Polymorphisms in *VEGF* and *KDR* genes in the development of endometriosis: a systematic review

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Abstract

Objectives: to review studies that used case-control design to verify the association of polymorphisms in VEGF and KDR genes in the development of endometriosis.

Methods: the systematic review selected articles published until September 1, 2015 from PubMed, MEDLINE, BVS, SciELO databases, considering the following key words: endometriosis and ("polymorphism" or "SNP" or "genetic polymorphism") and ("VEGF" OR "Vascular endothelial growth factor" or "VEGFR-2" or "Vascular endothelial growth factor-2" or "KDR" or "Kinase Insert Domain Receptor").

Results: 106 articles were identified, only 11 were eligible. Discrepant results were observed regarding polymorphisms in VEGF gene in the development of endometriosis, which can be explained by methodological differences, sample size, eligible control type, using the unadjusted risk estimates and the heterogeneity of the studied population. Only one study investigated polymorphisms in KDR gene in the development of endometriosis, however it was ineligible for this review.

Conclusions: to avoid discrepancy in the results, we suggest that the ideal control group should be formed by fertile women and free of gynecological diseases. Multicentric studies with adequate design, involving different population besides the combined analysis on polymorphisms in VEGF and KDR genes are still necessary to contribute in the understanding of this disease, which are social, clinical and economical problems.

Key words *Endometriosis, VEGF, KDR, Polymorphism, Infertility*

Introduction

Endometriosis is a gynecological disease characterized by the presence of functional endometrial tissue outside the uterine cavity, representing one of the most common gynecological disorders.[1] The real profile of patients with endometriosis is uncertain, although there is a consensus that it is present in at least 10% of the women at reproductive age,[2,3] 30 to 50% of infertile women[4] and 3 to 5% of postmenopausal women.[5]

Women with endometriosis may be asymptomatic (10.7%), however, most of them present symptoms in different intensities, the main ones are: dysmenorrhoea (52-97%), chronic pelvic pain (22-69%), infertility (25-59%), dyspareunia (44-71%) and intestinal symptoms (71%) and cyclic urinary (60%).[6-8] Endometriosis causes physical, mental and social consequences to women, bearing in mind that their psyche, interpersonal and martial relationships are affected by the problems of the symptoms, in particularly, by the difficulty of bearing children.[9]

There are several theories explaining about the appearance of endometriosis; however its etiology is still not clear. The most widely accepted theory until today is Sampson´s proposal,[10] who described that the endometrial tissue, by backward menstrual flow, returns to the uterine tubes and adheres to the peritoneal cavity. For this to occur, the angiogenesis process is essential and it is characterized by the growth of new blood vessels from pre-existing ones.[11] Among the pro-angiogenic factors, the vascular endothelial growth factor (*VEGF*) is highlighted and plays an important role in the development of endometriosis.[11] In 2008, our group observed an increased distribution of *VEGF* and its receptor *VEGFR-2* in samples of endometriosis of the ovary, bladder and the recto-sigmoid, when it was compared to control and the largest distribution of *VEGF* and *VEGFR-2* was observed in the endometriosis of the recto-sigmoid,which is the most severe.[12]

VEGF is encoded by a gene with the same name, located on chromosome 6p21.3, composed by eight exons,[13] while its receptor *VEGFR-2* is encoded by *KDR* gene (kinase insert domain receptor), located on chromosome 4q11-q12, composed by 30 exons.[14] Both genes are polymorphic, highlighting in the study on endometriosis, five single nucleotide polymorphisms (SNPs) in *VEGF* gene (rs699947, rs833061, rs1570360, rs2010963 and rs3025039) and three in *KDR* gene (rs1870377, rs2305948 and rs2071559), due to the high frequency which occurs in different populations, although interfering significantly in the activity or in the enzyme expression.[15-18]

Recently, our group observed a positive association for *VEGF* rs1570360 SNP in the development of endometriosis (OR: 1.90; CI95%: 1.23-2.97) in Brazilian women.[8] Several case-control studies also investigated the influence of SNPs in *VEGF* or *KDR* genes in the development of endometriosis.[8,17,19-37] However, the results of the analysis are still controversial. In this context, the aim of this study was to review all the studies that used case-control design to investigate the magnitude of SNPs association of *VEGF* and/or *KDR* genes susceptibility to develop endometriosis, in order to discuss the possible causes of the conflicting results already described.

Methods

To identify all the studies published until September 1, 2015 on the associations among SNPs of *VEGF* and/or *KDR* and the development of endometriosis, an evaluation on titles and abstracts of the studies was performed by two researchers in an independent form and "blinded" in PubMed, Medline, BVS and SciELO databases using the following keywords: ("endometriosis") *AND* ("polymorphism" *OR* "SNP" *OR* "genetic polymorphism" *OR* "genetic polymorphisms") *AND* ("*VEGF*" *OR* "Vascular endothelial growth factor" *OR* "*VEGFR-2*" *OR* "Vascular endothelial growth factor-2" *OR* "Vascular endothelial growth factor-2" *OR* "*KDR*" *OR* "Kinase Insert Domain Receptor"). The studies were selected according to the inclusion and exclusion criteria, pre-selected and described as followed.

The inclusion criteria for this review were case-control design studies and that evaluated associations among polymorphisms of *VEGF* and/or *KDR* genes and the development of endometriosis. As for the period of the publication, articles that were published until September 2015 were included without any restriction of languages.

The exclusion criteria of the study were: (I) the absence of a control population; (II) incomplete data associations; (III) duplicated data; (IV) meta-analysis, review, letters, commentaries or editorial articles; (V) those that did not analyze SNPs in *VEGF* and/or *KDR*; (VI) and did not include patients with endometriosis; (VII) those that did not have access to the complete text.

After performing a search strategy in PubMed, Medline, BVS and SciELO databases in articles which studied SNPs in *VEGF* and/or *KDR* genes with the development of endometriosis, the following information was extracted: the first author,

publication year, journal; country where the study was conducted; number of cases and controls; mean age of cases and controls with their respective deviation standard and/or interval; selection on criteria/eligibility and exclusion of cases of endometriosis and controls; endometriosis staging; control source; type of SNP; genotyping technique used to identify the studied SNPs; data of allelic and genotypic frequency of the SNPs and statistic data of *odds ratio* (OR) with its respective 95% confidence intervals (CI95%).

Evaluation on quality in the included studies

Two reviewers independently evaluated the methodological quality of 19 studies included in this review, using STROBE (Strengthening the Reporting of Observational Studies in Epidemiology), a criteria instrument.[38] Twenty and two evaluated items received a score from 0 to 1. These items were related to the title and the abstract of the article (item 1), the introduction (items 2 and 3), the methods (items 4 to 12), the results (items 13 to 17), the discussion (items 18 to 21) and the financial information (item 22). After evaluating all the criteria, each article received a score from 0 to 22 of each researcher. The final score was performed an average of the two grades transformed into percentage to better evaluate the quality of the articles. Previously, the reviewers described if the articles reached a percentage higher than 50%, they would be consi-dered satisfactory.

Results

Figure 1 shows a flowchart on the selection of articles for this review. The search strategy identified 100 publications on *VEGF* gene and 6 publications on *KDR*, no articles were found evaluating simultaneously the influence of SNPs in both genes with the development of endometriosis. After removing the duplicated articles, 34 were selected for reading the titles and abstracts. However, 14 studies were excluded: 5 because they were meta-analyzes, 2 because they were reviews, one because it was a criticism on the meta-analysis published before, one because it explored SNPs in *IL-10* gene instead of polymorphisms in *VEGF* and/or *KDR* genes, 4 because they studied patients with adenomyosis and one because it evaluated women with pterygium instead of patients with endometriosis. After the selection of the abstracts, a study was excluded because the text was not completely available.[19] After a thorough reading of 19 articles eligible for this review, the inclusion and exclusion criteria on cases and controls were evaluated and 8 studies were still excluded: 5 used endometriosis cases without histopathological confirmation of the disease;[20,25,28,30,34] 2 which included controls did not have surgery procedures (laparoscopy and/or laparotomy) in confirming the diagnosis of endometriosis;[22,29] and 1 which included controls only performed an ultrasound and was diagnosed negative for the disease.[17] Thus, 11 case-control studies were included in this review that evaluated SNPs only in *VEGF* gene susceptibility to develop endometriosis from 2005 to 2015.[8,21,23,24,26,27,31-33,36,37] In relation to evaluate the quality of 11 studies selected for this review, all the articles reached a percentage greater than 50%[38] and the average score ranged between 12 to 21 points (55-96%). The studies that had the lowest and the highest score, according to the STROBE criteria were Kim *et al*.[24] and Perini *et al*.,[8] respectively (Table 1).

In this review 4205 women´s data were included (2096 with endometriosis and 2109 controls) from Asian countries,[21,24,27,32] North America,[33] North Africa,[8] Europe,[23,26,37] Euro-Asian countries[31] and Africa.[36]

Table 1 describes the inclusion and exclusion criteria of the group with endometriosis, emphasizing that all the studies considered the women eligible who had surgical diagnosis of the disease with histopathological confirmation. Whereas the staging of the disease was found in 4 (36.3%) studies that included patients in stage I, II, III or IV;[24,31,33,36] 3 (27.3%) grouped into stages I-II or III-IV;[8,23,26] 2 (18.2%) included only women in stages I and II;[32,37] one (9.1%) included patients in stage III and IV[21] and another (9.1%) showed no information on surgical staging of the disease.[27] In relation to the exclusion criteria of the group with cases of endometriosis, 2 (18.2%) studies did not inform any criteria,[27,33] while others, a total of 9 (81.8%) considered patients who had a history of angiogenesis-dependent diseases or that it could be associated to polymorphisms of *VEGF*, and women who presented adenomyosis, a pelvic inflammatory disease, bilateral tubal occlusion and myoma.

For the control group (Table 2), all the studies included women with surgical diagnosis of endometriosis, although 3 (27.3%) studies included women who underwent surgery for tubal ligation,[8,26,32] 3 (27.3%) included women who underwent surgery to remove cyst in the ovary;[8,21,23] 2 (18.2%) included women who underwent surgery to remove myoma;[8,23] 2 (18.2%) included women who underwent hysterectomy[21,27] and 6 (54.5%) included

Figure 1

Flowchart of the selected articles included in the review.

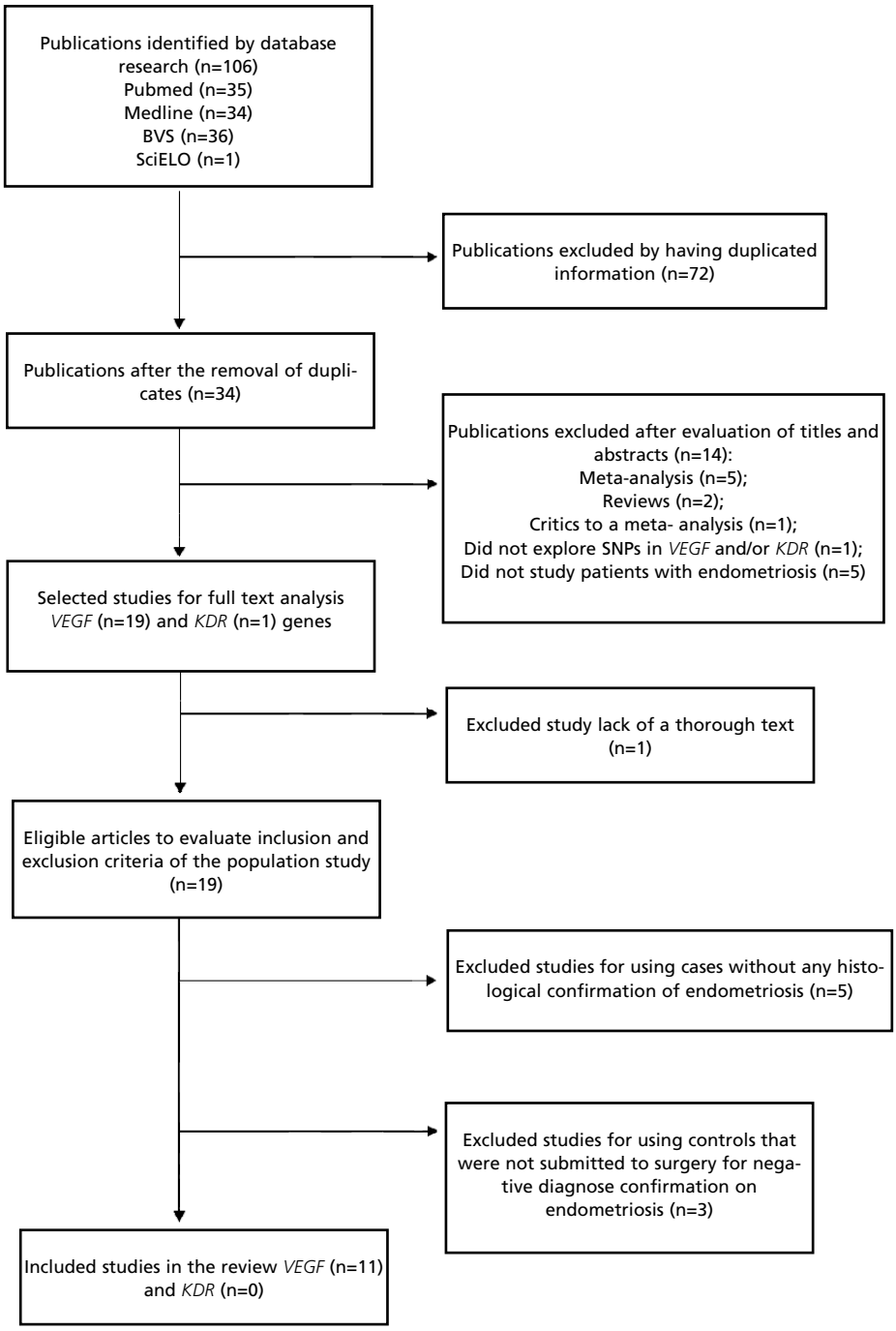

Table 1

Description of the inclusion and exclusion criteria of the case group, established by eligible studies for this review.

Reference, year	Population (N)*	Surgery**	Endometriosis staging**** (N)	Exclusion criteria	Quality Score Strobe (%)
Kim *et al*.[21] 2005	Korean (215)	Laparoscopy or laparotomy	III = 65 IV = 150	Rheumatoid arthritis, GCA, retinopathy diabetic, psoriasis, Behcet's disease and endometriosis I-II.	19 (86%)
Gentilini *et al*.[23] 2008	Italian (203)	Laparoscopy	I-II = 78 III-IV = 125	Pelvic inflammatory disease.	18 (82%)
Kim *et al*.[24] 2008	Korean (105)	Laparoscopy	I = 20; II = 41 III = 11; IV = 33	Amenorrhoea, previous surgery for endometriosis or other pelvic pathology for example myoma.	12 (55%)
Cosin *et al*.[26] 2009	Spanish (186)	Laparoscopy	I-II = 19 III-IV = 167	Suspicion of endometriosis, but without histopathological confirmation of the disease, Menorrhagia, hypermenorrhagia, who became pregnant or breast-feeding for the past 6 months.	20 (91%)
Liu *et al*.[27] 2009	Chinese (344)	Laparoscopy or laparotomy	No information	No information	19 (86%)
Altinkaya *et al*.[31] 2011	Turkish (98)	Yes***	I = 4; II = 18 III = 41; IV = 35	Rheumatoid arthritis, GCA, diabetic retinopathy, psoriasis or Behcet´s disease.	17 (77%)
Emamifar *et al*.[32] 2012	Iranian (480)	Yes	I-II	Endocrine therapy before surgery.	15 (68%)
Rotman *et al*.[33] 2013	American (24)	Laparoscopy	I = 3; II = 2 III = 2; IV = 17	No information	13 (59%)
Perini *et al*.[8] 2014	Brazilian (182)	Laparoscopy or laparotomy	I-II = 71, III-IV = 110, No information = 1	History of cancer or Adenomyosis.	21 (96%)
Henidi *et al*.[36] 2015	Tunisian (105)	Laparoscopy	I = 27; II = 36, III = 18; IV = 24	Rheumatoid arthritis, GCA, diabetic retinopathy, breast cancer, Behcet's disease, leiomyoma, Adenomyosis, myoma, carcinoma in situ of the cervix or ovarian cancer.	17 (77%)
Szczepańska *et al*.[37] 2015	Polish (154)	Laparoscopy	I = 83 II = 71	Mechanical distortion of the endometrial cavity for fibroids, tubal occlusion bilateral and factor of male infertility.	17 (77%)

GCA = giant cell arthritis; * N is the number of cases of endometriosis included in each article; ** Type of surgery for endometriosis diagnosis; ** Yes = not specified the type of surgery performed; *** Classification of endometriosis staging described by the American Society for Reproductive Medicine (1996, 1997), the American Society of Fertility (1985, 1997).

women who were submitted to surgery because of various problems such as appendicitis, carcinoma in situ of the cervix, dermoid cysts, parovarium cysts, serous cysts and mucinous tumors, dysmenorrhoea, pelvic pain, fibroma ovarian, dysfunctional bleeding uterine, hydrosalpinges, infertility and malformation of the uterine and normal pelvis.[8,21,23,27,31,37] The exclusion criteria for the control group was one (9.1%) study that did not inform any of the exclusion criteria.[33] The remaining 10 (90.9%) considered almost the same exclusion criteria of the group with cases of endometriosis.[8,21,23,24,26,27,31,32,36,37]

In Table 3 shows investigated SNPs characteristics in the studies eligible for this review. 5 SNPs in *VEGF* gene were studied being genotyped by PCR-RFLP techniques (81.8%) and PCR at real time (18.2%). SNP rs699947 was evaluated in 2 (18.2%) studies and the frequency of *VEGF -2578A* allele ranged between 19- 40% and 26-34% in cases and controls, respectively. Liu *et al*.[27] observed a negative association with endometriosis, while Perini *et al*.[8] found no association to the disease (Table 4). SNP rs833061 was evaluated in 8 (72.7%) studies and the frequency of *VEGF -460C* allele ranged between 3-53% in cases and 1-51% in controls, however no study found an association of SNP with the development of the disease.[8,21,26,27,31,32,36,37] Three (27.3%) studies evaluated SNP rs1570360 and the frequency of *VEGF -1154A* allele ranged between 16-28% and 16-27% in cases and controls, respectively (Table 3). However, Liu *et al*.[27] and Perini *et al*.[8] observed opposite effects, while Rotman *et al*.[33] found no association with the development of endometriosis (Table 4). SNP rs2010963 investigated 8 (72.7%) studies and the frequency of *VEGF +405C* allele ranged between 33-56% in cases and 31-95% in controls (Table 3), although 4 studies (Table 4) found no association with endometriosis,[8,26,36,37] 1 study observed a positive association in the presence of *VEGF +405G* allele 31 and, contrary 3 studies verified a positive association in the presence of allele or *+405C* genotype.[21,23,32] Six studies (54.5%) evaluated SNP rs3025039 and the frequency of *VEGF +936T* allele ranged between 13-25% and 10-17% in cases and controls, respectively (Table 3), however 4 studies found no association[8,23,27,37] and 2 verified a positive association with endometriosis in the presence of *+936T* allele.[26,36]

Table 2

Description of the inclusion and exclusion criteria of the control group, established by eligible studies for this review.

Reference, year	Control (N)	Source of controls	Surgery * (N)	Reason for surgery		Exclusion criteria
				Tubal ligation(N)	Others***(N)	
Kim *et al*.[21] 2005	219	Hospital basis	Laparoscopy or laparotomy	No	Yes (219)	Rheumatoid arthritis, GCA, diabetic retinopathy, psoriasis, Behcet's disease, endometriosis (I-II, leiomyoma, Adenomyosis, carcinoma *in situ* of the cervix or cancer of the ovary.
Gentilini *et al*.[23] 2008	140	Hospital basis	Laparoscopy	No	Yes (140)	Pelvic inflammatory disease.
Kim *et al*.[24] 2008	101	Hospital basis	Laparoscopy	No	No information	Amenorrhoea, previous surgery for endometriosis or other pelvic pathology, as an example myoma.
Cosin *et al*.[26] 2009	180	Hospital basis	Laparoscopy	Yes (180)	No	Menorrhagia, hypermenorrhagia, who became pregnant or breastfeeding in the last 6 months
Liu *et al*.[27] 2009	360	Based on Population	Laparoscopy or laparotomy	No	Yes (No information)	Malignant disease or endometriosis.
Altinkaya *et al*.[31] 2011	94	Hospital Basis	Laparoscopy	No	Yes (94)	Rheumatoid arthritis, GCA, diabetic retinopathy, psoriasis and Behçet's disease.
Emamifar *et al*.[32] 2012	600	Hospital Basis	Yes**	Yes (600)	No	Endocrine therapy before surgery, endometriosis, inflammatory disease and myoma.

continue

GCA = giant cell arthritis; * Type of surgery performed; ** Yes = not specified the type of surgery performed; *** Other reasons for conducting surgical procedure include appendicitis, carcinoma *in situ* of the cervix, dermoid cysts, parovarium cysts, serous cysts and mucinous tumors, dysmenorrhoea, pelvic pain, ovarian fibroma, dysfunctional uterine bleeding, hydrosalpinx, infertility and malformation of the uterine and normal pelvis.

Table 2 **concluded**

Description of the inclusion and exclusion criteria of the control group, established by eligible studies for this review.

Reference, year	Control (N)	Source of controls	Surgery * (N)	Reason for surgery		Exclusion criteria
				Tubal ligation(N)	Others***(N)	
Rotman *et al*.[33] 2013	18	Hospital Basis	Laparoscopy	No information	No information	No information
Perini *et al*.[8] 2014	112	Hospital Basis	Laparoscopy or laparotomy	Yes (51)	Yes (61)	History of cancer or Adenomyosis
Henidi *et al*.[36] 2015	150	Hospital Basis	Laparoscopy	No information	No information	Rheumatoid arthritis, GCA, diabetic retinopathy, breast cancer, Behcet's disease, leiomyoma, Adenomyosis, myoma, carcinoma *in situ* of the cervix or ovarian cancer.
Szczepańska *et al*.[37] 2015	135	Hospital Basis	Laparoscopy	No	Yes (135)	Signs of inflammation, past or present, pelvic abnormalities and bilateral tubal occlusion.

GCA = giant cell arthritis; * Type of surgery performed; ** Yes = not specified the type of surgery performed; *** Other reasons for conducting surgical procedure include appendicitis, carcinoma *in situ* of the cervix, dermoid cysts, parovarium cysts, serous cysts and mucinous tumors, dysmenorrhoea, pelvic pain, ovarian fibroma, dysfunctional uterine bleeding, hydrosalpinx, infertility and malformation of the uterine and normal pelvis.

Table 3

Polymorphisms characteristics of *VEGF* gene analyzed in eligible studies for this review.

rs	Site of the gene	Genotyping technique	Reference, year	Variant allele frequency	
				Case	Control
				-2578A	
rs699947	RP	TaqMan- real time PCR	Perini *et al*.[8] 2014	40	34
		PCR-RFLP	Liu *et al*.[27] 2009	19	26
				-460C	
rs833061	RP	TaqMan- real time PCR	Perini *et al*.[8] 2014	43	40
		PCR-RFLP	Kim *et al*.[21] 2005	28	26
		PCR-RFLP	Cosin *et al*.[26] 2009	47	49
		PCR-RFLP	Liu et al.[27] 2009	23	21
		PCR-RFLP	Altinkaya *et al*.[31] 2011	3	1
		PCR-RFLP	Emamifar *et al*.[32] 2012	33	31
		PCR-RFLP	Henidi *et al*.[36] 2015	40	42
		real time PCR	Szczepańska *et al*.[37] 2015	53	51

continue

RP = promoter region; 5'UTR = 5' untranslated region ; 3'UTR = 3' untranslated region; PCR-RFLP = polymerase chain reaction - restriction fragment length polymorphism.

Table 3 **concluded**

Polymorphisms characteristics of *VEGF* gene analyzed in eligible studies for this review.

rs	Site of the gene	Genotyping technique	Reference, year	Variant allele frequency	
				Case	Control
				-1154A	
rs1570360	RP	TaqMan- real time PCR	Perini *et al.*[8] 2014	27	16
		PCR-RFLP	Liu *et al.*[27] 2009	16	22
		PCR-RFLP	Rotman *et al.*[33] 2013	28	27
				+405C	
rs2010963	5'UTR	TaqMan- real time PCR	Perini *et al.*[8] 2014	33	40
		PCR-RFLP	Kim *et al.*[21] 2005	44	40
		PCR-RFLP	Gentilini *et al.*[23] 2008	40	31
		PCR-RFLP	Cosin *et al.*[26] 2009	34	31
		PCR-RFLP	Altinkaya *et al.*[31] 2011	55	95
		PCR-RFLP	Emamifar *et al.*[32] 2012	56	34
		PCR-RFLP	Henidi *et al.*[36] 2015	44	37
		real time PCR	Szczepańska *et al.*[37] 2015	26	28
				+936T	
rs3025039	3'UTR	TaqMan- real time PCR	Perini *et al.*[8] 2014	15	15
		PCR-RFLP	Kim *et al.*[21] 2005	20	No information
		PCR-RFLP	Cosin *et al.*[26] 2009	16	10
		PCR-RFLP	Liu *et al.*[27] 2009	17	17
		PCR-RFLP	Henidi *et al.*[36] 2015	25	13
		real time PCR	Szczepańska *et al.*[37] 2015	13	17

RP = promoter region; 5'UTR = 5' untranslated region ; 3'UTR = 3' untranslated region; PCR-RFLP = polymerase chain reaction - restriction fragment length polymorphism.

Table 4

Description of the results concerning the magnitude of association of the polymorphism in *VEGF* gene with endometriosis, according to studies eligible for this review.

Gene/SNP	Reference, year	Association	Allele or genotype associated	OR (CI95%)
VEGF -2578C>A (rs699947)	Liu *et al*.[27] 2009*	Yes	AA	0,34 (0,17 – 0,70)
	Perini *et al*.[8] 2014*	No	-	-
VEGF -460T>C (rs833061)	Perini *et al*.[8] 2014*, Kim *et al*.[21] 2005, Cosín *et al*.[26] 2009*, Liu *et al*.[27] 2009*, Altinkaya *et al*.[31] 2011, Emamifar *et al*.[32] 2011, Henidi *et al*.[36] 2015 and Szczepańska *et al*.[37] 2015	No	-	-
VEGF -1154G>A (rs1570360)	Perini *et al*.[8] 2014*	Yes	AA	6,17 (1,37 – 27,8)
	Liu *et al*.[27] 2009*	Yes	AA	0,26 (0,11 – 0,67)
	Rotman *et al*.[33] 2013	No	-	-
VEGF +405G>C (rs2010963)	Kim *et al*.[21] 2005	Yes	CC	1,99 (1,20 – 3,28)
	Gentilini *et al*.[23] 2008	Yes	C	1,80 (1,2 – 2,8)
	Altinkaya *et al*.[31] 2011	Yes	G	14,8 (7,38 – 29,7)
	Emamifar *et al*.[32] 2012	Yes	CC	3,78 (2,74 – 5,21)
	Perini *et al*.[8] 2014*, Cosín *et al*.[26] 2009*, Henidi *et al*.[36] 2015 and Szczepańska *et al*.[37] 2015	No	-	-
VEGF +936C>T (rs3025039)	Cosin *et al*.[26] 2009 *	Yes	T	1,75 (1,12 – 2,74)
	Henidi *et al*.[36] 2015	Yes	T	2,19 (1,39 – 3,46)
	Perini *et al*.[8] 2014*, Kim *et al*.[24] 2008, Liu *et al*.[27] 2009* and Szczepańska *et al*.[37] 2015	No	-	-

(-) = not applicable; OR = *odds ratio*; CI = confidence interval; *Authors that used statistical methods to calculate the adjusted OR.

Discussion

This review provides a detailed description of the studies that used a case-control design study to investigate the magnitude of association of the SNPs in *VEGF* and/or *KDR* genes susceptibility in the deve-lopment of endometriosis. The angiogenesis, through *VEGF-KDR* signals, is an important event in the development of the disease,[11,12] since endometriosis lesions are characterized by a dense vascularization and requires blood supply suitable for survival.[11] Furthermore, an increased expression of *VEGF* and *VEGFR-2* in samples of women with endometriosis was observed when compared to its control.[12]

The expression of *VEGF* and *KDR* genes as well as the activity of these proteins may be modified by the presence of polymorphisms in coding regions and non-coding genes.[16-18] SNPs that occur in promoter regions, 5'-UTR and 3'-UTR, affect potential regulatory elements, are sensitive to hypoxia and contribute to high variability of production in *VEGF* tissues.[18,39,40] rs699947, rs833061 and rs1570360 SNPs present in the promoter region of *VEGF* gene,

present higher activity of transcription and are associated to high levels of protein.[41] rs2010963 SNP located in region 5'-UTR of *VEGF* gene affects the efficiency of understanding and is related to the production of *VEGF* from the mononucleated cells of the peripheral blood.[42] So, rs3025039 SNP in region 3'-UTR of *VEGF* gene influences the concentration of *VEGF* plasmatic stock.[15] rs2305948 and rs1870377 SNPs located in the exons of *KDR* gene decrease the efficiency of *VEGF* binding to *KDR*.[18] The possible effect of rs7667298 SNP located in region 5'-UTR of *KDR* gene is unclear. However, this SNP is associated to rs2071559 SNP (*KDR-604T>C*) located in the promoter region. The presence of -604C allele reduces the transcriptional activity of *KDR*.[18] rs1531289 and rs7692791 SNPs located in introns of *KDR* gene were not yet studied in relation to the functional effects.

VEGF-KDR role in endometriosis physiopathology as well as the influence of SNPs to modulate the levels and activity of these proteins justify the high number of studies that attempted to describe the magnitude of SNPs association of *VEGF* and *KDR* genes susceptibility in endometriosis developing.[8,17,19-37] In 2013, Li *et al.*,[43] conducted a meta-analysis study involving 14 studies which investigated the magnitude of association of 5 SNPs in the *VEGF* gene (rs699947, rs833061, rs1570360, rs2010963 and rs3025039) in the susceptibility on endometriosis. The increased risk of endometriosis developing was observed in the presence of *VEGF + 936C>T* SNP, while *VEGF -2578C>A* and *-1154G>A* SNPs were protective factors for the development of the disease. Thus, *VEGF -460T>C* and *+405G>C* SNPs were not associated to the development of endometriosis.[43] For *KDR*, only one case-control design study evaluated the magnitude of association among five SNPs (rs1531289, rs2305948, rs1870377, rs7692791 and rs7667298) and the development of endometriosis in Chinese women, noticing a negative association in the presence of *1192T* allele (rs2305948).[17] However, this study was excluded from this review because included controls performed only an ultrasound to diagnose negative for endometriosis and this method of imaging does not exclude the presence of pelvic endometriosis.[44,45] Moreover, until the present moment there are no data in literatures that assess the combined form of SNPs in *VEGF* and *KDR* genes and the development of endometriosis.

The controversial results observed in the case-control studies can be explained by the sample size, by methodological differences in each study design, mainly, by the eligible control type, by the difference in SNPs frequency in different populations and by the use of the measured of association, the unadjusted *odds ratio* (OR) used to evaluate the magnitude of association of SNPs in the *VEGF* and *KDR* genes in the development of endometriosis. The stratification techniques and multiple regression are two statistical methods which can be used to exclude potential confounding variables and consequently produce the adjusted OR.[46,47] Among the 11 studies included in this review, only 3 (27.3%) used the adjusted OR (Table 4) to evaluate the magnitude of association of SNPs in *VEGF* gene and the development of endometriosis.[8,26,27] The manner in which the variables are measured may insert a bias and distort the estimated measurement of the effect.[48] The confounding variables occur when part of the association found is the result of the presence of a third variable that is related to the disease and the exposure of interest result in a change of the estimated risk.[49]

The analytical observational epidemiological studies of the case-control type are applied to evaluate exposures among similar groups, which present (cases) or not (controls) to the disease.[50] They are widely used in studies on rare diseases or in long period of induction and regular expositions,[51] such as in the case of endometriosis. In addition, case-control studies tend to contribute in locating important findings and efficient in terms of relatively short time with little financial resource.[48] However, case-control studies tend to be more susceptible to biases than any other analytical epidemiological designs and how individuals are recruited for the study can distort the estimated measurement of the effect.[51] The definition of the case group is a critical point in a case-control study, criteria for diagnosis and eligibility and two fundamental aspects for the selection of the subject. The aim is to ensure that all the true cases have equal probability of joining the group, and that no false case is selected, thus, it may distort the estimated measurement of the association in direction to a null hypothesis.[52] In 19 published studies which analyzed *VEGF* and *KDR* polymorphisms in susceptibility to the development of endometriosis, it was observed that the cases of endometriosis had surgical diagnosis.[8,17,20-34,36,37] In spite of the available images of the method presented a good accuracy in the endometriosis diagnosis, according to a consensus of the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), the gold standard to diagnose endometriosis is the videolaparoscopy with direct inspection of the pelvic cavity and visu-

alization of the implants, as well as the histopathological confirmation of the disease.[53,54] Thus, 11 articles were included in this review using the "gold standard" to select the cases (surgical and histopathological confirmation), considered to be a good diagnostic criteria and eligibility, ensuring that all 2096 women included were true cases of endometriosis.[8,21,23,24, 26,27,31,32,33,36,37]

The selection of the control group requires special care and perhaps is the main challenge to guarantee the validity of the study, considering the complexity of the investigated disease (endometriosis). The control group of studies included in this review was formed by women who could have other diseases, including gynecological disorders associated to the development of endometriosis, along with the process of angiogenesis and/or polymorphisms in *VEGF* gene.[17,39,55,56] Approximately 82% (n=9) of the studies included in this review used the control group that presented other gynecological diseases or had no information about the reason for a surgical procedure performed by a gynecologist (laparoscopy and/or laparotomy), being able to provide low risk estimates, considering these diseases and/or the reason why a woman underwent surgery may be associated to the exposure under investigated (SNPs of *VEGF* gene). It is fundamental that the control group is directly determined by the definition and selection of the case group sampled by the same source.[57] Among the 11 studies included in this review, about 91% (n=10) used controls of hospital basis (Table 2).[8,21,23,24,26,31-33,36,37] It has been described in the literature that about 11% of the women with endometriosis have no symptoms of the disease.[6,7] In addition, the average time between the onset of the symptoms and the diagnosis of endometriosis is extensively long, ranging between 7 to 12 years.[58] We emphasize that 4 articles were excluded from this review,[22,25,29,30] because they did not use adequate control to evaluate the magnitude of SNPs association to *VEGF* gene and the development of endometriosis. In a study conducted by Zhao *et al*.[25] the controls were women who reported not having endometriosis and only 27% had a negative diagnosis of endometriosis by laparoscopy or hysterectomy. Toktam *et al*.,[30] used control of hospital basis, however it was not informed whether the women had undergone surgical procedure, they only reported that they had gynecological problems. Lamp *et al*.[29] used healthy fertile women (with at least 2 children), without clinical suspicion of endometriosis and without performing a surgical procedure to exclude the presence of the disease. Ikuhashi *et al*.[22] used umbilical cord as control material obtained from newborn females.

The main limitations of the case-control studies are: the lack of validity due to the selected bias; the recruitment of controls are not representative to the population at risk; errors on the measurements associated to the function of different biases between cases and controls and the confounding variables that affect a causal association.[50] The differences in the allelic frequency pattern can be observed in the studies with ethnic groups, as well as environmental factors contributing to different phenotypic responses.[59] Furthermore, it should be taken into account the sample size to avoid making type I errors, stating that the risk factor is associated to the disease, but, in fact, it is not; or type II errors, stating that the risk factor is not associated with the disease, but, in fact, it is.[50] Among the 11 articles included in this review, only 3 (27%) of them informed the calculated sample used in the study.[21,23,26] Moreover, we emphasize the limited number of cases and controls included in Rotman *et al*.[33] study (24 cases and 18 controls) and Attar *et al*.[28] (52 cases and 60 controls) and the latter was excluded from this review because they used cases without histological confirmation of endometriosis.

According to Zondervan *et al*.,[57] the choice of the ideal control group for the study on genetic basis of endometriosis is free of diseases, whereas is submitted to surgery for tubal ligation. Among the 11 studies included in this review, approximately 27% (n=3) used women who underwent tubal ligation in the control group,[8,26,32] and only 2 (18%) studies were formed exclusively by a control group of women who underwent the procedure of tubal ligation.[26,32] Corroborating with these findings, we also suggest that the ideal control group perform case-control studies, which evaluates the genetic profile of women with endometriosis and should be informed by women submitted to tubal ligation, because it is possible to make a direct inspection of the pelvic cavity and exclude the presence of endometriosis lesions.

This review had the intention to describe and discuss the inclusion/exclusion criteria used to define the cases group with endometriosis and especially the controls group, in order to verify methodological differences that could contribute to the discrepancies in the results found in the literature. The analytical observational studies on case-control, when they are carefully designed can provide relevant information to identify risk factors, especially in cases of endometriosis, which is a complex disease and multifactorial. The epidemiological

analyzes which explore the molecular profile of women with endometriosis may contribute to the etiologic investigation and build instruments for the actions and interventions in the field of the public health.

In conclusion, we suggest broader studies with correct methodological design involving different populations as well as a combined analysis of SNPs in *VEGF* and/or *KDR* genes are still necessary in virtue of the fundamental role of these factors in the endometriosis development. We suggest a selection of a control group from a hospital basis, without history of infertility and gynecological disorders, such as the case of women undergoing tubal ligation procedures corresponds to the appropriate control type to evaluate the magnitude of SNPs association of *VEGF* and/or *KDR* genes in the endometriosis development.

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