

Genetic variations in the *SULF1* gene alter the risk of cervical cancer and precancerous lesions

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Abstract. Human papillomavirus (HPV) infection alone is not sufficient to explain the development of cervical cancer. Genetic variants have been linked to the development of precancerous lesions and cervical cancer. In this study, we aimed to evaluate the association of 10 single nucleotide polymorphisms (SNPs) of the Fas cell surface death receptor (*FAS*),

trinucleotide repeat containing 6C (*TNRC6C*), transmembrane channel like 8 (*TMC8*), DNA meiotic recombinase 1 (*DMC1*), deoxyuridine triphosphatase (*DUT*), sulfatase 1 (*SULF1*), 2'-5'-oligoadenylate synthetase 3 (*OAS3*), general transcription factor IIH subunit 4 (*GTF2H4*) and interferon gamma (*IFNG*) genes with susceptibility to precancerous lesions and cervical cancer. In total, 608 female participants, consisting of 199 patients with persistent low-grade precancerous lesions (CIN1), 100 with high-grade precancerous lesions (CIN2/3), 17 patients with cervical cancer and 292 healthy controls, were enrolled in this study. SNPs were tested for associations with each of the above-mentioned cervical group lesions or when considering an overall patient group. A significant difference for rs4737999 was observed between the controls and the overall patient group considering the recessive mode of inheritance [odds ratio (OR), 0.48; 95% confidence interval (CI), 0.24-0.96; P=0.033]. This effect was even stronger on the risk of CIN1 lesions. Carriers of the rs4737999 AA genotype were almost 3-fold less likely of having low grade lesions compared to the other genotypes. On the whole, this study provides evidence of an influence of the *SULF1* gene rs4737999 SNP in the development of precancerous lesions/cervical cancer.

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Abbreviations: SNP, single nucleotide polymorphism; FAS, Fas cell surface death receptor; TNRC6C, trinucleotide repeat containing 6C; TMC8, transmembrane channel like 8; DMC1, DNA meiotic recombinase 1; DUT, deoxyuridine triphosphatase; SULF1, sulfatase 1; OAS3, 2'-5'-oligoadenylate synthetase 3; GTF2H4, general transcription factor IIH subunit 4; IFNG, interferon gamma; HPV, human papillomavirus; TMC6, transmembrane channel like 6; OR, odds ratio; CI, confidence interval; TNF, tumor necrosis factor; HSPGs, heparan sulfate proteoglycans; 3'-UTR; 3'-untranslated region; CD95, cluster of differentiation 95; FASL, Fas ligand

Key words: sulfatase 1, DNA repair, viral infection, cell entry genes, cervical cancer

Introduction

Cervical cancer is estimated to be the fourth most frequent type of cancer among women worldwide (1) and the fourth leading cause of cancer-related mortality (1-3). Environmental factors, living habits and human papillomavirus (HPV) infection have been linked to the development of cervical cancer (4-6). Specifically, HPV infection is considered to be

one of the most important causal factors related to cervical cancer (7). However, HPV alone, appears to not be sufficient for the development of cervical cancer (8), as only a small amount of HPV-infected women finally develop cervical cancer (3,9).

A number studies have provided evidence of familial clustering of cervical cancer, supporting the existence of genetic effects (10-12). Moreover, several association studies have demonstrated a number of genetic variants that possibly confer susceptibility to cervical cancer by affecting immune responses, DNA repair or viral cell entry and infection (3,13,14). However, uncertainty for the effect size of genetic variants, particularly in different ethnic backgrounds still exists, as the results of different genetic studies have been conflicting (13).

Recently, Wang *et al*, genotyped 7,140 SNPs across 305 genes that were involved in HPV infection, cell entry and DNA repair, and reported that genes, among which general transcription factor IIH subunit 4 (*GTF2H4*), deoxyuridine triphosphatase (*DUT*), DNA meiotic recombinase 1 (*DMC1*), 2'-5-oligoadenylate synthetase 3 (*OAS3*), sulfatase 1 (*SULF1*), interferon gamma (*IFNG*), transmembrane channel like 6 (*TMC6*) and transmembrane channel like 8 (*TMC8*) were associated with the risk of HPV persistence and cervical pre-cancer/cancer (14). The loss of the expression of *DMC1* plays an important role in the development of cancers in human tissues, including cervical cancer lines (15). The *DUT* enzyme influences nucleotide metabolism by producing the immediate precursor of thymidine nucleotides, dUMP, and consequently decreasing the intracellular concentration of dUTP (16). As a result, uracil cannot be incorporated into DNA (16). *SULF1* is a heparin-degrading endosulfatase, which desulfates heparan sulfate proteoglycans (HSPGs) and blocks the binding of growth factors and their receptors, inhibiting as a result, the activation of growth factors and signaling pathways (17,18). *OAS3* is induced during viral infection and plays an important role on the antiviral intracellular innate immune response (19). *GTF2H4* is a general transcription factor that interacts with factors important in carcinogenesis and is involved in processes of DNA repair and transcriptional control (20). *IFNG* is regulatory cytokine, released by lymphocytes, that enhances cellular immune responses via increased T-cell cytotoxicity and natural killer (NK)-cell activity (21). The *TMC6* and *TMC8* genes (also referred to as *EVER1* and *EVER2* genes), are known for the development of Epidermodysplasia verruciformis, which is associated with a high sensitivity to HPV infections (22). The *TMC6* and *TMC8* proteins appear to regulate cellular zinc homeostasis in keratinocytes and lymphocyte (23).

In a previous analysis in a population from Northern Greece (3), we failed to detect a significant effect of two SNPs of the *EVER1/2* gene region (rs2290907 and rs16970849) and the *FAS-670* polymorphism (rs1800682) on precancerous lesions and cervical cancer. This was in contrast to a previous positive study by Castro *et al* (24). *FAS* belongs to the family of tumor necrosis factor (TNF) receptors (25,26). The downregulation of *FAS* leads to resistance to death signals, a phenomenon that has been observed in cervical cancer (27-30).

The present study was designed to replicate the findings reported by Wang *et al* (14) and Castro *et al* (24) in a different, from our previous study (3), Greek population of Central Greece. In particular, we examined the effects of 10 SNPs (rs1800682,

rs5757133, rs3784621, rs4737999, rs12302655, rs2894054, rs11177074, rs2290907, rs9893818 and [*FAS*, *DMC1*, *DUT*, *SULF1*, *OAS3*, *GTF2H4*, *IFNG*, *TMC6* and *TMC8* (2 SNPs)] on the risk of precancerous lesions and cervical cancer.

Materials and methods

Study population. A total of 608 women that had attended the Obstetrics and Gynaecology Clinic of the University Hospital of Larissa, Larissa, Greece participated in this study. The patient group consisted of 316 women with a histopathologically confirmed diagnosis of cervical cancer (n=17) or precancerous lesions, either high grade (CIN2/3, n=100) or persistent low grade (CIN1, n=199). The control group consisted of 292 age-matched women with normal annual cervical cytology screening.

The local Ethics Review Board of the University Hospital of Larissa approved the study protocol. Informed consent was obtained from all individual participants included in the study.

Isolation of DNA and genotyping. Genomic DNA was extracted from 200 μ l of EDTA-anti-coagulated whole blood, using a QIAamp[®] DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. SNPs were genotyped with TaqMan allele-specific PCR amplification technology on an ABI PRISM 7900 Sequence Detection System and analyzed with the Sequence Detection Software (SDS 2.1) (both from Applied Biosystems, Foster City, CA, USA) by laboratory personnel blinded to clinical status. In order to assess genotyping reproducibility, initially observed SNP allelic discrimination curves of all genotypes were confirmed by direct DNA sequencing on an ABI PRISM 3100 genetic analyzer (Applied Biosystems).

Statistical analysis. Hardy-Weinberg equilibrium was examined with the exact test Power calculation analysis performed using the CaTS Power Calculator (31). Genotype-disease association analysis was performed with binary logistic regression using the SNPStats platform (<http://bioinfo.iconco-logia.net/SNPstats/>) (32). Odds ratios (ORs), 95% confidence intervals (CIs) and P-values were calculated assuming the co-dominant (genotypic) model (AA vs. Ab vs. bb) and the recessive (AA + Ab vs. bb) modes of inheritance. Four phenotypic groups were searched for the association with the analyzed SNPs compared to the healthy controls: i) The cervical cancer group; ii) the group of patients with high-grade precancerous lesions (CIN2/3); iii) the group of patients with low-grade precancerous lesions (CIN1); and iv) an overall patient group with abnormal cervical changes (either cervical cancer or any type of precancerous lesions).

Results

The characteristics of the 10 studied SNPs (gene, chromosome, chromosomal position, minor allele and minor allele frequencies) are presented in Table I. The genotype call rate was $\geq 98.85\%$. All studied SNPs were found to follow the Hardy-Weinberg equilibrium either in the cases or the controls (exact test, $P > 0.01$) (33). Genotype call rate and P-value (exact test) for HWE, for each SNP, are presented and Table II.

Table I. Characteristics of SNPs genotyped in the current study.

SNP	rs number	Gene	Chromosome	Chromosome position	Minor allele	MAF CEU	MAF in our control group
1	rs1800682	<i>FAS</i>	10	90739943	C	0.45	0.44
2	rs2290907	<i>TNRC6C</i>	17	76093677	C	0.29	0.16
3	rs16970849	<i>TMC8</i>	17	73645503	A	0.14	0.04
4	rs5757133	<i>DMC1</i>	22	37277781	T	0.23	0.31
5	rs3784621	<i>DUT</i>	15	46420384	C	0.46	0.20
6	rs4737999	<i>SULF1</i>	8	70680589	A	0.20	0.27
7	rs9893818	<i>TMC8/TMC6</i>	17	73653762	A	0.04	0.00
8	rs12302655	<i>OAS3</i>	12	111858889	A	0.13	0.00
9	rs2894054	<i>GTF2H4</i>	6	30980253	T	0.09	0.12
10	rs11177074	<i>IFNG</i>	12	66830701	C	0.14	0.07

SNP, single nucleotide polymorphism; MAF, minor allele frequency; CEU, Utah residents with Northern and Western European ancestry; *FAS*, Fas cell surface death receptor; *TNRC6C*, trinucleotide repeat containing 6C; *TMC8*, transmembrane channel like 8; *DMC1*, DNA meiotic recombina-1; *DUT*, deoxyuridine triphosphatase; *SULF1*, sulfatase 1; *OAS3*, 2'-5'-oligoadenylate synthetase 3; *IFNG*, interferon gamma.

Table II. Genotype call rate and exact test for HWE of each SNP in the current study.

SNP	rs number	Gene	Genotype call rate (%)	Exact test (P-value) for HWE	
				Controls	Cases
1	rs1800682	<i>FAS</i>	99.18	0.096	1
2	rs2290907	<i>TNRC6C</i>	99.67	0.83	0.36
3	rs16970849	<i>TMC8</i>	99.34	1	1
4	rs5757133	<i>DMC1</i>	98.85	0.14	0.11
5	rs3784621	<i>DUT</i>	99.34	0.85	0.6
6	rs4737999	<i>SULF1</i>	99.34	0.29	0.12
7	rs9893818	<i>TMC8/TMC6</i>	99.34	NA	NA
8	rs12302655	<i>OAS3</i>	99.84	1	1
9	rs2894054	<i>GTF2H4</i>	99.67	0.089	0.78
10	rs11177074	<i>IFNG</i>	98.85	0.38	0.71

SNP, single nucleotide polymorphism; HWE, Hardy-Weinberg Equilibrium; NA, non-available; *FAS*, Fas cell surface death receptor; *TNRC6C*, trinucleotide repeat containing 6C; *TMC8*, transmembrane channel like 8; *DMC1*, DNA meiotic recombina-1; *DUT*, deoxyuridine triphosphatase; *SULF1*, sulfatase 1; *OAS3*, 2'-5'-oligoadenylate synthetase 3; *IFNG*, interferon gamma.

The allelic and genotypic frequencies of the studied SNPs in the control and the overall patient group, as well as in the cervical cancer, high-grade precancerous lesion and low-grade precancerous lesion groups are presented in Table III. Of note, as regards rs9893818, all successfully genotyped participants (100%) carried the CC genotype, whereas as regards rs12302655, >99.0% of the participants carried the wild-type genotype.

Power analysis revealed that our study had a statistical power of >80.0% to detect a genetic association with an OR

of 1.78, under the assumption of the multiplicative model, a minor allele frequency of 5% (the lowest in cases for the rs16970849), a type I error level of 0.05, in a sample size consisting of 292 controls and 316 cases (data not shown).

Binary logistic regression analysis demonstrated a significant effect of *SULF1* rs4737999 on the risk of the abnormal cervical changes. In particular, a significant difference was observed between the controls and the overall patient group (low-grade, high-grade and cervical cancer) considering the recessive mode of inheritance (OR, 0.48; 95% CI, 0.24-0.96; P=0.033). Individuals carrying the AA genotype had almost half a risk of having cervical cancer, and low- or high-grade lesions compared to those carrying either the GG or the GA genotypes. Moreover, this effect was even more potent on the risk of low-grade precancerous lesions (OR, 0.36; 95% CI, 0.14-0.92; P=0.042) and (OR, 0.35; 95% CI, 0.14-0.87; P=0.014) in the co-dominant and recessive models, respectively. Carriers of the rs4737999 AA genotype were almost 3-fold less likely of having low-grade lesions compared to carriers of the other genotypes. No other SNP was found to alter the risk of any examined phenotype (Table IV).

The main mechanism of *SULF1* gene is presented in Fig. 1. *SULF1* gene encodes the SULF1 protein. SULF1 is a heparin-degrading endosulfatase, which desulfates HSPGs and blocks the binding of growth factors and their receptors. Consequently, it inhibits the activation of growth factor and the signaling pathways.

Discussion

In the present study, we tried to replicate the findings of previous studies regarding the role of SNPs in DNA repair, viral infection and cell entry, and their effects on the risk of cervical cancer and precancerous lesions (14,24). In addition, we re-examined an independent Greek cohort in order to determine the influence of the rs1800682 (*FAS*), rs2290907 (*TMC6*) and rs16970849 (*TMC8*) gene variants (3). In the present study, we found that a specific variant of the *SULF1*

Table III. Allelic and genotype frequencies of SNPs in healthy controls and in cases (cervical cancer cases, cases with low grade and with high grade precancerous lesions).

SNP	Genotypes/ alleles	Controls n (%)	All cases n (%)	Cervical cancer n (%)	High-grade precancerous lesions, n (%)	Low-grade precancerous lesions, n (%)
rs1800682						
Genotype	C/C	63 (22)	49 (16)	2 (12)	13 (13)	34 (17)
	T/C	129 (44)	150 (48)	8 (47)	50 (51)	92 (47)
	T/T	98 (34)	114 (36)	7 (41)	36 (36)	71 (36)
	Missed	2	3	0	1	2
Allele	T	325 (56)	378 (60)	22 (65)	122 (62)	234 (59)
	C	255 (44)	248 (40)	12 (35)	76 (38)	160 (41)
rs2290907						
Genotype	C/C	8 (3)	4 (1)	1 (6)	1 (1)	2 (01)
	T/C	79 (27)	81 (26)	3 (0.18)	19 (0.19)	59 (0.30)
	T/T	204 (70)	230 (73)	13 (0.76)	80 (0.80)	137 (0.69)
	Missed	1	1	0	0	1
Allele	T	487 (84)	541 (86)	29 (85)	179 (90)	333 (84)
	C	95 (16)	89 (14)	5 (15)	21 (10)	63 (16)
rs16970849						
Genotype	G/A	24 (8)	30 (10)	0 (0)	10 (10)	20 (10)
	G/G	265 (92)	285 (90)	17 (100)	90 (90)	178 (90)
	A/A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Missed	3	1	0	0	1
Allele	G	554 (96)	600 (95)	34 (100)	190 (95)	376 (95)
	A	24 (4)	30 (5)	0 (0)	10 (5)	20 (5)
rs5757133						
Genotype	C/C	142 (49)	156 (50)	11 (65)	45 (45)	100 (51)
	C/T	114 (39)	120 (39)	5 (29)	43 (43)	72 (37)
	T/T	34 (12)	35 (0.11)	1 (6)	11 (11)	23 (12)
	Missed	2	5	0	1	4
Allele	C	398 (69)	432 (69)	27 (79)	133 (67)	272 (70)
	T	182 (31)	190 (31)	7 (21)	65 (33)	118 (30)
rs3784621						
Genotype	C/C	12 (4)	14 (4)	2 (12)	3 (3)	9 (5)
	T/C	92 (32)	97 (31)	4 (25)	30 (30)	63 (32)
	T/T	187 (64)	202 (65)	10 (62)	66 (67)	126 (64)
	Missed	1	3	1	1	1
Allele	T	466 (80)	501 (80)	24 (75)	162 (82)	315 (80)
	C	116 (20)	125 (20)	8 (25)	36 (18)	81 (20)
rs4737999						
Genotype	A/A	24 (8)	13 (4)	1 (6)	6 (6)	6 (3)
	G/A	106 (37)	125 (40)	7 (41)	37 (37)	81 (41)
	G/G	160 (55)	176 (56)	9 (53)	57 (57)	110 (56)
	Missed	2	2	0	0	2
Allele	G	426 (73)	477 (76)	25 (74)	151 (76)	301 (76)
	A	154 (27)	151 (24)	9 (26)	49 (24)	93 (24)
rs9893818						
Genotype	C/C	290 (100)	316 (100)	17 (100)	100 (100)	197 (100)
	C/A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	A/A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Missed	2	2	0	0	2
Allele	C	580 (100)	628 (100)	34 (100)	200 (100)	394 (100)
	A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table III. Continued.

SNP	Genotypes/ alleles	Controls n (%)	All cases n (%)	Cervical cancer n (%)	High-grade precancerous lesions, n (%)	Low-grade precancerous lesions, n (%)
rs12302655						
Genotype	G/G	292 (100)	313 (99)	17 (100)	99 (99)	197 (99)
	G/A	0 (0)	2 (1)	0 (0)	1 (1)	1 (1)
	A/A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Missed	0	1	0	0	1
Allele	G	584 (100)	628 (99.7)	34 (100)	199 (100)	395 (100)
	A	0 (0)	2 (0.3)	0 (0)	1 (0)	1 (0)
rs2894054						
Genotype	C/C	229 (79)	246 (78)	17 (100)	78 (78)	151 (76)
	C/T	54 (19)	67 (21)	0 (0)	22 (22)	45 (23)
	T/T	7 (02)	3 (1)	0 (0)	0 (0)	3 (2)
	Missed	2	0	0	0	0
Allele	C	512 (88)	559 (88)	34 (100)	178 (89)	347 (87)
	T	68 (12)	73 (12)	0 (0)	22 (11)	51 (13)
rs11177074						
Genotype	C/C	0 (0)	1 (0)	0 (0)	0 (0)	1 (1)
	T/C	42 (15)	52 (17)	3 (18)	18 (18)	31 (16)
	T/T	245 (85)	261 (83)	14 (82)	81 (0.82)	166 (84)
	Missed	5	2	0	1	1
Allele	T	532 (93)	574 (91)	31 (91)	180 (0.91)	363 (92)
	C	42 (7)	54 (9)	3 (9)	18 (9)	33 (8)

SNPs, single nucleotide polymorphisms. The rows indicating the 'missed' numbers indicate the number of failed samples (DNA from some participants failed to be genotyped and consequently there were a few missed genotypes). Percentages (%) have been calculated according to the total number of patients in each group.

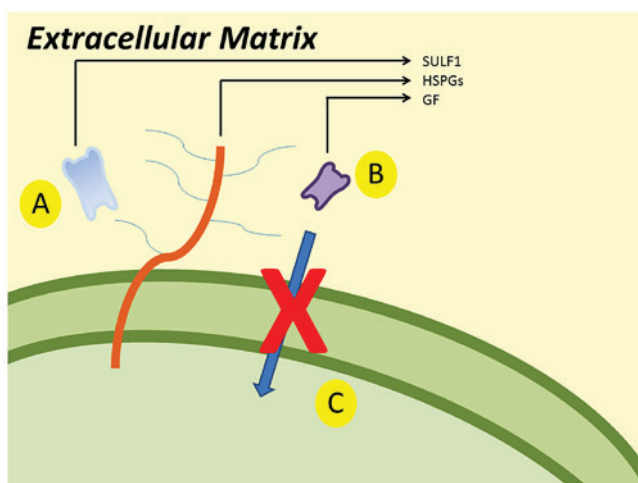


Figure 1. *SULF1* desulfates HSPGs (A) and blocks the binding of growth factors and their receptors (B). As a result it inhibits the activation of growth factor and the signaling pathways (C).

gene, rs4737999, was associated with a significantly decreased risk of developing precancerous lesions and cervical cancer.

The *SULF1* gene is located at the 8q13.3 region. It encodes the homonymous protein, a heparin-degrading endosulfatase, which desulfates HSPGs and blocks the binding of

growth factors and their receptors and as a result it inhibits the activation of growth factor and the signaling pathways (Fig. 1) (17,18). The expression of *SULF1* appears to be stable in normal tissues, whereas it is downregulated in several tumor cells (34). Moreover, the proliferation and migration of tumor cells can be inhibited by the re-expression of the *SULF1* gene (35).

In the study by Wang *et al* (14), the *SULF1* gene reached a statistically significant threshold for the CIN3/Cancer group compared to the controls ($P=0.0030$). Moreover, the *SULF1* gene was also associated with HPV persistence ($P=0.005$). Additionally, according to SNP-Based association analysis, when the CIN3/Cancer group was compared to the control group, 3 out of the 77 examined *SULF1* SNPs (rs4737999, rs4284050 and rs10108002) achieved statistical significance (P -value trend <0.05). In this analysis of Wang *et al*, the strongest association was reported for the rs4737999; this polymorphism also was associated with precancerous lesions and cervical cancer in the present study.

A number of SNPs of the *SULF1* gene have been found to influence the risk of cancer. The AA genotype of rs3802278, a SNP located in the 3'-untranslated region (3'-UTR) of the *SULF1* gene, was found to play a protective role against breast cancer (36). Moreover, rs2623047, a 5'-upstream gene variant in *SULF1*, has been associated with an increased risk of breast cancer, as well as with an earlier age of onset and the

Table IV. Single locus analysis.

SNP	Genotype	All cases (n=316) vs. controls (n=292)		Low-grade (n=199) vs. controls (n=292)		High-grade (n=100) vs. controls (n=292)		Cancer (n=17) vs. controls (n=292)	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs1800682	Codominant								
	T/T	1.00	0.16	1.00	0.47	1.00	0.15	1.00	0.54
	T/C	1.00 (0.70-1.43)		0.98 (0.66-1.48)		1.06 (0.64-1.74)		0.87 (0.30-2.48)	
	C/C	0.67 (0.42-1.06)		0.74 (0.44-1.25)		0.56 (0.28-1.14)		0.44 (0.09-2.21)	
rs2290907	Codominant								
	T/T	1.00	0.37	1.00	0.33	1.00	0.12	1.00	0.57
	T/C	0.91 (0.63-1.31)		1.11 (0.74-1.66)		0.61 (0.35-1.08)		0.60 (0.17-2.15)	
	C/C	0.44 (0.13-1.49)		0.37 (0.08-1.78)		0.32 (0.04-2.59)		1.96 (0.23-16.89)	
rs16970849	Codominant								
	T/T	1.00	0.19	1.00	0.16	1.00	0.27	1.00	0.51
	T/C	0.45 (0.14-1.53)		0.36 (0.08-1.72)		0.36 (0.04-2.89)		2.21 (0.26-18.77)	
	C/C								
rs5757133	Codominant								
	T/T	1.00	0.6	1.00	0.5	1.00	0.61	1.00	0.091
	T/C	1.16 (0.66-2.04)		1.24 (0.67-2.31)		1.23 (0.56-2.66)		0.00 (0.00-NA)	
	C/C								
rs3784621	Codominant								
	T/T	1.00	0.95	1.00	0.86	1.00	0.77	1.00	0.42
	T/C	0.96 (0.68-1.35)		0.90 (0.61-1.33)		1.19 (0.73-1.93)		0.57 (0.19-1.68)	
	C/C	0.94 (0.55-1.58)		0.96 (0.53-1.73)		1.02 (0.48-2.18)		0.38 (0.05-3.04)	
rs4737999	Codominant								
	T/T	1.00	0.86	1.00	0.98	1.00	0.87	1.00	0.42
	T/C	0.95 (0.58-1.58)		1.01 (0.57-1.77)		0.94 (0.46-1.94)		0.47 (0.06-3.66)	
	C/C								
rs4737999	Codominant								
	T/T	1.00	0.97	1.00	0.97	1.00	0.84	1.00	0.4
	T/C	0.98 (0.69-1.38)		1.02 (0.69-1.50)		0.92 (0.56-1.52)		0.81 (0.25-2.66)	
	C/C	1.08 (0.49-2.39)		1.11 (0.46-2.72)		0.71 (0.19-2.59)		3.12 (0.61-15.85)	
rs4737999	Codominant								
	T/T	1.00	0.83	1.00	0.82	1.00	0.62	1.00	0.19
	T/C	1.09 (0.49-2.39)		1.11 (0.46-2.68)		0.73 (0.20-2.63)		3.32 (0.68-16.29)	
	C/C								
rs4737999	Codominant								
	T/T	1.00	0.096	1.00	0.042	1.00	0.75	1.00	0.89
	T/C	1.07 (0.77-1.50)		1.11 (0.76-1.62)		0.98 (0.61-1.59)		1.17 (0.42-3.25)	
	C/C	0.49 (0.24-1.00)		0.36 (0.14-0.92)		0.70 (0.27-1.80)		0.74 (0.09-6.11)	
rs4737999	Codominant								
	T/T	1.00	0.033	1.00	0.014	1.00	0.45	1.00	0.71
	T/C	0.48 (0.24-0.96)		0.35 (0.14-0.87)		0.71 (0.28-1.78)		0.69 (0.09-5.45)	
	C/C								

Table IV. Continued.

SNP	Genotype	All cases (n=316) vs. controls (n=292)		Low-grade (n=199) vs. controls (n=292)		High-grade (n=100) vs. controls (n=292)		Cancer (n=17) vs. controls (n=292)	
		OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs9893818		NA		NA		NA		NA	
rs12302655	G/G	1.00	0.1	1.00	0.18	1.00	0.098	NA	NA
	G/A	NA (0.00-NA)		NA (0.00-NA)		NA (0.00-NA)		NA	
rs2894054									
Codominant	C/C	1.00	0.28	1.00	0.46	1.00	0.1	1.00	0.02
	T/C	1.15 (0.77-1.72)		1.26 (0.81-1.97)		1.20 (0.68-2.09)		0.00 (0.00-NA)	
Recessive	T/T	0.40 (0.10-1.56)		0.65 (0.17-2.55)		0.00 (0.00-NA)		0.00 (0.00-NA)	
	C/C-T/C	1.00	0.15	1.00	0.48	1.00	0.041	1.00	0.37
rs11177074	T/T	0.39 (0.10-1.51)		0.62 (0.16-2.42)		0.00 (0.00-NA)		0.00 (0.00-NA)	
Codominant	T/T	1.00	0.42	1.00	0.39	1.00	0.41	1.00	0.74
	T/C	1.16 (0.75-1.81)		1.09 (0.66-1.80)		1.30 (0.71-2.38)		1.25 (0.34-4.54)	
Recessive	C/C	NA (0.00-NA)		NA (0.00-NA)		NA		NA	
	T/T-T/C	1.00	0.25	1.00	0.18	NA	NA	NA	NA
	C/C	NA (0.00-NA)		NA (0.00-NA)		NA		NA	

SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio. Statistically significant values are shown in bold; NA, not available.

survival of ovarian cancer (37). Finally, rs6990375, a 3' prime UTR variant, has been associated with earlier age of ovarian cancer (38). The SNP rs4737999, that reached a statistically significant threshold in the present study, is an intronic non-coding variant located between exons 13 and 14. Therefore, the *SULF1* gene may represent an important locus linked to tumorigenesis, as SNPs located in the 5'-upstream region, in the 3'-UTR region or even in the middle of the gene have been found to alter the risk of cancer.

A number of studies have reported that the *FAS-670* gene promoter polymorphism is associated with cervical carcinogenesis (39-43). Moreover, the expression of the *FAS/FASL* genes and the CD95-CD95 ligand (*FAS/FASL*) interaction seem to confer susceptibility to the development of cervical cancer (3). However, the present study failed to detect any significant effect of the *FAS* gene SNPs on the risk of cervical cancer or any precancerous lesion. This is in accordance with the results of our previous study in another Greek cohort (3). It is possible that ethnic differences in *FAS* gene variability may account for the different results among populations (44).

In conclusion, the present study confirms the findings of previous reports regarding the role of *SULF1* in the risk of precancerous lesions and cervical cancer. This association may have prognostic and pharmacogenetic implications to precancerous lesions or cervical cancer, as *SULF1* may be considered as a therapeutic target or biomarker (45,46). Our findings need to be replicated in other populations of other ethnic backgrounds and in experimental models, in order to elucidate the possible role of polymorphic variants of the *SULF1* gene in the pathophysiology of mechanism of tumorigenesis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Eda and VS were involved in the conceptualization of the study, data curation, formal analysis and investigation, methodology, project administration, resources, software, study supervision, validation, writing of the original draft and writing, reviewing and editing the manuscript. AG, EP, MK, GX, ED, GG and ENK provided the patient blood samples and clinical information, and were involved in data validation, and in the writing, reviewing and editing of the manuscript. DAS and AT were involved in data investigation and validation, as well as in the writing, reviewing and editing of the manuscript. AD was involved in the conceptualization of the study, data curation, formal analysis and investigation, methodology, project administration, resources, software, study supervision, valida-

tion, writing of the original draft and writing, reviewing and editing the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The local Ethics Review Board of the University Hospital of Larissa approved the study protocol. Informed consent was obtained from all individual participants included in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. *CA Cancer J Clin* 66: 7-30, 2016.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. *CA Cancer J Clin* 61: 69-90, 2011.
3. Pavlidou E, Daponte A, Egea R, Dardiotis E, Hadjigeorgiou GM, Barbadilla A and Agorastos T: Genetic polymorphisms of *FAS* and *EVER* genes in a Greek population and their susceptibility to cervical cancer: A case control study. *BMC Cancer* 16: 923, 2016.
4. Schoell WM, Janicek MF and Mirhashemi R: Epidemiology and biology of cervical cancer. *Semin Surg Oncol* 16: 203-211, 1999.
5. Boda D, Docea AO, Calina D, Ilie MA, Caruntu C, Zurac S, Neagu M, Constantin C, Branisteanu DE, Voiculescu V, *et al*: Human papilloma virus: Apprehending the link with carcinogenesis and unveiling new research avenues (Review). *Int J Oncol* 52: 637-655, 2018.
6. Libra M, Scalisi A, Vella N, Clementi S, Sorio R, Stivala F, Spandidos DA and Mazzarino C: Uterine cervical carcinoma: Role of matrix metalloproteinases (Review). *Int J Oncol* 34: 897-903, 2009.
7. Bosch FX, Lorincz A, Muñoz N, Meijer CJLM and Shah KV: The causal relation between human papillomavirus and cervical cancer. *J Clin Pathol* 55: 244-265, 2002.
8. Baritaki S, Sifakis S, Huerta-Yepez S, Neonakis IK, Soufla G, Bonavida B and Spandidos DA: Overexpression of VEGF and TGF- β 1 mRNA in Pap smears correlates with progression of cervical intraepithelial neoplasia to cancer: Implication of YY1 in cervical tumorigenesis and HPV infection. *Int J Oncol* 31: 69-79, 2007.
9. Castellsagué X and Muñoz N: Chapter 3: Cofactors in human papillomavirus carcinogenesis - role of parity, oral contraceptives, and tobacco smoking. *J Natl Cancer Inst Monogr* 2003: 20-28, 2003.
10. Magnusson PK, Lichtenstein P and Gyllenstein UB: Heritability of cervical tumours. *Int J Cancer* 88: 698-701, 2000.
11. Mammas IN, Zafiroopoulos A and Spandidos DA: Involvement of the ras genes in female genital tract cancer. *Int J Oncol* 26: 1241-1255, 2005.
12. Koffa M, Koumantakis E, Ergazaki M, Malamouitsi V and Spandidos D: Detection of ras gene-mutations and hpv in lesions of the human female reproductive-tract. *Int J Oncol* 5: 189-195, 1994.
13. Chen X, Jiang J, Shen H and Hu Z: Genetic susceptibility of cervical cancer. *J Biomed Res* 25: 155-164, 2011.
14. Wang SS, Gonzalez P, Yu K, Porras C, Li Q, Safaiean M, Rodriguez AC, Sherman ME, Bratti C, Schiffman M, *et al*: Common genetic variants and risk for HPV persistence and progression to cervical cancer. *PLoS One* 5: e8667, 2010.

15. Harada H, Nagai H, Tsuneizumi M, Mikami I, Sugano S and Emi M: Identification of DMC1, a novel gene in the TOC region on 17q25.1 that shows loss of expression in multiple human cancers. *J Hum Genet* 46: 90-95, 2001.
16. Mol CD, Harris JM, McIntosh EM and Tainer JA: Human dUTP pyrophosphatase: Uracil recognition by a beta hairpin and active sites formed by three separate subunits. *Structure* 4: 1077-1092, 1996.
17. Ji W, Yang J, Wang D, Cao L, Tan W, Qian H, Sun B, Qian Q, Yin Z, Wu M, *et al*: hSulf-1 gene exhibits anticancer efficacy through negatively regulating VEGFR-2 signaling in human cancers. *PLoS One* 6: e23274, 2011.
18. Liu H, Fu X, Ji W, Liu K, Bao L, Yan Y, Wu M, Yang J and Su C: Human sulfatase-1 inhibits the migration and proliferation of SMMC-7721 hepatocellular carcinoma cells by downregulating the growth factor signaling. *Hepatol Res* 43: 516-525, 2013.
19. Thamizhmani R and Vijayachari P: Association of dengue virus infection susceptibility with polymorphisms of 2'-5'-oligoadenylate synthetase genes: a case-control study. *Braz J Infect Dis* 18: 548-550, 2014.
20. Gervais V, Lamour V, Jawhari A, Frindel F, Wasielewski E, Dubaele S, Egly JM, Thierry JC, Kieffer B and Poterszman A: TFIIH contains a PH domain involved in DNA nucleotide excision repair. *Nat Struct Mol Biol* 11: 616-622, 2004.
21. Sun Y, Lu Y, Pen Q, Li T, Xie L, Deng Y and Qin A: Interferon gamma +874 T/A polymorphism increases the risk of cervical cancer: Evidence from a meta-analysis. *Tumour Biol* 36: 4555-4564, 2015.
22. Orth G: Host defenses against human papillomaviruses: Lessons from epidermodysplasia verruciformis. *Curr Top Microbiol Immunol* 321: 59-83, 2008.
23. Lazarczyk M, Dalard C, Hayder M, Dupre L, Pignolet B, Majewski S, Vuillier F, Favre M and Liblau RS: EVER proteins, key elements of the natural anti-human papillomavirus barrier, are regulated upon T-cell activation. *PLoS One* 7: e39995, 2012.
24. Castro FA, Ivansson EL, Schmitt M, Joko-Pecirep I, Kjellberg L, Hildesheim A, Gyllenstein UB and Pawlita M: Contribution of TMC6 and TMC8 (EVER1 and EVER2) variants to cervical cancer susceptibility. *Int J Cancer* 130: 349-355, 2012.
25. Contreras DN, Krammer PH, Potkul RK, Bu P, Rossi JL, Kaufmann AM, Gissmann L and Qiao L: Cervical cancer cells induce apoptosis of cytotoxic T lymphocytes. *J Immunother* 23: 67-74, 2000.
26. Dybikowska A, Sliwinski W, Emerich J and Podhajska AJ: Evaluation of Fas gene promoter polymorphism in cervical cancer patients. *Int J Mol Med* 14: 475-478, 2004.
27. Butler LM, Hewett PJ, Butler WJ and Cowled PA: Down-regulation of Fas gene expression in colon cancer is not a result of allelic loss or gene rearrangement. *Br J Cancer* 77: 1454-1459, 1998.
28. Lee SH, Shin MS, Park WS, Kim SY, Dong SM, Pi JH, Lee HK, Kim HS, Jang JJ, Kim CS, *et al*: Alterations of Fas (APO-1/CD95) gene in transitional cell carcinomas of urinary bladder. *Cancer Res* 59: 3068-3072, 1999.
29. Shimonishi T, Isse K, Shibata F, Aburatani I, Tsuneyama K, Sabit H, Harada K, Miyazaki K and Nakanuma Y: Up-regulation of fas ligand at early stages and down-regulation of Fas at progressed stages of intrahepatic cholangiocarcinoma reflect evasion from immune surveillance. *Hepatology* 32: 761-769, 2000.
30. Huang Q, Wang J and Hu Y: FAS-670 gene polymorphism and cervical carcinogenesis risk: A meta-analysis. *Biomed Rep* 1: 889-894, 2013.
31. Skol AD, Scott LJ, Abecasis GR and Boehnke M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 38: 209-213, 2006.
32. Solé X, Guinó E, Valls J, Iñiesta R and Moreno V: SNPStats: A web tool for the analysis of association studies. *Bioinformatics* 22: 1928-1929, 2006.
33. Ziegler A, Van Steen K and Wellek S: Investigating Hardy-Weinberg equilibrium in case-control or cohort studies or meta-analysis. *Breast Cancer Res Treat* 128: 197-201, 2011.
34. Narita K, Staub J, Chien J, Meyer K, Bauer M, Friedl A, Ramakrishnan S and Shridhar V: HSulf-1 inhibits angiogenesis and tumorigenesis in vivo. *Cancer Res* 66: 6025-6032, 2006.
35. Lai J, Chien J, Staub J, Avula R, Greene EL, Matthews TA, Smith DI, Kaufmann SH, Roberts LR and Shridhar V: Loss of HSulf-1 up-regulates heparin-binding growth factor signaling in cancer. *J Biol Chem* 278: 23107-23117, 2003.
36. Zhou Q, Jiang Y, Yin W, Wang Y and Lu J: Single-nucleotide polymorphism in microRNA-binding site of SULF1 target gene as a protective factor against the susceptibility to breast cancer: A case-control study. *Oncotargets Ther* 9: 2749-2757, 2016.
37. Okolicsanyi RK, Faure M, Jacinto JM, Chacon-Cortes D, Chambers S, Youl PH, Haupt LM and Griffiths LR: Association of the SNP rs2623047 in the HSPG modification enzyme SULF1 with an Australian Caucasian breast cancer cohort. *Gene* 547: 50-54, 2014.
38. Han CH, Huang YJ, Lu KH, Liu Z, Mills GB, Wei Q and Wang LE: Polymorphisms in the SULF1 gene are associated with early age of onset and survival of ovarian cancer. *J Exp Clin Cancer Res* 30: 5, 2011.
39. Kordi Tamandani DM, Sobti RC and Shekari M: Association of Fas-670 gene polymorphism with risk of cervical cancer in North Indian population. *Clin Exp Obstet Gynecol* 35: 183-186, 2008.
40. Ueda M, Terai Y, Kanda K, Kanemura M, Takehara M, Yamaguchi H, Nishiyama K, Yasuda M and Ueki M: Fas gene promoter -670 polymorphism in gynecological cancer. *Int J Gynecol Cancer* 16 (Suppl 1): 179-182, 2006.
41. Nunobiki O, Ueda M, Toji E, Yamamoto M, Akashi K, Sato N, Izuma S, Torii K, Tanaka I, Okamoto Y, *et al*: Genetic polymorphism of cancer susceptibility genes and HPV infection in cervical carcinogenesis. *Pathol Res Int* 2011: 364069, 2011.
42. Lai HC, Lin WY, Lin YW, Chang CC, Yu MH, Chen CC and Chu TY: Genetic polymorphisms of FAS and FASL (CD95/CD95L) genes in cervical carcinogenesis: An analysis of haplotype and gene-gene interaction. *Gynecol Oncol* 99: 113-118, 2005.
43. Lai HC, Sytwu HK, Sun CA, Yu MH, Yu CP, Liu HS, Chang CC and Chu TY: Single nucleotide polymorphism at Fas promoter is associated with cervical carcinogenesis. *Int J Cancer* 103: 221-225, 2003.
44. Tan SC, Ismail MP, Duski DR, Othman NH and Ankathil R: FAS c.-671A>G polymorphism and cervical cancer risk: A case-control study and meta-analysis. *Cancer Genet* 211: 18-25, 2017.
45. Hammond E, Khurana A, Shridhar V and Dredge K: The role of heparanase and sulfatases in the modification of heparan sulfate proteoglycans within the tumor microenvironment and opportunities for novel cancer therapeutics. *Front Oncol* 4: 195, 2014.
46. Hur K, Han TS, Jung EJ, Yu J, Lee HJ, Kim WH, Goel A and Yang HK: Up-regulated expression of sulfatases (SULF1 and SULF2) as prognostic and metastasis predictive markers in human gastric cancer. *J Pathol* 228: 88-98, 2012.



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