Common Genetic Variation in *TP53* Is Associated with Lung Cancer Risk and Prognosis in African Americans and Somatic Mutations in Lung Tumors

Leah E. Mechanic,[1] Elise D. Bowman,[1] Judith A. Welsh,[1] Mohammed A. Khan,[1] Nobutoshi Hagiwara,[1,2] Lindsey Enewold,[3] Peter G. Shields,[3] Laurie Burdette,[4] Stephen Chanock,[5,6] and Curtis C. Harris[1]

[1]Laboratory of Human Carcinogenesis, National Cancer Institute, Center for Cancer Research, Bethesda, Maryland; [2]Department of Surgery I, Nippon Medical School Hospital, Tokyo, Japan; [3]Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, District of Columbia; [4]Advanced Technology Center, Intramural Research Support Program, Science Applications International Corporation-Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland; [5]Pediatric Oncology Branch, National Cancer Institute-Center for Cancer Research; and [6]Department of Cancer Epidemiology and Genetics, National Cancer Institute; Rockville, Maryland

Abstract

Lung cancer is primarily caused by tobacco smoking, but susceptibility is likely modified by common genetic variation. In response to many forms of cellular stress, including DNA damage, the p53 protein functions to induce cell cycle arrest, DNA repair, senescence, or apoptosis. We hypothesized that common *TP53* haplotypes modulate pathways of lung carcinogenesis and lung cancer susceptibility or prognosis. To investigate our hypothesis, 14 polymorphisms in *TP53*, including haplotype tagging and coding single nucleotide polymorphisms, were genotyped in two studies from the greater Baltimore, Maryland area. One study is a case-control study and the second is a case-only study for which *TP53* mutational spectra data are available. African Americans with Pro-T-A-G-G haplotypes of the combined *TP53* polymorphisms *TP53_01* (rs1042522), *TP53_65* (rs9895829), *TP53_66* (rs2909430), *TP53_16* (rs1625895), and *TP53_11* (rs12951053) had both an increased risk for lung cancer (odds ratio, 2.32; 95% confidence interval, 1.18-4.57) and a worsened lung cancer prognosis (hazards ratio, 2.38; 95% confidence interval, 1.38-4.10) compared with those with Arg-T-A-G-T haplotypes. No associations of *TP53* polymorphisms with lung cancer were observed in Caucasians. In the case-only study, several polymorphisms in *TP53* and *TP53* haplotypes, overlapping regions of *TP53* associated with risk and prognosis in African Americans, were associated with increased odds of somatic *TP53* mutation in lung tumors in Caucasians. In conclusion, common genetic variation in *TP53* could modulate lung cancer pathways, as suggested by the association with lung cancer in African Americans and somatic *TP53* mutation frequency in lung tumors. (Cancer Epidemiol Biomarkers Prev 2007;16(2):214–22)

Introduction

Lung cancer is the leading cause of cancer death worldwide (1). Although cigarette smoking is the predominant cause of lung cancer, not all smokers develop lung cancer (2), suggesting that particular individuals may be more susceptible to cigarette smoke. Familial aggregation studies have provided evidence for a genetic component to lung cancer risk (see refs. 3, 4 for review). Therefore, susceptibility to lung cancer may be due, in part, to interindividual genetic variation in the form of single nucleotide polymorphisms (SNP) or common allele variants.

Inactivation of the *TP53* tumor suppressor gene is a frequent and early event in lung carcinogenesis (5-9). The p53 protein functions to induce growth arrest, DNA repair, senescence, and apoptosis in response to cellular stress, including DNA damage (10-12). Consistent with the p53 tumor suppressor functions, mutations in p53 are present in >90% of small cell lung cancers and >50% of non–small cell lung cancers (7, 13-16).

Based on the role of the p53 protein in preventing tumor formation, genetic variability in the *TP53* gene may modulate lung cancer susceptibility. Many variants in *TP53* were identified on National Center for Biotechnology Information SNP database (17-19).[7] The biological activity associated with the most commonly studied polymorphism in *TP53*, Arg^{72}Pro, differs depending on the amino acid. Arginine at codon 72 of p53 more effectively induced p53-mediated apoptosis (20, 21), partially through targeting of p53 to the mitochondria (20). Meanwhile, the Pro^{72} p53 forms of p53, when compared with Arg^{72}, more efficiently induced cell cycle arrest (21, 22) and DNA repair (23). Several epidemiology studies examined the association of *TP53* Arg^{72}Pro polymorphisms with lung cancer, with inconsistent results (17-19).

Several studies observed that the Arg^{72}Pro polymorphism of *TP53* is in linkage disequilibrium (LD) with other potential susceptibility alleles in *TP53*. Therefore, the Arg^{72}Pro polymorphism may incompletely mark a larger susceptibility haplotype. Previously, we showed that the Arg^{72}Pro polymorphism in *TP53* was associated with an increased frequency of somatic mutations in lung tumors (24). Based on this observation, we expanded our study of the genetic variation in *TP53*. We hypothesized that variant *TP53* haplotypes modulate pathways of lung carcinogenesis, therefore effecting

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Requests for reprints: Curtis C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, NIH, 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

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[7] http://www.ncbi.nlm.nih.gov/SNP

lung cancer susceptibility and prognosis. To investigate this hypothesis, we examined 14 polymorphisms in *TP53* in a case-control study and a case-only study of lung cancer, both of which were conducted in the greater Baltimore area, and studied the association of *TP53* genotypes and haplotypes with lung cancer and somatic *TP53* mutations in lung tumors.

Materials and Methods

Study Population. Two study populations were used in this study, a case-control and case-only study, both of which were conducted in the greater Baltimore, Maryland area. Subjects were accrued from the same hospitals, but at different times and clinics. For the case-control population, lung cancer cases and controls were recruited from 1998 to 2003 as part of an ongoing study described previously (25, 26). Briefly, lung cancer patients were of Caucasian or African American descent, residing in Metropolitan Baltimore, the Maryland Eastern Shore, and recruited from seven hospitals in Baltimore. Hospital-based controls were frequency matched to cases by gender, ethnicity, age, smoking history, and hospital. Hospital-based controls were cancer-free patients recruited from the same hospitals as lung cancer cases and were recruited from internal medicine clinics, primary care, pulmonology, and cardiology clinics. Population controls were identified from Department of Motor Vehicles lists and matched to cases by age, gender, and ethnicity. Blood specimens were processed immediately after collection for isolation of blood components and stored at −70°C.

Lung cancer cases were recruited, in a separate study, from 1974 to 1999 to form a case-only study described previously (24). Inclusion in the study was based on the availability of paraffin-embedded tumor tissue for DNA sequencing for *TP53* mutations. Exons 5 to 8 of *TP53* were sequenced using the p53 GeneChip (Affymetrix, Santa Clara, CA), single-stranded conformation polymorphism and manual sequencing of DNA from paraffin-embedded tissues from surgical resections as described previously for this study (24).

Institutional Review Board approval was obtained from all participating institutions and the NIH. Informed consent was obtained from all participants.

DNA Genotyping. Blood samples were available from 99% of case-control participants. DNA for genotyping was extracted using 300 μL of isolated buffy coat using the FlexiGene kit following the manufacturer's instructions (Qiagen, Valencia, CA). DNA was available for genotyping for 80% of the participants from the case-only study and DNA was extracted from noninvolved tissue (82%) or tumor tissue (5%; ref. 24), or buffy coat (13%) following manufacturer's instructions (Qiagen).

Haplotype tagging SNPs and coding SNPs were selected from a subset of validated *TP53* SNPs from *TP53* sequencing all exons and conserved regions 5 kb upstream and 3 kb downstream of the gene using the SNP500 population[8] (27). Genotyping was done on the SNP500 population and a STATA module[9] (28) was used to select haplotype tagging SNPs from all polymorphisms with a minor allele frequency >5% after phase was inferred using Phase 2.0 (29). Genotyping assays on the selected haplotype tagging SNPs plus coding SNPs ($Arg^{72}Pro$, *TP53_01*, rs1042522; $Arg^{213}Arg$, *TP53_18*, rs1800372; and $Val^{217}Met$, *TP53_64*) were done as described by the National Cancer Institute Core Genotyping Facility[8] (Supplementary Table S1). Assays were designed for selected SNPs and were concordant with the sequencing results on the SNP500 population. At least 10% of the case-control and case-only samples from each genotyping assay was duplicated. Overall, the concordance between duplicates was 98% and each assay had >95% concordance.

Survival Determination. Date and cause of death were obtained through the National Death Index (NDI)[10] using the NDI Plus search, which provides cause of death codes. The NDI Retrieval Program is used to search the NDI file to determine whether a particular NDI death record qualifies as a possible record match with a particular user record. To qualify as a possible match, records must satisfy an algorithm based on at least one of seven matching criteria, including the Social Security number, exact month of birth, and first and last name, according to instructions from the NDI. A person scored with a 'no match' was presumed alive. Survival data are reported through December 31, 2004. Case survival was dichotomized as 'alive' or 'dead' based on survival status 5 years following diagnosis.

All survival analysis was done for lung cancer survival and all-cause mortality. Causes of death unrelated to lung cancer were censored from the study. Cause of death was evaluated from death certificate data obtained from NDI. Any mention of lung cancer, or another cancer death within 2 years of diagnosis, on a death certificate was treated as death from lung cancer.

Statistical Analysis. Differences in the characteristics of lung cancer cases and controls were compared by χ^2 for categorical values or by Student's *t* tests for continuous measures as indicated. Departures from Hardy-Weinberg equilibrium for *TP53* genotypes were evaluated by calculating the expected genotype frequencies based on observed allele frequencies and comparing expected frequencies with observed genotype frequencies using χ^2 tests. Never smokers were defined as those who smoked <100 cigarettes during their lifetime (case-control) or <6 months in duration (case-only). Former smokers were defined as those who reported quitting smoking ≥1 year before the date of diagnosis. Race was classified by self-report.

Unconditional logistic regression models were used to calculate adjusted odds ratios (OR) to assess the effect of *TP53* genotypes on the odds of lung cancer in the case-control study or odds of *TP53* mutation in lung tumors in the case-only study using PROC LOGISTIC in SAS (version 8.1; SAS Institute, Cary, NC) for those SNPs ≥5% in frequency. Models in the case-control study were adjusted for smoking status (never/former/current), age, and pack-years of smoking. Models in the case-only study were adjusted for age and pack-years of smoking. Due to the small number of never smokers in the case-only study, associations with *TP53* mutation were not adjusted for smoking status. Results in the case-only study were similar when adjusted for former or current smoking. Participants with missing values for any of the variables in a regression model were omitted from the analysis. Tests for trend were conducted by calculating *P* values for the β coefficient in unconditional logistic regression models with *TP53* genotype combinations coded as ordinal variables (P_{trend}).

Survival analysis was done for single *TP53* SNPs in the case-control study using COX proportional hazard modeling (Proc PHREG) in SAS. Models were adjusted for smoking status, pack-years of smoking, age, and stage (II-IV versus I). Results were similar in crude and adjusted models. Tests for violation of COX proportional hazards assumption were done by estimating the *P* value for interaction of individual *TP53* polymorphisms with time. All *P* values were nonsignificant ($P > 0.05$), except for *TP53_12* in African Americans. None of

[8] http://snp500cancer.nci.nih.gov
[9] http://www-gene.cimr.cam.ac.uk/clayton/software/stata/
[10] http://www.cdc.gov/nchs/ndi.htm

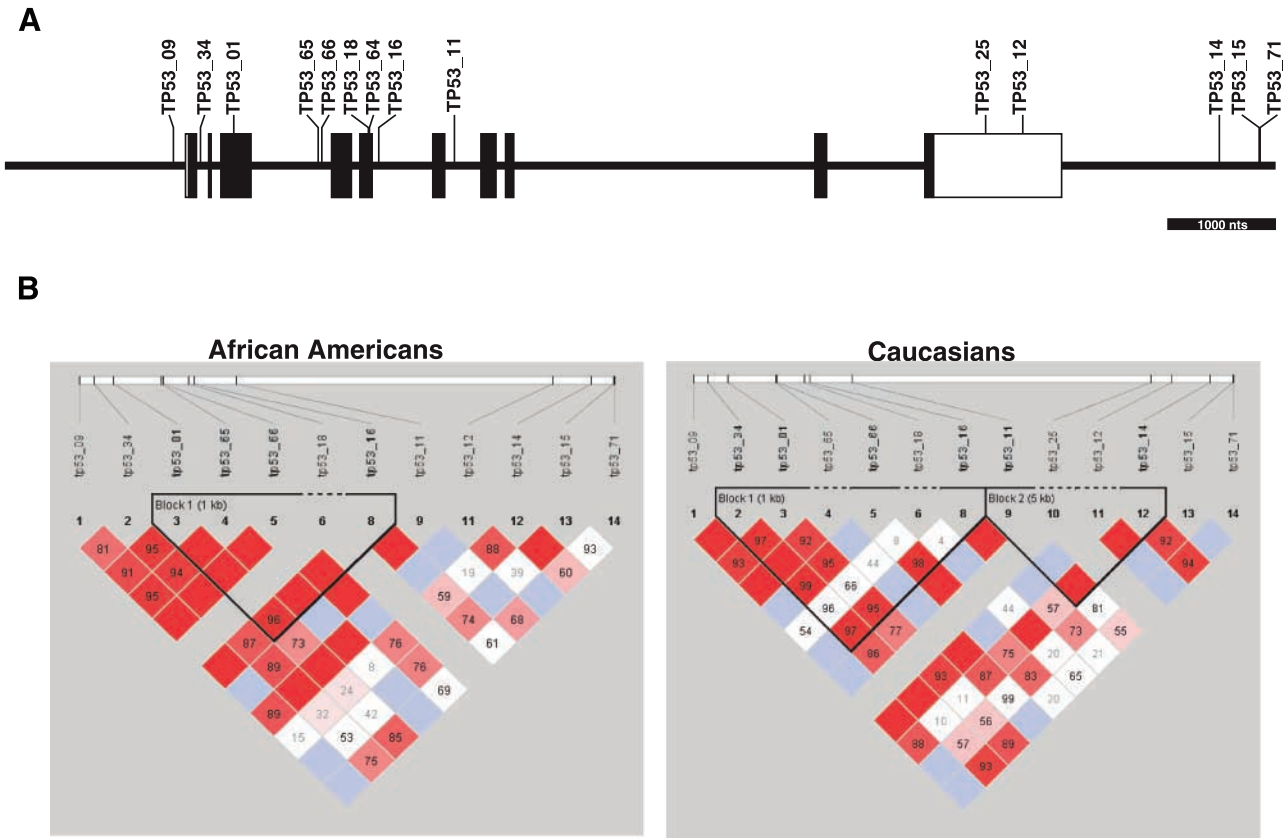

Figure 1. Position and linkage of haplotype tagging polymorphisms in *TP53*. **A,** diagram of the *TP53* gene and the position of polymorphisms genotyped. Figure was generated using SNP map (http://www.drgang.net/svg_map.html). Polymorphisms are labeled according to National Cancer Institute Core Genotyping Facility nomenclature (http://snp500cancer.nci.nih.gov). Corresponding rs numbers are listed in Supplementary Table S1. *Filled boxes,* coding exonic regions; *empty box,* noncoding region of the exon. *Line,* intronic regions. **B,** graphical representation of D' values for *TP53* polymorphisms was generated for case-control participants using Haploview (30) and the block definitions from Gabriel et al. (31).

the haplotype associations with survival reported included this polymorphism.

Haplotype analysis was done using individuals with ≥90% completeness of genotyping data for the polymorphisms selected and ≥5% frequency in our study population (African Americans or Caucasians). Haplotype blocks were determined in Haploview (30) using the block definitions from Gabriel et al. (31). Global permutation tests of association (1,000 permutations) of *TP53* haplotypes with lung cancer or *TP53* mutation were done using haplo.cc in haplo.stats module, implemented in R (32). Adjusted ORs for the association of individual *TP53* haplotypes associated with lung cancer or *TP53* mutation were estimated using haplo.glm in haplo.stats. Haplotype associations with lung cancer or all-cause survival,

Table 1. Characteristics of lung cancer cases, hospital, and population controls

	Cases, n = 443 (%)	Total controls, n = 547 (%)	Hospital controls, n = 240 (%)	Population controls, n = 307 (%)
Age (mean ± SD)	65.7 ± 10.3	64.8 ± 10.9	63.1 ± 12.1*	66.2 ± 9.7
Gender				
Male	220 (50)	261 (48)	112 (47)	149 (49)
Female	223 (50)	286 (52)	128 (53)	158 (52)
Race				
African American	120 (27)	204 (37)†	77 (32)	127 (41)†
Caucasians	323 (73)	343 (63)	163 (68)	180 (59)
Smoking status				
Never	35 (8)	175 (32)‡	53 (22)†	122 (40)†
Former	192 (43)	264 (49)	119 (50)	145 (48)
Current	215 (49)	105 (19)	67 (28)	38 (12)
Pack-years (mean ± SD)	41.5 ± 28.3	23.3 ± 28.5§	34.6 ± 33.7§	14.3 ± 19.5§

*Hospital controls were younger than cases (P = 0.005, t test for unequal variances).
†The race distribution was different in total controls (P = 0.001, χ^2 test) and population controls (P < 0.0001, χ^2 test) compared with lung cancer cases.
‡The distribution of smoking status differed in total controls, hospital controls, and population controls (P < 0.0001, χ^2 test) compared with lung cancer cases.
§Total controls, population controls (P < 0.0001, t test for unequal variances), and hospital controls (P = 0.007, t test for unequal variances) smoked fewer pack-years than lung cancer cases.

hazards ratios (HR), and 95% confidence intervals (95% CI) were determined using THESIAS (33), which did COX proportional hazards regression. All haplotype analysis assumed additive effects of haplotypes.

Results

Genotyping. Fourteen polymorphisms in *TP53* were genotyped in two study populations, an ongoing case-control study (25, 26) and a case-only study described previously (24, 34). No variation was observed for *TP53_25* (Ex11 +567, G>A) or *TP53_64* (Ex6 −24, G>A) in our case-control or case-only study (data not shown). The positions of the *TP53* polymorphisms are shown in Fig. 1A. The frequencies of all polymorphisms were similar in the Caucasian lung cases in both studies ($P > 0.05$, for all). All polymorphisms were in Hardy-Weinberg equilibrium in African American and Caucasian controls, except for *TP53_09* (IVS1 −112 G>A) in African American controls ($P = 0.014$). This violation is unlikely due to genotyping error because the concordance between duplicate samples was 98%, the violation was not observed in Caucasians, and samples were randomly distributed when genotyped. The violation may be partially driven by the rarity of this polymorphism. Given the violation, results using *TP53_09* are not reported. The frequencies of the *TP53_01* Arg^{72}Pro (35-42) and the *TP53_16* (*MspI*, IVS6 +62 G>A; refs. 35, 39, 43) polymorphisms in our population were consistent with

Table 2. Association of *TP53* polymorphisms with lung cancer in African Americans and Caucasians

Genotype	African American			Caucasian		
	Case, *n* (%)	Control, *n* (%)	OR (95% CI)*	Case, *n* (%)	Control, *n* (%)	OR (95% CI)*
TP53_34 IVS2 +38						
G/G	30 (27)	51 (27)	1	148 (51)	187 (58)	1
G/C	55 (49)	98 (52)	0.96 (0.51-1.81)	126 (44)	119 (37)	1.34 (0.94-1.92)
C/C	28 (25)	40 (21)	1.22 (0.57-2.59)	15 (5)	19 (6)	0.90 (0.42-1.93)
			$P_{trend} = 0.63$			$P_{trend} = 0.36$
G/C + C/C	83 (74)	138 (73)	1.03 (0.57-1.88)	141 (49)	138 (43)	1.28 (0.90-1.80)
TP53_01 Arg^{72}Pro						
Arg/Arg	16 (14)	46 (23)	1	166 (54)	193 (58)	1
Arg/Pro	57 (50)	83 (42)	2.48 (1.16-5.27)	125 (41)	122 (36)	1.23 (0.86-1.76)
Pro/Pro	42 (37)	67 (34)	1.84 (0.85-3.99)	16 (5)	20 (6)	0.87 (0.41-1.84)
			$P_{trend} = 0.25$			$P_{trend} = 0.59$
Arg/Pro + Pro/Pro	99 (87)	150 (76)	2.16 (1.07-4.36)	141 (46)	142 (39)	1.18 (0.84-1.66)
TP53_65 IVS4 −125						
T/T	97 (85)	159 (83)	1	269 (92)	304 (94)	1
T/C	15 (13)	30 (16)	0.96 (0.45-2.02)	25 (8)	21 (6)	1.48 (0.78-2.82)
C/C	2 (2)	3 (2)	1.82 (0.26-12.6)	0 (0)	0 (0)	ND
			$P_{trend} = 0.81$			
T/C + C/C	17 (15)	33 (18)	1.02 (0.50-2.08)	25 (8)	21 (6)	1.48 (0.78-2.82)
TP53_66 IVS4 −91						
A/A	65 (58)	101 (53)	1	225 (77)	254 (78)	1
A/G	39 (35)	75 (40)	0.69 (0.39-1.22)	62 (21)	68 (21)	1.17 (0.77-1.78)
G/G	8 (7)	13 (7)	0.97 (0.33-2.84)	7 (2)	5 (2)	1.08 (0.31-3.76)
			$P_{trend} = 0.41$			$P_{trend} = 0.51$
A/G + G/G	47 (42)	88 (47)	0.73 (0.42-1.25)	69 (23)	73 (23)	1.16 (0.77-1.74)
TP53_16 IVS6 +62						
G/G	62 (55)	97 (51)	1	217 (75)	243 (75)	1
G/A	43 (38)	75 (39)	0.75 (0.43-1.33)	68 (23)	75 (23)	1.12 (0.74-1.68)
A/A	8 (7)	18 (9)	0.55 (0.20-1.50)	6 (2)	5 (2)	0.93 (0.25-3.41)
			$P_{trend} = 0.17$			$P_{trend} = 0.69$
G/A + A/A	51 (45)	93 (48)	0.71 (0.41-1.22)	74 (25)	80 (25)	1.10 (0.74-1.64)
TP53_11 IVS7 +92						
T/T	78 (69)	155 (82)	1	247 (85)	281 (86)	1
T/G	31 (27)	30 (16)	2.31 (1.19-4.47)	42 (14)	45 (14)	0.91 (0.56-1.49)
G/G	4 (4)	4 (2)	1.49 (0.27-8.19)	3 (1)	1 (0)	1.97 (0.19-20.6)
			$P_{trend} = 0.03$			$P_{trend} = 0.91$
T/G + G/G	35 (31)	34 (18)	2.20 (1.17-4.14)	45 (15)	46 (14)	0.94 (0.58-1.52)
TP53_14 1474 3′STP						
C/C	45 (40)	81 (43)	1	92 (32)	116 (36)	1
C/T	48 (43)	76 (41)	0.99 (0.55-1.77)	144 (50)	147 (45)	1.17 (0.80-1.72)
T/T	19 (17)	30 (16)	1.13 (0.53-2.42)	55 (19)	63 (19)	1.02 (0.63-1.66)
			$P_{trend} = 0.80$			$P_{trend} = 0.81$
C/T + T/T	67 (60)	106 (57)	1.03 (0.60-1.77)	199 (69)	210 (64)	1.12 (0.78-1.62)
TP53_15 1846 3′STP						
C/C	104 (92)	164 (86)	1	201 (70)	230 (71)	1
C/T	9 (8)	25 (13)	0.47 (0.19-1.18)	82 (28)	91 (28)	1.04 (0.71-1.53)
T/T	0 (0)	1 (1)	ND	6 (2)	5 (2)	1.09 (0.29-4.03)
			$P_{trend} = 0.10$			$P_{trend} = 0.81$
C/T + T/T	9 (8)	26 (14)	0.47 (0.19-1.17)	88 (30)	96 (30)	1.05 (0.72-1.52)
TP53_71 1855 3′ STP						
A/A	88 (77)	163 (86)	1	264 (90)	296 (92)	1
A/G	26 (23)	26 (14)	1.63 (0.82-3.24)	28 (10)	25 (8)	1.19 (0.64-2.21)
G/G	0 (0)	1 (1)	ND	0 (0)	0 (0)	ND
			$P_{trend} = 0.19$			
A/G + G/G	26 (23)	27 (15)	1.61 (0.81-3.19)	28 (10)	25 (8)	1.19 (0.64-2.21)

Abbreviation: ND, not determined.
*Adjusted for age, smoking status (never, former, and current), and pack-years of smoking (continuous).

previous reports in African Americans and Caucasians (Supplementary Table S2). The frequency of all *TP53* polymorphisms examined, except *TP53_11* (IVS7 +92, T>G) and *TP53_14* (1474 3′STP, C>T), were significantly different in African American and Caucasian controls ($P < 0.001$ to $P < 0.01$).

Case-Control Population Characteristics. Hospital controls were younger than population controls and lung cancer cases ($P = 0.005$); lung cancer cases ($P < 0.0001$) and hospital controls ($P = 0.026$) had a lower proportion of African Americans than population controls; and lung cancer cases were more frequently current smokers ($P < 0.0001$) and smoked a higher number of pack-years ($P < 0.0001$; Table 1). Frequency matching was incomplete for some variables. The frequencies of all *TP53* polymorphisms were similar in both control groups (data not shown) and because both control groups represent the study base from which cases were derived, all further analyses were done comparing lung cancer cases with total controls adjusted for age, smoking status, and pack-years of smoking.

Association with Lung Cancer. The association of the individual *TP53* polymorphisms with lung cancer in African Americans and Caucasians was examined (Table 2). Among African American participants, individuals with *TP53* codon 72 Arg/Pro + Pro/Pro genotypes in comparison with those with *TP53* Arg/Arg[72] genotypes and individuals with *TP53_11* T/G + G/G genotypes (IVS7 +92) versus *TP53_11* T/T genotypes had a 2-fold increased odds of lung cancer. No other individual *TP53* polymorphisms were associated with lung cancer in African Americans or Caucasians (Table 2).

Haplotype analysis was done by examining the block structure of *TP53*. Two linkage blocks were observed in Caucasians and one in African Americans (Fig. 1B). The LD structure of *TP53* differed in African Americans and Caucasians. Block I encompassed slightly different SNPs for these ethnic/racial groups (Table 3). African American participants with Pro-T-A-G haplotypes (*TP53_01, TP53_65, TP53_66*, and *TP53_16*) had increased odds of lung cancer compared with participants with Arg-T-A-G haplotypes. In the analysis of single *TP53* SNPs associated with lung cancer, *TP53_01* and *TP53_11* were both associated with lung cancer in African Americans (Table 2). Haplotype analysis in African Americans was also done by extending the block I definition to include *TP53_11*. African American participants with Pro-T-A-G-G haplotypes had almost a 2-fold increase in the odds of lung cancer compared with those with Arg-T-A-G-T haplotypes (Table 3). Using this more precise haplotype definition resulted in a stronger association with lung cancer in African Americans. No other haplotypes in the linkage blocks were associated with lung cancer risk in either African Americans or Caucasians.

Lung Cancer Survival. The average amount of follow-up time for lung cancer patients in the case-control study was 26.1 months. Individual *TP53* polymorphisms were studied for association with lung cancer prognosis (Table 4). *TP53_11* in African American lung cancer cases was predictive of worsened lung cancer prognosis or worsened all-cause mortality. *TP53_15* was predictive of improved lung cancer prognosis in Caucasians or all-cause mortality. None of the other individual polymorphisms were associated with survival. Results were similar in unadjusted models (data not shown) or in models examining all-cause mortality instead of specifically lung cancer death (Table 4).

Given the observed associations of individual polymorphisms, we examined the association of combined *TP53* haplotypes with lung cancer survival using both the block definition and adding *TP53_11* based on association with risk and survival (Table 5). The same haplotypes, Pro-T-A-G (*TP53_01, TP53_65, TP53_66*, and *TP53_16*) or Pro-T-A-G-G (*TP53_01, TP53_65, TP53_66, TP53_16*, and *TP53_11*), which were associated with lung cancer risk in African Americans were associated with worsened lung cancer prognosis in both crude (data not shown) and adjusted models.

Case-Only Study Population Characteristics. To explore possible phenotypic effects of these polymorphisms, we examined the correlation between the *TP53* polymorphisms with a phenotypic measure—the frequency of somatic *TP53* mutations in lung tumors in a case-only study. Analysis of the case-only study was limited to Caucasians ($n = 188$) due to the small number of African Americans in the study. The characteristics of the Caucasian subset of the case-only study were similar to the population as a whole and results were similar when African American cases were included in the analysis (24, 34; data not shown). The average age was 65.3 ± 9.3 years. One third of the cases was female ($n = 61$). The population was mostly former ($n = 90$, 48%) or current ($n = 92$, 49%) smokers with an average number of pack-years of 60.0 ± 34.2.

Association with Somatic TP53 Mutations. Several of the individual germ-line *TP53* polymorphisms were associated with increased odds of somatic *TP53* mutations, including *TP53_34, TP53_01, TP53_16*, and *TP53_14* (Supplementary Table S2). All of the ORs, assessing the association of *TP53* polymorphisms with the presence of somatic mutations, were above 1. Haplotypes formed from block I SNPs (C-Pro-A-G versus G-Arg-A-G), block I SNPs plus *TP53_11* (C-Pro-A-G-T versus G-Arg-A-G-T), and the combination of the *TP53* SNPs associated with somatic *TP53* mutations (*TP53_34, TP53_01,*

Table 3. Association of *TP53* haplotypes with lung cancer in African Americans and Caucasians

Haplotype	Case Frequency*	Control Frequency*	OR (95% CI) †
African Americans			
Block I SNPs			
TP53_01, TP53_65, TP53_66, TP53_16			
Arg-T-A-G	0.38	0.43	1
Pro-T-G-A	0.24	0.27	0.92 (0.57-1.49)
Pro-T-A-G	0.27	0.19	1.61 (0.98-2.64)
Pro-C-A-G	0.08	0.10	1.10 (0.56-2.11)
Block I SNPs plus *TP53_11*			
TP53_01, TP53_65, TP53_66, TP53_16, TP53_11			
Arg-T-A-G-T	0.37	0.42	1.0
Pro-T-G-A-T	0.27	0.24	0.94 (0.58-1.52)
Pro-T-A-G-G	0.17	0.10	1.94 (1.06-3.57)
Pro-C-A-G-T	0.08	0.10	1.13 (0.58-2.19)
Pro-T-A-G-T	0.11	0.09	1.40 (0.70-2.81)
Caucasians			
Block I SNPs			
TP53_34, TP53_01, TP53_66, TP53_16			
G-Arg-A-G	0.72	0.74	1
C-Pro-G-A	0.12	0.11	1.00 (0.61-1.63)
C-Pro-A-G	0.12	0.10	1.18 (0.79-1.75)
Block I SNPs plus *TP53_11*			
TP53_34, TP53_01, TP53_66, TP53_16, TP53_11			
G-Arg-A-G-T	0.71	0.74	1
C-Pro-G-A-T	0.12	0.12	1.17 (0.80-1.71)
C-Pro-A-G-G	0.07	0.06	1.01 (0.61-1.63)
Block II SNPs			
TP53_11, TP53_14			
T-C	0.56	0.58	1
T-T	0.35	0.35	1.03 (0.80-1.33)
G-T	0.08	0.07	1.01 (0.63-1.60)

*Frequency of haplotypes in cases and controls (combined hospital and population controls) were determined using haplo.cc as described in Materials and Methods. Associations reported for haplotypes ≥5% frequency.
†ORs and 95% CIs were calculated using haplo.glm adjusted for age, smoking status, (never, former, and current), and pack-years of smoking (continuous) assuming additive effects of haplotypes.

Table 4. Association of *TP53* haplotypes with survival in African Americans and Caucasians

Haplotype	Event frequency*	Censored frequency*	Lung cancer HR (95% CI)†	All-cause HR (95% CI)†
African Americans				
Block I SNPs				
TP53_01, TP53_65, TP53_66, TP53_16				
Arg-T-A-G	0.36	0.42	1.0	1.0
Pro-T-G-A	0.23	0.26	1.04 (0.64-1.70)	1.02 (0.63-1.67)
Pro-T-A-G	0.29	0.24	1.67 (1.09-2.58)	1.62 (1.06-2.48)
Pro-C-A-G	0.10	0.06	1.37 (0.76-2.49)	1.31 (0.73-2.38)
Block I SNPs plus *TP53_11*				
TP53_01, TP53_65, TP53_66, TP53_16, TP53_11				
Arg-T-A-G-T	0.30	0.35	1.0	1.0
Pro-T-G-A-T	0.23	0.26	0.95 (0.56-1.60)	0.94 (0.56-1.57)
Pro-T-A-G-G	0.15	0.02	2.38 (1.38-4.10)	2.27 (1.33-3.88)
Pro-C-A-G-T	0.08	0.10	1.29 (0.71-2.34)	1.25 (0.69-2.26)
Pro-T-A-G-T	0.15	0.21	1.07 (0.59-1.96)	1.07 (0.59-1.94)
Caucasians				
Block I SNPs				
TP53_34, TP53_01, TP53_66, TP53_16				
G-Arg-A-G	0.74	0.70	1.0	1.0
C-Pro-G-A	0.10	0.13	0.83 (0.55-1.27)	0.97 (0.67-1.41)
C-Pro-A-G	0.12	0.13	1.04 (0.69-1.56)	1.02 (0.69-1.51)
Block I SNPs plus *TP53_11*				
TP53_34, TP53_01, TP53_66, TP53_16, TP53_11				
G-Arg-A-G-T	0.74	0.69	1.0	1.0
C-Pro-G-A-T	0.10	0.13	0.84 (0.55-1.28)	0.98 (0.67-1.42)
C-Pro-A-G-G	0.07	0.08	0.97 (0.59-1.59)	0.95 (0.59-1.53)
C-Pro-A-G-T	0.05	0.05	1.17 (0.65-2.11)	1.15 (0.66-2.03)
Block II SNPs				
TP53_11, TP53_14				
T-C	0.53	0.59	1.0	1.0
T-T	0.39	0.32	1.11 (0.86-1.45)	1.07 (0.83-1.38)
G-T	0.08	0.09	0.99 (0.62-1.58)	0.93 (0.59-1.46)

*Frequency of haplotypes were determined using THESIAS (33) based on lung cancer–specific mortality. Associations determined for haplotypes ≥5% frequency.
†HRs and 95% CIs were calculated using adjusted for age, smoking status (never, former, and current), stage (II-IV versus I), and pack-years of smoking (continuous) using THESIAS for lung cancer or all-cause mortality as described in Materials and Methods.

TP53_16, and *TP53*_14; C-Pro-G-T versus G-Arg-G-C) were associated with somatic *TP53* mutations in lung tumors (Table 6). Notably, the block I haplotypes associated with somatic *TP53* mutations in lung tumors in Caucasians overlapped with the block I haplotypes associated with lung cancer in African Americans.

Discussion

Susceptibility to lung cancer caused by smoking may be due, in part, to common genetic variation and other risk factors. In this study, we investigated the hypothesis that *TP53* haplotypes modulate mechanisms of lung carcinogenesis and lung cancer susceptibility or prognosis by studying 14 polymorphisms in *TP53* in a case-control and case-only study of lung cancer. African Americans with Pro-T-A-G-G haplotypes of the combined *TP53* polymorphisms *TP53_01* (rs1042522), *TP53_65* (rs9895829), *TP53_66* (rs2909430), *TP53_16* (rs1625895), and *TP53_11* (rs12951053) had an increased odds of lung cancer and worsened prognosis compared with those with Arg-T-A-G-T haplotypes. None of the individual *TP53* haplotypes was associated with lung cancer in Caucasians. Several *TP53* SNPs and haplotypes were associated with an increased frequency of somatic *TP53* mutations in lung tumors.

African American participants with Pro-T-A-G-G (*TP53_01, TP53_65, TP53_66, TP53_16*, and *TP53_11*) haplotypes had an increased odds of lung cancer and worsened lung cancer survival when compared with those with Arg-T-A-G-T haplotypes. A few previous studies examined the association of *TP53* haplotypes with lung cancer, focusing on three polymorphisms, *TP53_166* (16-bp insertion, IVS3 +41), *TP53_01* ($Arg^{72}Pro$), and *TP53_16* (G>A, IVS6 +62; refs. 37, 39). In one study, Pro^{72} genotypes were only associated with lung cancer in combination with the 16-bp insertion in intron 3 (39). In another study, several haplotypes, or combinations of susceptibility alleles, were associated with lung cancer in Caucasians (37). Despite differences in the alleles examined in these studies and our report, given the observed linkage between the three polymorphisms (35, 37, 39, 44, 45) and the position of the haplotype on *TP53* associated with lung cancer in our study, our results and previous studies suggest that this region of *TP53* may have a role in modulating lung cancer susceptibility. No previous studies examined the association of *TP53* haplotypes with lung cancer survival.

Our study is the first to describe the association of *TP53* haplotypes with lung cancer in African Americans. There are several possible explanations for the observed association only in African Americans; however, the reason is currently unknown. The association only in African Americans could be attributed to the higher frequency of particular haplotypes in African Americans (Table 3). However, given the larger number of Caucasians in our study, the power to detect associations was similar among African Americans and Caucasians. Another possible explanation for the differential effects of the *TP53* polymorphism on lung cancer could be related to differences in exposure because interaction of *TP53_01* with cigarette smoking was observed in several previous studies (46-48), although an interaction of *TP53_01* with smoking and lung cancer was not observed in this analysis. One possible exposure difference is menthol cigarette smoking. Menthol cigarette smoking is more prevalent in African Americans (69% versus 22%; ref. 49). Some studies suggested that smoking menthol cigarettes results in smoking larger puff volumes and higher nicotine exposures (50), and therefore, increased exposure to tobacco-specific carcinogens in African Americans. Finally, these results could be due to LD with another polymorphism, such as a rare *TP53*

Table 5. Association of individual *TP53* polymorphisms with lung cancer survival

Genotype	African Americans		Caucasians	
	HR (95% CI)*	P_{trend}	HR (95% CI)*	P_{trend}
Lung cancer mortality				
TP53_09	1.21 (0.69-2.12)	0.50	1.19 (0.67-2.12)	0.32
TP53_34	1.19 (0.83-1.70)	0.35	0.98 (0.73-1.31)	0.88
TP53_01	1.21 (0.85-1.71)	0.29	0.87 (0.66-1.14)	0.32
TP53_65	1.31 (0.77-2.21)	0.32	1.12 (0.62-2.04)	0.71
TP53_66	0.79 (0.53-1.18)	0.25	0.90 (0.62-1.30)	0.56
TP53_18	ND	ND	0.90 (0.33-2.44)	0.83
TP53_16	0.80 (0.54-1.19)	0.26	0.72 (0.48-1.08)	0.11
TP53_11	1.92 (1.21-3.05)	0.01	0.94 (0.62-1.44)	0.78
TP53_12	0.88 (0.37-2.08)	0.77	1.16 (0.64-2.10)	0.63
TP53_14	1.06 (0.76-1.48)	0.74	1.11 (0.87-1.41)	0.42
TP53_15	0.80 (0.32-2.00)	0.63	0.61 (0.42-0.89)	0.01
TP53_71	1.45 (0.84-2.50)	0.18	0.95 (0.50-1.82)	0.88
All-cause mortality				
TP53_09	1.16 (0.67-2.03)	0.60	1.15 (0.66-2.00)	0.63
TP53_34	1.18 (0.83-1.68)	0.35	1.02 (0.77-1.35)	0.87
TP53_01	1.18 (0.84-1.66)	0.33	0.92 (0.71-1.19)	0.52
TP53_65	1.25 (0.74-2.12)	0.40	1.09 (0.61-1.93)	0.77
TP53_66	0.80 (0.54-1.19)	0.28	1.00 (0.71-1.40)	0.99
TP53_18	ND	ND	1.05 (0.43-2.59)	0.91
TP53_16	0.80 (0.54-1.19)	0.27	0.84 (0.59-1.21)	0.36
TP53_11	1.92 (1.22-3.03)	0.01	0.90 (0.60-1.37)	0.62
TP53_12	0.84 (0.36-1.98)	0.70	1.13 (0.64-2.00)	0.68
TP53_14	1.07 (0.77-1.49)	0.68	1.05 (0.83-1.34)	0.66
TP53_15	0.78 (0.31-1.95)	0.59	0.70 (0.50-0.99)	0.04
TP53_71	1.39 (0.81-2.39)	0.23	0.95 (0.51-1.75)	0.86

*HRs and 95% CIs were estimated assuming additive effects of genotypes. Models were adjusted for age, smoking status (never, former, and current), pack-years, and stage (II-IV versus I) using Proc PHREG in SAS.

polymorphism. $Pro^{47}Ser$ (<5%) was observed only in African Americans and not observed in Caucasians (51, 52). The Ser^{47} variant of p53 had a reduced ability to transactivate p53 target genes, *p53AIP1* and *PUMA*, and induce apoptosis (52). The $Pro^{47}Ser$ was shown to be in LD with the $Arg^{72}Pro$ polymorphism of *TP53* (52), and therefore, could be in LD with the haplotype associated with lung cancer in our study.

Several previous studies examined the association of the *TP53* $Arg^{72}Pro$ polymorphism with lung cancer (see refs. 17-19 for review). In a 2003 meta-analysis, overall, the authors observed no significant association of the *TP53* $Arg^{72}Pro$ polymorphism with lung cancer (19). However, in the combined analysis, the point estimate for the OR for individuals with Pro/Pro genotypes was elevated. Moreover, in studies with larger population sizes (N = 900-2,500) and some more recent studies not covered by the meta-analysis, individuals with Pro genotypes had an increased odds of lung cancer (38, 41, 46-48, 53). These observations are consistent with our observed association in African Americans. Few previous studies examined the association of *TP53* polymorphisms with lung cancer in African Americans, and these studies were quite small (N = 25, 70, 121, 141; refs. 36, 37, 54, 55). In our study, only one haplotype (>1% frequency) containing Pro^{72} allele was associated with lung cancer and the Pro^{72} allele mapped to several additional common haplotypes, which were not associated with lung cancer (Table 2). Therefore, in our study, only examining the Pro^{72} allele imprecisely estimates the relationship between *TP53* haplotypes and lung cancer, possibly partially explaining the observed inconsistency in its associations with lung cancer (19).

As an extension of our previous study of *TP53_01* ($Arg^{72}Pro$) polymorphism with somatic *TP53* mutations, several additional *TP53* polymorphisms (*TP53_34*, *TP53_16*, and *TP53_14*) were associated with an increased frequency of somatic *TP53* mutations in lung tumors. Haplotypes of these combined polymorphisms were also associated with somatic *TP53* mutations. Several germ-line *TP53* polymorphisms (including *TP53_34*, *TP53_01*, *TP53_16*, and *TP53_14*) and haplotypes were associated with an increased odds of somatic *TP53* mutations in lung tumors. Our results suggest a combined influence of *TP53* alleles and need to be confirmed in additional studies.

The region of *TP53* between *TP53_01* ($Arg^{72}Pro$ in exon 4) to *TP53_16* (IVS6 +62) contained *TP53* germ-line polymorphisms and haplotypes associated both with lung cancer in African Americans and with somatic *TP53* mutations in lung tumors from Caucasians (Fig. 1A). This genetic region was identified in two different ethnic populations and associated with different outcomes, suggesting that a locus, or loci, within this region of *TP53* may be important in modulating lung cancer susceptibility. Consistent with this notion, several studies showed that the Arg^{72} versus Pro^{72} alters p53 function (17, 18, 20-23) and mutation in *TP53* often occurs in exons 5 to 8 in lung tumors (56).

Our case-control study was designed using two separate control groups (hospital and population based). Due to the limitation of our population size, in this analysis, we combined the control groups and did comparisons between lung cancer cases and total controls. In contrast to the population controls, hospital controls had a higher proportion of smokers. Moreover, controls recruited from hospitals have a higher frequency of illness than population controls. Combining these heterogeneous populations (population plus hospital-based controls) could result in biased associations because the distribution of risk factors for lung cancer (such as smoking) differs in these two populations. We believe that this has not affected the results presented in this study because the frequency of *TP53* polymorphisms was similar (not statistically different) in these two populations, and analyses were adjusted for smoking. Another source of heterogeneity between these two populations is the difference in race distribution in hospital and population controls. This potential source of bias was likely addressed in the stratification of all

Table 6. Association of *TP53* haplotypes with somatic *TP53* mutation in Caucasians

Haplotype	+ Any *TP53* mutation	− Any *TP53* mutation	OR (95% CI) †
	Frequency*	Frequency*	
Block I SNPs			
TP53_34, TP53_01, TP53_66, TP53_16			
G-Arg-A-G	0.57	0.74	1
C-Pro-G-A	0.17	0.13	1.80 (0.88-3.66)
C-Pro-A-G	0.21	0.11	2.31 (1.18-4.52)
Block I SNPs plus *TP53_11*			
TP53_34, TP53_01, TP53_66, TP53_16, TP53_11			
G-Arg-A-G-T	0.57	0.74	1
C-Pro-G-A-T	0.16	0.13	1.71 (0.82-3.53)
C-Pro-A-G-G	0.11	0.07	1.96 (0.85-4.50)
C-Pro-A-G-T	0.10	0.04	3.15 (1.18-8.37)
Block II SNPs			
TP53_11, TP53_14			
T-C	0.44	0.56	1
T-T	0.44	0.36	1.53 (0.92-2.54)
G-T	0.12	0.07	2.30 (0.94-5.60)
Combined SNPs associated with mutation			
TP53_34, TP53_01, TP53_16, TP53_14			
G-Arg-G-C	0.27	0.44	1
G-Arg-G-T	0.29	0.30	1.48 (0.71-3.07)
C-Pro-G-T	0.19	0.10	2.74 (1.25-6.05)
C-Pro-A-C	0.12	0.11	1.64 (0.60-4.46)

*Frequency of haplotypes in tumors with or without somatic *TP53* mutation were calculated using haplo.cc. Associations determined for haplotypes ≥5% frequency.

†ORs and 95% CIs were calculated using haplo.glm assuming additive effects of haplotypes. Models were adjusted for age and pack-years of smoking.

analysis by race. In addition, we feel that both study populations represent the study base from which the lung cancer cases were derived.

Our study has several limitations. Given that our case-control associations were only observed in one ethnic group, it is possible that the association may have been observed by chance. Although it is always formally possible that an association is driven by chance, given the biological plausibility of the association and consistency of the association with the observations in our case-only study, it is important to pursue these observations in follow-up studies. The *TP53* gene, as was reported previously (57), had low LD across the entire gene, resulting in many rare haplotypes. Therefore, our study had limited power to examine smoking interaction with *TP53* haplotypes. We observed an association of *TP53* haplotypes with lung cancer and prognosis, only in African American participants, but we were unable to examine the association of *TP53* polymorphisms with somatic *TP53* mutations in lung tumors from African Americans due to the small number of African American cases in our case-only study. Further studies will need to investigate the association of *TP53* haplotypes with lung cancer and with somatic *TP53* mutations in lung tumors in African Americans.

In conclusion, our study examined the association of *TP53* haplotypes with lung cancer in African Americans and Caucasians. We also investigated the association of *TP53* polymorphisms and haplotypes with somatic *TP53* mutations in lung tumors. Our results could suggest that *TP53* haplotypes may modulate lung cancer pathways, but results need to be confirmed in further studies.

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Common Genetic Variation in *TP53* Is Associated with Lung Cancer Risk and Prognosis in African Americans and Somatic Mutations in Lung Tumors

Leah E. Mechanic, Elise D. Bowman, Judith A. Welsh, et al.

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