European Journal of Obstetrics & Gynecology and Reproductive Biology: X 3 (2019) 100041

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology: X

journal homepage: www.elsevier.com/locate/eurox

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

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ARTICLE INFO

Article history:
Received 12 February 2019
Received in revised form 3 May 2019
Accepted 5 May 2019
Available online 6 May 2019

Keywords:
Endometriosis
Polymorphisms
MMP3
Infertility
Angiogenesis

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 276 G > 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 276 G > 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.

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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http://dx.doi.org/10.1016/j.eurox.2019.100041

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 276 G > A* single nucleotide polymorphism (SNP), within exon 2 [14]. *MMP3 276 G > 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>ASNP* and endometriosis. Therefore, the objective of this study was to investigate the role of *MMP3 276 G > 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 endometrium 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 where 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 276 G > 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 276 G > 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 276 G > A* SNP, a significant difference in genotype distribution was observed (*MMP3 276*GG + GA versus *276*AA). Patients carrying the *MMP3 276* A 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*

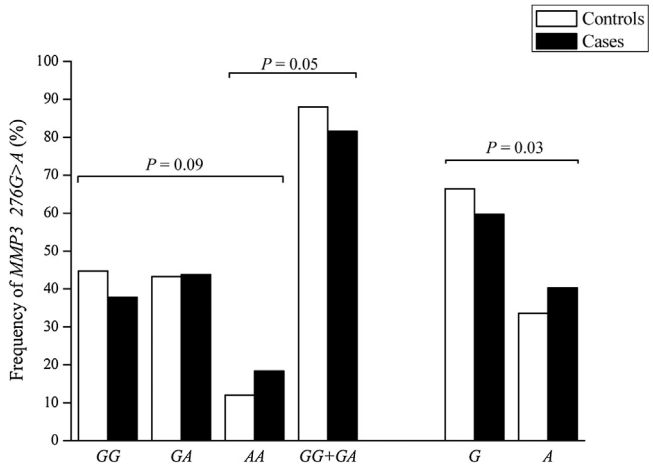

Fig. 1. Allelic and genotypic distribution of the *MMP3 276 G > A* polymorphism in the endometriosis cases and control group. *P*-value from Chi-square test (Pearson *P*-value).

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

MMP3 276 G > 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.

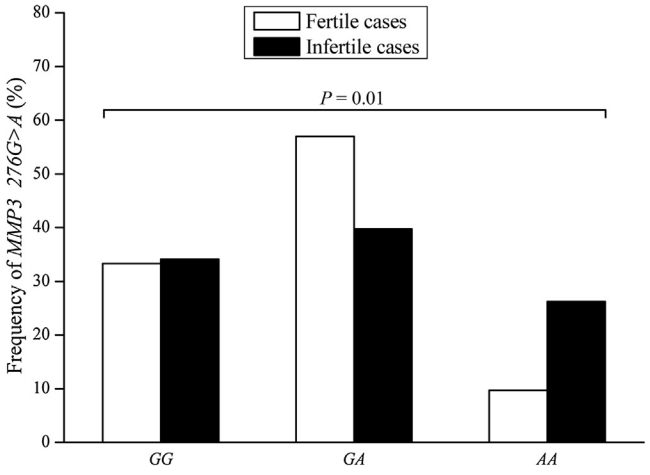

Fig. 2. Allelic and genotypic distribution of the *MMP3 276 G > A* polymorphism in fertile and infertile women with endometriosis. *P*-value from Chi-square test (Pearson *P*-value).

276 G > 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 276 AA* when compared to fertile women (9.7%) with endometriosis (Fig. 2). The presence of the *MMP3 276 AA* genotype (versus *MMP3 276 G G* and *276 G G + 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 276 G > 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 276 G > 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 genoma 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 276 G > 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 276 G > 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 276 G > A* SNP can be considered an endometriosis biomarker.

Conflict of interest

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

Acknowledgments

The authors thank all the staff of the three recruitment hospitals (FMUSP, HFSE and HMF) for their technical assistance and who have contributed to realization this study. This study was supported by the Brazilian agency (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ).

References

[1] Benagiano G, Brosens I, Lippi D. The history of endometriosis. Gynecol Obstet Invest. 2014;78:1–9.
[2] Eisenberg VH, Weil C, Chodick G, Shalev V. Epidemiology of endometriosis: a large population-based database study from a healthcare provider with 2 million members. BJOG 2018;125(January 1)55–62 Epub 2017 Jun 14.
[3] Facchin F, Barbara G, Saita E, Mosconi P, Roberto A, Fedele L, et al. Impact of endometriosis on quality of life and mental health: pelvic pain makes the difference. J Psychosom Obstet Gynaecol 2015;36(4):135–41.
[4] Koltermann KC, Schlotmann A, Schröder H, Willich SN, Reinhold T. Economic burden of deep infiltrating endometriosis of the bowel and the bladder in Germany: The statutory health insurance perspective. Z Evid Fortbild Qual Gesundhwes 2016;118–119(December):24–30.
[5] Gao M, Allebeck P, Mishra GD, Koupil I. Developmental origins of endometriosis: a Swedish cohort study. J Epidemiol Community Health 2019;(January):19.
[6] Taylor RN, Yu J, Torres PB, Schickedanz AC, Park JK, Mueller MD, et al. Mechanistic and therapeutic implications of angiogenesis in endometriosis. Reprod Sci 2009;16:140–6.
[7] Ueda M, Yamashita Y, Takehara M, Terai Y, Kumagai K, Ueki K, et al. Gene expression of adhesion molecules and matrix metalloproteinases in endometriosis. Gynecol Endocrinol 2002;16(October 5):391–402.
[8] Perini JA, Cardoso JV, Berardo PT, Vianna-Jorge R, Nasciutti LE, Bellodi-Privato M, et al. Role of vascular endothelial growth factor polymorphisms (-2578C&A, -460 T&C, -1154G&A, +405G&C and +936C&T) in endometriosis: a case-control study with Brazilians. BMC Womens Health 2014;14:117.
[9] Cardoso JV, Abrão MS, Vianna-Jorge R, Ferrari R, Berardo PT, Machado DE, et al. Combined effect of vascular endothelial growth factor and its receptor polymorphisms in endometriosis: a case-control study. EJOG 2017;209 (February)25–33 Epub 2016 Oct 29.
[10] Osteen KG, Yeaman GR, Bruner-Tran KL. Matrix metalloproteinase and endometriosis. Semin Reprod Med 2003;21:155–64.
[11] Johansson N, Saarialho-Kere U, Airola K, Herva R, Nissinen L, Westermarck J, et al. Collagenase-3 (MMP-13) is expressed by hypertrophic chondrocytes, periosteal cells, and osteoblasts during human fetal bone development. Dev Dyn 1997;208:387–97.
[12] Gilabert-Estelle's J, Ramo'n LA, Espanã F, Gilabert J, Vila V, Réganon E, et al. Expression of angiogenic factors in endometriosis: relationship to fibrinolytic and metalloproteinase systems. Hum Reprod 2007;22:2120–7.
[13] Mabrouk M, Elmakky A, Caramelli E, Farina A, Mignemi G, Venturoli S, et al. Performance of peripheral (serum and molecular) blood markers for diagnosis of endometriosis. Arch Gynecol Obstet 2012;285(May 5):1307–12.
[14] Chen Y, Nixon NB, Dawes PT, Mattey DL. Influence of variations across the MMP-1 and -3 genes on the serum levels of MMP-1 and -3 and disease activity in rheumatoid arthritis. Genes Immun 2012;13(January 1):29–37.
[15] Ye H, He Y, Wang J, Song T, Lan Z, Zhao Y, et al. Effect of matrix metalloproteinase promoter polymorphisms on endometriosis and adenomyosis risk: evidence from a meta-analysis. J Genet 2016;95(September 3):611–9.
[16] Lin Y, Liu J, Jin L, Jiang Y. Polymorphisms in matrix metalloproteinases 2, 3, risk by regulating enzyme activity in gastric and 8 increase recurrence and mortality adenocarcinoma. . Oncotarget 2017;20(November 62)105971–83 8.
[17] Vlahos NF, Kalampokas T, Fotiou S. Endometriosis and ovarian cancer: a review. Gynecol Endocrinol 2010;26(March 3):213–9.
[18] Machado DE, Berardo PT, Palmero CY, Nasciutti LE. Higher expression of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (flk-1) and metalloproteinase-9 (MMP-9) in a rat model of peritoneal endometriosis is similar to cancer diseases. J Exp Clin Cancer Res 2010;19(January 29):4.
[19] Baruch RR, Melinscak H, Lo J, Liu Y, Yeung O, Hurta RA. Altered matrix metalloproteinase expression associated with oncogene-mediated cellular transformation and metastasis formation. Cell Biol Int. 2001;25(5):411–20.
[20] Macer ML, Taylor HS. Endometriosis and infertility: a review of the pathogenesis and treatment of endometriosis-associated infertility. Obstet Gynecol Clin North Am 2012;39(December 4):535–49.
[21] Keller NR, Sierra-Rivera E, Eisenberg E, Osteen KG. Progesterone exposure prevents matrix metalloproteinase-3 (MMP-3) stimulation by interleukin-1α in human endometrial stromal cells. J Clin Endocrinol Metab 2000;85:1611–9.
[22] Lahav-Baratz S, Shiloh H, Koifman M, Kraiem Z, Wiener-Megnazi Z, Ishai D, et al. Early embryo-endometrial signaling modulates the regulation of matrix metalloproteinase-3. Fertil Steril 2004;82(October 3):1029–35.
[23] Rahmioglu N, Missmer SA, Montgomery GW, Zondervan KT. Insights into Assessing the Genetics of Endometriosis2. JCurr Obstet Gynecol Rep 2012;1 (September 3)124–37 Epub 2012 Jun 15.
[24] Newton-Cheh C, Hirschhorn JN. Genetic association studies of complex traits: design and analysis issues. Mutat Res 2005;573(1-2):54–69.