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The effect of *TP53* codon 72 and *RNASEL* codon 462 polymorphisms on the development of cervical cancer in Argentine women

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Epidemiological evidence suggests that genetic factors, such as variants in cancer suppressor genes, may play an important role in the etiology of cervical carcinoma. *TP53* is an outstanding cell cycle regulator, mutated in most human cancers, and *RNASEL* is thought to be involved in antiviral and apoptotic responses. To determine whether *TP53* Arg72Pro and *RNASEL* Arg462Gln polymorphisms are associated with susceptibility to cervical cancer, a case-control study of 98 cancer patients and 123 healthy controls was conducted. Cervical samples were genotyped for both polymorphisms by pyrosequencing technology. The association between cervical cancer risk and the studied SNPs was evaluated by logistic regression, and potential gene–gene interactions were studied by Multifactor Dimensionality Reduction analysis. In the single-locus analysis, only the heterozygous *TP53* Arg72Pro genotype was significantly associated with the risk of developing a cervical carcinoma, while the *RNASEL* polymorphism showed no association after age adjustment. In addition, the combination of both polymorphisms gives near-null information gain. Consequently, the effect provided by each single nucleotide polymorphism individually is considered higher than the effect resulting from the interaction between these two genes in cervical cancer risk. These results suggest that a heterozygous *TP53* Arg72Pro genotype may contribute to cervical cancer susceptibility.

Keywords Cervical cancer, *TP53*, *RNASEL*, polymorphisms, pyrosequencing

Cervical cancer is the second most frequent cancer in women worldwide, and in developing countries, it is the main cancer affecting women, representing 80% of total cervical cancer cases worldwide (1,2).

Invasive cervical cancer arises as the result of a combination between host and environmental factors, where persistent infection with high-risk human papilloma virus (HPV) plays a key role in the development and progression of cervical intraepithelial neoplasia and squamous cell carcinoma of the cervix (3). Because many women are infected with HPV but less than 1% develop invasive cervical cancer, other cofactors may be involved in the development of invasive cervical cancer. The wild-type *TP53* gene has tumor suppressor properties, and it is a target for several of the oncoproteins encoded by DNA tumor viruses. In cervical neoplasia, uncontrolled cell proliferation is produced by the two viral oncoproteins, E6 and E7, acting over *TP53* and *RB1* tumor suppressor genes, respectively (4). In the 1990s, several works demonstrated that the E6 oncoprotein encoded by HPV-16 and HPV-18 can complex the wild-type TP53 protein *in vitro*, stimulating its degradation through the ubiquitin-dependent protease system (5,6).

Two variants of the *TP53* gene have been recognized regarding a polymorphic site at codon 72: the arginine (*TP53Arg*) and the proline (*TP53Pro*) alleles. These isoforms display functional differences in their biological and biochemical activities. Some studies have shown that *TP53* codon 72 variants are functionally different *in vitro*, and the *TP53Arg* allele induces apoptosis five times more efficiently than the proline variant (7,8). Since the initial results reported by Storey et al. (9), the relationship of *TP53* codon 72 polymorphism and cervical cancer has been extensively studied.

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Originally, it was proposed that the *TP53Arg* variant was seven times more susceptible to degradation than the *TP53Pro* variant, suggesting that the homozygosity for arginine has a greater susceptibility for HPV-associated neoplasias (9). To date, more than 90 investigations have been published, mainly retrospective studies, but only a few have achieved the initial results obtained by Storey et al. As Koushik et al. pointed out (10), the different populations analyzed plus some aspects of study design and methodology may have contributed to the inconsistency in the reported results. A recent meta-analysis by Klug et al. analyzed the results obtained in 49 studies (including one from our group). They concluded that subgroup analyses showed that excess risks were mainly found because of errors in the methods of study, and not because of the existence of biological factors. When the analyses were restricted to "methodologically sound studies," no association was found between *TP53* codon 72 polymorphism and cervical cancer (11). Nevertheless, some other studies still find different degrees of relationship between the *TP53* codon 72 polymorphism and cervical disease (9–11).

Recently, polymorphisms in another candidate tumor suppressor gene, the *RNASEL* gene, were associated with an increased risk for cervical, head and neck, and breast cancers (12). The *RNASEL* gene is located in the long arm of chromosome 1 (1q25.3) in a cancer susceptibility locus called HPC1 (Hereditary Prostate Cancer on Chromosome 1q24–25), and its product is related to antiviral defense and proapoptotic functions (13). In addition to its antiviral function, the interferon-inducible *RNASEL* may play an important role during stress response through RNA degradation and apoptosis (14). This enzyme is constitutively expressed and mediates the interferon-regulated 2′–5′-linked oligoadenylate (2–5A) system. When triggered by an external stimulus or by double-stranded RNA, the interferon induces members of the family of oligo synthetases 2′–5′, which convert pyrophosphate in a series of small 2′–5′ linked oligoadenylates known as 2–5A (15).

RNASEL codon 462, which codes for a kinase domain, contains a G/A polymorphism. It has been shown that the variants at codon 462 exhibit functional differences in the *in vitro* activity, with the Arg allele three times more active than the Gln allele. Furthermore, the Gln variant has been found to be deficient in causing apoptosis in response to 2–5A cells, allowing cancer cells to overcome an important antiproliferative pathway (16,17). Considering that the antiviral and proapoptotic capacities of *RNASEL* could differ according to the allele present at codon 462, polymorphisms within this codon could be related to variations in the individual risk of acquiring a persistent HPV infection, and thereby an increased risk of developing cervical cancer.

One study showed an additive effect between *TP53* and *RNASEL* variants with respect to the age at onset of colorectal cancer. This study showed that the different apoptotic and antiviral capabilities of *TP53* and *RNASEL* variants can modify in an additive mode the disease phenotype, where wild-type alleles mediate the destruction of emerging tumors more efficiently than the rare variants (18).

Taking into consideration the controversy in relation to the effect of *TP53* codon 72 and cervical cancer development, data regarding *RNASEL* activity and gene polymorphisms related to cervical cancer predisposition, and the possible interaction between these two tumor suppressor genes, the aim of the present study was to analyze a series of cervical carcinomas and normal cervical samples to assess the effect of *TP53* codon 72, *RNASEL* codon 462, and both sequence variants in the development of squamous cervical cancer in a set of cervical samples obtained from Argentine women.

Materials and methods

A total of 221 cervical samples were collected from an anonymous cervical specimen data bank in La Plata, Argentina. A total of 123 samples had normal cytologies (obtained from women attending screening) and 98 samples corresponded to histologically diagnosed squamous cervical carcinomas. The mean age for the control group was 40 years (SD ± 10.3), and 44 years (SD ± 10.0) for the case group. Cervical specimens include exfoliated cells from the ectoendocervix, freshly frozen tissue biopsy samples, and formalin-fixed, paraffin-embedded tissue biopsy samples.

DNA extraction

Paraffin-embedded samples were washed twice with xylol and finally with 100% ethanol, resuspended in 250 μL of digestion buffer (50 mmol/L Tris–HCl pH 8.5; 1 mmol/L EDTA [ethylenediaminetetraacetic acid]; 1% Triton X-100 and 0.5% Tween 20) with 250 mg/mL proteinase K (Genbiotech, Buenos Aires, Argentina), and incubated for 24 hours at 56°C. Cervical exfoliated cell pellets and freshly frozen biopsy samples were suspended and washed twice with 1 mL of phosphate-buffered saline and incubated for 24 hours at 56°C in 400 μl of digestion buffer (50 mmol/L Tris–HCl pH 8.5; 1 mmol/L EDTA; 1% Triton X-100 and 0.5% Tween 20) containing 250 mg/mL of proteinase K (Genbiotech, Buenos Aires, Argentina). After protease digestion, the samples were kept for 10 minutes at 100°C for enzyme inactivation. DNA purification was performed by the salting-out procedure as described by Miller et al. (19). Finally, the DNA was suspended in distilled water at a final concentration of 1 ng/μL and stored at −20°C until used.

Human papillomavirus DNA detection and genotyping

Sample preparation, polymerase chain reaction (PCR) setup, and amplicon analysis were performed in separate rooms to prevent contamination. Human papillomavirus DNA was detected in cervical tissues by means of a nested PCR approach with MY09/11 as external and GP5+/6+ as internal primers, according to methods previously described (20,21). These two primer pairs are the most widely used for the detection of genital HPVs. For nested PCR, separate micropipettes and disposable gloves and filter tips were always used, and a negative control containing digestion buffer was included every five samples to prevent and detect carryover between samples. After an initial amplification with the primers My9 and My11, 5 μL of the amplicons were subjected to a second nested PCR reaction. This nested PCR was performed with the Gp5+ primer and

a 5′ biotinylated Gp6+ oligonucleotide. The biotinylated amplicons were analyzed in 2% agarose gels stained with Safer Green and visualized with a blue light transilluminator (450 nm).

For HPV genotyping, an enzyme immunoassay procedure was performed with the biotinylated PCR product. The PCR–enzyme immunoassay assay was performed according to the method described by Söderlund-Strand et al. (22), with some minor modifications. Briefly, 5 μL of the amplicons were captured on streptavidin-coated Maxi Sorp wells (Nunc, Thermo Fisher Scientific, Rochester, NY). The biotinylated Gp was denatured with NaOH, washed three times with wash solution (standard saline citrate 0.25×, 20% Tween 20) and hybridized with 5′ fluorescein-labeled oligoprobes corresponding to HPV-6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -66. Hybridization was carried out at 60°C for 20 minutes, with 5 pmol of each probe in a final volume of 100 μL of hybridization solution (5× standard saline citrate, 20% Tween 20). After hybridization, the wells were washed three times with 300 μL of wash solution at room temperature. After the washing steps, samples were incubated for 30 minutes with an antifluorescein monoclonal antibody (Millipore, Billerica, MA). After two additional washings, the reactions were revealed with 1-Step Turbo TMB-ELISA (Pierce Biotechnology, Rockford, IL). The color reaction was stopped after 10-minute development with 100 μL of 1 mol/L sulfuric acid and read in a microplate reader with a 404-nm filter.

TP53 and *RNASEL* fragment amplification

PCR oligonucleotides for *TP53* and *RNASEL* amplification were designed by FastPCR (University of Helsinki, Finland) software, version 5.4.6. The PCR oligonucleotides for *TP53* polymorphism were 5′-TCC CCC TTG CCG TCC CAA-3′ (TP53 PiroF) and 5′-CTG GTG CAG GGG CCG TCG GTG TAG-3′ (TP53 PiroRBiotin) for forward and reverse primers, and defined a 166 bp fragment. The forward and reverse oligonucleotides for *RNASEL* amplification were 5′-GTG TCA CCC TCT GTG AGC AGA C-3′ (*RNASEL* PiroFBiotin) and 5′-GCA GAT CCT GGT GGG TGT ATC C-3′ (*RNASEL* PiroR), respectively. This pair of primers defined a fragment of 110 bp. The reaction mixtures were performed in a final volume of 25 μL containing 5 μL of genomic DNA; 0.85 pmol/μL of each primer; 20 mmol/L of each deoxynucleoside triphosphate; PCR buffer 1× (50 mmol/L KCl and 10 mmol/L Tris–HCl, pH 8.3), and 0.1% Triton X-100); 1.2 mmol/L of $MgCl_2$, and 1.5 units of Taq DNA polymerase (Invitrogen, Carlsbad, CA). The amplification reaction for *TP53* was carried out with an initial denaturing step of 92°C for 3 minutes, followed by 35 cycles of 30 seconds at 92°C, 40 seconds at 63°C, and 40 seconds at 72°C, with a final elongation step of 72°C for 5 minutes. For *RNASEL* amplification, the cycling conditions were as follows: an initial denaturing step of 92°C for 3 minutes, followed by 35 cycles of 30 seconds at 92°C, 40 seconds at 61°C, and 40 seconds at 72°C, with a final elongation step of 72°C for 5 minutes.

The PCR products from both genes were verified on 2% agarose gels, stained with Safer Green, and visualized by Safe Imager (Invitrogen).

SNP genotyping

SNPs from *TP53* and *RNASEL* genes were genotyped by Pyrosequencing technology on a Biotage PSQ 96MA pyrosequencer. The relative levels of each allele for the analyzed SNPs were evaluated with the PSQ96MA single nucleotide polymorphism (SNP) analysis software (Biotage, Uppsala, Sweden).

Briefly, pyrosequencing was performed in a volume of 50 μL at 25°C. Biotinylated PCR products (20 μL) were resuspended in binding buffer, captured on streptavidin-coated beads, denaturated, and prepared for pyrosequencing by using the recommended protocol for the vacuum preparation tool (Biotage AB, Uppsala, Sweden). For each reaction, purified PCR products were incorporated in 45 μL of annealing buffer containing 0.3 μmol/L of sequencing primer (5′-CAG AGG CTG CTC CCC-3′ for *TP53* and 5′-GAT GAC AGG ACA TTT-3′ for *RNASEL*). Single-stranded DNA with annealed sequencing primer was added to the pyrosequencing reaction mixture containing 10 U of exonuclease-deficient (exo2) Klenow DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden), 40 mU of apyrase (Sigma Chemical, St. Louis, MO), 4 mg of purified luciferase/mL (BioThema, Dalarö, Sweden), 15 mU of recombinant ATP sulfurylase, 0.1 mol/L Tris–acetate (pH 7.75), 0.5 mmol/L EDTA, 5 mmol/L Mg–acetate, 0.1% (w/v) bovine serum albumin (BioThema), 1 mmol/L dithiothreitol, 10 mmol/L adenosine 59-phosphosulfate, 0.4 mg of poly(vinylpyrrolidone)/mL, and 100 mg of D-luciferin/mL (BioThema). The sequencing procedure was carried out by stepwise elongation of the primer strand upon sequential addition of the different deoxynucleoside triphosphates. Degradation of excess nucleotides by apyrase was carried out simultaneously.

Statistical analysis

Descriptive statistics were analyzed by SPSS software, version 15.0 (SPSS, Chicago, IL). Pearson's chi-square test was conducted to examine the Hardy–Weinberg equilibrium (HWE) and the independence of genotype frequencies between cases and controls. To determine the polymorphisms, risk-estimation unconditional logistic regression was used to calculate crude and age-adjusted odds ratios (ORs) and their relative 95% confidence intervals (CIs). Finally, Multifactor Dimensionality Reduction (MDR) software package, version 2.0 beta 8 (Computational Genetics Laboratory, Dartmouth Medical School, Hanover, NH), was used to detect gene–gene interactions. MDR is a nonparametric data mining strategy for detecting and characterizing nonlinear interactions among discrete attributes that would be predictive of an outcome (i.e., a disease). With MDR, multilocus genotypes are combined in high-risk and low-risk groups, reducing the dimensionality of the genotype predictors from *n* dimensions to only one. It applies data reduction techniques to address problems associated with testing for interactions in high-dimensional space. This advanced statistical tool also overcomes sample size limitations often encountered by parametric statistical methods (e.g., logistic regression analysis). MDR has greater than 80% statistical power and rigor to evaluate gene–gene interactions even in small sample sizes (23).

Results

In this study, which included 221 samples corresponding to 123 healthy tissue and 98 cervical cancer cases obtained from white Argentine women, both *TP53* and *RNASEL* polymorphisms were successfully genotyped in all samples. The global age ranged from 18 to 69 years, with a median age of 39.6 years (SD $\pm$ 10.3) for the control group, and 43.8 years (SD $\pm$ 10.0) for the case group. The specimens were also tested for the presence of HPV DNA. The prevalence of HPV ranged from 35.8% among healthy controls to 86.7% among squamous cell carcinomas (OR = 35.6; 95% CI 10.5–120.5; χ^2 = 582; P = 0.01).

TP53 and *RNASEL* allele frequencies

Gene and genotype frequencies for *TP53* and *RNASEL* polymorphisms are shown in Table 1. Alleles for both SNPs were uniformly distributed among controls and cases, showing no statistically significant differences (*TP53* χ^2 = 0.19, P = 0.66; *RNASEL* χ^2 = 1.96, P = 0.16).

TP53 and *RNASEL* genotype frequencies and risk for cervical cancer development

The distribution of genotypes for both polymorphisms fit well as expected by the HWE model in the control group. Single-locus analysis for the *TP53* polymorphism revealed a statistically significant difference in genotype distribution between cases and controls (χ^2 = 8.45; P = 0.015). Furthermore, age-adjusted logistic regression analysis showed that the heterozygous condition Arg72Pro (GC) was related to cervical cancer risk (OR = 2.28; 95% CI 1.20–4.34; P = 0.012) when compared with women homozygous for the Arg72 (G) allele. Interestingly, the same relationship was also found when the data were adjusted by HPV infection (OR = 2.54; 95% CI 1.15–5.58; P = 0.020).

On the other hand, analysis for the Arg462Gln polymorphism in the *RNASEL* gene revealed that the homozygous Gln462Gln (AA) genotype seemed inversely related to cervical cancer risk (OR = 0.27; 95% CI 0.10–0.73; P = 0.010) compared with the homozygous Arg462Arg (GG) genotype. However, the association between this polymorphism and cervical cancer disappeared when the data were adjusted by age (OR = 0.37; 95% CI 0.12–1.11; P = 0.078) and by age/HPV infection (OR = 0.43; 95% CI 0.12–1.58; P = 0.20).

Furthermore, analysis of both polymorphisms for the sample preservation method showed no differences between paraffin-embedded tissue and freshly frozen tissue in terms of the frequency of association of *TP53* and *RNASEL* genotypes and cervical cancer.

TP53 and *RNASEL* polymorphisms and risk for HPV infection

In spite of the association between both polymorphisms and cervical cancer risk, *TP53* and *RNASEL* genotypes were uniformly distributed among HPV-positive and HPV-negative women in the control group ($P > 0.05$). In addition, no significant association was found between *TP53* and *RNASEL* polymorphisms and high-risk HPV infection in the control group. In this sense, none of the studied polymorphisms was found to provide a higher risk of virus infection.

MDR and the risk of cervical cancer

MDR software was used to assess potential gene–gene interactions on cervical cancer risk on the basis of both SNPs genotyping data. As demonstrated by this analysis, the model conformed by *TP53/RNASEL* was statistically significant (average testing accuracy of 0.612 and cross-validation consistency of 10/10). The obtained statistical values for the whole data set were OR = 3.09, 95% CI 1.75–5.45, specificity = 0.75, and sensitivity = 0.51. Figure 1 summarizes the two-locus genotype combinations of *TP53* and *RNASEL* associated with cervical cancer risk. Global permutation test (set at 1,000 tests) and the entropy decomposition analysis of the interaction graph was further performed to assess

Table 1 Association between *TP53* and *RNASEL* polymorphisms and risk for cervical carcinoma

SNP	Genotype	Control (n = 123)	Squamous cell carcinoma (n = 98)	Adjusted OR (age) (95% CI)	Adjusted OR (age/HPV) (95% CI)
	Arg/Arg (GG)	69 (56.1%)	42 (42.9%)	1.00 (reference)	1.00 (reference)
TP53	Arg/Pro (GC)	44 (35.8%)	53 (54.1%)	2.28 (1.20–4.34)[a]	2.54 (1.12–5.59)[a]
Arg72Pro	Pro/Pro (CC)	10 (8.1%)	3 (3.1%)	0.47 (0.09–2.37)	0.52 (0.08–3.42)
	Allele frequency				
	Arg (G)	91 (73.9%)	69 (70.4%)	1.00 (reference)	
	Pro (C)	32 (26.0%)	29 (29.6%)	1.19 (0.66–2.16)	
RNASEL	Arg/Arg (GG)	44 (35.8%)	44 (44.9%)	1.00 (reference)	1.00 (reference)
Arg462Gln	Gln/Arg (AG)	57 (46.3%)	48 (49.0%)	1.02 (0.53–1.97)	1.02 (0.45–2.29)
	Gln/Gln (AA)	22 (17.9%)	6 (6.1%)	0.37 (0.12–1.11)	0.43 (0.12–1.59)
	Allele frequency				
	Arg (G)	73 (59.3%)	68 (69.4%)	1.00 (reference)	
	Gln (A)	50 (40.6%)	30 (30.6%)	0.64 (0.36–1.12)	

[a] $P < 0.05$ for the unconditional logistic regression analysis.

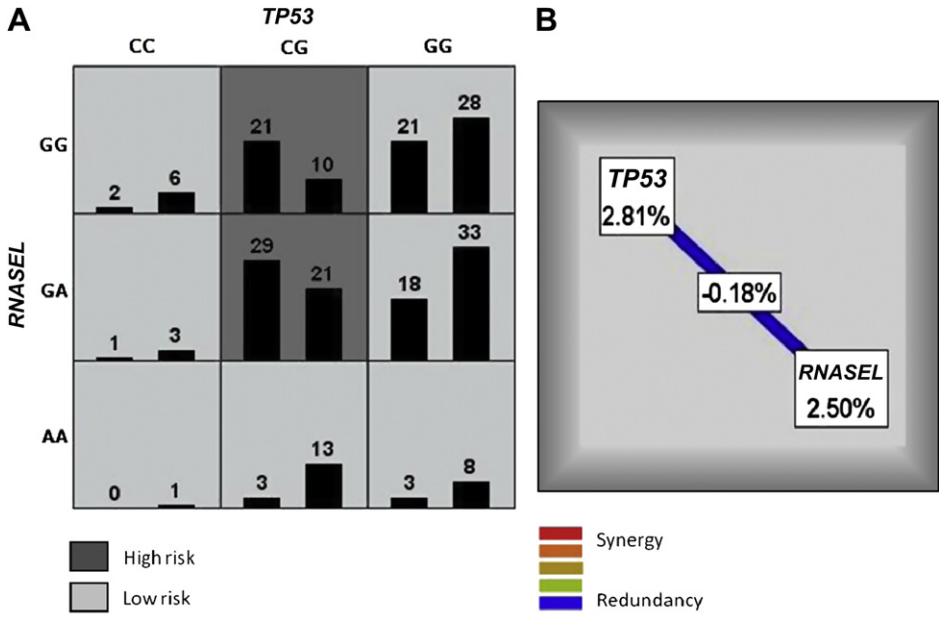

Figure 1 MDR analysis. (A) This graphical model is a summary of two-locus genotype combinations associated with high risk (dark shading) and with low risk (light shading) for cervical cancer, along with the corresponding distribution of cases (left bars in boxes) and controls (right bars in boxes). (B) The entropy decomposition analysis describes the percentage entropy that is explained by each SNP and the combination of the two loci within the studied population. Positive percentage entropy indicates information gain or synergy. However, negative percentage indicates redundancy or lack of information gain. Schematic coloration represents a continuum from synergy (red, representing nonadditive interaction) to redundancy (blue, representing lack of information gain). The effect provided by each SNP individually is higher than the effect resulting from the interaction between these two genes in cervical cancer risk. (A color figure can be found in the online version of this article.)

which types of effects were represented in this two-locus model. A negative entropy value of −0.018% indicated that there was a loss of information as a result of redundancy and/or correlation of both genes.

Discussion

In this study, the impact of *TP53* and *RNASEL* polymorphisms was evaluated in 221 samples corresponding to 98 cervical cancer cases and 123 healthy tissues obtained from white Argentine women. The *TP53* codon 72 polymorphism seemed to be associated with cervical cancer. There were more heterozygous genotypes in cases compared to controls. There was also a borderline association between *RNASEL* gene polymorphism and cervical cancer, although this association disappeared when the data were adjusted by age. As demonstrated by the MDR analysis, the combination of both polymorphisms gives near-null information gain as a result of redundancy and/or correlation of both genes. Consequently, the effect of each polymorphism is considered higher than the effect obtained through the interaction between these two genes in cervical cancer risk. This research is novel in its investigation of the role of the codon 462 polymorphism from *RNASEL* in cervical cancer risk; it is also novel in that its approach analyzes the interaction between *TP53* codon 72 and *RNASEL* codon 462 polymorphisms in cervical cancer development.

The association of *TP53* codon 72 and cervical cancer has been intensively studied since the report of Storey et al. (9). This topic has resulted in conflicting results and opposing points of view. Indeed, *in vitro* studies have shown that isoforms resulting from a codon 72 polymorphism display differential behavior in biological activities. These properties include those related to protein degradation and apoptosis induction (7,8). Transferring these results to population studies has not been conclusive, and articles published even into 2010 still show disagreement.

Koushik et al. (10) reported that *TP53* codon 72 heterogeneity could have arisen as a consequence of differences in the analyzed populations, poor study design, and, in particular, variability in methodology. Regarding the latter, some authors have raised concerns about the use of allele-specific PCR for diagnostic purposes. Apparently, allele-specific PCR might lose its specificity when 100 pg or less of genomic DNA is used; the sensitivity drops when the amount of DNA is lower than 10 pg per reaction (24,25). Considering this situation, and with an aim of significantly reducing genotype misclassification, the present work was entirely performed by pyrosequencing technology. This approach is a DNA sequencing methodology based on real-time detection of DNA synthesis, monitored by bioluminescence and currently considered the gold standard for single nucleotide polymorphism genotyping. This technique offers unambiguous SNP genotyping results as well as some redundant sequence information beyond the SNP position, which is useful for at least two reasons: viability of a positive control in the amplification process, and accurate calibration of peaks and reaction conditions (26,27). Pyrosequencing is emerging as the method of choice for SNP genotyping as a result of its

robustness in the sequencing process, which guarantees the absence—or at least the highest reduction—of genotype misclassification. As defined by Ahmadian et al., the unique property with pyrosequencing in typing SNPs is that each allele combination will give a specific pattern compared to the two other variants, making the genotyping process extremely accurate and easy (27,28).

While genotype misclassification may drive results that lead to false conclusions, another reason for misinterpretation in case-control studies is the departure of genotype frequencies from HWE in controls. In the present study, the control group fulfills the HWE criteria for both *TP53* and *RNASEL* polymorphisms. If the population is not in HWE, results should be treated with caution because the observed genotype distribution in the control group would not be representative of the population from which the cases were presumably taken, and therefore conclusions are of limited value (10,29,30). Sometimes departure of HWE has been wrongly considered as a consequence of biological errors or sampling mistakes. The possibility of evolutionary forces (mainly selection or migration) acting on the studied population should be considered, and departures from the equilibrium may not be solely the result of errors in methodology or sampling bias. However, either case is an inappropriate scenario to use populations lacking HWE equilibrium in controls because the obtained results will be questionable.

In the present study, the *TP53* Arg72Pro genotype was statistically associated with cervical cancer risk. Interestingly, this difference remained after data were adjusted for age and HPV infection. Similar findings were reported by Klug et al. (31) and Kim et al. (32), who also found an increased risk for the Arg72Pro genotype. The biological reason for these findings is unknown, although it is presumed that the heterotetramer formed in the heterozygous cells has less efficiency than a tetramer formed by homozygous ones (33).

Recently, *TP53* codon 72 polymorphism has been the subject of an important meta-analysis by Klug et al. (11). They did not find an increase in risk of *TP53* genotypes for cervical cancer. However, the authors excluded those studies with controls without HWE, not epidemiological studies, and those that took tumor samples or exfoliated cervical tissues for genotyping. It seems to us reasonable to exclude those studies where the control groups are not in HWE, although the exclusion on the basis of the latter two criteria is somewhat questionable. Finally, their conclusions had to be restricted to a few studies. What is clear from the pooled analysis is that inter-study heterogeneity is too wide to make comparisons. The authors could not make HPV or age adjustments as a result of high variability among studies. Also, the inclusion of all available data on the polymorphism is useful, but the use of unpublished information is not appropriate because it has not been peer reviewed. In the present study, sample selection was carefully performed to preserve homogeneity between cases and paired controls. In this sense, the homogeneity of the samples was the major advantage at the time of statistical analysis.

The fact that *TP53* polymorphism varies geographically adds an additional factor, making it clear that more and larger studies are required to reach firm conclusions.

Potential limitations to this study were sample size and the use of paraffin-embedded tissue instead of white blood cells to obtain DNA from samples. However, to our knowledge, there is no solid evidence that indicates that DNA from white blood cells is more representative than DNA obtained from normal exfoliated cells or tissue surrounding the tumor. Moreover, results obtained in the present study showed an increment of heterozygotes in the case group, avoiding the idea of the existence of loss of heterozygosity.

In addition to *TP53, RNASEL* polymorphisms have also been implicated in cervical cancer development. In the present study, there was a slight association of *RNASEL* Arg462Gln polymorphism with cervical cancer, but this association disappeared when the data were adjusted by age and age/HPV infection. These results could be originated by a valid result (i.e., the absence of association between RNASEL and squamous cell carcinoma) or the lack of statistical power. Madsen et al. (12) have recently shown a positive association between *RNASEL* and cervical cancer. The biological plausibility of such an association is given by functional studies that showed that *RNASEL* Gln462 allele had reduced ability to dimerize into its catalytic active form, showing a threefold decrease in the *RNASEL* activity (16,17,34). Also, the Gln462 allele was deficient in promoting apoptosis in a mouse *RNASEL*$^{-/-}$ cell line; meanwhile, other nucleotide substitutions analyzed showed comparable levels of *RNASEL* activity to wild-type protein (17). This situation supports the role of the Arg462Gln polymorphism in the apoptosis process and possibly in cervical cancer development. According to these results, there were no supporting data on *RNASEL* polymorphisms in cervical cancer by adjusted univariate analyses.

A second step in the analysis was to assess an interactive effect between both *TP53* and *RNASEL.* A novel methodology was used to create a univariate attribute (variable) that encodes every possible combination of genotypes and correlates it to disease outcomes (35). This approach has an important advantage: it can detect polymorphism interactions even in the absence of main effects. The model originated from constructive induction was statistically significant, indicating a potential interaction between both polymorphisms, after performing a permutation test. However, entropy analyses demonstrated that the combination of both polymorphisms gives near-null information gain, which means that information is lost as a result of redundancy and/or correlation of both genes. Consequently, each SNP may have a main effect that is independent of the other locus. Whether this is an artifact of sample size or a valid association should be confirmed in larger studies.

The search for genetic variants conferring risk to complex diseases like cancer has fundamental challenges. Although the *TP53* codon 72 polymorphism has been widely implicated in cervical cancer susceptibility, a role in other prognostic features cannot be ruled out, such as tumor aggressiveness, cancer survival, or mortality (36–44), attributes that are not commonly considered. A positive effect of *TP53* polymorphism on cancer susceptibility will have important implications. However, the magnitude of risk would be modest because of the joint effect of several factors.

In conclusion, this study showed that the heterozygous *TP53* Arg/Gln genotype modestly increases the risk of cervical cancer. This variation, along with the characterization of other genetic loci and discrimination of external factors, may lead to better risk assessment and, in the long term, a deeper understanding of the disease.

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