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ORIGINAL ARTICLE

Effect of Follicle Stimulating Hormone Receptor Gene Polymorphisms in Cervical Cancer Risk

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Abstract For the first time in the word, we investigated the association between five FSHR polymorphisms with the risk of cervical cancer among Tunisians. Study subjects comprised 112 Cervical Cancer (CC) patients and 164 control women. Genotyping of FSHR rs6166, rs1007541, rs11692782, rs2055571 and rs1394205 variants was done by realtime PCR, with defined clusters. The allelic distributions of the tested FSHR SNPs were comparable between CC patients and control women. In contrast, the heterozygous genotype of rs1007541 was associated with 1.8-fold increased risk of CC. Stratification according to FIGO staging revealed that the minor allele of rs1007541 was more frequent among advanced tumor stage patients, with 11-fold increased risk of CC [$P < 0.0001$; OR (95 % CI) = 11.32 (7.46–17.18)]. However, no significant allelic association was revealed in the rest of analyzed *FSHR* SNPs. Haploview analysis showed high Linkage disequilibrium (LD) between rs2055571 and rs1394205. Haplotype analysis revealed a lack of association between cases and controls. However, analysis of CC patient subgroups demonstrated enrichment of GGTAG haplotype in early tumor stage [$P = 0.025$; OR (95 % CI) = 0.07 (0.01–0.70)]. The FSHR variants and haplotypes may be a genetic markers for CC susceptibility and evolution among Tunisian women.

Keywords FSHR · Cervical cancer · FIGO stages · Polymorphisms · Haplotypes · Tunisians

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Introduction

Follicle stimulating hormone (FSH) is a major female sex hormone, and a major determinant of reproduction outcome, owing to its contribution to follicular growth and ovarian steroidogenesis [1, 2]. FSH exert its effects by binding its high affinity cell-bound receptor (FSHR) [3, 4], resulting in intracellular signaling events, characteristic of G-protein-coupled receptors (GPCR) [5, 6]. Structurally, FSHR consists of a large N-terminal extracellular domain, seven-transmembrane domains, and three inter-connected loops, and an intracellular C-terminal tail [7]. The C-terminal portion is located in the cytoplasm, and is rich in serine and threonine residues, which in turn act as substrates for FSHR phosphorylation [8]. FSHR is expressed in granulosa cells, and facilitates follicular maturation in response to the release of FSH from the pituitary. The FSHR gene spans a 54 kb region on chromosome 2p21-p16, and is organized into 10 exons and 9 introns [9, 11]. Single nucleotide polymorphisms (SNPs) in the promoter and the coding region of the FSHR gene were reported, of which the functional Asn680Ser substitution in exon 10 was associated with altered FSH levels. This was highlighted by the finding that 680Ser/Ser homozygous genotype correlated with augmented FSH levels [10]. Functionally, FSHR mutations were associated with ovarian dysgenesis, high gonadotropin levels with primary amenorrhea, thin uteri, small ovaries and sterility, largely due to inhibition of folliculogenesis before antral follicle formation [12–15]. The majority of studies have

focused almost exclusively the ovarian cancer but with inconsistent and inconclusive results [16–20]. However, none has objectified their involvement in the occurrence of cervical cancer (CC). Despite, CC is the third most commonly diagnosed cancer and the fourth leading cause of cancer death among women in the word [21]. Given that FSHR gene is involved in the biochemical pathway of estrogen production, it can be an obvious candidates for initial gynecological cancer. This is the first study in the world that aimed to evaluate five FSHR polymorphisms as a molecular marker of CC.

Subjects and Methods

Subjects

This retrospective case–control study was performed between October 2010 and August 2012 at Salah Azeiz Oncology Institute (SAI), Tunis, Tunisia. Study subjects comprised 112 patients with histological confirmed CC. Clinical data were collected through self-reported questionnaires, and tumor staging was according to International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org). Control subjects consisted of 164 age-matched women who were free of malignancy, drug allergy, hypertension, diabetes, or cardiovascular disease. Controls were recruited during a regular control in the family planning of Tunisian Military Hospital, Regional Hospital of Nefta, and Dispenser of Ettadhamen City. Cases and controls originated from different regions of Tunisia, and were asked to sign an informed consent form agreeing to participate in the study, and all ethics requirements were approved by local research ethics committees. Blood samples were taken from participants in EDTA-containing tube for total genomic DNA extraction prior to radiation therapy or chemotherapy.

FSHR Genotyping

Genomic DNA was extracted using QIAamp DNA Blood Mini kit, according to the instructions of the manufacturer (Qiagen GmbH, Hilden, Germany). We selected five SNPs in FSHR gene, based on minor allele frequency (MAF) of >5 % in Caucasians. FSHR genotyping was performed by the allelic (VIC- and FAM-labeled) discrimination method. TaqMan assays, as assay-on-demand, were ordered from Applied Biosystems (Foster City, CA). The reaction was performed in 6 μl volume on StepOne/StepOne Plus real-time PCR systems, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping reaction; concordance was >99 %.

Statistical Analysis

Statistical analysis was performed on SPSS v. 21.0 (SPSS Inc., Arnok, NY). Data were expressed as percentages of total for

Table 1 Characteristics of study participants

	Cases (n = 112)	Controls (n = 164)	P [1]
Environnemental characteristics			
Age (mean ± SD)	52.0 ± 1.2	52.2 ± 0.9	0.992
≥ 50 years	60 (53.58 %) [2]	66 (40.24 %)	0.036
Married status	108 (96.40 %)	150 (91.50 %)	0.163
Sexual partner: 0	2 (1.80 %)	9 (5.50 %)	0.218
≥ 1	110 (98.20 %)	155 (94.50 %)	
Post-menopausal	82 (73.20 %)	112 (68.30 %)	0.422
Oral contraceptive users	101(90.10 %)	135 (82.30 %)	0.081
Smokers	27 (24.00 %)	7 (4.30 %)	0.000
Family history of cancer	25 (22.30 %)	10 (6.09 %)	0.000
FIGO staging: Stage I	39 (34.80 %)	NA	NA
Stage II	41 (36.60 %)	NA	NA
Stage III	26 (23.20 %)	NA	NA
Stage IV	6 (5.40 %)	NA	NA
Histology: Squamous cell carcinoma	93 (83.03 %)	NA	NA
Adenocarcinoma	16 (14.27 %)	NA	NA
Sarcoma	3 (2.70 %)	NA	NA

FIGO, International Federation of Gynecology and Obstetrics; NA, not applicable

1. Student's t-test (continuous variables), Pearson χ^2 test (categorical variables)
2. Number of subjects (percent total within group)

Table 2 FSHR SNPs allelic distribution in patient and control groups

FSHR SNPs			MAF		HW[c]	χ^a	p_{value}	OR (95 % CI)
rs number	Location[b]	MA	Cases[a]	Controls[a]				
rs6166	48,962,782	**A**	0.892	0.865	0.381	0.099	0.753	1.05 (0.75–1.48)
rs1007541	48,981,895	**A**	0.312	0.207	0.709	3.366	0.066	1.60 (0.96–2.65)
rs11692782	49,064,754	**A**	0.651	0.786	0.001	2.606	0.106	1.34 (0.93–1.91)
rs2055571	49,117,675	**G**	1.017	0.890	0.005	2.175	0.140	1.29 (0.91–1.81)
rs1394205	49,154,446	**A**	0.366	0.371	0.210	0.008	0.930	1.01 (0.65–1.58)

Values in bold are statistically significant at the 5 % Level; χ2 : the Chi-Squared Test

[a] Study subjects included 112 CC cases and 164 control women

[b] Location on chromosome

[c] *HWE* Hardy-Weinberg Equilibrium, *MA* Minor Allele, *MAF* Minor Allele Frequency, *OR* Odds Ratio, Nominal Value of Comparison; $P > 0.05$ no Significant Association, Degree of freedom = 1

categorical variables, or as mean (±SD) for continuous variables. Differences in means were determined by Student's *t*-test, while Pearson's χ^2 or Fisher's exact test were used to assess inter-group significance. Allele frequencies were calculated by the gene-counting method, genotypes were tested for departures from Hardy–Weinberg equilibrium (HWE) in control subjects using Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview).

All analyses were conducted under additive genetic effect, as it is the conservative model, using SNPStats software (http://www.bioinfo.iconcologia.net/snpstats/). Linkage disequilibrium (LD) analysis was performed using Haploview 4.2, and haplotype reconstruction was performed by the expectation maximization method (Haploview 4.2). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95 % confidence intervals (CI) associated with the risk of cervical cancer, taking the control as the reference group (OR =1.00). Statistical significance was set at $P < 0.05$; statistically significant differences being designated as boldface in the tables.

Table 3 *FSHR* genotype distribution in cases and controls

SNPs	Genotypes	Cases[a] *(n,%)*	Controls[a]*(n,%)*	P_{value}	OR (95 % CI)
rs6166					
	G/G	34 (30.40 %)	57 (34.80 %)	-	**1.00 (Reference)**
	G/A	56 (50.00 %)	72(43.90 %)	0.600	1.30(0.75–2.26)
	A/A	22 (19.60 %)	35 (21.30 %)		1.05(0.53–2.08)
rs1007541					
	G/G	78 (69.60 %)	132 (80.50 %)	-	**1.00 (Reference)**
	G/A	33 (29.50 %)	30 (18.30 %)	0.097	**1.86(1.05–3.29)**
	A/A	1 (0.90 %)	2 (1.20 %)		0.85(0.08–9.48)
rs11692782					
	T/T	55 (49.10 %)	69 (42.10 %)	-	**1.00 (Reference)**
	T/A	41 (36.60 %)	61 (37.20 %)	0.320	0.84(0.50–1.43)
	A/A	16 (14.30 %)	34 (20.70 %)		0.59(0.30–1.18)
rs2055571					
	A/A	31 (27.70 %)	59 (36.00 %)	-	**1.00 (Reference)**
	A/G	48 (42.90 %)	65 (39.60 %)	0.330	1.41(0.79–2.49)
	G/G	33 (29.50 %)	40 (24.40 %)		1.57(0.83–2.96)
rs1394205					
	G/G	76 (67.90 %)	109 (66.50 %)	-	**1.00 (Reference)**
	G/A	31 (27.70 %)	47 (28.70 %)	0.970	0.95(0.55–1.62)
	A/A	5 (4.50 %)	8 (4.90 %)		0.90(0.28–2.85)

Values in bold are statistically significant at the 5 % Level

[a] Study subjects comprised 112 CC patients and 164 control women; N Number of Women, OR: Odds Ratio; nominal value of comparison; $P > 0.05$ no significant association, degree of freedom = 1

Results

Study Subjects

The demographic and clinical characteristics of CC cases and control women are presented in Table 1. The median age was 52 years for patients and healthy controls (range: 30–70 years), with most CC cases being in the 51–60 years' category. Among CC patients; 108 (96.40 %) were married, and 82 (73.20 %) were post-menopausal. In addition, 101 (90.10 %) used oral contraceptives, 27 (24.00 %) were smokers, and 25 (22.30 %) reported positive family history of cancer. Diagnoses of squamous cell carcinoma confirmed by histology as per FIGO revealed 39 (34.80 %) with stage I, 41 (36.60 %) with stage II, 26 (32.20 %) with stage III, and the remaining 6 (5.40 %) with stage IV. The majority of the histological types identified were squamous cell carcinoma 93 (83.03 %), followed by adenocarcinoma 16 (14.27 %), and sarcoma 3 (2.70 %).

Association Studies of FSHR Alleles and Genotypes

The allelic distribution of rs6166, rs1007541, rs11692782, rs2055571 and rs1394205 between CC patients and controls are shown in Table 2. Minor allele frequencies of the five analyzed SNPs were comparable between CC patients and healthy women (Table 2). Taking homozygous wild-type genotype as reference (OR =1.00), the genotype distribution of rs1007541 showed a positive association with CC, with the carriage of the heterozygous (G/A) genotype was associated with 1.8-fold increased risk of CC. In contrast, there was no significant association between CC and the genotypes of the remaining four SNPs (Table 3).

Association Studies According FIGO Stages, and CC Evolution

CC patients were stratified into two subgroups: early tumor stage group (stage I or stage II), and advanced tumor stage (stage III or stage IV). Results of *FSHR* allele and genotype frequencies distribution in these subgroups vs. controls are shown in Table 4. Taking homozygous wild-type genotype as reference (OR =1.00), the genotype distribution of the tested *FSHR* SNPs was similar between cases subgroups and controls. The minor allele of rs1007541 was more frequent among advanced tumor stage patients, with 11-fold increased risk of CC [$P < 0.0001$; OR (95 % CI) = 11.32 (7.46–17.18)].

Table 4 Genotype and allele frequency distribution of *FSHR* polymorphisms according FIGO stages

IL-6 SNPs		Controls	Early stages ($n = 80$)(%)	p_{value}	OR (CI 95 %)	Advanced stages ($n = 32$)(%)	p_{value}	OR (CI 95 %)
Rs6166	**G/G**	57 (34.80 %)	25 (31.20 %)	-	**1.00 (Reference)**	9 (28.10 %)	0.640	**1.00 (Reference)**
	G/A	72 (43.90 %)	42 (52.50 %)	0.410	1.33 (0.73–2.44)	14 (43.80 %)		1.23 (0.50–3.05)
	A/A	35 (21.30 %)	13 (16.20 %)		0.85 (0.38–1.87)	9 (28.10 %)		1.63 (0.59–4.50)
	A	142 (43 %)	68 (42 %)	0.862	1.03 (0.70–1.51)	32 (50 %)	0.322	1.30 (0.76–2.23)
Rs1007541	**G/G**	132 (80.50 %)	58 (72.50 %)	-	**1.00 (Reference)**	20 (62.50 %)	0.054	**1.00 (Reference)**
	G/A	30 (18.30 %)	21 (26.20 %)	0.360	1.59 (0.84–3.01)	12 (37.50 %)		2.64 (1.16–5.98)
	A/A	2 (1.20 %)	1 (1.20 %)		1.14 (0.10–12.80)	0 (0.00 %)		NA
	A	34 (10 %)	23 (14 %)	0.194	0.68 (0.39–1.21)	12 (19 %)	**<0.0001**	**11.32 (7.46–17.18)**
Rs11692782	**T/T**	69 (42.10 %)	38 (47.50 %)	-	**1.00 (Reference)**	17 (53.10 %)	0.380	**1.00 (Reference)**
	T/A	61 (37.20 %)	33 (41.20 %)	0.170	0.98 (0.55–1.75)	8 (25.00 %)		0.53 (0.21–1.32)
	A/A	34 (20.70 %)	9 (11.20 %)		0.48 (0.21–1.11)	1(21.90 %)		0.84 (0.32–2.21)
	A	129 (39 %)	51 (32 %)	0.108	1.38 (0.92–2.06)	22 (34 %)	0.454	1.23 (0.70–2.16)
Rs2055571	**A/A**	59 (36.00 %)	22 (27.50 %)	-	**1.00 (Reference)**	9 (28.10 %)	0.480	**1.00 (Reference)**
	A/G	65 (39.60 %)	36 (45.00 %)	0.410	1.49 (0.79–2.81)	12 (37.50 %)		1.21 (0.48–3.08)
	G/G	40 24.40 %)	22 (27.50 %)		1.47 (0.72–3.01)	11 (34.40 %)		1.80 (0.68–4.75)
	G	145 (44 %)	80 (50 %)	0.228	0.79 (0.54–1.15)	34 (53 %)	0.189	0.69 (0.40–1.19)
Rs1394205	**G/G**	109 (66.50 %)	54 (67.50 %)	-	**1.00 (Reference)**	22 (68.80 %)	0.900	**1.00 (Reference)**
	G/A	47 (28.70 %)	22 (27.50 %)	0.980	0.94 (0.52–1.73)	9 (28.10 %)		0.95 (0.41–2.21)
	A/A	8 (4.90 %)	4 (5.00 %)		1.01 (0.29–3.50)	1(3.10 %)		0.62 (0.07–5.20)
	A	63(19 %)	3 (19 %)	0.920	1.03 (0.63–1.66)	11 (17 %)	0.708	1.04 (0.56–2.31)

Values in bold are statistically significant at the 5 % level; ND: not defined

N number of women, *OR* odds ratio; nominal value of comparison; $P > 0.05$ no significant association, degree of freedom = 1

No significant allelic association was revealed in the rest of analyzed *FSHR* SNPs between early tumor stage and advanced tumor stage patients and healthy subjects. In addition, there was lack of association between all tested *FSHR* SNPs and the clinical progression of CC (early stages vs. advanced stages), which was based on the FIGO classification (Table 5).

Haploview Analysis

We evaluated the distribution of 5-locus *FSHR* haplotypes in CC cases and healthy controls by Haploview (Fig. 1). *FSHR* haplotypes containing rs6166-rs1007541-rs11692782-rs2055571-rs1394205 were constructed based on the prevalence of individual SNPs and LD between them (Fig. 1). High LD was demonstrated between rs2055571 and rs1394205, with weak or no LD between rs6166-rs1007541-rs11692782 (Fig.1). Of the 32 possible haplotypes, only 12 were found to be common (frequency > 2 %), and thus were included in further analysis. The distribution of the 12 common haplotypes was comparable between CC cases and control subjects (Table 6). However, analysis of CC patient subgroups demonstrated enrichment of GGTAG haplotype in early tumor stage CC women, suggesting "CC-protective" nature to this haplotype [$P = 0.025$; OR (95 % CI) = 0.07 (0.01–0.70)].

Discussion

Whereas laboratory and epidemiological studies suggested that CC is attributed to infection with oncogenic HPV [22], only a minority of HPV infections progress to malignancy [23]. As such, the progression to invasive cancer and cervical intraepithelial neoplasia is dictated by the concerted action of tumor-promoting factors, along with genetic factors, and pro-inflammatory cytokines [24, 25]. This is the first study which analyzed the association between FSHR polymorphisms and CC. Analysis of rs6166 (2117G > A), rs1007541 (1017G > A), rs11692782 (3465A > T), rs2055571 (171270G > A) and rs1394205 (−29G > A), revealed that allele frequencies of these SNPs were comparable between CC patients and healthy women. However, we noted positive association of only rs1007541 genotypes with CC. Our findings were in agreement with the recent study of Ahsan, which revealed that FSHR is expressed by the microvasculature of metastatic tumors. However, no significant differences between FSHR expression and staining intensity among lung, breast, prostate, colon, kidney, and leiomyosarcoma cancers [26]. This highlights the significance of FSHR as potential marker of cancer, including CC, and hence as target for cancer imaging and likely for therapy [26]. Insofar as FSHR genetic variants influence FSHR expression, resulting in high FSH serum levels and altered responsiveness of sex hormones

Table 5 Genotype and allele frequency distribution of *FSHR* polymorphisms according FIGO stages

IL-6 SNPs		Early stages (n,%)	Advanced stages (n,%)	p_{value}	OR (CI 95 %)
Rs6166	G/G	25 (31.20 %)	9 (28.10 %)	0.370	**1.00 (Reference)**
	G/A	42 (52.50 %)	14 (43.80 %)		1.08 (0.41–2.86)
	A/A	13 (16.20 %)	9 (28.10 %)		0.52 (0.17–1.63)
	A	68 (42 %)	32 (50 %)	0.307	1.35 (0.75–2.42)
Rs1007541	G/G	58 (72.50 %)	20 (62.50 %)	0.380	**1.00 (Reference)**
	G/A	21 (26.20 %)	12 (37.50 %)		0.60 (0.25–1.44)
	A/A	1 (1.20 %)	0 (0.00 %)		NA
	A	23 (14 %)	12 (19 %)	0.416	1.37 (0.63–2.96)
Rs11692782	T/T	38 (47.50 %)	17 (53.10 %)	0.170	**1.00 (Reference)**
	T/A	33 (41.20 %)	8 (25.00 %)		1.85 (0.71–4.82)
	A/A	9 (11.20 %)	1(21.90 %)		0.58 (0.18–1.80)
	A	51 (32 %)	22 (34 %)	0.718	1.11 (0.60–2.06)
Rs2055571	A/A	22 (27.50 %)	9 (28.10 %)	0.720	**1.00 (Reference)**
	A/G	36 (45.00 %)	12 (37.50 %)		1.50 (0.57–3.98)
	G/G	22 (27.50 %)	11 (34.40 %)		1.22 (0.42–3.53)
	G	80 (50 %)	34 (53 %)	0.671	0.88 (0.49–1.57)
Rs1394205	G/G	54 (67.50 %)	22 (68.80 %)	0.900	**1.00 (Reference)**
	G/A	22 (27.50 %)	9 (28.10 %)		1.00 (0.40–2.50)
	A/A	4 (5.00 %)	1(3.10 %)		1.63 (0.17–15.41)
	A	3 (19 %)	11 (17 %)	0.791	0.899 (0.42–1.92)

Values in bold are statistically significant at the 5 % level; ND: not defined

N number of women, *OR* odds ratio; nominal value of comparison; $P > 0.05$ no significant association, degree of freedom = 1

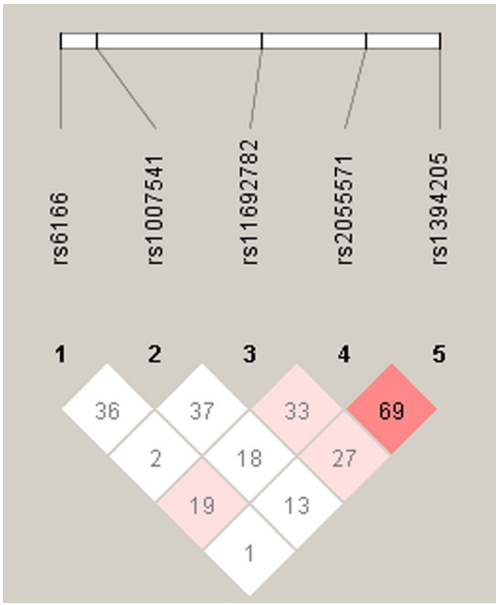

Fig. 1 Linkage disequilibrium (LD) map of the five *FSHR* SNPs genotyped using haploview. The positions of the tested SNPs are indicated above the haploview output. The LD between specific pair of F*SHR* SNPs is indicated by the color scheme, which represents LD relationships, based on D0 values (normalized linkage disequilibrium measure or D) multiplied by 100; D0 is calculated as D divided by the theoretical maximum for the observed allele frequencies. Values approaching zero indicate absence of LD, and those approaching 100 indicate complete LD. The square colored red represent varying degrees of LD < 1 and LOD (logarithm of odds) > 2 scores; darker shades indicating stronger LD

[27, 28], the contribution of FSHR genetic variants to altered risk of cancer was suggested. An earlier study reported on the association of rs6166 (exon 10) with altered FSHR sensitivity, which was paralleled by altered FSH plasma levels throughout the menstrual cycle [29]. In addition, minor allele homozygous genotype of rs6166 was proposed as a risk factor for mild resistance and low responsiveness to FSH action [30, 31]. We found no significant association of rs6166 with CC, which was reminiscent of results observed in testicular cancer [32], in polycystic ovary syndrome (PCOS) [33, 34] and ovarian cancer [35]. The role of rs6166 as determinant of sensitivity to FSH in ovarian cancer yielded mixed results [36, 38]. For example, rs6166 was associated with increased risk of serous and mucinous ovarian cancers subtypes [17], but with no significant association with tumor stages, grades, involvement of ascitic fluid, and the age of patients at first diagnosis [17]. While no significant association was found between rs6166 and premature ovarian failure [39], Ser/Ser variant was shown to be associated with ovarian dysfunction, and carriage of Asn/Ser genotype was linked with higher number of follicles and oocytes [40]. Recent metaanalysis including four case–control studies showed that rs6166 imparts increased risk of ovarian cancer, which was more pronounced in Asians than Caucasians [19]. A later metaanalysis that combined 16 cohort studies revealed that rs6166 is a significant predictor of the number of retrieved oocytes, notably in Asians. Other related outcomes such as exogenous FSH dose, OHSS, and pregnancy status were not influenced by Asn680Ser [20]. We demonstrated no of association rs1007541 with CC. Similar results were recently reported for women with PCOS [33]. Of the five tested FSHR SNPs, only heterozygous rs1007541 was positively associated with CC in Tunisia, in contrast to its association with PCOS [33]. On the other hand, rs11692782, rs2055571 and rs1394205 are not associated with altered risk of CC, which was in contrast to their association with PCOS [33]. While not directly related to CC, it was previously suggested that rs1394205 minor allele and minor allele-carrying genotypes, and rs2055571 minor allele are associated with potential hypertension among women [41]. There are a paucity of data regarding the associational and functional implications of FSHR gene variants in CC. Patient subgroup analysis as per FIGO staging revealed that rs1007541 minor allele is enriched in patients with advanced stages tumor involving. The allele and genotype distribution of the remaining FSHR SNPs were comparable between patients with early or advanced tumor stages when compared to healthy controls. This lack of association was also noted with the pathology results of patient with tumor in early tumor stage vs. patients in advanced tumor stage, suggesting that these FSHR SNPs were not implicated in the progression of CC. Future studies involving larger sample size are required to confirm, or alternatively rule out our conclusion. Since analysis of the presence of single variants within a haplotype is more informative than single variants in determining disease susceptibility, we analyzed the LD pattern between the tested FSHR variants

Table 6 Distribution of 5-locus *FSHR* haplotypes in CC cases and controls

Haplotype[a]	Cases	Controls	p_{value}	OR (95 % CI)
AGTGG	0.241	0.179	-	1.00
GGTAG	0.106	0.141	0.076	0.54 (0.27–1.06)
GGTGG	0.099	0.111	0.380	0.68 (0.29–1.61)
GGAGG	0.051	0.088	0.210	0.59 (0.26–1.35)
GGAAG	0.073	0.092	0.670	0.84 (0.37–1.90)
AGAAA	0.037	0.078	0.110	0.42 (0.15–1.22)
AGAAG	0.038	0.073	0.081	0.37 (0.12–1.12)
GGTAA	0.072	0.021	0.070	2.76 (0.93–8.23)
AGTAG	0.028	0.044	0.490	0.67 (0.22–2.06)
GATAG	0.055	0.021	0.340	2.00 (0.48–8.38)
AGAGG	0.053	0.015	0.150	3.22 (0.66–15.67)
GGAAA	0.019	0.024	0.340	0.36 (0.05–2.92)
Global haplotype association *p*-value = 0.045				

Boldface indicates statistically significant differences

[a] rs6166, rs1007541, rs11692782, rs2055571 and rs1394205 haplotypes frequency; Fisher's exact test. $P > 0.05$ no significant association, degree of freedom = 1

and CC. Moderate-weak LD was seen among the studied SNPs, with clear heterogeneity in the haplotypes obtained. The distribution of the obtained common haplotypes was comparable between CC cases and control subjects. Similar results were revealed among Bahraini women with PCOS [33]. However, haplotype analysis in subgroup cases demonstrated enrichment of GGTAG haplotype among women with tumor in early stage thus conferring disease protection.

Significant association was noted between the incidence of CC and the family history of cancer and smoking, in agreement with previous studies [42, 43]. While not addressed here, it is likely that the presence of a family cancer and smoking may synergize in suppressing local cervical immunity, thus facilitating the development and the progression of cancerous lesions [42]. It is strongly recommends cessation of smoking in at-risk women, in order to prevent progression of cervical lesions. Future controlled studies involving larger sample size and molecular approaches are required to clarify the exact contribution of modifiable and non-modifiable (including genetic) factors to the development and/or evolution of CC.

This is the first study investigating the association of common FSHR SNPs in CC susceptibility and evolution. The present study has some strengths as well as limitations. The women included in this study were of similar ethnicity, thus minimizing the possibility of admixture. In addition, only de novo CC cases prior to chemotherapy and/or radiotherapy were recruited, and the clinical and histological data (type of tumor and FIGO staging) were available for all CC cases. The study had also some limitations, mainly regarding the relatively limited sample size, which affected study power. Furthermore, the HPV status of study participants, thus reducing the role of HPV infection to purely speculative. Further study will be necessary to elucidate the relationship of those polymorphisms with gynecological diseases and the functional differences between the receptors.

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Compliance with Ethical Standards

Conflict of Interest None of the authors have any conflict of interest to declare.

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