Polymorphism Located in the Upstream Region of the RPS19 Gene (rs2305809) Is Associated With Cervical Cancer: A Case-control Study

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Cervical cancer (CC) is caused by persistent human papillomavirus (HPV) infection and affects women worldwide. The progression of an HPV persistent infection to CC is influenced by genetic factors. Three single nucleotide polymorphisms (SNPs) in TP53, NQO1 and RPS19 genes (rs1042522, rs1800566, rs2305809, respectively) were previously associated with CC in European and North American populations. The present case-control study aimed to investigate the association of the SNPs rs1042522, rs1800566, and rs2305809 with CC in an admixed population in southern Brazil. A total of 435 women (106 CC patients and 329 controls) were recruited for this study. All women were interviewed and underwent clinical sampling. SNPs rs1042522 and rs1800566 were evaluated by PCR-RFLP. SNP rs2305809 was determined by real-time PCR. The crude and adjusted ORs with 95% CI were estimated. The recessive genetic model (C/C + C/T) for rs2305809 was more frequent in the control group (79.9%) compared to the cases (69.8%), being associated with CC protection (adjusted OR = 0.49; 95% CI: 0.27-0.90). However, the other polymorphisms evaluated did not present significant differences between cases and controls. This study detected a protective association for the recessive genetic model in rs2305809. These results suggest a potential role of the RPS19 gene in CC.

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Key Words: Cervical cancer, Single nucleotide polymorphisms, Case-control study

INTRODUCTION

Cervical cancer (CC) is the fourth most common cancer in women, with approximately 528,000 new cases in the world each year.1 In 2016, 16,340 new cases of CC were reported in Brazil. It is one of the top five most common cancer types in all states of the country.2 Human papillomavirus (HPV) persistence is a key factor in the development of CC, inducing carcinogenesis by the integration of the whole genome into the cell host chromosome, transformation of the cervical cells and appearance of intraepithelial lesions that progress to CC.3 However, most HPV-infected women do not progress to CC, suggesting that other factors are related to this outcome. Socio-demographic (e.g., income, educational level, age, and multiparity) and behavioral (e.g., age at first intercourse, number of sexual partners) factors were already demonstrated to have a direct relationship to HPV exposure and persistence in the uterine cervix and progression to precancerous lesions.3 In addition, human genetic factors have also a pivotal influence in the development of CC.4,5 Several single nucleotide polymorphisms (SNPs) of the human genome have been associated with CC in the last years.4,5 These SNPs are present in genes of different cell pathways, such as tumor suppression, inflammation, apoptosis and cell cycle regulation, DNA repair, cell migration, cell signaling and viral entry into the cell.4,5
The TP53, NQO1, and RPS19 genes are related with DNA repair mechanism and cell general function (e.g., metabolism, antioxidant activities, ribosomal structure). Specifically, the gene coding for the human tumor protein p53 is one of the most investigated in recent association studies. The SNP rs1042522, which causes a nucleotide modification G>C with a consequent amino acid change in p53 (Arg72Pro), was reported to be associated with CC. However conflicting results have been observed in different populations and both alleles have been associated with the risk for CC. In addition, NAD (P) H: quinone oxidoreductase-1 gene (NQO1) has been also a preferential target of many genetic association studies since it codes an enzyme related to the cellular response to oxidative stress. The SNP rs1800566 causes a modification C>T (in the position 609 of NQO1 gene) and a consequent amino acid change Pro187Ser in NQO-1 protein, resulting in reduced enzymatic activity and predisposition of CC in women with HPV infection. Further, RPS19 codes for a 40S subunit ribosomal protein (S19E family) and probably has an extra-ribosomal function in cell differentiation and proliferation. Two previous studies demonstrated an association between an SNP located in the upstream region of the RPS19 gene (rs2305809) and the risk of CC in Central American and African women.

Few studies investigated the association of SNPs with CC in South America populations. Recently, we investigated some other SNPs previously associated to CC in genes related to immune response (IRF3), cell cycle (FANCA) and cell enzymes (DUT, FLJ35220, OAS3, and SULF1). However, we not found any association in any admixed population in southern Brazil. The present study evaluated the frequencies of other three SNPs (rs1042522 in TP53, rs1800566 in NQO1, and rs2305809 in RPS19) as well as the association of the respective alleles and genotypes with CC in the same admixed population in southern Brazil.

MATERIALS AND METHODS

1. Study design and population

The CC patients (n = 106) were recruited at the Center of High Complexity in Oncology (Centro de Assistência de Alta Complexidade em Oncologia) from 2012 to 2014. This center is located in the city of Ijui and treats cancer in women from the Northwest and Center regions of Rio Grande do Sul, the southernmost Brazilian state. These women had a medical history of HPV infection, presented CC diagnosis (squamous cell carcinoma or adenocarcinoma) and were receiving cancer treatment (chemo-, radio, and/or brachytherapy) when they were invited to participate of the study. The control group (n = 329) was selected by age-matched sampling from two previous cross-sectional studies conducted in the same region in the Rio Grande do Sul state. All these women presented normal cervical cytology at the moment of the recruitment. Socio-demographic and behavioral information was obtained through a questionnaire administered to all women. In the case group, some additional clinical information was also obtained from the patient records. This study was approved by University of Cruz Alta Research Ethics Committee (Number 54501216.4.0000.5322).

2. Clinical samples and human papillomavirus detection

All women underwent cell sampling with buccal (case group) or cervical (control group) exfoliation using cytobrush and stored in a buffer solution (EDTA pH 8.0 0.01M, SDS 0.03 M) at −20°C until analysis. DNA extraction and HPV detection/typing were performed as described in previous studies.

3. Single nucleotide polymorphism analysis

A fragment of 199 bp of the TP53 gene, nesting rs1042522, was amplified by PCR using primers previously described, while a fragment of 267 bp of the NQO1 gene, nesting rs1800566, was amplified by PCR using the following primers: forward (F 5' GGTAACGGCTAGTAGGGAAGGG -3') and reverse (R 5' ATTTGAAT TCGGGCGTCTGC -3'). The cycling conditions for both reactions were as follows: initial denaturation at 94°C for 5 minutes. 45 cycles of 94°C for 10 seconds, 55°C for 30 seconds and 72°C for 30 seconds. The SNPs rs1042522 and rs1800566 were evaluated by restriction digestion with BstUI and HinfI enzymes (Thermo Fisher Scientific, Dreieich, Germany), respectively. The results were analyzed by polyacrylamide gel electrophoresis stained with silver nitrate. SNP rs2305809 in RPS19 gene was evaluated using a TaqMan® SNP genotyping assay C_3060197_1 (Applied Biosystems, Foster City, CA, USA). Allelic discrimination real-time PCR was performed on the StepOnePlusSM with the following cycling conditions: 10 minutes at 95°C followed by 45 cycles of 15 seconds at 95°C and 1 minute at 60°C. Allelic discrimination was measured by end-point fluorescence using StepOneTM Software version 2.3 and TaqMan® Genotyping Software version 1.3 (Applied Biosystems, Waltham, MA, USA).

4. Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences ver. 18.0 (PASW; IBM Co., Armonk, NY, USA).
Student t-test for independent samples was used to detect statistical differences between quantitative variables. Allele and genotypes frequencies were determined by direct counting and Hardy-Weinberg equilibrium was evaluated by chi-square test. The genetic models used were additive (genotypes), recessive (Arg/Arg + Arg/Pro vs. Pro/Pro for rs1042522, Pro/Pro + Pro/Ser vs. Ser/Ser for rs1800566, and C/C + C/T vs. T/T for rs2305809) and dominant (Arg/Pro + Pro/Pro vs. Arg/Arg for rs1042522, Pro/Ser + Ser/Ser vs. Pro/Pro for rs1800566, and C/T + T/T vs. C/C for rs2305809). Associations between qualitative variables and CC were evaluated by bivariate analysis (Pearson’s chi-square test). The crude odds ratios (crudeOR) with 95% CI were estimated in order to detect the association of the SNPs with CC. Logistic regression analysis was conducted to control possible interferences of covariates that presented P-values ≤ 0.10 in the bivariate analysis of socio-demographic and behavioral variables. Therefore, the adjusted odds ratios (adjustedOR) with 95% CI were estimated. Statistical power calculations of the sample were performed using the software Epi Info™ version 7.1.5.2 considering minor allele frequencies (MAF) for rs1042522, rs1800566, and rs2305809 in control group. All P-values presented are two-tailed and those with values < 0.05 were considered significant.

RESULTS

Socio-demographic and behavioral data in the sample studied are presented in Table 1. In the comparison of these characteristics, cases and controls did not present statistically significant differences, except for parity (94.3% in cases vs. 55.5% in controls; P < 0.01), contraceptive oral use (17.9% vs. 32.5%; P < 0.01), to have ≥ 2 lifetime sexual partners (49.1% vs. 62.0%; P = 0.02) and sexual debut at ≤ 18 years-old (66.7% vs. 48.2%; P < 0.01). In the laboratory analysis, HPV detection was performed in all clinical cervical samples of the healthy women. HPV was detected in 78 (24.5%; mean age: 43.4 ± 12.3) women. Socio-demographic and behavioral data were compared between HPV positive and HPV negative women and there was no significant difference in all characteristics (data not shown). All samples were used in the comparative analysis between cases and controls because the observed frequency of HPV positive women is expected in the whole women population of this geographic

Table 1. Bivariate analysis of socio-demographic and behavioral data of the case and control groups

| Variable                                      | Case                 | Control               | P-value |
|-----------------------------------------------|----------------------|-----------------------|---------|
| Age (yr)                                      | 50.45 ± 14.38        | 48.12 ± 14.38         | 0.15    |
| Educational level                            |                      |                       |         |
| Complete primary education or less            | 72 (68.6)            | 188 (69.1)            | 0.92    |
| Secondary or higher education                 | 33 (31.4)            | 84 (30.9)             |         |
| Total household income (in Brazilian minimum monthly wage) | 51 (48.6)            | 98 (51.0)             | 0.68    |
| Household income ≥ 2 minimum salary          | 54 (51.4)            | 94 (49.0)             |         |
| Household income ≤ 1 minimum salary          |                      |                       |         |
| Smoking                                       |                      |                       |         |
| No                                           | 81 (77.1)            | 169 (85.8)            | 0.06    |
| Yes                                          | 24 (22.9)            | 28 (14.2)             |         |
| Parity                                       |                      |                       |         |
| No                                           | 6 (5.7)              | 142 (44.5)            | < 0.01  |
| Yes                                          | 100 (94.3)           | 177 (55.5)            |         |
| Contraceptive oral use                        |                      |                       |         |
| No                                           | 87 (82.1)            | 217 (65.9)            | < 0.01  |
| Yes                                          | 19 (17.9)            | 107 (32.5)            |         |
| Condom use in all sexual relations            |                      |                       |         |
| No                                           | 83 (78.3)            | 152 (77.6)            | 0.88    |
| Yes                                          | 23 (21.7)            | 44 (22.4)             |         |
| No. of lifetime sexual partners ≥ 2           |                      |                       |         |
| No                                           | 54 (50.9)            | 92 (38.0)             | 0.02    |
| Yes                                          | 52 (49.1)            | 150 (62.0)            |         |
| Sexual debut at ≤ 18 years-old               |                      |                       |         |
| No                                           | 35 (33.3)            | 99 (51.8)             | < 0.01  |
| Yes                                          | 70 (66.7)            | 92 (48.2)             |         |

Values are presented as mean ± SD or number (%). Student t-test was used to evaluate possible differences of age between groups. Totals do not coincide due to the lack of data.
Table 2. Allelic frequencies of gene TP53 (rs1042522), NQO1 (rs1800566), RPS19 (rs2305809) in patients with cervical cancer (cases) and healthy women (controls)

| Variable     | Case (%) | Control (%) | Total (%) | P-value |
|--------------|----------|-------------|-----------|---------|
| **TP53**     |          |             |           |         |
| Arg          | 135 (70.3)| 441 (71.4)  | 576 (71.1)| 0.78    |
| Pro          | 57 (29.7) | 177 (28.6)  | 234 (28.9)|         |
| **NQO1**     |          |             |           |         |
| Pro          | 131 (76.20)| 434 (72.6) | 565 (69.8)| 0.35    |
| Ser          | 41 (23.8) | 164 (27.4)  | 205 (25.3)|         |
| **RPS19**    |          |             |           |         |
| C            | 103 (48.6)| 364 (55.3)  | 467 (57.7)| 0.09    |
| T            | 109 (51.4)| 294 (44.7)  | 403 (42.3)|         |

Value are presented as number (%). *Genotyping results were obtained for the single nucleotide polymorphisms rs1048522 (n = 96 cases and n = 309 controls), rs1800566 (n = 86 and n = 299 controls), and rs2305809 (n = 106 cases and n = 329 controls). *Pearson’s chi-squared test.
Table 3. Analysis of genotypes and alleles of gene TP53 (rs1042522), NQO1 (rs1800566), RPS19 (rs2305809) in patients with cervical cancer (cases) and healthy women (controls)

| Genetic model | Case | Control | crude OR (95% CI) | P-value | adjusted OR (95% CI) | P-value |
|---------------|------|---------|------------------|---------|----------------------|---------|
| **TP53 (rs1042522)** |      |         |                  |         |                      |         |
| Additive |          |          |                  |         |                      |         |
| Arg/Arg | 47 (49.0) | 161 (52.1) | 1.00             | 1.00    |                      |         |
| Arg/Pro | 41 (42.7) | 119 (38.5) | 1.18 (0.73-1.91) | 0.50 | 1.11 (0.56-1.50) | 0.83    |
| Pro/Pro | 8 (8.3)  | 29 (9.4)  | 0.94 (0.40-2.20) | 0.80 | 0.94 (0.36-2.43) | 0.90    |
| Recessive |          |          |                  |         |                      |         |
| Arg/Arg + Arg/Pro | 88 (91.6) | 280 (90.6) | 1.13 (0.50-2.58) | 0.75 | 1.38 (0.68-2.79) | 0.35    |
| Dominant |          |          |                  |         |                      |         |
| Arg/Pro + Pro/Pro | 49 (51.0) | 148 (47.9) | 1.13 (0.72-1.79) | 0.59 | 1.22 (0.58-1.60) | 0.89    |
| **NQO1 (rs1800566)** |      |         |                  |         |                      |         |
| Additive |          |          |                  |         |                      |         |
| Pro/Pro | 49 (57.0) | 164 (54.9) | 1.00             | 1.00    |                      |         |
| Pro/Ser | 33 (38.4) | 106 (35.4) | 1.04 (0.69-1.53) | 0.87 | 1.05 (0.61-1.81) | 0.84    |
| Ser/Ser | 4 (4.7)  | 29 (9.7)  | 0.46 (0.16-1.37) | 0.16 | 0.37 (0.12-1.19) | 0.09    |
| Recessive |          |          |                  |         |                      |         |
| Pro/Pro + Pro/Ser | 82 (95.3) | 270 (90.3) | 2.20 (0.75-6.44) | 0.15 | 1.39 (0.75-2.58) | 0.30    |
| Dominant |          |          |                  |         |                      |         |
| Pro/Ser + Ser/Ser | 37 (43.0) | 135 (45.1) | 0.92 (0.56-1.49) | 0.73 | 0.75 (0.44-1.26) | 0.28    |
| **RPS19 (rs2305809)** |      |         |                  |         |                      |         |
| Additive |          |          |                  |         |                      |         |
| CC | 29 (27.4) | 101 (30.7) | 1.00             | 1.00    |                      |         |
| CT | 45 (42.5) | 162 (49.2) | 0.96 (0.57-1.64) | 0.90 | 0.64 (0.38-1.35) | 0.49    |
| TT | 32 (30.2) | 66 (20.1)  | 1.68 (0.93-3.04) | 0.08 | 1.82 (0.91-3.61) | 0.09    |
| Recessive |          |          |                  |         |                      |         |
| CC + CT | 74 (69.8) | 263 (79.9) | 0.58 (0.35-0.95) | 0.03 | 0.49 (0.27-0.90) | 0.02*   |
| Dominant |          |          |                  |         |                      |         |
| CC + TT | 77 (72.6) | 228 (69.3) | 1.17 (0.72-1.91) | 0.51 | 1.07 (0.61-1.80) | 0.80    |

Value are presented as number (%). a Genotyping results were obtained for the single nucleotide polymorphisms rs1048522 (n = 96 cases and n = 309 controls), rs1800566 (n = 86 and n = 299 controls), and rs2305809 (n = 106 cases and n = 329 controls). b Adjusted OR for parity, contraceptive oral use, age at first intercourse ≤ 18, number of lifetime sexual partners ≥ 2 and smoking in logistic regression analysis. *P-values < 0.05 were considered statistically significant. c Recessive genetic model (Arg/Arg + Arg/Pro vs. Pro/Pro) for rs1042522. Pro/Pro + Pro/Ser vs. Ser/Ser for rs1800566, and CC + CT vs. TT for rs2305809. d Dominant genetic model (Arg/Pro + Pro/Pro vs. Arg/Arg for rs1042522, Pro/Ser + Ser/Ser vs. Pro/Pro for rs1800566, and CT + TT vs. CC for rs2305809).

that the recessive genetic model (C/C + C/T) causes a protective effect to CC in the women population of this study. These conflicting data seem to be similar to those of SNP rs1042522 in TP53. More studies and meta-analysis reviews are necessary to define the real role of this SNP. Experimental evidence has shown that the ribosomal S19 protein (encoded by the RPS19 gene) has immunosuppressive properties, being upregulated in human ovarian and breast cancer cells and released from apoptotic cancer cells. In this way, this protein interacts with the complementary C5a receptor 1 expressed on tumor-infiltrating myeloid-derived suppressor cells. This interaction contributes to neoplastic growth since it facilitates the attraction of these cells to the tumor. Reducing RPS19 in cancer cells or blocking the C5a receptor 1-RPS19 interaction decreased RPS19-mediated immunosuppression, delayed the development of tumors, and impaired tumor growth in a transgenic model of breast cancer. Therefore, this set of factors can be influenced by the SNP rs2305809, which hypothetically act by regulating the gene expression of RPS19 protein or even the splicing process, contributing in the complex and cumulative frame to CC.

Other polymorphisms are located in non-coding and coding regions of the RPS19 gene and could be involved with the regulation processes in the expression of this gene. All of them should be evaluated for a better understanding of the relationship between RPS19 and CC. For example, the SNP investigated here (rs2305809) has a high linkage disequilibrium to the SNPs (rs4803512, rs6509002, rs5885798, rs7254214, rs7259596, and rs2075752) previously associated with Diamond-Blackfan anemia.

In conclusion, it was detected a protective association of the SNP rs2305809 in the recessive genetic model (C/C + C/T) with CC in women from southern Brazil. Prospective cohort studies...
will be necessary to ascertain this association observed in the present case-control study as well as to define the relative risk of this SNP for CC. In addition, studies in populations with different genetic backgrounds will be needed to confirm our findings since genetic influences of CC are complex.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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