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DROSHA rs10719 and *DICER1 rs3742330* polymorphisms in endometriosis and different diseases: Case-control and review studies.

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ABSTRACT

Objective: DROSHA and DICER1 enzymes participate in the main stages of microRNA synthesis. Polymorphisms can influence mRNAs stability and genes expression, and hence affect the binding of miRNAs. Thus, the present study evaluated the association of *DROSHA* and *DICER1* polymorphisms in the development of endometriosis and other diseases.

Methods: A total of 240 endometriosis cases and 242 controls were genotyped for the *DROSHA* rs10719 $G > A$ and *DICER1* rs3742330 $A > G$ polymorphisms using the *TaqMan* system. The association between polymorphisms and endometriosis was estimated by binary logistic regression. A literature review was also performed including all published articles (PubMed database) until December 2020, regarding the association of the studied polymorphisms and different diseases.

Results: DICER1 rs3742330GG was only found in endometriosis cases (2.1%) and deep infiltrative endometriosis (DIE) (2.5%). The *DICER1 rs3742330GG* genotype was significantly associated with endometriosis ($P < 0.05$), suggesting a tendency to present an increased risk for disease. *DROSHA rs10719A* and *DICER1 rs3742330G* allele frequencies varied among populations (6%–79% and 10.2%–55.1%, respectively). In the Brazilian population, the frequencies of these alleles were 42.3% and 7.3%, respectively. Both polymorphisms were risk factors for nonsyndromic orofacial clefts, tuberculosis, stroke ischemia and mortality after stroke, recurrent idiopathic pregnancy loss, and some types of cancer. Moreover, the *DICER1* rs3742330 polymorphism was a protective factor for precancerous cervical lesions, different types of cancer and tuberculosis.

Conclusions: The results suggest that only the *DICER1* rs3742330 $A > G$ polymorphism may be associated with susceptibility to endometriosis. The frequencies of both polymorphisms were significantly different among populations, and there were discrepancies in the risk associations with the development of diseases.

1. Introduction

Endometriosis is a multifactorial disease defined as the growth of functional endometrial tissue outside of the uterine cavity; it affects approximately 10% of women of reproductive age and 40–50% of infertile women (Benagiano et al. 2014; Eisenberg et al., 2018). The molecular mechanisms for the pathogenesis of endometriosis are not fully elucidated (Krishnamoorthy et al., 2017), although monozygotic and dizygotic twin studies showed a 47% genetic contribution to endometriosis development, supporting the hypothesis of genetic hereditability in the susceptibility to disease (Saha et al. 2015).

MicroRNAs (miRNA) are a class of small noncoding RNAs 20–24 nucleotides in length that anneal to complementary sequences in the 3′ untranslated region (3' UTR) of messenger RNAs (mRNAs), regulating posttranscriptional gene expression (Ambros 2004). MicroRNAs influence various eukaryotic cellular processes, such as proliferation,

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differentiation, migration, and oncogenesis (Gregory and Shiekhattar 2005). Two master RNase III enzymes, DROSHA and DICER, are involved in the generation of miRNAs. Initially, in the nucleus, longer precursors are processed into primary RNAs (pri-miRNAs) by the action of RNase II. Following this step, DROSHA cleaves pri-miRNAs into a 70-bp stem-loop structure called pre-miRNA, which is transported by the exportin-5 protein to the cytoplasm. Subsequently, DICER processes pre-miRNAs into mature miRNAs, which will be incorporated into the RNA-induced silencer complex (RISC) to promote repression of gene expression (Costa and Pacheco, 2016). Approximately 30% of human genes are regulated by miRNAs (Lewis, 2005).

Aghajanova and Giudice observed that the DICER1 enzyme was highly expressed in secretory endometrium from women with advanced endometriosis compared with mild endometriosis, which can lead to negative regulation of adhesion molecules and apoptosis-associated genes, increasing migratory functions in endometrial cells and resistance to apoptosis (Aghajanova and Giudice 2011). Recently, it was observed that transfection of endometriotic epithelial 12Z cells with DROSHA siRNA reduced the transcript and protein levels and was associated with enrichment in pri-miR-144-3p expression, a miRNA that can influence some factors relevant to endometriosis, such as prostaglandin-endoperoxide synthase 2 (PTGS2), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 expression levels (Nothnick et al. 2019).

The differences found in the gene expression levels of the two enzymes may be explained by single nucleotide polymorphisms (SNPs) occurrence in miRNA-coding genes (Yang et al. 2008; Horikawa et al. 2008; Lin et al. 2010). Two SNPs that stand out are *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G*, localized in the 3'UTRs of their respective genes, which can influence the stability and gene expression and can affect the binding of miRNAs (Han et al. 2013). The *rs10719* SNP is located in the miR-27b binding site within the 3'UTR of DROSHA, which is related to fibrosis, an important characteristic of endometriosis (Wang et al. 2012). Furthermore, the *rs3742330* SNP appears to be within miR-632, miR-3622a-5p and miR-5582-5p potential target sequences and is able to influence cell apoptosis, proliferation, migration and invasion (Cheng et al. 2018).

To the best of our knowledge, there are no data in the literature evaluating the association of these SNPs in the development of endometriosis. It can be hypothesized that DROSHA and DICER1 contribute to influencing the processes involved in endometriosis, such as angiogenesis and inflammation (Bjorkman and Taylor, 2019). Therefore, the main objective of this study was to investigate the roles of *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs in endometriosis susceptibility. Moreover, a literature review was also carried out with all published articles to define the association of these SNPs with other diseases/conditions.

2. Methods and materials

2.1. Study design

The current study was approved by the Human Research Ethics Committees of *Hospital Federal dos Servidores do Estado* (HFSE 414/2011) and *Hospital Moncorvo Filho* (HMF 1.244.294/2015). The procedures followed in this study were in accordance with the Declaration of Helsinki of 1964. All study subjects were recruited between 2011 and 2018 and signed the informed consent form.

Endometriosis cases ($n = 240$) were considered eligible when diagnosed by laparoscopy ($n = 108$) or laparotomy ($n = 34$) or both ($n = 6$), with histological confirmation of the ectopic implants or those diagnosed only by magnetic resonance imaging (MRI) ($n = 92$). All cases without surgical diagnosis of endometriosis were diagnosed with deep infiltrative endometriosis (DIE) by MRI (Ito et al. 2016) and had a clinical history of the disease. Indications for surgery were women with suspected endometriomas, infertile women with persistent pain even during medical treatment, and/or women with functional impairment of the large bowel and/or urinary tract. Patients with surgical diagnosis were stratified in I–II and III-IV stages according to the revised American Fertility Society (ASRM - American Society for Reproductive Medicine, 1997). In addition, according to the classification proposed by Nisolle and Donnez (Nisolle and Donnez 1997), patients with visible ectopic implants were classified as having superficial endometriosis (SUP), ovarian endometrioma (OMA), or DIE. Women with both peritoneal and ovarian lesions were considered to have OMA; peritoneal and ovarian lesions associated with infiltrative endometriosis were considered to have DIE (Chapron et al. 2012).

The control group ($n = 242$) was women underwent laparoscopy or laparotomy for tubal ligation or treatment of certain benign diseases, such as ovarian cysts and hydrosalpinx. In addition, all women were submitted preparatory to diagnostic imaging but with no suspicion of endometriosis.

Women in both groups had diagnostic imaging, and there was no suspicion of adenomyosis. Women with hypertension-related chronic kidney disease, rheumatoid arthritis or any previous history of cancer were also excluded from this study.

As described in our previous study (Cardoso et al. 2016), women with severe and incapacitating pain were considered to have clinical symptoms of endometriosis. Patients who did not become pregnant after one year of regular sexual intercourse without contraceptive use were considered infertile (Perini et al. 2014).

2.2. Genotyping

DNA extractions were obtained from the subjects' peripheral blood using QIAamp® DNA Blood mini kit (Qiagen, Düsseldorf, Germany) following the manufacturer's instructions. Genotyping of the *DROSHA rs10719 G > A* (Chr.5:31401340) and *DICER1 rs3742330 A > G* (Chr.14:95087025) SNPs were performed by StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) using *TaqMan* probes (C_7761648_10 and C_27475447_10, respectively). Real-Time PCR conditions are described in Pinto et al., 2019.

2.3. Literature review

Published articles involving the association of *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs with other diseases/conditions were identified using the PubMed database, following the combined key words: ("*DROSHA rs10719*" or "*DICER1 rs3742330*") and ("polymorphism" or "SNP" or "genetic polymorphism" or "variants").

Case-control studies published until December 2020, with full texts available and with all frequency data for the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs, were included in the review. The exclusion criteria were reviews or meta-analyses and those in a language other than English.

The following data were extracted from all selected full-text articles: author; publication year; population; number of controls; type of studied disease/condition; frequency data of genotypes and alleles of controls; and association data. The methodological quality of all articles was evaluated by two reviewers independently following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria instrument, and a percentage greater than 50% was considered satisfactory (Von 2007).

2.4. Statistical analysis

Statistical analyses were performed with software IBM® SPSS® Statistics (version 20.0, Statistical Package for Social Sciences Inc) for Windows and a value of $P < 0.05$ was considered statistically significant. Sample calculation was performed using the Epi Info 7 program, version 7.1.3. (http://wwwn.cdc.gov/epiinfo/html/downloads.htm), to detect a difference between the case and control groups, assuming a power of

0.8 and 5% type I error, and at least 240 women in each group should have been recruited.

The *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs were tested for Hardy–Weinberg equilibrium (HWE) using the goodness-of-fit Chi-Square test and allele and genotype distributions were performed by direct counting. Categorical variables were assessed using chi-square test ($\chi 2$) or, when appropriate, the Fisher's exact test, whereas continuous variables were assessed using Student's *t*-test. The risk associations for endometriosis were estimated by the odds ratio (OR) with their respective 95% confidence intervals (95% CI), with adjustment for confounding factors could potentially influence the risk for disease ($P = 0.20$).

Chi-square test was also performed to verify whether the distributions of the *DROSHA rs10719 G > A* and *DICER rs3742330 A > G* genotypes in the studied population were similar to the frequencies of other published studies in other countries with healthy populations (controls).

3. Results

Table 1 describes the main characteristics of the studied population. Patients with endometriosis were younger and had a lower BMI than controls. Approximately 20% of women with endometriosis had a family history of endometriosis and presented a high frequency of all clinical symptoms of the disease. A total of 12 (5%) endometriosis cases were asymptomatic. Most women with endometriosis had advanced stage (III-IV) and DIE (Table 1), with 74 (30.9%) of them presenting both DIE and OMA, 8 (3.3%) presenting both DIE and SUP, 6 (2.5%) presenting both SUP and OMA, and 18 (7.5%) presenting DIE, OMA and SUP. Additionally, 19 (7.9%), 56 (23.3%) and 59 (24.6%) women presented with only SUP, OMA or DIE, respectively.

Analyses of allele and genotype frequencies of the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs in the control group, endometriosis cases and only DIE cases are shown in Table 2. The rates of successful genotyping of the SNPs were 97% for *DROSHA rs10719 G > A* and 98% for *DICER1 rs3742330 A > G*. The observed frequencies of both SNPs for the control group agreed with HWE; however, deviation from HWE was observed in the case group, in which higher than expected frequencies of genotype homozygote variants were observed (*DROSHA rs10719 G > A*: observed, 21.9% and expected, 17.3%; *DICER1 rs3742330 A > G*: observed, 2.1% and expected, 0.6%) (Table 2).

The *DICER1 rs3742330GG* variant genotype was significantly associated with endometriosis, either considering all cases or DIE, showing a tendency to present a risk for endometriosis. However, it was not possible to perform a logistic regression model, as this genotype was not found in the control group (Table 2). The hypothesis of the role of the *DICER1 rs3742330* SNP in miRNA biogenesis and endometriosis is shown in Fig. 1. No significant differences were detected in allele or genotype distributions of the *DICER1 rs3742330* and *DROSHA rs10719* SNPs between controls and other types of endometriosis (SUP and OMA) or stages (I-II and III-IV) of the disease (data not shown). Regarding the *DROSHA rs10719* SNP, there was no significant difference between the cases and controls, considering either all cases or DIE only cases (Table 2). Considering the symptoms of the disease, there were no significant differences between the groups studied for either SNP (data not shown).

Table 3 describes the SNPs frequencies in healthy women from different populations (China, Iran, Korean, Turkey, Poland and Serbian) and their associations with different diseases/conditions. The frequency of the *DROSHA* allele varied from 6% to 79%, and that of the *DICER1 rs3742330G* allele varied from 10.2% to 55.1%. The frequency of the *DROSHA* genotype varied from 20.2% to 56.6%, and that of the *DICER1* genotype varied from 1.3% to 49.1%. For both SNPs, the population with the highest frequency was Chinese. The population with the lowest frequency for the *DROSHA rs10719* SNP was Serbian, and that for the *DICER1 rs3742330* SNP was Turkish. Only the study by Cheng et al. 2018 (Chinese) found a frequency similar to the present study for the *DICER1 rs3742330* SNP ($P = 0.12$) and the study by Nikolić et al., 2017 (Serbian) for the *DROSHA rs10719* SNP ($P = 0.25$).

Regarding the *DROSHA rs10719* SNP, two studies observed an increased risk of developing nonsyndromic orofacial clefts (Xu et al. 2018) and Alzheimer's disease (Görücü Yilmaz et al., 2016) in the presence of the *A* allele. Six studies found a positive association of the *G* allele with the development of preeclampsia (Rezaei et al. 2019; Rezaei et al. 2018), stroke (Kim et al. 2018), primary hypertension (Zhang et al. 2017), colorectal cancer (Cho et al. 2015), and bladder cancer (Yuan et al. 2013).

The *DICER1 rs3742330 G* allele was negatively associated with the development of different types of cancer (Mohammadpour-Gharehbagh et al., 2020; Kim et al. 2019, Song et al. 2017; Kim et al. 2016; Nikolic et al., 2017), precancerous cervical lesions (Huang et al. 2018) and tuberculosis (Song et al. 2013). In addition, eight articles observed positive effects on the development of tuberculosis (Cheng et al. 2018), stroke ischemia and mortality after stroke (Kim et al. 2018), pre-eclampsia (Eskandari et al. 2018), recurrent idiopathic pregnancy loss (Jung et al., 2014) schizophrenia (Zhou et al. 2013) and some types of cancer (Osuch-Wojcikiewicz et al. 2015; Cho et al. 2015; Liao et al. 2018).

Table 1
General characteristics of the studied population ($N = 482$).

Variables	Controls ($N = 242$)	Cases ($N = 240$)	*P*-value***
	Mean ± SD		
Age (year)	41 ± 9.9	36 ± 7.0	< 0.001
BMI	28.6 ± 6.2	26.5 ± 5.4	< 0.001
Family history of endometriosis[a]	N (%)		
Yes	26 (11.1%)	47 (20.4%)	0.005
Symptoms[b]			
Dysmenorrhea	85 (35.6%)	197 (82.1%)	< 0.001
Deep dyspareunia	67 (28.0%)	157 (67.1%)	
Non-cyclic chronic pelvic pain	40 (16.7%)	129 (53.8%)	
Cyclical intestinal complaints[c]	23 (11.0%)	120 (51.7%)	
Cyclical urinary complaints[c]	7 (3.3%)	57 (24.6%)	
Infertility	29 (13.4%)	91 (47.9%)	
Endometriosis stages			
I-II	–	60 (40.5%)	–
III-IV	–	88 (59.5%)	
Endometriosis classification			
SUP	–	19 (7.9%)	–
OMA	–	62 (25.8%)	
DIE	–	159 (66.3%)	

SD, standard deviation.
BMI, body mass index.
*** *P*-value from Chi-square test.
[a] Family history only in first-degree relatives.
[b] The same woman can have more than one symptom.
[c] Pain or bleeding during the menstrual period.

4. Discussion

Processes involving endometriosis, such as proliferation, differentiation and cell migration, may be downregulated by miRNAs (Gregory and Shiekhattar 2005). Their biogenesis involves different enzymatic processes, with DROSHA and DICER1 being present in the two essential steps of miRNA synthesis (Costa and Pacheco, 2016). Here, it was hypothesized that SNPs of *DROSHA* and *DICER1* may affect the accessibility of miRNA target sites and contribute to the development of endometriosis.

The enzyme DICER1 appears to be related to vascular growth and angiogenesis (Suárez et al., 2007; Chen et al. 2016), processes that are crucial in the stabilization and maintenance of endometriotic lesions (Machado et al. 2010). The association of SNPs in vascular endothelial growth factor (*VEGF*) and kinase insert domain receptor (*KDR*) genes

Table 2
Analyses of allele and genotype frequencies of the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs in the control group, endometriosis cases and only DIE cases.

SNPs	Controls	Cases	*P*-value[b]	ORa (95% CI)	DIE	*P*-value[c]	ORa (95% CI)
DROSHA rs10719	(N = 233)	(*N* = 233)			(*N* = 154)		
GG	83 (35.6)	90 (38.6)		1[a]	61 (39.6)		1[a]
GA	103 (44.2)	92 (39.5)	0.35	0.76 (0.49–1.16)	60 (39.0)	0.32	0.74 (0.46–1.21)
AA	47 (20.2)	51 (21.9)	0.99	0.98 (0.56–1.69)	33 (21.4)	0.87	0.96 (0.52–1.78)
G	269 (57.7)	272 (58.4)		1[a]	182 (59.1)		1[a]
A	197 (42.3)	194 (41.6)	0.65	0.94 (0.71–1.24)	126 (40.9)	0.60	0.92 (0.68–1.25)
DICER1 rs3742330	**(*N* = 236)**	**(*N* = 238)**			**(*N* = 157)**		
AA	202 (85.6)	205 (86.1)		1[a]	135 (86.0)		1[a]
AG	34 (14.4)	28 (11.8)	0.44	0.88 (0.50–1.56)	18 (11.5)	0.45	0.87 (0.46–1.64)
GG	0 (0.0)	5 (2.1)	**0.03**	–	4 (2.5)	**0.02**	–
A	438 (92.8)	438 (92.0)		1[a]	288 (91.7)		1[a]
G	34 (7.2)	38 (8.0)	0.48	1.2 (0.73–1.99)	26 (8.3)	0.42	1.25 (0.72–2.18)

DIE, deep infiltrative endometriosis.
ORa, odds ratio adjusted by age and BMI.
CI, Confidence Interval.
[a] Reference group.
[b] Chi-Square Test or Fisher's exact test compared controls and cases.
[c] Chi-Square Test or Fisher's exact test compared controls and DIE cases.

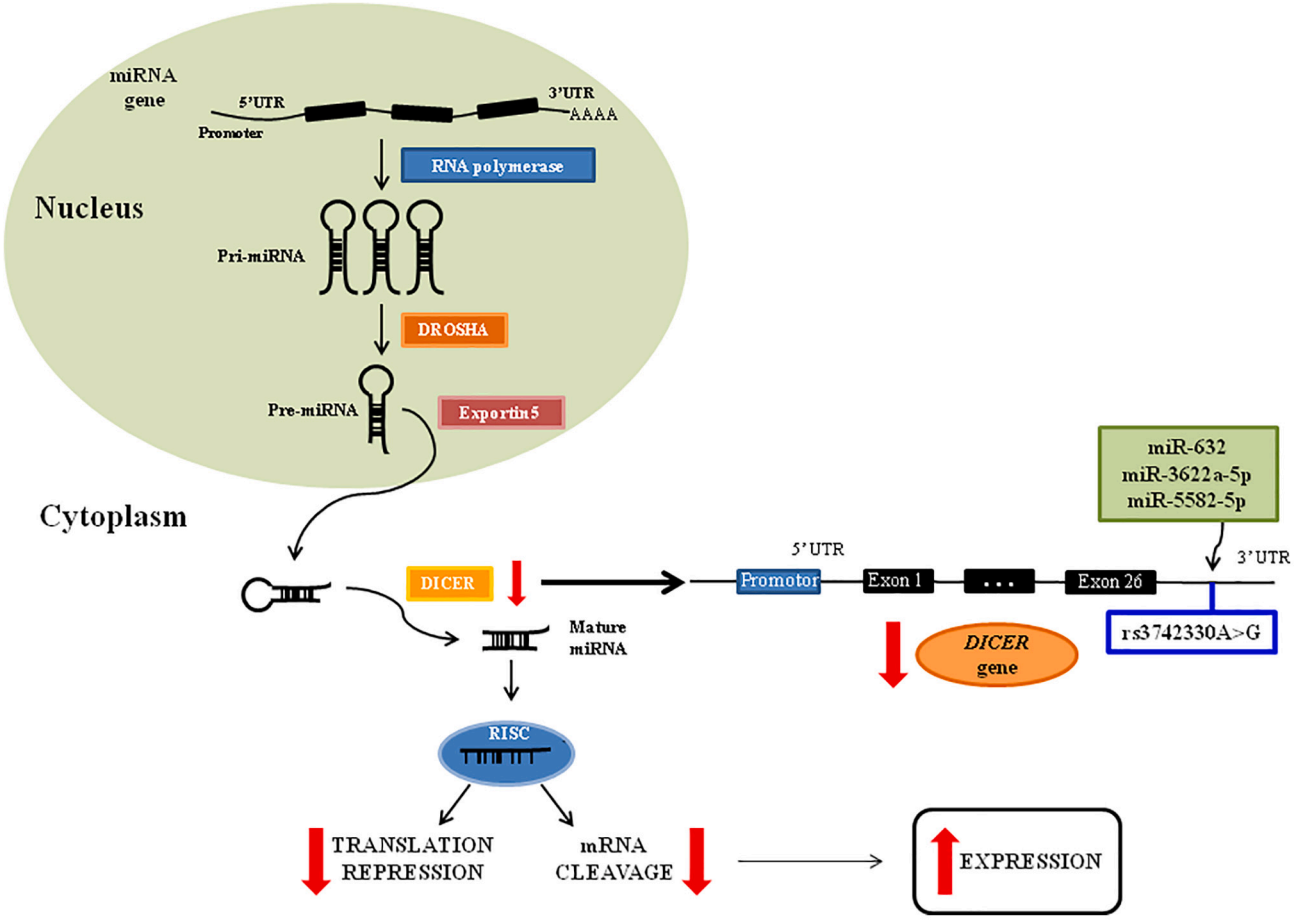

Fig. 1. Hypothesis of the role of the *DICER1* rs3742330 *A > G* SNP in miRNA biogenesis in the development of endometriosis.

and the development of endometriosis was previously described (Perini et al. 2014; Cardoso et al. 2016; Cardoso et al., 2016). Positive regulation of the VEGF receptor (VEGFR)-2 and VEGFR1 by DICER silencing is probably due to a direct effect of this loss via miRNA regulation of mRNA stability or translation interference, suggesting a critical role of the DICER enzyme in angiogenesis (Suárez et al., 2007). Based on this and the results of this study, we hypothesize the role of the *DICER1* rs3742330 *A > G* SNP in miRNA biogenesis and, consequently, in angiogenesis related to endometriosis (Fig. 1). The presence of the *DICER1 rs3742330 G* variant favors the binding of miR-632, miR-3622a-

Table 3
Allele and genotype frequencies of the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* polymorphisms in healthy women (controls) from different populations and their associations with several diseases/conditions.

Polymorphisms	Population	N[a]	Genotype Frequency[a] (AA)	Allele Frequency[a] (A)	*P*-value**	Diseases/Conditions	Association	References
DROSHA rs10719 G > A	Iranian	205	47.8	69.1	< 0.001	Pre eclampsia	Risk (G)	Rezaei et al. 2019
		205	45	70	< 0.001		Risk (G)	Rezaei et al., 2018
		120	52	70	< 0.001	Azoospermia	No association (G)	Moghbelinejad et al., 2018
	Chinese	287	40.07	63.41	< 0.001	Tuberculosis	No association (G)	Song et al. 2013
		310	23.87	49.03	0.12		No association	Cheng et al. 2018
		403	56.6	23.8	< 0.001	Post-Stroke Mortality	Risk (G)	Kim et al. 2018
		502	49.4	69.8	< 0.001	Gastric cancer	No association (G)	Song et al. 2017
		596	55.5	73.6	< 0.001	Orofacial Clefts	Risk	Xu et al., 2018
		621	52.8	72.1	< 0.001	Primary hypertension	Risk (G)	Zhang et al. 2017
		684	56.8	75,7	< 0.001	Bladder cancer	Risk (G)	Yuan et al. 2013
		252	48.4	68.8	< 0.001	Schezophrenia	No association	Zhou et al. 2013
		419	44.87	69.7	< 0.001	Semen Quality	No association (G)	Qin, 2012
	Korean	209	52.6	73.7	< 0.001	Hepatocellular carcinoma	No association (G)	Kim et al. 2016
		400	52.8	73.8	< 0.001	Colorectal cancer	Risk (G)	Cho et al. 2015
		238	46.2	68.9	< 0.001	Pregnancy loss	No association (G)	Jung, 2014
		236	46.6	69.1	< 0.001	Idiopathic primary ovarian insufficiency	No association	Rah et al., 2013
	Turkish	109	6.4	26.6	< 0.001	Alzheimer	Risk	Gorucu et al., 2016
	Brazilian	242	20.2	42.3	–	Endometriosis	No association	Present study
DICER rs3742330 A > G	Chinese	532	49.4	55.1	< 0.001	Diabetes mellitus type 2	No association	Wen et al. 2019
		287	8.01	26.48	< 0.001	Tuberculosis	Protection	Song et al. 2013
		310	3.55	20.48	< 0.001		Risk	Cheng et al. 2018
		296	15.2	42.4	< 0.001	Precancerous cervical lesion	Protection	Huang et al. 2018
		96	7.3	30.2	< 0.001	Gastric Cancer	Risk	Liao et al. 2018
		502	15.7	40.2	< 0.001		Protection	Song et al. 2017
		252	17.9	41.1	< 0.001	Schizophrenia	Risk	Zhou et al. 2013
		328	15.24	38.1	< 0.001	Pre eclampsia	No association	Huang et al., 2018
	Iranian	219	3	20	< 0.001		Risk	Eskandari et al. 2018
		130	5.4	21	< 0.001	Papillary thyroid carcinoma	Protection	Mohammadpour-Gharehbagh et al., 2020
		120	14.4	30	< 0.001	Azoospermia	No association	Moghbelinejad et al., 2018
	Korean	1400	19.1	44	< 0.001	Colorectal cancer	Protection	Kim et al. 2019
		400	18.4	41.1	< 0.001		Risk	Cho et al. 2015
		403	18.6	40.9	< 0.001	Ischemic Stroke Susceptibility and Post-Stroke Mortality	Risk	Kim et al. 2018
		209	20.1	43.1	< 0.001	Hepatocellular carcinoma	Protection	Kim et al. 2016
		233	14.7	41.4	< 0.001	Pregnancy loss	Risk	Jung et al., 2014
		236	14.8	41.9	< 0.001	Idiopathic primary ovarian insufficiency	No association	Rah et al., 2013
	Turkish	79	6.3	17.8	< 0.001	Endometrial Cancer	No association	Oz, 2018
	Serbian	318	1.9	10.2	0.25	Prostate cancer	Protection	Nikolic et al., 2017
	Polish	170	2.4	32.6	< 0.001	Larynx Cancer	Risk	Osuch-Wojcikiewicz et al. 2015
	Brazilian	242	0	7.2	–	Endometriosis	No association	Present study

** *P*-value from Chi-square test was performed assuming the other studies' frequencies as reference.
[a] Only control group.

5p and miR-5582-5p in the 3'UTR, decreasing *DICER1* expression (Cheng et al. 2018). This reduction leads to less RNA cleavage and translation repression and increased cell migration, invasion and angiogenesis which may influence the development of endometriosis (Bjorkman and Taylor, 2019). The *DICER1 rs3742330 GG* variant genotype was observed only in cases of endometriosis. The variant *DICER1 rs3742330 G* allele creates three new binding sites for miRNAs in the 3´-UTR of *DICER1*, leading to degradation of the mRNA (Eskandari et al. 2018). Moreover, this SNP appears to be a target site of miR-632, miR-3622a-5p and miR-5582-5p, which may influence cell proliferation, migration and invasion (Cheng et al. 2018) and the expression of enzymes involved in the development of endometriosis, such as growth factors, MMPs and inflammatory cytokines (Zhang et al. 2019; Yu et al., 2019, Mei et al., 2018). The *MMP-3276 G > A* SNP, which leads to an increase in MMP-3 activity, was associated with a higher risk of developing endometriosis in Brazilian women (Cardoso et al., 2019).

The miRNome of endometriosis has shown the involvement of miRNAs in endometriosis pathogenesis (Saare et al., 2014, Wright et al. 2017). Bjorkman and colleagues reviewed six studies, 4 from China and 2 from the USA, and observed 173 dysregulated circulating miRNAs in plasma or serum samples from women with endometriosis compared with the control group. Among them, 5 miRNAs (let-7 family, miR-145, miR-199a, miR-125b and miR-451) were highlighted for being overexpressed and were considered potential biomarkers for the diagnosis of endometriosis (Bjorkman and Taylor, 2019). To our knowledge, there are no studies about miR-632, miR-3622a-5p and miR-5582-5p in endometriosis, and new information is necessary regarding their role in the pathogenesis of the disease.

The increase in the expression of miR-27b-3p, a miRNA located in the 3'UTR of DROSHA, was observed in the eutopic endometrium of women with endometriosis compared with women without the disease. In addition, the inhibition of miR-27b-3p downregulated markers of fibrosis in human endometrial stromal cells (HESCs), such as collagenase (Col)-1, matrix metalloproteinase (MMP)-2 and MMP-9 (Kim et al. 2017). In the present study, the *DROSHA rs10719* SNP was not associated with endometriosis. Importantly, fibrogenesis is an important process involved in ectopic lesions (Zeng et al. 2018); however, the difference in *DROSHA* expression found in a previous study was observed in HESCs (Kim et al. 2017).

Although endometriosis is considered a benign disease, it shares some characteristics with malignant diseases and is able to connect, invade and damage other tissues, leading to the development of advanced lesions (Vlahos et al. 2010; Machado et al. 2010). It has been observed that the presence of the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs may increase the risk of diseases associated with angiogenesis, such as some types of cancer (Yuan et al. 2013; Cho et al. 2015) and recurrent idiopathic pregnancy loss (Yong et al., 2014). In the present study, the *DICER1* rs3742330 *A > G* SNP presented a tendency to increase the risk of advanced endometriosis, whereas the *DROSHA* rs10719 *G > A* SNP was not associated with advanced endometriosis. Prodromaki and colleagues (2015) demonstrated that DICER1 may be involved in the progression of human non-small cell lung carcinomas (NSCLC) to advanced stages, corroborating other published studies with different types of cancer (Merritt et al. 2008; Karube et al. 2005; Sugito et al. 2006; Chiosea et al. 2006). As angiogenesis is crucial for the pathogenesis of the disease and the *DICER1 rs3742330 A > G* SNP seems to be related to angiogenesis, other studies evaluating this SNP in the context of susceptibility to endometriosis have become important to better understand this relationship.

To the best of the authors' knowledge, this is the first study that evaluated *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* SNPs in terms of susceptibility to endometriosis. The main limitations of this study were (i) the lower frequency of *DICER1 rs3742330*, which may have contributed to the lack of association, and (ii) the presence of other diseases in both groups, since endometriosis may share some pathogenic mechanisms with some benign uterine diseases and thus can also be affected by the studied polymorphisms. Due to the heterogeneity of the Brazilian population, extrapolation of data derived from well-defined ethnic groups is not appropriate for the majority of Brazilians. There were significant differences in the frequency distributions of the *DROSHA rs10719 G > A* and *DICER1 rs3742330 A > G* alleles and genotypes across the different populations. Two studies found frequencies similar to ours for *DICER1 rs3742330* (Cheng et al. 2018) and *DROSHA rs10719* SNPs (Nikolić et al., 2017). Moreover, positive associations were observed in 10 studies in the development of several conditions for both polymorphisms (Cheng et al. 2018; Kim et al. 2018; Eskandari et al. 2018; Yong et al., 2014; Zhou et al. 2013; Osuch-Wojcikiewicz et al. 2015; Cho et al. 2015; Liao et al. 2018; Xu et al. 2018; Gorucu et al., 2016), and negative associations were observed in 7 studies only for the *DICER1 rs3742330* polymorphism (Mahammadpour-Gharehbagh et al., 2019; Kim et al. 2019; Song et al. 2017; Kim et al. 2016; Nikolic et al., 2017; Huang et al. 2018; Song et al. 2013). These discrepancies can be attributed to the interactions of genetic and/or nongenetic factors of each studied population (Angioni et al. 2020). These considerations notwithstanding, the study of this variant involved in gene expression regulation is important to help understand the etiopathogenesis of endometriosis since hereditary susceptibility may be involved in the development of the disease.

5. Conclusion

The results suggest that the *DICER1* rs3742330 SNP may be involved in the pathogenesis of endometriosis in Brazilian women. However, the *DROSHA* rs10719 SNP does not seem to influence the development of disease. Regarding the literature review, discrepancies in the frequencies of the studied SNPs were observed between the other populations and the present study, and the results of the association analysis were controversial.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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