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LETTER TO THE EDITOR

Polymorphisms in Genes Related to Cervical Cancer in A Brazilian Population: A Case-Control Study

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To the editor:

Cervical cancer (CC) is the fourth most common cancer in women, with approximately 528,000 new cases in the world each year, 80% of them in developing countries [1]. It is well recognized that persistent infection of human papillomavirus (HPV) is the main cause of precursor lesions that progress to CC, but only a small proportion of these HPV infected women develop the disease. In this sense, polymorphisms in human genes have also been associated with CC [2]. Genome-wide studies investigated the association of human single nucleotide polymorphisms (SNPs) with HPV persistent infection, progression to cervical intraepithelial neoplasia (CIN) and CC in Latin American women [3, 4]. More than seven thousand SNPs were investigated in genes related to immune response, DNA repair, viral replication and entry into the host cell. Association to persistent HPV progression to CIN and CC was observed with SNPs in genes of DNA repair (*EXO1*, *CYBA*, *FANCA*, *XRCC1*, *GTF2H4*, *DUT*, *FLJ35220*, and *DMC1*), immune response (IRF) and virus entry into the cell (SULF1 and OAS3) [3, 4].

The present case-control study evaluated the frequency of nine SNPs (all of them previously demonstrated to have significant association with HPV persistence and/or cancer) and the association with CC in a population in South Brazil. The selected SNPs were located in genes of DNA repair (rs4149963 in *EXO1*, rs3784621 in *DUT*, rs4603608 in *FLJ35220* and rs2239359 in *FANCA*), immune response (rs7251 in the *IRF*), and virus entry into the host cell (rs4737999, rs10108002, rs4284050 in *SULF1*, and rs12302655 in *OAS3*).

The population sample of this study was 109 CC patients (mean age 50.3 ± 14.3 years; range 25–88 years), recruited during treatment at the Center of High Complexity in Oncology (*Centro de Assistência de Alta Complexidade em Oncologia - CACON*), located in the city of Ijuí in the Brazil's southernmost state (Rio Grande do Sul), from 2012 to 2016; and 220 controls (mean 49.5 ± 13.2 years; range 21–82 years) recruited at the Women's Health Center (*Centro de Saúde da Mulher*), a primary public health care clinic located in the city of Cruz Alta (also in Rio Grande do Sul State, Brazil) from 2012 to 2013. This last women group was previously characterized in cross-sectional epidemiological study [5]. Biological samples were obtained from the mouth in the CC patients (cases) and from the endocervix in the healthy controls. Buccal and endocervical cells were obtained by exfoliation using cytobrush and after stored in a buffer solution (EDTA pH = 8.0 0.01 M, SDS 0.03 M) at −20 °C until analysis.

Total DNA was extracted from peripheral blood cells by silica adsorption method. *EXO1* (rs4149963), *DUT* (rs3784621), *FLJ35220* (rs4603608), *FANCA* (rs2239359), *IRF3* (rs7251), *SULF1* (rs4737999, rs10108002 and rs4284050) and *OAS3* (rs12302655) SNPs were genotyped using TaqMan® specific SNP genotyping assays (Life Technologies Co, Carlsbab, CA, USA). Allelic discrimination real-time polymerase chain reactions (PCR) were performed on the StepOnePlus™ system according to conditions informed by this manufacturer. Thermal cycling conditions were: 10 min at 95 °C followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Allelic discrimination was performed by measuring end-point fluorescence using

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Table 1 Alleles and genotypes of polymorphisms in genes *EXO1* (rs4149963), *DUT* (rs3784621), *FLJ35220* (rs4603608) and *FANCA* (rs2239359) in patients with cervical cancer (cases) and healthy women (controls)

Variables	Cases ($n = 109$)		Controls ($n = 220$)		OR (CI 95%)	*p*-value
	n	%	n	%		
EXO1 rs4149963						
Alleles ($n = 650$)						
C	176	83.0	378	86.3	1.00 (Ref).	
T	36	17.0	60	13.7	1.29 (0.82–2.02)	0.270
Genotypes ($n = 325$)						
CC	72	67.9	162	74.0	1.00 (Ref.)	
CT	32	30.2	54	24.7	1.33 (0.79–2.24)	0.276
TT	2	1.9	3	1.4	1.50 (0.25–9.17)	0.661
DUT rs3784621						
Alleles ($n = 658$)						
T	151	69.3	299	68	1.00 (Ref.)	
C	67	30.7	141	32	0.94 (0.66–1.34)	0.733
Genotypes ($n = 329$)						
TT	53	48.6	107	48.6	1.00 (Ref.)	
CT	45	41.3	85	38.6	1.07 (0.66–1.74)	0.790
CC	11	10.1	28	12.7	0.79 (0.37–1.72)	0.556
FLJ35220 rs4603608						
Alleles (n = 658)						
C	113	51.8	204	46.4	1.00 (Ref.)	
T	105	48.2	236	53.6	0.80 (0.58–1.11)	0.186
Genotypes (n = 329)						
CC	31	28.4	50	22.7	1.00 (Ref.)	
CT	51	46.8	104	47.3	0.79 (0.45–1.38)	0.411
TT	27	24.8	66	30	0.66 (0.35–1.24)	0.198
FANCA rs2239359						
Alleles ($n = 656$)						
T	108	49.5	210	47.9	1.00 (Ref.)	
C	110	50.5	228	52.1	0.94 (0.68–1.30)	0.700
Genotypes ($n = 328$)						
TT	25	22.9	48	21.9	1.00 (Ref.)	
CT	58	53.2	114	52.1	0.98 (0.55–1.74)	0.937
CC	26	23.9	57	26	0.88 (0.45–1.71)	0.698

Ref. Reference category

StepOne™ Software (Version 2.3, Life Technologies Co, Carlsbab, CA, USA) and TaqMan® Genotyper Software (Version 1.3, Life Technologies Co, Carlsbab, CA, USA).

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 18.0, Chicago, IL). The Student's t-test for independent samples was used to verify possible

Table 2 Alleles and genotypes of polymorphisms in genes *IRF3* (rs7251), *SULF1* (rs4737999, rs10108002 and rs4284050) and *OAS3* (rs12302655) in patients with cervical cancer (cases) and healthy women (controls)

Variables	Cases ($n = 109$)		Controls ($n = 220$)		OR	*p*-value
	N	%	n	%	(95% CI)	
IRF3 rs7251						
Allele ($n = 640$)						
G	77	37.7	170	39.0	1.00 (Ref.)	
C	127	62.3	266	61.0	1.05 (0.75–1.48)	0.763
Genotype ($n = 320$)						
GG	13	12.7	38	17.4	1.00 (Ref.)	
CG	51	50.0	94	43.1	1.59 (0.78–3.25)	0.207
CC	38	37.3	86	39.4	1.29 (0.62–2.70)	0.496
SULF1 rs4737999						
Allele (n = 658)						
G	152	69.7	313	71.1	1.00 (Ref.)	
A	66	30.3	127	28.9	1.07 (0.75–1.53)	0.708
Genotype (n = 329)						
GG	58	53.2	112	50.9	1.00 (Ref.)	
AG	36	33.0	89	40.5	0.78 (0.47–1.29)	0.333
AA	15	13.8	19	8.6	1.52 (0.72–3.22)	0.269
SULF1 rs10108002						
Allele ($n = 648$)						
C	138	66.3	316	71.8	1.00 (Ref.)	
T	70	33.7	124	28.2	1.29 (0.91–1.84)	0.156
Genotype ($n = 324$)						
CC	47	45.2	115	52.3	1.00 (Ref.)	
CT	44	42.3	86	39.1	1.25 (0.76–2.06)	0.376
TT	13	12.5	19	8.6	1.67 (0.77–3.66)	0.197
SULF1 rs4284050						
Allele (n = 658)						
C	133	61.0	267	60.7	1.00 (Ref.)	
A	85	39.0	173	39.3	0.99 (0.71–1.38)	0.936
Genotype (n = 329)						
CC	43	39.4	72	32.7	1.00 (Ref.)	
AC	47	43.1	123	55.9	0.64 (0.39–1.06)	0.083
AA	19	17.4	25	11.4	1.27 (0.63–2.58)	0.504
OAS3 rs12302655						
Allele (n = 658)						
G	214	98.2	425	96.6	1.00 (Ref.)	
A	4	1.8	15	3.4	0.53 (0.17–1.62)	0.264
Genotype (n = 329)						
GG	105	96.3	207	94.1	1.00 (Ref.)	
AG	4	3.7	11	5.0	0.72 (0.22–2.31)	0.577
AA	0	0.0	2	0.9	0.39 (0.02–8.27)	0.548

Ref. Reference category

statistical differences between quantitative variables. Allele and genotypes frequencies were determined by direct counting and Hardy-Weinberg equilibrium was evaluated by chi-square test. Associations between qualitative variables and CC were evaluated by bivariate analysis (Pearson's chi-square test). Odds ratios (OR) with 95% confidence intervals (CI) were estimated in order to detect the association of the SNPs with CC. All p values calculated were two-tailed and those with values <0.05 were considered significant. The whole study was approved by University of Cruz Alta Research Ethics Committee.

The allele frequencies of the SNPs in DNA repair genes (rs4149963 in *EXO1*, rs3784621 in *DUT*, rs4603608 in *FLJ35220*, rs2239359 in *FANCA*), as well as the respective genotype frequencies, are shown in Table 1. The allele and respective genotype frequencies of the remaining SNPs (rs7251 in the *IRF*, rs4737999, rs10108002, rs4284050 in *SULF1*, and rs12302655 in *OAS3*) are shown in Table 2. All the genotypes frequencies observed in the sample for these four SNPs are in Hardy-Weinberg equilibrium with the exception of the SNP rs12302655 ($p < 0.05$). There were no significant differences in the comparison of the alleles and genotypes frequencies between cases and control groups (Tables 1 and 2).

Polymorphisms in genes from proteins of tumor-related cell pathways have already been studied for their contribution to CC development, but the whole picture remains complex with inconsistent data [3, 4]. Previous genome-wide study detected four main SNPs (rs4149963 in *EXO1*, rs3784621 in *DUT*, rs4603608 in *FLJ35220* and rs2239359 in *FANCA*) associated with CC in Latin American women [3]. However, we could not detect any association in a South Brazil sample. America countries like Costa Rica and Brazil are ethnically admixed, however Costa Rican population is predominantly originated of people with Iberian and indigenous ancestry. On oppose, southern Brazil population is strongly composed by Italian, German and other European ancestries due to waves of immigration from the 17th to the 20th centuries. Therefore, this could be an explanation for the difference in the allelic profile of the polymorphisms studied and the lack of the association between the studied SNPs and CC in the southern Brazil population.

In conclusion, there were no associations between the SNPs in *EXO1*, *DUT*, *FLJ35220 FANCA*, *IRF3, SULF1,* and *OAS3* genes with CC in a sample of women from southern Brazil. To the best of our knowledge, there are few reports in the literature that aimed to detect association of SNPs and CC. Therefore, the present study contributes to a better understanding of this issue in the Brazilian population and will fill some gaps that exist with respect to the influence of the human genetic in CC.

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Author Contributions Boeira, Coser, Simon, and Lunge designed the study. Boeira and Wolf managed lab work and the data analyses. Boeira, Coser, Wolf, Simon, Lunge contributed to literature review and discussion.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there is no conflict of interest.

References

1. IARC, International Agency for Research on Cancer. Pharmaceuticals: a review of human carcinogens. Lyon, 2012. (IARC monographs on the evaluation of carcinogenic risks to humans, v. 100E)
2. Magnusson PK, Sparén P, Gyllensten UB (1999). Genetic link to cervical tumours. Nature. 1;400(6739):29–30
3. Wang SS, Bratti MC, Rodríguez AC, Herrero R, Burk RD, Porras C, González P, Sherman ME, Wacholder S, Lan ZE, Schiffman M, Chanock SJ, Hildesheim A (2009) Common variants in immune and DNA repair genes and risk for human papillomavirus persistence and progression to cervical cancer. J Infect Dis 199(1):20–30
4. Wang SS, Gonzalez P, Yu K, Porras C, Li Q, Safaeian M, Rodriguez AC, Sherman ME, Bratti C, Schiffman M, Wacholder S, Burk RD, Herrero R, Chanock SJ, Hildesheim A (2010) Common genetic variants and risk for HPV persistence and progression to cervical cancer. PLoS One 5(1):e8667
5. Coser J, Boeira Tda R, Wolf JM et al (2016) Cervical human papillomavirus infection and persistence: a clinic-based study in the countryside from South Brazil. Braz J Infect Dis 20(1):61–68