The Role of Apurinic/Apyrimidinic Endonuclease DNA Repair Gene in Endometriosis

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Abstract. *Background/Aim: The altered cellular repair capacity plays a critical role in genomic instability and carcinogenesis. We aimed at evaluating the contribution of the polymorphic variant in apurinic/apyriminidinic endonuclease (APEX1) gene to its mRNA and protein levels and the risk of endometriosis. Patients and Methods: In the current case-control study, 153 endometriosis patients and 636 non-endometriosis controls were recruited. APEX1 Asp^{148}Glu (rs1130409) genotyping was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). At the same time, twenty eight endometriosis tissue samples with different genotypes were examined regarding their expression levels of APEX1 mRNA and protein by quantitative reverse transcription-polymerase chain reaction (q-PCR) and western blotting, respectively. Results: Compared with wild-type TT genotype, TG and GG genotypes of APEX1 Asp^{148}Glu had a risk of endometriosis of 0.93- and 0.87-fold. The results from in vivo transcriptional (RNA) and translational (protein) level analysis revealed that the APEX1 mRNA and protein were of similar levels among the endometriosis tissues of people carrying TT, TG, or GG genotypes. There was no joint effect of APEX1 Asp^{148}Glu genotype with menarche, pregnancy, smoking or alcohol drinking lifestyles on endometriosis risk. Conclusion: The APEX1 Asp^{148}Glu genotype correlates well with its mRNA and protein expression among endometriosis patients and may not serve as a sensitive marker for prediction of endometriosis risk in Taiwan.*

Worldwide, approximately 10% of females at reproductive ages suffer from endometriosis, which is an hormone-dependent inflammatory benign gynecological disease defined as that functional endometrial tissue is unusually present outside the uterus (1, 2). In clinical practice, the main manifestations are dysmenorrhea, chronic pelvic pain, painful intercourse and infertility (3). Although the mechanisms underlying endometriosis are still unrevealed, mounting evidence has shown that endometriosis is a multi-factor, multi-step disease affected by inflammation, hormonal regulation, genetic and environmental interactions (4-6).

Among the cancer hallmarks, genomic instability may also be closely related to the etiology of endometriosis and several polymorphic biomarkers on DNA repair genes have been found (7-10). The DNA repair capacity of a cell is vital to the integrity of its genome and, thus, to its regular functions and that of the organism (11). Therefore, any subtle mutations or polymorphisms in the DNA repair genes, which contribute to the loss of its function, are thought to play a critical role in carcinogenesis (12-14). Since endometriosis also has the deregulated proliferation property similar to cancers, it is reasonable that genetic variants of the DNA repair genes might also be involved in the initiation and development of endometriosis.

Among the known DNA repair pathways, base excision repair (BER) pathway are in charge of removing the DNA adducts induced mainly by oxidation and alkylation and, thus, protecting cells against the cytotoxic effects at the first line (15, 16). Immediately after the DNA insults, DNA glycosylases recognize and excise the altered bases of purine

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or pyrimidine leaving some abasic sites. Consequently, apurinic/apyrimidinic endonucleases (APEX1, also known as APE, APE1, APEX, HAP1 and Ref-1) incise the DNA 5' to the abasic sites. Then the continued processes undergo a short-patch (when the gap is only one nucleotide) or long-patch (when the gap is two or more nucleotides) sub-pathways of the BER system (17). From the above background we know that the virtual human APEX1 is uniquely responsible to the repair of apurinic/apyrimidinic sites at altered DNA produced either spontaneously hydrolyzing the 5'-phosphodiester bone of the apurinic/apyrimidinic site or after enzymatic removal of damaged bases. In addition to its apurinic/apyrimidinic endonuclease activity, APEX1 can also act as a 3'-phosphodiesterase initiating repair of DNA strand breaks with 3'-blocking damage, which are produced either directly by reactive oxygen species or indirectly through the enzymatic removal of damaged bases (18, 19). Furthermore, APEX1 also reduces-oxidizes activators for several transcription factors reported to be important in carcinogenesis, such as activator protein (Fos/Jun), hypoxia-inducible factor 1, cAMP-responsive element binding protein and p53 (20-22). In a knockout mice model, APEX1 deficiency induced embryonic lethality that strengthened the importance of APEX1 in maintaining the genomic integrity and viability not only of cells but of the whole organism (23).

It has been shown that genetic polymorphisms in DNA repair genes have conferred predisposition to many types of cancer and, as mentioned above, there exist a few studies that investigated the association between the genotypes of some DNA repair genes and the endometriosis risk, such as *XRCC1, XRCC3, BLHX, TP53* and *XRCC4* (7, 8, 10, 14). However, no study has yet confirmed the association between the polymorphisms of *APEX1*, which is a central gene in human BER, and the risk of endometriosis. In recent years, several epidemiological studies have examined the contribution of *APEX1* genotypes to several types of cancer, including bladder (24), lung (25-27), gastric (28), prostate (29), colorectal (30) and head and neck cancer (31). We assumed that the genotypes, together with phenotypes of *APEX1*, may also contribute to the personal endometriosis risk determination. Thus, the present case-control study aimed at revealing the relationship between *APEX1* genotype/phenotype and risk for endometriosis in a moderate Taiwan female population. For the *APEX1* genotype, we chose the most common polymorphism in literature, *APEX1* Asp^{148}Glu (rs1130409), to investigate the association between *APEX1* genetic polymorphism with endometriosis risk. For the *APEX1* phenotype, the mRNA and protein expression levels of various *APEX1* genotypes *in vivo* were examined by reversed transcription PCR and western blotting assays, respectively. To the best of our knowledge, this is the first study to evaluate the contribution of the *APEX1* Asp^{148}Glu genotype and its phenotype to endometriosis susceptibility.

Materials and Methods

Study population. One hundred and fifty-three patients diagnosed with endometriosis were recruited at the outpatient clinics of general surgery during 2000-2010 at Chung Shan Medical University Hospital in Taiwan. The endometriosis patients were diagnosed by laparoscopy, classified according to the American Society for Reproductive Medicine and confirmed histologically. Patients with pathological confirmation or clinical suspicion of leiomyoma, adenomyosis or invasive carcinoma of the uterine cervix or ovary were excluded from this study. No patient had received hormone therapy during the preceding 12 months. The mean age of the endometriosis patients was 40.3±4.9 years, while 55 of them (35.9%) did not have a child or full pregnancy. The basal follicle-stimulating hormone (FSH) level was 7.2±1.4 IU/l. The non-endometriosis statuses were confirmed after detail ultrasonography. All operations were performed by the experienced surgeon Dr. Yin and his colleagues. According to the revised American Fertility Society classification, 32 (20.9%) had minimal or mild endometriosis (stage I-II) and 121 (79.1%) had moderate or severe endometriosis (stage III-IV). All women accepted to provide their peripheral blood sampling for genotype analyses with their informed consent. The experiment was approved by the Ethical Committee and Institutional Review Board of the Chung Shan Medical University Hospital. Six hundred and thirty-six non-endometriosis healthy volunteers were selected as the controls by matching for age and habits after initial random sampling from the Health Examination Cohort of China Medical University Hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin or any familial disease. Both groups completed a questionnaire, which included the individual smoking and drinking habits. Smokers were defined as daily or almost daily smokers who had smoked at least five packs of cigarettes in their lifetime. Smokers were asked for the age of initiation, whether they were currently smoking or had already quit and, if so, when they had quit and, on average, how many cigarettes they smoked or had smoked daily. The non-drinkers included those with social drinking behavior of less than 200 ml per week and less than twice per month.

Genotyping protocol. The total genomic DNA of each participant was extracted from the leucocytes of peripheral blood using a QIAamp Blood Mini Kit (Qiagen, Taipei, Taiwan) as previously published (27). The primers used for *APEX1* Asp^{148}Glu were: forward 5'-CCAGCTGAACTTCAGGAGCT-3', and reverse 5'-CTCGGCCTGCATTAGGTACA-3'. The following cycling conditions were performed: started with one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s and 72˚C for 30 s; and a final extension at 72˚C for 10 min. The resultant 350-bp PCR product was mixed with 2 U *MnlI* and the enzyme digestion was carried out for 2 h at 37˚C. The G form PCR products could be further digested, while the T form could not. Two fragments of 252 and 98 bps were present if the product was G form digestible. Then, 10 μl of product was loaded into a 3% agarose gel electrophoresis and recognized under UV light with ethidium bromide staining.

APEX1 mRNA expression pattern. To evaluate the correlation between the *APEX1* mRNA expression and *APEX1* genotype, 28 surgically removed tissue samples adjacent to endometriosis with different genotypes were subjected to extraction of total RNA using the Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Total RNA

was measured by real-time quantitative RT-PCR using the FTC-3000 real-time quantitative PCR instrument series (Funglyn Biotech Inc., Toronto, Canada). *GAPDH* was used as an internal quantitative control. The primers used for amplification of *APEX1* mRNA were 5'-GCCCACTCAAAGTTTCTTAC-3' as forward one and 5'-TGTGCCACATTGAGGTCTCC-3' as reverse, while the primers for *GAPDH* were 5'-GAAATCCCATCACCATCTTCCAGG-3' as forward and 5'-GAGCCCCAGCCTTCTCCATG-3' as reverse. Fold changes were normalized by the levels of *GAPDH* expression and each assay was performed at least in triplicate.

APEX1 protein expression pattern. The endometriosis or control specimens were homogenized in RIPA lysis buffer (Upstate Inc., Lake Placid, NY, USA), the homogenates were then centrifuged at 10,000 g for 30 min at 4˚C and the supernatants were used for Western blotting. The samples were denatured by heating at 95˚C for 10 min, separated in a 10% SDS-PAGE gel and the resultants were transferred to a nitrocellulose membrane (Bio-Rad, CA, USA). The membrane was blocked with 5% non-fat milk and incubated overnight at 4˚C with a first anti-APEX1 antibody (1:1000; Santa Cruz Biotechnology, CA, USA) and then with the secondary horseradish peroxidase-conjugated goat anti-mouse IgG antibody (Chemicon, Temecula, CA, USA) for 1 h at room temperature. After reaction with the ECL solution (Amersham, Arlington Heights, IL, USA), the bound antibodies were visualized using a chemiluminescence imaging system (Syngene, Cambridge, UK). Finally, the blots were incubated at 56˚C for 18 min in stripping buffer (0.0626 M Tris-HCl, pH 6.7, 2% SDS, 0.1 M mercaptoethanol) and re-probed with a monoclonal mouse anti-α-tubulin antibody (Sigma, St. Louis, MO, USA) as the loading control. The optical densities of each specific band were measured using a computer-assisted imaging analysis system (Gene Tools Match software; Syngene).

Statistical analysis. To ensure that the controls used were representative of the general population and exclude the possibility of genotyping error, the deviation of the genotype frequencies of *APEX1* single nucleotide polymorphisms in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Chi-square test was used to compare the distribution of the *APEX1* genotypes between cases and controls. The associations between the *APEX1* polymorphisms and endometriosis risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis with the adjustment for possible confounders. The expression levels of mRNA and protein were examined by unpaired Student's *t*-test. Values of $p<0.05$ were considered statistically significant; all statistical tests were two-sided.

Results

Basic comparisons of the cases and controls. The characteristics of the control and endometriosis subjects are presented in Table I. No differences between the case and control group were found regarding age, menarche age, smoking or alcohol drinking status ($p>0.05$). Noticeably, there were less females having full pregnancy among cases than in the controls (64.1% *versus* 75.9%) and the significance level ($p=0.0041$) suggested that full pregnancy may be one of the protective factors for endometriosis.

Association of APEX1 genotypes with endometriosis risk. The genotypic distributions of the APEX1 $Asp^{148}Glu$ polymorphism in endometriosis cases and controls are presented in Table II. The crude ORs for women carrying TG and GG genotypes were 0.93 (95% CI=0.64-1.36) and 0.87 (95% CI=0.51-1.48) respectively, compared to those carrying the TT wild-type genotype. After the adjustment of the confounding factors (age, full pregnancy, smoking and alcohol drinking status) the ORs for women carrying TG and GG genotypes became 0.94 (95% CI=0.62-1.33) and 0.86 (95% CI=0.49-1.42), respectively, compared to those carrying the TT wild-type genotype. The *p* for trend was not significant ($p=0.8532$). In the dominant (TG plus GG *versus* TT) or recessive (GG *versus* TT plus TG) analyzing models, the association between *APEX1* $Asp^{148}Glu$ polymorphism with endometriosis risk was not statistically significant either (Table II). To sum up, these results indicated that individuals carrying a variant G allele at *APEX1* $Asp^{148}Glu$ may not have a higher risk of endometriosis.

Correlation of the APEX1 $Asp^{148}Glu$ genotype with the expression levels of the APEX1 mRNA and protein. The frequencies of the TT, TG and GG genotypes of the *APEX1* $Asp^{148}Glu$ of the 28 surgically-removed endometriosis tissue samples, were 13, 11 and 4, respectively. The effects of these three genotypes on the transcriptional expression of mRNA levels and translational expression of protein levels were evaluated by quantitative RT-PCR and western blotting, respectively (Figure 1). There was no obvious difference found among the mRNA or protein levels of various genotypes (Figure 1).

Interaction of APEX1 genotypes with pregnancy, menarche, smoking and alcohol drinking status. It is reasonable that the genetic variation of a low-penetrance gene, such as *APEX1* in this paper, may not significantly contribute to the susceptibility of endometriosis as much as the environmental factors or the individual smoking lifestyle to smoking-related cancers. The environmental factors for endometriosis among Taiwan females are not clear. Therefore, we are interested in analyzing the interaction of the *APEX1* $Asp^{148}Glu$ genotype with some factors including pregnancy, menarche, smoking and alcohol drinking status. As shown in Table III, the frequencies of various *APEX1* $Asp^{148}Glu$ genotypes were not significantly different between endometriosis and non-endometriosis control groups among those who have early menarche (≤12.8 years old) or late menarche (>12.8 years old) ($p=0.7673$ and 0.3030, respectively) (Table III). As show in Table IV, the frequencies of various *APEX1* $Asp^{148}Glu$ genotypes were not significantly different between endometriosis and non-endometriosis control groups among non-smokers or smokers ($p=0.3634$ and 0.2765, respectively) (Table IV). The frequencies of various *APEX1*

Table I. *Distributions of selected characteristics among endometriosis cases and control subjects.*

Characteristics	Cases (n=153)		Controls (n=636)		*p*-Value
	N	%	N	%	
Age (year) (mean±SD)	40.3±4.9		41.2±4.5		0.8865
Age at menarche					
≤12.8	85	55.6%	318	50.0%	0.2418
>12.8	68	44.4%	318	50.0%	
Full pregnancy					
No	55	35.9%	153	24.1%	0.0041*
Yes	98	64.1%	483	75.9%	
Smoking status					
Non-smokers	113	73.9%	476	74.8%	0.8361
Smokers	40	26.1%	160	25.2%	
Alcohol drinking status					
Non-drinkers	116	75.8%	463	72.8%	0.4772
Drinkers	37	24.2%	173	27.2%	
Stages					
I or II	32	20.9%			
III or IV	121	79.1%			

*Statistically significant; SD, standard deviation.

Table II. *Distributions of genotypic frequencies and their association with risk of endometriosis.*

	Cases (%)	Controls (%)	Crude OR (95% CI)	Adjusted ORa (95% CI)	*p*-Value
APEX1 Asp148Glu (rs1130409)					
TT	72 (47.1)	285 (44.8)	1.00 (ref)	1.00 (ref)	
TG	60 (39.2)	255 (40.1)	0.93 (0.64-1.36)	0.94 (0.62-1.33)	0.7706
GG	21 (13.7)	96 (15.1)	0.87 (0.51-1.48)	0.86 (0.49-1.42)	0.6878
p for trend					0.8532
(TG+GG) *vs.* TT			0.91 (0.64-1.30)	0.88 (0.65-1.34)	0.6514
GG *vs.* (TT+TG)			0.89 (0.54-1.49)	0.86 (0.55-1.47)	0.7999

[a]Adjusted by age, pregnancy, smoking and alcohol drinking status; CI, confidence interval; OR, odds ratio.

$Asp^{148}Glu$ genotypes were not significantly different between endometriosis and non-endometriosis control groups among those with or without full pregnancy and alcohol drinkers or non-drinkers (data not shown).

Discussion

Endometriosis, which has the same features of malignant tumors, is one of the serious gynecological diseases among females. The DNA repair systems act as gatekeepers for genome integrity by preventing the accumulated genetic mutations. Mounting evidence from cancer genomic studies showed that the genomic instability of cancer cells is a common event found in the steps of both cancer initiation and progression. In the current study, the association of *APEX1* genotype and endometriosis risk was firstly investigated in Taiwanese women, where the prevalence of endometriosis was 2.7% during 1998 to 2008 (32). The *APEX1* $Asp^{148}Glu$ is the most common polymorphism studied resulting in amino acid alteration (33). Previous reports have shown a controversial contribution of the *APEX1* $Asp^{148}Glu$ genotype to the risk of other types of cancer. For instance, the association of the *APEX1* $Asp^{148}Glu$ genotype with lung cancer risk, although well studied, remains inconclusive (27, 34-37). Up to now, there has been no report investigating the association of *APEX1* genotype with endometriosis risk.

The percentage of women with full pregnancy experience in the endometriosis group was lower than that of the control (64.1% *vs.* 75.9%) (Table I) supporting the concept that endometriosis is suspected to contribute to infertility (38). From the results of the *APEX1* $Asp^{148}Glu$ genotyping, we found that individuals carrying variant TG or GG genotypes were not of higher risk for endometriosis compared with

those carrying the wild-type TT genotype (Table II). We have also investigated the correlation of the *APEX1* Asp^{148}Glu genotype from the angles of transcriptional (*APEX1* mRNA) and translational (APEX1 protein) expression levels using tissues collected from endometriosis patients. The results showed that the endometrial tissues from women with TT, TG or GG *APEX1* Asp^{148}Glu genotypes were of similar level of *APEX1* mRNA and protein levels (Figure 1). It is not surprising that the amino-acid substitution variants on *APEX1* Asp^{148}Glu lead to similar resultant levels of mRNA and protein. Further efforts could be made on the (i) genotyping of polymorphisms in the promoter region, such as the promoter -141T/G (rs1760944) (25); (ii) functional analysis of overall base excision repair capacity in the primarily cultured cells from tissues with different genotypes since, compared with wild-type mice, the APEX1 heterozygous animals were reported to have accelerated DNA damage adducts accumulated in mitochondrial DNA and spontaneous mutagenesis (39); (iii) enlargement of sample size that will be helpful for enhancing the analyzing power of overall or stratified comparisons.

It is widely accepted that endometriosis is a multi-step and multi-factorial disease and factors, such as hormone exposure, inflammation, familial predisposition, growth factors, diet, altered immune system, environmental factors and oxidative stress status, may contribute to the initiation and progression of the disease and its possible transformation into endometrial cancer. However, the majority of the mechanisms involved are unknown. As for endometriosis and endometrial cancer, early menarche, low parity, late menopause, infertility, organochlorinated persistent pollutants have been implicated to be risk factors for them (40-42). Some of the etiology of these factors was closely related to the difference estrogen exposure status, and it is believed that longer estrogen exposure period may contribute to higher disease susceptibility. However, the collections of precise records and the investigations of their direct and indirect relationship are both time- and effort-consuming and their association(s) with endometriosis had not been well-established. Thus, in Tables III and IV, we present the evaluation of the joint effect of the polymorphism of *APEX1* genes stratified by menarche age and smoking status. We show that *APEX1* Asp^{148}Glu genotypes had no obvious preferential distribution among each subgroup suggesting that the *APEX1* Asp^{148}Glu genotype did not enhance the risk of endometriosis to either those with early or late menarche (Table III), or to smokers or non-smokers (Table IV). Concerning women's full pregnancy experience and alcohol drinking, the effect is not obvious either (data not shown). As mentioned above, the limited sample size may restrict the reliability and feasibility of stratification and interaction analyses.

In the present study no significant association of the *APEX1* genotype was found. However, we could not ignore

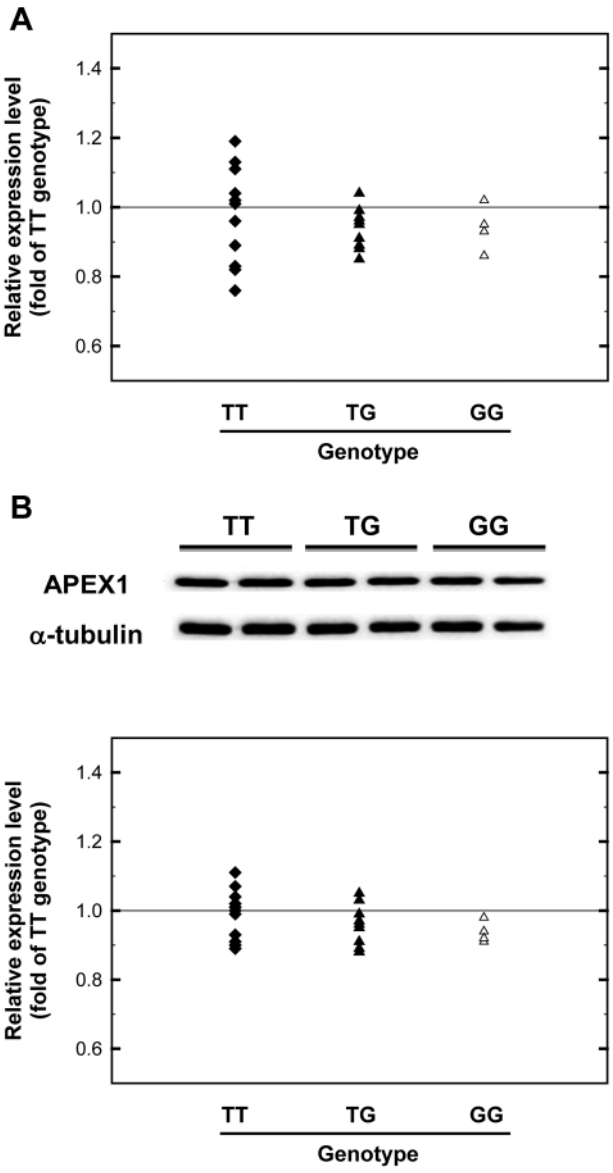

Figure 1. *Analysis of APEX1 mRNA and protein expression levels. (A), Quantitative RT-PCR for APEX1 from the endometriosis tissue samples of TT, TG and GG genotypes was performed (GAPDH was used as internal quantitative control). Fold alterations were normalized by the levels of control GAPDH expression and each assay was performed in at least three times; (B), Western blotting analysis was performed from the endometriosis tissue samples of TT, TG and GG genotypes and quantitated for statistical comparison. α-tubulin was used as the internal standard. No statistical difference was found by the Student's t-test.*

the contribution of APEX1 in the etiopathology of endometriosis. It is possible that some other polymorphic sites, such as the promoter -141T/G mentioned previously, may be related to an alteration in the expression level of mRNA, protein and, consequently, its function, which could lead to an enhanced risk of endometriosis. To make the

Table III. *Association between APEX1 genotype and risk of endometriosis stratified by menarche age.*

APEX1 genotype	Early menarche		Late menarche	
	Cases/controls	aOR (95% CI)[a]	Cases/controls	aOR (95% CI)[a]
Asp[148]Glu (rs1130409)				
TT	38/147	1.00 (ref)	34/138	1.00 (ref)
TG	32/125	0.98 (0.61-1.65)	28/130	0.88 (0.51-1.58)
GG	15/46	1.25 (0.62-2.48)	6/50	0.49 (0.22-1.19)
p for trend		0.7673		0.3030

aOR, Adjusted odds ratio; CI, confidence interval. [a]Data were adjusted for pregnancy, smoking and alcohol drinking status.

Table IV. *Association between APEX1 genotype and risk of endometriosis stratified by personal smoking status.*

APEX1 genotype	Non-smokers		Smokers	
	Cases/controls	aOR (95% CI)[a]	Cases/controls	aOR (95% CI)[a]
Asp[148]Glu (rs1130409)				
TT	53/200	1.00 (ref)	19/85	1.00 (ref)
TG	47/197	0.94 (0.61-1.40)	13/58	0.94 (0.43-2.01)
GG	13/79	0.69 (0.38-1.15)	8/17	1.80 (0.68-4.89)
p for trend		0.3634		0.2765

aOR, Adjusted odds ratio; CI, confidence interval. [a]Data were adjusted for age, pregnancy, and alcohol drinking status.

predictive, preventive, personalized and participatory medicine and therapy for endometriosis feasible and to lower the speed and the prevalence of several types of cancer and endometriosis in developed countries, the environmental factors which may cause lots of oxidative damage related to BER, such as the shoddy oil used in cosmic and in daily diet, smoking and alcohol drinking, could be adaptively prohibited step by step in our society.

In conclusion, the current study provided evidence from DNA, RNA and protein levels showing that *APEX1* Asp[148]Glu was not associated with endometriosis in Taiwan. Further understanding of the role of DNA repair genes, such as APEX1, along with genotypic and phenotypic evidence, is in urgent need to reveal the etiology of endometriosis.

Conflicts of Interest

The Authors declare no conflicts of interest.

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References

1 Cramer DW, Wilson E, Stillman RJ, Berger MJ, Belisle S, Schiff I, Albrecht B, Gibson M, Stadel BV and Schoenbaum SC: The relation of endometriosis to menstrual characteristics, smoking, and exercise. JAMA *255*: 1904-1908, 1986.

2 Giudice LC and Kao LC: Endometriosis. Lancet *364*: 1789-1799, 2004.

3 Bulun SE: Endometriosis. N Engl J Med *360*: 268-279, 2009.

4 Falconer H, D'Hooghe T and Fried G: Endometriosis and genetic polymorphisms. Obstet Gynecol Surv *62*: 616-628, 2007.

5 Olive DL and Schwartz LB: Endometriosis. N Engl J Med *328*: 1759-1769, 1993.

6 Rizner TL: Estrogen metabolism and action in endometriosis. Mol Cell Endocrinol *307*: 8-18, 2009.

7 Monteiro MS, Vilas Boas DB, Gigliotti CB and Salvadori DM: Association among XRCC1, XRCC3, and BLHX gene polymorphisms and chromosome instability in lymphocytes from patients with endometriosis and ovarian cancer. Genet Mol Res *13*: 636-648, 2014.

8 Gallegos-Arreola MP, Valencia-Rodriguez LE, Puebla-Perez AM, Figuera LE and Zuniga-Gonzalez GM: The TP53 16-bp duplication polymorphism is enriched in endometriosis patients. Gynecol Obstet Invest *73*: 118-123, 2012.

9 Bau DT, Hsieh YY, Wan L, Wang RF, Liao CC, Lee CC, Lin CC, Tsai CH and Tsai FJ: Polymorphism of XRCC1 codon arg 399 Gln is associated with higher susceptibility to endometriosis. Chin J Physiol *50*: 326-329, 2007.

10 Hsieh YY, Bau DT, Chang CC, Tsai CH, Chen CP and Tsai FJ: XRCC4 codon 247*A and XRCC4 promoter -1394*T related genotypes but not XRCC4 intron 3 gene polymorphism are

associated with higher susceptibility for endometriosis. Mol Reprod Dev *75*: 946-951, 2008.
11 Sugimura T, Kumimoto H, Tohnai I, Fukui T, Matsuo K, Tsurusako S, Mitsudo K, Ueda M, Tajima K and Ishizaki K: Gene-environment interaction involved in oral carcinogenesis: molecular epidemiological study for metabolic and DNA repair gene polymorphisms. J Oral Pathol Med *35*: 11-18, 2006.
12 Miller KL, Karagas MR, Kraft P, Hunter DJ, Catalano PJ, Byler SH and Nelson HH: XPA, haplotypes, and risk of basal and squamous cell carcinoma. Carcinogenesis *27*: 1670-1675, 2006.
13 Bau DT, Fu YP, Chen ST, Cheng TC, Yu JC, Wu PE and Shen CY: Breast cancer risk and the DNA double-strand break end-joining capacity of nonhomologous end-joining genes are affected by BRCA1. Cancer Res *64*: 5013-5019, 2004.
14 Bau DT, Mau YC, Ding SL, Wu PE and Shen CY: DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis *28*: 1726-1730, 2007.
15 Fleck O and Nielsen O: DNA repair. J Cell Sci *117*: 515-517, 2004.
16 Hoeijmakers JH: Genome maintenance mechanisms for preventing cancer. Nature *411*: 366-374, 2001.
17 Krokan HE, Nilsen H, Skorpen F, Otterlei M and Slupphaug G: Base excision repair of DNA in mammalian cells. FEBS Lett *476*: 73-77, 2000.
18 Fortini P, Pascucci B, Parlanti E, D'Errico M, Simonelli V and Dogliotti E: The base excision repair: mechanisms and its relevance for cancer susceptibility. Biochimie *85*: 1053-1071, 2003.
19 Izumi T, Hazra TK, Boldogh I, Tomkinson AE, Park MS, Ikeda S and Mitra S: Requirement for human AP endonuclease 1 for repair of 3'-blocking damage at DNA single-strand breaks induced by reactive oxygen species. Carcinogenesis *21*: 1329-1334, 2000.
20 Evans AR, Limp-Foster M and Kelley MR: Going APE over ref-1. Mutat Res *461*: 83-108, 2000.
21 Tell G, Damante G, Caldwell D and Kelley MR: The intracellular localization of APE1/Ref-1: more than a passive phenomenon? Antioxid Redox Signal *7*: 367-384, 2005.
22 Jayaraman L, Murthy KG, Zhu C, Curran T, Xanthoudakis S and Prives C: Identification of redox/repair protein Ref-1 as a potent activator of p53. Genes Dev *11*: 558-570, 1997.
23 Burdak-Rothkamm S, Rube CE, Nguyen TP, Ludwig D, Feldmann K, Wiegel T and Rube C: Radiosensitivity of tumor cell lines after pretreatment with the EGFR tyrosine kinase inhibitor ZD1839 (Iressa). Strahlenther Onkol *181*: 197-204, 2005.
24 Gangwar R, Ahirwar D, Mandhani A and Mittal RD: Influence of XPD and APE1 DNA repair gene polymorphism on bladder cancer susceptibility in north India. Urology *73*: 675-680, 2009.
25 Li Z, Guan W, Li MX, Zhong ZY, Qian CY, Yang XQ, Liao L, Li ZP and Wang D: Genetic polymorphism of DNA base-excision repair genes (APE1, OGG1 and XRCC1) and their correlation with risk of lung cancer in a Chinese population. Arch Med Res *42*: 226-234, 2011.
26 Lo YL, Jou YS, Hsiao CF, Chang GC, Tsai YH, Su WC, Chen KY, Chen YM, Huang MS, Hu CY, Chen CJ and Hsiung CA: A polymorphism in the APE1 gene promoter is associated with lung cancer risk. Cancer Epidemiol Biomarkers Prev *18*: 223-229, 2009.
27 Chen WC, Tsai CW, Hsia TC, Chang WS, Lin LY, Liang SJ, Tu CY, Cheng WE, Chen HJ, Wang SM and Bau DT: The contribution of DNA apurinic/apyrimidinic endonuclease genotype and smoking habit to Taiwan lung cancer risk. Anticancer Res *33*: 2775-2778, 2013.
28 Gu D, Wang M, Wang S, Zhang Z and Chen J: The DNA repair gene APE1 T1349G polymorphism and risk of gastric cancer in a Chinese population. PLoS One *6*: e28971, 2011.
29 Kuasne H, Rodrigues IS, Losi-Guembarovski R, Reis MB, Fuganti PE, Gregorio EP, Libos Junior F, Matsuda HM, Rodrigues MA, Kishima MO and Colus IM: Base excision repair genes XRCC1 and APEX1 and the risk for prostate cancer. Mol Biol Rep *38*: 1585-1591, 2011.
30 Kasahara M, Osawa K, Yoshida K, Miyaishi A, Osawa Y, Inoue N, Tsutou A, Tabuchi Y, Tanaka K, Yamamoto M, Shimada E and Takahashi J: Association of MUTYH Gln324His and APEX1 Asp148Glu with colorectal cancer and smoking in a Japanese population. J Exp Clin Cancer Res *27*: 49, 2008.
31 Mahjabeen I, Baig RM, Sabir M and Kayani MA: Genetic and expressional variations of APEX1 are associated with increased risk of head and neck cancer. Mutagenesis *28*: 213-218, 2013.
32 Fang RC, Tsai YT, Lai JN, Yeh CH and Wu CT: The traditional chinese medicine prescription pattern of endometriosis patients in taiwan: a population-based study. Evid Based Complement Alternat Med *2012*: 591391, 2012.
33 Hung RJ, Hall J, Brennan P and Boffetta P: Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol *162*: 925-942, 2005.
34 Chen W, Wang Q, Liu M and Ding XB: The association of APE1 Asp148Glu gene polymorphisms and lung cancer risk: an updated meta-analysis. Tumour Biol *35*: 3597-3603, 2014.
35 Pan H, Niu W, He L, Wang B, Cao J, Zhao F, Liu Y, Li S and Wu H: Contributory role of five common polymorphisms of RAGE and APE1 genes in lung cancer among Han Chinese. PLoS One *8*: e69018, 2013.
36 Ito H, Matsuo K, Hamajima N, Mitsudomi T, Sugiura T, Saito T, Yasue T, Lee KM, Kang D, Yoo KY, Sato S, Ueda R and Tajima K: Gene-environment interactions between the smoking habit and polymorphisms in the DNA repair genes, APE1 Asp148Glu and XRCC1 Arg399Gln, in Japanese lung cancer risk. Carcinogenesis *25*: 1395-1401, 2004.
37 Kiyohara C, Takayama K and Nakanishi Y: Association of genetic polymorphisms in the base excision repair pathway with lung cancer risk: a meta-analysis. Lung Cancer *54*: 267-283, 2006.
38 Holoch KJ and Lessey BA: Endometriosis and infertility. Clin Obstet Gynecol *53*: 429-438, 2010.
39 Vogel KS, Perez M, Momand JR, Acevedo-Torres K, Hildreth K, Garcia RA, Torres-Ramos CA, Ayala-Torres S, Prihoda TJ, McMahan CA and Walter CA: Age-related instability in spermatogenic cell nuclear and mitochondrial DNA obtained from Apex1 heterozygous mice. Mol Reprod Dev *78*: 906-919, 2011.
40 Verit FF and Yucel O: Endometriosis, leiomyoma and adenomyosis: the risk of gynecologic malignancy. Asian Pac J Cancer Prev *14*: 5589-5597, 2013.
41 Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T and Tsugane S: Urinary phthalate monoesters and endometriosis in infertile Japanese women. Sci Total Environ *408*: 37-42, 2009.
42 Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, Ferro A, Ingelido AM, Maggi A, Panici PB and De Felip E: Endometriosis and organochlorinated environmental pollutants: a case-control study on Italian women of reproductive age. Environ Health Perspect *117*: 1070-1075, 2009.

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