

Null Results in Brief

MDM2 SNP309 and SNP354 Are Not Associated with Lung Cancer Risk

Sharon R. Pine,¹ Leah E. Mechanic,¹ Elise D. Bowman,¹ Judith A. Welsh,¹ Stephen C. Chanock,² Peter G. Shields,³ and Curtis C. Harris¹

¹Laboratory of Human Carcinogenesis, National Cancer Institute, Center for Cancer Research, NIH; ²Section on Genomic Variation, Pediatric Oncology Branch, National Cancer Institute, Bethesda, Maryland; and ³Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, District of Columbia

Abstract

A single nucleotide polymorphism (SNP) in the *MDM2* promoter (a T to G exchange at nucleotide 309) has been found to be associated with tumor formation. Publication of this null report is important because an association between *MDM2* SNP309 and lung cancer was previously reported in two independent studies. Our findings suggest that *MDM2*

SNP309 is not a strong factor in lung carcinogenesis. In addition, this is the first *MDM2* SNP309 report on a population consisting of Caucasians in the United States and African-Americans. A strength of the study design is that the controls consist of both population and hospital controls. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1559–61)

Introduction

MDM2 is a ubiquitin ligase that inhibits p53 and promotes its degradation (1). The variant G allele of *MDM2* SNP309 heightens *MDM2* protein levels and causes an attenuation of the p53 response pathway (2, 3) and may represent a cancer predisposing allele. The *MDM2* SNP309 polymorphism was associated with a decreased age at the time of cancer diagnosis in Li-Fraumeni syndrome, sporadic sarcoma (3), and in colorectal patients with p53 wild-type tumors (4). The G allele of *MDM2* SNP309 was associated with an increased risk of esophageal squamous cell carcinoma (5) but not breast or ovarian cancer (6). *MDM2* SNP354 (E354E) was found to be associated with breast cancer risk (7).

The association of *MDM2* SNP309 with lung cancer was reported, but the results were contradictory (7–9). This is the first report of *MDM2* polymorphisms and lung cancer risk in the United States and includes Caucasians and African-Americans.

Materials and Methods

Study Population. The case-control study design, inclusion and matching criteria, and demographics were described (10). Ninety-seven percent of the 1,224 study participants had sufficient DNA for analysis. Of these, 504 were lung cancer patients, 317 were hospital controls, and 363 were population controls. Four hundred four (33%) were African-Americans, and the remainder were Caucasians. The majority were current ($n = 384$, 32%) or former ($n = 575$, 49%) smokers. All but four samples were successfully genotyped for the *MDM2* SNP309

polymorphism. Nine hundred ninety samples were genotyped previously for *MDM2* SNP354, when fewer participants were enrolled in the ongoing study, and 98% (971) were successfully genotyped. The race, age, gender, and smoking status distributions for those with SNP354 genotype data were the same as the larger study population. The study was approved by the participating institutions' Review Boards.

Genotyping. SNP309 (rs2279744) was determined with a MGB Eclipse by Design assay (Nanogen, Inc., San Diego, CA; ref. 7). SNP354 (rs769412) was genotyped at the National Cancer Institute Genotyping Core Facility (<http://snp500cancer.nci.nih.gov>). All samples and 10% duplicates were blinded and randomized among the cases and controls. There was 100% and 98% concordance among the *MDM2* SNP309 and SNP354 duplicates, respectively. TP53-01 (R72P, rs1042522) genotypes were determined by the National Cancer Institute Genotyping Core Facility (11).

Statistical Analysis. We assessed the associations between *MDM2* SNP309 and SNP354 genotypes and risk of lung cancer after controlling for age, pack-years, and race by computing odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression analysis. The frequency distributions of the polymorphisms and the ORs were similar in the hospital- and population-based controls; therefore, the control groups were combined. Comparisons of mean age at lung diagnosis were tested using two-tailed *t* tests. Calculations were done using STATA software version 9 (STATA Corp., College Station, TX). Power analysis was done by assuming a dominant allele model using PS: Power and Sample Size Calculation version 2.1.31 by W.D. Dupont and W.D. Plummer (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>; ref. 12).

Results

The *MDM2* SNP309 genotype frequencies among the control groups were in Hardy-Weinberg equilibrium for the African-Americans (hospital, $P = 0.25$; population, $P = 0.21$) and Caucasians (hospital, $P = 0.97$; population, $P = 0.58$). For *MDM2* SNP354, there was a violation of Hardy-Weinberg

Received 3/20/06; accepted 5/30/06.

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.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

S.R. Pine and L.E. Mechanic contributed equally to this work.

Requests for reprints: Curtis C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, Room 3068, Building 37, 37 Convent Drive, Bethesda, MD 20892-4258. Phone: 301-496-2048; Fax: 301-496-0497. E-mail: curtis_harris@nih.gov

Copyright © 2006 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-06-0217

Table 1. Association of MDM2 SNP309 and MDM2 SNP354 with lung cancer

Polymorphism	Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	
MDM2 SNP309	Overall*				
		T/T	261 (51.8)	385 (57.0)	1.00
		T/G	187 (37.1)	234 (34.6)	0.93 (0.70-1.91)
		G/G	56 (11.1)	57 (8.4)	1.22 (0.78-1.91)
		T/G + G/G	243 (48.2)	291 (43.0)	0.98 (0.75-1.28)
	African-Americans [†]	T/T	111 (83.5)	203 (79.6)	1.00
		T/G	20 (15.0)	47 (18.4)	0.70 (0.38-1.32)
		G/G	2 (1.5)	5 (2.0)	0.52 (0.09-3.05)
		T/G + G/G	22 (16.5)	52 (20.4)	0.68 (0.37-1.25)
	Caucasians [†]	T/T	150 (40.4)	182 (43.2)	1.00
	T/G	167 (45.0)	187 (44.4)	1.07 (0.79-1.45)	
	G/G	54 (14.6)	52 (12.4)	1.25 (0.81-1.95)	
	T/G + G/G	221 (59.6)	239 (56.8)	1.11 (0.84-1.48)	
MDM2 SNP354	Overall [†]				
		A/A	375 (86.8)	474 (87.9)	1.00
		A/G	51 (11.8)	58 (10.8)	1.22 (0.80-1.86)
		G/G	6 (1.4)	7 (1.3)	1.69 (0.54-5.30)
		A/G + G/G	57 (13.2)	65 (12.1)	1.26 (0.84-1.89)
	African-Americans [†]	A/A	95 (81.2)	168 (84.0)	1.00
		A/G	18 (15.4)	26 (13.0)	0.99 (0.47-2.09)
		G/G	4 (3.4)	6 (3.0)	2.23 (0.54-9.26)
		A/G + G/G	22 (18.8)	32 (16.0)	1.15 (0.58-2.27)
	Caucasians [†]	A/A	280 (88.9)	306 (90.3)	1.00
		A/G	33 (10.5)	32 (9.4)	1.31 (0.77-2.23)
		G/G	2 (0.6)	1 (0.3)	2.58 (0.22-30.09)
		A/G + G/G	35 (11.1)	33 (9.7)	1.34 (0.80-2.27)

*Adjusted for age, race, and pack-years of smoking.

[†]Adjusted for age and pack-years of smoking.

equilibrium among the African-American population controls ($P = 0.02$). A genotyping error cannot explain the violation because concordance among the duplicates was high, and there was Hardy-Weinberg equilibrium among the other control groups (African-American hospital, $P = 0.66$; Caucasian hospital, $P = 1.00$; Caucasian population, $P = 0.89$). There was a significant difference in genotype frequencies between African-Americans and Caucasians (SNP309, $P < 0.001$; SNP354, $P = 0.01$), but there was no significant difference between cases and controls [SNP309, $P = 0.66$ (African-Americans) and $P = 0.58$ (Caucasians); SNP354, $P = 0.82$ (African-Americans) and $P = 0.75$ (Caucasians)].

The variant MDM2 SNP309 and SNP354 polymorphisms exhibited no apparent relationship with the risk of lung cancer (Table 1). The variant alleles were also not associated with lung cancer when participants were stratified by pack-years of smoking (Table 2), smoking status (never, former, or current), gender, or by the TP53 R72P polymorphism

(data not shown). The average age at diagnosis was not significantly different between those with no G allele and those with at least one G allele (African-Americans, $P = 0.94$; Caucasians, $P = 0.58$). MDM2 SNP309 was recently reported to be associated with an earlier age of colorectal cancer diagnosis among patients whose tumors have a wild-type TP53 gene (4). We could not study age at diagnosis by tumor TP53 status but investigated the relationship between the MDM2 SNP309 genotype and the TP53 R72P polymorphism. Among patients with the TP53 R/R genotype, the mean age at diagnosis was 65.7 ± 9.8 for patients with the T/T genotype and 66.6 ± 11.2 for patients with the T/G or G/G genotype ($P = 0.57$). Among patients with at least one TP53 P allele, the mean age at diagnosis was 64.6 ± 10.2 for patients with the T/T genotype and 67.0 ± 9.4 for patients with the T/G or G/G genotype ($P = 0.07$). There was also no difference when stratified by race (data not shown).

Table 2. Association of MDM2 SNP309 with lung cancer stratified by pack-years of smoking

	Genotype	Overall*			African-Americans [†]			Caucasians [†]		
		Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
MDM2 SNP309										
	≤10 pack-years									
	T/T	31	188	1.00	15	114	1.00	16	74	1.00
	T/G	24	84	1.53 (0.82-2.86)	6	22	1.93 (0.67-5.61)	18	62	1.39 (0.65-2.95)
	G/G	9	23	1.95 (0.79-4.84)	0	2	—	9	21	1.97 (0.76-5.11)
	T/G + G/G	33	107	1.62 (0.90-2.91)	6	24	1.77 (0.61-5.10)	27	83	1.54 (0.77-3.09)
11-44 pack-years	T/T	73	80	1.00	38	47	1.00	35	33	1.00
	T/G	34	46	0.83 (0.53-1.32)	3	12	0.51 (0.20-1.28)	31	34	1.05 (0.61-1.80)
	G/G	7	12	0.88 (0.41-1.86)	0	1	—	7	11	1.10 (0.48-2.49)
	T/G + G/G	41	58	0.84 (0.55-1.30)	3	13	0.49 (0.21-1.18)	38	55	1.06 (0.63-1.76)
≥45 pack-years	T/T	83	66	1.00	32	27	1.00	51	39	1.00
	T/G	56	41	0.82 (0.53-1.26)	5	6	0.60 (0.18-1.94)	51	35	0.85 (0.54-1.35)
	G/G	19	9	1.19 (0.60-2.34)	1	2	—	18	7	1.16 (0.58-2.32)
	T/G + G/G	75	50	0.88 (0.58-1.32)	6	8	0.69 (0.22-2.17)	69	42	0.91 (0.59-1.41)

*Adjusted for race and age.

[†]Adjusted for age.

Discussion

The *MDM2* SNP309 and SNP354 polymorphisms were not associated with lung carcinogenesis in African-Americans or Caucasians in our study. Reports have shown an association between the variant allele of *MDM2* SNP309 and the risk of other tumor types or accelerated tumor formation (3, 5). Our study results indicate that the mechanistic effects of the *MDM2* SNP309 polymorphism may not be generalized to all tissue types.

We had an 80% power ($\alpha = 0.05$, two-sided test) to detect a minimum OR of 1.7 and 1.2 in African-Americans and Caucasians, respectively, for the *MDM2* SNP309 G allele. Thus, our study had sufficient power to detect an OR of 1.83 (95% CI, 1.45-2.32) and 1.62 (95% CI, 1.06-2.50) reported in other studies (8, 9). Similarly, we had an 80% power ($\alpha = 0.05$, two-sided test) to detect a minimum OR of 1.6, 2.3, and 1.9 for the *MDM2* SNP354 G allele, overall, in African-Americans, and Caucasians, respectively.

The results of this study were similar to one report that did not observe an association between *MDM2* SPN309 and lung cancer (7). However, two independent reports showed an association (8, 9). Differences in ethnicity between our population and that reported by Zhang et al. could provide a partial explanation. In the study by Lind et al., the controls were current or former smokers, whereas our study controls included nonsmokers. The cases reported by Lind et al. were all surgical; therefore, stage I may have been more represented in their population than in ours. Lastly, there was a strong association with women in the study reported by Lind et al. In our population, there was no interaction between the *MDM2* SNP309 and smoking or gender. We also did not see an association between *MDM2* SNP309 and lung cancer when only the surgical cases were examined. The overall results reported to date, including ours, suggest that *MDM2* SNP309 may not be a strong indicator of lung cancer risk.

Acknowledgments

We thank Stefan Ambs for his thoughtful discussions and critical comments; Dorothea Dudek-Creaven for editorial assistance; Karen MacPherson for bibliographic assistance; Donna Perlmutter, Anthony Alberg, Christopher Loffredo, Raymond Jones, Leoni Leondaridis, Glennwood Trivers, and the Surgery and Pathology Departments from participating hospitals for their contributions; and Audrey Salabes, John Cottrell, and Drs. Rex Yung and Mark Krasna for their contributions to patient accrual and tissue collection.

References

1. Michael D, Oren M. The p53-2 module and the ubiquitin system. *Semin Cancer Biol* 2003;13:49-58.
2. Arva NC, Gopen TR, Talbott KE, et al. A chromatin-associated and transcriptionally inactive p53-2 complex occurs in *mdm2* SNP309 homozygous cells. *J Biol Chem* 2005;280:26776-87.
3. Bond GL, Hu W, Bond EE, et al. A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 2004;119:591-602.
4. Menin C, Scaini MC, De Salvo GL, et al. Association between *MDM2*-309 and age at colorectal cancer diagnosis according to p53 mutation status. *J Natl Cancer Inst* 2006;98:285-8.
5. Hong Y, Miao X, Zhang X, et al. The role of P53 and *MDM2* polymorphisms in the risk of esophageal squamous cell carcinoma. *Cancer Res* 2005;65:9582-7.
6. Campbell IG, Eccles DM, Choong DY. No association of the *MDM2* SNP309 polymorphism with risk of breast or ovarian cancer. *Cancer Lett* 2005.
7. Hu Z, Ma H, Lu D, et al. Genetic variants in the *MDM2* promoter and lung cancer risk in a Chinese population. *Int J Cancer* 2006;118:1275-8.
8. Lind H, Zienolddiny S, Ekstrom PO, Skaug V, Haugen A. Association of a functional polymorphism in the promoter of the *MDM2* gene with risk of nonsmall cell lung cancer. *Int J Cancer* 2006;119:718-21.
9. Zhang X, Miao X, Guo Y, et al. Genetic polymorphisms in cell cycle regulatory genes *MDM2* and *TP53* are associated with susceptibility to lung cancer. *Hum Mutat* 2006;27:110-7.
10. Zheng YL, Loffredo CA, Yu Z, et al. Bleomycin-induced chromosome breaks as a risk marker for lung cancer: a case-control study with population and hospital controls. *Carcinogenesis* 2003;24:269-74.
11. Mechanic LE, Marrogi AJ, Welsh JA, et al. Polymorphisms in *XPD* and *TP53* and mutation in human lung cancer. *Carcinogenesis* 2005;26:597-604.
12. Dupont WD, Plummer WD, Jr. Power and sample size calculations for studies involving linear regression. *Control Clin Trials* 1998;19:589-601.