EXPERT REVIEWS

Genetic polymorphisms and endometrial cancer risk

Expert Rev. Anticancer Ther. 8(7), 1159–1167 (2008)

Larissa A Meyer,
Shannon N Westin,
Karen H Lu,
Michael R Milam†

†*Author for correspondence*
Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center,1155 Herman P. Pressler St, CPB 6.3244, Unit 1362, Houston, TX 77030, USA
Tel.: +1 713 563 4563
Fax: +1 713 792 7586
mrmilam@mdanderson.org

For most sporadic cancers, genetic susceptibility results from the additive effect of multiple genetic variants, each of which contributes a modest risk individually. The study of genetic single nucleotide polymorphisms (SNPs) may help explain the differences in individual cancer susceptibility and may assist in identifying novel markers of risk that can be utilized to create more effective and tailored cancer prevention strategies. Genetic polymorphisms in functionally critical genes have been suggested as risk factors for the development of a variety of cancers, including endometrial cancer. Candidate SNPs may be involved in DNA damage repair, steroid metabolism, carcinogen metabolism, cell-cycle control, apoptosis and steroid receptor activation pathways. In this review, recent findings of genetic association studies exploring genetic polymorphisms and their association with endometrial cancer are reported. In addition, the challenges of genetic association studies, such as power and bias, and the need for validation of promising findings are explored.

KEYWORDS: endometrial cancer • polymorphism

Endometrial cancer is the most common gynecologic malignancy among women in the USA and many developed countries. In 2008, an estimated 40,100 women will be diagnosed with endometrial cancer, making this the fourth most common malignancy in females [1]. The clinical picture of endometrial cancer is heterogeneous and likely reflects the complex etiology of the disease. Endometrial cancer has traditionally been classified into three types: type I, type II and familial [2]. Type I cancers are usually low grade and associated with estrogen exposure and typically occur in younger, obese or perimenopausal women. Type II tumors, which encompass approximately 10% of cases, present in older women and are typically high-grade tumors and may have papillary serous, clear cell, or malignant mixed mullerian tumor histology. Familial disease is used to describe cases where a single germline DNA mutation is responsible for the great increase in individual cancer susceptibility. For endometrial cancer, the most common familial disease is Lynch Syndrome, also known as hereditary nonpolyposis colorectal cancer, which accounts for up to 5% of endometrial cancer cases [3].

While early identification of endometrial cancer can lead to an 80–90% cure rate, advanced and recurrent tumors are difficult to treat successfully. This has encouraged the search for identifiable risk factors for women prior to the development of a malignancy.

Risk factors for women with type II disease are not well understood. However, traditional risk factors for women with type I endometrial cancer have been understood for over 30 years and include prior exogenous estrogen exposure, obesity, nulliparity and diabetes [4–10]. Recent studies have explored the estrogen- and insulin-related pathways in more detail, suggesting that diet, insulin-associated factors (such as adiponectin) and physical activity may also play a role in the development of endometrial cancer [9,11–14]. Although obesity is a key factor in endometrial cancer risk, the overall incidence of endometrial cancer at this time does not appear to be increasing at the same rate as the rate of obesity in adults. Data from the National Health and Nutrition Survey have suggested that, although the prevalence of obesity has increased to 32.9% in 2003/2004 from 15.0% in 1976–1980 [101], the risk of a 50-year-old woman of any race developing uterine cancer is still less than 0.3% [102].

The reality is that not every individual with the described medical and lifestyle risk factors will go on to develop endometrial cancer, while others develop endometrial cancer in the absence of known risk factors. Clearly, further

 10.1586/14737140.8.7.1159 ISSN 1473-7140

studies are needed to risk stratify those women who are at high risk for cancer of the endometrium by traditional risk factors and to potentially identify new risk profiles to capture those who will develop endometrial cancer in the absence of traditional risk factors.

Cancer results from an interplay of the environment and the internal milieu of an individual, which includes one's genetic makeup. The sequencing of the human genome ushered in a new era of investigation that focused on the genetic determinants of complex diseases, including cancer [15,16]. With the exception of a few hereditary cancer syndromes where mutations in single genes account for inherited susceptibility, for most cancers, susceptibility results from the additive effect of multiple genetic variants that each individually contribute a modest risk [17]. Successful identification of genetic variants, such as single nucleotide polymorphisms (SNPs), that contribute to the development of endometrial cancer, may lead to the development of clinical tests to identify susceptible genetic profiles through a simple blood test or endometrial biopsy. The ultimate goal of such endeavors would be to tailor preventative strategies to individuals at risk. The potential benefit that arises from the identification of women at risk for endometrial cancer has been emphasized in recent literature. For example, significant risk reduction after prophylactic hysterectomy has been demonstrated in select women with Lynch Syndrome, a hereditary cancer susceptibility syndrome whereby women are at significantly increased lifetime risk of developing specific cancers, of which the most common are colon, endometrial and ovarian cancer [18–21].

Single nucleotide polymorphisms

A recent focus of research endeavors has been SNPs, aiming to explore the differences in individual cancer susceptibility. SNPs are the most common known form of human genetic variation and are defined as stable single-base substitutions with a frequency of greater than 1% in at least one population [22]. The specific population studied is important in genetic association studies and must be considered when the results of different studies on the same polymorphisms are being compared. Because the distribution of certain genetic polymorphisms can differ based on ethnic or racial background, attention to the SNP distribution of the control populations is paramount.

For cancer research, the focus has been on SNPs that alter the function or expression of a gene, as this enables researchers to attempt to explain observed associations with a pathogenic mechanism [22]. In fact, genetic polymorphisms in functionally critical genes have been suggested as risk factors for the development of a variety of cancers, including endometrial cancer [23]. Candidate SNPs may be involved in DNA damage repair, steroid metabolism, carcinogen metabolism, cell-cycle control, apoptosis and steroid receptor activation pathways [17, 24].

Risk stratification of endometrial cancer

Case-control studies

Family-based linkage studies are limited in their ability to determine the genetic contribution of polygenic diseases such as cancer. Genetic association studies, which are in essence, case-control studies, provide an efficient way to identify common genetic variants that may confer a modest disease risk [17]. In such studies, age- and race- or ethnicity-matched controls are evaluated for the prevalence of a particular genetic marker. Greater prevalence of a marker in affected individuals is considered evidence to support an association between the disease phenotype and the SNP of interest [25].

The measure of association calculated in genetic association studies is an odds ratio (OR). Although ORs cannot directly measure relative risk, when the probability of disease is low (<10%), the OR approximates the true relative risk [26]. Given that the probability of endometrial cancer is low in the general population, ORs are typically used in genetic association studies and will be reported in this paper as an estimate of relative risk of developing the disease of interest, endometrial cancer.

When evaluating the findings of genetic association studies, the importance of sample size and the need for validation and replication of the original findings cannot be underestimated. In general, genetic association studies are plagued by the challenge of achieving adequate power. While the sample size needed ultimately depends on the hypothesized strength of association, most of these studies are underpowered. This makes it difficult to ascertain whether a negative study is truly negative or just underpowered to detect a difference in association. Publication bias and time-lag bias also play a role as positive studies are more likely to be published and are generally published more quickly [27].

Interstudy heterogeneity is frequent in genetic association studies and the results of the first study often only modestly correlate with subsequent research on the same association [27]. The first study often reports a stronger genetic effect than validation studies. Ioannidis and colleagues suggest that both bias and genuine population diversity may explain why early association studies often display this upward bias in estimates of effects and believe that a systematic meta-analysis approach may help estimate population-wide effects of the genetic risk factors studied [27].

The use of genetic association studies to identify genetic polymorphisms is one scientific approach to investigate how genetic variance may contribute to endometrial cancer susceptibility and is the focus of this review. Early studies have utilized a candidate gene approach where the gene and SNPs selected for study are chosen based on biological plausibility. Given the known association between unopposed estrogen and endometrial cancer, studies evaluating polymorphisms of estrogen and progesterone receptor genes with endometrial cancer have been performed. Likewise, estrogen-metabolizing genes and sex-steroid receptor genes have been targets of interest. Polymorphisms in genes

involving cellular proliferation and differentiation, regulation of cell cycling and DNA-damage repair have also been investigated in relation to endometrial cancer risk.

Sex hormone receptors & metabolic genes

The endometrium is known to be affected by a variety of hormonal factors. It is estimated that 80% of endometrial cancers are estrogen related and arise via progression from precancerous hyperplasia [28,29]. Polymorphisms in estrogen receptor (ER)-α have been investigated for an association with the development of endometrial cancer. Estrogen interacts via specific ERs, ERα and ERβ, on the target cells in the endometrium [30]. Therefore, the ER is a logical target for endometrial cancer association studies.

In a population-based study from Sweden including 154 cases and 204 controls, restriction fragment polymorphisms for *Xba*I, *Pvu*II and an upstream TA repeat polymorphism were investigated [31]. The *Xba*I allele demonstrated a reduced risk of endometrial cancer with a multivariate OR = 0.52; 95% confidence interval (CI): 0.21–1.29, for the XX genotype compared with the xx genotype. The *Pvu*II PP genotype was associated with a nonsignificant decrease in risk for endometrial cancer with a multivariate OR: 0.70 (95% CI: 0.34–1.44) for the PP genotype as compared with the pp genotype. Women with two short TA (<19 repeats) alleles, although not significant, were found to have a higher risk of endometrial cancer than women with two long alleles (OR: 1.54; 95% CI: 0.73–3.27). A study of Japanese women had similar findings with the *Pvu*II PP genotype, demonstrating a decreased risk of endometrial cancer with a multivariate OR: 0.23 (95% CI: 0.07–0.82) and the *Xba*I XX genotype with an OR of 0.26 (95% CI: 0.09–0.79) [32].

The cytochrome P-450 17A1 gene (*CYP17A1*) is involved in the early steps of estrogen biosynthesis. The 34 T→C (A1→A2) variant in CYP17A1 has been reported to be associated with a decreased risk of endometrial cancer in several studies [33,34]. In a case-control study of 184 cases and 554 controls nested within the Nurses' Health study cohort, women with the A2 allele of CYP17 were at decreased risk of endometrial cancer (A2/A2 genotype: OR: 0.43; 95% CI: 0.23–0.80) [35]. In a small multi-ethnic case-control study, the risk of endometrial cancer among women who had taken estrogen replacement therapy was significantly increased for those with the A1 variant (OR: 4.10; 95% CI: 1.64–10.3), but not for those with the A2 variant [36]. A recent large case-control study from Poland questioned the above findings [37]. In this study of 497 endometrial cancer cases and 1024 controls, no significant association with endometrial cancer was found (OR: 1.12; 95% CI: 0.96–1.30).

Aromatase (*CYP19A1*) controls the terminal and rate-limiting step in the estrogen biosynthesis pathway [38]. It is known to be a highly polymorphic gene [39]. Two studies have found an association between the risk of developing endometrial cancer and the TTTA polymorphisms of the *CYP19A1* gene. In a case-control study nested within the Nurses' Health study, an increased risk (OR: 1.92;95% CI: 1.17–3.14) of endometrial cancer was found for women who had two longer alleles (>seven repeats) in the intron 4 *TTTA* polymorphism after adjusting for confounding factors [40]. Similarly, a hospital-based case-control study of 136 patients with endometrial cancer and 116 controls from Russia found an increased risk in women with longer repeats (>seven) with an OR of 3.26 (95% CI: 1.53–6.93) [34,41].

A report from the Shanghai Endometrial Cancer study described findings from other *CYP19A1* polymorphisms [42]. In this case-control study, the haplotype TCATC was associated with a decreased risk of endometrial cancer with an OR of 0.76 (95% CI: 0.62–0.92). Additionally, for SNP rs1870050 in the promoter region of the gene, an OR of 0.81 (95% CI: 0.68–0.97) was calculated for the heterozygote genotype AC and a further decreased OR of 0.58 (95% CI: 0.42–0.80) for homozygotes (CC).

CYP1A1 is one of the major cytochrome P450 isoforms responsible for the catabolism of 17β-estradiol (E2) and estrone (E1) via 2-hydroxylation in extrahepatic tissues, including the uterus [43]. A case-control study of 150 endometrial cancer patients and 165 age-matched controls by Hirata *et al.* investigated the role of genetic polymorphisms of genes encoding for several enzymes involved in the metabolism, detoxification and bioavailability of estrogen [44]. In this study, the authors reported a decrease in the frequency of the CYP1a1 m1 (T/C) polymorphism among women with endometrial cancer when compared with controls (OR: 0.42; 95% CI 0.27–0.69). McGrath *et al.* performed a large case-control study nested within the Nurses' Health Study in which the same MspI restriction site polymorphism T→C transition as above was evaluated, along with a C→A change at nucleotide 4887(Thr461Asn) and a A→G change at nucleotide 4889 (Ile462Val), were analyzed [45]. This study included 456 women with endometrial cancer and 1134 controls matched by year of birth, menopausal status and postmenopausal hormone use at the time of the blood draw. The authors did not find a significant association for any of the three polymorphisms and endometrial cancer risk (TABLE 1).

Progesterone counteracts estrogen-dependent endometrial cancer development. Thus, genes within the progesterone pathway are good candidates for polymorphisms that may be associated with endometrial cancer susceptibility [46]. In a nested case-control study from the Nurses' Health Study, a functional polymorphism in the promoter region of the progesterone receptor gene was studied [46]. In comparison with the +331 G/G wild-type genotype, women with either the +331 G/A or +331 A/A genotypes were associated with an increased OR: 1.90 (95% CI: 1.10–3.29).

The androgen receptor gene encodes for a nuclear transcription factor that is thought to impact the growth of the endometrium and the development of uterine diseases [47]. A study that pooled cases and controls from the Women's Health Study and the Nurses' Health Study evaluated the association

Table 1. Summary of genetic polymorphisms associated with endometrial cancer.

Gene	Proposed mechanism	Genetic polymorphism	Cases/controls (n)	Odds ratio	95% CI	Ref.
Estrogen receptor α	Affects estrogen receptor binding	*Xba*I XX	154/205	0.52	(0.21–1.29)	[31]
		*Xba*I XX	92/65	0.26	(0.09–0.79)	[32]
		*Pvu*II PP	154/205	0.70	(0.34–1.44)	[31]
		*Pvu*II PP	92/65	0.23	(0.07–0.82)	[32]
		TA repeat (<19 repeats)	154/205	1.54	(0.73–3.27)	[31]
CYP17A1	Early steps of estrogen biosynthesis	34 T→C (A2/A2)	184/554	0.43	(0.23–0.80)	[35]
		(A2/A2)	497/1024	1.12	(0.96–1.30)	[37]
		(A1/AI) + estrogen therapy	51/391	4.10	(1.64–10.3)	[36]
CYP19A1 (aromatase)	Rate-limiting step of estrogen biosynthesis	TTTA (>7 repeats)	222/666	1.92	(1.17–3.14)	[40]
		TTTA (>7 repeats)	136/116	3.26	(1.53–6.93)	[34]
		TCATC	1040/1031	0.76	(0.62–0.92)	[42]
	In promoter region of aromatase gene	Rs1870050 CC	1040/1031	0.58	(0.42–0.80)	[42]
CYP1A1	Phase I enzyme that metabolizes estrogen	m1 TT→TC+CC	150/165	0.42	(0.27–0.69)	[44]
		m1TT→TC+CC	328/834	1.19	(0.87–1.62)	[45]
		Ile/Ile→Ile/Val+Val/Val	364/891	0.78	(0.40–1.11)	[45]
		Thr/Thr→Thr/Asn+Asn/Asn	369/865	0.86	(0.59–1.27)	[45]
Progesterone receptor	Promoter region of receptor	+331 G/A +331 A/A	187/397	1.90	(1.10–3.29)	[46]
Androgen receptor	Nuclear transcription factor that mediates steroid hormone action	CAG repeats >22 repeats	137/411 (pooled study)	0.76	(0.59–0.98)	[48]
p53	Cell-cycle arrest and apoptosis	Codon 72 Arg/Arg	108/95	1.86	(1.06–3.26)	[58]
		Arg/Pro and Pro/Pro	95/285	3.56	(2.10–6.00)	[24]
MDM2	Negative regulator of p53	SNP309 G/G	73/79	2.76	(1.06–7.20)	[59]
p21	Downstream mediator of p53	Codon 31 Ser/Ser	95/285	2.68	(1.59–4.51)	[24]
STK15 (aurora-A)	Serine threonine kinase involved in chromosome segregation	F31I AA	193/218	10.2	(2.23–46.50)	[23]
CHEK2	Cell-cycle arrest and activation of DNA repair	TAG1 AA	705/1565	2.29	(1.28–4.08)	[63]
Cyclin D1	Cell-cycle control (G1 to S phase)	G870A AA	77/154	3.16	(1.18–8.43)	[65]
Lymphotoxin-α	Maturation and recruitment of natural killer cells	*LTα* 252AG or 804CA	110/220	0.54	(0.33–0.87)	[68]

CI: Confidence interval.

between the functional CAG repeat polymorphism in this receptor and endometrial cancer in a Caucasian population [48]. They demonstrated that women with an average repeat allele length of 22 or more repeats had a decreased risk of endometrial cancer (OR: 0.76; 95% CI: 0.59–0.98) when compared with women with fewer than 22 repeats. The risk for women with at least one allele of more than 27 repeats was reduced even further (OR: 0.60; 95% CI: 0.36–0.99). In

comparison, a much smaller case-control study of Japanese women found the opposite. In their study of 58 endometrial cancer patients and 89 controls, the length of CAG repeats was longer compared with the healthy controls ($p < 0.001$) [49]. This may be a reflection of CAG repeats being prevalent at different levels in women based on ethnic or racial backgrounds.

While the studies described above did not differentiate the specific type of endometrial cancer, Berstein and colleagues attempted to investigate whether the allelic polymorphisms related to certain steroid metabolism genes were more associated with either the development of type I or type II endometrial cancer in a study of 156 endometrial cancer patients [50]. Based on case history and characteristics of the person and the tumor, approximately two-thirds of the group were characterized as having type I histology and a third with type II histology. The authors did not find any differences in the distribution of CYP17 (17α-hydroxylase/17,20-lyase) and CYP1B1(estrogen 4-hydroxylase) genotypes but did find that patients with type II endometrial cancer were more likely to have the high activity (HH) genotype of catechol-*O*-methyltransferase (COMT) than those with type I disease (OR: 2.9, $p = 0.05$).

Checkpoints & cell cycle

p53 & downstream genes

The TP53 tumor suppressor gene is a natural target for investigation, given its significant role in carcinogenesis, evidenced by the fact that over half of all human tumors carry inactivating mutations [51,52]. The p53 protein product regulates the G1 to S transition in the cell cycle and entry into a DNA damage repair pathway [53]. Under cellular stress, p53 participates in cell-cycle arrest to allow for DNA repair or it can lead to cellular apoptosis. Thus, mutant p53 can lead to genetically unstable cells and increased cancer risk [52]. Polymorphisms in codon 72 of exon 4 ([CCC] proline versus [CGC] arginine), first described in 1987, were thought to carry different oncogenic risks [54]. In relation to uterine cancer risk, the findings have been controversial. Several studies have failed to show a correlation between p53 codon 72 and uterine cancer risk [55–57]. Ueda *et al.* described an increased risk for uterine cancer (OR: 1.86; 95% CI:1.06–3.26) for homozygous Arg/Arg genotype in comparison to Arg/Pro and Pro/Pro genotypes in a Japanese population [58]. The converse was observed in a case-control study in a Korean population where both the Arg/Pro heterozygotes and Pro/Pro homozygotes were significantly associated with endometrial cancer with an OR of 3.56 (95% CI:2.1–6.0) in comparison to the Arg homozygous genotype [24].

Proteins that interact within the p53 stress-response pathway have also been investigated. One study suggested an association between a polymorphism of MDM2, a E3 ubiquitin ligase that directly binds to p53 and suppresses p53 activity through ubiquitinization and the risk of developing sporadic endometrial cancer [59]. The polymorphism SNP309, located in the promoter region of the *MDM2* gene, was found to functionally increase MDM2 protein levels and impair p53 tumor suppressor activity [60]. The SNP309 T to G base substitution (10% homozygous GG in the general population) was associated with an age-adjusted OR of 2.76 (95% CI:1.06,7.20) of uterine cancer compared with individuals with T/T and T/G genotypes [59]. p21, a downstream mediator of p53, inhibits cyclin-dependent kinases and contributes to cell-cycle control. In codon 31 of p21, a C to A base pair transversion changes the amino acid sequence from serine to arginine (31Ser→31 Arg) [53]. In a Korean population, the Ser/Ser genotype was associated with a history of endometrial cancer with an OR of 2.68 (95% CI:1.59–4.51) [24]. While each individual SNP may confer only a modestly increased risk of cancer development, in combination, the effects may be increased. For example, Roh *et al.* reported that individuals with both the at-risk genotypes for p53 and p21 had a more significant association with endometrial cancer risk than either polymorphism alone (OR: 9.55; 95% CI: 4.3–21.2) [24].

STK15

STK 15 is a serine threonine kinase that assists in chromosomal separation and mitotic spindle stability through interaction with the centrosome during mitosis. A pilot study of 193 cases and 218 controls found that, after adjustment for age, race and smoking status for the F31I SNP, the homozygous variant phenotype (AA) was associated with a significantly increased uterine cancer risk (OR: 10.2; 95% CI: 2.23–46.5) in a Caucasian population when compared with the (TT) reference population [23].

CHEK2

Checkpoint kinase 2 (CHEK2) is a multifunctional kinase involved in the cellular response to DNA damage, promoting cell-cycle arrest and activation of DNA repair via phosphorylation of multiple cellular substrates, including p53, Cdc2 and BRCA1 [61,62]. In a Swedish population-based case-control study, 14 SNPs of CHEK2 were evaluated [63]. SNP Rs8135424 (G/A) was found to have a mildly elevated association with endometrial cancer (OR: 1.26; 95% CI: 1.06–1.51). The rare allele (AA), on the other hand, was associated with an OR of 2.29 (95% CI: 1.28–4.08).

Cyclin D1

Cyclin D1 plays a role in cell-cycle regulation by participating in the transition from the G1–S phase and is frequently found to be overexpressed in neoplastic processes [64]. In a hospital-based case-control study in Korea, where subjects were matched for year of birth, menopausal status and current hormone therapy status, the G870G→A polymorphism was evaluated in relation to the risk of developing endometrial cancer. The AA genotype was found to have an increased association with endometrial cancer with an OR of 3.16 (95% CI: 1.18–8.43) [65].

Immune regulators

Lymphotoxin-α

Lymphotoxin-α (LTα), a multifunctional cytokine homologous to TNF, is thought to be involved in the maturation and recruitment of natural killer (NK) cells [66,67]. Given the role of NK cells and antitumor immune surveillance, Niwa *et al.* investigated the association of LTα polymorphisms with endometrial cancer in a study of 110 endometrial cancer cases and 220 controls in a Japanese population [68]. Women with the LTα *252AG* and *804CA* genotype were associated with a decreased risk of endometrial cancer (OR: 0.51; 95% CI: 0.31–0.86) compared with the homozygous GG or AA groups. For women with just one of the variant alleles, the decreased risk of endometrial cancer remained (OR: 0.54; 95% CI: 0.33–0.87).

Novel approaches to SNP analysis & cancer risk

SNPs & epigenetic mechanisms

Approximately 30% of endometrial cancers demonstrate microsatellite instability via the mechanism of loss of DNA mismatch repair [69]. Epigenetic silencing of MLH1 has been reported as the most common cause of defects in DNA mismatch repair [70,71]. Using eight MLH1 SNPs, Chen *et al.* performed a case-control study on endometrial cancer patients whose tumors revealed MLH1 methylation compared with controls whose tumors lacked MLH1 methylation [72]. This study represents a unique application of SNP analysis by demonstrating a heritable predisposition to epigenetic silencing of MLH1 through the identification of risk alleles for MLH1 methylation. In this study, the SNP rs1800734 in the MLH1 CpG island at -93 was significantly associated with MLH1 methylation in women within the St Louis cohort of endometrial cancer patients ($p = 0.005$) . This finding was validated in a cohort of colon cancer patients, but not in a smaller cohort of endometrial cancer patients from Columbus (50% of the power of the St Louis cohort), which failed to find an association [72]. However, with the combination of the three study populations, (St Louis endometrial cancer cohort, Columbus colon cancer cohort and Columbus endometrial cancer cohort), the combined OR was 1.61 (95% CI: 1.20–2.16).

Summary & conclusions

Endometrial cancer, the most common gynecologic malignancy among women in the USA, is an excellent target for research focusing on prevention and early detection of disease. Since every individual with the described medical and lifestyle risk factors does not go on to develop endometrial cancer, while others develop endometrial cancer in the absence of such risk factors, novel methods are needed to risk-stratify women. Identifying markers of risk through genetic variants such as SNPs can contribute to prevention strategies for those with susceptible genetic profiles.

The candidate gene approach, whereby genes are selected for study based upon the biologic pathways associated with cancer development or pathways associated with risk factors for the development of endometrial cancer, is an economic and efficient approach to SNP selection. All of the genetic polymorphisms reviewed in this paper were chosen based on biologic plausibility. These case-control studies represent the current findings of associations demonstrated between genetic polymorphisms and endometrial cancer among limited populations. While the findings are important, this field of research is in a preliminary phase and the results need to be validated in larger and more diverse patient populations prior to wide-scale application.

Expert commentary

Although early investigations of genetic polymorphisms associated with endometrial cancer risk have been promising, there is much work that remains to identify adequate markers of risk to classify subpopulations who may benefit from prevention strategies and risk-reductive surgery. Although case-control genetic association studies are an efficient way to investigate candidate single nucleotide genetic polymorphisms associated with the risk of developing a malignancy, few findings have been reliably reproduced. This may be due to false-positive findings or type I errors. An alternative situation is that subsequent studies that negate the findings are the result of type II errors, or false-negatives secondary to a lack of statistical power [17]. In addition, many genetic polymorphisms are population specific and may not be associated with cancer in people from different ethnic backgrounds. This may limit the broad application of positive study findings to diverse populations. Validation studies will have the greatest chance of success if performed on large ethnically stable populations, given the variations of SNPs among different ethnic groups.

Genetic association studies may be performed on multiple ethnic populations but benefit from specific strategies such as multi-ethnic tagging, as described by Haiman *et al.* [73]. Although this strategy will likely miss a variant common in only one specific group, it enables the identification of a wider range of risk alleles and provides increased power to detect variants common in several ethnic groups.

Despite a publication bias, many negative studies on genetic polymorphisms and endometrial cancer are still published. The results of such studies must also be interpreted with caution as it may be difficult to determine if the results are truly negative or just underpowered to detect a positive result.

Five-year view

The scientific community must build on the early work on genetic polymorphisms and their association with endometrial cancer. To date, much work has used the candidate gene approach, taking into account previously known information and pathways. While there are certainly many more studies that

can be performed using this methodology, novel genes may also be identified via other methods. Newer techniques such as analyzing haplotypes or full candidate pathways may lead to important findings in a more efficient manner. Multiplex-based platforms are generating thousands of SNP results for large case-control studies. Previously prohibitive for routine investigation, the cost of genome-wide studies is decreasing and the technology is improving, which will make such studies easier to perform in the coming years. We anticipate that soon studies will complete genome-wide scans for endometrial cancer.

Regardless of the research method used, validation of reported associations is of paramount importance. Given the large number of potential false-positive findings and the small numbers of patients in single institutions, cooperative, multicenter studies will be essential to provide the statistical power necessary for their validation.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- There is great potential to create prevention strategies to decrease the incidence of endometrial cancer with identification of adequate markers of risk.
- Validation of promising markers in large-scale studies is necessary and will require cooperation across institutions.
- While the candidate gene approach remains a rational way to investigate polymorphisms and their association with endometrial cancer, the technological advances making genome-wide association studies may help identify novel markers for risk of developing endometrial cancer.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1 Jemal A, Siegel R, Ward E *et al.* Cancer statistics, 2008. *CA Cancer J. Clin.* 58, 71–96 (2008).

2 Sorosky JI. Endometrial cancer. *Obstet. Gynecol.* 111, 436–447 (2008).

3 Gruber SB, Thompson WD. A population-based study of endometrial cancer and familial risk in younger women. Cancer and Steroid Hormone Study Group. *Cancer Epidemiol. Biomarkers Prev.* 5, 411–417 (1996).

4 Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. *N. Engl. J. Med.* 293, 1164–1167 (1975).

5 Ziel HK, Finkle WD. Increased risk of endometrial carcinoma among users of conjugated estrogens. *N. Engl. J. Med.* 293, 1167–1170 (1975).

6 Ziel HK, Finkle WD. Association of estrone with the development of endometrial carcinoma. *Am. J. Obstet. Gynecol.* 124, 735–740 (1976).

7 Kelsey JL, LiVolsi VA, Holford TR *et al.* A case-control study of cancer of the endometrium. *Am. J. Epidemiol.* 116, 333–342 (1982).

8 Schwartz Z, Dgani R, Flugelman MY *et al.* A novel approach to the analysis of risk factors in endometrial carcinoma. *Gynecol. Oncol.* 21, 228–234 (1985).

9 Parazzini F, La Vecchia C, Bocciolone L, Franceschi S. The epidemiology of endometrial cancer. *Gynecol. Oncol.* 41, 1–16 (1991).

10 Brinton LA, Berman ML, Mortel R *et al.* Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study. *Am. J. Obstet. Gynecol.* 167, 1317–1325 (1992).

11 Friberg E, Mantzoros CS, Wolk A. Physical activity and risk of endometrial cancer: a population-based prospective cohort study. *Cancer Epidemiol. Biomarkers Prev.* 15, 2136–2140 (2006).

12 Cust AE, Kaaks R, Friedenreich C *et al.* Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. *J. Clin. Endocrinol. Metab.* 92, 255–263 (2007).

13 Bandera EV, Kushi LH, Moore DF *et al.* Dietary lipids and endometrial cancer: the current epidemiologic evidence. *Cancer Causes Control* 18, 687–703 (2007).

14 Soliman PT, Wu D, Tortolero-Luna G *et al.* Association between adiponectin, insulin resistance, and endometrial cancer. *Cancer* 106, 2376–2381 (2006).

15 Lander ES, Linton LM, Birren B *et al.* Initial sequencing and analysis of the human genome. *Nature* 409, 860–921 (2001).

16 Venter JC, Adams MD, Myers EW *et al.* The sequence of the human genome. *Science* 291, 1304–1351 (2001).

17 Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. *Nat. Rev. Cancer* 4, 850–860 (2004).

•• **Provides an excellent background into the use of genetic polymorphisms for association studies looking for cancer susceptibility.**

18 Hampel H, Frankel W, Panescu J *et al.* Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. *Cancer Res.* 66, 7810–7817 (2006).

19 Schmeler KM, Lynch HT, Chen LM *et al.* Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. *N. Engl. J. Med.* 354, 261–269 (2006).

20 Chen LM, Yang KY, Little SE *et al.* Gynecologic cancer prevention in Lynch syndrome/hereditary nonpolyposis colorectal cancer families. *Obstet. Gynecol.* 110, 18–25 (2007).

21 Lu KH. Hereditary gynecologic cancers: differential diagnosis, surveillance, management and surgical prophylaxis. *Fam. Cancer* 7, 53–58 (2008).

22 Taylor JG, Choi EH, Foster CB, Chanock SJ. Using genetic variation to study human disease. *Trends Mol. Med.* 7, 507–512 (2001).

23 Milam MR, Gu J, Yang H *et al*. STK15 F31I polymorphism is associated with increased uterine cancer risk: a pilot study. *Gynecol. Oncol.* 107, 71–74 (2007).

24 Roh JW, Kim JW, Park NH *et al*. p53 and p21 genetic polymorphisms and susceptibility to endometrial cancer. *Gynecol. Oncol.* 93, 499–505 (2004).

25 Collins FS, Guyer MS, Charkravarti A. Variations on a theme: cataloging human DNA sequence variation. *Science* 278, 1580–1581 (1997).

26 Grimes DA, Schulz KF. Making sense of odds and odds ratios. *Obstet. Gynecol.* 111, 423–426 (2008).

27 Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat. Genet.* 29, 306–309 (2001).

• **Good reference for discussing issues related to the replication and validation of genetic association studies.**

28 Emons G, Fleckenstein G, Hinney B *et al*. Hormonal interactions in endometrial cancer. *Endocr. Relat. Cancer* 7, 227–242 (2000).

29 Sherman ME. Theories of endometrial carcinogenesis: a multidisciplinary approach. *Mod. Pathol.* 13, 295–308 (2000).

30 Kang S, Roh JW, Kim JW. Single nucleotide polymorphism: a new risk factor for endometrial cancer? *Future Oncol.* 1, 323–330 (2005).

31 Weiderpass E, Persson I, Melhus H *et al*. Estrogen receptor α gene polymorphisms and endometrial cancer risk. *Carcinogenesis* 21, 623–627 (2000).

32 Iwamoto I, Fujino T, Douchi T, Nagata Y. Association of estrogen receptor α and β3-adrenergic receptor polymorphisms with endometrial cancer. *Obstet. Gynecol.* 102, 506–511 (2003).

33 Berstein LM, Imyanitov EN, Gamajunova VB *et al*. *CYP17* genetic polymorphism in endometrial cancer: are only steroids involved? *Cancer Lett.* 180, 47–53 (2002).

34 Berstein LM, Imyanitov EN, Kovalevskij AJ *et al*. *CYP17* and *CYP19* genetic polymorphisms in endometrial cancer: association with intratumoral aromatase activity. *Cancer Lett.* 207, 191–196 (2004).

35 Haiman CA, Hankinson SE, Colditz GA *et al*. A polymorphism in *CYP17* and endometrial cancer risk. *Cancer Res.* 61, 3955–3960 (2001).

36 McKean-Cowdin R, Feigelson HS, Pike MC *et al*. Risk of endometrial cancer and estrogen replacement therapy history by *CYP17* genotype. *Cancer Res.* 61, 848–849 (2001).

37 Gaudet MM, Lacey JV Jr, Lissowska J *et al*. Genetic variation in *CYP17* and endometrial cancer risk. *Hum. Genet.* 123, 155–162 (2008).

38 Simpson ER, Mahendroo MS, Means GD *et al*. Tissue-specific promoters regulate aromatase cytochrome P450 expression. *J. Steroid Biochem. Mol. Biol.* 44, 321–330 (1993).

39 Haiman CA, Stram DO, Pike MC *et al*. A comprehensive haplotype analysis of *CYP19* and breast cancer risk: the Multiethnic Cohort. *Hum. Mol. Genet.* 12, 2679–2692 (2003).

40 Paynter RA, Hankinson SE, Colditz GA *et al*. *CYP19* (aromatase) haplotypes and endometrial cancer risk. *Int. J. Cancer* 116, 267–274 (2005).

41 Olson SH, Bandera EV, Orlow I. Variants in estrogen biosynthesis genes, sex steroid hormone levels, and endometrial cancer: a HuGE review. *Am. J. Epidemiol.* 165, 235–245 (2007).

42 Tao MH, Cai Q, Zhang ZF *et al*. Polymorphisms in the *CYP19A1* (aromatase) gene and endometrial cancer risk in Chinese women. *Cancer Epidemiol. Biomarkers Prev.* 16, 943–949 (2007).

43 Lee AJ, Cai MX, Thomas PE *et al*. Characterization of the oxidative metabolites of 17β-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology* 144, 3382–3398 (2003).

44 Hirata H, Hinoda Y, Okayama N *et al*. *CYP1A1*, *SULT1A1*, and *SULT1E1* polymorphisms are risk factors for endometrial cancer susceptibility. *Cancer* 112, 1964–1973 (2008).

45 McGrath M, Hankinson SE, De Vivo I. Cytochrome P450 1A1, cigarette smoking, and risk of endometrial cancer (United States). *Cancer Causes Control* 18, 1123–1130 (2007).

46 De Vivo I, Huggins GS, Hankinson SE *et al*. A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. *Proc. Natl Acad. Sci. USA* 99, 12263–12268 (2002).

47 Ito K, Suzuki T, Akahira J *et al*. Expression of androgen receptor and 5α-reductases in the human normal endometrium and its disorders. *Int. J. Cancer* 99, 652–657 (2002).

48 McGrath M, Lee IM, Hankinson SE *et al*. Androgen receptor polymorphisms and endometrial cancer risk. *Int. J. Cancer* 118, 1261–1268 (2006).

49 Sasaki M, Sakuragi N, Dahiya, R. The CAG repeats in exon 1 of the androgen receptor gene are significantly longer in endometrial cancer patients. *Biochem. Biophys. Res. Commun.* 305, 1105–1108 (2003).

50 Berstein L, Zimarina T, Imyanitov E *et al*. Hormonal imbalance in two types of endometrial cancer and genetic polymorphism of steroidogenic enzymes. *Maturitas* 54, 352–355 (2006).

51 Hollstein M, Sidransky D, Vogelstein B, Harris CC. *p53* mutations in human cancers. *Science* 253, 49–53 (1991).

52 Soussi T. The *p53* tumor suppressor gene: from molecular biology to clinical investigation. *Ann. NY Acad. Sci.* 910, 121–139 (2000).

53 Lukas J, Groshen S, Saffari B *et al*. *WAF1/Cip1* gene polymorphism and expression in carcinomas of the breast, ovary, and endometrium. *Am. J. Pathol.* 150, 167–175 (1997).

54 Matlashewski GJ, Tuck S, Pim D *et al*. Primary structure polymorphism at amino acid residue 72 of human *p53*. *Mol. Cell Biol.* 7, 961–963 (1987).

55 Peller S, Halperin R, Schneider D *et al*. Polymorphisms of the *p53* gene in women with ovarian or endometrial carcinoma. *Oncol. Rep.* 6, 193–197 (1999).

56 Esteller M, Garcia A, Martinez-Palones JM *et al*. Susceptibility to endometrial cancer: influence of allelism at *p53*, glutathione S-transferase (*GSTM1* and *GSTT1*) and cytochrome P-450 (*CYP1A1*) loci. *Br. J. Cancer* 75, 1385–1388 (1997).

57 Agorastos T, Masouridou S, Lambropoulos AF *et al*. P53 codon 72 polymorphism and correlation with ovarian and endometrial cancer in Greek women. *Eur. J. Cancer Prev.* 13, 277–280 (2004).

58 Ueda M, Terai Y, Kanda K *et al*. Germline polymorphism of p53 codon 72 in gynecological cancer. *Gynecol. Oncol.* 100, 173–178 (2006).

59 Walsh CS, Miller CW, Karlan BY, Koeffler HP. Association between a functional single nucleotide polymorphism in the *MDM2* gene and sporadic endometrial cancer risk. *Gynecol. Oncol.* 104, 660–664 (2007).

60 Bond GL, Hu W, Bond EE *et al*. A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* 119, 591–602 (2004).

61 Shieh SY, Ahn J, Tamai K *et al*. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev.* 14, 289–300 (2000).

62 Antoni L, Sodha N, Collins I, Garrett MD. CHK2 kinase: cancer susceptibility and cancer therapy - two sides of the same coin? *Nat. Rev. Cancer* 7, 925–936 (2007).

63 Einarsdottir K, Humphreys K, Bonnard C *et al*. Effect of ATM, CHEK2 and ERBB2 TAGSNPs and haplotypes on endometrial cancer risk. *Hum. Mol. Genet.* 16, 154–164 (2007).

64 Donnellan R, Chetty R. Cyclin D1 and human neoplasia. *Mol. Pathol.* 51, 1–7 (1998).

65 Kang S, Kim JW, Park NH *et al*. Cyclin D1 polymorphism and the risk of endometrial cancer. *Gynecol. Oncol.* 97, 431–435 (2005).

66 Paul NL, Ruddle NH. Lymphotoxin. *Annu. Rev. Immunol.* 6, 407–438 (1988).

67 Ito D, Back TC, Shakhov AN *et al*. Mice with a targeted mutation in lymphotoxin-α exhibit enhanced tumor growth and metastasis: impaired NK cell development and recruitment. *J. Immunol.* 163, 2809–2815 (1999).

68 Niwa Y, Ito H, Matsuo K *et al*. Lymphotoxin-α polymorphisms and the risk of endometrial cancer in Japanese subjects. *Gynecol. Oncol.* 104, 586–590 (2007).

69 Goodfellow PJ, Buttin BM, Herzog TJ *et al*. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. *Proc. Natl Acad. Sci. USA* 100, 5908–5913 (2003).

70 Esteller M, Levine R, Baylin SB *et al*. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* 17, 2413–2417 (1998).

71 Simpkins SB, Bocker T, Swisher EM *et al*. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. *Hum. Mol. Genet.* 8, 661–666 (1999).

72 Chen H, Taylor NP, Sotamaa KM *et al*. Evidence for heritable predisposition to epigenetic silencing of MLH1. *Int. J. Cancer* 120, 1684–1688 (2007).

73 Haiman CA, Hsu C, de Bakker P *et al*. Comprehensive Association Testing of Common Genetic Variation in DNA Repair Pathway Genes in Relationship with Breast Cancer Risk in Multiple Populations. *Hum. Mol. Genet.* 17, 825–384 (2008).

Websites

101 Department of Health and Human Services. Centers for Disease Control & Prevention. Overweight and Obesity. www.cdc.gov/nccdphp/dnpa/obesity/index.htm (Accessed February 22, 2008).

102 Surveillance, Epidemiologyand End Results (SEER) Program. DevCan database: Probability of Developing Cancer For Corpus and Uterus, NOS Cancer by Race, Females. SEER 17 Registries for 2002–2004". NCI, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2006, based on November 2005 submission. www.seer.cancer.gov (Accessed February 22, 2008).

Affiliations

- Michael R Milam, MD, MPH
Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center, 1155 Herman P. Pressler St, CPB 6.3244, Unit 1362, Houston, TX 77030, USA
Tel.: +1 713 563 4563
Fax: +1 713 792 7586
mrmilam@mdanderson.org
- Larissa A Meyer, MD
Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center, 1155 Herman P. Pressler St, CPB 6.3244, Unit 1362, Houston, TX 77030, USA
Tel.: +1 713 563 4577
Fax: +1 713 792 7586
lmeyer@mdanderson.org
- Shannon N Westin, MD, MPH
Fellow, Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center, 1155 Herman P. Pressler St, CPB 6.3244, Unit 1362, Houston, TX 77030, USA
Tel.: +1 713 563 4575
Fax: +1 713 792 7586
swestin@mdanderson.org
- Karen H Lu, MD
Professor, Department of Gynecologic Oncology, University of Texas MD Anderson Cancer Center, 1155 Herman P. Pressler St, CPB 6.3244, Unit 1362, Houston, TX 77030, USA
Tel.: +1 713 745 8902
Fax: +1 713 792 7586
khlu@mdanderson.org