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RESEARCH ARTICLE

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HPV E6/E7mRNA association with interleukin 10 (rs1800872) polymorphism in a group of Macedonian women

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Abstract

Interleukin 10 (IL-10) is an immunosuppressive cytokine and its genetic variants could have an indirect impact on viral biology and human papillomavirus (HPV) E6/E7 messenger RNA (mRNA) expression as well. This study evaluates the association between IL-10-592 C/A (rs1800872) single-nucleotide polymorphism and HPV E6/E7 mRNA expression in a group of women from the Republic of North Macedonia. Using a commercial test, 272 women's cervical samples were analyzed for HPV E6/E7 mRNA and HPV DNA presence. The cases were stratified into three groups: double-positive ($n = 108$, positive for both tests), negative ($n = 51$, negative for HPV E6/E7 mRNA and HPV DNA positive), and the control group ($n = 113$, negative for both tests). The IL-10-592 C/A polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism. The results showed the CC genotype and the C allele frequencies of IL-10-592C/A were significantly higher in double-positive (59.3% and 78.2%) compared to negative group (39.2% and 65.7%), ($p = 0.018$, confidence interval [CI] = 2.25; 1.14–4.45 and $p = 0.016$, CI = 1.88; 1.11–3.16, respectively). The CC genotype and C allele of rs1800872 polymorphism were shown to be associated with HPV E6/E7 mRNA but not with HPV DNA positivity, which implies a possible role of this polymorphism in the course of the infection only after HPV onset, and lack of association with the susceptibility to HPV.

KEYWORDS
cervical cancer, cervical intraepithelial lesions, E6/E7 mRNA, IL-10 polymorphism

1 | INTRODUCTION

Cervical carcinogenesis is strongly associated with persistent infection with high-risk (HR) human papillomavirus (HPV),[1,2] usually accompanied by HPV E6 and E7 messenger RNA (mRNA) expression.[3] E6 and E7 viral proteins are very important in cervical carcinogenesis due to their interference in steps of the cellular control mechanisms. Hence, HPV E6/E7 mRNA testing has been considered as a more specific test for HPV persistence and a useful biomarker for predicting the condition that could undergo cell transformation.[4,5] HPV E6 and E7 oncogene active transcription can be monitored directly with the detection of HPV E6/E7 viral mRNA transcripts or proteins.[6–8]

Since the HPV infection is the initial prerequisite for cervical lesions and most infections resolve spontaneously, its persistence depends on many additional factors. The host's immunogenetics, particularly cytokines' variants could underlie HPV persistence as well as HPV E6/E7 mRNA expression. Interleukin 10 (IL-10) acts as an immunosuppressive cytokine and influences viral clearance or viral persistence.[9–11] It is a Th2 cytokine that acts as an immune response

modulator, but the modulation has been described to be paradoxical in cervical cancer. Namely, in presence of HPV oncoproteins E6 and E7, IL-10 has an anti-inflammatory function but later it promotes tumor growth during the high E6 oncoprotein activity.

Previous studies have described different IL-10 production to be dependent on its genetic variant.[12] Hence, it could be expected that some variants promote more its anti-inflammatory effect and influence HPV persistence, or cell clearance from this infection.[13] In the previous study of Duvlis et al.,[14] it was found that IL-10-592 (rs180072) CC genotype and C allele variant located in the promoter of the IL-10 gene, are associated with cervical intraepithelial neoplasia (CIN) lesions and cervical cancer (CCa), but the exact mechanism of the impact of this variant on CIN and CCa development and HPV persistence is still unknown. This study was conducted to investigate a possible correlation between IL-10-592 C/A polymorphism and HPV E6/E7 mRNA positivity in a group of Macedonian women and whether the finding could be an additional predictive marker for women susceptible to CCa development.

2 | METHODS

2.1 | Sample description

The study group consisted of 272 women aged 17–67 years, who underwent a routine cervical cancer screening program. Endocervical swabs collected by a gynecologist from the University Clinic for Gynecology and Obstetrics, Skopje were provided to the Laboratory for Virology and molecular diagnostics at the Institute of Public Health of the Republic of North Macedonia, Skopje. All participants had signed informed consent and the study protocol was approved by the Ethical Committee of the Institute of public health of the Republic of North Macedonia and Medical Faculty, University "Ss. Cyril and Methodius," Skopje (no. 03-5515/4). All the samples were submitted to both testing: HPV E6/E7 mRNA expression and detection of HPV DNA as well as genotyping for the IL-10 c.-592 C/A variant.

The study group was divided into three subgroups according to the results from the HPV testing: a positive group with positive status for both tests: HPV E6/E7 mRNA expression and HPV DNA (n = 108); a negative group of patients with a negative status on the HPV E6/E7 mRNA expression but the positive status of HPV DNA (n = 51); and a control group consisted of samples with negative status for both tests (n = 113).

2.2 | Nucleic acid isolation

Total nucleic acid was extracted from the pellet after centrifuging the cervical specimen preserved in PreservCyt/ThinPrep solution (Cytyc Corporation) or in viral transport medium (phosphate-buffered saline) according to the NucliSens protocol using the miniMAG platform (bioMerieux). The nucleic acids were eluted in a 55 µl elution buffer and were further processed for HPV DNA testing as well as mRNA detection.

2.3 | HPV DNA detection and genotyping

Seeplex® HPV4A ACE screening, assay Seegene, is based on dual priming oligonucleotide technology that enables high specific priming, stable annealing, and blocking of nonspecific annealing of primers/probes to the target during the polymerase chain reaction (PCR) reaction. It was used as a commercial test for the first line HPV DNA screening. The test enables differentiation between low-risk HPV types (6 and 11) and the 16 most frequent high risk (HR) HPV types (26, 31, 33, 35, 39*, 45, 51, 52*, 53, 56, 58, 59, 66, 68*, 73, and 82) but does not allow identification of the specific type with the exception of genotyping for the two HRHPV types: types 16 (500 bp band) and 18 (360 bp) and the two low-risk types: HPV 11 and 6 (260 bp). The others are referred to as HR types (450 bp).

2.4 | HPV mRNA detection

HPV mRNA was detected with the PreTect HPV Proofer test according to the manufacturer's instructions. Briefly, in three prepared premixes of U1A/HPV16, HPV18/31, and HPV33/45 with primer/molecular beacon mixes in supplied strip tubes, the reaction was initialized by the addition of enzymes and measured in real-time in the isothermal condition of the NucliSENS EasyQ analyzer at 41°C. Data analysis was performed using the PreTect Analysis Software.

The PreTect HPV Proofer test includes primer pairs targeting U1A mRNA as intrinsic control to determine the sample validity. Human U1 small ribonucleoprotein (U1A mRNA) was used as an RNA integrity/adequacy internal control. When the U1A amplification was not detected, the test result was denoted invalid. To evaluate the run validity, positive controls for U1A/HPV16, HPV18/HPV31, and HPV33/45 were included.

2.5 | IL-10-592 C/A genotyping

The IL-10-592 (rs1800872) polymorphism was analyzed using PCR-restriction fragment length polymorphism, with primer set for the promoter region of this gene designed using primer design software – Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and used in previous study[14] (sense: GGGGTCATGGT GAGCACTAC and antisense: CAAGCAGCCCTTCCATTTTA).

The PCR product encompassing 230 bp was digested with the 1U of RsaI restriction enzyme (Invitrogen, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instruction: 2 h on 37°C. The different genotypes were confirmed with sample sequencing on ABI PRISM 310 Genetic Analyzer (Thermo Fisher Scientific) (Supporting Information).

2.6 | Statistical analysis

The genotype distribution and allelic frequencies were analyzed using Pearson's χ^2 test and Fisher's exact test, or its extension, considering $p \leq 0.05$ as a significance threshold. The odds ratio (OR) and the confidence interval (CI) of 95% by the Speckle image statistical analysis statistics method (https://www.quantitativeskills.com/sisa/statistics/twoby2.htm) were calculated to estimate the probability of association of the studied polymorphisms with positive HPV E6/E7 mRNA expression.

The ORs were performed for rs1800872 polymorphism under the allelic model (C vs. A), homozygous model (CC vs. AA), recessive genetic models (AA vs. CC + AC), heterozygous model (CC vs. AA), and dominant inheritance model (AA + AC vs. CC), respectively.

3 | RESULTS

Genotype distribution analysis showed that the CC genotype was significantly ($p = 0.018$) more common (OR = 2.25) in the positive group (59.3%) compared to the negative group (39.2%), and compared to negative and control together ($p = 0.04$), but not compared to the control group (49.6%) alone (Table 1). The OR of the CC genotype versus the CA and AA genotype in the positive group to be associated with a positive HPV E6/E7 mRNA expression test was 2.25 (95% CI: 1.14–4.45) compared to the negative group and OR: 1.68 (95% CI: 1.03–2.75) compared to the rest groups together (Table 2).

The frequency of the C allele was 78.2% in the positive group, 65.7% in the negative group, and 72.6% in the control group. The C allele was significantly more frequent in the positive group ($p = 0.016$) compared to the negative group and with borderline significance compared to the other groups ($p = 0.04$). Regarding the allele distribution, OR of the C allele to be associated with the HPV E6/E7 mRNA expression test was 1.88 (95% CI: 1.11–3.16) when the positive group was compared to the negative group; and 1.5 (95% CI: 1.01–2.25) compared with both (negative and control group together), respectively. This OR was insignificant when compared to the control group alone. The gene and the allele frequencies of the IL-10-592 C/A variants are shown in Figure 1.

4 | DISCUSSION

The genetic background of the host strongly influences human response to infections and recently has been extensively explored to predict the infection outcome.[13] A common polymorphism -592 C/A in the IL-10 promoter region (rs1800872), was shown to be associated with susceptibility to various malignancies[15] and significantly associated with different infections outcomes. Some studies found an association of the CC genotype with a decreased risk of *Mycobacterium tuberculosis* manifestation [16] as well as with the worst outcome of enterovirus 71

TABLE 1 Genotype and allele distribution of rs1800872 variants in double-positive, negative, and control groups

	Genotype distribution (%)				Allele frequency (%)		
Groups	CC, *n* (%)	AC, *n* (%)	AA, *n* (%)	Total, *n* (%)	C, *n* (%)	A, *n* (%)	Total, *n* (%)
Positive[a]	64 (59.3)	41 (38.0)	3 (2.7)	108	169 (78.2)	47 (21.8)	216
Negative[b]	20 (39.2)	27 (53.0)	4 (7.8)	51	67 (65.7)	35(34.3)	102
Control	56 (49.6)	52 (46.0)	5 (4.4)	113	164 (72.6)	62 (27.4)	226
Total	140 (46.9)	120 (48.5)	12 (4.6)	272	400 (71.2)	144 (28.8)	544

Abbreviations: HPV, human papillomavirus; mRNA, messenger RNA.
[a]Positive: Dual positive (HPV E6/E7 mRNA and HPV DNA positive).
[b]Negative: Only HPV DNA positive.

TABLE 2 Statistical significance of rs1800872 genotypes frequency compared among the three stratified groups

rs (1800872)	P1	OR	(95%CI)	P2	OR	(95% CI)	P3	OR	(95% CI)
C/A	0.016	1.88	1.11–3.16	0.04	1.5	1.01–2.25	0.166	1.36	0.88–2.1
CC/AC	0.034	2.1	1.05–4.24	0.058	1.62	0.98–2.68	0.18	1.45	0.84–2.49
CC/AA	0.05	4.27	0.88–20.69	0.016	2.53	0.66–9.73	0.38	1.90	0.44–8.33
CC/AA + AC	0.018	2.25	1.14–4.45	0.037	1.68	1.03–2.75	0.148	1.48	0.87–2.52
AC + CC/AA	0.15	2.98	0.64–13.84	0.289	2.03	0.54–7.68	0.51	1.62	0.38–6.95

Note: P1 = HPV dual positive (positive group) versus HPV DNA positive (negative group); P2 = HPV dual positive (positive group) versus HPV DNA positive (negative group) + control group; and P3 = HPV dual positive (positive group) versus control group.
Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

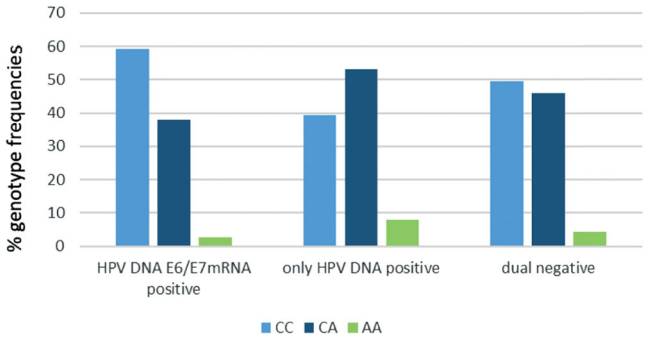

FIGURE 1 Genotype frequencies of rs1800872 in the three groups. HPV, human papillomavirus; mRNA, messenger RNA.

infections.[17] Also, many studies explain the impact of IL-10 on HPV infection.[12,18] Still, the exact mechanism of how local or serum levels of IL-10 influence HPV infection is not clear, but its impact on estimating HPV persistence through its association with increased production of viral E6/E7 is well documented.[19] In a previous study that described the interaction between IL-10 and HPV, IL-10 induces some expression of viral E6 and E7, and vice versa, HPV E2, E6, and E7 proteins induce IL-10 expression, resulting in a vicious cycle.[18] This event further leads to infection progression to CIN lesions or CCa.[20,21] In addition to this evidence, is finding that the protein levels of HPV16 E7 increased after treatment with IL-10 in all HPV16-positive cells[22]; hence, it could be concluded that there is a close association between the host's IL-10 and the viral oncogene expressions. However, it is still unknown whether the genetic variant could additionally influence this association.

This study investigates the association between a variant of IL-10-592C/A with HPV E6/E7 mRNA presence. Unfortunately, it was done without quantification of the IL-10 local or serum levels, which are about to be investigated in further studies. Up to now, there are no published results on whether the IL-10 gene variants additionally affect the HPV E6/E7 mRNA expression, so in this study was assumed that the IL-10-592 C/A polymorphism could be an additional factor associated with this expression.

The results from this study showed that the CC genotype and C allele are significantly more frequent in HPV double-positive cases compared to HPV E6/E7 mRNA negative group only, indicating their positive association with HPV E6/E7 mRNA expression but not with HPV DNA positivity alone. The lower frequency of CC genotype and C allele in the negative group of cases (HPV DNA positive cases without E6/E7 mRNA expression) suggests that the variant does not have an impact on the onset of the infection or this finding might be attributed to the transient nature of HPV DNA infection. In this scenario, the variant does not influence this stage that is usually accompanied without HPV E6/E7 mRNA expression and the HPV DNA is usually in an epigenetic state in this stage. Another explanation could be that the CC genotype might have a higher stimulating effect on HPV E6/E7 mRNA expression related to reports from a study by Men et al.,[23] where these genotypes are associated with a higher level of IL-10 in peripheral blood associated with higher immunosuppression.

The CC genotype of this single-nucleotide polymorphism (SNP) was confirmed to be associated with a risk for many different cancers in a metastudy in the Asian population done by Ding et al.[15] and AA of the same SNP to be associated with a lower risk of cervical cancer in a recent meta-study by Wang et al.[24] The results from this study could be complementary to results obtained in a previous study of the same polymorphism,[14] in Macedonian women where the CC genotype was associated with cervical cancer and CIN lesions, but now, this effect was mirrored through viral E6/E7 oncogene expression.

Surely, there is a limitation in this study regarding the small number of samples investigated and therefore both findings warrant confirmation in wider epidemiological and molecular studies with a higher number of participants.

In conclusion, the C/CC variant of IL-10-592C/A SNP may be a marker of susceptibility to HPV persistence, through possible influence of higher rate of HPV E6/E7 mRNA expression, but only after the onset of infection, as well as a risk factor for developing cervical cancer. This genotype is not associated with susceptibility to onset of HPV infection given the absence of association with HPV DNA alone.

The identification of immunogenic predictors could be useful for the predictive selection of women who may undergo HPV persistence and progression to CIN, in the context of avoiding overload in diagnosis, optimizing treatment services, and improving the cancer prevention strategy in general.

AUTHOR CONTRIBUTIONS

S. Duvlis designed the study and wrote the first draft, performed the experiments, reviewed, editing, and finalized the manuscript. D. Dabeskiselected women for testing and evaluated clinical investigations. S. Memetiand D. Osmani made the statistical analyzes and supported the research. M. Hiljadnikova-Bajro participated in data analysis, reviewing, and editing of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available in the UKIM repository, http://hdl.handle.net/20.500.12188/17427.

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REFERENCES

1. zur Hausen H. Papillomavirus infections—a major cause of human cancers. *Biochim Biophys Acta*. 1996;1288(2):F55-F78. doi:10.1016/0304-419x(96)00020-0

2. Thomison J, 3rd, Thomas LK, Shroyer KR. Human papillomavirus: molecular and cytologic/histologic aspects related to cervical intraepithelial neoplasia and carcinoma. *Hum Pathol*. 2008;39(2):154-166. doi:10.1016/j.humpath.2007.11.002
3. Pal A, Kundu R. Human papillomavirus E6 and E7: the cervical cancer hallmarks and targets for therapy. *Front Microbiol*. 2019;10:3116. doi:10.3389/fmicb.2019.03116
4. Sørbye SW, Fismen S, Gutteberg T, Mortensen ES. Triage of women with minor cervical lesions: data suggesting a "test and treat" approach for HPV E6/E7 mRNA testing. *PLoS One*. 2010;5(9):e12724. doi:10.1371/journal.pone.0012724
5. Sørbye SW, Fismen S, Gutteberg TJ, Mortensen ES, Skjeldestad FE. Primary cervical cancer screening with an HPV mRNA test: a prospective cohort study. *BMJ Open*. 2016;6(8):e011981. doi:10.1136/bmjopen-2016-011981
6. Cuschieri K, Wentzensen N. Human papillomavirus mRNA and p16 detection as biomarkers for the improved diagnosis of cervical neoplasia. *Cancer Epidemiol Biomarkers Prev*. 2008;17(10):2536-2545. doi:10.1158/1055-9965.epi-08-0306
7. Lie AK, Kristensen G. Human papillomavirus E6/E7 mRNA testing as a predictive marker for cervical carcinoma. *Expert Rev Mol Diagn*. 2008;8(4):405-415. doi:10.1586/14737159.8.4.405
8. Schweizer J, Lu PS, Mahoney CW, et al. Feasibility study of a human papillomavirus E6 oncoprotein test for diagnosis of cervical precancer and cancer. *J Clin Microbiol*. 2010;48(12):4646-4648. doi:10.1128/jcm.01315-10
9. Lowe PR, Galley HF, Abdel-Fattah A, Webster NR. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. *Crit Care Med*. 2003;31(1):34-38. doi:10.1097/00003246-200301000-00005
10. Hinds DA, Stuve LL, Nilsen GB, et al. Whole-genome patterns of common DNA variation in three human populations. *Science*. 2005;307(5712):1072-1079. doi:10.1126/science.1105436
11. Langsenlehner U, Krippl P, Renner W, et al. Interleukin-10 promoter polymorphism is associated with decreased breast cancer risk. *Breast Cancer Res Treat*. 2005;90(2):113-115. doi:10.1007/s10549-004-3607-7
12. Berti FCB, Pereira APL, Cebinelli GCM, Trugilo KP, Brajão de Oliveira K. The role of interleukin 10 in human papilloma virus infection and progression to cervical carcinoma. *Cytokine Growth Factor Rev*. 2017;34:1-13. doi:10.1016/j.cytogfr.2017.03.002
13. Kachuri L, Francis SS, Morrison ML, et al. The landscape of host genetic factors involved in immune response to common viral infections. *Genome Med*. 2020;12(1):93. doi:10.1186/s13073-020-00790-x
14. Duvlis S, Dabeski D, Noveski P, Ivkovski L, Plaseska-Karanfilska D. Association of IL-10 (rs1800872) and IL-4R (rs1805010) polymorphisms with cervical intraepithelial lesions and cervical carcinomas. *J BUON*. 2020;25(1):132-140.
15. Ding Q, Shi Y, Fan B, et al. The interleukin-10 promoter polymorphism rs1800872 (-592C>A), contributes to cancer susceptibility: meta-analysis of 16,785 cases and 19,713 controls. *PLoS One*. 2013;8(2):e57246. doi:10.1371/journal.pone.0057246
16. Shin HD, Park BL, Kim YH, Cheong HS, Lee IH, Park SK. Common interleukin 10 polymorphism associated with decreased risk of tuberculosis. *Exp Mol Med*. 2005;37(2):128-132. doi:10.1038/emm.2005.17
17. Zhao N, Chen HL, Chen ZZ, Li J, Chen ZB. IL-10-592 polymorphism is associated with IL-10 expression and severity of enterovirus 71 infection in Chinese children. *J Clin Virol*. 2017;95:42-46. doi:10.1016/j.jcv.2017.08.005
18. Berti FCB, Pereira APL, Trugilo KP, et al. IL-10 gene polymorphism c.-592C>A increases HPV infection susceptibility and influences IL-10 levels in HPV infected women. *Infect Genet Evol*.2017;53:128-134. doi:10.1016/j.meegid.2017.05.020
19. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med*. 2006;12(11):1301-1309. doi:10.1038/nm1492
20. Moscicki AB, Schiffman M, Burchell A, et al. Updating the natural history of human papillomavirus and anogenital cancers. *Vaccine*. 2012;30(suppl 5):24-33.doi:10.1016/j.vaccine.2012.05.089
21. Schiffman M, Solomon D. Clinical practice. Cervical-cancer screening with human papillomavirus and cytologic cotesting. *N Engl J Med*. 2013;369(24):2324-2331. doi:10.1056/NEJMcp1210379
22. Arany I, Grattendick KG, Tyring SK. Interleukin-10 induces transcription of the early promoter of human papillomavirus type 16 (HPV16) through the 5′-segment of the upstream regulatory region (URR). *Antiviral Res*. 2002;55(2):331-339. doi:10.1016/s0166-3542(02)00070-0
23. Men T, Yu C, Wang D, et al. The impact of interleukin-10 (IL-10) gene 4 polymorphisms on peripheral blood IL-10 variation and prostate cancer risk based on published studies. *Oncotarget*. 2013;11(8 28):45994-46005. doi:10.18632/oncotarget.17522
24. Wang K, Jiao Z, Chen H, et al. The association between rs1800872 polymorphism in interleukin-10 and risk of cervical cancer: a meta-analysis. *Medicine*. 2021;100(3):e23892. doi:10.1097/MD.0000000000023892

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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