科技部補助專題研究計畫報告

建構一個以生物標記為基礎的預測模式來早期推算多囊性卵巢症候群婦女罹患子宮內膜癌的機率(L03)

報 告 類 別 ： 精簡報告
計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 108-2629-B-182A-004-
執 行 期 間 ： 108年08月01日至110年07月31日
執 行 單 位 ： 長庚醫療財團法人婦產科

計 畫 主 持 人 ： 張嘉琳

計畫參與人員： 碩士級-專任助理：蔡孟樺

本研究具有政策應用參考價值：■否 □是，建議提供機關
（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
本研究具影響公共利益之重大發現：□否 □是

中 華 民 國 110 年 11 月 10 日

中 文 摘 要 ： 宮內膜癌是婦科惡性腫瘤中最常見之一。在過去的數十年中，子宮內膜癌的發病 率在一直在上升，特別是在生育年齡的婦女。在這些年輕婦女中，有罹患多囊性卵巢症後群（PCOS）的婦女比起沒有罹患多囊性卵巢症後群的婦女，得子宮內膜癌的風險又增加3 至8 倍。因此，我們認為高胰島素血症，血脂異常和異常內分泌荷爾蒙代謝可能是引發多囊性卵巢症後群患者易罹患子宮內膜癌之潛在機制。基於多囊性卵巢症後群和子宮內膜癌均具有高度基因變異相關背景，我們將以此為基礎，試著創建一個以結合多囊性卵巢症後群，早發性子宮內膜癌常見突變基因和內分泌生物標誌的預測平台，來早期估算有多囊性卵巢症後群之婦女未來可能罹患子宮內膜癌的風險機率。在目標一中，我們將使用來自組織庫的福馬林固定石蠟包埋樣品（formalin-fixedparaffin embedded, FFPE, samples）以及來自患者的新收集的子宮內膜增生和癌症組織來鑑定子宮內膜癌中的癌症突變基因。在這個目標，我們將確定傾向於發生在多囊性卵巢症後群的年輕患者常見的子宮內膜癌癌症風險突變基因，和這些突變與發展成子宮內膜癌之間的相關性。在目標二中，我們將研究從液體衛生棉萃取得基因樣品來檢測子宮內膜細胞異常突變基因的可行性。在這個概念驗證分析中，我們將整合分析來自液體衛生棉取樣和體細胞基因組的突變基因群以及血清中的異常內分泌荷爾蒙代謝生物標誌，以此架建一個用於可預測多囊性卵巢症後群患者得子宮內膜癌之機率平台。
總體而言，這項研究不僅可以讓人們能更清楚瞭解多囊性卵巢症後群患者易罹患子宮內膜癌的相關突變基因和異常內分泌荷爾蒙代謝病理，而且能為多囊性卵巢症後群患者提供一個以保存子宮生育能力為前提的精準醫學醫療管理。

中 文 關 鍵 詞 ： 多囊性卵巢症後群, 子宮內膜癌, 生物標記, 早期偵測

英 文 摘 要 ： Endometrial cancer is the most common malignancy of gynecological tract. The incidence of endometrial cancer has been increasing over the last few decades, especially in the reproductive-aged women. Among reproductive-aged women, those with polycystic ovary syndrome (PCOS) have a 3- to 8-fold increase in the risk of developing endometrial cancer when compared to women without PCOS. Because a large proportion of PCOS women are presented with overweight/obese or defective insulin sensitivity and because PCOS and endometrial cancer are associated with the genetic background of patients, we hypothesize that PCOS susceptibility associated genetic variants and mutations in key endometrial cancer risk genes -could contribute to the pathogenesis of PCOS associated endometrial cancer. Because PCOS is characterized by an aberrant endocrine profile, we further hypothesize that PCOS-associated endometrial cancer is associated with the interplay of genetic background and PCOS specific endocrine aberrations. Based on this understanding, we propose to develop a prediction biomarker panel that estimates the risk of endometrium cancer in PCOS

women by combining the forecasting power of PCOS-specific genetic alterations, genetic variants and endocrine biomarkers.

英文關鍵詞：biomarker, risk predicting, endometrial cancer, PCOS

Background and Significant

Endometrial cancer is the most common gynecologic cancer and lacks an early detection method

Endometrial cancer is the most common malignancy of gynecological tract. In developed countries, women have a 2–3% lifetime risk of developing this malignancy. An estimated 60,050 new cases are expected to occur in the United States in 2016, and an estimated 10,170 women are expected to die of the disease (http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2016/; http://www.ncbi.nlm.nih.gov/books/NBK65786/). The incidence of endometrial cancer has been increasing over the last few decades, presumably because of the growing obesity epidemic [1]. Unfortunately, most young cases (~85%) cannot be diagnosed at the early stage in spite of common use of transvaginal ultrasound screening [2-4]. It is obvious that current screening procedure is inadequate to improve the care of endometrial cancer patients, especially those at their reproductive age. New approaches that can predict the risk of endometrium cancer and / or efficiently detect endometrial cancer at its early stages of development are urgently needed.

Polycystic ovary syndrome (PCOS) is a strong risk factor for endometrial cancer

Although the exact mechanisms underlying endometrial carcinogenesis are not well understood, risk factors such as obesity and insulin resistance have been implicated in the development of this malignant disease [5-9]. Earlier studies have shown that the risk of endometrial cancer increases 1.59-fold per 5 kg/m^2 change in body mass [5]. On the other hand, regular exercise is associated with a 38-46% decrease in endometrial cancer risk [10].

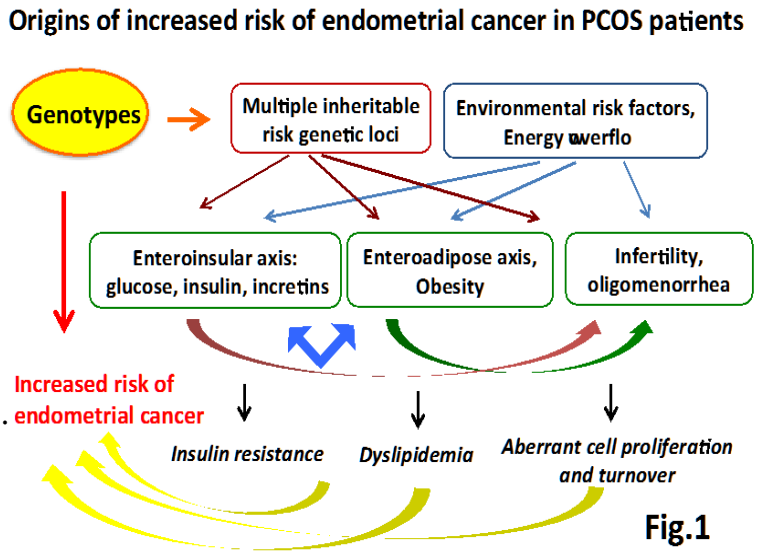


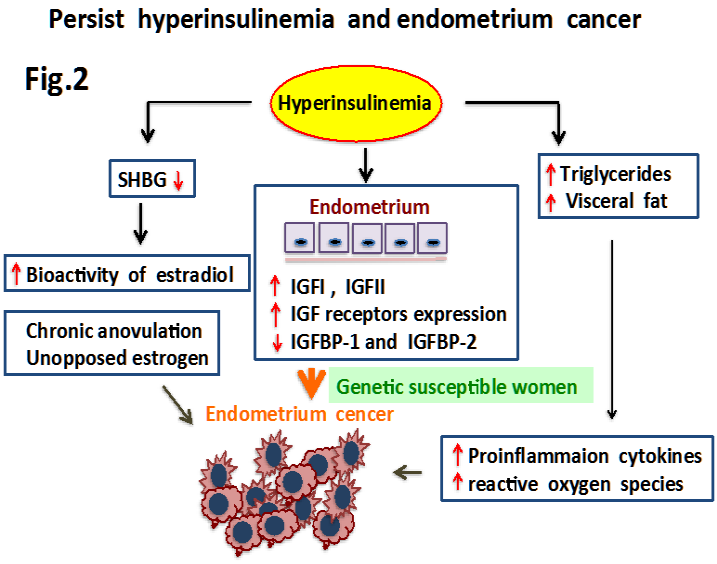
Fig.1

In addition to obesity and insulin resistance, polycystic ovary syndrome (PCOS) represents a significant risk factor for the development of endometrial cancer [2]. PCOS affects about 5-12% of women worldwide, and is the most common endocrine abnormality of reproductive-age women [11-14]. PCOS was first reported by Stein and Leventhal in 1935 as Stein-Leventhal syndrome, and is characterized by a complex phenotype, including oligo- or anovulation, hyperandrogenism, hirsutism, amenorrhea, obesity, and/or enlarged polycystic appearing ovaries [15-19]. While defective reproduction is the main outcome of PCOS, >42% of women with PCOS in developed countries are overweight/obese, and have a high risk of developing type 2 diabetes (T2D), atherosclerosis and cardiovascular events [18, 20-22]. Importantly, women with PCOS have a 3- to 8-fold increase in the risk of developing endometrial cancer when compared to women without PCOS. In Caucasian PCOS patients, the lifetime risk is ~9%, and women with recurrent and/or metastatic endometrial cancer have a median

survival of 7–12 months despite systemic treatments with endocrine and combination chemotherapy [23]. This enhanced risk is specific for endometrial cancer because the risk of ovarian and breast cancers is not significantly increased overall in PCOS patients [24, 25]. With this background, a popular hypothesis suggested that hyperinsulinemia, dyslipidemia and obesity in PCOS patients not only act on the ovary to increase theca cell proliferation and testosterone synthesis, but also contribute to the development of PCOS-associated endometrial dysplasia and cancer [9] (Fig. 1).

Mitogenic insulin signaling pathway could enhance malignant cell growth

Under chronic hyperinsulinemia condition, insulin can directly promote cell proliferation and survival, and reduce apoptosis through activation of the *ras–raf*–MAPK and PI3K–AKT pathways [29, 30]. Indirectly, insulin may exert its pro-survival effects by enhancing growth factor-dependent cell proliferation [29, 31, 32]. For example, insulin can (1) increase the synthesis of sex hormones and reduce sex hormone-binding globulin (SHBG) level, which increases estrogen bioavailability [31-33] and (2) increase the bioactivity of insulin-like growth factors (IGF-I and IGF-II) by enhancing hepatic IGF synthesis or by reducing hepatic production of the IGF-binding proteins 1 (IGFBP-1) and 2 (IGFBP-2) [31-33]. Likewise, obesity, which is the most common cause of insulin resistance, could promote cell survival by elevating endogenous estrogen production [34]. In addition, dyslipidemia and obesity could lead to a low-grade inflammatory condition in which overproduction of proinflammatory cytokines enhances malignant transformation [26] (Fig. 2).



Because dysregulation of tyrosine-kinase receptor-mediated mitogenic pathways has been implicated in the development of many types of cancers, high levels of insulin and IGFs could enhance malignant cells growth in PCOS patients (Fig. 2) [30, 35-37]. Specifically, endometrial stromal cells have been shown to produce IGF-I and IGF-II, and these growth factors act as mitogens in uterine cells by increasing the proliferation rate [38]. IGFs are also potent simulators of endometrial cancer cell growth, and levels of IGF-I receptor and IGF-II receptor mRNA have been shown to increase in endometrial cancer samples compared to normal endometrium samples [39-41]. Furthermore, clinical conditions that have an increased risk of endometrial cancer (i.e., PCOS and obesity) are characterized by the absence or decreased expression of IGFBP-1. In addition, insulin may enhance IGF signaling in the uterus by inhibiting IGFBP-1 expression [38, 42-44]. Consistent with these findings, genetic variations within in IGF-II, IGFBP-3 and insulin receptor substrate-2 (IRS-2) have been shown to influence endometrial cancer risk [45, 46]. Therefore, the interaction of insulin, IGF

and steroid hormones in the endometrial tissue of PCOS patients may activate a molecular chain of events that eventually lead from a phenotypically normal cell to one harboring neoplastic traits.

Molecular signatures of endometrial cancer in PCOS patients

It is well known that molecular mechanisms that drive insulin resistance in PCOS differ from those in other common insulin-resistant states, such as T2D and obesity [11]. PCOS is associated with a unique profile of hormonal changes which are manifested as elevation of a plethora of endocrine hormones including, androgens, insulin, glucose-dependent insulinotropic peptide (GIP), irisin, SHBG, and anti-mullerian hormone (AMH) [47]. In addition, strong evidence suggested that (1) adipocytes and adipose functions are aberrant in most PCOS patients, and the hormonal environment favors insulin resistance and subclinical inflammation [17, 24]. Studies of isolated endometrial cell populations from PCOS women have shown that endometrial stromal cells play a paracrine role in the regulation of epithelium-derived endometrial cancer development in PCOS patients, and that inflammation and pro-oncogenic changes occur independent of BMI [48]. Consistently, it has been shown that (1) genes in the insulin and IGF signaling pathways are frequently mutated in endometrial cancer, (2) increased plasma levels of insulin and IGFs are associated with endometrial cancer risk in PCOS patients, and (3) the expression of total insulin receptor (IR) and insulin receptor isoform alpha (IR-A) are upregulated in endometrial carcinoma [8, 34, 48-51]. Thus, the unique endocrine environment in PCOS patients could play a critical role in carcinogenesis of PCOS-associated endometrial cancer [17, 24].

There is a strong genetic component in the development of endometrial cancer

Endometrial carcinomas can be classified based on histopathological characteristics (e.g., endometrioid, serous, or clear-cell adenocarcinoma), or as type I or II on the basis of clinical and endocrine features [52]. However, there is actually substantial heterogeneity in biological, pathological, genetic, and molecular features among these endometrial cancer subtypes. Like many other cancers, the development of endometrial cancer appears to have a strong genetic component [53, 54]. Genetic studies in the last decade indicated that endometrial cancer can be roughly divided into two molecular types: Type A: estrogen receptor (ER+), progesterone receptor (PR+) and TP53(-), which is mainly found in young patients, and Type B: (ER-), (PR-), and TP53(+), which occurs mainly in old-age patients [54]. Type A is also characterized by frequent loss of tumor suppressor PTEN, mutations in KRAS, FGFR2 and PIK3CA,

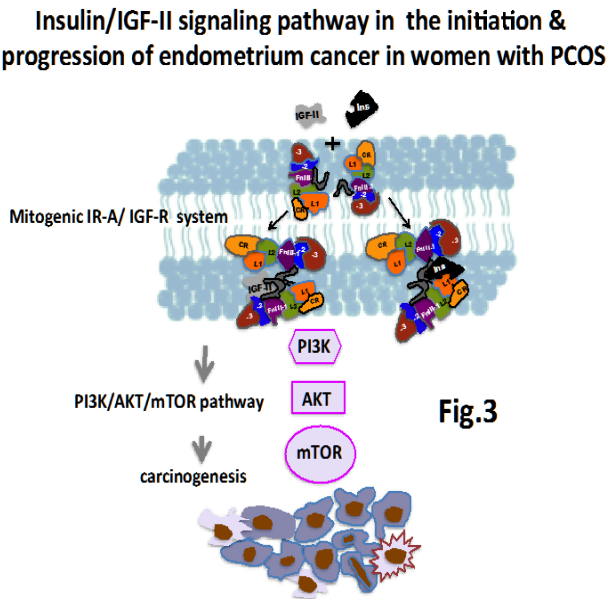


Fig.3

and microsatellite instability. In addition, mutations in PIK3CA and PTEN, a major negative regulator of the phosphatidylinositol 3-kinase (PI3K) pathway, appeared to occur in a large fraction of endometrial cancers, whereas aberrations in the PI3K pathway often occur with co-mutations of multiple cancer genes such as TP53, KRAS, AKT, and CTNNB1. Likewise, germline mutations in mismatch repair (MMR) genes (e.g., MLH1, MSH2, and MSH6) appear to contribute to the development of endometrial cancer in young patients [53].

In more recent molecular studies, it has become clear that endometrial carcinomas could harbor a wide spectrum of genetic mutations. For example, studies of endometrioid tumors indicated that there are at least four principle categories of endometrial cancers: (1) POLE ultramutated, (2) microsatellite instability hypermutated, (3) copy-number low, and (4) copy-number high [55]. A recent whole-exome sequencing study also revealed 12 potential endometrial cancer ‘‘driver’’ genes that functionally contribute to endometrial tumorigenesis [56]. These driver genes include 10 tumor-suppressor candidates (ARID1A, INHBA, KMO, TTLL5, GRM8, IGFBP3, AKTIP, PHKA2, TRPS1, and WNT11) and two oncogene candidates (ERBB3 and RPS6KC1). Because PI3K/AKT pathway was aberrant in more than 90% of endometrial cancer [55], and because PCOS, T2D and obesity are all closely associated with insulin resistance [6], it is believed that endometrial cancer could be a disease driven by aberrant PI3K mutations (Fig. 3). Theoretically, endometrial cancer could be rooted in an excess insulin/IGF-I or IGF-II signaling which interacts with insulin/IGF receptors on endometrial cells, followed by the formation of an activated dimeric receptor complex (i.e., IR-A, IR-B, and IGF1R) and subsequent activation of PI3K-AKT-mTOR signaling pathway. This process could lead to increased endometrial cell proliferation and the accumulation of mutations in key driver genes, thereby leading to carcinogenic transformation (Fig. 3).

Levels of irisin and glucose-dependent insulinotropic peptide (GIP) are novel biomarkers of PCOS

To better understand the molecular attributes of PCOS patients, we have studied the levels of a variety of serum hormones in lean and obese PCOS patients. Importantly, **we have recently reported that serum levels of irisin, a newly discovered muscle-derived brown adipose-differentiation factor, and glucose-dependent insulinotropic peptide (GIP) are significantly elevated in PCOS patients [47].**

Because serum levels of the newly identified irisin have been shown to be abnormal in patients with T2D or gestational diabetes mellitus (GDM) [57-66], and because GIP induces obesity in transgenic animals, we hypothesized that the development of PCOS could be partly attributed to dysregulation of these hormonal factors. Consistent with our hypothesis, we found that the “irisin-resistant” phenotype is parallel with insulin resistance in PCOS patients [47]. In addition, we documented that the level of GIP, which plays a particularly important role in the regulation of the deposition of triglycerides in the adipose, muscle and liver tissues, is abnormally elevated in PCOS patients when compared to those of control women regardless of whether they are obese/overweight or not. Analysis of the effect size indicated that both fasting irisin and glucose-induced GIP response are significant risk factors for PCOS with odds ratios of 6.63 and 4.21, respectively. Importantly, our

findings have been independently verified by several laboratories [67-70].

More recently, we have expanded the study, and investigated the hormonal profile of a total of 156 normal and 444 PCOS patients (please see preliminary study). Consistent with our earlier finding, levels of irisin are significantly increased in PCOS patients when compared with normal volunteers. On the other hand, the level of a newly identified white adipose tissue-derived glucose-mobilizing hormone, asprosin, is not altered in PCOS patients [71, 72].

Because the levels of irisin and GIP are positively correlated with insulin resistance in PCOS patients, and because increased GIP levels may lead to dyslipidemia, we hypothesize that elevated levels of GIP and irisin in PCOS patients could also contribute to the development of endometrial cancer in these patients. Theoretically, the elevated irisin and GIP could enhance the mitogenic action of insulin in uterine tissues by promoting adipogenesis and associated proinflammatory environment. Alternatively, the elevated GIP and irisin signaling could facilitate endometrial carcinogenesis by actually acting upstream of the insulin signaling pathway by stimulating insulin expression and secretion.

Identification of specific endometrial cancer biomarkers and a refined sample collection method are needed to develop an early prediction tool for endometrial cancer in PCOS patients

Currently, endometrial cancer is mainly detected by the sign/symptoms, and followed by hysteroscopy, endometrium curratage, and pathological endometrial biopsy diagnosis. These approaches are inadequate for early prediction and prevention. Because many PCOS patients with endometrial cancer are at their reproductive age, an effective and sensitive endometrial cancer prediction method is urgently needed in order to preempt the progression of endometrial cancer and preserve the fertility in these patients.

Because PCOS patients have a particularly high risk of developing endometrial cancer, we have hypothesized that endometrial cancer in PCOS patients is associated with a unique set of genetic and endocrine biomarkers, and that the identification of these biomarkers represents a promising strategy for devising an early screening method of endometrial cancer in PCOS patients [73]. To test these hypotheses, we propose to integrate recent advances in cancer genetics and the power of next generation sequencing (NGS) to systemically define clinically relevant genetic biomarker in endometrial cancer of PCOS patients as well as endometrial cancer-associated endocrine biomarkers in PCOS patients **(Aim 1)**. We will also perform a feasibility study to investigate the efficiency of detecting endometrial cancer genetic biomarkers in endometrial tissue samples collected noninvasively by always infinity pads sampling **(Aim 2)**. By combining the convenience of at home collection of endometrial samples with always infinity pads and the power of genetic profiling, the proposed study represents an expedited route to understand the development of endometrial cancer in PCOS patients, and to develop a novel screening method for early prediction of endometrium cancer in a high-risk population.

Always infinity pads is a convenient noninvasive device for uterine tissue sampling

In the last three decades, Pap smear tests have revolutionized the management of patients with

human papillomavirus and cervical cancers. However, cervical cytology is not sensitive for detecting upper female reproductive tract cancers, and is not a good screening test for endometrial cancer or other noncervical gynecologic cancer [74]. Among the various uterine sampling methods, the use of Always Infinity Pads could be the most convenient approach [75]. always infinity pads have been widely marketed for over 60 years and are currently used by up to 50% of menstruating women in developed countries. In addition to this long history of usage and acceptance, the safety of pads has been well documented [76]. As early as 1957, it has been proposed that tissue and blood samples in pads represent a source of material for the detection of cancers of endometrial or ovarian origins, and for the detection of infectious agents. Earlier studies have shown that the amount of DNA isolated from vaginal pad is sufficient for the detection of (1) human papillomaviruses, (2) tumor cells in the vagina of women with serous ovarian cancer based on the analysis of TP53 [77], and (3) aberrant methylation pattern in endometrial cancer candidate genes [75, 78]. Likewise, studies of dry pad samples indicated that this method has reasonably high sensitivity and specificity for the detection of high-grade cervical intraepithelial neoplasia [79].

Because pads can collect large amount of DNA during each menses, the detection of rare endometrial cancer cells in menses would pose little technical challenges when we incorporate the power
of quantitative PCR (qPCR) and NGS into the screening procedure, and have a clearly defined panel of endometrial cancer-associated genes. In addition to early detection, the proposed screening method could offer potential therapeutic targets (e.g., the identification and inhibition of the EGFR, VEGFR, FGFR2 and PI3K/PTEN/AKT/mTOR signaling pathways by existing targeted therapies) for efficient treatment of growing endometrial tumors [54].

Previous and current Studies

Select SNPs in insulin signaling pathway genes are associated with the development of PCOS

Because PCOS exhibits familial aggregation, and because insulin resistance and beta-cell dysfunction in women with PCOS is heritable [80], it has been hypothesized that this disease could have a strong heritable component. However, earlier GWAS studies revealed only a handful of low-significance PCOS-associated loci at 2p16.3 (near luteinising hormone/ choriogonadotropin receptor; *LHCGR*), 2p21 (near thyroid associated protein; *THADA*), and 9q33.3 (DENN/MADD domain containing 1A; *DENNDIA*) [12]. Although these studies

25 targeted genetic risk variants in 12 susceptible loci

Genes	SNP_ID	Genotype (Anc/Der)	Derived Allele Frequency	Ancestral Allele Frequency
CDKAL1	rs7754840	G/C	0.41	0.59
CDKAL1	rs10806920	T/A	0.30	0.70
CYB5R4	rs1325471	A/G	0.66	0.34
GAD2	rs2839670	A/C	0.65	0.35
GIP	rs3895874	G/A	0.50	0.50
GIP	rs2291725	T/C	0.47	0.54
HHEX	rs1111875	A/G	0.56	0.44
HNF1B	rs757210	A/G	0.36	0.64
KCNQ1	rs2237892	C/T	0.17	0.83
KLF14	rs972283	G/A	0.34	0.66
NRIP1	rs2142218	C/T	0.58	0.42
NRIP1	rs2823002	T/A	0.57	0.43
NRIP1	rs7280064	C/T	0.34	0.66
NRIP1	rs6517179	T/C	0.60	0.40
PPARG	rs2920502	C/G	0.61	0.39
PPARG	rs2920500	A/G	0.41	0.59
PPARG	rs2972164	C/T	0.58	0.42
PRDM16	rs2651886	G/C	0.36	0.64
PRDM16	rs2817145	A/T	0.54	0.46
PRDM16	rs2500297	G/A	0.73	0.28
PRDM16	rs1875448	G/A	0.61	0.39
PRDM16	rs2500302	G/A	0.60	0.40
PRDM16	rs10797383	T/C	0.43	0.57
PRDM16	rs2651888	A/C	0.55	0.45
RBL1	rs1744765	T/C	0.24	0.77

Fig.4

indicated that genetic variants may play little role in the development of PCOS, we suspected that earlier GWAS studies could be mired by inherent heterogeneity issues of samples. We reasoned that an integrated investigation focusing on high priority SNPs could be a better approach to reveal (1) potential genetic risk factors and (2) important genotype-phenotype relationships among PCOS patients. In an ongoing study in my laboratory, we have focused on SNPs within genes that have been implicated in the regulation of insulin resistance and adipogenesis because these pathological features are overtly related to the development of PCOS. In this study, we have collected DNA samples from more than 600 PCOS patients and 300 control patients, and genotyped SNPs within 43 candidate genes using the Applied Biosystems TaqMan® SNP Genotyping Assays. These genes and loci were selected based on their relevance to PCOS, glucose and lipid metabolism, or have high genome-wide significance for BMI, weight gain, adipogenesis, obesity, or T2D in multiple clinical studies. These SNPs include those from *GIP, CDKAL1, CYB5R4, GAD2, PPARG, FTO, GIPR, CAPN10, BDNF, ETV5, FAIM2,GNPDA2, KTCD15, LYPLAL1, MC4R, MSRA, MTCH2, NEGR1, SEC16B, SH2B1, TFAP2B, TMEM18, ARAP1, AP3S2, C2CD4A/B, GLIS3, GRB14, PEPD, FITM2-R3HDML-HNF4A, HMG20A, HNF4A, KCNK16, MAEA, GCC1-PAX4, PSMD6, SLC30A8, ST6GAL1, TCF7L2, VPS26A,* and *ZFAND3*. Most of these loci are related to pathways (e.g., mTOR, insulin and IGF signaling and PI3K/AKT) that have been implicated in the development ofdiabetes, obesity, PCOS, and/or endometrial cancer [37]. So far, we have genotyped 25 SNPs in 12 susceptible loci in over 300 PCOS and control patients (Fig.4). In this ongoing study we have investigated the relationship between these targeted variations and BMI, BAI, SBP, DBP, waist circumference, hip circumference, waist/hip ratio, F-M score, levels of free-testosterone, DHEA-S, ASD, TSH, prolactin, LH, FSH, estradiol, testosterone, C-peptide, GIP, glucose AUC, insulin AUC, insulin Ab, Apo-A1, Apo-B, HDL-C, VLDL-C, LDL-C, T-CHOL/HDL-C, LDL-C/HDL-C, T-cholesterol, triglyceride, non-HDL-C, SHBG, adiponectin, and irisin as well as OGTT sugar (O, 1, 2, and 3 hr), OGTT insulin (0, 1,
2, and 3 hr), HOMA-IR, beta-cell function, QUICK, ISI Matsuda, ISI 0,120, and insulenic index.

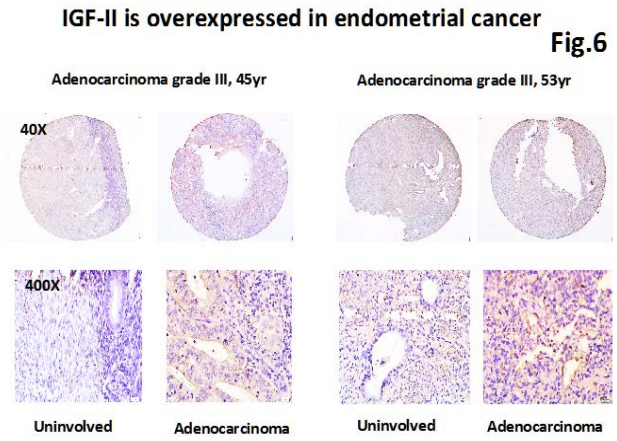
In addition, allele frequencies, Mendelian errors, sex-assignment discrepancies, and Hardy- Weinberg Equilibrium were analyzed to root out sample switches, duplications, or contamination. These studies revealed that 11 of the 12 candidate loci have significant associations with a variety of anthropogenic or clinical phenotypes of PCOS patients and (2) 23 of the 25 candidate SNPs have significant associations with at

Significant associations between risk variants in candidate genes and metabolic phenotypes in PCOS patients

CDKAL1	TSH, testosterone, insulenic index, adiponectin
CYB5R4	Ins 1hr, 2hr, HOMA-IR, QUICK, ASD, testosterone
GAD2	Ins 0hr, HOMA-IR, Insulenic index, LDL, LDL/HDL, T-CHO/HDL, Irisin
GIP	F-M score, LH
HHEX	Ins 1hr, C-peptide 1hr, LDL, T-CHO, Irisin
KCNQ1	Sugar 1hr
KLF14	Sugar 1hr, HDL, adiponectin
NRIP1	BMI, Ins 1hr, 2hr, Matsuda ISI, C-peptide 0hr, 1hr, estradiol, testosterone
PPARG	Irisin, testosterone
PRDM16	TSH, Ins 0hr, 2hr, T-CHO/HDL, SBP, DBP, estradiol, SHBG, adiponectin, QUICK, HOMA-IR
RBL1	Sugar 1hr, lipid levels

Fig.5

least one of the phenotypes ($p<0.01$) (Fig. 5). For example, the diabetes-associated CDKAL1 rs7754840 and rs10806920 variants are associated with testosterone level and insulenic index in PCOS patients. The identification of these novel genotype-phenotype relationships not only provides a better understanding of the etiology of PCOS but also allows future prediction of the risk of PCOS among women. Importantly, these studies also indicate that these PCOS-related SNPs may play a role in the development of endometrial cancer in PCOS patients.



***IGF-II and insulin receptor isoform alpha (IR-A) are overexpressed in* endometrial cancer**

To characterize the role of insulin signaling in endometrial cancer, we have analyzed the expression of IGFs and insulin receptors in a variety of human endometrial cancer tissues (Biochain Company). Importantly, we have found that immuno-reactive IGF-II is abundantly expressed in endometrium gland and stromal cell of endometrial adenocarcinoma tissues when compared to normal endometrial tissues

(Fig 6). Likewise, the expression of immuno-reactive IR-A in endometrioid cancers is more widespread and intense than that of normal endometrial tissues. These data clearly suggested that there is an aberrant upregulation of insulin and IGF signaling in a wide spectrum of endometrial tumor tissues, and hyperinsulinemia could stimulate cell proliferation and enhance oncogenesis in PCOS patients.

The serum levels of adipose tissue-derived irisin, but not asprosin, are significantly increased in PCOS patients

To identify potential biomarkers of PCOS patients, we have also analyzed the level of a novel white adipose tissue-derived hepatic glucose release hormone, asprosin, in a large cohort of PCOS patients [71, 72]. Recent studies have shown that asprosin is a glucogenic factor, and its level is elevated in humans and animals with insulin resistance. Accordingly, we hypothesized that asprosin may play a role in the manifestation of PCOS. As expected, irisin,

Table 1. Metabolic profiles and the irisin level of PCOS patients

	PCOS (n=444)	Normal(n=156)	P value
	Mean ± SD	Mean ± SD	
Age	25±4.67	27.42±4.61	P<0.05
F-M score	8.34±4.59	3.43±1.85	P<0.05
free-testo (pg/ml)	1.83±0.79	1.21±0.41	P<0.05
LH(mIU/L)	6.98±4.83	5.03±3.39	P<0.05
FSH (mIU/mL)	5.68±1.87	6.41±1.58	P<0.05
Estradiol (pg/mL)	44.2±50.15	37.52±15.06	N.S.
Testosterone (ng/mL)	0.56±0.2	0.39±0.14	P<0.05
Sugar-0hr	83.88±13.95	84.15±13.92	N.S.
Sugar-1hr	132.38±42.49	118.24±43.47	P<0.05
Sugar-2hr	111.89±34.65	103.73±35.52	P<0.05
Sugar-3hr	86.86±29.62	81.64±27.32	N.S.
Insulin-0Hr	11.22±8.47	6.51±3.38	P<0.05
Insulin-1Hr	92.66±81.46	53.76±38.41	P<0.05
Insulin-2Hr	81.38±76.47	43.38±32.33	P<0.05
Insulin-3Hr	39.21±60.28	17.91±17.32	P<0.05
HOMA-IR	2.43±2.39	1.4±0.97	P<0.05
QUICKI	0.35±0.04	0.38±0.04	P<0.05
ISImatuda	7.29±5.77	10.71±5.84	P<0.05
HDL-C (mg/dL)	54.17±13.57	56.59±12.27	N.S.
VLDL-C (mg/dL)	19.31±11.42	15.48±13.14	P<0.05
LDL-C (mg/dL)	103.44±28.51	96.33±22.68	P<0.05
T-Cholesterol	177.25±31.1	168.28±28.54	P<0.05
Triglyceride	97.77±63.51	77.43±70.1	P<0.05
Irisin (ng/ml)	78.99±38.79	69.96±34.77	P<0.05
Asprosin (ng/ml)	65.72±110.43	50.01±93.36	N.S.

the F-M score, LH, FSH, testosterone, fasting sugar, fasting insulin and select lipids are characteristically elevated in PCOS patients (Table 1). However, the asprosin level in PCOS and normal patients are similar, suggesting that irisin is a unique PCOS biomarker whereas asprosin does not have a role in the development of PCOS.

Research Design and Methods

To identify endometrial cancer-risk gene mutations that specifically occur in endometrial cancer tissues of PCOS patients, and the genotype-phenotype relationships

Hypothesis: *The development of endometrial cancers in PCOS patients is associated with specific mutations in endometrial cancer-risk genes, and PCOS-related SNPs.*

Rationale

Because many PCOS patients who develop endometrial cancer are at their reproductive age before the family planning is completed, they suffer not only aggressive cancer progression but also the loss of ability to carry out a pregnancy in the future due to hysterectomy. Therefore, novel methods that can forecast the risk of endometrial cancer in reproductive-aged women would not only allow these patients to be properly treated but also enhance the prospectus of carrying out a pregnancy in the future by sparing unnecessary hysterectomy. The major goal of this Aim is to identify (1) endometrial cancer-risk gene mutations that are prone to occur specifically in endometrial dysplasia/cancers of PCOS patients, and (2) the relationships between key mutations and the development of endometrial hyperplasia/cancer in PCOS patients.

Because mutations in key cancer-risk genes are essential steps in the process of carcinogenesis of most cancers, and that cancer progression is in parallel with the accumulation of cancer type-specific mutations (i.e., mutational load) in risk genes, it is important to differentiate endometrial cancer driver mutations and random mutations that accumulated during the process of carcinogenesis.

To achieve this goal, we will perform three distinct sets of studies in order to reveal: (1) the repertoire of mutations in endometrial cancer-risk genes that frequently occur in endometrial cancers of PCOS patients, (2) relationships between the newly identified PCOS-associated

Candidate biomarkers for the prediction of endometrium cancer development in women with PCOS

Tabel 2.			Genetic Biomarkers		Serum Biomarkers
Published endometrial cancer risk genes; PTEN-PIK3-mTOR, Ras-MAPK-ERK, beta-catenin, and other pathways			Genes implicated in endometrial cancer cell proliferation	PCOS-associated SNPs and Genes	Published PCOS endocrine biomarkers
AKT1	HSPA2	PPP2R1A	IGF1	CDKAL1	androgens
AKTIP	INHBA	PR	IGF2	CYB5R4	triglycerides
AMPK	KMO	PTEN	IGFBP1	GAD2	GIP
APC	KRAS	RAF	IGFBP3	GIP	irisin
ARID1A	MAPK	RASSF1A	IRS2	HHEX	insulin
BRAF	MLH1	RNF43	PPAR	KCNQ1	AMH
CDH13	MLH2	RPS6KC1	SNAI1	KLF14	
CTNNB1	MLH6	SOCS2	TGFB1	NRIP1	
EGFR	MLL2	SOX17	TGFBR1	PPARG	
ER	MTOR	TP53	TWIST1	PRDM16	
ERBB3	NRAS	TRPS1	VEGF	RBL1	
FBXW7	P16	TTLL5			
FGFR2	PHKA2	WNT11			
GRM8	PI3K				
HER2	PIK3CA				

polymorphisms and endometrial cancer , and (3) relationships between serum PCOS biomarkers and endometrial cancer . By combining these three sets of information, we would be able to calculate the life-time risk of endometrial cancer in PCOS patients, and generate a prediction model that comes with high sensitivity and specificity. In Aim 1a, we will test the hypothesis using formalin-fixed paraffin embedded (FFPE) endometrial dysplasia/cancer samples that have been collected and curated in Tissue Banks, and focus on published endometrial cancer-risk genes and genes that have been implicated in the proliferation of endometrial cells (Table 2; e.g., PPAR, VEGF, TGFB1, TGFBR1, SNAI1, IR-A, IGF-II and IGF-II receptor). The Tissue Bank at CGMH contains more than 5000 endometrium biospecimens from patients with a variety of endometrial pathology.

Because carcinogenesis could involve a wide spectrum of mutations in risk genes, we will identify both known mutations in risk genes using qPCR and sporadic *de novo* mutations in target genes using NGS in order to obtain a comprehensive inventory of potential endometrial cancer-related mutations. By sorting the relationships between mutations and disease progression, we will identify the most critical mutations that predispose the development of endometrial cancer in PCOS patients. Furthermore, we will investigate the role of PCOS-associated variants in endometrial cancer based on genotyping of a selected group of PCOS-associated SNPs (Figs, 4 and 5). All genotype-phenotype relationships that are identified using FFPE samples in Aim 1a will be then verified using freshly collected endometrial hyperplasia/cancer tissues from PCOS and control patients.

Material and Method

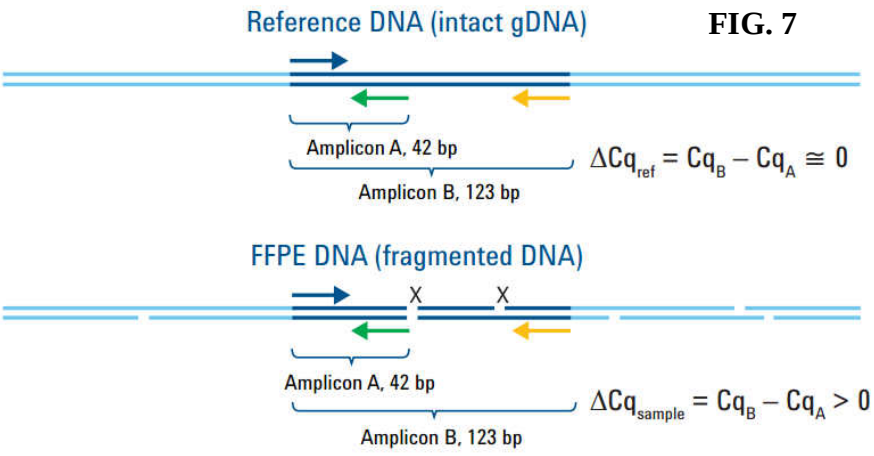
FFPE 切片樣本的篩選與 DNA extraction

我們將從長庚紀念醫院 (CGMH) 病理科的組織銀行中獲取病患的 FFPE 子宮內膜和其他婦科癌症樣本。 FFPE 樣本於時間 2006 年至 2017 年期間收取自醫院病患，包括來自患有或不患有 PCOS、子宮內膜增生、子宮內膜息肉、子宮內膜發育不良和子宮內膜癌的患者的子宮組織。每種組織類型預計篩選樣本 500 個做為實驗樣本。為了從 FFPE 組織中有效回收高質量基因組 DNA (gDNA)，使用 QIAamp® DNA FFPE Tissue Kit (Qiagen) 萃取 FFPE 樣本中的 gDNA。

FFPE 樣本 QC 與建庫試劑的選擇

針對 house-keeping gene 設計兩段 PCR product，藉由與 Control DNA 在 qPCR Ct 的差值推估 FFPE DNA 的完整度，並估計樣本中 amplifiable DNA 量。

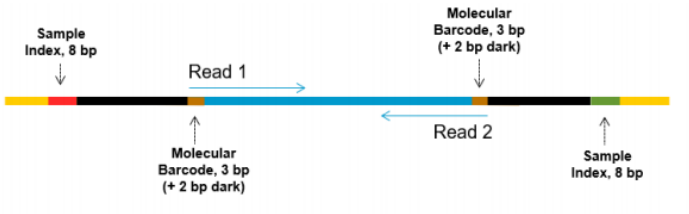
FIG. 7



不同品質的 FFPE 樣本需要最佳化其建庫與定序條件，QC 結果與建庫條件互相配合，使用 Agilent NGS FFPE QC Kit 將 FFPE gDNA 樣本進行定性與定量後，分類後，個別調整建庫條件至最佳化 (包含 DNA input amount、Fragmentation time、PCR cycle 等)，如果樣本數充足，可在此階段剔除品質較不佳之 FFPE 樣本。

不同品質的 FFPE 樣本需要最佳化其建庫與定序條件，SureSelect XT HS2 DNA System 是經過 FFPE-optimized 的建庫試劑，只需 10 ng 以上 DNA 即可建庫，包含有雙端分子標籤 (Duplex Molecular Barcodes)，可以有效地去除因 PCR 或是定序儀所產生的 duplicate reads、並檢測辨識與排除 PCR 或定序而產生的錯誤 (random error)，library 兩端都接上 unique index，達成雙鑑別獨特配對 (UDI)，能有效消弭 Illumina 在高通量定序系統的樣品標籤誤配現象 (index hopping) (FIG. 8)。以上特點對於癌症樣本的低頻率變異(<1% VAF)檢出有極大的幫助。

FIG. 8 SureSelect XT HS2 sequencing library 結構



Content of SureSelect XT HS2 sequencing library. Each fragment contains one target insert (blue) surrounded by the Illumina paired-end sequencing elements (black), unique dual sample indexes (red and green), duplex molecular barcodes (brown) and the library PCR primers (yellow).

Result

FFPE 樣本 QC 結果與後續進行定序量的預估

為了創建一個以結合多囊性卵巢症後群，早發性子宮內膜癌常見突變基因和內分泌生物標誌的預測平台，來早期估算有多囊性卵巢症後群之婦女未來可能罹患子宮內膜癌的風險機率，我們將患者的 FFPE 樣本利用次世代定序來鑑定子宮內膜癌中的癌症中我們感興趣的 57 個突變基因位點。本試驗共取得 124 支 FFPE gDNA 樣本，其中包含子宮內膜息肉、子宮內膜增生與子宮內膜三組 FFPE gDNA 作為實驗樣本，其中有 5 支樣本量不足，執行了 119 支樣本 QC。據 QC 結果將 119 支樣本依照樣本品質、總量、濃度來做分類 (Table.3)。ΔΔCq 值越高代表 gDNA 的破碎度越高或是 DNA crosslinked 程度越高，根據操作手冊當 ΔΔ Cq>2，定序則所需的定序量 (sequencing throughput) 相較 Class 1 的樣本需要增加 5~10 倍或以上，每個樣品的最低定序量為 39.923 Mbp (約 200 倍 Mean coverage)，所以 Table.3 中 Class 4、Class 5 與 Class 6 類樣本的定序量，估計其定序量至少 400 Mbp (約 2000 倍 Mean coverage) 以上，才足夠後續分析需求。

Class	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6
Sample No.	**55**	**19**	**11**	**18**	**4**	**12**
Quality (ΔΔCq<2)	O	O	O	X	X	X
DNA Amount (>400ng)	O	O	X	O	O	X
Concentration(>28.5/μl)	O	X	X	O	X	X

Table.3 119支樣本依照樣本品質、總量、濃度的分類結果

Pilot study 的結果

為了最佳化次世代定序所得到的結果，我們在 Pilot study 挑選 15 支 class 1 的 FFPE 樣本與 1 支 NA12878 control cell line gDNA 以 SureSelect DNA Design 進行客製化 panel 設計並搭配 SureSelect XT HS2 DNA System 進行建庫，定序儀為 NovaSeq 6000，總定序量約 20Gb，每個樣本平均定序量為 1.25Gb (換算 raw mean coverage 為 6200 倍)；去除 duplicated reads 後，平均 unique reads 佔 total reads 70%，Mean target coverage 可達 1724 倍。16 支樣本 Aligned base%平均值為 99.5%，表示 reads 能有效比對回 GRCh38 參考序列，代表 customized probe 的設計時依據正確的參考序列並有良好的設計。% On target 的平均值為 80.9%，即抓取的 reads~80%皆落在指定的設計區間，此數值與其他品牌試劑效果相當。Target base at 0X (unique reads)為 0%，顯示所有設計區域皆可抓取到對應之 DNA 並定序。Target base at 30X (unique reads) 為 99.9%，顯示 99.9%區域可進行有效的 Germline mutation 分析。Target base at 100X 為 99.7%、Target base at 500X 為 95.0%，顯示 95.0%以上區域可有效地進行~5%低頻率變異分析。

Pilot study 的結果-FFPE DNA 樣本與 Control DNA 樣本的比較

比較 Class 1 的 FFPE 樣本與 NA12878 的各項 performance。On-Target%的項目 FFPE 樣本平均值為 80.8%、NA12878 為 83.4%；在 Target base at 250X (unique reads)以上時，有 1%的差距，500 倍以上開始有 5%以上的差異，以上顯示 FFPE 樣本因 DNA 完整度較差導致某些易感區域的覆蓋度較低。後續將藉由設計與實驗的優化加以改善 (Table.4)。

Analysis	Average of 15 FFPEs	NA12878
On-Target	80.8%	83.4%
PCT_TARGET_BASES_1X	100.0%	100.0%
PCT_TARGET_BASES_2X	100.0%	100.0%
PCT_TARGET_BASES_10X	100.0%	100.0%
PCT_TARGET_BASES_20X	99.9%	100.0%
PCT_TARGET_BASES_30X	99.9%	99.9%
PCT_TARGET_BASES_40X	99.9%	99.9%
PCT_TARGET_BASES_50X	99.8%	99.9%
PCT_TARGET_BASES_100X	99.7%	99.8%
PCT_TARGET_BASES_250X	98.8%	99.7%
PCT_TARGET_BASES_500X	94.7%	99.0%
PCT_TARGET_BASES_1000X	75.0%	95.5%
PCT_TARGET_BASES_2500X	16.2%	48.8%

Table.4 FFPE DNA 樣本與 Control DNA 樣本比較表

Discussions

後續將根據 Pilot study 進行設計與實驗的優化，本次 Pilot study 中 16 支樣本 Fold 80 base penalty 平均值為 1.89，其定義為 The fold over-coverage necessary to raise 80% of bases in "non-zero-cvg" targets to the mean coverage level in those targets. 顯示不同設計區域其抓取的效率有一定程度的差異，capture probe 的設計必須要考量到 probe 長度、二級結構、overlapping%、GC%與 hybridization 條件(stringency)的配合。於是，在總數 779 個設計區間中，覆蓋度最低的 8 個區間，其%GC 為 28.9，整體%在 20%~80%間，大部分落在 35~55%間，我們分析了每個設計區間的覆蓋度與 GC%，發現有一定的相關性，，若將%GC 與 Median Mean Depth of 16 samples 繪製散佈圖 (Fig.9)，可見其呈現一定相關性，於是我們針對下一階段的 customized probe design 進行優化，將覆蓋度依照低至高進行排序後，將低覆蓋度區域的探針數目，相較前一版本依據需求增加 1~10 倍。實驗流程中 capture probe 的 hybridization 溫度由預設的 65°C 調整為 62.5°C，並延長總時間一小時。**以上是本研究截至目前的實驗進程，後續次世代定序的分析結果出來後將進行報告內容更新。**

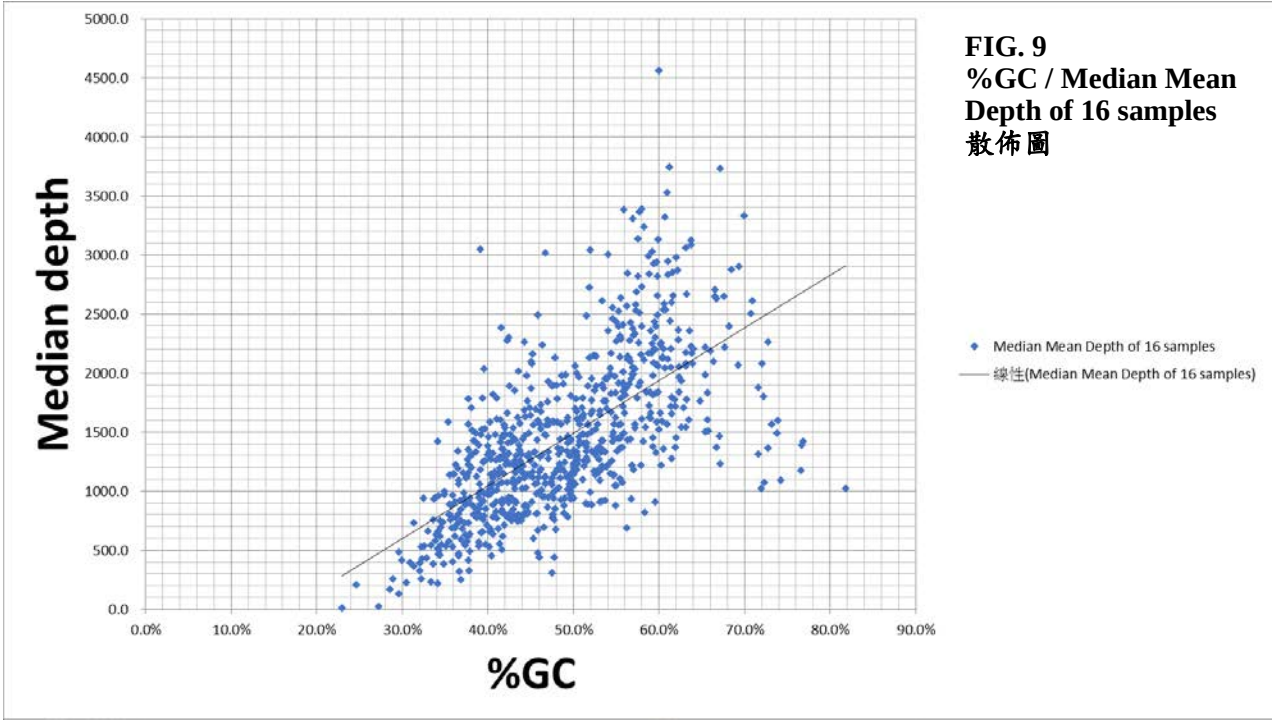


FIG. 9
%GC / Median Mean Depth of 16 samples 散佈圖

References.

1. Ferlay, J., et al., *Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008.* Int J Cancer, 2010. 127(12): p. 2893-917.
2. Amant, F., et al., *Endometrial cancer.* Lancet, 2005. 366(9484): p. 491-505.
3. Llaurado, M., et al., *Molecular bases of endometrial cancer: new roles for new actors in the diagnosis and the therapy of the disease.* Mol Cell Endocrinol, 2012. 358(2): p. 244-55.
4. Murali, R., R.A. Soslow, and B. Weigelt, *Classification of endometrial carcinoma: more than two types.* Lancet Oncol, 2014. 15(7): p. e268-78.
5. Aune, D., et al., *Anthropometric factors and endometrial cancer risk: a systematic review and dose-response meta-analysis of prospective studies.* Ann Oncol, 2015. 26(8): p. 1635-48.
6. Burzawa, J.K., et al., *Prospective evaluation of insulin resistance among endometrial cancer patients.* Am J Obstet Gynecol, 2011. 204(4): p. 355 e1-7.
7. Haoula, Z., M. Salman, and W. Atiomo, *Evaluating the association between endometrial cancer and polycystic ovary syndrome.* Hum Reprod, 2012.
8. Oh, J.C., et al., *Increased plasma levels of insulin-like growth factor 2 and insulin-like growth factor binding protein 3 are associated with endometrial cancer risk.* Cancer Epidemiol Biomarkers Prev, 2004. 13(5): p. 748-52.
9. Shafiee, M.N., et al., *Reviewing the molecular mechanisms which increase endometrial cancer (EC) risk in women with polycystic ovarian syndrome (PCOS): time for paradigm shift?* Gynecol Oncol, 2013. 131(2): p. 489-92.
10. Schouten, L.J., R.A. Goldbohm, and P.A. van den Brandt, *Anthropometry, physical activity, and endometrial cancer risk: results from the Netherlands Cohort Study.* J Natl Cancer Inst, 2004. 96(21): p. 1635-8.
11. Azziz, R., et al., *Polycystic ovary syndrome.* Nat Rev Dis Primers, 2016. 2: p. 16057.
12. Goodarzi, M.O., et al., *Polycystic ovary syndrome: etiology, pathogenesis and diagnosis.* Nat Rev Endocrinol, 2011. 7(4): p. 219-31.
13. March, W.A., et al., *The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria.* Hum Reprod, 2010. 25(2): p. 544-51.
14. Diamanti-Kandarakis, E., et al., *A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile.* J Clin Endocrinol Metab, 1999. 84(11): p. 4006-11.
15. Fauser, B.C., et al., *Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group.* Fertil Steril, 2012. 97(1): p. 28-38 e25.
16. Genuth, S., et al., *Follow-up report on the diagnosis of diabetes mellitus.* Diabetes Care, 2003. 26(11): p. 3160-7.
17. Diamanti-Kandarakis, E. and A. Dunaif, *Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications.* Endocr Rev, 2012. 33(6): p. 981-1030.

18. Legro, R.S., et al., *Diagnosis and treatment of polycystic ovary syndrome: an endocrine society clinical practice guideline.* J Clin Endocrinol Metab, 2013. 98(12): p. 4565-92.
19. Jayasena, C.N. and S. Franks, *The management of patients with polycystic ovary syndrome.* Nat Rev Endocrinol, 2014. 10(10): p. 624-36.
20. Apridonidze, T., et al., *Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome.* J Clin Endocrinol Metab, 2005. 90(4): p. 1929-35.
21. Shaw, L.J., et al., *Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health--National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation.* J Clin Endocrinol Metab, 2008. 93(4): p. 1276-84.
22. Legro, R.S., et al., *Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study.* J Clin Endocrinol Metab, 2005. 90(6): p. 3236-42.
23. Oza, A.M., et al., *Phase II study of temsirolimus in women with recurrent or metastatic endometrial cancer: a trial of the NCIC Clinical Trials Group.* J Clin Oncol, 2011. 29(24): p. 3278-85.
24. Barry, J.A., M.M. Azizia, and P.J. Hardiman, *Risk of endometrial, ovarian and breast cancer in women with polycystic ovary syndrome: a systematic review and meta-analysis.* Hum Reprod Update, 2014. 20(5): p. 748-58.
25. Fearnley, E.J., et al., *Polycystic ovary syndrome increases the risk of endometrial cancer in women aged less than 50 years: an Australian case-control study.* Cancer Causes Control, 2010. 21(12): p. 2303-8.
26. Li, X. and R. Shao, *PCOS and obesity: insulin resistance might be a common etiology for the development of type I endometrial carcinoma.* Am J Cancer Res, 2014. 4(1): p. 73-9.
27. Calle, E.E., et al., *Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults.* N Engl J Med, 2003. 348(17): p. 1625-38.
28. Renehan, A.G., et al., *Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies.* Lancet, 2008. 371(9612): p. 569-78.
29. Arcidiacono, B., et al., *Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms.* Exp Diabetes Res, 2012. 2012: p. 789174.
30. Inoue, M. and S. Tsugane, *Insulin resistance and cancer: epidemiological evidence.* Endocr Relat Cancer, 2012. 19(5): p. F1-8.
31. Sciacca, L., et al., *In IGF-I receptor-deficient leiomyosarcoma cells autocrine IGF-II induces cell invasion and protection from apoptosis via the insulin receptor isoform A.* Oncogene, 2002. 21(54): p. 8240-50.
32. Wang, C.F., et al., *Effects of insulin, insulin-like growth factor-I and -II on proliferation and intracellular signaling in endometrial carcinoma cells with different expression levels of insulin receptor isoform A.* Chin Med J (Engl), 2013. 126(8): p. 1560-6.
33. Avnet, S., et al., *Insulin receptor isoform A and insulin-like growth factor II as additional*

treatment targets in human osteosarcoma. Cancer Res, 2009. 69(6): p. 2443-52.
34. Bishop, E.A., et al., *Insulin exerts direct effects on carcinogenic transformation of human endometrial organotypic cultures.* Cancer Invest, 2014. 32(3): p. 63-70.
35. Arcaro, A., et al., *Novel role for insulin as an autocrine growth factor for malignant brain tumour cells.* Biochem J, 2007. 406(1): p. 57-66.
36. Chettouh, H., et al., *Mitogenic insulin receptor-A is overexpressed in human hepatocellular carcinoma due to EGFR-mediated dysregulation of RNA splicing factors.* Cancer Res, 2013. 73(13): p. 3974-86.
37. Shao, R., et al., *Direct effects of metformin in the endometrium: a hypothetical mechanism for the treatment of women with PCOS and endometrial carcinoma.* J Exp Clin Cancer Res, 2014. 33: p. 41.
38. Rutanen, E.M., *Insulin-like growth factors in endometrial function.* Gynecol Endocrinol, 1998. 12(6): p. 399-406.
39. Kleinman, D., et al., *Regulation of endometrial cancer cell growth by insulin-like growth factors and the luteinizing hormone-releasing hormone antagonist SB-75.* Regul Pept, 1993. 48(1-2): p. 91-8.
40. Talavera, F., et al., *Insulin-like growth factor I receptors in normal and neoplastic human endometrium.* Cancer Res, 1990. 50(10): p. 3019-24.
41. Roy, R.N., et al., *Discordant expression of insulin-like growth factors and their receptor messenger ribonucleic acids in endometrial carcinomas relative to normal endometrium.* Mol Cell Endocrinol, 1999. 153(1-2): p. 19-27.
42. Giudice, L.C., B.A. Dsupin, and J.C. Irwin, *Steroid and peptide regulation of insulin-like growth factor-binding proteins secreted by human endometrial stromal cells is dependent on stromal differentiation.* J Clin Endocrinol Metab, 1992. 75(5): p. 1235-41.
43. Lathi, R.B., et al., *Dose-dependent insulin regulation of insulin-like growth factor binding protein-1 in human endometrial stromal cells is mediated by distinct signaling pathways.* J Clin Endocrinol Metab, 2005. 90(3): p. 1599-606.
44. Rutanen, E.M., et al., *Suppressed expression of insulin-like growth factor binding protein-1 mRNA in the endometrium: a molecular mechanism associating endometrial cancer with its risk factors.* Int J Cancer, 1994. 59(3): p. 307-12.
45. Cayan, F., et al., *Insulin receptor substrate-2 gene polymorphism: is it associated with endometrial cancer?* Gynecol Endocrinol, 2010. 26(5): p. 378-82.
46. McGrath, M., et al., *Common genetic variation within IGFI, IGFII, IGFBP-1, and IGFBP-3 and endometrial cancer risk.* Gynecol Oncol, 2011. 120(2): p. 174-8.
47. Chang, C.L., et al., *Circulating irisin and glucose-dependent insulinotropic peptide are associated with the development of polycystic ovary syndrome.* J Clin Endocrinol Metab, 2014. 99(12): p. E2539-48.
48. Galazis, N., et al., *Proteomic biomarkers of endometrial cancer risk in women with polycystic*

ovary syndrome: a systematic review and biomarker database integration. Gynecol Endocrinol, 2013. 29(7): p. 638-44.

49. Ouyang, H., et al., *The insulin-like growth factor II receptor gene is mutated in genetically unstable cancers of the endometrium, stomach, and colorectum.* Cancer Res, 1997. 57(10): p. 1851-4.
50. Wang, C.F., et al., *Overexpression of the insulin receptor isoform A promotes endometrial carcinoma cell growth.* PLoS One, 2013. 8(8): p. e69001.
51. Frasca, F., et al., *Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells.* Mol Cell Biol, 1999. 19(5): p. 3278-88.
52. Creasman, W.T., et al., *Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer.* Int J Gynaecol Obstet, 2006. 95 Suppl 1: p. S105-43.
53. Berends, M.J., et al., *Toward new strategies to select young endometrial cancer patients for mismatch repair gene mutation analysis.* J Clin Oncol, 2003. 21(23): p. 4364-70.
54. Dedes, K.J., et al., *Emerging therapeutic targets in endometrial cancer.* Nat Rev Clin Oncol, 2011. 8(5): p. 261-71.
55. Kandoth, C., et al., *Integrated genomic characterization of endometrial carcinoma.* Nature, 2013. 497(7447): p. 67-73.
56. Liang, H., et al., *Whole-exome sequencing combined with functional genomics reveals novel candidate driver cancer genes in endometrial cancer.* Genome Res, 2012. 22(11): p. 2120-9.
57. Bostrom, P., et al., *A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis.* Nature, 2012. 481(7382): p. 463-8.
58. Moreno-Navarrete, J.M., et al., *Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance.* J Clin Endocrinol Metab, 2013. 98(4): p. E769-78.
59. Pedersen, B.K. and M.A. Febbraio, *Muscles, exercise and obesity: skeletal muscle as a secretory organ.* Nat Rev Endocrinol, 2012. 8(8): p. 457-65.
60. Pardo, M., et al., *Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index.* Int J Endocrinol, 2014. 2014: p. 857270.
61. Liu, J.J., et al., *Lower circulating irisin is associated with type 2 diabetes mellitus.* J Diabetes Complications, 2013.
62. Choi, Y.K., et al., *Serum irisin levels in new-onset type 2 diabetes.* Diabetes Res Clin Pract, 2013.
63. Hee Park, K., et al., *Circulating irisin in relation to insulin resistance and the metabolic syndrome.* J Clin Endocrinol Metab, 2013. 98(12): p. 4899-907.
64. Bostrom, P.A. and J.M. Fernandez-Real, *Metabolism: Irisin, the metabolic syndrome and follistatin in humans.* Nat Rev Endocrinol, 2014. 10(1): p. 11-2.
65. Kuzmicki, M., et al., *Serum irisin concentration in women with gestational diabetes.* Gynecol

Endocrinol, 2014: p. 1-4.
66. Yuksel, M.A., et al., *Maternal serum and fetal cord blood irisin levels in gestational diabetes mellitus.* Diabetes Res Clin Pract, 2014. 104(1): p. 171-5.
67. Adamska, A., et al., *Serum irisin and its regulation by hyperinsulinemia in women with polycystic ovary syndrome.* Endocr J, 2016. 63(12): p. 1107-1112.
68. Li, H., et al., *Free androgen index and Irisin in polycystic ovary syndrome.* J Endocrinol Invest, 2016. 39(5): p. 549-56.
69. Pukajlo, K., et al., *Irisin plasma concentration in PCOS and healthy subjects is related to body fat content and android fat distribution.* Gynecol Endocrinol, 2015. 31(11): p. 907-11.
70. Li, M., et al., *Elevated circulating levels of irisin and the effect of metformin treatment in women with polycystic ovary syndrome.* J Clin Endocrinol Metab, 2015. 100(4): p. 1485-93.
71. Greenhill, C., *Liver: Asprosin - new hormone involved in hepatic glucose release.* Nat Rev Endocrinol, 2016. 12(6): p. 312.
72. Romere, C., et al., *Asprosin, a Fasting-Induced Glucogenic Protein Hormone.* Cell, 2016. 165(3): p. 566-79.
73. Berg, A., et al., *Molecular profiling of endometrial carcinoma precursor, primary and metastatic lesions suggests different targets for treatment in obese compared to non-obese patients.* Oncotarget, 2015. 6(2): p. 1327-39.
74. Bakkum-Gamez, J.N., *Repurposing the vaginal tampon for endometrial cancer detection.* Biomark Med, 2015. 9(8): p. 715-7.
75. Bakkum-Gamez, J.N., et al., *Detection of endometrial cancer via molecular analysis of DNA collected with vaginal tampons.* Gynecol Oncol, 2015. 137(1): p. 14-22.
76. Shehin, S.E., et al., *Clinical safety-in-use study of a new tampon design.* Infect Dis Obstet Gynecol, 2003. 11(2): p. 89-99.
77. Erickson, B.K., et al., *Detection of somatic TP53 mutations in tampons of patients with high-grade serous ovarian cancer.* Obstet Gynecol, 2014. 124(5): p. 881-5.
78. Fiegl, H., et al., *Methylated DNA collected by tampons--a new tool to detect endometrial cancer.* Cancer Epidemiol Biomarkers Prev, 2004. 13(5): p. 882-8.
79. Khan, A.M., P. Sasieni, and A. Singer, *A prospective double-blind cross-sectional study of the accuracy of the use of dry vaginal tampons for self-sampling of human papillomaviruses.* BJOG, 2014. 122(3): p. 388-94.
80. Colilla, S., N.J. Cox, and D.A. Ehrmann, *Heritability of insulin secretion and insulin action in women with polycystic ovary syndrome and their first degree relatives.* J Clin Endocrinol Metab, 2001. 86(5): p. 2027-31.
81. Fan, H.C., et al., *Non-invasive prenatal measurement of the fetal genome.* Nature, 2012. 487(7407): p. 320-4.
82. Ge, H., et al., *Noninvasive prenatal detection for pathogenic CNVs: the application in alpha-thalassemia.* PLoS ONE, 2013. 8(6): p. e67464.

83. Liao, G.J., et al., *Noninvasive prenatal diagnosis of fetal trisomy 21 by allelic ratio analysis using targeted massively parallel sequencing of maternal plasma DNA.* PLoS ONE, 2012. 7(5): p. e38154.
84. Ueda, S.M., et al., *Trends in demographic and clinical characteristics in women diagnosed with corpus cancer and their potential impact on the increasing number of deaths.* Am J Obstet Gynecol, 2008. 198(2): p. 218 e1-6.

108年度專題研究計畫成果彙整表

計畫主持人：張嘉琳	**計畫編號：**108-2629-B-182A-004-
計畫名稱：建構一個以生物標記為基礎的預測模式來早期推算多囊性卵巢症候群婦女罹患子宮內膜癌的機率(L03)	

成果項目			量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
國外	學術性論文	期刊論文	0	篇	
		研討會論文	0		
		專書	0	本	
		專書論文	0	章	
		技術報告	0	篇	
		其他	0	篇	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	1		碩士級專任助理-蔡孟樺
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士級研究人員	0		
		專任人員	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					