Association between the ELAVL1 gene single nucleotide polymorphisms and the Genetic Susceptibility to cervical cancer by high resolution melting in a Tunisian population

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Abstract

Background Human papillomavirus is the major cause of cervical cancer, but only few cases develop into cancer. Nevertheless, HuR (ELAVL1) gene has been implicated in the oncogenesis of certain cancers. The correlation between ELAVL1 gene and the risk of cervical cancer remains unclear. Therefore, this study investigated the effect of ELAVL1 gene polymorphisms (SNPs) in cervical cancer development in Tunisian women.

Method ELAVL1 gene SNPs: ELAVL1 rs12983784 T>C, ELAVL1 rs14394 T>C, ELAVL1 rs74369359 G>T, ELAVL1 rs35986520 G>A, ELAVL1 rs10402477 C>T, ELAVL1 rs12985234 A>G and ELAVL1 rs2042920 T>G, were genotyped by High resolution melting (HRM). SNPStats software was used to perform linkage disequilibrium (LD) and haplotype analysis.

Results Comparing the cervical cancer patients with healthy control participants, the SNPs rs12983784 (P = 0.032), rs74369359 (p =< 10^{-3}) and rs10402477 (P = 0.001) were associated with an increased cervical cancer risk. Contrary to the SNPs rs14394, rs7469359, rs35986520, rs12985234 and rs2042920 (p>0.05). The haplotype analysis of the seven SNPs of ELAVL1 gene showed that there is no association between the different haplotypes and a possible risk of cervical cancer disease. Moreover, there was a significant Linkage disequilibrium between rs35986520 and rs2042920 (D’=0.9972) and between rs2042920 and rs10402477 (D’=0.9977).

Conclusion Our results indicated that genetic variants in the ELAVL1 gene might be associated with susceptibility to cervical cancer in the Tunisian population.

Keywords Cervical cancer · HuR · Polymorphism · RNA-binding protein · HRM

Abbreviations

HPV human papillomavirus.  
SNP single nucleotide polymorphism.  
HRM High resolution melting.  
OR odds ratio.  
ARE adenylate and uridylate (AU)-rich elements.  
HuR Hu antigen R; ELA V Like1.  
ELAVL1 Embryonic Lethal, Abnormal Vision, Drosophila)-Like 1 (Hu Antigen R).  
RRM RNA recognition motifs.  
3′-UTR three prime untranslated region.


**Introduction**

Cervical cancer was the fourth most frequent type of malignancy in women worldwide, and the second most predominant cause of cancer-associated mortality among women in developing countries in 2018; with an estimated 604,237 women diagnosed with cervical cancer globally, representing 6.5% of all female cancers (Sung, Ferlay et al. 2021).

In addition, cervical cancer is the fourth leading cause of cancer deaths in women in Tunisia, causing at least 185 deaths each year among the 4.5 million Tunisian women aged 15 years and older who are at risk for the disease (https://www.uicc.org/new-global-cancer-data-globocan-2020).

Several epidemiological studies have proven that infections by certain types of Human papillomavirus (HPV) are closely linked to cervical cancer development. Those studies considered HPV as the major risk factor for the disease [36], Spence, Franco et al. 2005. In addition, environmental risk factors such as smoking (Sugawara, Tsuji et al. 2019), occupational history (Charbotel, Massardier-Pilonchery et al. 2013), oral contraceptive use (Asthana, Busa et al. 2020), diet (Medina-Contreras, Luvían-Morales et al. 2020) and sexual behavior contribute to the onset and the progression of cervical cancer. Genetic factors intensify cervical cancer development as well (Bahrami, Hasanzadeh et al. 2018, Tang, Chen et al. 2019, Chen, Yang et al. 2020).

Polymorphisms can be associated with the expression of several oncogenic factors. Taking into account the importance of the regulation of many growth factors, proto-oncogenic factors and cytokines, in the development of a cell cancer, by differential RNA stability, many events must occur precisely to generate the protein product accurately and efficiently. An estimate ranging from 8 to 16% of several human protein-coding genes has a 3'UTR Adenylate and uridylate (AU)-rich elements (AREs) sequence. Those AREs are defined by their ability to promote rapid deadenylation dependent mRNA decay, mRNA stabilization or translational silencing (Rothamel, Arcos et al. 2021). The ARE may therefore be considered as a crucial regulator of mRNA function at several steps during the lifetime of a transcript. The ARE binding protein HuR (Hu antigen R; ELAVLike1) which is a ubiquitously expressed member of the ELAV Like family of RNA binding proteins, stabilizes some ARE-containing mRNAs in cells. In cell cancer, HuR promotes cancer-related gene expression (Pauzas, Gyvyte et al. 2020, Yang, Yu et al. 2021). Several studies have shown that HuR levels are upregulated in tumour tissues. Although HuR and its associated RNA-binding factors have been investigated in different types of cancer, it is still unclear how this phenomenon occurs in cervical cancer (Lim, Kim et al. 2007, Wang, Guo et al. 2013, Tifaoui, Maudelonde et al. 2018, Xue, Li et al. 2019, Filippova and Nabors [9] Pauzas, Gyvyte et al. 2020).

In the present study, we aim to investigate genetic polymorphisms in ELAVL1 gene with cervical cancer risk in Tunisian population. Among the numerous SNPs, we selected seven polymorphisms (rs1298378, rs14394, rs74369359, rs35986520, rs10402477, rs12985234 and rs2042920) (Supporting information; Table S1), which were investigated in association with breast cancer (Upadhyay, Sanduja et al. 2013). This is the first study dealing with an association between these SNPs and cervical cancer among Tunisian women.

Research in this field may identify new biomarkers and targets that can be used in the future therapeutic approaches of this disease.

**Materials and methods**

**Study population**

A total of 171 female participants were recruited from the Department of Gynaecology and Obstetrics at Tahar Sfar University Hospital, Mahdia, Tunisia. The study group was made up of 71 patients with cervical cancer and 100 women, who were not suffering from any type of cancer or any other serious disease and who served as a healthy control group. Peripheral blood samples were collected from all participants.

The patients and control subjects were from the same geographical area and belonged to the same ethnic group. They were divided into two age group ≤50 and >50. Each participant was interviewed and a short questionnaire about social and demographic features such as age, occupational exposure history, family cancer history, pregnancy and delivery history, sexual behaviour and the use of oral contraceptives was carried out to investigate the increased risk of cervical cancer. An approval of the Ethical Committee (CER-SVS/ISBM 007/2021) and a written informed consent from all participants in the study were obtained.

**The case group:** this group included patients with confirmed cervical cancer consulting reproductive health care in the Obstetrics and Gynaecology Department. Their ages ranged from 17 to 76 years. This group contains 58 patients suffering from cervical squamous cell carcinoma, 7 women carrying cervical adenocarcinoma and 6 cases of other pathological types of cervical cancer. According to the International Federation of Gynaecology and Obstetrics (FIGO), our patients were staged as following: 41 patients were staged I-II, 18 women were staged III-IV and 12 cases were missing (Supporting information; Table S2).
The control group this group included women with no individual history of cancer. Participants in this group were randomly selected and they were matched with those in the case group by age and geographic location.

DNA extraction from peripheral blood lymphocytes

Peripheral venous blood was collected from each participant using a k-EDTA anticoagulated tube. A salting out method was performed to extract the genomic DNA from the blood samples (Miller, Dykes et al. 1988).

5 ml of each sample were placed into glass tubes. Then 5 ml of sterilized water were added. The tubes were centrifuged for 15 min at 3000 rpm. The supernatant was discarded, 20 µl Tris HCl (1 M), 20 µl NaCl (5 M), 40 µl EDTA (0.5 M), 20 µl PK and 120 µl SDS (10%) were added to the pellets. The samples were vortexed for 1 min. These tubes were incubated in a bain marie at 55 °C for 2 H. Then we added 666 µl NaCl to each tube, and finally placed them in the refrigerator at +4 °C for 15 min. The tubes were centrifuged for 15 min at 3000 rpm. The supernatant was transferred quickly to fresh microfuge tube and DNA was precipitated by the addition of 15 ml of ethanol. Precipitated DNA was retrieved using a heat sealed, thin-end glass pipette, washed twice with 0, 5 ml of ethanol (70%) and finally dissolved in a sterilized water. The precipitate was completely dissolved and stored at -80 °C. A Thermo Nano-Drop Nd-1000 Spectrophotometer quantified the extracted DNA.

Genetic polymorphism testing

A High Resolution Melting curve analysis (HRM) using HOT FIREPol® DNA polymerase and EvaGreen® dye (Solis BioDyne, Tartu, Estonia) with a LightCycler 480 system (Roche Diagnostics Mannheim, Germany) using Applied Biosystems High Resolution Melt Software, was performed on double-stranded (ds) DNA samples. The DNA was amplified using a real-time platform prior to the HRM melt phase. The HRM process is a slow denaturation of the dsDNA from 50–95 °C in conjunction with an intercalating of a fluorescent dye. The fluorescence level drops when the two strands ‘melt’ apart. The shape of the curves depends upon the characteristics of the dsDNA, which relate to whether it is homozygous wild type, homozygous mutant or heterozygous wild type and mutant. HRM permits to distinguish between PCR products varying by a minimum of one base (Fig. 1).

Statistical analysis

All data were analysed with SPSS software 25.0. We performed a univariate analysis to assess the association between ELAVL1 gene polymorphisms and genetic susceptibility of cervical cancer which was evaluated using
SNPStats software (http://bioinfo.iconcologia.net/SNPstats) was used to analyse linkage disequilibrium (LD) and to calculate haplotype frequencies for each SNP locus in the case group and control group. p < 0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of study subjects

In this study, the distribution of clinical and demographic characteristics of the healthy women and patients are presented in Table 1. No significant differences were observed in the frequency of distribution of age (p = 0.906), education (p = 0.273), occupational status (p = 0.083), marital status (p = 0.306), menopausal status (p = 0.144), age at menarche (p = 0.503), childbirth (p = 0.392) and the use of oral contraceptives (p = 0.411) in patients with cervical cancer and in healthy controls. No correlation was found between those characteristics and the risk of cervical cancer in the observed Tunisian population.

The study subjects who had experienced more than five pregnancies had a significantly higher frequency in the case group than in healthy control group (53.2% vs. 46.8%). Women who had fewer than two pregnancies had a higher frequency in the control group than in patients (67.9% vs. 32.1%), which proves that a high number of pregnancies is a risk factor for cervical cancer (p = 0.034).

Moreover, the frequency of women with a family history of different types of cancers was found to be higher in the patient group than in the healthy control group (51.2% vs. 48.8%).

However, women without a family history of cancer had a significantly higher frequency in controls than in the case group (67% vs. 33%). This confirms that a family history of cancer is also a significant risk factor for developing cervical cancer (p = 0.016).

Genotype frequencies of Single Nucleotide Polymorphisms of ELAVL1 gene in Patients with cervical cancer and healthy control women

The distribution of genotype frequencies of the seven SNPs (rs12983784, rs14394, rs74369359, rs35986520, rs10402477, rs12985234 and rs2042920) is summarized in Table 2.

In the present study, we compared genotypic frequencies of the selected SNPs between controls and patients and, found that the distribution of genotypes was different.
Genotypic frequencies of ELA VL1 gene SNPs (rs14394, rs35986520, rs1298534 and rs2042920) were not significantly different between the two study groups of healthy women and patients (p > 0.05).

The observed genotypic frequency TC of rs12983784 SNP in patients was significantly higher than that in controls (63.6% vs. 36.4%) which suggests that TC genotype increased cervical cancer risk. In contrast, TT genotypic frequency was significantly higher in the control group than in patients (62.9% vs. 37.1%) which indicates a possible protective effect of this genotype.

GT genotype of the rs74369359 SNP showed a statistically significant difference among patients (90.9% vs. 9.1%), but the GG genotype is statistically higher in the control group (63.4% vs. 36.8%). While GT genotype was found to be a risk factor for cervical cancer, GG genotype might be a protective factor. The same result was obtained with the CT genotype of the SNP rs10402477 that was associated with an increased risk of the disease by a higher manifestation in the patient group (91.7% vs. 8.3%). CC genotype frequency was significantly higher in the control group (63.4% vs. 36.6%) and it might be considered as a protective factor against cervical cancer in the Tunisian population.

### Table 2 Results of the ELAVL1 gene polymorphisms genotype by Group

| SNPs/Genotypes     | Controls, n = 100 (%) | Cases, n = 71 (%) | p-value |
|---------------------|-----------------------|------------------|---------|
| rs12983784          |                       |                  |         |
| TT                  | 88 (62.9)             | 52 (37.1)        |         |
| TC                  | 8 (36.4)              | 14 (63.6)        |         |
| CC                  | 4 (44.4)              | 5 (55.6)         | 0.032   |
| rs14394             |                       |                  |         |
| TT                  | 49 (59.8)             | 33 (40.2)        |         |
| TC                  | 37 (56.1)             | 29 (43.9)        |         |
| CC                  | 14 (60.9)             | 9 (39.1)         | 0.913   |
| rs74369359          |                       |                  |         |
| GG                  | 98 (63.2)             | 56 (36.8)        |         |
| GT                  | 1 (9.1)               | 11 (90.9)        |         |
| TT                  | 1 (20)                | 4 (80)           | 0       |
| rs35986520          |                       |                  |         |
| GG                  | 85 (59.9)             | 57 (40.1)        |         |
| GT                  | 13 (50)               | 13 (50)          |         |
| AA                  | 2 (66.7)              | 1 (33.3)         | 0.540   |
| rs10402477          |                       |                  |         |
| CC                  | 97 (63.4)             | 56 (36.6)        |         |
| CT                  | 1 (8.3)               | 11 (91.7)        |         |
| TT                  | 2 (33.3)              | 4 (66.7)         | 0.001   |
| rs1298534           |                       |                  |         |
| AA                  | 72 (61)               | 46 (39)          |         |
| AG                  | 18 (50)               | 18 (50)          |         |
| GG                  | 10 (58.8)             | 7 (41.2)         | 0.494   |
| rs2042920           |                       |                  |         |
| TT                  | 53 (55.8)             | 42 (44.2)        |         |
| TG                  | 39 (62.9)             | 23 (37.1)        |         |
| GG                  | 8 (57.1)              | 6 (42.9)         | 0.569   |

A-adenine, C-cytosine, G-guanine and T-thymidine

### Table 3 Binary logistic regression analysis for variables associated with cervical cancer risk

| Variable                  | p      | aOR   | 95% CI       | LCL  | UCL  |
|---------------------------|--------|-------|--------------|------|------|
| Age                       | 0.068  | 0.388 | 0.140        | 1.074|
| Menopausal status         | 0.046  | 2.598 | 1.019        | 6.624|
| Family history of cancer  | 0.013* | 2.402 | 1.207        | 4.778|
| ELAVL1 rs74369359 G>T     | 0.007* | 5.528 | 1.606        | 19.032|
| ELAVL1 rs10402477 C>T     | 0.006* | 3.991 | 1.496        | 10.644|

*p < 0.05 are statistically significant. LCL = Lower confidence limit, UCL = Upper confidence limit, CI = Confidence interval, aOR = Adjusted odds ratio

### Table 4 Allele frequency analysis of ELAVL1 SNPs (n=171)

| SNPs/Genotypes | Alleles | T   | C   | p-value |
|----------------|---------|-----|-----|---------|
| rs12983784     | Case    | 118 (83%) | 24 (17%)  | 0.021   |
|                | Control | 184 (92%) | 16 (8%)   | 0.00081 |
| rs14394        | Case    | 95 (67%)  | 47 (33%)  | 0.59    |
|                | Control | 135 (68%) | 65 (32%)  | 0.12    |
| rs74369359     | Case    | 124 (87%) | 18 (13%)  | 0.0095  |
|                | Control | 197 (98%) | 3 (2%)    | 0.015   |
| rs35986520     | Case    | 127 (89%) | 15 (11%)  | 0.56    |
|                | Control | 183 (92%) | 17 (8%)   | 0.14    |
| rs10402477     | Case    | 123 (87%) | 19 (13%)  | 0.015   |
|                | Control | 195 (98%) | 5 (2%)    | 0.00038 |
| rs1298534      | Case    | 109 (77%) | 33 (23%)  | 0.0078  |
|                | Control | 162 (98%) | 38 (19%)  | 0.00015 |
| rs2042920      | Case    | 107 (75%) | 35 (25%)  | 0.33    |
|                | Control | 145 (72%) | 55 (28%)  | 0.8     |

Genotypic frequencies of ELAVL1 gene SNPs (rs14394, rs35986520, rs1298534 and rs2042920) were not significantly different between the two study groups of healthy women and patients (p>0.05).

The observed genotypic frequency TC of rs12983784 SNP in patients was significantly higher than that in controls (63.6% vs. 36.4%) which suggests that TC genotype increased cervical cancer risk. In contrast, TT genotypic frequency was significantly higher in the control group than in patients (62.9% vs. 37.1%) which indicates a possible protective effect of this genotype.

GT genotype of the rs74369359 SNP showed a statistically significant difference among patients (90.9% vs. 9.1%), but the GG genotype is statistically higher in the control group (63.2% vs. 36.8%). While GT genotype was found to be a risk factor for cervical cancer, GG genotype might be a protective factor. The same result was obtained with the CT genotype of the SNP rs10402477 that was associated with an increased risk of the disease by a higher manifestation in the patient group (91.7% vs. 8.3%). CC genotype frequency was significantly higher in the control group (63.4% vs. 36.6%) and it might be considered as a protective factor against cervical cancer in the Tunisian population.
Out of the seven SNPs evaluated in ELAVL1 gene, it was statistically demonstrated that only ELAVL1 gene SNPs rs12983784 (p = 0.032), rs74369359 (p = 0) and rs10402477 (p = 0.001) were associated with an increased risk of cervical cancer in Tunisian women.

The binary logistic regression revealed that menopausal status (aOR = 2, 95% CI = 1.019, 6), family history of cancer (aOR = 2, 95% CI = 1, 4), ELAVL1 rs74369359 G>T (aOR = 5, 95% CI = 1, 19.032) and ELAVL1 rs10402477 C>T (aOR = 3, 95% CI = 1, 10) were the most significant risk factors out of all factors that were conducted in this study. For the variable age (aOR = 0% CI = 0, 1.074), the risk of cervical cancer is likely lower in women within an age >50 (Table 3).

Association between SNPs, haplotypes and cervical cancer risk

Single-locus association analyses of allelic frequency in each group (case and controls) for the ELAVL1 SNPs are presented in Table 4. There were no significant differences of allele frequencies between overall cervical cancer patients and controls at any of those analysed SNPs rs14394, rs35986520 and rs2042920 (p > 0.05). However, statistically significant allele frequencies were found between patients and controls presenting rs12983784, rs74369359, rs10402477 and rs1298534 SNPs (p < 0.05).

For the rs12983784 SNP, the frequency of the allele “T” was significantly higher than the frequency of allele “C” for all subjects. Furthermore, the frequency of the allele “G” of the rs74369359 SNP was significantly higher than the frequency of allele “T” in both cases and controls. Then, for the SNP rs10402477, the allele “C” presented a higher frequency in all subjects than the allele “T”. Finally, the allele “A” of the SNP rs1298534 was more frequent than the allele “G” in our study subjects.

Since haplotype analysis of the ELAVL1 gene may further define the role of this gene in cervical cancer disease, haplotype analysis including all seven analysed SNPs was performed for all the case patients and the control subjects. We computed the linkage disequilibrium (LD) between nearby variants and it is described by D' [18], Bohmanova, Sargolzaei et al. 2010. The analysed SNPs rs12983784, rs14394, rs74369359, rs35986520, rs10402477, rs12985234 and rs2042920 together constitute a relatively weak LD block, and there was a significant linkage between rs35986520 and rs2042920 (D’ = 0.9972). Similarly between rs2042920 and rs10402477 (D’ = 0.9977) indicating a strong LD between these markers, with a weak linkage between rs14394 and rs2042920 (D’ = 0.6825) (Supporting information; Table S3).

The association analysis results between haplotypes and cervical cancer risk are shown in Supporting information; Table S4, which represent results of possible haplotypes formed from our seven SNPs. Global haplotype analysis for the studied SNPs and their association with cervical cancer risk among Tunisian women was relatively far from the statistically significant level (p = 0.099). Apart from the rare haplotype (p = 0.006, aOR = 0.12, 95% CI = 0.03–0.52), no significant results were found.

Discussion

In this study, we observed that the risk of developing cervical cancer was higher among women who had experienced more than five pregnancies. Because of the hormonal changes and immune system modulation during pregnancy, women could be more vulnerable to cancer (Shah, Imami et al. 2018, Jørgensen, Persson et al. 2019). Moreover, the fact that women have become pregnant several times would indicate that they are more sexually active and therefore more exposed to the HPV infection.

Furthermore, menopausal status may also be a potential factor. Post-menopausal women were at higher risk than those who were not yet at menopause. This is in agreement with other research papers. A study carried out by Gyllensten et al. showed that post-menopausal women were at a higher risk of contracting HPV virus (Gyllensten, Gustavsson et al. 2012). Another similar study conducted by Singh B and Nalini N. estimated the incidence of cervical cancer in post-menopausal women at 16% [26].

There is growing evidence that having relatives with any type of cancer puts patients at a higher risk than healthy control women with no family history of the disease. We can not be sure whether this is linked to genes, or due to common shared family habits like diet, healthcare, daily routine, socio-economic status or exposure to environmental risk factors.

The increased incidence of cervical cancer in the Tunisian population shows an urgent need to identify multiple genetic lesions leading to aberrant gene expression programs that are responsible for the cancer phenotype.

Recently, several studies have investigated genes coding for proteins, which regulate a variety of biological processes like cell growth, proliferation, apoptosis, and metabolic pathways that could be potentially useful in cancer diagnosis, prognosis, and therapy (Terasaka, Kim et al. 2019, Xie, Wang et al. [39]).

From this point of view, the study of polymorphisms in genes involved in carcinogenic mechanisms is promising, especially genes coding for RNA-binding proteins (RBPs).
One such RBP is ELAVL1 also known as HuR (Szabo, Dalmu et al. 1991).

The RBP Elavl1/HuR is believed to have ubiquitous expression patterns in most tissues (Ma, Cheng et al. 1996, Lu and Schneider [15] and has three distinct and highly conserved RNA- binding domains belonging to the RNA-recognition motif (RRM)family (Wächter, Köhn et al. 2013). ELAVL1 has been shown to be primarily localized at the nucleus but can translocate to the cytoplasm via phosphorylation of Y200, S202, and S221, located in the hinge region of the protein between the second and third RRM [6].

Furthermore, HuR could contribute to the aberrant gene over expression and promotes the tumorigenesis, by binding selectively to poly-U elements and AU-rich elements (AREs) in the 3'-UTR of target mRNAs for post-transcriptional regulation (Bakheet, Hitti et al. 2018).

The ELAVL1 gene located in chromosome 19p13.2 [17] has been implicated in the occurrence and development of various human cancers. A great deal of research in the literature, has highlighted its oncogenic effects (López de Silanes, Lal et al. 2005, Li, Huang et al. 2020, Ni, He et al. 2020, Palomo-Irigoyen, Pérez-Andrés et al. 2020).

Fang Xue’s study indicated that miRNA-139-3p inhibited the progression of ovarian cancer cells via inhibiting the expression of ELAVL1 (Xue, Li et al. 2019).

A previous study of Ming-Jun Fan and his colleagues observed that ELAVL1 was upregulated in cervical cancer cells and was reported to promote cancer cell growth through regulating RNA in the cell cytoplasm (Fan, He et al. 2020).

The present study investigated a possible association between ELAVL1 gene SNPs and the risk of cervical cancer among Tunisian women.

The investigated SNPs were genotyped according to the difference in melting curves established by HRM. This method of post-PCR is used for identifying genetic variants in suitable regions of interest in our candidate gene.

As a result of this case-control study, no association was found between the ELAVL1 gene SNPs (rs14394, rs35986520, rs12983534 and rs2042920) and the risk of developing cervical cancer. Conversely, women carrying ELAVL1 gene SNPs (rs12983784, rs74369359 and rs10402477) have a high risk for this disease).

Rohit Upadhyay’s report indicated that according to db SNP (https://www.ncbi.nlm.nih.gov/snp/), the ELAVL1 gene has more than 400 SNPs, most of them having less than 5% MAF (Minor allele frequency). However, the selected SNPs had a MAF> 5% and therefore they were chosen for our study (Upadhyay, Sanduja et al. 2013).

A study of Rothamendal showed that 3'UTR binding confers enrichment and transcript stability and demonstrated that ELAVL1 mediates the RNA stability of genes that regulate pathways which are essential to pathogen sensing and cytokine production (Wanke, Devanna et al. 2018).

Multiple diseases, especially cancer arise from anomalies in this region, which affect the expression of one or more genes. According to previous studies and to our findings, the possible explanation is that ELAVL1 single nucleotide polymorphisms in the 3'UTR region could be in the AU-rich region, which leads to deregulation of ARE-binding proteins and cause tumorigenesis. Thus, women with ELAVL1 gene SNPs rs12983784, rs74369359 and rs10402477, which are located in the 3'UTR region, presented high risk of cervical cancer.

In the present study, we assessed the association between rs35986520 and rs2042920 SNPs and between rs2042920 and rs10402477 SNPs, using a haplotype-based case-control analysis. This study suggested that rs2042920 had no impact on cervical cancer risk in Tunisian women. As rs10402477 SNP was higher in patients compared with control subjects, it is obvious that this SNP is a genetic risk factor for cervical cancer among Tunisian women.

The same results were obtained for rs35986520, which was in st rongLD withrs2042920; no significant association was seen with cervical cancer in Tunisian participants.

A possible explanation for the observed results is a possible functional combination of SNP alleles that could alter the ELAVL1 function. The presence of several simultaneous SNPs could alter more the interaction between ELAVL1 and AREs. In summary, the current study detected the genotypes of patients with cervical cancer and healthy controls in a Tunisian population using the HRM. A significant association was observed between the ELAVL1 gene SNPs (rs12983784, rs74369359 and rs10402477) and the risk of cervical cancer. This finding provides evidence that ELAVL1 gene SNPs may be considered as a promising marker of genetic susceptibility to cervical cancer in the Tunisian population.

Further studies are needed to confirm our results on genetic variation in ELAVL1 as a novel prognostic marker and therapeutic target of cervical cancer.

In the same way, expanding our sample size, providing more clinical analysis and adding gene expression data from tumour tissues of the ELAVL1 gene, should be considered in the future to confirm these findings.

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Declarations

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Ethical approval Approval for the Ethical standards of the Ethics Committee for Research in Life Sciences and Health of the ISBM (CER-SVS/ISBM 007/2021) was obtained.

Consent to participate Informed written consent was obtained from all women according to the ethical standards of the Ethics Committee for Research in Life Sciences and Health of the ISBM (CER-SVS/ISBM).

Consent for publication Informed consent was obtained from all participants for whom identifying information is included in this article.

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