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/apyrimidinic 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 Asp148Glu (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 Asp148Glu 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 Asp148Glu genotype with menarche, pregnancy, smoking or alcohol drinking lifestyles on endometriosis risk. Conclusion: The APEX1 Asp148Glu 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 multifactor, 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
To the best of our knowledge, this is the first study to evaluate the relationship between endometriosis in a moderate Taiwan female population. For transcription PCR and western blotting assays, respectively. APEX1 polymorphism in literature, thus, the present case-control study aimed at revealing the genotypes, together with phenotypes of APEX1, XRCC3, BLHX, TP53, and head and neck cancer (31). We assumed that the non-carcinogenes, 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 established 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 Asp148Glu (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 Asp148Glu 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 status was 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 Asp148Glu were: forward 5’-CCAAGCTGAACTTCAGGAGCT-3’, and reverse 5’-CTCAGGCTGCATTAGGTTCA-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
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-penetration 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...
Asp148Glu 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 Asp148Glu is the most common polymorphism studied resulting in amino acid alteration (33). Previous reports have shown a controversial contribution of the APEX1 Asp148Glu genotype to the risk of other types of cancer. For instance, the association of the APEX1 Asp148Glu 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 Asp148Glu genotyping, we found that individuals carrying variant TG or GG genotypes were not of higher risk for endometriosis compared with

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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=153)</th>
<th>Controls (n=636)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Age (year) (mean±SD)</td>
<td>40.3±4.9</td>
<td>41.2±4.5</td>
<td>0.8865</td>
</tr>
<tr>
<td>Age at menarche</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤12.8</td>
<td>85</td>
<td>55.6%</td>
<td>318</td>
</tr>
<tr>
<td>&gt;12.8</td>
<td>68</td>
<td>44.4%</td>
<td>318</td>
</tr>
<tr>
<td>Full pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>55</td>
<td>35.9%</td>
<td>153</td>
</tr>
<tr>
<td>Yes</td>
<td>98</td>
<td>64.1%</td>
<td>483</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>113</td>
<td>73.9%</td>
<td>476</td>
</tr>
<tr>
<td>Smokers</td>
<td>40</td>
<td>26.1%</td>
<td>160</td>
</tr>
<tr>
<td>Alcohol drinking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinkers</td>
<td>116</td>
<td>75.8%</td>
<td>463</td>
</tr>
<tr>
<td>Drinkers</td>
<td>37</td>
<td>24.2%</td>
<td>173</td>
</tr>
<tr>
<td>Stages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II</td>
<td>32</td>
<td>20.9%</td>
<td></td>
</tr>
<tr>
<td>III or IV</td>
<td>121</td>
<td>79.1%</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically significant; SD, standard deviation.

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

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

aAdjusted by age, pregnancy, smoking and alcohol drinking status; CI, confidence interval; OR, odds ratio.
those carrying the wild-type TT genotype (Table II). We have also investigated the correlation of the APEX1 Asp<sup>148</sup>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<sup>148</sup>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<sup>148</sup>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<sup>148</sup>Glu genotypes had no obvious preferential distribution among each subgroup suggesting that the APEX1 Asp<sup>148</sup>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 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

![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.](image-url)
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.

Acknowledgements

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References

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