

Association of VEGF +405G>C polymorphism with endometriosis

Maria Celestina Vanaja¹, Roya Rozati², Khaja Nassaruddin³, Satti Vishnupriya⁵

¹M.Sc. Research Scholar, MHRT Hospital and Research Centre, Owaisi Hospital and Research Centre Hyderabad, ²M.D., A.I.I.M.S, Delhi, F.R.C.O.G., London, Chief Fertility Specialist, Research Director of MHRT-Hospital and Research Centre, Professor and Head Owaisi Hospital, Deccan College of Medical Sciences, ³C.T.O. of Bioserve Biotechnologies India Pvt Ltd, Hyderabad, 500076, ⁴M.Sc, M.Phil. Scientist R and D, Bioserve Biotechnologies India Pvt Ltd, Hyderabad, 500076, ⁵Head, Department of Genetics, Osmania University, Hyderabad

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
 - 3.1. Molecular analysis of the VEGF genotype
 - 3.2. Statistical analysis
4. Results
 - 4.1. Genotype frequencies
 - 4.2. Allele frequencies
5. Discussion
6. Acknowledgements
7. References

1. ABSTRACT

The present study was designed to explore the association between the SNP +405G>C of the VEGF gene with the risk of endometriosis, and endometriosis associated with adenomyosis and chocolate cysts. Following extraction of genomic DNA, genotyping of the +405 G>C polymorphisms of the VEGF gene was performed by PCR - RFLP analysis. The genotype ($X^2=21.713$, 2 df, $P < 0.0001$) and allele ($X^2=10.697$, 1 df, $P = 0.0011$) frequencies of endometriosis patients were significantly different from those of the control women. The genotype and allele frequencies significantly differed in all the clinical subgroups of endometriosis patients. The significant differences in allele frequencies were the result of an increased proportion of homozygote GG genotype carriers. No significant difference was observed between the clinical subgroups with respect to the genotype and allele frequencies of the VEGF +405G>C polymorphism. These findings suggest that the VEGF +405 G>C polymorphism is associated with the risk of endometriosis, and endometriosis associated with adenomyosis and chocolate cysts.

2. INTRODUCTION

Endometriosis is a common, chronic estrogen-dependent gynecologic disorder associated with pelvic pain and infertility. The prevalence of pelvic endometriosis approaches 10% among women; in women with pelvic pain, infertility, or both, the frequency is 35%-50% (1, 2). Retrograde menstruation has been suggested as the crucial element in the development of endometriosis, the factors that allow the implantation and propagation of endometriotic lesions are yet largely unknown (3). Genetic alterations that might contribute to lesion initiation and progression have been identified in endometriotic lesions (4). It is logical to suspect that somatic genetic factors might contribute to the development of endometriosis (5). The actual cellular and molecular mechanisms responsible for endometriosis are unclear. However, the cause is almost certainly multifactorial involving environmental, immunological, endocrine and genetic processes (6).

Histologically, endometriosis is a benign disease but it can behave like a malignancy in terms of growth, infiltration and adherence to the surrounding tissues (7).

To survive and develop into an endometriotic lesion, the ectopic tissue must induce new vessel formation to connect to the vascular system (8). Vascular endothelial growth factor (VEGF) is a key mediator in neoangiogenesis as it stimulates endothelial cell proliferation and migration, and increases vascular permeability (9, 10); VEGF has even been proposed as a diagnostic and therapeutic target in endometriosis (11-13)

Angiogenesis is a process involving new blood vessel development from preexisting vasculature. The VEGF is one of the most potent endothelial cell mitogens and plays a crucial role in both angiogenesis and lymphogenesis. (14). The VEGF gene is located on chromosome 6p21.3 (15) and consists of 8 exons exhibiting alternate splicing to form a family of proteins. The promoter region and 5' untranslated region of the VEGF gene were first screened for polymorphisms by Watson *et al.* (16). VEGF has at least 3 receptors (VEGFR-1, VEGFR-2, and neuropilin) Selected single-nucleotide polymorphisms (SNPs) in the VEGF gene have been found to be associated with differences in VEGF protein production (17-21).

Some researchers have demonstrated higher peritoneal concentrations of VEGF in women with advanced stage endometriosis than in women with minimal to mild endometriosis or no disease (22, 23), others have shown increased VEGF mRNA and protein expression in the eutopic endometrium from subjects with endometriosis (24,25) Recently, it has been demonstrated that the use of antihuman VEGF effectively interferes with the maintenance and growth of endometriosis by inhibiting angiogenesis in a nude mouse model(26). Several studies have investigated the association of VEGF gene polymorphisms with diseases in which angiogenesis plays a major role in pathogenesis, such as diabetic retinopathy (27), renal cell carcinoma (28), acute renal allograft rejection (29), prostate cancer (30), and malignant melanoma (31). The results of these studies, however are were mixed.

Since the +405 site is located adjacent to the +410 estrogen response element and carriage of the -460/+405 polymorphism significantly alters VEGF promoter activity and responsiveness (32), it is possible that the +405 polymorphism itself influences transcriptional activity perhaps by alteration of the response to estrogen. The present study was designed to explore the association between the SNP +405G>C in the VEGF gene with the risk of endometriosis, and endometriosis associated with adenomyosis and chocolate cysts in South Indian women.

3. MATERIALS AND METHODS

This is a prospective case-control study, in which women undergoing infertility treatment at the following 2 two centers were recruited:

Maternal Health and Research Trust (MHRT), and Owaisi Hospital and Research Centre (OHRC), Hyderabad.

These centers receive cases from all over South India. Venous blood was obtained from 626 patients who had undergone diagnostic laparoscopy or laparotomy. A total of 302 patients showed surgical and histological evidence of endometriosis while 324 patients without the disease served as controls. All the subjects were of reproductive age (22-46 years). The demographic and clinical characteristics of the cases and controls are shown in Table 4.

The indications for laparoscopy included chronic pelvic pain, infertility, ovarian cysts and myomas. Prior to the operation, the patients had not received any endocrine therapy such as therapy involving GnRH analogues, danazol or estrogen-progestin that may have masked the presence of the disease.

The patients were informed that their blood would be used for research purpose and they provided written consent. The ethics committee of our institute approved the research protocol for this study.

The patients who had endometriosis were classified into six clinical subgroups:

1. Patients with mild Endometriosis (n=122)
2. Patients with severe endometriosis (n=180)
3. Patients with endometriosis and associated with adenomyosis (n=191)
4. Patients who had only endometriosis (n=111) but no other gynecological disease (n=111)
5. Patients with chocolate cysts ((n=225)
6. Patients without chocolate cysts (n=77)

The stage of endometriosis was assigned according to the revised American Society for Reproductive Medicine scoring system(33). (Stages I and II were grouped as mild while stages III and IV were grouped as severe)

The classification described by the American Fertility Society(AFS) is based on the size and location of the endometriotic lesion.

1. Minimal (Stage I): small spots of endometriosis seen at laparoscopy but no clinical symptom.
2. Mild (Stage II): Scattered fresh superficial lesions seen. Scarring, retraction and adhexal adhesions were not observed.
3. Moderate (Stage III): Ovaries are involved, with some scarring and retraction. They contain endometriomas that are not larger than 2cm in size. There is minimal peritubal and periovarian adhesion.
4. Severe (Stage IV): Ovaries are involved with the width of endometriomas exceeding 2cm.

Endometriosis is diagnosed by laparoscopy; the symptoms of endometriosis are infertility, dyspareunia, dysmenorrhoea, and abdominal pain.

Adenomyosis is also known as uterine endometriosis; it is a common condition in which

Table 1. Genotype frequencies of VEGF polymorphisms in Healthy Control Women, Endometriosis patients and each clinical subgroup of endometriosis patients

Total No: of patients:626	+405GC Genotype Polymorphism			X ² -Value	P-Value ²
	GG	GC	CC		
Healthy control ¹ women n=324(%)	134	167	23		
Patients with endometriosis n=302 (%)	178	101	23	21.713	< 0.0001
Re-AFS stage I+II mild n=122(%)	77	36	9	18.332	0.0001
Re-AFS stage II+III severe n=180(%)	101	65	14	11.461	0.0032
With adenomyosis and/or leiomyomas n= 191 (%)	116	57	18	23.118	< 0.0001
Without adenomyosis and/or leiomyomas n= 111 (%)	62	44	5	7.136	0.0282
With chocolate cysts n=225(%)	132	75	18	18.344	0.0001
Without chocolate cysts n=77(%)	46	26	5	8.801	0.0123

¹ The Control group was used as the reference group, ²P< 0.05 considered as statistically significant

endometrial islands are found in the wall of the uterus. More than one third of the hysterectomy specimens from woman aged 40 years and above reveal the presence of adenomyosis. This disease often coexists with pelvic endometriosis. It is diagnosed by ultrasonography of the uterus. The symptoms include menorrhagia, dysmenorrhoea, dyspareunia, pelvic discomfort, and back ache.

Chocolate cysts are also known as endometrial cysts or endometrioma. The presence of endometrial tissue in the ovary leads to the formation of a cyst known as chocolate cyst. It shows obvious thickening of tunica albuginea and vascular red adhesions are well marked on the under surface of the ovary. The inner surface of the cyst wall is vascular and contains areas of dark brown tissue.

3.1. Molecular analysis of the VEGF genotype

Genomic DNA was extracted from venous blood with a Qiagen kit according to the manufacturer's instructions. Genotyping of the +405 G>C polymorphism was carried out using the PCR– RFLP method. The PCR primers for the +405 G>C polymorphisms were as follows:

1. 5 -TTGCTTGCCATTCCCCACTTGA- 3 (forward) and
2. 5 -CCGAAGCGAGAACAGCCCAGAA-3 (reverse)

An aliquot containing 1 µl of genomic DNA was mixed with 1 µl of each primer, 2 µl dNTPs and 1 µl of Premix Ex Taq polymerase (Bioserve Biotechnologies Hyderabad, India.) in a total volume of 25 µl of PCR buffer (provided by the manufacturer)

The PCR procedure was as follows: an initial denaturation step at 94°C for 5 min; followed by 40 cycles of amplification involving denaturation at 94°C for 30 s, annealing at 62°C for 60 s and a final extension step at 72°C for 60 s. The PCR products were digested with the restriction enzyme BsmFI (New England Biolabs) at 65°C overnight for the +405 SNP and analyzed using 2% agarose gel electrophoresis, and identified using ethidium bromide staining. The +405G allele was cut into 2 fragments of 273 and 196 bp, while the +405C allele remained uncut (469 bp). (Figure 1).

3.2. Statistical analysis

Statistical analysis was performed using the Medcalc statistical software (<http://www.medcalc.be>). The

frequency of genotypes and alleles in the different groups was evaluated by X² with a 2 x3 contingency table (for genotypes) or a 2 x2 table (for alleles), and the OR and 95% confidence intervals (CIs) were calculated. P-values less than 0.05 were considered statistically significant.

4. RESULTS

The genotypic and allelic frequencies of healthy controls as well as the groups of patients divided in the clinical subgroups of VEGF+405G>C are shown in Table 1. The populations were found under Hardy-Weinberg Equilibrium for this locus.

4.1. Genotype frequencies

The genotype frequencies of the VEGF +405G>C polymorphism in healthy control women, endometriosis patients and in each clinical subgroup of endometriosis patients are given in Table 1. A significant difference of the VEGF +405G>C polymorphism genotypes was found between patients with endometriosis and in each clinical subgroup of endometriosis patients.

1. Patients with endometriosis (n=302, X²=21.713, P< 0.0001)
2. Patients with mild endometriosis (n=122, X²=18.332 , P=0.0001)
3. Patients with severe endometriosis (n=180, X²=11.461, P=0.0032)
4. Patients with endometriosis associated with adenomyosis (n=191, X²=23.118, P<0.0001)
5. Patients who had only endometriosis but no other gynecological disease (n=111, X²=7.136, P=0.0282)
6. Patients with chocolate cysts (n=225, X²=18.334, P=0.0001)
7. Patients without chocolate cysts ((n=77, X²=8.801, P=0.0123)

4.2. Allele frequencies

The allele frequencies of the VEGF +405G>C polymorphism in healthy control women, endometriosis patients and each clinical subgroup of endometriosis patients are given in Table 2.

1. Patients with endometriosis (n=302, X²=10.697, P= 0.0011, OR=0.66, 95%CI=0.51-0.84)
2. Patients with mild Endometriosis (n=122, X²=9.242, P= 0.0024, OR=0.58, 95%CI=0.41-0.82)

Table 2. Allele distributions of *VEGF* +405G>C polymorphism in Healthy Control Women, Endometriosis patients and each clinical subgroup of endometriosis patients

Total No: of patients:626	GC Allele Polymorphism		X ² -Value	P-Value ²	Odds Ratio ³ (95% CI ⁴)
	G	C			
Healthy control ¹ women n=324(%)	435	213			
Patients with endometriosis n=302 (%)	457	147	10.697	0.0011	0.66 (0.51- 0.84)
Re-AFS stage I+II mild n=122(%)	190	54	9.242	0.0024	0.58 (0.41- 0.82)
Re-AFS stage II+III severe n=180(%)	267	93	5.093	0.0240	0.71 (0.53-0.95)
With adenomyosis and/or leiomyomas n= 191 (%)	289	93	7.960	0.0048	0.66 (0.49 -0.87)
Without adenomyosis and/or leiomyomas n= 111%	168	54	5.283	0.0215	0.66 (0.46 - 0.93)
With chocolate cysts n=225(%)	339	111	8.203	0.0042	0.66 (0.51 - 0.87)
Without chocolate cysts n=77%	118	36	4.805	0.0284	0.62 (0.41- 0.93)

¹The Control group was used as the reference group, ²P< 0.05 considered as statistically significant, ³OR = odds ratio, ⁴CI = confidence interval

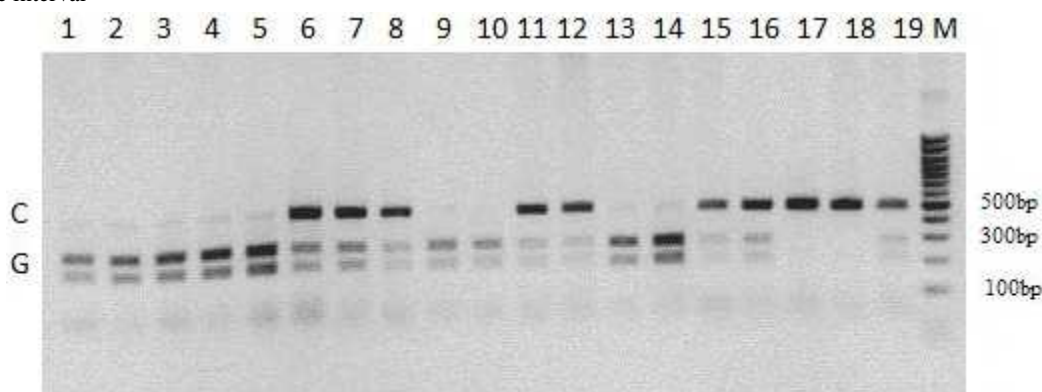


Figure 1. The +405G allele was cut into two fragments of 273 and 196 bp, while the +405C allele remained uncut (469 bp).

3. Patients with severe endometriosis (n=180, X²=5.093, P=0.0240, OR=0.71, 95%CI=0.53-0.95)

4. Patients with endometriosis associated with adenomyosis (n=191, X²=7.960, P= 0.0048, OR=0.66, 95%CI=0.49-0.87)

5. Patients who had only endometriosis but no other gynecological disease (n=111, X²=5.283, P=0.0215, OR=0.66, 95%CI=0.46-0.93)

6. Patients with chocolate cysts (n=225, X²=8.203, P=0.0042, OR=0.66, 95%CI=0.51-0.87)

7. Patients without chocolate cysts ((n=77, X²=4.805, P< 0.0284, OR=0.62, 95%CI=0.41-0.93)

The allele frequencies in all the patients with endometriosis and in each clinical subgroup of endometriosis patients were found to be significantly different from those of the control women. The significant differences in allele frequencies were found to be as a result of an increased proportion of homozygote GG genotype carriers and were not due to heterozygote GC carriers.

We further analyzed genotypes and allele frequencies for the *VEGF* +405 G>C polymorphism among the clinical subgroups of patients with endometriosis. No significant difference was observed in the genotype and allele frequencies of *VEGF* +405G/C polymorphism between the groups with Re-AFS stage I+II (mild endometriosis) and Re-

AFS stage III+IV (severe endometriosis), with and without chocolate cysts and with and without adenomyosis. (Table 3)

5. DISCUSSION

Endometriosis is a common gynecological disorder in women of reproductive age, characterized by pelvic pain and infertility. Approximately 5–10% of women of reproductive age are estimated to have endometriosis. Although the exact etiological features and pathogenesis of endometriosis are unclear, environmental and genetic factors have been implicated in the development of this disease. There is accumulating evidence to support the hypothesis that angiogenesis is of pivotal importance in the development of endometriosis. The present study was designed to explore the association between the SNP +405G>C of the *VEGF* gene with the risk of endometriosis in South Indian women.

In our study we analyzed the + 405 G>C polymorphism of the *VEGF* gene and found that the genotype distribution of the +405G>C polymorphism significantly differs between patients with and without endometriosis and in each clinical subgroup of patients with endometriosis. Women with the +405 GG genotype had a significantly higher risk of endometriosis than women without this genotype

Table 3. Distribution of genotypes and allele frequencies for the VEGF +405G>C polymorphism between the clinical subgroups of patients with endometriosis

	Genotype	X ² -Value	P-Value	AlleleG	AlleleC	X ² -Value	P-Value ¹		
	GG	GC	CC						
Re-AFS ² stage I+II mild n=122	77	36	9	-----	-----	190	54	-----	-----
Re-AFS stage II+III severe n=180	101	65	14	-----	-----	267	93	-----	-----
Significance: mild versus severe	-----	-----	-----	1.568	0.4565	-----	-----	0.891	0.3453
With adenomyosis n= 191	116	57	18	-----	-----	289	93	-----	-----
Without adenomyosis n=111	62	44	5	-----	-----	168	54	-----	-----
Significance: with versus without	-----	-----	-----	4.529	0.1039	-----	-----	0.009	0.9263
With chocolate cysts n=225	132	75	18	-----	-----	339	111	-----	-----
Without chocolate cysts n=77a	46	26	5	-----	-----	118	36	-----	-----
Significance with versus without	-----	-----	-----	0.185	0.9115	-----	-----	0.045	0.8311

¹P< 0.05 considered as statistically significant, ²Re-AFS = Revised American Fertility Society, The P-value was evaluated by X² test with a 2 X 3 contingency table for genotypes frequencies and 2 X 2 table for allele frequencies

Table 4. Demographic and clinical characteristics of cases and controls

Characteristics	Study Group Cases/Controls	Cases ¹ (n=302)	Controls ¹ (n=324)
Age (yrs)	302/324	27.4 ± 5.2	28.9 ± 4.9
BMI (kg/ m ²)	302/324	22.7 ± 3.0	23.1 ± 3.4
Age at Menarche (yrs)	302/324	13.4 ± 1.3	12.5 ± 2.3
Menstrual Cycles Regular Irregular	302/324	283 (94%) 19(6%)	314 (97%) 10 (3%)
Type of Infertility Primary Secondary	302/324	238 (79%) 64 (21%)	----- -----
Duration of Infertility	302/324	5.2 ± 2.8	

¹Values are given as the mean ± SD or n (%)

We found a higher frequency of the +405 G allele in patients with endometriosis and in each clinical subgroup of patients suffering with endometriosis. Therefore, we report the possible involvement of the +405G allele in the 5¹ untranslated region of the VEGF might have a certain etiological significance in endometriosis.

An association has been reported between endometriosis and the +405 G>C VEGF gene polymorphisms. These 5 independent studies from South India, Korea, Italy, Australia and Iran (34-38). A clear discordance was found between the results on comparing their findings. The frequency of the +405 C/C genotype in patients with severe endometriosis was more than that of in the control group in study by Kim *et al.* (2005) The study performed by Gentilini *et al.* (2008) showed that the minor C allele is associated with the disease. Bhanoori *et al.* (2005) reported significant difference between the prevalence of endometriosis and +405G>C polymorphism. The studies performed by Zhen Zhen Zhao *et al.* 2008 and Memariani T. *et al.* 2010 also provide evidence supporting an association between these VEGF polymorphisms and endometriosis susceptibility.

Our data is in complete agreement with the study reported by Bhanoori *et al.* (2005). We have evaluated our control group extensively to understand the impact of the sampling design on the reported association. Our controls consisted of women in whom absence of endometriosis was laparoscopically confirmed, whereas in other studies healthy individuals were enrolled as controls (Bhanoori *et al.* 2005; Kim *et*

al., 2005). Secondly the sample size of other studies was comparatively smaller than the sample size of the present study.

We feel that our study design is more accurate because women who underwent laparoscopy and in whom no endometriosis was found served as the control group. In our study the patients with endometriosis were classified into 6 clinical subgroups. In the endometriosis group a significant difference was found in the genotype and allele frequencies regardless of whether the patients had the complications of adenomyosis, or chocolate cysts or the clinical stage was mild or severe.

We further analyzed the genotypes and allele frequencies for the VEGF +405G>C polymorphism among the clinical subgroups of patients with endometriosis. No significant difference was observed in the genotype and allele frequencies of the VEGF +405 polymorphism between the groups with Re-AFS stage I+II (mild endometriosis) and Re-AFS stage III+IV (severe endometriosis), with or without chocolate cysts and with or without adenomyosis (Table 3).

Our study strongly supports the findings that +405 G allele in the 5¹ untranslated region of the VEGF is associated, with the development and growth of endometriosis, and endometriosis associated with adenomyosis and chocolate cysts in South Indian women. This is the second study from South India that reports that the +405G allele in the 5¹ untranslated region of VEGF is associated, with the development and growth of endometriosis in South Indian women. Since our results are in agreement with the previous study of

Bhanoori *et al.*(34), it is possible that this accordance is due to the fact that the subjects had the same ethnicity.

6. ACKNOWLEDGMENTS

The authors are grateful to Bioserve Technologies Pvt limited India, for providing the necessary equipment for performing this investigation. We wish to thank, MHRT and OHRC, Hyderabad, India, for providing the samples and for their support throughout this study. We also wish to thank the Department of Biotechnology, India, for the funding to perform the above work.

7. REFERENCES

- Giudice LC, Kao LC: Endometriosis. *Lancet* 364, 1789-1799 (2004)
- Ryan IP, Taylor RN: Endometriosis and infertility: new concepts. *Obstet Gynecol Surv* 5, 365-371 (1997)
- Sampson JA: Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 14, 422-469 (1927)
- Jiang X, Morland SJ, Hitchcock A, Thomas EJ and Campbell IG: Allelotyping of endometriosis with adjacent ovarian carcinoma reveals evidence of a common lineage. *Cancer Res* 58, 1707-1712 (1998)
- Treloar SA, O'Connor DT, O'Connor VM and Martin NG: Genetic influences on endometriosis in an Australian twin sample. *Fertil Steril* 71, 701-710 (1999)
- Olive DL and Schwartz LB: Endometriosis. *N Engl J Med* 328, 1759-1769 (1993)
- Varma R, Rollason T, Gupta JK and Maher ER: Endometriosis and the neoplastic process. *Reproduction* 127, 293-304 (2004)
- Nisolle M and Donnez J: Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* 68, 585-596 (1997)
- Risau W: Mechanisms of angiogenesis. *Nature* 386, 671-674 (1997)
- Ferrara N: Molecular and biological properties of vascular endothelial growth factor. *J Mol Med* 77, 527-543 (1999)
- M. Louise Hull, D. Stephen Charnock-Jones, Clement L. K. Chan, Kaylon L. Bruner- Tran, Kevin G. Osteen, Brian D. M. Tom, Tai-Ping D. Fan and Stephen K. Smith: Antiangiogenic agents are effective inhibitors of endometriosis. *J Clin Endocrinol Metab* 88, 2889-2899 (2003)
- Bedaiwy MA and Falcone T: Laboratory testing for endometriosis. *Clin Chim Acta* 340, 41- 56 (2004)
- Annemiek W. Nap, Arjan W. Griffioen, Gerard A. J. Dunselman, Jessica C. A. Bouma-Ter Steege, Victor L. J. L. Thijssen, Johannes L. H. Evers and Patrick G Groothuis: Antiangiogenesis therapy for endometriosis. *J Clin Endocrinol Metab* 89, 1089-1095(2004)
- Carmeliet P and Jain RK: Angiogenesis in cancer and other diseases. *Nature* 407, 249-257 (2000)
- Vincenti V, Cassano C, Rocchi M and Persico G: Assignment of the vascular endothelial growth factor gene to human chromosome 6p21.3. *Circulation* 93, 1493-1495 (1996)
- Watson CJ, Webb NJ, Bottomley MJ, and Brenchley PE: Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine* 12, :1232 – 5 (2000)
- Hamamichi Y, Ichida F, Yu X, Hirono KI, Uese KI, Hashimoto I, Tsubata S, Yoshida T, Futatani T, Kanegane H, Miyawaki T: Neutrophils and mononuclear cells express vascular endothelial growth factor in acute Kawasaki disease:possible role in progression of coronary artery lesions. *Pediatr Res* 49, 74-80 (2001)
- Ferrara N, Gerber HP, LeCouter J: The biology of VEGF and its receptors. *Nat Med*, 9, 669-76 (2003)
- Takuya Awata, Kiyooki Inoue, Susumu Kurihara, Tomoko Ohkubo, Masaki Watanabe, Kouichi Inukai, Ikuo Inoue, and Shigehiro Katayama: A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. *Diabetes* 51, 1635-9 (2002)
- Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV: Novel polymorphisms in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene. *Hum Immunol* 60, 1245-9 (1999)
- Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E.:A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res* 37, 443-8 (2000)
- McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Muller KH, Sharkey AM and Smith SK: Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. *J Clin Invest* 98, 482 489 (1996)
- Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N, Jaffe RB and Taylor RN: Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J Clin Endocrinol Metab* 81, 3112-3118 (1996)

24. Donnez J, Smoes P, Gillerot S, Casanas-Roux F and Nisolle M : Vascular endothelial growth factor (VEGF) in endometriosis. *Hum Reprod* 13, 1686–1690 (1998)
25. Tan XJ, Lang JH, Liu DY, Shen K, Leng JH and Zhu L: Expression of vascular endothelial growth factor and thrombospondin-1 mRNA in patients with endometriosis. *Fertil Steril* 78, 148–153 (2002)
26. Nap AW, Griffioen AW, Dunselman GA, Bouma-Ter Steege JC, Thijssen VL, Evers JL and Groothuis PG : Antiangiogenesis therapy for endometriosis. *J Clin Endocrinol Metab* 89, 1089–1095 (2004)
27. Takuya Awata, Kiyoaki Inoue, Susumu Kurihara, Tomoko Ohkubo, Masaki Watanabe, Kouichi Inukai, Ikuo Inoue, and Shigehiro Katayama.: A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. *Diabetes* 51, 1635–9 (2002)
28. Akihiko Abe , Kazunari Sato, Tomonori Habuchi ,Lizhong Wang, Zhenhua Li, Norihiko Tsuchiya, Chikara Ohyama, Shigeru Satoh, Osama Ogawa, and Tetsuro Kato: Single nucleotide polymorphisms in the 31 untranslated region of vascular endothelial growth factor gene in Japanese population with or without renal cell carcinoma. *Tohoku J Exp Med* 198, 181 – 90 (2002)
29. Majid Shahbazi, Anthony A. Fryer, Vera Pravica, Iain J. Brogan, Helen M. Ramsay, Ian V. Hutchinson and Paul N. Harden: Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol* 13, :260–4 (2002)
30. Sarah L. McCarron, Stephen Edwards, Philip R. Evans, Roz Gibbs, David P. Dearnaley, Anna Dowe, Christine Southgate, The Cancer Research Campaign/British Prostate Group United Kingdom Familial Prostate Cancer Study Collaborators, Douglas F. Easton, Rosalind A. Eeles, and W. Martin Howell: Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* 62, 3369 – 72 (2002)
31. Howell WM, Bateman AC, Turner SJ, Collins A, Theaker JM: Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumour development in cutaneous malignant melanoma. *Genes Immun* 3, 229 – 32(2002)
32. Stevens A, Soden J, Brenchley PE, Ralph S, Ray DW: Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promote. *Cancer Res* 63, 812–816 (2003)
33. American Society for Reproductive Medicine: Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil Steril*, 67, 817- 821 (1997)
34. Manjula Bhanoori, K.Arvind Babu, N.G.Pavankumar Reddy, K.Lakshmi Rao, Krina Zondervan, Mamata Deenadaya, Stephen Kennedy and S.Shivaji: The vascular endothelial growth factor (VEGF) 1405G>C 51-untranslated region polymorphism and increased risk of endometriosis in South Indian women: a case control study. *Human Reproduction* 20, No.7, 1844–1849, (2005)
35. Sung Hoon Kim <http://humrep.oxfordjournals.org/content/20/10/2904.abstract> - aff-1, Young Min Choi , Seon Ha Choung , Jong Kwan Jun , Jung Gu Kim and Shin Yong Moon: Vascular endothelial growth factor gene +405 C/G polymorphism is associated with susceptibility to advanced stage endometriosis. *Human Reproduction* 20, No.10, 2904–2908, (2005)
36. Davide Gentilini, Edgardo Somigliana, Paola Vignani, Michele Vignali, Mauro Busacca, and Anna Maria Di Blasio: The vascular endothelial growth factor +405G.>C polymorphism in endometriosis. *Human Reproduction* 23, No.1, 211–215, (2008)
37. Zhen Zhen Zhao, Dale R. Nyholt, Shane Thomas, Susan A. Treloar and Grant W. Montgomery: Polymorphisms in the vascular endothelial growth factor gene and the risk of familial endometriosis. *Molecular Human Reproduction* .14, No.9, 531–538, 2008.
38. Memariani, Toktam, Salimi Nejad, Kioomars, Kamali, Kourosh , Shervin, Adel, Mohajer-Maghari, Behrokh, Akhondi Mohhamad Mehdi, Khorram Khorshi Hamid Reza: Association of Vascular Endothelial Growth Factor (VEGF) +405 G>C Polymorphism with Endometriosis in an Iranian Population. *J Reprod Infertil* 11, (1):33-37 (2010)

Abbreviations: VEGF: Vascular Endothelial Growth Factor, PCR: Polymerase Chain Reaction , Re-AFS: revised American Fertility Society

Key Words: Angiogenesis, Endometriosis, Polymorphism, VEGF, Laparoscopy

Send correspondence to: Maria Celestina Vanaja, Dept of Reproductive Medicine, MHRT-Hospital and Research Centre, 8-2-120/86/1/A, Road # 3, Banjara Hills, Hyderabad, 500 034, A.P, India, Tel: 91-9966902487, Fax:: 040-23552525, E-mail: drmariacelestinavanaja@gmail.com