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HPV E6/E7mRNA association with Interleukin - 10 592C/A variant in group of Macedonian women

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02 - Viral and molecular biology

Background/Objectives:

Interleukin 10 (IL-10) is an immunosuppressive cytokine and its genetic variant could have an indirect impact on viral biology and HPV E6/E7 mRNA expression as well. In the study, we evaluate the association between IL10 -592 C/A polymorphism and HPV E6/E7 mRNA expression in a group of women from R North Macedonia.

Methods:

Using commertial tests we analyzed 272 women's cervical samples for HPV E6/E7 mRNA and HPV DNA presence respectively. 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 IL10-592 C/A polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism.

IL-10-592 Genotyping

- The IL-10-592 polymorphism was analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)., using designed a primer for the promoter region of this gene using primer design software - Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) sense: GGGGTCATGGTGAGCACTAC and antisense: AAAGTTGGGGACACACAAGC (Table 1).
- The PCR product was digested with the RsaI restriction enzyme according to the manufacturer's instruction: 2h on 37°C. The pattern after digestion was analyzed on 2.5 agarose gel.

HPV DNA and HPV E6/E7 mRNA detection

HPV DNA and E6/E7 mRNA detecting and typing were performed using Seeplex® HPV4A ACE screening, assay Seegene, (Korea), and PreTect HPV Proofer (PreTect AS, Norway) tests respectively

Results:

The CC genotype and the C allele frequencies of IL10-592C/A were significantly higher in double-positive (59.3% and 78.2%) compared to negative group (39.2% and 65.7%), (p=0.01, CI=0.44;0.22-0.87- dominant model; and p=0.01, CI=0.53; 0.3-0.8) respectively and compared to negative and control groups together

Conclusions:

The CC genotype and C allele of IL10-592 showed to be associated with HPV E6/E7 mRNA but not with HPV DNA positivity, which could mean this polymorphism could affect the course of the infection only after HPV onset and it is not associated with susceptibility to HPV.

Table 1.

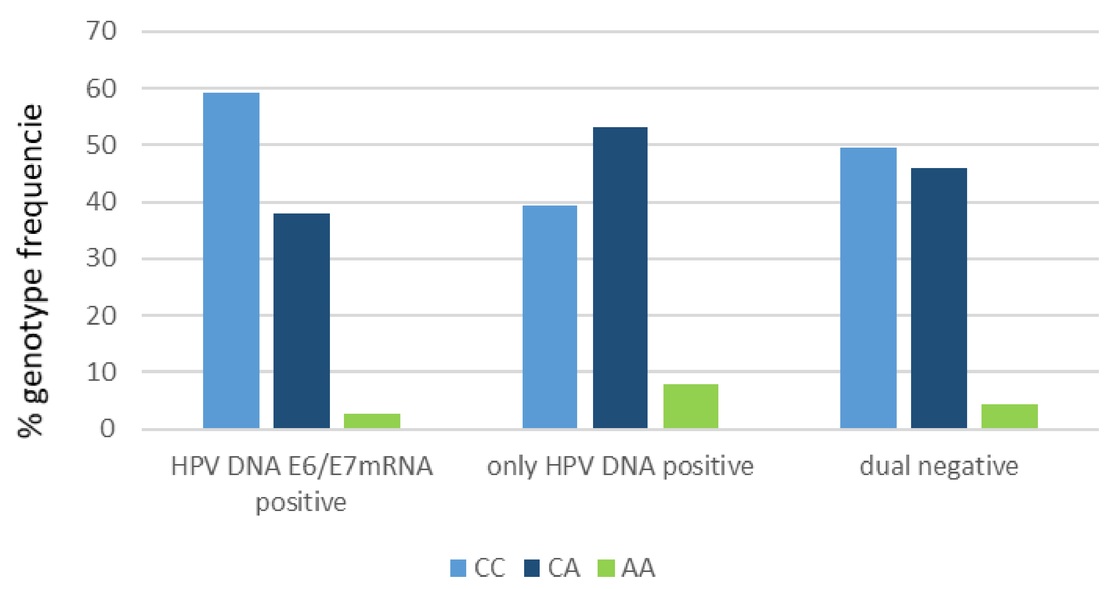
Groups	Genotype distribution (%)				Allele frequency (%)		
	CC n (%)	AC n (%)	AA n (%)	Total n (%)	C n (%)	A n (%)	Total n (%)
*positive	64 (59.3)	41 (38.0)	3 (2.7)	108	169 (78.2)	47 (21.8)	216
*negative	20 (39.2)	27 (53.0)	4 (7.8)	51	67 (65.7)	35(34.3)	102
control	56 (49.6)	52 (46.0)	5 (4.4)	113	164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

Table 2. Statistically significance of variant frequencies of genotypes compared in the three stratified groups.

rs	P1	OR	(95%CI)	P2	OR	(95%CI)	P3	OR	(95%CI)
C A	0.01	1.18	1.11-3.16	0.04	1.5	1.01-2.25	0.16	1.35	(0.87-2.1)
CC AC	ref 0.034	2.1	(1.04-4.23)	0.05	1.62	0.982.68	0.18	1.44	(0.84-2.49)
AA	0.05	4.26	(0.88-20.69	0.16			0.38	1.90	(0.43- 8.33)
AA+AC/CC	0.018	2.25	(1.14-4.45)	0.03	1.68	1.03-2.75	0.14	1.48	(0.86-2.52)
AA/AC+CC	0.14			0.28			0.51		

The analysis of genotype
P1= HPV dual positive (positive group) vs HPV DNA positive (negative group)
P2= HPV dual positive (positive group) vs HPV DNA positive (negative group) +control group
P3= HPV dual positive (positive group) vs control group

Interleukin 10 - 592 CC variant compared with CA and AA variant association with on HPV E6/E7 mRNA expression



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