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Genetic variation in COX-2 -1195 and the risk of endometriosis and adenomyosis

Y. Wang[1], Y. Qu[2], W. Song[3]

[1]Department of Obstetrics and Gynecology, Tangshan Worker's Hospital, Tangshan Hebei
[2]Department of Histology and Embryology, Basic Medical College of Hebei United University, Tangshan Hebei
[3]Department of Radiotherapy and Chemotherapy, Tangshan Worker's Hospital, Tangshan Hebei (China)

Summary

Aim: This study aims to explore the relationship between *COX-2* gene polymorphism and the hereditary susceptibility of endometriosis and adenomyosis. *Materials and Methods:* Gene polymorphism in *COX-2* gene was genotyped in 170 cases of endometriosis, 150 cases of adenomyosis, and 240 matched non-endometriosis and non-adenomyosis controls. *Results:* Genotypic frequencies of GG, AG, and AA in *COX-2* locus in endometriosis and adenomyosis were 16.5%, 51.2%, 32.4% and 16.0%, 49.3%, 34.7%, respectively. They were both significantly different from those in the control group (24.6%, 53.3%, and 22.1%) ($p < 0.05$). An allele frequency in endometriosis and adenomyosis were significantly higher than that in the control group. The risk of endometriosis or adenomyosis for those carrying two A alleles were 2.19 and 2.41 times to non-A genotype. *Conclusion:* Genetic variation of G to A at -1195 locus in the promoter region of *COX-2* gene increases the risk of endometriosis and adenomyosis, and the genetic susceptibility of these two diseases are similar.

Key words: Endometriosis; Adenomyosis; COX-2; genetic susceptibility; Single nucleotide polymorphisms (SNPs).

Introduction

Endometriosis and adenomyosis are two common gynecological diseases that usually manifest dysmenorrhea and infertility clinically [1, 2]. Their incidences in women in reproductive age are more than ten percent. To date, the exact etiology and pathogenesis of these two diseases are still not clear. Although both the etiology and pathological mechanisms are not similar in these two diseases, a certain genetic predisposition does exist indeed [3, 4]. In recent years, it is very enthusiastic to the polymorphism studies on these two diseases, and a lot of relationships between some genetic variants and these two diseases have been revealed. For example, Juo *et al.* found neither the CYP1A1 nor CYP17 genes, estrogen-metabolizing genes, had no significant association with either of the two diseases [5], meanwhile, Govindan *et al.* indicated a significant association of C allele of estrogen receptor (ER) alpha gene with endometriosis [6]. Liu *et al.* reported -1154G/A and -2578C/A in the vascular endothelial growth factor (VEGF) gene maybe decreased risk of endometriosis in Chinese women [7]. Still some other cytokine-related genes, such as TNF and IL [8-10], and matrix metalloproteinase gene family, such as MMP-2, MMP-7 and MMP-9 [11, 12] were investigated, and some certain relationships have been revealed. However, up to now, the study on genetic variants in cyclooxygenase-2(COX-2) gene in endometriosis and adenomyosis is relatively rare.

COX-2 is an important inducible enzyme in the inflammatory process. It is an important rate-limiting enzyme in the synthesis of prostaglandin (PG), which is an important mediator in the inflammatory process [13]. Normally, COX-2 does not express in most tissues. However, if the cells receive a variety of stimuli, such as inflammatory mediators, growth factors, cytokines and tumor promoters, COX-2 will be quickly synthesized and involved in the inflammatory processes and tumor's occurrence [14]. COX-2 overexpression has been confirmed in eutopic and ectopic endometrium of patients affected by endometriosis and/or adenomyosis in previous studies [15]. Therefore COX-2 is regarded as an important factor in the development of endometriosis and/or adenomyosis and its mechanism is that the two diseases are estrogen-dependent. Estrogen promotes the production of PG through the upregulation of COX-2, and establishes a positive feedback loop that the high concentration of peritoneal prostaglandin E2 (PGE2) increases the cyclic adenosine monophosphate (cAMP) level in cells. cAMP-dependent alternative pathway stimulates the production of P450 aromatase in ectopic endometrial cells and regulates the synthesis of estrogen and play an important role in the occurrence and development of endometriosis and adenomyosis [16, 17]. Recently, The Korean scholars Kim *et al.* reported the relationship between the *COX-2* -765 G>C genetic variation and endometriosis and concluded that C allele may be a protective factor from endometriosis [18]. In this

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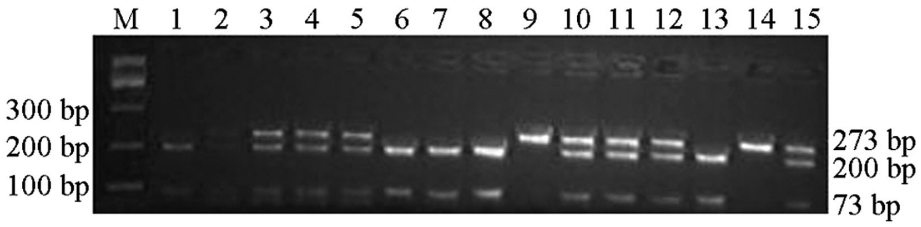

Figure 1. — Genotyping of *COX-2* -1195 by PCR-RFLP. One band of 273 bp represents genotype AA (lane 9 and 14), two bands of 200 and 73 bp represent genotype GG (lane 1, 6, 7, 8, and 13) and three bands of 273, 200, and 73 bp represent genotype GA (lane 2, 3, 4, 5, 10, 11, 12, and 15). M, DNA marker.

study, a case-control analyzing method was used to explore the distribution of the SNP locus at -1195bp in the promoter region of *COX-2* gene in the Han women with endometriosis and adenomyosis in Tangshan, China, with the aim to supply some molecular theoretical basis for revealing the pathogenesis of endometriosis and adenomyosis.

Table 1. — *Distribution of age in the three groups.*

Group	N	Age(years)		*F*	*p*
		Mean	SEM		
Endometriosis	170	39.82	6.06		
Adenomyosis	150	41.32	5.13	1.845	0.159
Control	240	39.40	6.41		

Materials and Methods

Subjects

The study included 170 cases of endometriosis, 150 cases of adenomyosis, and 240 matched non-endometriosis and non-adenomyosis controls that were enrolled. Patients with endometriosis or adenomyosis enrolled were from the Tangshan Han women hospitalized in the Department of Obstetrics and Gynecology of Tangshan Workers' Hospital and underwent a laparoscopy or laparotomy treatment from January 2007 to March 2011. All postoperative specimens were confirmed pathologically as ovarian chocolate cysts, pelvic endometriosis or adenomyosis and were classified as Stage II-IV using the revised American Fertility Society (AFS) classification of endometriosis. Patients in the control group were randomly selected from the non-endometriosis and non-adenomyosis patients treated in the same hospital during the same period. The age, menstrual history, reproductive history, family history, and other basic information of each subject was recorded. Patients complicated with other medical, surgical or endocrine diseases were excluded in this study. All subjects had not been treated with hormone therapy in the latest three months. Four ml peripheral venous blood sample from each subject was collected in a sodium citrate anticoagulant tube and stored at - 80ºC. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Tangshan Worker's Hospital. Written informed consent was also obtained from all participants.

Genomic DNA extraction

Genomic DNA was extracted from blood samples using proteinase K digestion and saturated phenol-chloroform extraction method and dissolved in TE buffer. The DNA concentration was measured with a spectrophotometer.

Polymerase chain reaction (PCR)

PCR-based restriction fragment length polymorphism (PCR-RFLP) was used for genotyping. The following specific gene primers pair was used: forward: 5′-CCC TGA GCA CTA CCC ATC AT-3′ and reverse: 5′-GCC CTT CAT AGG AGA TAC TGG-3′. The PCR product size was 273 bp. The PCR reaction system contained 100 ng DNA template, 0.1 mM primer, 0.2 mM dNTP, 1.0 IU Taq DNA polymerase , 1×buffer and 1.5 mM $MgCl_2$. The reaction was run in the program of 95°C pre-denatured for two minutes, 35 cycles of 94°C 30 s, 60°C 30 s, and 72°C 45 s, and another seven minutes extending at 72°C. Then the PCR products were kept in 4°C for the next experiment.

Enzyme digestion

One mg of the purified PCR production was used for enzyme digestion. The reaction also required two ml 10×buffer, 0.25 IU restriction enzyme PvuII and double-distilled water to a total volume of 20 ml. Enzyme digestion was performed at 37°C for four hours and then the production was analyzed with 2% agarose gel electrophoresis. Gel imaging system determined the digesting results.

Sequence analysis

Some of the PCR products were purified by agarose gel extraction and sequenced by an ABI 377 automatic sequencer in order to verify the mutations at the *COX-2* -1195 locus. AA was homozygous, GA was heterozygous, and the allele frequency = (2 × AA+GA) / (2×total cases). Genotyping was carried out blinded in order to ensure its reliability. In addition, repeated experiments were carried out by another researcher using 10% of the specimens randomly selected. The results were consistent with the first experimental results.

Statistical analysis

SPSS v17.0 software was used for statistical analysis in this work. Comparison of the age was carried out with analysis of variance. Comparison of genotypes was performed with Chi-square test. The odds ratio (OR) and 95% confidence interval (CI) calculated with non-conditional logistic regression model were used to indicate the strength of the association of genotypes with endometriosis and adenomyosis.

Results

Clinical characteristics

The age of each group showed no statistical difference (Table 1).

PCR-Restriction fragment length polymorphism (RFLP)

COX-2-1195 genotypes of the three groups were analyzed by PCR-RFLP. Genotype GG presented two bands of 200 bp and 73 bp, genotype GA presented three bands of

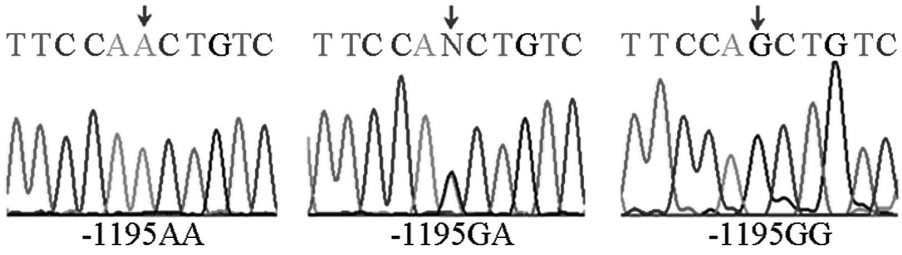

Figure 2. — Sequence analysis of the COX-2–1195 G>A polymorphism. Arrow identifies the position of nucleotide variation.

Table 2. — *Genotype frequencies of COX-2 in the two groups and their association with endometriosis.*

Group	N	Genotype (%)			A allele (%)
		GG	AG	AA	
Control	240	59 (24.6)	128 (53.3)	53 (22.1)	48.8
Case	320	52 (16.3)	161 (50.3)	107 (33.4)	58.6
χ^2			2.520	10.668	10.713
P			0.112	0.001	0.001
OR (95% CI)		1.00	1.43 (0.92~2.21)	2.29 (1.39~3.77)	

Table 3. — *Analysis of genotype frequencies of COX-2 among different groups.*

Group	Control	Endometriosis			Adenomyosis		
Genotypes	n = 240 (%)	n = 170 (%)	*p*	OR (95% CI)	n = 150 (%)	*p*	OR (95% CI)
GG	59 (24.6)	28 (16.5)			24 (16.0)		
GA	128 (53.3)	87 (51.2)	0.181	1.43 (0.85-2.42)	74 (49.3)	0.214	1.42 (0.82-2.47)
AA	53 (22.1)	55 (32.4)	0.009	2.19 (1.22-3.93)	52 (34.7)	0.005	2.41 (1.31-4.43)
A allele	234 (48.8)	197 (57.9)	0.009		178 (59.3)	0.004	

273, 200, and 73 bp, and genotype AA presented only a 273 bp band (Figure 1). The sequence analysis of some PCR products randomly selected from the three groups confirmed the results obtained from PCR-RFLP (Figure 2).

Relationship between COX-2 gene and endometriosis disease

Genotypic frequencies of GG, GA, and AA at -1195 bp in the promoter region of *COX-2* gene of the control group were 24.6%, 53.3%, and 22.1%, respectively. However, in the cases group including endometriosis and adenomyosis, their genotypic frequencies were 16.3%, 50.3% and 33.4%, respectively (Table 2). There were significant differences in the frequencies between the two groups (χ^2 =11.235, p = 0.004). According to the Hardy-Weinberg genetic equilibrium law, the distributions of the three genotypes in both the control group and the cases group are in line with the law, prompting that the subjects selected in this study was random in genetics. Genotypic frequency of AG showed no significant difference between the two groups (p = 0.112) but genotypic frequency of AA was significantly higher in the case group than in the control group (p = 0.001). In the case group, A allele frequency was significantly higher than that in control group. Non-conditional logistic regression analysis showed that the risk of endometriosis was 1.43 (95% CI = 0.92~2.21) for those carrying an A allele and it was 2.29 (95% CI =1.39~3.77) for those carrying two A alleles compared with GG genotype. It was markedly different between them (p = 0.001), indicating that A allele at -1195 bp in the promoter region of *COX-2* gene significantly increased the risk of endometriosis disease.

Relationship between COX-2 gene and endometriosis or adenomyosis

The distributions of the three genotypic frequencies in the three groups are in line with the Hardy-Weinberg genetic equilibrium law, indicating that subjects in this study are random in genetics. Genotypic frequencies of GG, GA, and AA at -1195 bp in the promoter region of *COX-2* gene were respectively 24.6%, 53.3%, and 22.1% in the control group, 16.5%, 51.2%, and 32.4% in the endometriosis group, and 16.0%, 49.3%, and 34.7% in the adenomyosis group (Table 3). They were dramatically higher in the endometriosis group and adenomyosis group than in the control group (χ^2 =7.159, p = 0.028 and χ^2 =8.909, p = 0.012, respectively) but showed no significant difference between the endometriosis group and the adenomyosis group (χ^2 = 0.192, p = 0.908). Difference in the AG genotypic frequency was not significant, while difference in the AA genotypic frequency was significant between the endometriosis group and the adenomyosis group. A allele frequency in both the endometriosis group and the adenomyosis group were higher than in the con-

trol group. The risk of endometriosis of those carrying two A alleles was 2.19 times to the GG genotype (95% CI =1.22~3.93), and the risk of adenomyosis of those carrying two A alleles was 2.41 times to the GG genotype (95% CI =1.31~4.43).

Discussion

The human COX-2 gene, mapped to chromosome 1q25.2–q25.3, is about 8.3 kilobase pairs in size and contains ten exons [19]. There are about 17 sites of the single nucleotide polymorphisms (SNPs) in COX-2 gene have been studied in some benign and malignant diseases, but most of them are non-functional [20-22]. As for *COX-2* -1195, a meta-analysis based on 25 case-control studies by Tang *et al.*, including a total of 9,482 cancer cases and 12,206 controls, indicated that the variant of COX-2 -1195G>A moderately increased risk of cancers (AA/AG versus GG, OR = 1.15, 95% CI: 1.02-1.31) [23]. Dasdemir *et al.* reported COX-2-1195 AG genotype was significantly high in patients with migraine [24]. However, to the authors' knowledge, the relationship between the G >A genetic variation at -1195 in the *COX-2* gene promoter region and endometriosis or adenomyosis has not yet been reported.

In this study, genotypic frequencies of GG, GA, and AA at -1195 bp in the promoter region of *COX-2* gene were 24.6%, 53.3%, and 22.1% in the control group, 16.5%, 51.2%, and 32.4% in the endometriosis group, and 16.0%, 49.3%, and 34.7% in the adenomyosis group. The genotypic frequencies in the control group were consistent with the literature reported by Zhang *et al.* that the distribution frequencies of GG, GA and AA at -1195 bp in the promoter region of *COX-2* gene were 22.5%, 53.4% and 24.1% in Han people [25]. The present authors found that *COX-2* -1195 AA genotypic frequency and A allele frequencies in the case group were significantly higher than those in the control group, but they were not quite different between the endometriosis group and the adenomyosis group. Carrying A alleles would increase the risk of adenomyosis disease, which was consistent with the functions of -1195 G>A polymorphism. According to the previous investigations, polymorphisms of -1195G>A can form a binding site for c-Myb and increases the *COX-2* gene promoter activity significantly, just as its carcinogenesis, regulate the balance among cell division, survival, and differentiation [26, 27], resulting in endometriosis. So the authors conjectured that -1195 G>A genetic variation may play an important role in the occurrence of endometriosis and adenomyosis, which have a similar pathogenesis.

Altogether, *COX-2* -1195 A allele would significantly increase the risk of endometriosis and adenomyosis, which may be an important factor that affects the individual genetic susceptibility to endometriosis and adenomyosis, whose genetic susceptibility is similar.

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References

[1] Ballard K., Lowton K., Wright J.: "What's the delay? A qualitative study of women's experiences of reaching a diagnosis of endometriosis". *Fertil. Steril*., 2006, *86*, 1296.

[2] Bhattacharya S., Porter M., Amalraj E., Templeton A., Hamilton M., Lee A.J., *et al*.: "The epidemiology of infertility in the North East of Scotland". *Hum. Repro*d., 2009, *24*, 3096.

[3] Tempfer C.B., Simoni M., Destenaves B., Fauser B.C.: "Functional genetic polymorphisms and female reproductive disorders: Part II-endometriosis". *Hum. Reprod. Update*, 2009, *15*, 97.

[4] Costa I.R., Silva R.C., Frare A.B., Silva C.T., Bordin B.M., Souza S.R., *et al*.: "Polymorphism of the progesterone receptor gene associated with endometriosis in patients from Goiás, Brazil". *Genet. Molecul. Res*., 2011, *10*, 1364.

[5] Juo S.H., Wang T.N., Lee J.N., Wu M.T., Long C.Y., Tsai E.M.: "CYP17, CYP1A1 and COMT polymorphisms and the risk of adenomyosis and endometriosis in Taiwanese women". *Hum. Reprod*., 2006, *21*, 1498.

[6] Govindan S., Shaik N.A., Vedicherla B., Kodati V., Rao K.P., Hasan Q.: "Estrogen receptor-alpha gene (T/C) Pvu II polymorphism in endometriosis and uterine fibroids". *Dis. Markers*, 2009, *26*, 149.

[7] Liu Q., Li Y., Zhao J., Sun D.L., Duan Y.N., Wang N., *et al*.: "Association of polymorphisms -1154G/A and -2578C/A in the vascular endothelial growth factor gene with decreased risk of endometriosis in Chinese women". *Hum. Reprod*., 2009, *24*, 2660.

[8] Gentilini D., Somigliana E., Vigano P., Vignali M., Busacca M., Di Blasio A.M.: "The vascular endothelial growth factor +405G>C polymorphism in endometriosis". *Hum. Reprod*., 2008, *23*, 211.

[9] Lee G.H., Choi Y.M., Kim S.H., Hong M.A., Oh S.T., Lim Y.T., *et al*.: "Association of tumor necrosis factor-{alpha} gene polymorphisms with advanced stage endometriosis". *Hum. Reprod*., 2008, *23*, 977.

[10] Zhang X., Hei P., Deng L., Lin J.: "Interleukin-10 gene promoter polymorphisms and their protein production in peritoneal fluid in patients with endometriosis". *Mol. Hum. Repro*d., 2007, *13*, 135.

[11] Shan K., Lian-Fu Z., Hui D., Wei G., Na W., Xia J., *et al*.: "Polymorphisms in the promoter regions of the matrix metalloproteinases-7, -9 and the risk of endometriosis and adenomyosis in China". *Mol. Hum. Reprod*., 2006, *12*, 35.

[12] Saare M., Lamp M., Kaart T., Karro H., Kadastik U., Metspalu A., *et al*.: "Polymorphisms in MMP-2 and MMP-9 promoter regions are associated with endometriosis". *Fertil. Steril*., 2010, *94*, 1560.

[13] Singh J., Hamid R., Reddy B.S.: "Dietary fat and colon cancer: modulation of cyclooxygenase-2 by types and amount of dietary fat during the postinitiation stage of colon carcinogenesis". *Cancer Res*., 1997, *57*, 3465.

[14] Moran E.M.: "Epidemiological and clinical aspects of nonsteroidal antiinflammatory drugs and cancer risks". *J. Environ. Pathol. Toxicol. Onco*l. 2002, *21*, 193.

[15] Fagotti A., Ferrandina G., Fanfani F., Legge F., Lauriola L., Gessi M., *et al*.: "Analysis of cyclooxygenase-2 (COX-2) expression in different sites of endometriosis and correlation with clinico-pathological parameters". *Hum. Reprod.*, 2004, *19*, 393.

[16] Li X., Gong X., Zhu L., Leng J., Fan Q., Sun D., *et al*.: "Stretch magnitude- and frequency-dependent cyclooxygenase 2 and prostaglandin E2 up-regulation in human endometrial stromal cells: possible implications in endometriosis". *Exp. Biol. Med. (Maywood)*., 2012, *237*, 1350.

[17] Machado D.E., Berardo P.T., Landgraf R.G., Fernandes P.D., Palmero C., Alves L.M., *et al*.: "A selective cyclooxygenase-2 inhibitor suppresses the growth of endometriosis with an antiangiogenic effect in a rat model". *Fertil. Steril*., 2010, *93*, 2674.

[18] Kim H.Y., Cho S., Choi Y.S., Yang H.I., Lee K.E., Seo S.K., *et al*.: "Cyclooxygenase-2 (COX-2) gene -765 G/C polymorphism and advanced-stage endometriosis in Korean women". *Am. J. Reprod. Immunol*., 2012, *68*, 238.

[19] Fritsche E., Baek S.J., King L.M., Zeldin D.C., Eling T.E., Bell D.A.: "Functional characterization of cyclooxygenase-2 polymorphisms". *J. Pharmacol. Exp. Ther*. 2001, *299*, 468.

[20] Pereira C., Medeiros R.M., Dinis-Ribeiro M.J.: "Review Cyclooxygenase polymorphisms in gastric and colorectal carcinogenesis: are conclusive results available"? *Eur. J. Gastroenterol. Hepatol*., 2009, *21*, 76.

[21] Humar B., Giovanoli O., Wolf A., Attenhofer M., Bendik I., Meier R., *et al*.: "Germline alterations in the cyclooxygenase-2 gene are not associated with the development of extracolonic manifestations in a large swiss familial adenomatous polyposis kindred". *Int. J. Cancer*, 2000, *87*, 812.

[22] Spirio L.N., Dixon D.A., Robertson J., Robertson M., Barrows J., Traer E., *et al*.: "The inducible prostaglandin biosynthetic enzyme, cyclooxygenase 2, is not mutated in patients with attenuated adenomatous polyposis coli". *Cancer Res*., 1998, *58*, 4909.

[23] Tang Z., Nie Z.L., Pan Y., Zhang L., Gao L., Zhang Q., *et al*.: "The Cox-2 -1195 G>A polymorphism and cancer risk: a meta-analysis of 25 case-control studies". *Mutagenesis*, 2011, *26*, 729.

[24] Dasdemir S., Cetinkaya Y., Gencer M., Ozkok E., Aydin M., Cakmakoglu B.: "Cox-2 gene variants in migraine". *Gene*, 2013, *518*, 292.

[25] Zhang X., Miao X., Tan W., Ning B., Liu Z., Hong Y., *et al*.: "Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer". *Gastroenterology*, 2005, *129*, 565.

[26] Shin W.G., Kim H.J., Cho S.J., Kim H.S., Kim K.H., Jang M.K., *et al*.: "The COX-2-1195AA Genotype Is Associated with Diffuse-Type Gastric Cancer in Korea". *Gut. Liver*, 2012, *6*, 321.

[27] Ramsay R.G., Barton A.L., Gonda T.J.: "Targeting c-Myb expression in human disease". *Expert Opin. Ther. Targets*, 2003, *7*, 235.

Address reprint requests to:
W. SONG, M.D.
Department of Radiotherapy and Chemotherapy,
Tangshan Worker's Hospital,
Tangshan Hebei 063000 (China)
e-mail: wenguangsongcn@126.com