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New candidate genes associated to endometriosis

Denise Maria Christofolini[a,b], Fernanda Abani Mafra[c], Michelle Cristina Catto[b], Bianca Bianco[a,b] and Caio Parente Barbosa[a,b]

[a]Instituto Ideia Fértil de Saúde Reprodutiva, Santo André, Brazil; [b]Discipline of Sexual and Reproductive Health and Populational Genetics, Department of Collective Health, Faculdade de Medicina do ABC, Santo André, Brazil; [c]Center for Applied Genomics, The Children's Hospital of Philadelphia, Philadelphia, USA

ABSTRACT

A previous GWAS study performed on Brazilian pooled samples indicated some SNPs (single nucleotide polymorphisms) differentially frequent in infertile patients with endometriosis and controls. Some of them were located in the genes whose biological function suggests that they could be associated with endometriosis pathogenesis; thus, the purpose here was to confirm GWAS findings in a larger group of cases and controls in order to associate the results with the pathogenesis of endometriosis. Then, a genetic association study comprising 394 infertile women with endometriosis and 650 fertile control women was conducted. TaqMan allelic discrimination assays were used to investigate the frequency of three SNPs in the genes *KAZN* (rs10928050), *LAMA5* (rs2427284), and *TAC3* (rs733629). The analysis revealed a significant association of *KAZN* rs10928050 ($p = .015$) and *LAMA5* rs2427284 ($p = .0059$) SNPs with endometriosis-related infertility, while *TAC3* rs733629 showed no difference between cases and controls. As a conclusion, it was possible to observe that individual genotyping of a larger sample of patients and controls confirmed the association among *KAZN* and *LAMA5* with endometriosis-related infertility and revealed new candidate genes contributing to the condition.

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Molecular biomarker; polymorphism; infertility; endometriosis; genotyping

Introduction

Endometriosis is a benign, estrogen-dependent gynecological disease that affects 10 to 16.3% of the women in reproductive age [1,2]. It is characterized by the growth of stromal and glandular tissue of endometrial origin outside the uterine cavity [3,4]. The characteristic symptoms of this disease are dysmenorrhea, dyspareunia, chronic pelvic pain, menstrual irregularities and infertility [5]. However, 16% of the patients may be asymptomatic [6].

Genetic factors have been correlated with susceptibility to endometriosis since the incidence of the disease is seven times more frequent in women with cases of endometriosis in the family than in sporadic cases [7].

In a previous Genome-Wide Association Study (GWAS), three gene polymorphisms were differentially incident in pooled samples from patients with endometriosis and controls [8]. One of the polymorphisms was located in the *KAZN* gene, which encodes a protein associated with cell adhesion, cytoskeletal organization and epidermal differentiation, cellular junctions and signal transduction. Variations in this gene could justify the adhesion of the endometrial cells outside the uterine cavity [9].

Another polymorphism was located in the *TAC3* gene, which decodes a taquinines' family member peptides, primarily expressed in the peripheral nervous system. Di Spiezio Sardo et al found increased levels of this protein in samples of endometriotic tissue. The authors associate this finding with pain reported by patients with endometriosis [10].

In addition, a polymorphism was identified in the *LAMA5* gene that encodes a laminin, one of the extracellular matrix glycoproteins, which are the largest non-collagen constituents of the membranes. Laminins have been implicated in a variety of biological processes including cell adhesion, differentiation, migration, signaling, and metastasis. Ozhan et al, evaluated the serum of 60 women with endometriosis in search of biomarkers and observed that in addition to CA-125, laminin 1 and syntaxin 5 proteins had increased levels when compared to the control group [11].

Previous studies on the proteins encoded by the genes in question point to the association of the biological function of the gene and endometriosis [1]. Thus, the aim of the study was to confirm the involvement of selected variants in the pathogenesis of endometriosis.

Material and methods

Patients

394 women with endometriosis and infertility (mean age 35.1 ± 3.9 years) were evaluated in order to compound the case group. They were recruited from the Reproduction and Human Genetics Center of the Faculdade de Medicina do ABC (FMABC), Santo André, Brazil. They were diagnosed with endometriosis by laparoscopy and were classified according to the American Society of Reproductive Medicine [12], with mandatory histological confirmation of the disease.

The investigation into the cause of infertility included a hormonal and biochemical profile, testing for sexually transmitted diseases, imaging examinations, investigation of genetic and/or

CONTACT Denise Maria Christofolini denise.morf@gmail.com Faculdade de Medicina do ABC Rua Lauro Gomes, 2000, Santo André/SP, CEP 09060-870, Brazil.

Table 1: Genotype and allele frequencies of rs733629, rs10928050 and rs2427284 SNPs in infertile women with endometriosis and controls.

			Genotypes			Alleles				
SNP	Group	*n*	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*p**	OR (95% IC)	HWE
rs733629			**TT**	**TC**	**CC**	**T**	**C**			
C/T (FWD)	EDT (I-IV)	394	333 (85)	58 (15)	3 (1)	724 (92)	64 (8)	.57	0.91 (0.66 – 1.25)	1
Ancestral Allele: T	EDT I/II	197	164 (83)	32 (16)	1 (1)	360 (91)	34 (9)	.89	0.97 (0.65 – 1.45)	1
Gene: *TAC3*	EDT III/IV	197	169 (86)	26 (13)	2 (1)	364 (92)	30 (8)	.44	0.85 (0.56 – 1.29)	0.31
	Control	650	540 (83)	105 (16)	5 (1)	1185 (91)	115 (9)			0.73
rs10928050			**AA**	**AG**	**GG**	**A**	**G**			
A/G (FWD)	EDT (I-IV)	394	252 (64)	126 (32)	16 (4)	630 (80)	158 (20)	**.015**	1.34 (1.06–1.69)	1
Ancestral Allele: A	EDT I/II	197	123 (62)	63 (32)	11 (6)	309 (78)	85 (22)	**.009**	1.47 (1.10–1.96)	0.41
Gene: *KAZN*	EDT III/IV	197	129 (65)	63 (32)	5 (3)	321 (81)	73 (19)	.210	1.22 (0.90–1.65)	0.49
	Control	650	454 (70)	185 (28)	11(2)	1093 (84)	207 (16)			0.14
rs2427284			**GG**	**GA**	**AA**	**G**	**A**			
A/G (FWD)	EDT (I-IV)	394	356 (90)	33 (8)	5 (1)	745 (95)	43 (5)	0.100	0.75 (0.53–1.07)	0.003
Ancestral Allele: G	EDT I/II	197	172 (87)	21 (11)	4 (2)	365 (93)	29 (7)	0.990	1.00 (0.66–1.50)	0.012
Gene: *LAMA5*	EDT III/IV	197	184 (93)	12 (6)	1 (1)	380 (96)	14 (4)	**0.005**	0.49 (0.28–0.86)	0.210
	Control	650	562 (86)	80 (12)	8 (1)	1204 (93)	96 (7)			0.018

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval.
*p-Value of allelic frequencies versus control group; HWE-Hardy–Weinberg Equilibrium; Bold means significant p values ($p < .05$).

immunological abnormalities, hysterosalpingography, hysteroscopy, laparoscopy, and semen analysis of the partner.

Finally, 394 cases were selected to participate in this case-control study, and it is subdivided according to the stage of the disease: 197 with minimal/mild endometriosis (stage I and II) and 197 with moderate/severe endometriosis (stage III and IV).

A group of 650 fertile women without endometriosis (mean age 39.7 ± 3.2 years) was selected in the Family Planning Outpatient Clinic of FMABC, among the group submitted to tubal ligation to compose the control group. The absence of endometriosis in all controls was confirmed by inspection of the pelvic cavity by laparoscopy. The samples included in the study were selected for the same criteria as the GWAS set [8].

The study was approved by the local ethics committee and all participants signed the informed consent after explaining the objectives of the study (process number 310094).

SNP selection

The SNP-array genotyping of pooled DNA samples previously published by our group considered the following standard quality control steps: SNPs with minor allele frequency (MAFs) > 0.05, missing rates <0.05, or p values >.0001 for the Hardy–Weinberg equilibrium test were included. Principal component analysis (PCA) was applied considering all valid SNPs to calculate genetic distances and account for population stratification. 148 SNPs presented p values <.0001 and after literature review three of them were selected for further association analysis in the replication stage based on their biological function that could be possibly associated with the endometriosis pathogenesis.

Molecular analysis

A sample of peripheral blood (4 ml) was collected from the patients and controls in EDTA-containing tubes for genomic DNA extraction. Salting out method was used as described previously [13]. Genotyping of the polymorphisms rs10928050, rs2427284, and rs733629 was performed using the respective c_2463632_10, c_16232618_10, and c_620360_20 TaqMan assays and TaqMan Master Mix genotyping (Thermo Fisher), with 50 ng of DNA per reaction. PCR conditions were as recommended by the manufacturer. Reactions were carried out in thermocycler Step One Real-Time PCR System (Applied Biosystems, Carlsbad, California, USA).

Statistical analysis

Statistical analyzes were carried out using SPSS for Windows 11.0 (SPSS, Inc., Chicago, IL). Chi-square test was used to compare allelic and genotypic frequencies between the groups, to estimate Hardy–Weinberg. The odds ratio (OR) and range with 95% confidence interval (95% CI) were calculated for the presence of the reference genotype using a logistic regression model. A p values< .05 was considered statistically significant.

Results

The 650 samples selected to compose the control group and the 394 samples of the case group were genotyped for the three gene polymorphisms indicated by GWAS: *TAC3* (rs733629), *KAZN* (rs10928050), and *LAMA5* (rs2427284). The results obtained were arranged in Table 1 and were described as below:

It was observed for *TAC3* rs733629 that there were no differences in genotype and allelic distribution considering endometriosis grades I to IV samples, grouped or discriminated according to the endometriosis grade compared to the frequencies observed in control group.

Considering the polymorphism *KAZN* rs10928050 it was observed a statistically significant difference concerning allelic distribution between groups with endometriosis and controls ($p = .015$). However, when endometriosis subgroups were compared to controls, it was possible to observe that the statistical difference was obtained only to Endometriosis grades I and II ($p = .009$).

The third polymorphism analyzed was the rs2427284 located in the *LAMA5,* and showed no difference among the total group of endometriosis samples when compared to controls. On the other hand, when endometriosis subgroups allelic distribution was compared to the ones obtained in the control group, a statistically significant difference was observed considering endometriosis grades III and IV and control group ($p = .005$).

Discussion

The present study addressed genes and metabolic pathways that may be physiologically associated to endometriosis, based on the evidence of a previous GWAS SNP study performed with a pooled sample obtained from infertile Brazilian women with endometriosis

Uno et al. conducted one of the first GWAS studies in endometriosis. Among their findings, two SNPs (rs16826658 and rs7521902) located in the chromosomal region 1p36, presented $p = 1.66 \times 10^{-6}$ with OR = 1.20 (95% CI 1.11–1.29) in the combined analysis [14]. This chromosomal region also contains the *KAZN* gene, identified in our GWAS analysis [8] and evaluated in the present study.

As mentioned, the *KAZN* encodes a protein responsible for the cell adhesion found within the desmosomes associated with periplakin, a protein that is part of a skin desquamation process. In the present study, the variant G of the rs10928050 polymorphism in *KAZN* was observed more frequently in the endometriosis groups when compared to the control group, in the different degrees of the disease. Nucleotide substitution promotes the replacement of the amino acid isoleucine by methionine at position 83 of the protein (dbSNP home page - NCBI, accessed on 2017). The variant form may be associated with adhesion of the endometrial cells outside the uterine cavity, characteristic of the disease.

A previous study with women with endometriosis and controls, comparing the levels of gene expression in the eutopic endometrium found that five genes from the eighty-four human angiogenesis-connected genes, evaluated, showed increased expression in the endometrium of patients with advanced endometriosis (III and IV). Among then was *LAMA5* [15].

Here, we also observed a significant association among *LAMA5* (rs2427284) SNP and moderate/severe endometriosis. Exchange of bases promotes the substitution of phenylalanine by serine at the position 1716 of the protein (dbSNP home page - NCBI, accessed in 2017). It is possible that the presence of polymorphism modifies laminin function, favoring the development of endometrial tissue outside the uterine cavity, by modifying the adhesion of the endometrial cells out of their original site, leading to more severe forms of the disease. The laminins are formed by three non-identical alpha, beta, and gamma chains encoded by different genes [16]. A study comparing the expression of gamma-laminin-332, in eutopic and ectopic endometrium of patients with and without endometriosis, showed that increased expression of laminin in the ectopic endometrium of women with endometriosis, suggested a possible role of this protein in the adhesion, migration, and invasion of endometrial cells necessary for the development of the disease [16].

In the present study, the association between rs733629 polymorphism in the *TAC3* gene and endometriosis was not confirmed. This variant, although intragenic, is located in an intronic region of the gene (dbSNP home page - NCBI, accessed on 2017), which may have contributed to the maintenance of this protein's function. However, it is not possible to rule out the possibility that other polymorphisms of this gene are associated with the condition.

An important factor to be considered in the studies on endometriosis is the heterogeneity among the populations evaluated. Many studies include patients with various symptoms such as the presence of pain, infertility, the combination of pain and infertility and, in some cases, the absence of symptoms. Our group questions whether this variation of symptoms presented by the patients would not represent the action of different genes on the spectrum of the same disease. In this study all patients had endometriosis and infertility was their main complaint. On the other side, the control group was specially selected, composed of fertile women free of endometriosis. In this way, despite the homogeneity of the sample, we can't distinguish whether the finding was associated with endometriosis or infertility. Thus, as future perspective we propose the evaluation of the variants here indicated and other variants of the same genes in other populations, as women with endometriosis and fertile, and then confirm the role of these genes in the pathogenesis of the disease.

Conclusions

The results obtained in this study confirmed the association of *LAMA5* and *KAZN* polymorphisms with endometriosis in Brazilian infertile women and reveals new candidate genes contributing to endometriosis development and progression.

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Disclosure statement

The authors disclose no conflict of interest.

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References

[1] Nyholt DR, Low SK, Anderson CA, et al. Genome-wide association meta-analysis identifies new endometriosis risk loci. Nat Genet. 2012;44:1355–1359.
[2] Painter JN, Anderson CA, Nyholt DR, et al. Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. Nat Genet. 2011;43:51–54.
[3] Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol. 1927;14:422–469.
[4] Poliness AE, Healey MG, Brennecke SP, et al. Proteomic approaches in endometriosis research. Proteomics. 2004;4:1897–1902.
[5] Barlow DH, Kennedy S. Endometriosis: new genetic approaches and therapy. Annu Rev Med. 2005;56:345–356.
[6] Barbosa CP, Souza AM, Bianco B, et al. Frequency of endometriotic lesions in peritoneum samples from asymptomatic fertile women and correlation with CA125 values. Sao Paulo Med J. 2009;127:342–345.
[7] Matalliotakis IM, Arici A, Cakmak H, et al. Familial aggregation of endometriosis in the Yale Series. Arch Gynecol Obstet. 2008;278:507–511. Dec
[8] Bianco B, André GM, Vilarino FL, et al. The possible role of genetic variants in autoimmune-related genes in the development of endometriosis. Hum Immunol. 2012;73:306–315.
[9] Le Pennec S, Konopka T, Gacquer D, et al. Intratumor heterogeneity and clonal evolution in an aggressive papillary thyroid cancer and matched metastases. Endocr Relat Cancer. 2015;22:205–216. Apr
[10] Di Spiezio Sardo A, Florio P, Fernandez LM, et al. The potential role of endometrial nerve fibers in the pathogenesis of pain during endometrial biopsy at office hysteroscopy. Reprod Sci. 2015;22:124–131.
[11] Ozhan E, Kokcu A, Yanik K, et al. Investigation of diagnostic potentials of nine different biomarkers in endometriosis. Eur J Obstet Gynecol Reprod Biol. 2014;178:128–133.

[12] American Society for Reproductive Medicine. Revised American Society for reproductive Medicine classification of endometriosis: 1996. Fertil Steril. 1997; 67:817–821.

[13] Lahiri DK, Bye S, Nurnberger JI, Jr, et al. A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested. J Biochem Biophys Methods. 1992;25:193–205.

[14] Uno S, Zembutsu H, Hirasawa A, et al. A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. Nat Genet. 2010;42:707–710.

[15] Laudanski P, Lemancewicz A, Kuc P, et al. Chemokines profiling of patients with preterm birth. Mediators Inflamm. 2014;2014:185758.

[16] Locci R, Nisolle M, Angioni S, et al. Expression of the gamma 2 chain of laminin-332 in eutopic and ectopic endometrium of patients with endometriosis. Reprod Biol Endocrinol. 2013;11:94.