POLYMORPHISMS OF TLR 4 AND 9 AS BIOMARKERS FOR CERVICAL DYSPLASIA AND HPV INFECTION IN A GROUP OF PUERTO RICAN WOMEN

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ABSTRACT

Introduction:
Cervical cancer, a leading cause of mortality globally, is caused by Human Papillomavirus (HPV) in over *90% of cases.* Puerto Rican women have higher incidence than other populations. Epigenetics and immune related host factors may explain this health disparity. Toll like receptors (TLRs) are involved in the immune response of cancer and inflammation. Single nucleotide polymorphisms(SNPs) of TLR4 and TLR9 have been related to HPV infection and cervical cancer. We aimed to correlate the presence of 8 SNPs with cervical dysplasia and HPV infection. Our hypothesis is that increased susceptibility to HPV infection and cervical dysplasia is due to these polymorphisms.

Methods:
We obtained 210 cervicovaginal samples from *protocol #10510114 for our study.* Information about cervical dysplasia, HPV genotype and sociodemographic data was available. We measured expression of 8 SNPs using PCR, then determined allele's frequency and analyzed its correlation with degree of cervical dysplasia and HPV positivity.
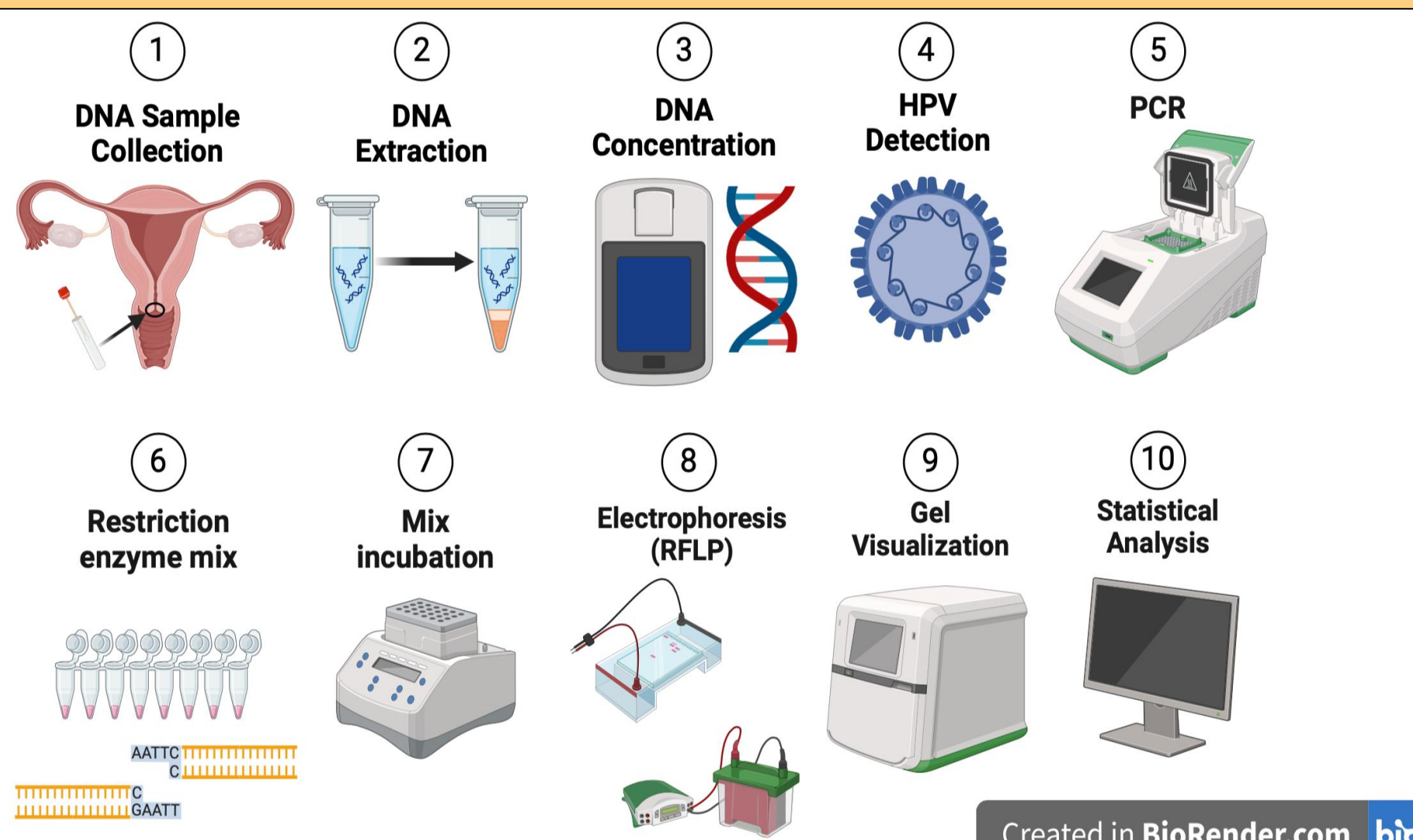
Results:
Women who expressed a NCOI of AA had 3.11 times higher odds of having dysplasia compared to women who expressed a NCOI of AG(p=0.03). Women exhibiting a CG EarI profile had 2.21 higher odds of being HPV-positive compared to women with a GG EarI. (p=0.03).

Conclusions:
We found 2 SNPs of TLR 4 to be significantly associated with severe dysplasia (NCOI) and HR-HPV infection(EARL). 35.71% of severe dysplasia patients had LR-HPV infection, raising concern for different epidemiology in PR. Future studies with other SNP's and better representation of dysplasia and cancer cases will be done to explore its use as potential biomarkers.

INTRODUCTION

- Cervical cancer remains a leading cause of mortality among women aged 20-50 years, ranking as the fourth most common cancer in women globally despite being amenable to primary and secondary prevention strategies.
- Cervical Cancer incidence in Puerto Rico was the highest among all states of the US (12.1 per 100,000) from 2016-2020 and was the seventh most common cancer in women in Puerto Rico from 2014-2018.
- Toll-like receptors (TLRs) are part of the innate immune system as pathogen recognition receptors but are also linked to cancer biology and inflammation. TLRs are being studied for their role in HPV entry into squamous cells, especially certain SNPs of genes 4 and 9.
- The objectives of this study are to characterize TLR 4 and TLR 9 SNP haplotypes in cervicovaginal lavage samples in a group of Puerto Rican Women attending a general gynecology clinic and correlate with dysplasia grade.

Age-Adjusted Incidence Rates by Health Region, in Puerto Rico. Cervix Uteri , 2014-2018

Age-Adjusted Incidence Rates by Health Region, in Puerto Rico. Cervix Uteri , 2014-2018

RESULTS

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TLR4 and TLR9 genotype analyses. PCR, PCR-RFLP or Allele-Specific PCR (AS-PCR) was used to analyze SNPs for *TLR4* (rs4986790, rs10759931, rs11536889, rs1927911) and *TLR9* (rs187084, rs5743836, rs352140, rs352139). Then a digestion with the restriction enzymes was done in which we used: NcoI, KpnI, EarI, StyI, AflII, BstNI and BstUI respectively, for rs352139 an AS-PCR.

DNA Extraction from vaginal lavage and HPV genotyping.,
n= 210 cervicovaginal samples with high-resolution kit to detect 24 HR and LR HPV genotypes: 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74.

Age, BMI, pregnancy sociodemographic and clinical characteristics of the cases

Table 1a: Sociodemographic Characteristics	N(%) n=210
Age[1]	
Mean ± SD	39 ± 11
Median (p25 – p75)	37 (29, 48)
BMI Groups[1]	
Under/Normal	64 (30.48)
Overweight	67 (31.90)
Obese	79 (37.62)
Pregnant[1]	
Yes	43 (20.48)
No	167 (79.52)
Drinker[1]	
Yes	70 (33.33)
No	140 (66.67)
Smoker[1]	
Yes	19 (09.05)
No	191 (90.95)

Table 1b: Clinical Characteristics	N(%) n=210
Dysplasia Diagnosis	
Yes	86 (40.95)
No	124 (59.05)
Dysplasia Severity	
None	124 (59.05)
Mild	44 (20.95)
Severe	42 (20.00)
HPV Positivity[3]	
Positive (known subtypes)	115 (55.56)
Positive (unknown subtypes)	16 (07.73)
Negative	76 (36.71)
HPV Subtype Risk[2]	
No Risk	76 (36.71)
Low Risk	12 (05.80)
High Risk	73 (35.27)
Low & High Risk	30 (14.49)
Unknown Risk	16 (07.73)

Table 1. Demographic characteristics (1a) and Clinical characteristics (1b).

NcoI restriction enzyme ratios from Cervical Dysplasia on the samples.

Dysplasia	Un-adjusted	Adjusted	
		Model 1[2]	Model 2[3]
	OR (95% CI)	OR (95% CI)	OR (95% CI)
NcoI			
AA	3.16 (1.12 - 8.89)*	3.11 (1.09 - 8.86)*	2.73 (0.94 – 7.95)
AG	REFERENCE	REFERENCE	REFERENCE
HPV Risk			
High	1.70 (0.95 - 3.03)	1.68 (0.93 - 3.01)	0.68 (0.20 - 2.32)
None/Low	REFERENCE	REFERENCE	REFERENCE

Table 2. Odds Ratios (95% Confidence Intervals)[1] for Cervical Dysplasia, according to risk factors (n=194).

LEGEND for *table 2*:
[1] Estimates were obtained from logistic regression models for the binary cervical dysplasia outcome (yes/no).
[2] Model 1 adjusted for age and smoking (smoker/nonsmoker).
[3] Model 2 adjusted for age, smoking (smoker/nonsmoker), and HPV positivity (yes/no).
* Statistically significant results p-value ≤ 0.05

EarI restriction enzyme ratios from Cervical Dysplasia on the samples.

Positive HPV	Un-adjusted	Adjusted	
		Model 1[2]	Model 2[3]
	OR (95% CI)	OR (95% CI)	OR (95% CI)
EarI			
CC	3.22 (0.35 - 29.62)	3.35 (0.36 - 30.94)	3.52 (0.38 - 32.62)
CG	2.19 (1.08 - 4.42)*	2.21 (1.09 - 4.50)*	2.19 (1.07 - 4.46)*
GG	REFERENCE	REFERENCE	REFERENCE

Table 3. Odds Ratios (95% Confidence Intervals)[1] for HPV positivity, according to EarI expression (n=115).

LEGEND for *table 3*:
[1] Estimates were obtained from logistic regression models for the binary cervical dysplasia outcome (yes/no).
[2] Model 1 adjusted for age and smoking (smoker/nonsmoker).
[3] Model 2 adjusted for age, smoking (smoker/nonsmoker), and severity.
* Statistically significant results p-value ≤ 0.05

RESULTS

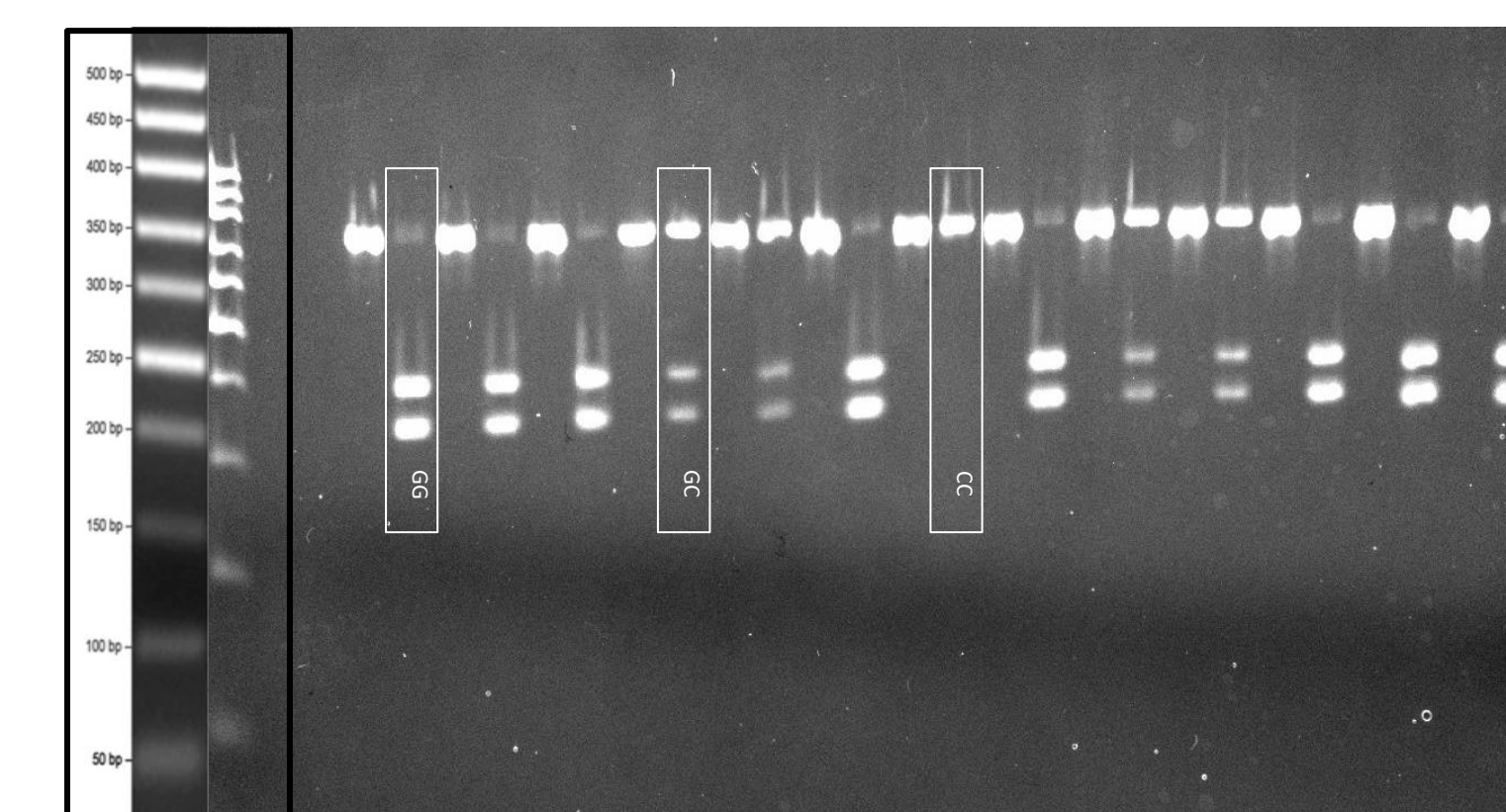*PCR-RFLP results of a horizontal electrophoresis.*

Figure 1. Agarose gel electrophoresis for EarI SNP rs11536889, 2.5% agarose gel in 1xTBE buffer.

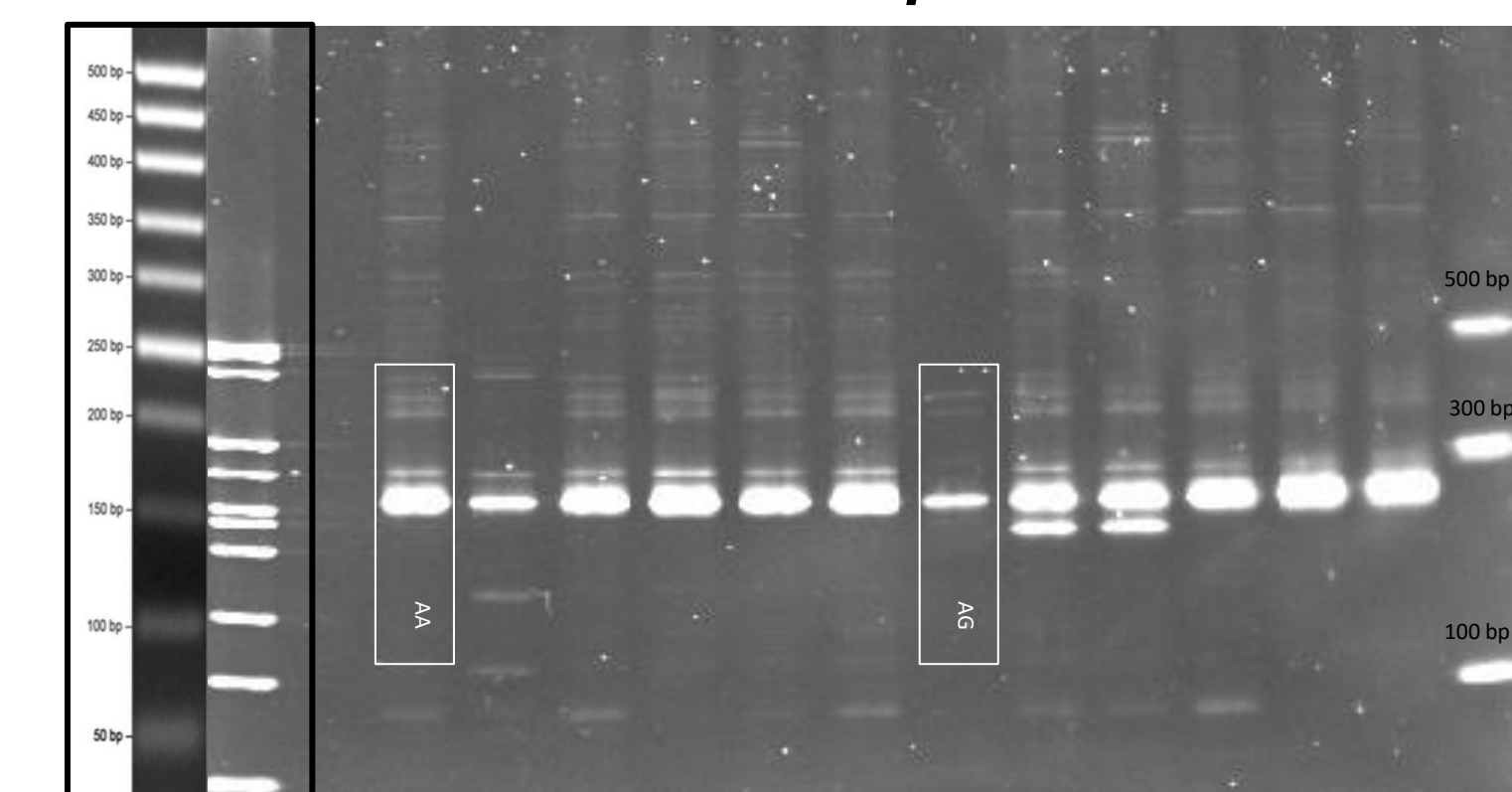*PCR-RFLP results of a vertical electrophoresis.*

Figure 2. Polyacrylamide gel electrophoresis for NcoI SNP rs4986790, 15% polyacrylamide gel in 1xTBE buffer.

CONCLUSIONS

- When considering dysplasia severity, our study revealed a statistically significant correlations between two polymorphisms and the disease among our sample of Puerto Rican women.

- In particular, the samples with the NcoI the (AA) genotype had 3.11 times higher odds of dysplasia compared to those with the (AG) allele (p=0.03).

- Despite being a polymorphism of TLR4 causing an aspartate to glycine amino acid exchange, the SNP (rs4985790) showed no statistically significant associations with cervical cancer in a study of Lithuanian patients with stage I-IV cervical cancer.

- Additionally, we found that women with a (CG) genotype in EarI profile had 2.21 times higher odds of being HPV-positive compared to those with the (GG) genotype in the EarI profile. Such polymorphisms could serve as genetic markers in diseases caused by TLR4-ligands, such as HPV infection and cervical cancer.

- Another notable observation was the detection of Low-Risk HPV in 35.71% of patients with Severe Dysplasia, raising concerns about varying HPV epidemiology in Puerto Rico.

ACKNOWLEDGEMENTS