

Short Communication

The p53 Arg72Pro Polymorphism, Human Papillomavirus, and Invasive Squamous Cell Cervical Cancer¹

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Abstract

A. Storey *et al.* [Nature (Lond.), 393: 229–234, 1998] reported a 7-fold increased risk of cervical cancer associated with having an Arg/Arg polymorphism at codon 72 of p53 compared with the Pro/Arg heterozygotes (odds ratio, 7.4; 95% confidence interval, 2.1–29.4). Complementary *in vitro* studies suggested that the HPV E6 oncoprotein more readily targets the arginine form, as opposed to the proline form, of p53 for degradation. We investigated the impact of this polymorphism in a population-based case-control study of invasive cervical cancer. Using a PCR assay to detect the p53 codon 72 polymorphism, we tested blood samples from 111 women with invasive squamous cell cancer of the cervix identified by a population-based registry and 164 random-digit telephone-dialed controls. The distribution of the genotype among control women was 38% heterozygous, 7% proline homozygous, and 55% arginine homozygous, and among the cases was 38%, 6%, and 56%, respectively. There was no increased risk of squamous cell invasive cervical cancer associated with homozygosity for the arginine allele (odds ratio, 1.0; 95% confidence interval, 0.6–1.7). Furthermore, there was no modification of this result by human papillomavirus (HPV) DNA status of the tumor, age, or smoking status. Among controls, there was no association between the polymorphism and HPV-16 L1 seropositivity. However, among case subjects, the codon 72 polymorphism may be related to HPV 16L1 seropositivity status.

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Introduction

HPV³ is almost certainly the primary etiologic agent of cervical cancer, yet few women infected with HPV go on to develop cancer. In a recent report, Storey *et al.* (1) describe a 7-fold increased risk of cervical cancer associated with the p53 Arg72Pro polymorphism. Complementary *in vitro* studies suggested that the HPV E6 oncoprotein, which binds p53 (2) and promotes its degradation (3), might more readily target the arginine form as opposed to the proline form of p53 for degradation. We investigated the association between the p53 Arg72Pro polymorphism and the risk of invasive squamous cell cancer of the cervix in a sample of participants from a large population-based case-control study.

Materials and Methods

The methods of the larger study, described in detail in Daling *et al.* (4), are briefly outlined here. Case subjects were identified by the Cancer Surveillance System, a population-based cancer registry serving western Washington State that is part of the SEER Program. The SEER Program is part of the United States National Cancer Institute and has monitored cancer incidence and mortality in a 10% sample of the United States population in nine geographic areas since 1973. Population-based control subjects were identified by using random-digit dialing and were frequency matched to case subjects by age. All of the subjects were residents of an urban, three-county area that included Seattle. The interview response rate in the parent study was 65% for case subjects and 72% for control subjects. We restricted the present investigation to white women to reduce the potential for our results to be influenced by differences in the genotypic frequency by race. The present study represents 18.0% percent of the white cases and 15.9% of the white controls in the parent study. The mean age of cases was 43.0 and that of controls was 43.6.

Serum and buffy coat samples were collected at the in-person interview, and archival tumor tissue was obtained for case subjects. Previously published methods were used to determine HPV-16 seroprevalence by a virus-like particle ELISA (5) and HPV DNA prevalence in archival tumor tissue by PCR (4). Among those who gave a buffy-coat sample at interview and met the study restrictions (*i.e.*, white race for all of the subjects and squamous cell histology for case subjects), a sample was randomly chosen for DNA extraction. The present study made use of DNA extracted from peripheral leukocytes from 111 case and 164 control subjects.

The PCR assay used to detect the two codon 72 alleles of p53 was performed as described by Storey *et al.* (1), with minor modifications. DNA was extracted from 1 ml of buffy coat sample using the QIAamp Blood Kit (QIAGEN, Chatsworth,

³ The abbreviations used are: HPV, human papillomavirus; SEER, Surveillance, Epidemiology and End Results (Program); OR, odds ratio; CI, confidence interval.

Table 1 Risk of invasive squamous cell cervical cancer associated with the p53, Arg72Pro polymorphism

p53 Allele	Cases		Controls		OR ^a	95% CI
	n = 111	%	n = 164	%		
Arg/Pro	42	37.8	62	37.8	1.0	
Pro/Pro	7	6.3	11	6.7	0.9	0.3–2.6
Arg/Arg	62	55.9	91	55.5	1.0	0.6–1.7

^a p53 Arg/Pro was considered as the baseline when calculating ORs.

CA) following the instructions provided by the manufacturer. p53 arginine and proline sequences were amplified from each DNA sample in separate reactions (30 cycles) using 30 ng of genomic DNA as template, 12.5 pmol of each primer, and 1.25 units each of Amplitaq DNA polymerase (PE Applied Biosystems, Foster City, CA) and platinum Taq antibody (Life Technologies, Gaithersburg, MD). Annealing temperatures and MgCl₂ concentrations were 55°C/1.0 mM and 64°C/1.5 mM for the arginine and proline amplifications, respectively. Genomic DNA extracted from a vulvar carcinoma cell line, A431 (6), or from the peripheral blood of an individual whose genotype was determined by sequencing served as positive controls for amplification of the p53 proline and arginine alleles, respectively.

The relative risk of cancer was estimated using the OR approximation by exponentiation of coefficients obtained from multiple logistic regression analysis using EGRET (7). The χ^2 test was used to compare the proportions of the p53 codon 72 genotypes between HPV-16 DNA positive cases and controls (with unknown HPV DNA status) and between HPV-16 L1 serology status of cases and controls (Epi Info; Ref. 8).

Results

The prevalence of the homozygous arginine allele was virtually identical in case and control subjects in this population (Table 1). The genotype frequencies in both populations are in Hardy-Weinberg equilibrium. Overall, there was neither an increased risk of invasive squamous cell cervical cancer associated with the arginine homozygous allele compared with the heterozygous allele in this study (OR, 1.0; 95% CI, 0.6–1.7) nor a decreased risk associated with the proline homozygous allele (OR, 0.9; 95% CI, 0.3–2.6). Among the 45 cases positive for HPV 16 DNA, the distribution of the p53 codon 72 alleles was not different from the distribution seen among the controls (χ^2 on 2 df, 1.3; $P = 0.518$). Furthermore, there was no increase in the relative risk of cervical cancer associated with arginine homozygosity as compared with heterozygosity between subgroups defined by age [<45 years (OR, 0.8; 95% CI, 0.4–1.6) and ≥ 45 years (OR, 1.3; 95% CI, 0.6–2.8)], cigarette smoking status [never (OR, 1.2; 95% CI, 0.6–2.7), former (OR, 0.9; 95% CI, 0.3–2.4), and current (OR, 0.8; 95% CI, 0.3–2.2)], or HPV-16 L1 serology [seronegative (OR, 0.6; 95% CI, 0.3–1.3) and seropositive (OR, 1.5; 95% CI, 0.7–3.0)].

There was a difference in the distribution of the genotypes among case subjects according to their HPV-16 L1 serological status (Table 2). Nearly 64% of seropositive case subjects were homozygous for the arginine allele compared with only 44% of the seronegative case subjects (χ^2 on 2 df, 5.70; $P = 0.058$). In contrast, there was no difference in the distribution of the Arg/Arg genotype among control subjects when grouped as HPV-16 L1 seronegative or seropositive (χ^2 on 2 df, 1.06; $P = 0.588$).

Table 2 Prevalence of the p53 Arg72Pro polymorphism by HPV-16 L1 serology among cases and controls^a

p53 Allele	Seronegative		Seropositive	
	n	%	n	%
Cases				
Arg/Pro	23	51.1	19	28.8
Pro/Pro	2	4.4	5	7.6
Arg/Arg	20	44.4	42	63.6
Controls				
Arg/Pro	30	37.5	32	38.1
Pro/Pro	7	8.8	4	4.8
Arg/Arg	43	53.8	48	57.1

^a The distribution of the p53 codon72 polymorphism was marginally different among cases (χ^2 on 2 df, 5.70; $P, 0.058$) but not different among the controls (χ^2 on 2 df, 1.06; $P, 0.588$).

Discussion

Many groups have attempted to repeat the findings of Storey *et al.* (1) because it was thought that the potential for increased degradation of p53 by the E6 oncoprotein might contribute substantially to the risk of progression to cervical cancer. Most subsequent reports, however, found no increased risk of cervical cancer associated with the p53 Arg72Pro polymorphism (9–19). In these reports, the relative risk of invasive cervical cancer associated with the Arg/Arg genotype compared with the heterozygous genotype ranged from 0.7 (95% CI, 0.3–1.3; Ref. 9) to 1.6 (95% CI, 0.6–4.4; Ref. 15). The distribution of the Arg/Arg polymorphism ranged from 40% (19) to 63% (9) among control women in these studies. Specifically, the prevalence of the Arg/Arg genotype was 50% or greater in control subjects from studies conducted in the United Kingdom (9–11), Norway (12), Sweden (13), the United States (14), the Netherlands (15), Hungary (16), Italy (17), Germany (18), and in the present United States study (63%, 57%, 57%, 54%, 50%, 52%, 57%, 60%, 56%, 56%, and 56%, respectively). The distribution of the Arg/Arg genotype was less than 50% among control subjects in the Japanese (19) and Costa Rican (14) populations (36% and 45%, respectively), as well as in the initial United Kingdom report (37%; Ref. 1).

In the only published study to support the initial findings, Zehbe *et al.* (20) reported an increased risk of invasive cervical cancer associated with the Arg/Arg genotype compared with the heterozygous genotype of 3.8 (95% CI, 1.1–15.1) among Italian women and 2.5 (95% CI, 1.0–7.0) among Swedish women. The prevalence of the Arg/Arg genotype was 53% in Italian controls and 50% in Swedish controls, so that this study is consistent with the majority of findings with respect to controls. However, the prevalence of the Arg/Arg genotype among case groups in this study (79% among the Italian case group and 73% among the Swedish group), was most similar to that found in the initial study (76%). The prevalence of Arg/Arg among the cases in the negative studies ranged from 40% (19) to 68% (15). Therefore, the findings of an increased risk of cervical cancer reported by Zehbe *et al.* (20) and by Storey *et al.* (1) may be attributable to small, nonrepresentative groups of cases and controls. Further, both of these studies used tumor tissue to examine the p53 Arg72Pro polymorphism for the case group; therefore, their results may be attributable to somatic changes in tumor tissue.

Unlike some of the previous studies (1, 7, 10, 13, 19, 20) that relied on tumor tissue from the invasive cervical cancer cases as a source of DNA for genotyping, we used peripheral leukocytes for both case and control subjects as the DNA source. Therefore, our results can be attributed neither to so-

matic changes in tumor tissue nor to PCR artifacts from differential amplification of the longer proline allele from formalin-fixed tissue. This strengthens the validity of our conclusion that the p53 Arg72Pro polymorphism is not associated with the risk of cervical carcinoma in our population.

Our data suggest that the Arg/Arg polymorphism may be related to antibody response to the HPV-16 L1 protein among cervical cancer patients. Although not quite statistically significant, this difference in seroprevalence could suggest that the Arg/Arg genotype might affect the ability of the host to clear an infection with HPV-16. This finding needs to be confirmed by other epidemiological studies in well-defined populations among different racial and ethnic groups. It is unclear what the mechanism might be, although in our study and other studies, a higher seroprevalence of HPV-16 L1 has been reported in women with cancer compared with controls (21). Overall, however, our data and those of others (7–19) do not support the hypothesis that the p53 Arg72Pro polymorphism modifies the risk of cervical cancer.

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