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Investigating the role of neurotransmitter system and neurotrophic factor-related genes in human papillomavirus-associated cervical lesions

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Abstract

Human papillomavirus (HPV) infection plays a crucial role in cervical carcinogenesis. The link between the autonomic nervous system and tumor biology is increasingly being recognized. Understanding how neural signaling pathways interact with HPV oncogenesis could open new avenues for therapeutic intervention. We aim to study the contribution of the autonomic nervous system-related genes to the HPV-associated cervical lesions. A population of 140 HPV-infected women presenting cervical lesions was compared to a control population. Genes/variants under study were: *BDNF*/rs6265, *NTRK2*/rs2289656, *NGF*/rs6330, *SLC6A4*/5-HTT variable number tandem repeats intron 2, *HTR2A*/rs6313, *ADRBR2*/rs1042713, and *CHRNA5*/rs16969968. Samples were genotyped using polymerase chain reaction (PCR), PCR-restriction fragment length polymorphism, and endpoint genotyping. Statistical analysis revealed a genetic contribution for *SCL6A4*, *ADRB2*, and *CHRNA5*. *SCL6A4* showed statistically significant association in the codominant (p=0.003) and dominant models (p=0.024, odds ratio [OR] = 2.301). *ADRB2* showed statistically significant association in the codominant (p<0.001), dominant (p=0.024, OR = 4.728), recessive (p<0.001, OR = 4.856), and allelic models (p<0.001, OR = 4.091), while *CHRNA5* showed statistically significant association in the dominant model (p=0.030, OR = 0.529). We conclude that there is a genetic contribution of the adrenergic (*ADRB2*), cholinergic (*CHRNA5*), and serotonergic (*SLC6A4*) systems to cervical lesions associated with HPV infections.

Keywords: Human papillomavirus infection; Autonomic nervous system; Genetics

1. Introduction

Cervical cancer, a malignancy of the uterus, accounted for approximately 6.9% of all malignancies in females in 2022, making it the fourth most common cancer in women and a significant global public health challenge.[1] While high-risk human papillomavirus (HPV) infection is a primary trigger of cervical cancer, it is important to recognize that HPV infection alone is not sufficient to drive the development of this cancer or its pre-cursors.[2-4] Factors contributing to its incidence include early sexual activity, multiple partners, inadequate clinical follow-up, and tobacco use.[4]

Chronic stress is linked to tumorigenesis and cancer progression,[5,6] as well as the persistence of HPV infection.[7] It disrupts the hypothalamic-pituitary-adrenal axis (HPA), increasing cortisol, epinephrine, and norepinephrine levels.[8] Catecholamines, key mediators of the stress response, act through β-adrenergic receptors and are released through nicotinic cholinergic receptor activation in the adrenal glands and peripheral sympathetic ganglia.[9,10] Dysregulation of this system is associated with conditions, such as depression and anxiety, which involve serotonergic signaling.[11,12] Serotonin receptors and solute carrier family proteins, essential for serotonin transport, may modulate the sympathetic stress response.[13-15]

A previous study demonstrated that chronic stress, through the release of norepinephrine, can protect cervical cancer cells from anoikis. This protective effect is mediated by the activation of the beta-adrenergic receptor 2 (β2-AR)/cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signaling pathway.[16] The nicotinic acetylcholine receptor system, including *CHRNA5*, plays a role in activating corticotropin-releasing hormone neurons, which regulate the HPA axis. This axis controls cortisol secretion in response to psychological stress. Dysregulation of this system has been associated with impaired immune surveillance, increased inflammation, and tumor-supportive environments.[17] Specifically, rs16969968 has been associated with altered HPA axis activation, heightened stress sensitivity, anxiety-related traits, and an enhanced cortisol response under stress. The *SLC6A4* gene, particularly the variable number tandem repeat (VNTR) polymorphism in intron 2, can influence serotonin transporter expression levels. *HTR2A* plays a critical role in mood and anxiety regulation as well as HPA axis function. Activation of the 5-hydroxytryptamine receptor 2A stimulates the release of corticotropin-releasing hormone, subsequently regulating cortisol secretion.[18] While these genes are well-documented in psychiatric disorders, their role in cancer is an emerging area of research. Although direct evidence in cancer is limited, altered serotonin uptake could potentially affect tumor cell proliferation or modulate immune responses within the tumor microenvironment.

Peripheral nerves play a crucial role in the early stages of tumorigenesis.[19-23] Adrenergic nerve denervation or inhibition of adrenergic signaling delays tumor formation in prostate cancer,[24] while in pancreatic ductal adenocarcinoma, sympathetic neuron-derived catecholamines promote neoplastic lesions through adrenergic receptors. Similarly, stomach denervation reduces gut cancer incidence,[25] and sensory nerve ablation in basal cell carcinoma models decreases tumor size.[26] Tumors interact with nerves through neurotransmitters, such as epinephrine, norepinephrine, serotonin, and acetylcholine,[27] while overexpressing neurotrophic factors, such as nerve growth factor (NGF), which drive growth through tropomyosin receptor kinase (Trk) A-mediated signaling.[28-34] Studies have shown that NGF promotes the proliferation and metastasis of cervical cancer cells through the Hippo/Yes-associated protein (YAP) signaling pathway.[35] The brain-derived neurotrophic factor/TrkB signaling pathway activates downstream cascades such as the phosphoinositide 3-kinase/protein kinase B pathway, which contributes to tumor proliferation and resistance to anoikis.[36] Notably, *NTRK2* gene fusions are linked to a subtype of cervical cancer.[37]

We aim to investigate the association between neurotransmitter systems and neurotrophic factor-related genes in HPV-infected women by examining genetic variations in key components of the nervous system. Specifically, we focus on genes involved in neurotransmitter systems – adrenergic (*ADRB2*/rs1042713), cholinergic (*CHRNA5*/rs16969968), and serotonergic (*SLC6A4*/5-HTT VNTR intron 2 and *HTR2A*/rs6313) – as well as genes encoding neurotrophic factors, which play critical roles in neuronal growth, survival, development, and function (*BDNF*/rs6265, *NTRK2*/rs2289656, and *NGF*/rs6330).

2. Materials and methods

2.1. Study participants

A total of 140 women infected with HPV and presenting cervical lesions were included in this study. Women were followed in the Portuguese Oncological Institutes in Lisbon and Porto. All women underwent cytologic and histopathologic analyses. HPV detection was performed using polymerase chain reaction (PCR) using the PGMY09/11 primer set[38] (STABVIDA, Portugal) and hybridization using the Digene Hybrid Capture 2 (QIAGEN, United States) methods. Inclusion criteria for the HPV group were women older than 18 years who were HPV positive and presented with cervical lesions. Pregnant women were excluded. The pathological group's age ranged

from 24 to 77 years, with a median age of 45 years. The control group consisted of 609 healthy Caucasian women with an age range of 18 – 85 years and a median age of 54 years. Inclusion criteria for the control group were women older than 18 years, with the absence of cervical lesions and no history of cervical lesions. Exclusion criteria were pregnant women. DNA extracts were obtained between 1996 and 2003 and are now categorized as archived samples. We were not able to obtain all the parameters from all the women (control and disease); as a result, the number of patients and controls differs between analyses.

2.2. DNA extraction

Whole blood samples were obtained from the patients and controls. Genomic DNA was isolated from 2 mL of whole blood using the NZY Tissue gDNA isolation kit, which is a spin column silica-based method (NZYtech/MB13503, Portugal). The samples were processed following the manufacturer's instructions.

2.3. Genotyping

Genotyping was performed with PCR-based methods. Conventional PCR was used for VNTR detection, PCR-restriction fragment length polymorphism (RFLP) was used if the target sequence for the restriction enzyme was available, and other single nucleotide polymorphisms (SNPs) were analyzed using endpoint genotyping. Primers and conditions for conventional PCR and PCR-RFLP are listed in Table 1. The specific fragment pattern for each genotype is explained below.

2.3.1. SLC6A4 genotyping

A PCR amplification flanking the 5-HTT VNTR intron 2 variation was performed. The amplicons were visualized in an agarose gel with 299 base pair (bp) representing the allele with an additional VNTR repeat (12 repeats) and 265 bp representing the allele without an additional VNTR repeat (10 repeats) (Figure 2).

2.3.2. ADRB2 genotyping

A PCR amplification flanking the rs1042713 SNP was performed. An amplicon of 308 bp was visualized in an agarose gel. Subsequent restriction with *Neisseria coli* restriction enzyme I facilitated genotyping, producing the following fragment patterns: AA (308 bp), AG (308 bp, 291 bp, and 17 bp), and GG (291 bp and 17 bp) (Figure 3).

2.3.3. HTR2A genotyping

A PCR amplification flanking the rs6313 SNP was performed. An amplicon of 344 bp was visualized in an agarose gel. Subsequent restriction with *Moraxella* species restriction enzyme I facilitated genotyping, producing the following fragment patterns: TT (344 bp), CT (344 bp, 217 bp, and 127 bp), and CC (217 bp and 127 bp).

Table 1. Primers and conditions for conventional polymerase chain reaction and polymerase chain reaction-restriction fragment length polymorphism

Gene	Polymerase chain reaction primers	Polymerase chain reaction conditions
ADRB2	Forward: 5'CCTTCTTGCTGGCACCCCAA3' Reverse: 5'GGAAGTCCAAAACTCGCACCA3'	40 cycles 94°C – 30 s 57° C – 30 s 72°C – 45 s
SLC6A4	Forward: 5×GTCAGTATCACAGGCTGCGAG3×Reverse: 5×TGTTCCTAGTCTTACGCCAG3×	40 cycles 94°C – 30 s 57°C – 30 s 72°C – 45 s
HTR2A	Forward: 5'TCTGCTACAAGTTCTGGCTT3' Reverse: 5'CTGCAGCTTTTTCTCTAGGG3'	40 cycles 94°C – 30 s 50°C – 30 s 72°C –45 s

BDNF/rs6265, *NTRK2*/rs2289656, *NGF*/rs6330, and *CHRNA5*/rs16969968 were analyzed using endpoint genotyping in a LightCycler® 408 (Roche, Switzerland) thermocycler, using standardized PCR conditions (One pre-incubation at 95°C for 10 min, 30 cycles of amplification at 95°C for 10 s, followed by annealing at 60°C for 1 min and extension at 72°C for 1 s). The following TaqMan assays were purchased from Thermo Fisher Scientific (United States): *BDNF*/rs6265 (Assay ID: C__11592758_10), *NTRK2*/rs2289656 (Assay ID: C__15882271_20), *NGF*/rs6330 (Assay ID: C__2525309_10), and *CHRNA5*/rs16969968 (Assay ID: C__26000428_20).

2.4. Statistical analysis

All statistical tests were conducted using Statistical Package for the Social Sciences® 28.0 software (IBM Corp., United States). Group differences were assessed using Pearson's Chi-square or Fisher's tests. When more than 20% of cells had an expected count of <5, correction for Chi-square was conducted using Fisher's Exact test (2 × 2 tables). Statistical significance was defined as a $p<0.05$. Odds ratios (OR) were calculated, and the corresponding confidence intervals (CI) had a 95% level of confidence. The OR and the CIs were obtained with a focus on the HPV group.

3. Results

The analysis was conducted following the flow diagram of Figure 1, in order to investigate the relationship between

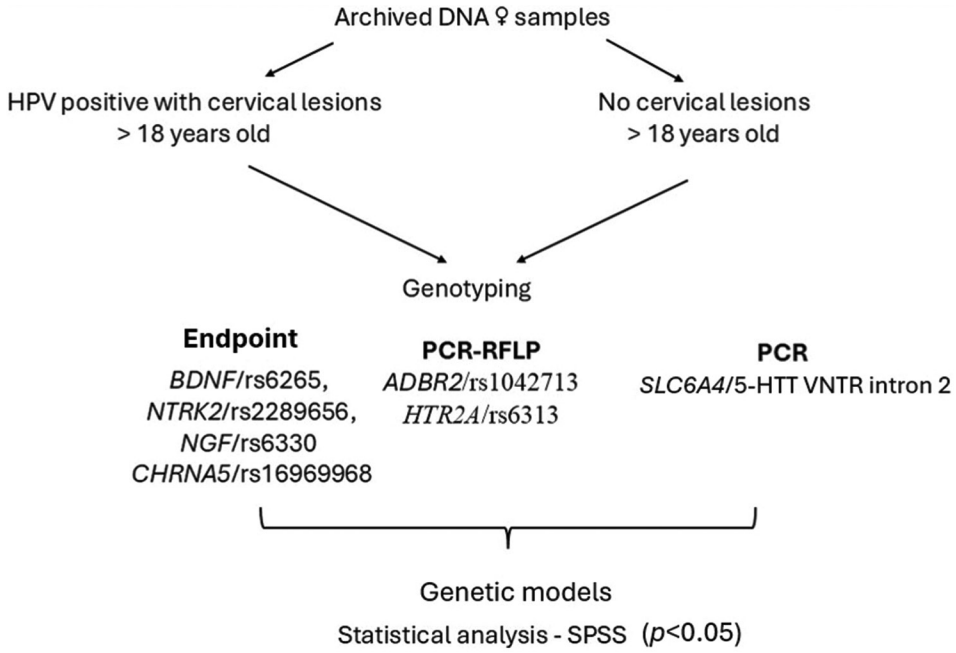


Figure 1. Flow diagram of sample processing
Abbreviations: PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism; SPSS: Statistical Package for Social Sciences; VNTR: Variable number tandem repeats

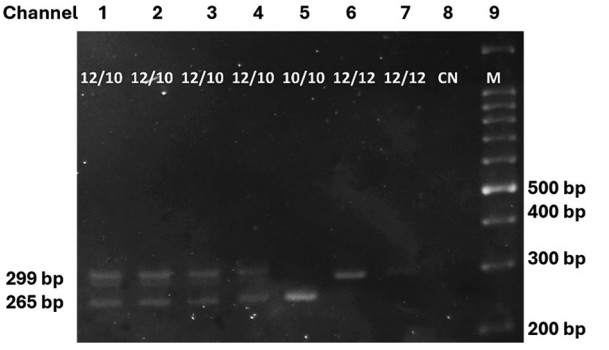


Figure 2. Image of an agarose gel with a representative genotyping of the *SLC6A4* gene. Channels 1 – 7 show amplification products from individuals under study. Channel 8 (CN) is the negative control, and channel 9 (M) is the 100 base pair (bp) molecular weight marker (100 bp DNA ladder). Note: 12/12 refers to homozygous normal (299 bp), 12/10 refers to heterozygous (299 bp and 265 bp), and 10/10 refers to homozygous mutated (265 bp)

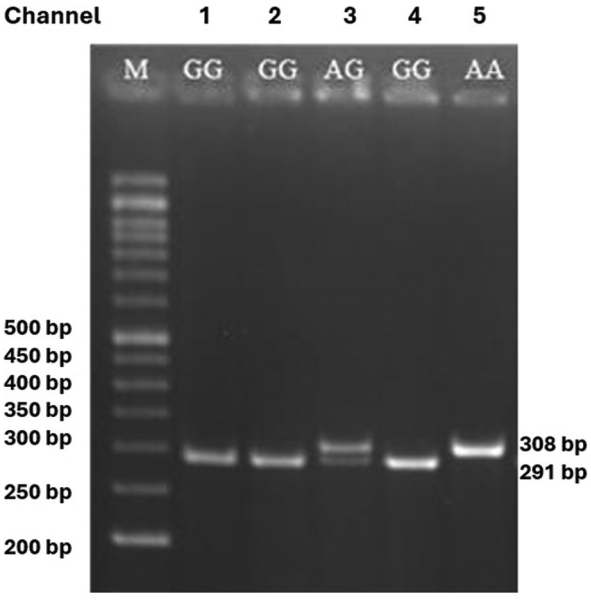


Figure 3. Image of an agarose gel with a representative electrophoretic genotyping of the *ADRB2* gene. Channels 1 – 5 show the restriction products from individuals under study. Channel 1 (M) shows the 100 base pair (bp) molecular weight marker (100 bp DNA ladder). Note: G/G indicates homozygous normal (291 bp), A/G indicates heterozygous (308 and 291 bp), and A/A indicates homozygous mutated (308 bp)

the seven genes and cervical lesions associated with HPV infection. In this study, we used codominant, dominant, and recessive genetic models to investigate allelic contribution to the development of cervical lesions. As the inheritance pattern for each variant is not known, codominant models will provide an indication, while dominant/recessive models will refine the analysis. The allelic model is used because of its ability to provide clear, focused insights into the relationship between genetic variants and cervical lesions. Ultimately, we want to understand how genetic variants influence disease risk.

3.1. Analysis using the codominant model

Under the codominant model, we initially compared the distribution of genotypes across HPV-infected individuals and controls (Table 2). Only significant results are shown. Among the genes studied, only *SLC6A4* and *ADRB2* displayed dissimilar genotype distributions between the two female populations (p=0.003 and p<0.001, respectively).

Table 2. Comparison of genotype distribution using the codominant model

Genes	Human papillomavirus, *n* (%)	Controls, *n* (%)	*p*[a]
SLC6A4			
12/12	10 (25.6)	219 (44.2)	0.003
12/10	27 (69.2.)	205 (41.4)	
10/10	2 (5.1)	71 (14.3)	
ADRB2			
GG	2 (6.5)	45 (24.6)	<0.001
AG	10 (32.3)	93 (50.8)	
AA	19 (61.3)	45 (24.6)	

Note: [a]Chi-square test, only significant values at p<0.05 are presented.

3.2. Analysis using the allelic model

For a clearer understanding of the risk or protective effects of each allele, we examined the allele distribution between the two populations (Table 3). Only significant results are shown. Notably, *ADRB2* (p<0.001) exhibited a distinct allele distribution, with allele A demonstrating a risk factor (OR = 4.091, 95% CI: 2.051 – 8.160).

3.3. Analysis using dominant, overdominant, and recessive models

We evaluated the genotype distribution using the dominant, overdominant, and recessive models (Table 4). Only genes with significant results are shown. Only for significant outcomes were ORs calculated.

The genotype distributions varied for the *SLC6A4*, *ADRB2*, and *CHRNA5* genes. In the case of *SLC6A4*, the dominant (p=0.024, OR = 2.301, 95% CI: 1.098 – 4.824) and overdominant (p<0.001, OR = 3.183, 95% CI: 1.576 – 6.430) models revealed a risk factor of 10 alleles. In addition, allele A of *ADRB2* emerged as a risk factor, with two genetic models showing significant results (p=0.024, OR = 4.728, 95% CI: 1.085 – 20.603 for the dominant

Table 3. Comparison of allele distribution using the allelic model

Gene	Human papillomavirus, *n* (%)	Controls, *n* (%)	*p*[a]	Odds ratio (confidence interval)
ADRB2				
Allele G	14 (22.6)	183 (50.0)	<0.001	0.244 (0.123 – 0.488)
Allele A	48 (77.4)	183 (50.0)		4.091 (2.051 – 8.160)

Note: [a]Chi-square test, only significant values at p<0.05 are presented.

Table 4. Comparison of genotype distribution using the dominant, overdominant, and recessive models

Genes	Human papillomavirus, *n* (%)		Controls, *n* (%)		*p*[a]	Odds ratio (confidence interval)
SLC6A4						
10/10 and 12/10 versus 12/12	29 (74.4)	10 (25.6)	276 (55.8)	219 (44.2)	0.024*	2.301 (1.098 – 4.824)
12/10 versus 12/12 and 10/10	27 (69.2)	12 (30.8)	205 (41.4)	290 (58.6)	<0.001*	3.183 (1.576 – 6.430)
10/10 versus 12/10and 12/12	2 (5.1)	37 (94.9)	71 (14.3)	424 (85.7)	0.107	NA
ADRB2						
AG and AA versus GG	29 (93.5)	2 (6.5)	138 (75.4)	45 (24.6)	0.024*	4.728 (1.085 – 20.603)
AG versus GG and AA	10 (32.3)	21 (67.7)	93 (50.8)	90 (49.2)	0.056	NA
AA versus GG and AG	19 (61.3)	12 (38.7)	45 (24.6)	138 (75.4)	<0.001*	4.856 (2.188 – 10.776)
CHRNA5						
AG and AA versus GG	32 (50.8)	31 (49.2)	121 (66.1)	62 (33.9)	0.030*	0.529 (0.296 – 0.946)
AG versus GG and AA	21 (33.3)	42 (66.7)	90 (49.2)	93 (50.8)	0.029*	0.517 (0.284 – 0.940)
AA versus AG and GG	11 (17.5)	52 (82.5)	31 (16.9)	152 (83.1)	0.925	NA

Note: [a]Chi-square test, *indicates significance at p<0.05.
Abbreviation: NA: Not applicable.

model, and $p<0.001$, OR = 4.856, 95% CI: 2.188 – 10.776 for the recessive model). Regarding *CHRNA5*, the variant allele (A) has a protective effect in the dominant ($p=0.030$, OR = 0.529, 95% CI: 0.296 – 0.946) and overdominant ($p=0.029$, OR = 0.517, 95% CI: 0.284 – 0.940) models.

4. Discussion

In this study, our investigation revealed associations between HPV-related cervical lesions and three out of the seven candidate genes under consideration. All candidate genes were linked to chronic stress-responsive mechanisms. Chronic stress has been associated with tumor progression,[5,6,39,40] including gynecological cancers.[41] Multiple physiological systems, including the autonomic nervous system and the HPA, are engaged during the stress response. This response is initiated by the production of key mediators, such as the catecholamines norepinephrine and epinephrine, which are released by the sympathetic nervous system and adrenal medulla. Norepinephrine, epinephrine, and cortisol are regarded as the primary stress hormones, and their levels are elevated during chronic stress. Catecholamines exert their effects through adrenergic receptors coupled to G-proteins, which activate the cAMP-dependent PKA system, subsequently triggering several downstream signaling pathways, including those related to cell growth.[42,43] Among adrenergic receptors, β2-ARs are predominant, mediating the majority of cellular responses to external stimuli. The ADRB2 gene encodes these receptors, which are located on cell membranes, and function pre-synaptically to stimulate the release of epinephrine.[44] β-blockers, which inhibit β-adrenergic receptors, have been shown to offer therapeutic benefits in managing several types of tumors.[45,46] Given the central role of catecholamines and their interaction with β2-AR in the stress response, we hypothesized that genetic variations in the *ADRB2* gene could influence susceptibility to HPV infection and cervical lesions. Our study identified an association between the AA genotype and the A allele of *ADRB2* with an increased risk of HPV infection and cervical lesions. Dominant and recessive models both showed a risk effect, which was slightly higher for the recessive model. In multifactorial conditions such as cervical lesion development, the distinction between dominant and recessive genetic models is often blurred because the complex interplay of multiple factors shapes the risk associated with genetic variants. However, this study shows us that at least one A allele is sufficient for susceptibility. The A allele corresponds to a missense mutation in which glycine (Gly) is replaced by arginine (Arg) at codon 16 of the intron-less *ADRB2* gene. According to the "dynamic model of receptor regulation" proposed by Liggett,[47] endogenous catecholamines dynamically desensitize β2-AR in their basal state, a process that is more pronounced in the Gly variant compared to the Arg variant. Furthermore, after exposure to isoprenaline, the Gly variant exhibits significantly enhanced receptor downregulation.[48] This suggests that individuals with the AA (Arg) genotype may exhibit a stronger physiological response to stress compared to those with the GG (Gly) genotype.

Our results also demonstrate a protective effect of the presence of the variant allele (A) in the *CHRNA5* gene. *CHRNA5* encodes a subunit of the nicotinic acetylcholine receptor. In the adrenal medulla, acetylcholine released from the splanchnic sympathetic nerves activates acetylcholine receptors on the membrane of chromaffin cells, which release catecholamines into the bloodstream. As mentioned above, catecholamines are one of the main mediators of the stress response. Rs16969968 is a missense variant resulting in an amino acid substitution at codon 398 (D398N) of *CHRNA5*. *In vitro* functional studies have shown that nicotinic receptors that contain this variant have a reduced response to agonists.[49] Therefore, since the variant allele is functionally less active, it may promote a lower release of catecholamines, protecting individuals from the physiological response to chronic stress.

Multiple studies have demonstrated that serotonin and serotonergic drugs influence various components of the HPA axis and the stress response.[50-52] The *SLC6A4* gene encodes an integral membrane protein (SERT) whose primary function in the central nervous system is to regulate serotonergic signaling by transporting serotonin molecules from the synaptic cleft back into the pre-synaptic terminal for reuse. A loss of SERT function has been associated with an increased sympathetic stress response.[53-55] SERT is highly expressed in chromaffin cells of the adrenal medulla, where it plays a local role within the adrenal gland in regulating the sympathetic stress response.[14,56] Evidence suggests that SERT coordinates serotonergic regulation of catecholamine exocytosis through 5-hydroxytryptamine receptor 1A-mediated inhibition of catecholamine secretion, contributing to the stress response. Although serotonin is not synthesized in adrenergic chromaffin cells, SERT facilitates its accumulation.[56] In this study, we analyzed a known polymorphic variation of this gene, 5-HTT VNTR, which consists of a VNTR in the second intron. Only the two most common alleles were identified, those with 10 or 12 tandem repeats of a 17 bp sequence. The 10 allele has been linked to lower transcriptional activity compared to the 12 allele.[57,58] Our findings revealed that the presence of the 10 allele is a risk factor. We propose that the *SLC6A4* genotype may influence catecholamine secretion, thereby

playing a role in the stress response, with implications for HPV infection and cervical lesions.

Although neurotrophins are known to play a role in gynecological cancers, including cervical cancer,[59] we found no evidence of any genetic contribution regarding the studied variants in the neurotrophic factors genes and cervical lesions associated with HPV infection. Previous studies reported higher expressions of *BDNF* and *NTRK2* in cervical cancer tissue.[36,60] However, we believe that possible slight changes in gene expression associated with these variants are not sufficient to promote cervical lesions.

A limitation of our study is the use of archived samples. We were not able to collect more data on the individuals that may be important in this context, such as medical history related to chronic stress conditions, genetic syndromes predisposing to cancer, co-morbidities, progression to cancer, type of cervical lesion, HPV genotype, and follow-up information. Without this information, it may be difficult to control for confounders, potentially leading to biased associations between variables of interest and outcomes. In addition, archived samples represent a specific point in time that may not reflect present population dynamics, disease prevalence, or environmental exposures. The incidence of cervical lesions has changed over time as a result of HPV vaccination programs around the world. Another limitation of this work is the lack of information regarding vitamin imbalances and nutraceutical supplementation, since it may play a significant role in women's health. Alpha-lipoic acid has shown promising effects in cancer treatment through its multifaceted roles in cellular metabolism and oxidative stress modulation. Research indicates that this acid can inhibit cancer cell proliferation and induce apoptosis across various cancer types.[61] Inositol has also been shown to exert anti-cancer effects through various biochemical pathways, making it a promising candidate for cancer prevention and treatment.[62] Finally, omega-3 fatty acids and their anti-inflammatory effects may help alleviate the side effects of cancer treatments.[63]

This study further supports the link between stress and tumorigenesis, reinforcing the role of chronic stress in tumorigenesis, particularly in gynecological cancers, by highlighting the involvement of specific genetic variations in stress-responsive mechanisms. The findings align with and expand upon previous research showing the activation of the autonomic nervous system and HPA in the stress response, with downstream effects on tumor biology. Regarding *ADRB2*, the identification of the A allele as a risk factor for HPV infection and cervical lesions emphasizes the physiological importance of catecholamine-mediated stress responses in disease progression. Insights into this polymorphism provide evidence of a stronger stress response mediated by the A variant, supporting the dynamic receptor regulation model proposed by Liggett.[47] The discovery of a protective effect associated with the *CHRNA5* variant allele highlights how genetic variation can modulate the release of catecholamines during stress. This adds a new layer of understanding to how reduced receptor activity impacts the stress response and its link to HPV-related lesions. The finding that the 10-repeat allele (*SLC6A4*) increases risk provides novel evidence for how serotonergic signaling, particularly through SERT, regulates catecholamine secretion and contributes to the physiological stress response in cervical lesion development. By integrating findings from adrenergic, cholinergic, and serotonergic systems, this study establishes a comprehensive view of how these interconnected systems contribute to the stress response and its downstream effects on HPV infection and cervical lesion progression. The study emphasizes the intricate role of neurotransmitter-related genetic variants in modulating the stress response, suggesting potential therapeutic targets. These findings pave the way for personalized approaches to managing HPV infection and cervical cancer, as genetic variations in stress-response genes may serve as biomarkers for individual susceptibility. The study suggests that interventions targeting the stress response, such as β-blockers or serotonergic drugs, could hold therapeutic potential for HPV-related lesions, further advancing precision medicine.

However, we cannot rule out the involvement of other tumorigenic pathways that are unrelated to chronic stress. For instance, *ADRB2* is linked to immune cell infiltration, suggesting its role in the immune response and tumor microenvironment regulation, and enhances the ETS variant transcription factor 1-c-KIT signaling pathway, promoting tumor progression.[64,65] *SLC6A4* evidence indicates that the SERT transporter is overexpressed in non-small cell lung cancer, where its activity correlates with poor prognosis due to activation of the c-*Myc* oncogene.[66] *CHRNA5* also plays a pivotal role in cancer development and progression by modulating key signaling pathways such as mitogen-activated protein kinase/extracellular signal-regulated kinase, signal transducer and activator of transcription 3, and YAP.[67-70]

5. Conclusion

Our research elucidates that genetic polymorphisms within the adrenergic, cholinergic, and serotonergic systems contribute significantly to the pathogenesis of tumorigenesis in cervical lesions associated with cervical carcinoma. This investigation substantiates the role of the autonomic nervous system in the biology of tumors, in conjunction with the genetic frameworks underpinning mechanisms pertinent to chronic stress. By accentuating and contributing to the understanding of the

genetic factors involved in the pathophysiology of HPV infection and neoplastic lesions, we posit that this inquiry cultivates avenues for the exploration of novel therapeutic interventions that modulate the neurotransmitter systems of the nervous system, representing a progressive advancement in the realm of personalized medicine by delineating individual predispositions to the disease.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Ângela Inácio, Rui Medeiros, Manuel Bicho
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Supervision: Ângela Inácio
Validation: Laura Aguiar
Writing – original draft: Angela Inácio
Writing – review & editing: Laura Aguiar, Maria Clara Bicho

Ethics approval and consent to participate

Ethical review and approval were waived for this study because this observational study was performed with archived and anonymized samples. At the time, there was no law regarding genetic studies, since the regulation only came out in 2005. The study was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from each of the subjects who participated in this study.

Consent for publication

Informed consent of all the human subjects of this study made possible to publish their data in this paper.

Availability of data

All data from the genotype analysis will be shared upon reasonable request to the corresponding author.

Further disclosure

Results of this study were presented partly at the 36th IPVC24, in Edinburgh, United Kingdom, November 12 – 15, 2024.

References

1. WHO. *Cancer Today*; 2024. Available from: https://gco.iarc.who.int/today/en [Last accessed on 2025 Feb 20].
2. Mlynarczyk-Bonikowska B, Rudnicka L. HPV Infections-classification, pathogenesis, and potential new therapies. *Int J Mol Sci*. 2024;25(14):7616.
   doi: 10.3390/ijms25147616
3. Muñoz N, Castellsagué X, Berrington de González A, Gissmann L. Chapter 1: HPV in the etiology of human cancer. *Vaccine*. 2006;24(Suppl 3):1-10.
   doi: 10.1016/j.vaccine.2006.05.115
4. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. *Lancet*. 2019;393(10167):169-182.
   doi: 10.1016/S0140-6736(18)32470-X
5. Dai S, Mo Y, Wang Y, *et al*. Chronic stress promotes cancer development. *Front Oncol*. 2020;19(10):1492.
   doi: 10.3389/fonc.2020.01492
6. Wang C, Shen Y, Ni J, Hu W, Yang Y. Effect of chronic stress on tumorigenesis and development. *Cell Mol Life Sci*. 2022;79(9):485.
   doi: 10.1007/s00018-022-04455-3
7. Kuebler U, Fischer S, Mernone L, Breymann C, Abbruzzese E, Ehlert U. Is stress related to the presence and persistence of oncogenic human papillomavirus infection in young women? *BMC Cancer*. 2021;21(1):419.
   doi: 10.1186/s12885-021-08010-4
8. Oyola M, Handa R. Hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes: Sex differences in regulation of stress responsivity. *Physiol Behav*. 2017;176(3):139-148.
   doi: 10.1080/10253890.2017.1369523
9. Krizanova O, Babula P, Pacak K. Stress, catecholaminergic system and cancer. *Stress*. 2016;19(4):419-428.
   doi: 10.1080/10253890.2016.1203415
10. Sam C, Bordoni B. Physiology, acetylcholine. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2021.
11. Caspi A, Sugden K, Moffitt TE, *et al*. Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science*. 2003;301(5631):386-389.
    doi: 10.1126/science.1083968
12. Paine NJ, Watkins LL, Blumenthal JA, Kuhn CM, Sherwood A. Associations of depressive and anxiety symptoms

with 24-hour urinary catecholamines in individuals with untreated high blood pressure. *Psychosom Med*. 2016;77(2):136-144.

doi: 10.1097/PSY.0000000000000144

13. Shioda N, Imai Y, Yabuki Y, Sugimoto W, Yamaguchi K, Wang Y. Dopamine D2L receptor deficiency causes stress vulnerability through 5-HT1A receptor dysfunction in serotonergic neurons. *J Neurosci*. 2019;39(38):7551-7563.

doi: 10.1523/JNEUROSCI.0079-19.2019

14. Brindley RL, Bauer MB, Walker LA, *et al*. Adrenal serotonin derives from accumulation by the antidepressant-sensitive serotonin transporter. *Pharmacol Res*. 2019;140:56-66.

doi: 10.1016/j.phrs.2018.06.008

15. Cao Y. Tumorigenesis as a process of gradual loss of original cell identity and gain of properties of neural precursor/progenitor cells. *Cell Biosci*. 2017;7(1):61.

doi: 10.1186/s13578-017-0188-9

16. Li Y, Yang S, Sadaoui NC, *et al*. Sustained adrenergic activation of YAP1 induces anoikis resistance in cervical cancer cells. *iScience*. 2020;24;23(7):101289.

doi: 10.1016/j.isci.2020.101289

17. Lutfy K, Aimiuwu O, Mangubat M, *et al*. Nicotine stimulates secretion of corticosterone via both CRH and AVP receptors. *J Neurochem*. 2012;120(6):1108-1116.

doi: 10.1111/j.1471-4159.2011.07633.x

18. Weisstaub NV, Zhou M, Lira A, *et al*. Cortical 5-HT2A receptor signaling modulates anxiety-like behaviors in mice. *Science*. 2006;313(5786):536-540.

doi: 10.1126/science.1123432

19. Boilly B, Faulkner S, Jobling P, Hondermarck H. Nerve dependence: From regeneration to cancer. *Cancer Cell*. 2017;31(3):342-354.

doi: 10.1016/j.ccell.2017.02.005

20. Mancino M, Ametller E, Gascón P, Almendro V. The neuronal influence on tumor progression *Biochim Biophys*. 2011;1816(2):105-118.

doi: 10.1016/j.bbcan.2011.04.005

21. Benzaquen D, Lawrence YR, Taussky D, Zwahlen D, Oehler C, Champion A. The crosstalk between nerves and cancer-a poorly understood phenomenon and new possibilities. *Cancers* (*Basel*). 2024;6(10):1875.

doi: 10.3390/cancers16101875

22. Bernstock JD, Gerstl JVE, Chen JA, *et al*. The case for neurosurgical intervention in cancer neuroscience. *Neurosurgery*. 2025;96(1):10-17.

doi: 10.1227/neu.0000000000003039

23. Le TT, Oudin MJ. Understanding and modeling nerve-cancer interactions. *Dis Model Mech*. 2023;16(1):dmm049729.

doi: 10.1242/dmm.049729

24. Magnon C, Hall SJ, Lin J, *et al*. Autonomic nerve development contributes to prostate cancer progression. *Science*. 2013;341(6142):1236361.

doi: 10.1126/science.1236361

25. Zhao CM, Hayakawa Y, Kodama Y, *et al*. Denervation suppresses gastric tumorigenesis. *Sci Transl Med*. 2014;6(250):250ra115.

doi: 10.1126/scitranslmed.3009569

26. Peterson SC, Eberl M, Vagnozzi AN, *et al*. Basal cell carcinoma preferentially arises from stem cells within hair follicle and mechanosensory niches. *Cell Stem Cell*. 2015;16(4):400-412.

doi: 10.1016/j.stem.2015.02.006

27. Roda N, Blandano G, Pelicci PG. Blood vessels and peripheral nerves as key players in cancer progression and therapy resistance. *Cancers* (*Basel*). 2021;13(17):4471.

doi: 10.3390/cancers13174471

28. Silverman DA, Martinez VK, Dougherty PM, Myers JN, Calin GA, Amit M. Cancer-associated neurogenesis and nerve-cancer cross-talk. *Cancer Res*. 2021;81(6):1431-1440.

doi: 10.1158/0008-5472.CAN-20-2793

29. Adriaenssens E, Vanhecke E, Saule P, *et al*. Nerve growth factor is a potential therapeutic target in breast cancer. *Cancer Res*. 2008;68(2):346-351.

doi: 10.1158/0008-5472.CAN-07-1183

30. Watanabe T, Katayama Y, Kimura S, Yoshino A. Control of proliferation and survival of C6 glioma cells with modification of the nerve growth factor autocrine system. *J Neurooncol*. 1999;41(2):121-128.

doi: 10.1023/a:1006127624487

31. Demir IE, Tieftrunk E, Schorn S, Friess H, Ceyhan GO. Nerve growth factor & TrkA as novel therapeutic targets in cancer. *Biochim Biophys Acta*. 2016;1866(1):37-50.

doi: 10.1016/j.bbcan.2016.05.003

32. Bapat AA, Munoz RM, Von Hoff DD, Han H. Blocking nerve growth factor signaling reduces the neural invasion potential of pancreatic cancer cells. *PLoS One*. 2016;11(10):e0165586.

doi: 10.1371/journal.pone.0165586

33. Lei Y, Tang L, Xie Y, *et al*. Gold nanoclusters-assisted delivery of NGF siRNA for effective treatment of pancreatic cancer. *Nat Commun*. 2017;8:15130.

doi: 10.1038/ncomms15130

34. Brodeur GM. Neuroblastoma: Biological insights into a clinical enigma. *Nat Rev Cancer*. 2003;3(3):203-216.

doi: 10.1038/nrc1014

35. Wang L, Li J, Wang R, *et al*. NGF signaling interacts with the Hippo/YAP pathway to regulate cervical cancer progression. *Front Oncol*. 2021;11:688794.

doi: 10.3389/fonc.2021.688794

36. Yuan Y, Ye HQ, Ren QC. Proliferative role of BDNF/TrkB signaling is associated with anoikis resistance in cervical cancer. *Oncol Rep*. 2018;40(2):621-634.

doi: 10.3892/or.2018.6515

37. Aziz AUR, Zhang J, Zhang C, Yu X, Wang D. The mutual interplay between NTRK fusion genes and human papillomavirus infection in cervical cancer progression. *Mol Clin Oncol*. 2024;21(4):67.

doi: 10.3892/mco.2024.2765

38. Gravitt PE, Peyton CL, Alessi TQ, *et al*. Improved amplification of genital human papillomaviruses. *J Clin Microbiol*. 2000;38(1):357-361.

doi: 10.1128/JCM.38.1.357-361.2000

39. Moreno-Smith M, Lutgendorf SK, Sood AK. Impact of stress on cancer metastasis. *Futur Oncol*. 2010;6(12):1863-1881.

doi: 10.2217/fon.10.142

40. Iftikhar A, Islam M, Shepherd S, Jones S, Ellis I. Cancer and stress: Does it make a difference to the patient when these two challenges collide? *Cancers* (*Basel*). 2021;13(2):1-29.

doi: 10.3390/cancers13020163

41. Chen G, Qiu L, Gao J, *et al*. Stress hormones: Emerging targets in gynecological cancers. *Front Cell Dev Biol*. 2021;9:699487.

doi: 10.3389/fcell.2021.699487

42. Lai LP, Mitchell J. β2-adrenergic receptors expressed on murine chondrocytes stimulate cellular growth and inhibit the expression of Indian hedgehog and collagen type XJ. *Cell Biochem*. 2008;104(2):545-553.

doi: 10.1002/jcb.21646

43. Pullar CE, Isseroff RR. The beta 2-adrenergic receptor activates pro-migratory and pro-proliferative pathways in dermal fibroblasts via divergent mechanisms. *J Cell Sci*. 2006;119(3):592-602.

doi: 10.1242/jcs.02772

44. Floras JS. Epinephrine and the genesis of hypertension. *Hypertension*. 1992;19(1):1-18.

doi: 10.1161/01.hyp.19.1.1

45. Carlos-Escalante JA, de Jesús-Sánchez M, Rivas-Castro A, Pichardo-Rojas PS, Arce C, Wegman-Ostrosky T. The use of antihypertensive drugs as coadjuvant therapy in cancer. *Front Oncol*. 2021;11:660943.

doi: 10.3389/fonc.2021.660943

46. Fan Y, Khan NH, Farhan Ali Khan M, *et al*. Association of hypertension and breast cancer: Antihypertensive drugs as an effective adjunctive in breast cancer therapy. *Cancer Manag Res*. 2022;14:1323-1329.

doi: 10.2147/CMAR.S350854

47. Liggett SB. The pharmacogenetics of beta2-adrenergic receptors: Relevance to asthma. *J Allergy Clin Immunol*. 2000;105(2):S487-S492.

doi: 10.1016/s0091-6749(00)90048-4

48. Green SA, Liggett SB. A proline-rich region of the third intracellular loop imparts phenotypic β1- versus β2-adrenergic receptor coupling and sequestration. *J Biol Chem*. 1994;269(42):26215-26219.

doi: 10.1016/s0021-9258(18)47181-5

49. Bierut LJ, Stitzel JA, Wang JC, *et al*. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry*. 2008;165(9):1163-1171.

doi: 10.1176/appi.ajp.2008.07111711

50. Carhart-Harris RL, Nutt DJ. Serotonin and brain function: A tale of two receptors. *J Psychopharmacol*. 2017;31(9):1091-1120.

doi: 10.1177/0269881117725915

51. Siwiec M, Hess G. The use of serotonin type 7 receptor antagonists as a pharmacological intervention in chronic stress. Insights from animal studies. *Int J Biochem Cell Biol*. 2024;175:106647.

doi: 10.1016/j.biocel.2024.106647

52. Bauer MB, Currie KPM. Serotonin and the serotonin transporter in the adrenal gland. *Vitam Horm*. 2024;124:39-78.

doi: 10.1016/bs.vh.2023.06.002

53. Tjurmina OA, Armando I, Saavedra JM, Goldstein DS, Murphy DL. Exaggerated adrenomedullary response to immobilization in mice with targeted disruption of the serotonin transporter gene. *Endocrinology*. 2002;143(12):4520-4526.

doi: 10.1210/en.2002-220416

54. Murphy DL, Lesch KP. Targeting the murine serotonin transporter: Insights into human neurobiology. *Nat Rev Neurosci*. 2008;9(2):85-96.

doi: 10.1038/nrn2284

55. Brindley RL, Bauer MB, Blakely RD, Currie KPM. An interplay between the serotonin transporter (SERT) and 5-HT receptors controls stimulus-secretion coupling in sympathoadrenal chromaffin cells. *Neuropharmacology*. 2016;110:438-448.

doi: 10.1016/j.neuropharm.2016.08.015

56. Brindley RL, Bauer MB, Blakely RD, Currie KPM. Serotonin and serotonin transporters in the adrenal medulla: A

potential hub for modulation of the sympathetic stress response. *ACS Chem Neurosci*. 2017;8(5):943-954.

doi: 10.1021/acschemneuro.7b00026

57. Hranilovic D, Stefulj J, Schwab S, *et al*. Serotonin transporter promoter and intron 2 polymorphisms: Relationship between allelic variants and gene expression. *Biol Psychiatry*. 2004;55(11):1090-1094.

doi: 10.1016/j.biopsych.2004.01.029

58. Lovejoy EA, Scott AC, Fiskerstrand CE, Bubb VJ, Quinn JP. The serotonin transporter intronic VNTR enhancer correlated with a predisposition to affective disorders has distinct regulatory elements within the domain based on the primary DNA sequence of the repeat unit. *Eur J Neurosci*. 2003;17(2):417-420.

doi: 10.1046/j.1460-9568.2003.02446.x

59. Garrido MP, Torres I, Vega M, Romero C. Angiogenesis in gynecological cancers: Role of neurotrophins. *Front Oncol*. 2019;9:913.

doi: 10.3389/fonc.2019.00913

60. Moon A, Won KY, Lee JY, Kang I, Lee SK, Lee J. Expression of BDNF, TrkB, and p53 in early-stage squamous cell carcinoma of the uterine cervix. *Pathology*. 2011;43(5): 453-458.

doi: 10.1097/PAT.0b013e3283484a3a

61. Laganà AS, Monti N, Fedeli V, Gullo G, Bizzarri M. Does Alpha-lipoic acid improve effects on polycystic ovary syndrome? *Eur Rev Med Pharmacol Sci*. 2022;26(4): 1241-1247.

doi: 10.26355/eurrev_202202_28116

62. Coldebella D, Buzzaccarini G, Ferrari J, *et al.* Inositols administration: Further insights on their biological role. *Ital J Gynaecol Obstet*. 2023;35(1):30-36.

63. Habib N, Buzzaccarini G, Centini G, *et al*. Impact of lifestyle and diet on endometriosis: A fresh look to a busy corner. *Prz Menopauzalny*. 2022;21(2):124-132.

doi: 10.5114/pm.2022.116437

64. Chen S, Wu F, Zhang J, Zhu J, Zhou X, Zhi X. ADRB2 regulates the proliferation and metastasis of gastrointestinal stromal tumor cells by enhancing the ETV1-c-KIT signaling. *J Oncol*. 2023;2023:6413796.

doi: 10.1155/2023/6413796

65. Ji L, Xu F, Zhang J, *et al*. ADRB2 expression predicts the clinical outcomes and is associated with immune cells infiltration in lung adenocarcinoma. *Sci Rep*. 2022;12(1):15994.

doi: 10.1038/s41598-022-19991-y

66. Tu Y, Yao S, Chen Q, Li W, Song Y, Zhang P. 5-Hydroxytryptamine activates a 5-HT/c-Myc/SLC6A4 signaling loop in non-small cell lung cancer. *Biochim Biophys*. 2022;1866(4):130093.

doi: 10.1016/j.bbagen.2022.130093

67. Li Q, Li J, Wang J, *et al*. PLEK2 mediates metastasis and invasion via α5-nAChR activation in nicotine-induced lung adenocarcinoma. *Mol Carcinog*. 2024;63(2):253-265.

doi: 10.1002/mc.23649

68. Kang G, Jiao Y, Pan P, *et al*. α5-nAChR/STAT3/CD47 axis contributed to nicotine-related lung adenocarcinoma progression and immune escape. *Carcinogenesis*. 2023;44(10-11):773-784.

doi: 10.1093/carcin/bgad061

69. Feng C, Mao W, Yuan C, Dong P, Liu Y. Correction: Nicotine-induced CHRNA5 activation modulates CES1 expression, impacting head and neck squamous cell carcinoma recurrence and metastasis via MEK/ERK pathway. *Cell Death Dis*. 2024;12(12):922.

doi: 10.1038/s41419-024-07261-w

70. Fu Y, Ci H, Du W, Dong Q, Jia H. CHRNA5 contributes to hepatocellular carcinoma progression by regulating YAP activity. *Pharmaceutics*. 2022;12(2):275.

doi: 10.3390/pharmaceutics14020275