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RESEARCH ARTICLE

Polymorphisms in cyclooxygenase-2 gene in endometrial cancer patients

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Abstract The enzyme cyclooxygenase 2 is an inducible enzyme expressed at sites of inflammation and in a variety of malignant solid tumors such as endometrial cancer (EC). In EC patients, its over-expression is correlated with progressive disease and poor prognosis. The expression is encoded by a polymorphic gene, called PTGS2. The aim of the current study was to test the hypothesis that rs5275 polymorphism of PTGS2 influence the prognosis of EC patients. This paper is a retrospective cohort study. Clinical and pathological data were extrapolated and genotypes were assessed on formalin-fixed and paraffin-embedded non-tumor tissues. A total of 159 type I EC patients were included in the final analysis. Univariate analysis indicated that patients with rs5275 genotype CC have a lower risk to develop a grade (G) 2–3 endometrial cancer. rs5275 effect on EC grading was confirmed by multivariate analysis also after data adjusting for age, BMI, parity, hypertension, and diabetes. Adjusted odds ratio (OR) confirmed that patients with rs5275 genotype CC have a risk 80 % lower (OR=0.20, P=0.009) to develop a G2 and/or G3 EC in comparison with patients with TT or TC genotype. Differentiation of the type 1 EC is significantly and independently influenced by rs5275 polymorphism. rs5275 CC patients have a lower risk to present a G2–G3 EC.

Keywords Endometrial cancer · rs5275 · COX 2 · PTGS2 · Grading

Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in developed countries, and 287,000 new cancer cases and 74,000 cancer deaths worldwide were recorded in the 2008 [1–4].

Two types of EC has been reported. Type I is the most common EC, it is an endometrioid adenocarcinoma that can be classified into highly differentiated (G1), moderately differentiated (G2), or undifferentiated (G3) according to FIGO classification. Usually, type I EC is a G1 tumour that is diagnosed at an early stage and is characterized by a good prognosis. Type II EC includes more aggressive histotype and is associated with worse prognosis [1]. Prolonged hyperestrogenism, unopposed by progesterone, has a pivotal role in the pathogenesis of type I EC. Hyperestrogenism characterizes early menarche, late menopause, nulliparity, estrogen only hormone replacement therapy and obesity that are well-known risk factors for type I EC [5, 6].

Single nucleotide polymorphisms (SNPs) of many genes related to sex steroid hormone metabolism pathway has been

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associated with an increased risk for EC [7]. SNPs of aromatase (CYP19A1) influenced susceptibility to EC, particularly among older and obese patients [7]. However, very little of the genetic risk related to these SNPs is actually known.

Recent data suggested that chronic inflammation cooperates with hyperestrogenism in the development of type I EC [8, 9]. Consequences of chronic inflammation are cell proliferation, inhibition of apoptosis, and desoxyribonucloproteic acid (DNA) damage [10]. Mediators of the inflammatory response, such as the enzyme cyclooxygenase (COX), produce prostaglandins (PGs) which together with inflammatory cytokines may suppress the cell-mediated immune responses and promote angiogenesis [10].

COX is the late-limiting enzyme that catalyzes the initial step in the biosynthesis of PGs from arachidonic acid [11]. Two different COX isoforms exist. COX-1 is a constitutionally expressed isoform, while COX-2 is an inducible enzyme expressed at sites of inflammation and in a variety of malignant solid tumors, such as colorectal, gastric, esophageal, pancreas, lung, breast, and EC [12]. The expression of COX-2 was increased in EC, and was associated with the inhibition of apoptosis and the promotion of angiogenesis [13].

In several studies, COX-2 expression was associated with tumor cell differentiation and prognosis. It was demonstrated that in hepatocellular, lung, and ovarian carcinoma [14–16], well-differentiated tumors have higher COX-2 concentration than poorly differentiated ones.

Opposite data were obtained by other studies conducted on oral, hypopharyngeal and bladder cancer. In these cases COX-2 expression was demonstrated to be significantly higher in poorly differentiated tumors and associated with poor prognosis and advanced stage [17–19]

COX-2 is encoded by a polymorphic gene, called PTGS2. COX-2 expression might be influenced by variant alleles located in the promoter region, next to binding sites for transcription factors [14, 15], and in the 3′-untranslated region, in regions involved with the maintenance of mRNA stability [16, 20].

The association between the T8473C (rs5275) polymorphism of COX-2 gene and the increased risk of tumors, often characterized by COX-2 overexpression [21–33], was described in several original articles.

Rs5275 SNP resides within the 3′UTR sequence of COX-2 gene and that region was described to target COX-2 mRNA for degradation through micro-RNA-mediated regulation. In vitro miR-542-3p was demonstrated to regulate COX-2 expression. In particular, in Hela and colorectal cancer cell, miR-542-3p binds transcripts derived from rs5275 T allele and consequently promotes COX-2 mRNA decay, while the presence of C allele interferes with this miRNA allowing mRNA stabilization and COX-2 higher expression [34].

Although the role of COX-2 in the pathogenesis of EC is well known, no data investigated the association between PTGS2 SNPs and EC prognosis. Based on previous assumptions, this study was aimed to evaluate the association between the rs5275 SNP of PTGS2 and the clinical and pathological characteristics of patients with type I EC in order to assess its prognostic value on clinical outcomes.

Methods

Patient samples

The study was designed following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [35]. The Local Ethical Committee approved the study design, and all patients provided written informed consent to use personal non-sensitive data at hospital admission.

Clinical charts of EC patients treated and followed at the IRCCS - Santa Maria Nuova Hospital of Reggio Emilia (Italy) from 1997 to 2010 were checked for inclusion and exclusion criteria. Tissue samples of the included patients were retrieved, prepared, and stored in the tissue storage system of the Department of Pathology. Genomic DNA extraction and purification, and rs5275 genotyping were then performed in the Laboratory of Molecular Biology.

Clinical, pathological, and genetic data were recorded in an electronic separate, anonymous, password-protected database. All relevant data were extrapolated and used for final analysis.

Patients with histological diagnosis of type I, endometrioid EC who received upfront adequate surgery treatment were electively included in the protocol study. Exclusion criteria were as follows: histological diagnosis of type II (non endometrioid tumors) EC, inadequate EC management according to internal and international guidelines [36, 37], neoadjuvant chemotherapy performed before surgery, an age less than 18 years, non-Caucasian ancestry, a follow-up length shorter than 6 months, inadequate follow-up according to internal guidelines, absence of written informed consent, diagnosis of a previous or concurrent cancer(s), and unavailable follow-up data.

An “adequate” management was considered as follows: total extrafascial hysterectomy (TEH) with bilateral salpingo-oophorectomy (BSO) was the standard staging procedure, whereas radical hysterectomy (RH) was performed only in stage II EC patients with gross cervical involvement; pelvic with/without paraaortic lymph node dissection were performed in case of myometrial invasion greater than 50 percent, large tumor (>2 cm in diameter) or filling the endometrial cavity. Vaginal brachytherapy alone was administered to patients at stage IA G3 and IB G1 or G2 without negative prognostic factors. External beam radiotherapy plus vaginal brachytherapy was administered to patients at stage IA G3 and IB G1 and G2 with negative prognostic factors, and to patients at stage IB G3 and to all patients at stage II, III, and IV. Chemotherapy was administered to patients at

stage III C and IV. In all cases, chemoradiotherapy consisted of paclitaxel 175 mg/m^2 (P) and carboplatin AUC5 (C) on day 1 every 3 weeks, for a total of four to six cycles, and it was followed by external pelvic radiation therapy (1.8 Gy/d, d1-5) at a total dose of 45 Gy plus vaginal brachytherapy (3×5 Gy) [36, 38].

A follow-up was defined "adequate" in case of adherence to the following schedule: type I EC at stage IA and grading G1/G2—physical and gynecological examination, and transvaginal ultrasound every 6 months for the first 2 years, and then every 12 months for at least 3 years; type I EC at stage IB and/or any grading G3 tumor—physical and gynecological examination, and transvaginal ultrasound every 6 months for the first 5 years. Further investigations such as abdominal ultrasound, chest X-ray, computed tomography scan, and serum CA 125 levels were performed if clinically indicated.

The same pathologist (M.C.G.) with long-time expertise in gynecological oncology reviewed all the histological samples in order to confirm formally the diagnosis.

DNA extraction and genotyping

Two operators (E.F, B.C.) performed the genomic DNA extraction and purification from five 5-μm-thick slices of formalin-fixed and paraffin-embedded non-tumor tissue using the BiOstic® FFPE Tissue DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA). DNA was quantified using Nanodrop 2000 (Thermo Scientific).

Rs5275 genotyping was performed in service by LGC Genomics/KBioscience (Hertfordshire, UK) using KASP™ genotyping assay, which employs a competitive allele-specific PCR (http://www.Kbioscience.co.uk). The major and minor alleles of rs5275 SNP were defined as T and C, respectively.

Immunohistochemistry

Histological slides were evaluated for the expression of COX-2.

Immunohistochemistry was performed on formalin fixed, paraffin embedded, 5-μm-thick EC tissue sections. Staining was performed using a Benchmark XT autoimmunostainer (Ventana Medical Systems, Tucson, Arizona).

After deparaffinization and hydration, Heat-Induced Epitope Retrieval (HIER) was performed on slides inside the system using CC2 buffer (low pH buffer). Rabbit polyclonal primary anti-COX-2 antibody (Novus Biologicals, Littleton, Colorado) was used at dilution of 1:50 and applied to tissue sections and incubated at room temperature for 24 min. Detection was performed using the Ultraview DAB detection Kit (Ventana Medical Systems, Tucson, Arizona), which uses 3,3′-diaminobenzidine (DAB) as chromogen. A Ventana amplification kit was used for increasing the signal.

As positive control, a section of colon carcinoma, with a clear cytoplasmic positivity, was used.

As negative control, a section of myometrial healthy tissues was used.

Statistical analysis

Parametric data were expressed as median and range. Fisher test was used to investigate univariate and multivariate association between rs5275 and clinical and pathological parameters.

The overall survival (OS) was computed as the time period from the date of surgery to either the date of death or last follow up, whichever occurred first. Cox regression hazard model was used to evaluate the effect of each parameter on overall survival, and results were presented as hazard ratio (HR). For statistical analysis, R-2.15.1 software was used. Significant statements referred *P* values of two-tailed tests that were less than 0.05.

Results

After patients' selection for the inclusion and exclusion criteria, a total of 159 EC patients were studied and included in the final analysis. Total extrafascial hysterectomy was performed in 150 (94.3 %) patients, whereas radical hysterectomy was performed in 9 (5.7 %) patients. Salpingo-oophorectomy was performed in 151 (95 %) patients. Omentectomy and appendicectomy were performed in 17 (10.7 %) and 4 (2.5 %) patients, respectively. Eighty-two (51.6 %) patients received pelvic lymphadenectomy and only 8 (5 %) patients received lombo-aortic lymphadenectomy. In all patients, a complete resection of the disease was obtained.

After a median follow-up time of 67 months (range, 7 to 151 months), a total of 9 patients died. For the patients who died, the median overall survival was 44 months (range, 7 to 61 months).

In our population, the frequency of major allele T of rs5275 polymorphism was 67.6 %. Genotypes TT, TC, and CC were found, respectively, in 45.2 % (72/159 patients), 44.7 % (71/159 patients), and 10.1 % (16/159 patients) of the studied population. That genotypic distribution of polymorphism resulted in Hardy-Weinberg equilibrium (P=0.81).

Table 1 shows the univariate association between rs5275 polymorphism and clinical and pathological characteristics. An influence of rs5275 CC genotype on EC grading was detected (P=0.02). A significant (P=0.03) association between TC genotype and the patients' age was also observed. However, no significance was detected considering homozygous genotypes. Our data didn't show any significant effect of rs5275 on overall survival (Table 1).

Table 1 Study of the association between rs5275 genotypes and principal clinical and pathological characteristics of the type I EC population analyzed

		Rs5275 genotype						Overall survival		
Characteristics	Patients (*n*%) (*N*=159)	TT (*n*%) (*N*=72)	*P* value	TC (*n*%) (*N*=71)	*P* value	CC (*n*%) (N=16)	*P* value	# Death	HR	*P* value
Age										
≤64	82 (51.6)	30 (41.7)		43 (60.6)		9 (56.2)		2		
>64	77 (48.4)	42 (58.3)	–	28 (39.4)	*0.0247**	7 (43.8)	0.2920	7	4.02	0.082
BMI										
≤28	85 (53.5)	39 (54.1)		39 (54.9)		7 (43.7)		3		
>28	74 (46.5)	33 (45.9)	–	32 (45.1)	0.927	9 (56.2)	0.452	6	2.60	0.177
Parity										
No	24 (15.1)	12 (16.6)		9 (12.7)		3 (18.7)		1		
Yes	135 (84.9)	60 (83.4)	–	62 (87.3)	0.501	13 (81.2)	0.841	8	1.51	0.699
Hypertension										
No	76 (47.8)	37 (51.4)		33 (46.5)		6 (37.5)		2		
Yes	83 (52.2)	35 (48.6)	–	38 (53.5)	0.557	10 (62.5)	0.318	7	4.06	0.081
Diabetes mellitus										
No	128 (80.5)	60 (83.3)		57 (80.3)		11 (68.7)		7		
Yes	31 (19.5)	12 (16.7)	–	14 (19.7)	0.636	5 (31.3)	0.189	2	1.25	0.779
Figo stage[1]										
I–II	145 (91.2)	65 (90.3)		64 (90.1)		16 (100)		7		
III–IV	14 (8.8)	7 (9.7)	–	7 (9.9)	0.978	0 (0)	nd	2	3.03	0.166
Grading										
G1	69 (43.4)	30 (41.8)		27 (38.0)		12 (75.0)		3		
G2–G3	90 (56.6)	42 (59.2)	–	44 (62.0)	0.2069	4 (25.0)	*0.0216**	6	1.54	0.541
Myometrial invasion										
<50 %	89 (56.0)	35 (48.6)		42 (59.2)		12 (75.0)		2		
>50 %	70 (44.0)	37 (51.4)	–	29 (40.8)	0.2069	4 (25.0)	0.0642	7	4.58	0.058
Adjuvant treatment										
None	109 (68.6)	45 (62.5)		48 (67.6)		16 (100)		6		
Radiotherapy	38 (23.9)	21 (29.2)		17 (23.9)		0 (0)		1	0.52	0.542
Chemo-Radiotherapy	12 (7.5)	6 (8.3)	–	6 (8.5)	0.5223	0 (0)	nd	2	3.30	0.143
Lymph node metastasis (108 lymphadenectomy)	108	48		51		9				
No	100 (92.6)	43 (89.6)		48 (94.1)		9 (100)		8		
Yes	8 (7.4)	5 (10.4)	–	3 (5.9)	0.414	0 (0)	nd	1	1.59	0.662
Rs5275 genotype										
TT	72 (45.3)							4		
TC	71 (44.6)							3	0.73	0.687
CC	16 (10.1)							2	2.51	0.289

Last three columns of the table indicate that none of the characteristics analyzed can be significantly associated to patients' overall survival

An analysis of the best-fitting genetic model that support a potential association between rs5275 polymorphism and the tumor grading was performed (Table 2), and univariate analysis suggested the adequacy of the recessive model. Genetic recessive model indicated that only in patients with both C allele, the rs5275 genotype was significantly associated with a lower risk to develop a grade G2-G3 poorly differentiated EC while the presence of an heterozygous genotype with only a C allele was not sufficient to substantially influence the risk.

Rs5275 effect on EC grading was confirmed by multivariate analysis also after data adjustment for age, BMI, parity, hypertension, and diabetes (Table 3). Adjusted OR confirmed that patients with rs5275 genotype CC have an 80 % lower risk (OR 0.20, *P*=0.009) to develop a G2 and/or G3 EC in comparison with patients with TT or TC genotype (Table 3).

Table 2 Analysis of the best-fitting genetic model that support an association between rs5275 polymorphism and the grade of type I EC tumors

	Grade G1 (*N*=69) (*n*%)	Grade G2–G3 (*N*=90) (*n*%)	OR (95%CI)	*P* value
Genotype				
T/T	30 (43.5)	42 (46.7)	1	–
C/T	27 (39.1)	44 (48.9)	1.16 (0.59–2.28)	0.657
C/C	12 (17.4)	4 (4.4)	0.24 (0.06–0.76)	*0.022**
Dominant model				
T/T	30 (43.5)	42 (46.7)	1	–
C/T+C/C	39 (56.5)	48 (53.3)	0.89 (0.47–1.65)	0.689
Recessive model				
T/T+C/T	57 (82.6)	86 (95.5)	1	–
C/C	12 (17.4)	4 (4.4)	0.22 (0.06–0.67)	*0.012**

ORs and *P* value are obtained from an unadjusted Fisher test

Immunohistochemistry was used to evaluate COX-2 protein localization in EC tissues and its expression in patients with different rs5275 genotypes. Staining confirmed COX-2 protein expression in tumor tissues (Fig. 1a–g) while myometrial healthy tissue (Fig. 1h) resulted unstained.

To evaluate the effect of polymorphism on COX-2 protein expression in EC tissues, excluding possible influences by tumor grading, only samples from G1 EC with different rs5275 genotypes were examined, (Fig. 1a–c). Immunohistological cytoplasmic staining of COX-2 resulted to be variable among G1 EC samples. In particular, rs5275 TT genotype tissues (Fig. 1a) showed generally a higher staining compared with TC and CC samples who exhibited respectively medium, with varying degrees of intensity in the cytoplasm of tumour cells (TC genotype) (Fig. 1b) and low or absent staining (CC genotype) (Fig. 1c).

Immunohistochemistry was also used to investigate the expression of COX-2 protein in different grades of endometrioid cancer. To do that, excluding possible rs5275 SNP effects, only TT genotype EC tissues with different grading were examined (Fig. 1d–f). Images demonstrated that the intensity of staining was correlated with tumor grade, and the highest and omogeneous staining was observed in G3 tumour cells (Fig 1d).

Discussion

To our knowledge, this was the first study aimed to test the hypothesis that the COX-2 encoding polymorphic gene PTGS2 influences the histopathological variables of type I EC. In particular, current findings demonstrated that the tumor grading of EC varies significantly according to rs5275 genotypes. In fact, CC genotyped patients showed a lower risk to

Table 3 Study of the association of clinical and genetic features with the grade of type I EC tumors

Features	Status	Grade G1 (*n*%) (*N*=69)	Grade G2–G3 (*n*%) (*N*=90)	Crude OR (95 % CI)	Crude *P* value	Adjusted OR (95 % CI)	Adjusted *P* value
Age (years)	≤64	40	42	–		1	–
	>64	29	48	1.58 (0.84–2.98)	0.158	1.36 (0.68–2.72)	0.376
BMI	≤28	40	45			1	–
	>28	29	45	1.38 (0.73–2.61)	0.319	1.37(0.68–2.76)	0.378
Parity	No	13	11			1	–
	Yes	56	79	1.67 (0.70–4.06)	0.251	1.46 (0.59–3.67)	0.413
Hypertension	No	38	38			1	–
	Yes	31	52	1.68 (0.89–3.17)	0.109	1.53 (0.75–3.16)	0.241
Diabetes mellitus	No	56	72			1	–
	Yes	13	18	1.08 (0.49–2.42)	0.855	0.94 (0.39–2.32)	0.892
rs5275_recessive model	T/T+C/T	57	86			1	–
	C/C	12	4	0.22 (0.06–0.67)	*0.012**	0.20 (0.05–0.63)	*0.009**

Crude ORs and *P* values are obtained using unadjusted Fisher tests. Adjusted ORs and *P* values are obtained from multivariate analysis were all features were fitted together

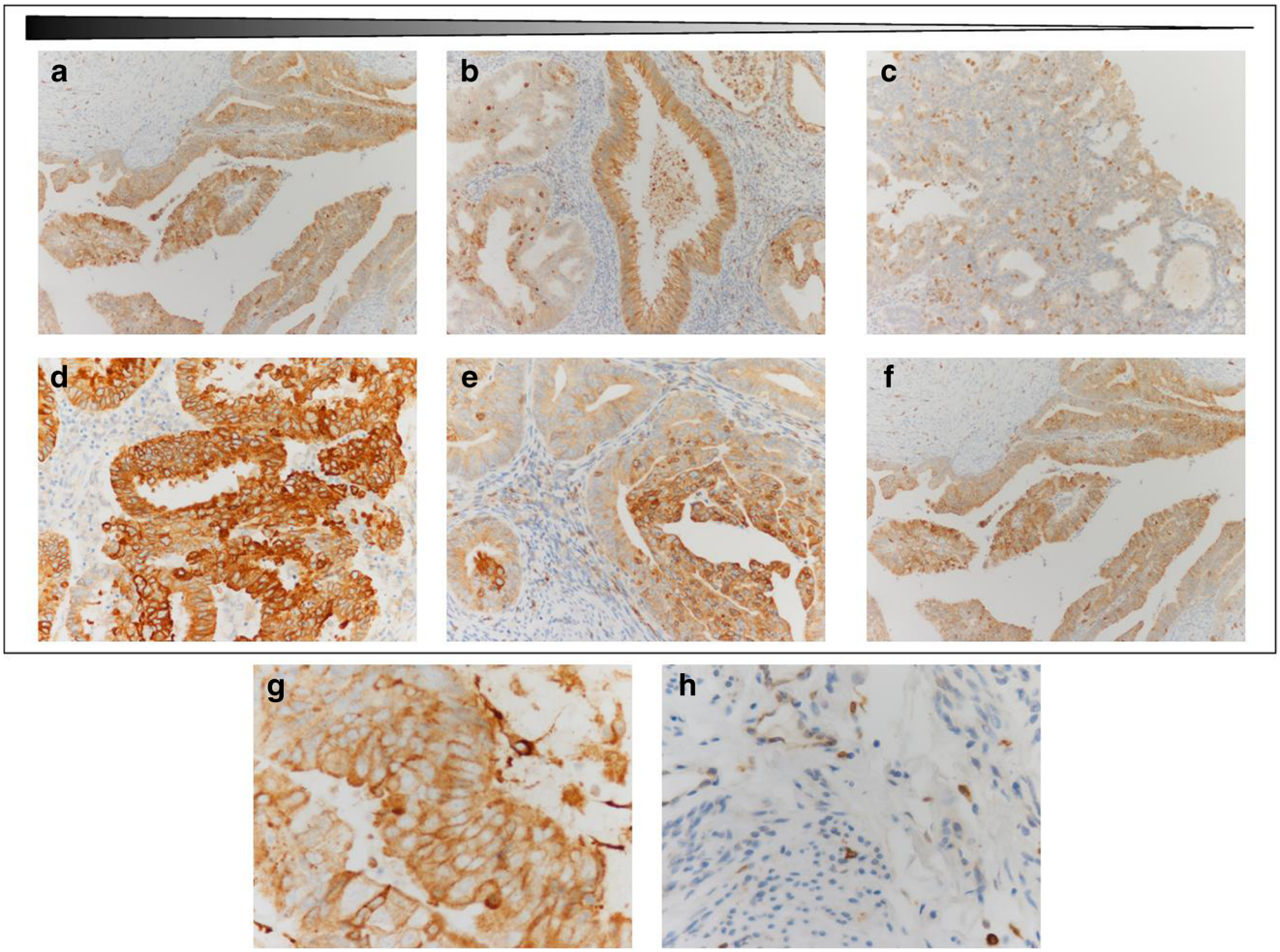

Fig. 1 Immunohistochemistry in EC FFPE tissues. **a** Immunohistochemical stain for COX-2 in G1 EC tissue with rs5275 TT genotype, ×200 magnification . **b** Immunohistochemical stain for COX-2 in G1 EC tissue with rs5275 TC genotype, ×200 magnification. **c** Immunohistochemical stain for COX-2 in G1 EC tissue with rs5275 CC genotype, ×200 magnification. **d** Immunohistochemical stain for COX-2 in G3 EC tissue with rs5275 TT genotype, ×200 magnification. **e** Immunohistochemical stain for COX-2 in G2 EC tissue with rs5275 TT genotype, ×200 magnification. **F** Immunohistochemical stain for COX-2 in G1 EC tissue with rs5275 TT genotype, ×200 magnification. **g** Positive control: Immunohistochemical stain for COX-2 in colorectal cancer tissue, ×400 magnification. **h** Negative control: Immunohistochemical stain for COX-2 in myometrial healthy tissue, ×400 magnification

have G2–G3 poorly differentiated EC. On the other hand, rs5275 SNP genotype could be hypothesize to be an independent protective factor for high-grade type I EC.

In order to explore that influence, our population was studied according to well-known risk factors such as age, BMI, parity, diabetes, and hypertension. Age was significantly associated with EC grade at univariate analysis; older patients presented more G2–G3 EC. However, at multivariate analysis, only rs5275 genotype showed an influence on EC grade.

Tumor grade represents a well-established risk factor, according to tumor grade staging procedure such as systematic lymphadenectomy, and adjuvant treatment are performed or not [38]. Moreover, with the widespread of fertility sparing surgery that can be proposed only in case of type 1 G1 EC at stage 1 [38], to know that the risk of G2-G3 EC is genetically reduced might be reassuring.

Although the role of COX-2 in the pathogenesis of EC is well known, no data exist about rs5275 genotype and EC. Some information comes from studies on breast and ovarian cancer. Breast cancer is an estrogen-dependent tumor, such as EC type 1, in which the main secretory factor inducing aromatase expression in the surrounding stromal cells is the prostaglandin E2 (PGE2) that is synthetized by COX-2 [39]. In breast cancer, the presence of rs5275 T in a certain haplotype might be a risk factor in relation to the growth of untreated breast cancers [40].

Moreover, CC rs5275 genotype was significantly associated with a reduced risk of ovarian cancer particularly among carriers of the CC genotype who were users of non-aspirin non-steroidal anti-inflammatory drugs [32]. About non-gynecological cancers, CC genotype was associated with a reduced risk of lung cancer [41]. On contrary, variant allele

C of rs5275 was significantly associated with an increased risk of gastric cancer particularly in females and no smokers [42], and was associated with an increased risk of esophageal adenocarcinoma in Caucasian population [43]. In addition, C allele was found to be significantly associated with diagnosis of hereditary non-syndromic colorectal cancer and an earlier age of diagnosis. Interestingly, female CC rs5275 carriers were diagnosed less when young, but by 60 years of age were the most at risk group [33].

In Hela and colorectal cancer cells, rs5275 was demonstrated to favor COX-2 mRNA stabilization and consequent COX-2 higher expression interfering with miR-542-3p binding [34], but to our knowledge, the effect of this miRNA on COX-2 expression was not investigated in other tissues in association to rs5275 SNP. Several studies associated COX-2 expression to the grade of differentiation of tumor cells, but contrasting data could suggest a various involvement of COX-2 protein in different sites of cancer [14–19].

Our preliminary data obtained by immunohistochemistry suggested a correlation between COX-2 protein expression and the grade of differentiation in endometrioid cancer. A higher intensity of staining was observed in G3, poorly-differentiated, EC tumors.

In the current study, rs5275 CC genotype was significantly associated with a higher frequency of G1 well-differentiated tumors. Preliminary data obtained from immunohistochemistry suggested a reduced level of COX-2 protein in tumor tissues from patients with CC genotype in comparison with grade equivalent cancer tissues from patients with TT/CT genotypes.

Basing on these initial observations, we suppose that type 1 EC tissues of patients with rs5275 TT/CT genotype could be characterized by COX-2 up-regulation that resulting in enhanced synthesis of prostaglandins, high levels of inflammation and cell proliferation could support the development of less-differentiated tumors. However, further experiment would be necessary to confirm our hypothesis, and no data are available about the way the polymorphism rs5275 can regulate COX-2 expression in endometrial cancer.

The strength of our study concern the selection of a very homogeneous population of type I EC patients who received an upfront surgery with adequate management and follow-up length. The centralization of treatment, of follow-up, and of pathology review are further study strengths that ensured a uniformity of treatment, of staging procedures, post-treatment monitoring, and of histological classification. Instead, in other available studies, the treatment and follow-up protocols widely varied [44, 45]. Moreover, our study has also important limitations. Firstly, it had a retrospective design, and the potential and related biases/confounders are well known. Secondly, it might be underpowered due to the small cohort studied, and current sample size might not be sufficient to detect a synergistic effect in a replicate study.

In conclusion, current preliminary analysis demonstrates that the differentiation of the type 1 EC is significantly and independently influenced by rs5275 polymorphism. Rs5275 CC patients have a lower risk to present a G2-G3 EC. However, further large multicenter studies need to confirm our results, and to assess whether the knowledge of the rs5275 polymorphism might be useful for patients' selection and for management personalization of type 1 EC patients.

Conflicts of interest None

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