The Association of Flap Endonuclease 1 Genotypes with the Susceptibility of Endometriosis

AN-KUO CHOU[1*], MING-YI SHEN[2,3*], FANG-YU CHEN[2*], CHIEH-LUN HSIAO[3,4],
LIANG-CHUN SHIH[4], WEN-SHIN CHANG[4], CHIA-WEN TSAI[4], TSUNG-HO YING[5],
MING-HSIEN WU[6], CHUNG-YU HUANG[7] and DA-TIAN BAU[3,4,8]

[1]Department of Anesthesiology, China Medical University Hospital, Taichung, Taiwan, R.O.C.;
[2]Department of Medical Research, China Medical University Hospital,
Taichung, Taiwan Hospital, Taichung, Taiwan, R.O.C.;
[3]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;
[4]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, R.O.C.;
[5]Department of Obstetrics and Gynecology, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.;
[6]Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;
[7]Taoyuan Armed Forces General Hospital, Taoyuan, Taiwan, R.O.C.;
[8]Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.

Abstract. *Background/Aim: Flap endonuclease 1 (FEN1), a protein with multiple functions in genome stability maintenance, is important in cancer prevention. The two functional germline variants of FEN1, rs174538 and rs4246215, regarding cancer susceptibility have been reported in lung, breast, liver, esophageal, gastric, colorectal cancer, glioma and leukemia, but not endometriosis. In this study, we firstly aimed at evaluating the contribution of FEN1 genotypes to endometriosis risk in a representative Taiwan population. Materials and Methods: In total, 153 patients with endometriosis and 636 non-cancer healthy controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. Results: The genotypes of FEN1 rs174538, but not those of rs4246215, were differently distributed between the endometriosis and control groups. In detail, the AA of FEN1 rs174538 genotypes were significantly less frequently found among endometriosis patients than among controls (odds ratio [OR]=0.43, 95% confidence interval [CI]=0.24-0.78, p=0.0125). The A allele at FEN1 rs174538 was also significantly less frequent among cases than controls (OR=0.65, 95%CI=0.50-0.86, p=0.0021). As for age of first menarche, those with first menarche at the age >12.8 carrying the FEN1 rs174538 AA genotype conferred lower OR of 0.29 (95%CI=0.11-0.78, p=0.0381) for endometriosis. Regarding the full pregnancy status, those without having had a full-term pregnancy carrying the FEN1 rs174538 AA genotype were of lower risk (ORs=0.12, 95%CI=0.03-0.53, p=0.0050). Conclusion: The FEN1 rs174538 A allele is a novel protective biomarker for endometriosis and this genotype may have interactions with age- and hormone-related factors on the development of endometriosis.*

All over the world, about 10% of females at reproductive ages suffer from endometriosis, which is a hormone-dependent inflammatory benign gynaecological disease where functional endometrial tissue is unusually present outside the uterus (1, 2). In clinical, the main manifestations are dysmenorrhea, chronic pelvic pain, pain during intercourse and infertility (3). Although the mechanisms underlying endometriosis are still unrevealed, mounting evidence has shown that endometriosis is a multi-factorial, multi-step disease affected by inflammation, hormonal imbalance, genetic and environmental interactions (4-6).

It is believed that alterations in DNA repair genes are closely-related to genomic instability and carcinogenesis. Among these DNA repair genes, Flap endonuclease 1

(*FEN1*) is a multiple-function endonuclease in charge of removal of DNA adducts caused by alkylating agents and UV irradiation (7). As for the base excision repair (BER) pathway, FEN1 could efficiently remove 5'flap as an endonuclease (8, 9). During the DNA replication process, FEN1 could promote the maturation of Okazaki fragments in the lagging strand (8, 9). Additionally, FEN1 could also act as a 5'exonuclease and a gap-dependent endonuclease, promoting the DNA fragmentation during programmed cell death (10, 11). In cells with functionally impaired yeast FEN1 (known as RAD27 in yeast), significantly elevated spontaneous mutation rates are observed (11-13). In mice models, the knockout of FEN1 can lead to increased genomic instability and carcinogenesis (14). In human cancer cells, *FEN1* mutations can result in reduced nuclease activity (15). Therefore, dysregulated expression of FEN1 resulting from genetic variations may contribute to an increased risk for the development of cancer.

In literature, most investigations of *FEN1* genomic variations focused on the two common polymorphisms with minor allelic frequencies larger than 5%, promoter -69G>A (rs174538) and 3'UTR 4150G>T (rs4246215), which were both effective on influencing the expression level of FEN1 and enzyme activity (16). The polymorphic genotypes of rs174538 and/or rs4246215 are associated with the risk of several types of cancer such as esophageal (17, 18), lung (16), gastrointestinal (18, 19), gallbladder (20), breast cancer (21-23), glioma (24) and leukemia (25). However, the contribution of *FEN1* genomic variation to endometriosis has never been investigated. The purpose of the present study was, therefore, to analyze the genetic polymorphisms of *FEN1* promoter -69G>A (rs174538) and 3'UTR 4150G>T (rs4246215) in a population consisting of 153 endometriosis and 636 non-endometriosis cases, in order to investigate the association between these two *FEN1* genotypes and endometriosis.

Materials and Methods

Study population and sample collection. One hundred and fifty-three endometriosis patients were recruited at the outpatient clinics of general surgery during 2000-2010 at Chung Shan Medical University Hospital in Taiwan. These 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 and 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

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

Characteristics	Cases (n=153)		Controls (n=636)		*p*-Value
	N	%	N	%	
Age (yr) (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.

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 provided 3 to 5 ml of peripheral blood for genotyping analyses with written informed consent. The experiment was approved by the Ethical Committee and Institutional Review Board of the Chung Shan Medical University Hospital. At the same period, 636 non-endometriosis healthy volunteers were selected as controls *via* a matching system 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 another or of 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. The selected characteristics of the control and patient groups are summarized and compared in Table I.

Genotyping methodology. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan), stored at −80°C, diluted and aliquoted for genotyping as working stock at −20°C (26, 27). Genotyping for *FEN1* rs174538 and rs4246215 of all subjects was carried-out by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assays, as previously reported (28, 29). The primers were designed by Terry Fox Cancer Research team and the PCR-

Table II. *Summary of the rs numbers, designed primer sequences, restriction enzymes, amplicon lengths before and after enzyme digestion for FEN1 rs174538 and rs4246215.*

rs number	Paired primer sequences	Restriction enzyme	Amplicon length (bp)	Enzymatic fragment sizes (bp)
rs174538	F: 5'-CCTAAGGAGTTCATGGCAAG-3'	*Sal* I	307	G: 307
	R: 5'-AATCGCAGGACTACAAGTCC-3'		A: 223+84	
rs4246215	F: 5'-GGTGGAGAGAGGATTCTAAG-3'	*BcoD* I	382	T: 382
	R: 5'-CATCTGCTAAGATGCGCCTT-3'		G: 234+148	

Table III. *Distribution of the FEN1 genotypic and allelic frequencies among 636 healthy controls and 153 endometriosis patients.*

Genotype	Controls	%	Cases	%	*p*-Value[a]	OR (95%CI)[b]
Promoter rs174538						
GG	245	38.5%	76	49.7%	0.0125*	1.00 (Reference)
AG	279	43.9%	62	40.5%		0.72 (0.49-1.04)
AA	112	17.6%	15	9.8%		0.43 (0.24-0.78)*
AG+AA	391	61.5%	77	50.3%		0.63 (0.45-0.91)*
G allele	765	60.5%	214	69.9%	0.0021*	1.00 (Reference)
A allele	503	39.5%	92	30.1%		0.65 (0.50-0.86)*
3' UTR rs4246215						
GG	235	36.9%	53	34.6%	0.8677	1.00 (Reference)
GT	289	45.5%	72	47.1%		1.10 (0.74-1.64)
TT	112	17.6%	28	18.3%		1.11 (0.67-1.85)
GT+TT	401	63.1%	100	65.4%		1.11 (0.76-1.60)
G allele	759	59.7%	178	58.2%	0.6315	1.00 (Reference)
T allele	513	40.3%	128	41.8%		1.06 (0.83-1.37)

[a]Based on Pearson's chi-square test; [b]OR: Odds ratio; CI: confidence interval. *Statistically significant.

RFLP conditions for *FEN1* rs174538 and rs4246215 are summarized in Table II. The success rate of PCR-RFLP was 100%, and the genotypes of 5% of the participants randomly selected from controls and cases were analyzed by PCR direct sequencing (Genomics BioSci & Tech Co., Taipei, Taiwan). The consistency between direct sequencing and PCR-RFLP was 100%.

Statistical analysis. The descriptive statistics of patients and controls were presented as the mean±standard deviations (SDs) or as percentages. The Pearson's Chi-square test or Fisher's exact test (when the number in any cell was less than five, such as "2" homo-variants in case group of no full pregnancy) was used to compare the distribution of the *FEN1* genotypes. Associations were evaluated by odds ratios (ORs) with 95% confidence intervals (95%CIs). Statistical tests were deemed significant when the *p*-value was less than 0.05.

Results

The frequency distributions for the age, age at menarche, full pregnancy, smoking and alcohol drinking status, and clinical stages of 153 endometriosis patients and 636 non-endometriosis healthy controls are shown in Table I. The

characteristics of patients and controls were well-matched according to age, age at menarche, smoking and alcohol drinking status (*p*>0.05), while the percentages of full pregnancy were less among endometriosis patients (64.1%) than among non-endometriosis subjects (75.9%) (Table I).

The genotypic and allelic frequencies for the *FEN1* rs174538 and rs4246215 among endometriosis patients and non-endometriosis healthy controls are shown in Table III. The genotypic frequency distributions for *FEN1* rs174538 were significantly different between endometriosis and control groups (*p* for trend=0.0125), while those for *FEN1* rs4246215 polymorphism were not significantly different (p for trend=0.8677) (Table III). In detail, those who carried AA, AG plus AA genotypes had a significantly reduced risk of endometriosis with ORs of 0.43 and 0.63 respectively compared to those with the wild-type GG genotype (95%CI=0.24-0.78 and 0.45-0.91, respectively). From the results of allelic frequency analysis, we can find that the A allele of *FEN1* rs174538 seemed to be a protective factor for endometriosis (*p*=0.0021, OR=0.65, 95%CI=0.50-0.86), while the T allele of *FEN1* rs4246215 was not (*p*=0.6315,

Table IV. *Distribution of the FEN1 promoter rs174538 and 3' UTR rs4246215 genotypes stratified by age at menarche and full pregnancy.*

Characteristics	Promoter rs174538				3' UTR rs4246215			
	Controls n (%)	Cases n (%)	P_{trend}[a]	OR (95%CI)[b]	Controls n (%)	Cases n (%)	P_{trend}[a]	OR (95%CI)[b]
Menarche age								
≤12.8 years			0.1395				0.8288	
Wild-type	116 (36.5)	41 (48.2)		1.00 (Reference)	116 (36.5)	30 (36.3)		1.00 (Reference)
Hetero-variant	153 (48.1)	34 (40.0)		0.63 (0.38-1.05)	151 (47.5)	39 (45.9)		1.00 (0.59-1.70)
Homo-variant	49 (15.4)	10 (11.8)		0.58 (0.27-1.24)	51 (16.0)	16 (18.8)		1.21 (0.61-2.42)
Hetero- + homo-variants	198 (63.5)	44 (51.8)		0.63 (0.39-1.02)	202 (63.5)	55 (63.7)		1.05 (0.64-1.74)
>12.8 years			0.0381*				0.7408	
Wild-type	129 (40.6)	35 (51.5)		1.00 (Reference)	119 (37.4)	23 (33.8)		1.00 (Reference)
Hetero-variant	126 (39.6)	28 (41.2)		0.82 (0.47-1.43)	138 (43.4)	33 (48.5)		1.24 (0.69-2.22)
Homo-variant	63 (19.8)	5 (7.3)		0.29 (0.11-0.78)*	61 (19.2)	12 (17.7)		1.02 (0.47-2.18)
Hetero- + homo-variants	189 (59.4)	33 (48.6)		0.64 (0.38-1.09)	199 (62.6)	45 (66.2)		1.17 (0.67-2.03)
Full Pregnancy								
No			0.0050*				0.9801	
Wild-type	57 (37.3)	31 (56.4)		1.00 (Reference)	58 (37.9)	21 (38.2)		1.00 (Reference)
Hetero-variant	65 (42.5)	22 (40.0)		0.62 (0.32-1.19)	66 (43.1)	23 (41.8)		0.96 (0.48-1.92)
Homo-variant	31 (20.2)	2 (3.6)		0.12 (0.03-0.53)*	29 (19.0)	11 (20.0)		1.05 (0.45-2.46)
Hetero- + homo-variants	96 (62.7)	24 (43.6)		0.46 (0.25-0.86)*	95 (62.1)	34 (61.8)		0.99 (0.52-1.86)
Yes			0.3986				0.7343	
Wild-type	188 (38.9)	45 (45.9)		1.00 (Reference)	177 (36.6)	32 (32.7)		1.00 (Reference)
Hetero-variant	214 (44.3)	40 (40.8)		0.78 (0.49-1.25)	223 (46.2)	49 (50.0)		1.22 (0.75-1.98)
Homo-variant	81 (16.8)	13 (13.3)		0.67 (0.34-1.31)	83 (17.2)	17 (17.3)		1.13 (0.60-2.16)
Hetero- + homo-variants	295 (61.1)	53 (54.1)		0.75 (0.48-1.16)	306 (63.4)	66 (67.3)		1.19 (0.75-1.89)

[a]P for trend based on chi-square test. [b]OR: Odds ratio; CI: confidence interval. *Statistically significant.

OR=1.06, 95%CI=0.83-1.37). The conclusion that can be deduced from Table III is that the *FEN1* AA genotypes (A allele) seemed to be a protective factor for endometriosis among Taiwanese women.

Because age- and hormone-related factors are risk factors for developing endometriosis, the contribution of the *FEN1* genotype to endometriosis stratified by age at menarche and full pregnancy status were further analyzed and presented in Table IV. Since the average age of first menarche for the 318th and 319th subjects in the control and patient groups was 12.8 years, we have further stratified the groups into ≤12.8 and >12.8year-old sub-groups. Noticeably, in the later-menarche (>12.8 years) group, subjects with homo-variant AA genotypes for *FEN1* rs174538 had lower risks for developing endometriosis than those with the wild-type GG genotype (p for trend=0.0381, OR=0.29, 95%CI=0.11-0.78), while the combined AG with AA group did not have the similar results (OR=0.64, 95%CI=0.38-1.09). On the contrary, this was not the case for the earlier-menarche (>12.8 years) group (Table IV). As for the full pregnancy status, those women with no full pregnancy and of AA or AG plus AA genotypes for *FEN1* rs174538 were less likely to develop endometriosis than those with the homozygous GG

genotype (*p* for trend=0.0050, OR=0.12 and 0.46, 95%CI=0.03-0.53 and 0.25-0.86 for AA and AG plus AA, respectively), but this was not the case for those with full pregnancy (Table III). As for the *FEN1* rs4246215, there was no difference between the comparisons of stratified menarche age or full pregnancy status groups. In summary, stratified analyses revealed an interaction between the age of first menarche and no full pregnancy among *FEN1* rs174538 genotypes in the endometriosis susceptibility.

Discussion

In the current study, our group firstly examined the contribution of *FEN1* genotypes to endometriosis susceptibility. According to that, FEN1 plays a multiple role in DNA repair, DNA replication, and cell apoptosis, and is very possible that hereditary genomic variations may determine susceptibility to the development of cancer and endometriosis. In literature, polymorphic *FEN1* genotypes, such as rs174538 and rs4246215, may determine FEN1 enzymatic activity and the rate of tumorigenesis at cell and animal levels (14, 15), and may be linked to the risk of cancers and endometriosis. In the current study, we found that

the AA genotype or A allele at *FEN1* rs174538 was significantly associated with a lower susceptibility to endometriosis among Taiwanese women (Table III). These findings are consistent with researches that have identified the A allele to be a protective factor for many types of cancer, including lung (16), gastrointestinal (19), esophageal (18), breast cancer (21), glioma (24) and childhood leukemia (25). As far as we are concerned, the current study was the first to provide evidence that the *FEN1* genotype, rs174538 but not rs4246215, is associated with endometriosis risk (Table III).

Since the age- and hormone-related factors are environmental risk factors that contribute to endometriosis, we have also analyzed the gene-environment interactions *via* analyzing the distributions of the *FEN1* rs174538 genotype and endometriosis status among the investigated subjects according to their age at first menarche and their full pregnancy status. Interestingly, in the later-menarche (>12.8 years) group, women with the AA genotype for *FEN1* rs174538 had lower risks for developing endometriosis than those with the wild-type GG genotype (Table IV). On the contrary, no such age difference was observed in analyses of the *FEN1* rs4246215 genotype. The mechanisms of FEN1 protein involved in the etiopathology and progression of endometriosis has never been studied. Although no statistical significance was found in the full pregnancy group in Table IV, we could not exclude the possibility our findings may not be the same when thousands of patients will be analyzed. Also, as for the positive findings, only five women with a homo-variant AA genotype had a first menarche at an age older than 12.8 years, an extremely small number (Table IV). The enlarged sample size may provide us with a more realistic answer. In the present article, we can only conclude that the protective impact of the A allele at *FEN1* rs174538 with respect to endometriosis risk (Table III), and very possibly, the genotypes may have a gene-environment interaction with age- and hormone-related factors (Table IV). While the complete underlying mechanisms have not been discovered, sexual hormones and steroids reportedly play an important part in controlling the proliferation of cancer cells. For instance, in supporting the idea of gender differences in susceptibility to cancer, 17-β estrogen was found to have a stronger inhibitory effect than testosterone on human monoblastic U937 cells (30). At the molecular level, further measurement and analysis of the DNA repair capacity, cell proliferation and apoptosis rates among samples from endometriosis patients with differential *FEN1* genotypes and age- and hormone-related status may help us further understand the development and gene-environment interaction in endometriosis.

In conclusion, the present study documented evidence of a positive association between the genotypes of *FEN1* and endometriosis and examined the age- and hormone-related interactions with the genotype, in order to determine endometriosis susceptibility. The presence of the A allele of rs174538 was not only a novel detectable and predictive biomarker for endometriosis but a possible anti-endometriosis therapeutic target.

Conflicts of Interest

The Authors declare that there are no conflicts of interest with any person or company.

Acknowledgements

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 Murray JM, Tavassoli M, al-Harithy R, Sheldrick KS, Lehmann AR, Carr AM and Watts FZ: Structural and functional conservation of the human homolog of the Schizosaccharomyces pombe rad2 gene, which is required for chromosome segregation and recovery from DNA damage. Mol Cell Biol *14*: 4878-4888, 1994.

8 Lieber MR: The FEN-1 family of structure-specific nucleases in eukaryotic DNA replication, recombination and repair. Bioessays *19*: 233-240, 1997.

9 Shen B, Singh P, Liu R, Qiu J, Zheng L, Finger LD and Alas S: Multiple but dissectible functions of FEN-1 nucleases in nucleic acid processing, genome stability and diseases. Bioessays *27*: 717-729, 2005.

10 Liu Y, Kao HI and Bambara RA: Flap endonuclease 1: a central component of DNA metabolism. Annu Rev Biochem *73*: 589-615, 2004.

11 Zheng L, Zhou M, Chai Q, Parrish J, Xue D, Patrick SM, Turchi JJ, Yannone SM, Chen D and Shen B: Novel function of the flap endonuclease 1 complex in processing stalled DNA replication forks. EMBO Rep *6*: 83-89, 2005.

12 Tishkoff DX, Filosi N, Gaida GM and Kolodner RD: A novel mutation avoidance mechanism dependent on S. cerevisiae RAD27 is distinct from DNA mismatch repair. Cell *88*: 253-263, 1997.

13 Parrish JZ, Yang C, Shen B and Xue D: CRN-1, a Caenorhabditis elegans FEN-1 homologue, cooperates with CPS-6/EndoG to promote apoptotic DNA degradation. EMBO J *22*: 3451-3460, 2003.

14 Kucherlapati M, Yang K, Kuraguchi M, Zhao J, Lia M, Heyer J, Kane MF, Fan K, Russell R, Brown AM, Kneitz B, Edelmann W, Kolodner RD, Lipkin M and Kucherlapati R: Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. Proc Natl Acad Sci USA *99*: 9924-9929, 2002.

15 Zheng L, Dai H, Zhou M, Li M, Singh P, Qiu J, Tsark W, Huang Q, Kernstine K, Zhang X, Lin D and Shen B: Fen1 mutations result in autoimmunity, chronic inflammation and cancers. Nat Med *13*: 812-819, 2007.

16 Yang M, Guo H, Wu C, He Y, Yu D, Zhou L, Wang F, Xu J, Tan W, Wang G, Shen B, Yuan J, Wu T and Lin D: Functional FEN1 polymorphisms are associated with DNA damage levels and lung cancer risk. Hum Mutat *30*: 1320-1328, 2009.

17 Sang Y, Bo L, Gu H, Yang W and Chen Y: Flap endonuclease-1 rs174538 G>A polymorphisms are associated with the risk of esophageal cancer in a Chinese population. Thorac Cancer *8*: 192-196, 2017.

18 Li WQ, Hu N, Hyland PL, Gao Y, Wang ZM, Yu K, Su H, Wang CY, Wang LM, Chanock SJ, Burdett L, Ding T, Qiao YL, Fan JH, Wang Y, Xu Y, Shi JX, Gu F, Wheeler W, Xiong XQ, Giffen C, Tucker MA, Dawsey SM, Freedman ND, Abnet CC, Goldstein AM and Taylor PR: Genetic variants in DNA repair pathway genes and risk of esophageal squamous cell carcinoma and gastric adenocarcinoma in a Chinese population. Carcinogenesis *34*: 1536-1542, 2013.

19 Liu L, Zhou C, Zhou L, Peng L, Li D, Zhang X, Zhou M, Kuang P, Yuan Q, Song X and Yang M: Functional FEN1 genetic variants contribute to risk of hepatocellular carcinoma, esophageal cancer, gastric cancer and colorectal cancer. Carcinogenesis *33*: 119-123, 2012.

20 Jiao X, Wu Y, Zhou L, He J, Yang C, Zhang P, Hu R, Luo C, Du J, Fu J, Shi J, He R, Li D and Jun W: Variants and haplotypes in Flap endonuclease 1 and risk of gallbladder cancer and gallstones: a population-based study in China. Sci Rep *5*: 18160, 2015.

21 Lv Z, Liu W, Li D, Liu L, Wei J, Zhang J, Ge Y, Wang Z, Chen H, Zhou C, Yuan Q, Zhou L and Yang M: Association of functional FEN1 genetic variants and haplotypes and breast cancer risk. Gene *538*: 42-45, 2014.

22 Mitra AK, Singh N, Singh A, Garg VK, Agarwal A, Sharma M, Chaturvedi R and Rath SK: Association of polymorphisms in base excision repair genes with the risk of breast cancer: a case-control study in North Indian women. Oncol Res *17*: 127-135, 2008.

23 Lin S, Wang M, Liu X, Lu Y, Gong Z, Guo Y, Yang P, Tian T, Dai C, Zheng Y, Xu P, Li S, Zhu Y and Dai Z: FEN1 gene variants confer reduced risk of breast cancer in chinese women: A case-control study. Oncotarget *7*: 78110-78118, 2016.

24 Chen YD, Zhang X, Qiu XG, Li J, Yuan Q, Jiang T and Yang M: Functional FEN1 genetic variants and haplotypes are associated with glioma risk. J Neurooncol *111*: 145-151, 2013.

25 Pei JS, Chang WS, Hsu PC, Tsai CW, Hsu CM, Ji HX, Hsiao CL, Hsu YN and Bau DT: The Association of Flap Endonuclease 1 Genotypes with the Risk of Childhood Leukemia. Cancer Genomics Proteomics *13*: 69-74, 2016.

26 Lai YL, Gong CL, Fu CK, Yueh TC, Tsai CW, Chang WS, Hsiao CL, Yen ST, Li HT, Jeng LB, Wang SC and Bau DT: The Contribution of Matrix Metalloproteinase-1 Genotypes to Hepatocellular Carcinoma Susceptibility in Taiwan. Cancer Genomics Proteomics *14*: 119-125, 2017.

27 Hsu CM, Chang WS, Hwang JJ, Wang JY, Hsiao YL, Tsai CW, Liu JC, Ying TH and Bau DT: The role of apurinic/apyrimidinic endonuclease DNA repair gene in endometriosis. Cancer Genomics Proteomics *11*: 295-301, 2014.

28 Liao CH, Chang WS, Hu PS, Wu HC, Hsu SW, Liu YF, Liu SP, Hung HS, Bau DT and Tsai CW: The Contribution of MMP-7 Promoter Polymorphisms in Renal Cell Carcinoma. In Vivo *31*: 631-635, 2017.

29 Hung YW, Tsai CW, Wu CN, Shih LC, Chen YY, Liu YF, Hung HS, Shen MY, Chang WS and Bau DT: The Contribution of Matrix Metalloproteinase-8 Promoter Polymorphism to Oral Cancer Susceptibility. In Vivo *31*: 585-590, 2017.

30 Mossuz P, Cousin F, Castinel A, Chauvet M, Sotto MF, Polack B, Sotto JJ and Kolodie L: Effects of two sex steroids (17beta estradiol and testosterone) on proliferation and clonal growth of the human monoblastic leukemia cell line, U937. Leuk Res *22*: 1063-1072, 1998.