	1.25 (0.65–2.38)
	Mutant ^c	8/8	1.00 ^b	26/10	1.95 (0.53–1.15)
Interaction ^d					1.65 (0.38–7.19)
P for interaction					0.5
Low fruit intake	Wild-type	59/41	1.00 ^b	41/62	0.78 (0.32–1.91)
	Mutant ^c	16/7	1.00 ^b	16/7	0.91 (0.07–12.52)
Interaction ^d					2.52 (0.60–10.58)
P for interaction					0.2
Low cruciferous vegetable intake	Wild-type	55/49	1.00 ^b	45/53	1.16 (0.56–2.39)
	Mutant ^c	15/9	1.00 ^b	17/5	2.45 (0.47–13.00)
Interaction ^d					3.94 (0.89–17.39)
P for interaction					0.07
Low green vegetable and tomato intake	Wild-type	59/65	1.00 ^b	41/38	1.52 (0.76–3.01)
	Mutant ^c	16/8	1.00 ^b	16/6	1.92 (0.27–13.58)
Interaction ^d					2.28 (0.52–9.91)
P for interaction					0.3

^a Odds ratios are adjusted for country.

^b Unexposed subjects are the reference category.

^c Heterozygous or homozygous.

^d Odds ratio of the term of interaction between polymorphism and each environmental factor.

active site of the repair protein and AGT activity might be influenced by amino acid substitutions in this region. The AGT activity has been previously measured using an *in vitro* assay with DNA containing *O*⁶-methylguanine as the substrate in lymphocyte protein extracts prepared from 145 of the individuals studied here (13). The presence of the codon 143 and 178 variant alleles did not correlate with a significant change in the mean enzyme activity in either the cases or controls (*P*s of difference, Mann-Whitney test, 0.31 and 0.27, respectively). In that analysis, a weak, nonsignificant difference in AGT activity was detected between cases and controls. The AGT genotype shows a stronger association with lung cancer risk than measurement of its enzymatic activity in peripheral blood lymphocytes at the time of disease. However, the functional consequences of these polymorphisms remain to be established. Because the reaction of AGT leads to its inactivation, any substrate for the protein acts as an irreversible inhibitor. The presence of the codon 160Arg variant, although having only a small effect on the activity of AGT toward methylated DNA *in vitro* (3), reduces the inactivation of the AGT protein by the inhibitor *O*⁶-benzylguanine with at least a 20-fold increase in the ED₅₀ (14), suggesting that the ability to inactivate is also a critical factor in the response of AGT to environmental carcinogens. Although comparable data on the effect of codon 143 and codon 178 variants on inactivation of AGT are not available, it has been shown that several amino acid substitutions within the active site pocket of AGT can influence its inactivation, and it remains to be determined whether these natural variants can also impart resistance (15). In conclusion, individual susceptibility from polymorphisms in genes involved in critical steps of carcinogenesis may play a particularly important role in circumstances of low-level exposure to environmental carcinogens (16): our results support this notion in the case of lung cancer development among non-smokers exposed to second-hand smoke.

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