

Identification of novel mutations in endometrial cancer patients by whole-exome sequencing

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Abstract. The aim of the present study was to identify genomic alterations in Taiwanese endometrial cancer patients. This information is vitally important in Taiwan, where endometrial cancer is the second most common gynecological cancer. We performed whole-exome sequencing on DNA from 14 tumor tissue samples from Taiwanese endometrial cancer patients. We used the Genome Analysis Tool kit software package for data analysis, and the dbSNP, Catalogue of Somatic Mutations in Cancer (COSMIC) and The Cancer Genome Atlas (TCGA) databases for comparisons. Variants were validated via Sanger sequencing. We identified 143 non-synonymous mutations in 756 canonical cancer-related genes and 1,271 non-synonymous mutations in non-canonical cancer-related genes in 14 endometrial samples. *PTEN*, *KRAS* and *PIK3R1* were the most frequently mutated canonical cancer-related genes. Our results revealed nine potential driver genes (*MAPT*, *IL24*, *MCM6*, *TSC1*, *BIRC2*, *CIITA*, *DST*, *CASP8* and *NOTCH2*) and 21 potential passenger genes (*ARMCX4*, *IGSF10*, *VPS13C*, *DCT*, *DNAH14*, *TLN1*, *ZNF605*, *ZSCAN29*, *MOCOS*, *CMYA5*, *PCDH17*, *UGT1A8*, *CYFIP2*, *MACF1*, *NUDT5*, *JAKMIP1*, *PCDHGB4*, *FAM178A*, *SNX6*, *IMP4* and *PCMTD1*). The detected molecular aberrations led to putative activation of the mTOR, Wnt, MAPK, VEGF and ErbB pathways, as well as aberrant DNA repair, cell cycle control and apoptosis pathways. We characterized the mutational landscape and genetic alterations in multiple cellular pathways of endometrial cancer in the Taiwanese population.

Introduction

Endometrial cancer is the most common malignancy of the female genital system in developed countries. In Taiwan, endometrial cancer ranks second among gynecological cancers in its incidence. Risk factors for endometrial cancer are both environmental and genetic. One major environmental risk factor is endogenous or exogenous estrogen exposure: many sources of exogenous estrogen exist, including oral contraceptives and hormone replacement therapy (1). Some genetic variants involved in the steroid hormone biosynthesis and metabolism pathways may contribute to hyperestrogenic status, which is associated with endometrial cancer risk (2,3). Use of oral contraceptives provides significant long-term protection against endometrial cancer (4). Long-term sequential estrogen-plus-progestin therapy during menopause increases the risk of endometrial cancer, whereas short-term estrogen-plus-progestin use in menopause decreases the risk of endometrial cancer (5). Endometrial cancer patients who received extended adjuvant tamoxifen do not appear to be at greater risk for endometrial cancer (6).

Next-generation sequencing (NGS) will play an important role in anticancer drug development (7) and targeted therapy (8). At present, there are multiple NGS instruments in use, such as the Illumina HiSeq and MiSeq (Illumina, Inc., San Diego, CA, USA), the Ion Torrent Proton and Personal Genome Machine (Life Technologies, Carlsbad, CA, USA), as well as the Roche 454 Sequencer and GS Junior (Roche Applied Biosystems, Nutley, NJ, USA). The Cancer Genome Atlas (TCGA) project used NGS techniques to identify hundreds of somatically altered genes (9-12). This technical advance is rapidly altering the routine practice of molecular pathology, from single-gene tests (i.e., Sanger sequencing to assess *KRAS* mutations in colorectal cancer) to multiplexed NGS assays. Several NGS approaches have been commonly implemented clinically in oncology, including hybrid capture-based panels, multiplexed polymerase chain reaction (PCR)-based panels and comprehensive genome/transcriptome/exome sequencing (13-16). Whole genome sequencing offers high throughput, high accuracy (<1 error per 100 kb) and affordable cost (<\$5,000 USD in reagents) (17). Recently, the American College of Medical Genetics and

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Table I. Summary of sequencing alignment and coverage statistics in the exomes of 14 endometrial tumors.

Patient ID	Total raw reads	Total effective reads	Reads mapped to genome	Total effective yield (Mb)	Average read length (bp)	Covered $\geq 20\times$ (%)	Average sequencing depth on target
F105T	20,976,408	14,817,961	14,725,603	45974.96	200.01	43.06	19.43
F139T	18,071,410	12,999,809	12,909,672	43438.89	200.01	51.07	21.78
F141T	18,308,457	13,180,222	13,077,252	45838.97	200.10	44.58	19.06
F61	21,965,842	15,337,823	15,247,340	47366.56	200.00	45.76	20.01
F114T	18,690,475	13,546,119	13,433,300	46105.01	200.16	48.37	20.01
F123	20,115,993	14,018,792	13,919,598	46067.53	199.99	43.44	18.83
F132	21,510,383	14,710,924	14,621,813	45507.75	199.83	45.5	20.56
F134	20,126,153	14,005,289	13,909,284	45658.48	199.98	42.77	18.94
F146	26,158,898	18,289,158	18,193,144	50310.04	200.03	46.31	21.53
F147T	19,394,632	14,099,623	13,962,761	45656.60	200.17	48.56	20.92
F150T	20,623,512	14,156,494	14,080,096	45931.50	199.85	48.22	20.58
F152T	19,092,410	14,153,725	14,048,858	46229.92	200.28	50.62	21.11
F92T	18,726,658	13,243,029	13,164,997	45153.23	200.09	43.91	18.60
03-3812T	17,550,923	12,789,944	12,748,550	44627.55	200.18	52.99	21.17
Average	20,093,725	14,239,208	14,145,876	45990.50	200	47.03	20

Genomics (ACMG) recommended reporting pathogenic findings in 56 genes with low coverage. Low sequencing coverage may contribute to false negative clinical exome results (18).

Based on histological subtype, endometrial cancer is classified into two major types (I and II). Type I (endometrioid) carcinoma is the most frequent type of endometrial cancer, accounting for over 80% of cases. Type II (non-endometrioid) carcinoma comprises a minority of endometrial cancer cases. Whole-exome sequencing (WES) has led to the discovery of *ARID1A* as a novel regulator of PI3K pathway activity in endometrioid endometrial cancer (19). Through an integrated genomic, transcriptomic and proteomic analysis of endometrial cancer, TCGA research has suggested a novel molecular classification for two histological subtypes (12).

We performed WES on 14 patients with endometrial cancers. Our results identify cancer driver and passenger genes, and adhere to ACMG recommendations for the examination and reporting of secondary genetic findings during clinical genomic testing.

Materials and methods

Patients and samples. Fourteen patients with endometrial cancer were recruited for this study. DNA was extracted using the QIAamp DNA Micro kit (Qiagen, Heidelberg, Germany) following the manufacturer's protocol. Extracted DNA samples were quantified using NanoDrop 2000 spectrophotometer (Thermal Fisher Scientific, Waltham, MA, USA) and a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). This study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUH-IRB-970488).

Whole-exome sequencing. To generate standard exome capture libraries, we used the Agilent SureSelect XT Reagent kit protocol for the Illumina HiSeq paired-end sequencing library (cat. no. G9611A). In all cases, the SureSelect XT Human All Exon Version 4 (51 Mb) probe set was used. We

used 50 ng genomic DNA for library construction with the Agilent SureSelect XT Reagent kit. The adapter-ligated sample was purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA) and analyzed on a Bioanalyzer DNA 1000 chip. Part of the sample (750 ng) was prepared for hybridization with the capture baits, and the sample was hybridized for 90 min at 65°C, captured with the Dynabeads MyOne Streptavidin T1 (Life Technologies) and purified using Agencourt AMPure XP beads. The Agilent protocol was used to add index tags by post-hybridization amplification. Finally, all samples were sequenced on an Illumina HiSeq system using 100 PE protocol. Metadata were deposited in the NCBI Sequence Read Archive under the accession no. SRP099176.

Data analysis. To filter low-quality reads, we used the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit) to process the raw read data files. There were two steps of sequence quality processing. The command 'fastq_quality_filter -Q33 -q 30 -p 70'. '-q 30' indicated that the minimum quality score was 30. '-p 70' meant that bases must have a '-q' quality score of 70% or greater. Sequences were retained if both forward and reverse sequencing reads passed the first step.

An efficient sequence alignment tool, Bowtie2, was used to align the retained reads with human genome (Grch38.p2) (20). Based on the results of sequence alignment, reads having only one chromosome location were retained for further analysis. The Genome Analysis Tool kit, is a widely used discovery tool to identify genetic variants based on the results of sequence alignment (21).

Several databases and tools were used to annotate identified genetic variants. dbSNP (b144) is an archive of genetic variation within different species that provides information about each genetic variant (22). ClinVar is a database of clinically significant genetic variants (23). COSMIC (v73) collects somatic mutation information for human cancers (24). The TCGA project collects genomics, methylomic and transcriptomic data across cancer types (25). Integrated mutation

Table II. Overview of our approach to identifying driver and passenger genes in endometrial cancer.

Type of prioritization filter	Remaining variants (n)		
	Canonical cancer-related genes	Non-canonical cancer-related genes	Total
1. All variants	2,936 variants in 586 genes	60,259 variants in 13,214 genes	63,185 variants in 13,800 genes
2. Non-synonymous variants	726 variants in 352 genes	14,970 variants in 6,974 genes	15,716 variants in 7,326 genes
3. The occurrence of variants $\leq 50\%$	603 variants in 322 genes	11,293 variants in 6,110 genes	12,561 variants in 6,432 genes
4. Global minor allele frequency $< 1\%$ in dbSNP v144 or NA	413 variants in 272 genes	6,286 variants in 4,264 genes	6,699 variants in 4,536 genes
5. ClinVar or PolyPhen-2 and SIFT predicted the variants to be pathogenic or possibly/probably functionally impaired	143 variants in 129 genes	1,316 variants in 1,188 genes	1,459 variants in 1,317 genes
6. DrGaP identified driver genes ($P < 0.01$)	NA	1,271 variants in 1,144 genes	1,271 variants in 1,144 genes

prediction software (PolyPhen-2 and SIFT) were used for analyses of the identified variants (26,27). DrGaP was used to identify driver genes and driver signaling pathways (28).

Confirmation by Sanger sequencing. Potential mutations identified by whole-exome sequencing were confirmed by PCR and Sanger sequencing. Specific PCR primers were designed using Primer3 software (primers can be provided on request). The products were sequenced directly with the ABI PRISM terminator cycle sequencing kit v3.1 on an ABI 3130 DNA sequencer (Applied Biosystems, Carlsbad, CA, USA).

Results

Whole-exome sequence analysis and coverage. Using massive parallel sequencing on a HiSeq platform, we generated ~45 billion bases of effective sequence data with an average read length of 200 bases. After mapping to the human reference genome (Grch38.p2) using the Bowtie 2 alignment tool, we obtained an average depth of coverage for the target regions of 20x for each sample (Table I). The false positive and false negative rates were estimated to be 2.75% (12/437) and 19.69% (76/386), respectively, after confirmed by target sequencing of cancer-related genes (29). Table II provides an overview of our approach to identify variants.

Mutational landscape in the 756 canonical cancer-related genes. The 143 non-synonymous mutations identified in the present study occurred in 129 genes, and included 141 missense mutations, one nonsense mutation and one frame shift mutation (details can be provided on request). The most frequently mutated genes were *PTEN* (35.71%; 5/14), *KRAS* and *PIK3R1* (14.29%, 2/14).

Sixty-eight variants already existed in the dbSNP, COSMIC, or TCGA databases and 75 variants in 71 genes did not. In addition, we found 13 different mutations in the same codon of 12 canonical cancer-related genes compared with the TCGA database (*CDK8*, *DPYD*, *EPHA3*, *EPHA6*, *FLNA*, *MAPK1*, *MAPK7*, *PLK1*, *PTEN*, *RAPGEF2*, *RFC4* and *ZNF521*) (details can be provided on request).

We ordered the 75 new variants according to sequencing depth. Next, we selected 12 variants for Sanger sequencing. Six variants had relatively high depth (208x, 156x, 131x, 105x, 97x and 89x). Several novel mutations were present only in cancer tissue, including *MAPT* p.C432Y, *IL24* p.G192W, *MCM6* p.R263H, *TSC1* p.D1054G and *BIRC2* p.P152S. However, *KAT6B* p.S1693N was also detected in paired non-cancerous tissues. Six variants had low depth (22x, 21x, 21x, 20x, 20x and 20x). Novel mutations present only in cancer tissue including *CIITA* p.E1063D, *DST* p.Q2014H, *CASP8* p.L219M and *NOTCH2* p.M2054I. *FLNA* p.V528L and *HSPAIL* p.P93H were also detected in paired non-cancerous tissues (Fig. 1).

Mutational landscape in the non-canonical cancer-related genes. The 1,271 non-synonymous mutations identified in this study, including 1,270 missense mutations and one nonsense mutation, were located at 1,144 genes. In total, 723 variants had previously been reported in the dbSNP, COSMIC or TCGA databases, while 548 variants in 516 genes had not (details can be provided on request).

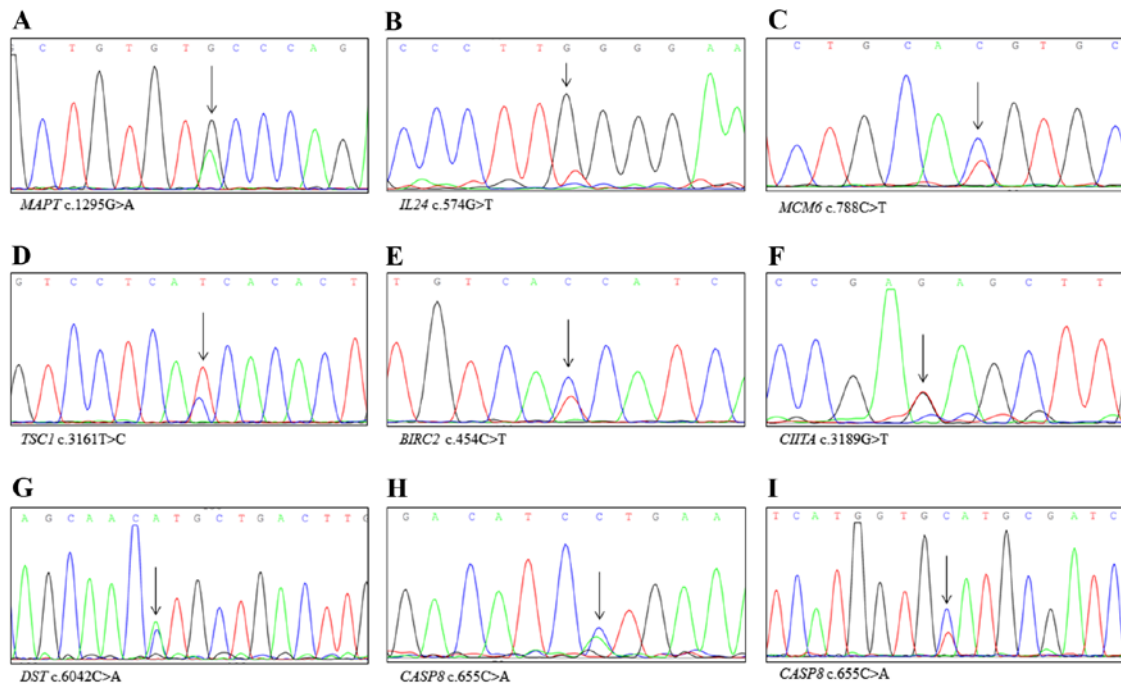


Figure 1. Confirmatory analysis by Sanger sequencing of canonical cancer related genes detected via WES. (A) *MAPT*, (B) *IL24*, (C) *MCM6*, (D) *TSC1*, (E) *BIRC2*, (F) *CIITA*, (G) *DST*, (H) *CASP8* and (I) *NOTCH2*.

However, nearly all mutations had been reported in the 542 TCGA endometrial cancer samples. Only three mutations had never been reported (*ARMCX4*, *CUTA* and *SAP30L*). We selected these mutations for Sanger sequencing. *CUTA* p.T187R and *SAP30L* p.H77Y were also detected in paired non-cancerous tissues; *ARMCX4* p.P2056H was the sole novel mutation that was detected only in cancer tissue (Fig. 2). We also found 49 different mutations in the same codon of 49 non-canonical cancer-related genes compared with the TCGA database (details can be provided on request).

The 1,144 genes were divided into five groups according to mutation frequency. Fifteen non-canonical cancer-related genes had high frequencies of mutation (35.71–50%; 5–7/14) and four variants in three genes (*IGSF10*, *FBXL13* and *PRUNE2*) were novel. We selected these new genetic variants for Sanger sequencing. We detected *IGSF10* p.R455W, *FBXL13* p.G313A and *PRUNE2* p.S2439I in paired non-cancerous tissues, while *IGSF10* p.K188Q was detected only in cancer tissue (Fig. 2).

Six genes were mutated in 4 (28.57%) of the 14 patients. One gene (*VPSI3C*) had a new genetic variant. Sanger sequencing confirmed that *VPSI3C* p.V2017M was a novel mutation detected only in cancer tissue (Fig. 2).

Twenty-two genes were mutated in 3 (21.43%) of the 14 patients, and there were 12 new genetic variants in 8 genes. We carried out Sanger sequencing on the new variants, and found that *ARAP2* p.Y1491C, *DNAH14* p.D4225V, *TLN1* p.D1325E, *TREH* p.M196T and *ZSCAN29* p.G304V were also present in paired non-cancerous tissues. Sanger sequencing also confirmed that *DCT* p.Y548S, *DNAH14* p.G1566C, *TLN1* p.A555V, *ZNF605* p.P283Q, p.E652A and *ZSCAN29* p.P71S were novel mutations present only in cancer tissue (Fig. 2). One variant (*TRANK1* p.W2445L) had an incorrect base call.

One hundred and nine genes were mutated in 2 (14.29%) of the 14 patients, and there were 73 new genetic variants in 54

genes. We ordered the 73 new variants according to sequencing depth. Next, we selected 10 variants for Sanger sequencing. Five variants had high depth (163x, 108x, 104x, 99x and 92x). Several novel mutations were detected only in cancer tissue, including *MOCOS* p.I320T, *CMYA5* p.P2149H and *PCDH17* p.P976H (Fig. 2). *MAGEE2* p.F249L and *CRYBG3* p.Q632E were also detected in paired non-cancerous tissues. Five variants had low depth (all 20x). Novel mutations present only in cancer tissue including *UGT1A8* p.Y290C and *CYFIP2* p.C959Y (Fig. 2), while *APOL4* p.A316V and *TRIM26* p.Q197H were also detected in paired non-cancerous tissues. One variant (*MYO3B* p.G230W) had an incorrect base call.

Among low-frequency mutations (7.14%; 1/14) in 992 non-canonical cancer-related genes, there were 458 new genetic variants in 450 genes. We ordered these 458 variants according to sequencing depth. Next, we selected 10 variants for Sanger sequencing. Five variants had high depth (286x, 244x, 223x, 197x and 187x). Several novel mutations were detected only in cancer tissue, including *MACF1* p.Q181H, *NUDT5* p.G135S, *JAKMIP1* p.R352M and *PCDHGB4* p.L154I (Fig. 2). *MYH10* p.L1048X was also detected in paired non-cancerous tissues. Five variants had low depth (all 20x). Novel mutations detected only in cancer tissue included *FAM178A* p.T86I, *SNX6* p.L38R, *IMP4* p.T163M and *PCMTD1* p.A41D (Fig. 2). *TNFAIP8L3* p.A38T was also detected in the paired non-cancerous tissues.

ACMG gene. Of the 14 endometrial cancer samples, 28.57% (4/14) harbored ACMG mutations, including *RYR2*, *MSH6* and *TSC1*, but did not have mutations in the remaining 53 genes. Mutations in the *RYR2* gene were detected in 14.29% (2/14) of endometrial cancer specimens, with mutations at p.G1209R (rs770286824) and p.F4960L (a novel mutation) identified in our endometrial cancer samples. Both the *MSH6* mutation at p.Q572H (rs745772518) and the *TSC1* mutation

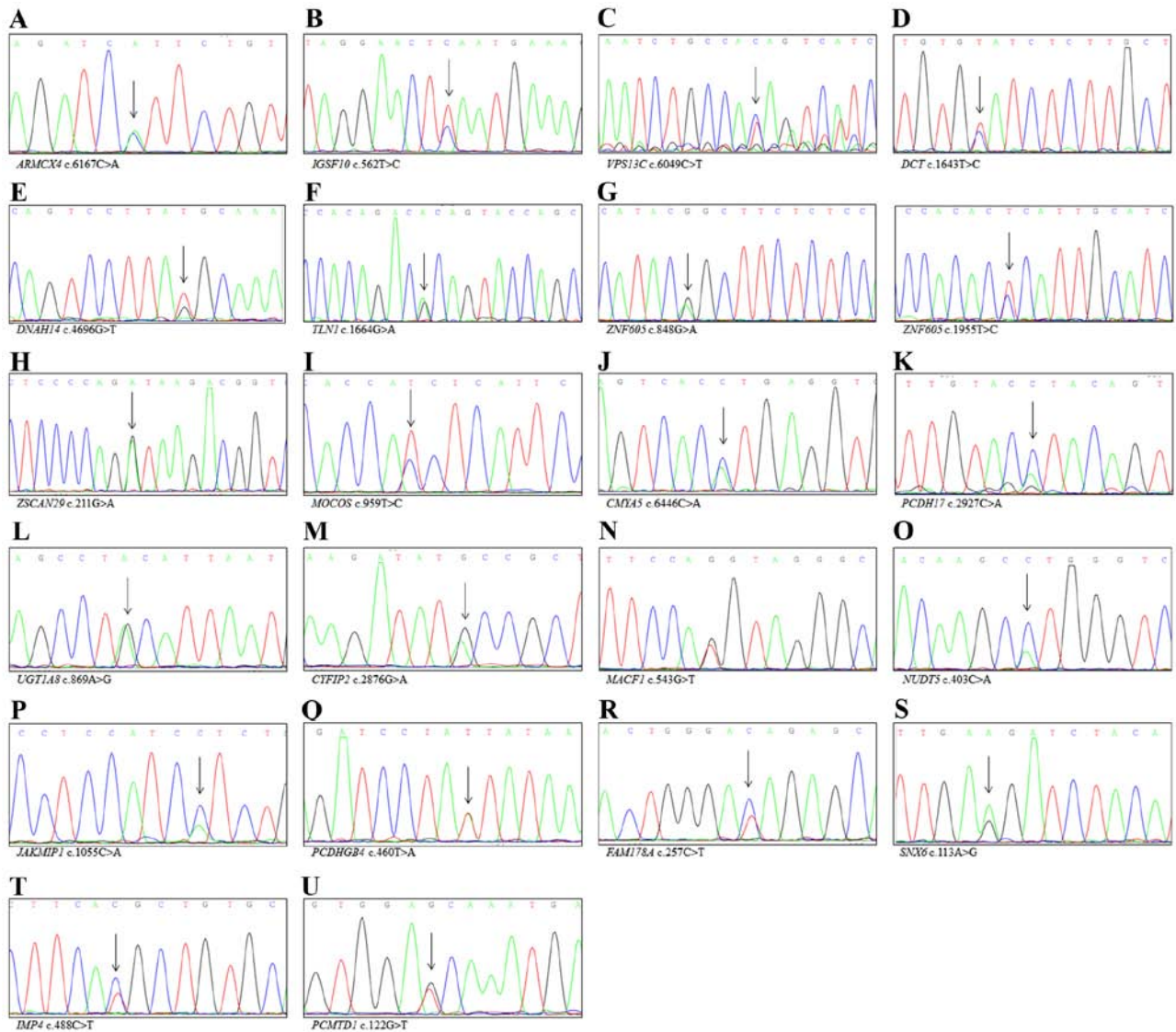


Figure 2. Confirmatory analysis by Sanger sequencing of non-canonical cancer related genes detected via WES. (A) *ARM CX4*, (B) *IGSF10*, (C) *VPS13C*, (D) *DCT*, (E) *DNAH14*, (F) *TLN1*, (G) *ZNF605*, (H) *ZSCAN29*, (I) *MOCOS*, (J) *CMYA5*, (K) *PCDH17*, (L) *UGT1A8*, (M) *CYFIP2*, (N) *MACF1*, (O) *NUDT5*, (P) *JAKMIP1*, (Q) *PCDHGB4*, (R) *FAM178A*, (S) *SNX6*, (T) *IMP4* and (U) *PCMTD1*.

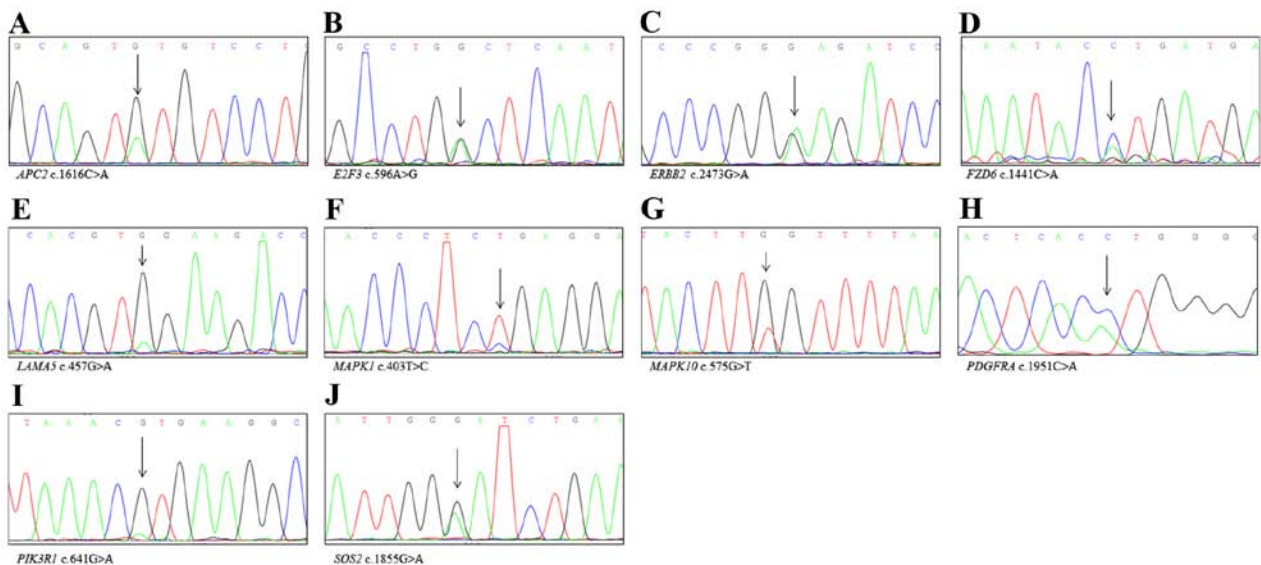


Figure 3. Confirmatory analysis by Sanger sequencing of KEGG cancer pathway (hsa05200) detected via WES. (A) *APC2*, (B) *E2F3*, (C) *ERBB2*, (D) *FZD6*, (E) *LAMA5*, (F) *MAPK1*, (G) *MAPK10*, (H) *PDGFRA*, (I) *PIK3R1* and (J) *SOS2*.

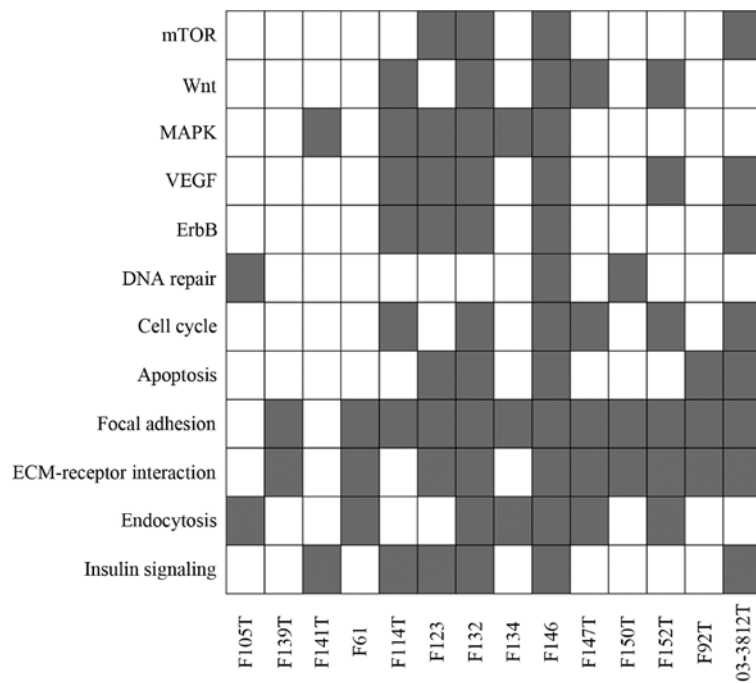


Figure 4. Altered pathways for 14 Taiwanese patient samples. The definition of altered pathway is at least 1 altered gene in the pathway.

at p.D1054G were detected in 7.14% (1/14) of endometrial cancer samples.

Altered pathways. Functional annotation of the 1,273 mutated genes was performed using the DrGaP tool. Thirty-seven of the 1,273 mutated genes were found in the Kyoto Encyclopedia of Genes and Genomics (KEGG) cancer pathways (hsa05200), including *APC2*, *BCR*, *BIRC2*, *CASP8*, *CCNA1*, *CREBBP*, *CTNNA2*, *CTNNA3*, *CTNNB1*, *E2F3*, *ERBB2*, *FGFR2*, *FOXO1*, *FZD6*, *HHIP*, *ITGA2*, *ITGA3*, *KRAS*, *LAMA1*, *LAMA2*, *LAMA3*, *LAMA5*, *LAMC1*, *LAMC2*, *MAPK1*, *MAPK10*, *MSH6*, *MTOR*, *PDGFRA*, *PIK3CA*, *PIK3R1*, *PLCG2*, *PTEN*, *SOS2*, *STAT5A*, *TCF7* and *TRAF5*. We used Sanger sequencing validation to confirm new variants (Fig. 3). Moreover, we identified several cellular pathways that were altered in endometrial cancer tissues and we found that each sample had at least two pathways involved in the carcinogenesis of endometrial cancer (Fig. 4).

Discussion

The present study described somatic mutation in the whole endometrial cancer exome. We identified several cancer driver and passengers genes from canonical and non-canonical cancer-related genes. Overall, 35.71% of endometrial cancer cases harbored *PTEN* mutations. Mutations were also found in other canonical cancer-related genes, including *KRAS* and *PIK3R1* (14.29% each). To the best of our knowledge, several sequencing variants have not been reported, including canonical cancer-related genes (*MAPT*, *IL24*, *MCM6*, *TSC1*, *BIRC2*, *CIITA*, *DST*, *CASP8* and *NOTCH2*) and non-canonical cancer-related genes (*ARMCX4*, *IGSF10*, *VPS13C*, *DCT*, *DNAH14*, *TLN1*, *ZNF605*, *ZSCAN29*, *MOCOS*, *CMYA5*, *PCDH17*, *UGT1A8*, *CYFIP2*, *MACF1*, *NUDT5*, *JAKMIP1*, *PCDHGB4*, *FAM178A*, *SNX6*, *IMP4* and *PCMTD1*).

Two canonical cancer-related genes (*TSC1* and *BIRC2*) were found in the Oncomine Cancer Research Panel, which was used in the National Cancer Institute Match Trial (30). *TSC1* is a tumor suppressor gene that encodes a growth inhibitory protein (hamartin) thought to play a role in the stabilization of tuberin. *TSC1* is a gene involved in the mTOR pathway. Mutations in *TSC1*, *TSC2* and *MTOR* have been associated with response to rapalogs in patients with metastatic renal cell carcinoma (31). We identified a novel mutation, p.D1054G, in one endometrial cancer patient.

BIRC2 is an oncogene that encodes c-IAP1, which is a member of the apoptosis inhibitor family. Members of this family inhibit apoptosis by binding to TRAF1 and TRAF2 and likely interfering with activation of ICE-like proteases. Previously, Choschick *et al* (32) reported *BIRC2* amplification in uterine cervix cancer. In the present study, we identified a novel mutation, p.P152S, in a patient with endometrial cancer.

The genetic alterations we found involved the mTOR, Wnt, MAPK, VEGF and ErbB pathways, as well as aberrant DNA repair, cell cycle control and apoptosis pathways. These pathways have previously been shown to be involved in the multistep development of endometrial cancer, and clinical trials of drugs for endometrial cancer that target these pathways have been carried out (33). We also found genetic alterations in the steroid hormone biosynthesis pathway (hsa00140), including *AKR1C3*, *CYP3A4*, *CYP3A43*, *HSD11B1*, *SULT1E1* and *UGT1A8*.

NGS technology allows for the detection of mutations and copy number variants. Although the hybrid capture method was used, we failed to assess copy number alterations because we sequenced only tumor samples. Sequencing coverage is uneven across the genome owing to variability introduced by the hybridization-capture step, and the development of a robust algorithm is challenging (34). On the other hand, multiplex PCR-based method fail to detection copy number changes and/or gene fusions (13).

In summary, we performed WES of endometrial cancer samples and identified several potential cancer driver and passenger cancer genes (*MAPT*, *IL24*, *MCM6*, *TSC1*, *BIRC2*, *CIITA*, *DST*, *CASP8*, *NOTCH2*, *ARMCX4*, *IGSF10*, *VPS13C*, *DCT*, *DNAH14*, *TLN1*, *ZNF605*, *ZSCAN29*, *MOCOS*, *CMYA5*, *PCDH17*, *UGT1A8*, *CYFIP2*, *MACF1*, *NUDT5*, *JAKMIP1*, *PCDHGB4*, *FAM178A*, *SNX6*, *IMP4* and *PCMTD1*). The major limitation of this study was the small sample size.

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References

- Sénéchal C, Cottreau E, de Pauw A, Elan C, Dagousset I, Fourchotte V, Gauthier-Villars M, Lae M, Stoppa-Lyonnet D and Buecher B: Environmental and genetic risk factors for endometrial carcinoma. *Bull Cancer* 102: 256-269, 2015 (In French).
- Ashton KA, Proietto A, Otton G, Symonds I, McEvoy M, Attia J, Gilbert M, Hamann U and Scott RJ: Polymorphisms in genes of the steroid hormone biosynthesis and metabolism pathways and endometrial cancer risk. *Cancer Epidemiol* 34: 328-337, 2010.
- Xu J, Lin X, Zhu H, Zhang Z and Yang B: Genetic variation of the CYP17 and susceptibility to endometrial cancer: A meta-analysis. *Mol Biol Rep* 40: 5085-5091, 2013.
- Collaborative Group on Epidemiological Studies on Endometrial Cancer: Endometrial cancer and oral contraceptives: An individual participant meta-analysis of 27 276 women with endometrial cancer from 36 epidemiological studies. *Lancet Oncol* 16: 1061-1070, 2015.
- Trabert B, Wentzensen N, Yang HP, Sherman ME, Hollenbeck AR, Park Y and Brinton LA: Is estrogen plus progestin menopausal hormone therapy safe with respect to endometrial cancer risk? *Int J Cancer* 132: 417-426, 2013.
- Brinton LA, Westhoff CL, Scoccia B, Lamb EJ, Trabert B, Niwa S and Moghissi KS: Fertility drugs and endometrial cancer risk: Results from an extended follow-up of a large infertility cohort. *Hum Reprod* 28: 2813-2821, 2013.
- Ong M, Carreira S, Goodall J, Mateo J, Figueiredo I, Rodrigues DN, Perkins G, Seed G, Yap TA, Attard G, *et al*: Validation and utilisation of high-coverage next-generation sequencing to deliver the pharmacological audit trail. *Br J Cancer* 111: 828-836, 2014.
- André F, Bachelot T, Commo F, Campone M, Arnedos M, Dieras V, Lacroix-Triki M, Lacroix L, Cohen P, Gentien D, *et al*: Comparative genomic hybridisation array and DNA sequencing to direct treatment of metastatic breast cancer: A multicentre, prospective trial (SAFIR01/UNICANCER). *Lancet Oncol* 15: 267-274, 2014.
- Cancer Genome Atlas Research Network: Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455: 1061-1068, 2008.
- Bell D, Berchuck A, Birrer M, Chien J, Cramer DW, Dao F, Dhir R, DiSaia P, Gabra H, Glenn P, *et al*: Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. *Nature* 474: 609-615, 2011.
- Cancer Genome Atlas Network: Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487: 330-337, 2012.
- Kandoth C, Schultz N, Cherniack AD, Akbani R, Liu Y, Shen H, Robertson AG, Pashtan I, Shen R, Benz CC, *et al*: Cancer Genome Atlas Research Network: Integrated genomic characterization of endometrial carcinoma. *Nature* 497: 67-73, 2013.
- Beadling C, Neff TL, Heinrich MC, Rhodes K, Thornton M, Leamon J, Andersen M and Corless CL: Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. *J Mol Diagn* 15: 171-176, 2013.
- Grasso C, Butler T, Rhodes K, Quist M, Neff TL, Moore S, Tomlins SA, Reinig E, Beadling C, Andersen M, *et al*: Assessing copy number alterations in targeted, amplicon-based next-generation sequencing data. *J Mol Diagn* 17: 53-63, 2015.
- Zheng Z, Liebers M, Zhelyazkova B, Cao Y, Panditi D, Lynch KD, Chen J, Robinson HE, Shim HS, Chmielecki J, *et al*: Anchored multiplex PCR for targeted next-generation sequencing. *Nat Med* 20: 1479-1484, 2014.
- Samorodnitsky E, Datta J, Jewell BM, Hagopian R, Miya J, Wing MR, Damodaran S, Lippus JM, Reeser JW, Bhatt D, *et al*: Comparison of custom capture for targeted next-generation DNA sequencing. *J Mol Diagn* 17: 64-75, 2015.
- Drmanac R, Peters BA, Church GM, Reid CA and Xu X: Accurate whole genome sequencing as the ultimate genetic test. *Clin Chem* 61: 305-306, 2015.
- Park JY, Clark P, Londin E, Sponziello M, Kricka LJ and Fortina P: Clinical exome performance for reporting secondary genetic findings. *Clin Chem* 61: 213-220, 2015.
- Liang H, Cheung LW, Li J, Ju Z, Yu S, Stemke-Hale K, Dogruluk T, Lu Y, Liu X, Gu C, *et al*: Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer. *Genome Res* 22: 2120-2129, 2012.
- Langmead B, Trapnell C, Pop M and Salzberg SL: Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10: R25, 2009.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, *et al*: The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20: 1297-1303, 2010.
- Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM and Sirotkin K: dbSNP: The NCBI database of genetic variation. *Nucleic Acids Res* 29: 308-311, 2001.
- Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM and Maglott DR: ClinVar: Public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res* 42 (D1): D980-D985, 2014.
- Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, *et al*: COSMIC: Exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 43 (D1): D805-D811, 2015.
- Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al*: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 6: pii, 2013.
- Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS and Sunyaev SR: A method and server for predicting damaging missense mutations. *Nat Methods* 7: 248-249, 2010.
- Kumar P, Henikoff S and Ng PC: Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 4: 1073-1081, 2009.
- Hua X, Xu H, Yang Y, Zhu J, Liu P and Lu Y: DrGaP: A powerful tool for identifying driver genes and pathways in cancer sequencing studies. *Am J Hum Genet* 93: 439-451, 2013.
- Chang YS, Huang HD, Yeh KT and Chang JG: Genetic alterations in endometrial cancer by targeted next-generation sequencing. *Exp Mol Pathol* 100: 8-12, 2016.
- Hovelson DH, McDaniel AS, Cani AK, Johnson B, Rhodes K, Williams PD, Bandla S, Bien G, Chopra P, Hyland F, *et al*: Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia* 17: 385-399, 2015.
- Kwiatkowski DJ, Choueiri TK, Fay AP, Rini BI, Thorner AR, de Velasco G, Tyburczy ME, Hamieh L, Albages L, Agarwal N, *et al*: Mutations in TSC1, TSC2, and MTOR are associated with response to rapalogs in patients with metastatic renal cell carcinoma. *Clin Cancer Res* 22: 2445-2452, 2016.
- Choschzick M, Tabibzade AM, Gieseck F, Woelber L, Jaenicke F, Sauter G and Simon R: BIRC2 amplification in squamous cell carcinomas of the uterine cervix. *Virchows Arch* 461: 123-128, 2012.
- Ma XY, Ma CX and Wang JH: Endometrial carcinogenesis and molecular signaling pathways. *Am J Mol Biol* 4: 134-149, 2014.
- Nam JY, Kim NK, Kim SC, Joung JG, Xi R, Lee S, Park PJ and Park WY: Evaluation of somatic copy number estimation tools for whole-exome sequencing data. *Brief Bioinform* 17: 185-192, 2016.