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Matrix metalloproteinases 3 polymorphism increases the risk of developing advanced endometriosis and infertility: A case-control study



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ABSTRACT

Objective: Endometriosis has a complex and multifactorial pathology, and it is considered one of the main causes of infertility nowadays. The angiogenic process, which involves remodeling of extracellular matrix, is crucial for the development of this disease, mainly by the action of the matrix metalloproteinase 3 (MMP-3). It is known that genetic factors can influence endometriosis, thus; we investigated the role of *MMP3* 276G>A polymorphism as a risk factor for the development of the disease and its symptoms.

Study Design: This case-control study included 283 women with endometriosis (cases) and 217 women without the disease (controls) who were submitted to laparoscopic or laparotomy surgery. Real-time polymerase chain reaction performed by *TaqMan* system was applied for all polymorphisms. A multivariate logistic regression was performed to evaluate the association between polymorphism and endometriosis or clinical and gynecological characteristics of the disease, using their respective odds ratios (OR) and 95% confidence intervals (CI).

Results: The allelic frequency of the *MMP3* 276G>A polymorphism was 33.6% in controls and 40.3% in endometriosis cases. The allelic distribution was significantly different between the two ($P=0.03$). The variant genotype of *MMP3* 276AA was associated with increased endometriosis risk in the advanced endometriosis cases (OR: 2.08, 95% CI: 1.05 – 4.07 and OR: 1.87, 95% CI: 1.01 – 3.45). Regarding the symptoms, endometriosis-related infertile women had a positive association with the presence of *MMP3* 276G>A polymorphism (OR: 3.13, 95% CI: 1.08–9.08 and OR: 3.30, 95% CI: 1.31 – 8.33).

Conclusions: These findings suggest that the *MMP3* 276A polymorphism is involved with advanced endometriosis cases and infertility, and these associations may implicate in the behavior of disease.

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Introduction

Endometriosis is a complex gynecological disease characterized by the presence of functional endometrial tissue outside the uterus [1]. It affects approximately 10% of women at reproductive age [2]

and directly disturbs their quality of life. This can be because of the delay and elevated cost of the treatment and diagnosis, beyond the incapacitating symptoms [3,4], of which infertility stands out. A recent study conducted in a Swedish cohort with 3406 women, followed by thirty-nine years, showed an increased rate of endometriosis among women with previous infertility problems [5].

Even though endometriosis' etiology is not completely understood, there is strong evidence that angiogenesis is essential for the establishment and growth of endometriotic lesions [6], and genetic components can be involved in this process [7]. Our previous studies have demonstrated that polymorphisms in genes

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that regulates the angiogenic process, such as VEGF and KDR, can be involved in endometriosis risk [8,9]. Another important factor for the angiogenesis is the matrix metalloproteinases (MMPs) family, which consists of enzymes involved in extracellular matrix remodeling [10], allowing the angiogenic process. The MMP-3 (stromelysin-1), encoded by *MMP3*, is a key member of the MMP's family, responsible for laminin, fibronectin, gelatins (I, -V) collagens and cartilage proteoglycans degradation [11]. Some studies have already shown that MMP3 is overexpressed both in endometrial tissue [12] and peripheral blood samples [13].

MMP3 gene is located on chromosome 11, and one of the most studied variants of this gene is the *MMP3* 276G>A single nucleotide polymorphism (SNP), within exon 2 [14]. *MMP3* 276G>A causes an amino acid change (Glu>Lys) at residue 45, that can lead to an interference in MMP-3 interaction with amino acids in this region, therefore, affecting on MMP-3 activation and function [14]. Then, this SNP causes in conformational changes that result in a quick autocatalytic activation to generate a fully active enzyme by removing a portion of the prodomain [14].

To date, there is no data in the literature regarding the association between the *MMP3* 276G>A SNP and endometriosis. Therefore, the objective of this study was to investigate the role of *MMP3* 276G>A polymorphism as a risk factor for the development of endometriosis and its possible associations with endometriosis' symptoms.

Materials and Methods

Study design

This case-control study was approved by the Human Research Ethics Committees of Hospital das Clínicas da Universidade de São Paulo (FMUSP 910/2011), Hospital Federal dos Servidores do Estado (HFSE 414/2011) and Hospital Moncorvo Filho (HMF 1.244.294/2015). All patients were recruited at three Brazilian public hospitals, when assigned for laparoscopic or laparotomy procedures, regardless of the therapeutic indication. Subjects diagnosed with endometriotic lesions by surgery and histologically confirmed were considered as cases (n=283). In the control group, had no visible ectopic endometriosis sites during surgical laparoscopy or laparotomy, that was proposed in order to perform a tubal ligation or treatment of benign diseases (n=217). Women presenting previous diagnostic of adenomyosis, cancer, rheumatoid arthritis or hypertension-related chronic kidney disease were excluded from the study. All participants signed informed consent and their epidemiological, gynecological and clinical data were already published in our previous article [9].

As demonstrated in our previous study [9], endometriosis cases were divided into two groups according to the revised American Fertility Society classification: early stage (women with minimal or mild endometriosis - stage I and II, respectively; n=109) and advanced stage (women with moderate or severe endometriosis - stage III and IV, respectively; n=164). Besides, 10 participants had no information about the staging.

Only women with severe and incapacitating pain were considered as symptomatic cases. Infertility was defined as not being able to get pregnant after one year of regular intercourse and without contraceptive methods use [8,9]. In the present study, the number of infertile women with endometriosis was 126 (44.5%).

Polymorphisms genotyping

A blood sample was obtained from the participants for subsequent DNA extraction, as previously described [8], and to genotyping of the *MMP3* 276G>A (rs679620) polymorphism. Polymorphism amplification and detection was carried by 7500

Real-Time System (Applied Biosystems, Foster City, CA, USA) using *TaqMan* probe (C_3047717_1_). All PCR conditions were described by Perini et al, 2014 [8].

Statistical analysis

All statistical analyses were performed with SPSS (Statistical Package for Social Sciences Inc., Chicago, IL, USA) program version 20.0 and were considered as statistically significant when value of *P* value <0.05 was considered statistically significant.

Hardy-Weinberg equilibrium for the *MMP3* 276G>A SNP was calculated by the goodness-of-fit Chi-Square test. Allelic and genotypic distribution of this polymorphism was performed by gene direct counting and their frequencies were compared using the Chi-Square Test or, when appropriate, the Fisher's exact test. Odds ratio (OR) and 95% confidence interval (CI) were calculated by a multivariate logistic regression model to evaluate the possible associations between SNP and endometriosis or between SNP and gynecological and clinical features of endometriosis. As a final regression model, each variable was introduced in the univariate analysis considering its biological significance and its statistical significance with a *P*-value of 0.20 input and a *P*-value of 0.05 output. Age and Body Mass Index (BMI) were considered as confounding factors in this model.

Results

The mean age and mean BMI in the endometriosis cases were 34.9 ± 7.2 and 24.5 ± 4.6 , respectively, and in the control group were 37.5 ± 8.4 and 27.9 ± 5.8 , respectively. *MMP3* 276G>A SNP was in Hardy-Weinberg equilibrium and the frequency of variant allele *MMP3* 276A (Fig. 1) in the endometriosis cases (40.3%) was statistically higher than the control group (33.6%). Assuming a recessive model for the *MMP3* 276G>A SNP, a significant difference in genotype distribution was observed (*MMP3* 276GG + GA versus 276AA). Patients carrying the *MMP3* 276A allele or the AA genotype had higher risk of developing endometriosis than the *MMP3* 276GG genotype (Table 1). The variables age, BMI, educational attainment, menopausal status and family history of endometriosis were assessed in logistic regression models according to our previous study [9]. After adjustment by significant co-factors that remained in logistic regression model (age and BMI), the *MMP3* 276A allele and the 276AA genotype presented a borderline significant difference. Conversely, *MMP3*

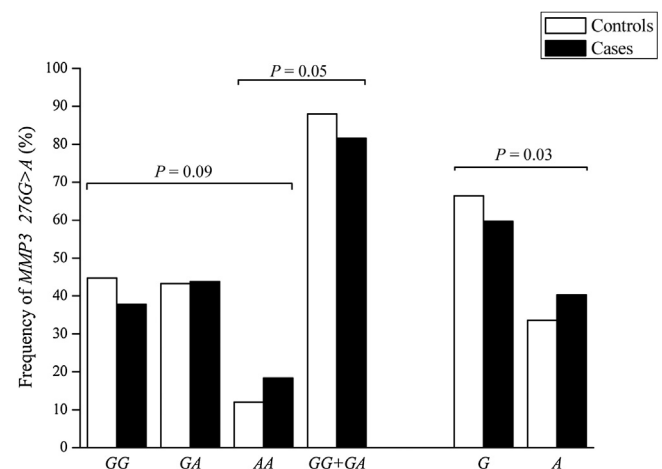


Fig. 1. Allelic and genotypic distribution of the *MMP3* 276G>A polymorphism in the endometriosis cases and control group. *P*-value from Chi-square test (Pearson *P*-value).

Table 1
Association analyses of the *MMP3* 276G>A polymorphism with endometriosis risk.

<i>MMP3</i> 276G>A	Controls (n=217)	Cases (n=283)	ORc (95% CI)	ORa (95% CI)	Cases III/IV (n=164)	ORc (95% CI) ^b	ORa (95% CI) ^b
Genotype							
GG	97 (44.7)	107 (37.8)	1 ^a	1 ^a	59 (36.0)	1 ^a	1 ^a
GA	94 (43.3)	124 (43.8)	1.20 (0.82 – 1.76)	1.28 (0.82 – 2.00)	69 (42.0)	1.21 (0.77 – 1.89)	1.18 (0.71 – 1.98)
AA	26 (12.0)	52 (18.4)	1.81 (1.05 – 3.13)	1.79 (0.96 – 3.34)	36 (22.0)	2.28 (1.25 – 4.15)	2.08 (1.05 – 4.07)
GG+GA	191 (88.0)	231 (81.6)	1 ^a	1 ^a	128 (78.0)	1 ^a	1 ^a
AA	26 (12.0)	52 (18.4)	1.65 (0.99 – 2.75)	1.52 (0.87 – 2.65)	36 (22.0)	2.07 (1.19 – 3.59)	1.87 (1.01 – 3.45)
Allele							
G	288 (66.4)	338 (59.7)	1 ^a	1 ^a	187 (57.0)	1 ^a	1 ^a
A	146 (33.6)	228 (40.3)	1.33 (1.03 – 1.73)	1.15 (0.99 – 1.33)	141 (43.0)	1.49 (1.11 – 2.00)	1.19 (1.01 – 1.41)

ORc = crude odds ratio; ORa = odds ratio adjusted by age and BMI; CI = Confidence Interval; Cases III/IV = Staging of moderate and/or advanced endometriosis (III/IV).

^a Reference group.

^b Controls vs Cases III/IV.

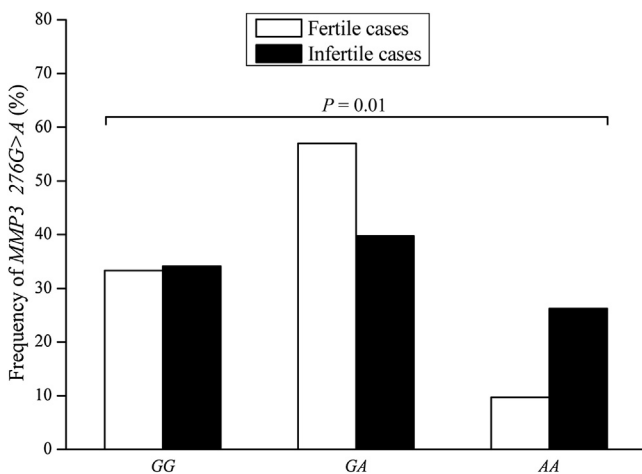


Fig. 2. Allelic and genotypic distribution of the *MMP3* 276G>A polymorphism in fertile and infertile women with endometriosis. P-value from Chi-square test (Pearson P-value).

276G>A SNP presented an increased risk of the advanced stage of endometriosis in either the crude or adjusted OR (Table 1).

Considering the symptoms of endometriosis, we observed that infertile women with endometriosis (26.2%) had a high frequency of the variant genotype *MMP3* 276AA when compared to fertile women (9.7%) with endometriosis (Fig. 2). The presence of the *MMP3* 276AA genotype (versus *MMP3* 276GG and 276GG+GA) was positively associated with endometriosis-related infertility (OR: 3.13; 95% CI: 1.08 – 9.08 and OR: 3.30, 95% CI: 1.31 – 8.33, adjusted by age and BMI). Regarding other endometriosis symptoms (dysmenorrhea, deep dyspareunia, non-cyclic chronic pelvic pain, intestinal and urinary cyclical complaints) no significant differences were found between the two groups (data not shown).

Comment

Our results revealed a positive association between the *MMP3* 276A allele and advanced stage endometriosis. No other study has investigated the association of this SNP in the disease's susceptibility; however, various studies have showed that SNPs involved in genes that take part in extracellular matrix remodeling have been associated with endometriosis and with advanced stage of the disease [16,15]. The *MMP3* 276G>A SNP is localized in a coding region that causes a change of Glutamine to Lysine, leading to an increase *MMP3* activity [14], which can consequently lead to increased angiogenesis and thus the development of endometriosis. Therefore, the *MMP3* 276G>A

SNP has previously been reported in association with gastric adenocarcinoma [16].

Endometriosis is a benign disease; however, it shares several characteristics with malignant cancer [17]. One of these invasive properties is related to the increase of tissue proteolytic activity, resulting in the development of advanced endometriosis [18]. In these cases, overexpression of MMPs can be related with this aggressive behavior. It has been showed an association between *MMP3* gene expression and the expression level of RAS proteins in metastasis formation [19]. Besides that, *MMP3* can activate collagenases and release cell surface molecules, like E-cadherin, an important contributor to cancer development [20]. These observations reinforce the role of *MMP3* in advanced endometriosis.

Endometriosis can negatively affect the reproductive process; however, this cause-and-effect relation was not established yet [5]. In this study, it was observed an increased risk of endometriosis-related infertility in the presence of the *MMP3* 276G>A SNP. Under normal physiological conditions, progesterone levels increase during fertilization to maintain endometrium integrity. It is known that the progesterone inhibits MMPs expression [21], and that withdrawal of this hormone may increase MMPs production. An *in vitro* study conducted by Lahav-Baratz et al. (2004) observed high expression and activity of *MMP-3* in stromal cell culture of endometrial tissue from infertile patients; however, the *MMP-3* activity could not be detected after 2 weeks of progesterone administration [22]. Since that 276A SNP increases *MMP3* activity [14] and *MMP-3* 276AA genotype women were more likely to be infertile, it is possible to suggest that the invasive action of *MMP* in the endometrium may predispose to infertility. Thus, the relationship between *MMP3* SNP in endometriosis-associated infertility needs to be further investigated.

Endometriosis is a multifactorial disease and results from genome wide studies are more informative than those of each polymorphism separately investigated [23]. Nevertheless, is relevant to extract data from distinct populations, with different environmental backgrounds, to construct a database to comprehension of complex diseases [24], such as endometriosis. As far as we know, the present study is the first one to focus on the possible contribution of the *MMP3* 276G>A SNP to the susceptibility to endometriosis or endometriosis-related infertility. Despite the limited number of subjects, this study was conducted in three reference centers for endometriosis treatment and had only controls with surgical diagnosis of absence of endometriosis, which increases credibility and ensures representativeness of data.

In conclusion, we observed that *MMP3* 276G>A SNP was positively associated with advanced stage of endometriosis and with endometriosis-associated infertility. Further studies are needed to associate this SNPs with the expression of gene in endometrial tissue, and thus confirm whether *MMP3* 276G>A SNP can be considered an endometriosis biomarker.

Conflict of interest

All the authors declare that there is no conflict of interest.

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