Endometrial cancer gene panels: clinical diagnostic vs research germline DNA testing

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Endometrial cancer is the most common gynecological cancer, but is nevertheless uncommon enough to have value as a signature cancer for some hereditary cancer syndromes. Commercial multigene testing panels include up to 13 different genes annotated for germline DNA testing of patients with endometrial cancer. Many other genes have been reported as relevant to familial endometrial cancer from directed genome-wide sequencing studies or multigene panel testing, or research. This review assesses the evidence supporting association with endometrial cancer risk for 32 genes implicated in hereditary endometrial cancer, and presents a summary of rare germline variants in these 32 genes detected by analysis of quasi-population-based endometrial cancer patients from The Cancer Genome Atlas project. This comprehensive investigation has led to the conclusion that convincing evidence currently exists to support clinical testing of only six of these genes for diagnosis of hereditary endometrial cancer. Testing of endometrial cancer patients for the remaining genes should be considered in the context of research studies, as a means to better establish the level of endometrial cancer risk, if any, associated with genetic variants that are deleterious to gene or protein function. It is acknowledged that clinical testing of endometrial cancer patients for several genes included on commercial panels may provide actionable findings in relation to risk of other cancers, but these should be considered secondary or incidental findings and not conclusive evidence for diagnosis of inherited endometrial cancer. In summary, this review and analysis provides a comprehensive report of current evidence to guide the selection of genes for clinical and research gene testing of germline DNA from endometrial cancer patients.

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Endometrial cancer is the most common gynecological cancer, affecting 1 in 37 women in the United States¹ and 1 in 49 in Australia² during their lifetime, signifying a 2 to 3% lifetime risk. Historically, endometrial cancers have been divided into two cellular classifications: type I tumors are associated with unopposed estrogen stimulation and are mostly endometrioid adenocarcinomas, whereas type II tumors are estrogen independent consisting of predominantly serous carcinomas. The endometrioid subtype accounts for 80% of endometrial cancers,

the serous subtype accounts for 10%, and the remainder include mucinous, clear-cell, squamous cell, and mixed adenocarcinomas.³ Recently, large-scale molecular analysis of endometrial tumors grouped endometrial cancers into four molecular classifications: *POLE*-ultramutated, microsatellite instability (MSI)-hypermethylated, copy-number low, and copy-number high.⁴

Factors that have been linked to an increase in endometrial cancer risk of all subtypes combined include long-term exposure to unopposed estrogens, age at menarche, age at menopause, nulliparity, obesity, diabetes,⁵ and long-term use of tamoxifen as a treatment for breast cancer.⁶ Individuals who have a first-degree relative with endometrial cancer are at increased risk of the disease, with a relative risk (RR) of 1.8 (1.7–2.0) estimated from a meta-analysis of 16 different studies of varying design and age range.⁷ Familial risk of endometrial cancer may, in part, be due to shared environmental risk factors, for

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example, obesity due to shared lifestyle choices. However, it is clear that familial risk of endometrial cancer is influenced by genetic factors, ranging from inheritance of multiple low-risk cancer predisposition variants to single, very high-risk variants. To date, several common single-nucleotide polymorphisms (SNPs) have been shown to be convincingly associated with endometrial cancer risk from large-scale candidate locus analysis and genome-wide association analyses. These include 'low-risk' SNPs at/near *HNF1B*,^{8,9} the *TERT-CLPTM1L* cancer risk region,¹⁰ the *CYP19A1* locus encoding the aromatase enzyme pivotal to estrogen biosynthesis,¹¹ *ESR1* encoding the estrogen receptor,¹² *SH2B3*,¹³ *SOX4*,¹⁴ and also *KLF5*, *AKT1*, *EIF2AK4*, *HEY2/NCOA7*, and at the *MYC* multicancer locus.¹⁵ Consistent with findings for most other cancers, the endometrial cancer risks associated with these common variants are considered modest (odds ratios ranging from 0.84 to 1.27). Taken together, loci identified to date are expected to account for ~5% of the familial RR of endometrial cancer. Of more direct clinical significance, some endometrial cancer cases are attributable to pathogenic (disease-causing) changes in hereditary cancer predisposition syndrome genes, which confer a moderate to high lifetime risk of endometrial and other syndromic cancers. Testing of germline DNA for pathogenic variants in these syndrome genes has been undertaken largely in the familial cancer setting, and results are used to direct genetic counseling and clinical management of cancer patients and their relatives.

In recent years, many service providers have modified their germline DNA screening strategies for the genetically heterogeneous cancers and cancer syndromes from stepwise molecular analysis of a limited number of highly relevant genes to targeted multigene panel testing using next-generation sequencing. This paradigm shift has been mainly driven by advances in sequencing technology, implementation of optimized analysis pipelines, and significantly reduced sequencing costs, which have collectively resulted in more efficient, sensitive, and cost-effective genetic testing. When targeting a specific cancer type such as endometrial cancer with multiplex gene panel testing, a major challenge is gene selection. The test should be a way of identifying disease etiology and should provide actionable data with the potential to lead to clinical options for the patients and their genetic carrier relatives, such as risk-reducing surgery, targeted therapy, increased surveillance, and/or chemoprevention. This has to be balanced with generating unwanted or uninterpretable information that is not clinically useful. However, there are increasing numbers of genes purported to be associated with modest (<2-fold) or moderate (2–4-fold) cancer risks,¹⁶ often based on limited data. Indeed, the need for clinical validation of genomic tests has been highlighted recently, in relation to breast cancer risk prediction.¹⁷ The inclusion of tenuously risk-

associated genes on a multigene panel is subject for debate, as identification of variants considered deleterious to protein function in these genes will often lead to their inclusion in clinical reports, despite the dearth of evidence of their association with risk of specific cancer types, and even sometimes with any cancer type. Such practice confounds genetic counseling and clinical management of patients and their relatives.^{18–20}

To our knowledge, only one commercial gene panel is marketed specifically for germline DNA testing when the diagnosis is endometrial cancer. The Endometrial Cancer Panel from GeneDx currently includes 12 genes: *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* (dosage analysis only), phosphatase and tensin homolog (*PTEN*), *BRCA1*, *BRCA2*, *MUTYH*, *CHEK2*, *TP53*, and *POLD1*. The GYNplus panel of 13 genes marketed by Ambry Genetics for ovarian and endometrial cancer germline DNA testing includes four additional genes currently annotated for ovarian cancer risk only (*PALB2*, *BRIP1*, *RAD51C*, and *RAD51D*), but not *MUTYH*, *CHEK2*, and *POLD1*. There are also other nonspecific cancer panels marketed for germline DNA testing of patients with multiple cancer types including endometrial cancer, two of which (Myriad myRisk, ColorGenomics), have annotated *STK11* as an additional gene relevant for endometrial cancer risk. Here we review the evidence to support the inclusion of these genes in testing panels marketed for diagnostic genetic testing of germline DNA from endometrial cancer patients and their relatives. We also review the evidence to support clinical or research germline testing of additional genes implicated in familial or unselected endometrial cancer from directed exome sequencing of familial cases, or multigene panel testing. Also, for all these genes, known or purported to be associated with risk of developing endometrial cancer, we include an analysis of germline variants identified in 543 quasi-population-based endometrial cancer patients from The Cancer Genome Atlas Project (TCGA),⁴ to provide additional support for our recommendations based on the review of the literature.

Genes on panels marketed for diagnostic genetic testing of endometrial cancer patients

MLH1, *MSH2/EPCAM*, *MSH6*, and *PMS2*

Pathogenic variants that disrupt the function of the mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, or *PMS2* confer a predisposition to Lynch syndrome, a dominantly inherited susceptibility to colorectal, endometrial, and other cancers. In addition, certain partial 3' deletions of *EPCAM*, a gene that lies directly upstream of *MSH2*, cause epigenetic silencing of *MSH2* leading to tissue-specific *MSH2* deficiency in *EPCAM*-expressing tissues.²¹ When

the MMR system is lost in a cell, the replication machinery has the inability to remove erroneous nucleotide incorporations during DNA replication, which leads to the accumulation of somatic changes throughout the genome. MSI is a consequence (hypermutable at simple, tandemly repeated sequences), which drives tumorigenesis by disrupting genes that are important for normal cellular function, such as those with roles in apoptosis and DNA repair.²² MSI in tumor DNA is thus the molecular signature of an MMR defect and arises via the following mechanisms: (1) constitutional loss of one MMR allele and subsequent somatic loss of the wild-type allele, as in Lynch syndrome; (2) hypermethylation of the *MLH1* gene promoter in combination with a second somatic hit to the other allele, estimated to occur in 20% of sporadic endometrial cancers;²³ (3) biallelic somatic loss of at least one MMR gene.^{24–26} The histology of Lynch syndrome-associated endometrial cancer is heterogeneous, with the tumors being both endometrioid and non-endometrioid. Histologic subtypes include endometrioid adenocarcinoma, clear-cell carcinoma, uterine serous papillary carcinoma, and uterine carcinosarcoma.^{27,28} Although there has been some debate in the literature to suggest that carriers of a pathogenic MMR gene variant mostly develop endometrial cancer of non-endometrioid subtype, larger population-based studies have not provided convincing support for this hypothesis.^{23,29}

Germline defects in MMR genes underlie the etiology of < 5% of endometrial cancer at a population level (summarized in Buchanan *et al*³⁰). Women with an MMR defect have a high endometrial cancer lifetime risk comparable to the risk of colon cancer,³¹ with the lifetime risk being influenced by which MMR gene is disrupted. The cumulative risk of endometrial cancer for women to age 70 years is estimated to be 18% (9–34%) for *MLH1*, 30% (18–45%) for *MSH2* pathogenic variant carriers,³² 26% (18–36%) for *MSH6* pathogenic variant carriers³³ (although the risk has been estimated to be as high as 71%³⁴), and 12–15% for *PMS2* pathogenic variant carriers.^{35,36} A cohort study of *EPCAM* deletion-positive Lynch syndrome patients estimates the endometrial cancer risk to be lower than for patients with a pathogenic change in an MMR gene, as only three of 92 women with 3' deletion of *EPCAM* developed endometrial cancer, which equates to a cumulative lifetime risk of 12% (0–27%).³⁷ The *EPCAM* deletions in all three patients extended close to the *MSH2* gene promoter, suggesting that an increased risk of endometrial cancer is dependent on the location of the *EPCAM* deletion in relation to *MSH2*.

The clinical management recommendations for those identified to have a Lynch syndrome-associated pathogenic genetic variant include colonoscopy every 1 to 2 years, and (in some centers) gynecological examination (with transvaginal ultrasound, and aspiration biopsy) on a yearly basis.³⁸

Risk-reducing gynecological surgery is an option for women from age 35 and generally recommended after childbearing is completed.³⁸ The high risks of endometrial cancer for women with a germline defect in *MLH1*, *MSH2*, *MSH6*, and *PMS2*—in addition to *EPCAM* dosage analysis—make these genes obvious inclusions on an endometrial cancer-focused panel.

***POLD1* (and *POLE*)**

The *POLD1* and *POLE* genes encode subunits of DNA polymerases δ and ϵ , respectively, which are part of the DNA replication machinery and are involved in the recognition and removal of mispaired bases that occur during DNA replication. Palles *et al*³⁹ analyzed the exomic variants of several individuals with multiple colorectal adenomas and family histories of cancer that were not accounted for by pathogenic variants in *APC*, *MUTYH*, or the MMR genes. They identified index cases and family members with microsatellite-stable tumors and germline substitutions in the exonuclease-active sites of *POLD1* (c.1433G>A (p.Ser478Asn)) and *POLE* (c.1270C>G (p.Leu424Val)). Analysis indicated that the amino-acid substitutions would perturb normal polymerase proofreading activity, thus reducing the capacity to correct mispaired bases that are erroneously inserted during DNA replication, thereby accelerating the accumulation of somatic genetic changes in cells. Tumors from these patients exhibited an ultramutant tumor phenotype, consistent with the molecular signature of tumors with somatically acquired variants in the exonuclease domain of *POLE*. The germline *POLD1* variant c.1433G>A (p.Ser478Asn) was associated with endometrial cancer, as it was identified in two families with multiple cases of endometrial cancer diagnosed between 33 and 52 years. In total, seven women had endometrial cancer: four were heterozygous for c.1433G>A (p.Ser478Asn), one was an obligate variant carrier, and two had not been genotyped. Genotyping for c.1433G>A (p.Ser478Asn) was then performed for 386 unselected endometrial cancer cases without a reported family history of CRC, but no carriers were detected in this cohort. The germline *POLE* variant c.1270C>G (p.Leu424Val) was identified in 13 probands with multiple adenomas and CRC. Interestingly, there were no extracolonic tumors reported in the probands or their family members.

Subsequently, Valle *et al*⁴⁰ studied a Spanish cohort comprising 858 familial and early-onset CRC cases using a combination of variant-specific genotyping (for *POLE* c.1270C>G (p.Leu424Val), and *POLD1* c.1433G>A (p.Ser478Asn)) and, in a subset, limited exon sequencing. A novel *POLD1* exonuclease domain variant (c.1421 T>C (p.Leu474Pro)) was identified in a family with endometrial, colorectal cancer, brain, gastric, and bladder cancer. One woman heterozygous for the variant and her obligate

carrier sister had developed endometrial cancer in their 50s. Consistent with previously identified *POLD1* families, a colon tumor and an endometrial tumor from two separate individuals in this family were MMR proficient.

Rohlin *et al*⁴¹ sequenced the exomes of several members of a large family with a dominant family history of MMR-proficient cancer that included endometrial, colorectal, ovarian, pancreatic, brain, and gastric cancer. They took an agnostic approach to converge on a novel *POLE* exonuclease domain variant (c.1089C>A (p.Asn363Lys)) as the cause of the cancers in this family. Two women, both heterozygous for the variant, developed endometrial cancer and another malignancy; one developed endometrial and ovarian cancer at the age of 45 years, and the other developed colorectal cancer at the age of 43 years followed by endometrial and ovarian cancer at the age of 50 years.

Elsayed *et al*⁴² genotyped a Dutch cohort comprising 1188 familial CRC and polyposis cases for *POLE* c.1421 T>C (p.Leu424Val) and *POLD1* c.1433G>A (p.Ser478Asn). Three index cases were identified to have *POLE* c.1421 T>C (p.Leu424Val). One case was a woman who developed a cecal tumor, colon polyps, and endometrial cancer. The endometrial tumor was MMR proficient, but the cecal tumor showed loss of MSH6 protein expression and MSI, which is characteristic of a germline defect in *MSH6*. Cecal tumor DNA sequencing revealed a hypermutator phenotype and two pathogenic somatic mutations in *MSH6*, thus explaining the immunohistochemical and molecular characteristics of the tumor. This suggests that the increased rate of somatic genetic variant accumulation resulting from compromised polymerase proofreading could, at times, precipitate concomitant biallelic somatic loss of an MMR gene, which would thereby initiate MMR-deficient tumorigenesis. This hypothesis is supported, at least for endometrial cancer, by a study of 544 endometrial tumors, which showed that *POLE* somatic exonuclease domain variants occur with equal frequency in microsatellite-stable and -unstable tumors (5.9% and 5.2%, respectively), and that *POLE* variants can be concomitant with somatic genetic variant in MMR genes in a subset of MSI tumors.⁴³ Therefore, individuals with a pathogenic germline variant in *POLD1* or *POLE* can present with both microsatellite-stable and -unstable endometrial tumor phenotypes.

Subsequently, Spier *et al*⁴⁴ screened *POLE* and seven other polymerase genes in 266 German patients with multiple colorectal adenomas or Amsterdam criteria-positive family history, and for whom no causative germline alteration in *APC*, *MUTYH*, or MMR genes had been identified.⁴⁴ The *POLE* c.1421 T>C (p.Leu424Val) variant was identified in 1.5% of all cases, increasing to 4% for familial cases, and 7% for familial polyposis cases specifically. An additional nine putative pathogenic *POLE* variants were identified. While no endometrial

cancer cases were reported in *POLE*-positive individuals, a range of other tumors and diseases were, including ovarian cancer at the age of 33 years. With regard to the variants in other polymerase genes reported as potentially pathogenic, the *POLD3* variant c.1154G>A (p.Arg385His) was identified in one proband from an Amsterdam-positive family, and his endometrial cancer-affected mother. Further study of *POLD3* may be of interest with respect to endometrial cancer, but the detection of this variant in control populations at frequency 0.1% suggests that it is unlikely to be proven to be associated with a high risk of cancer.

Most recently, Bellido *et al*⁴⁵ presented data for pathogenic variants identified in the exonuclease domain of *POLE* and *POLD1* in 529 kindreds, 444 with familial non-polyposis colorectal cancer, and 88 with polyposis, a review of existing and newly generated data. Four *POLD1* variants with strong evidence of pathogenicity were identified in non-polyposis colorectal cancer families: c.946G>C (p.Asp316His); c.947A>G (p.Asp316Gly); c.1225C>T (p.Arg409Trp); and c.1421 T>C (p.Leu474Pro). Of note, endometrial cancer at the age of 36 and 57 years was reported in two carriers of the c.947A>G (p.Asp316Gly) variant. Further the phenotypic spectrum of *POLE/POLD1*-associated spectrum was widened to include brain tumors.

Taken together, these reports support the hypothesis that pathogenic germline variants in the exonuclease proofreading domains of *POLE* (exons 9–14) and *POLD1* (exons 8–13) that are deleterious to polymerase proofreading function predispose to endometrial cancer. To date, three likely pathogenic *POLD1* variants (c.947A>G (p.Asp316Gly); c.1433G>A (p.Ser478Asn); c.1421 T>C (p.Leu474Pro)) have been identified in nine women with endometrial cancer (proven or obligate carriers) from four families, and two likely pathogenic *POLE* variants (c.1421 T>C (p.Leu424Val); c.1089C>A (p.Asn363Lys)) in three endometrial cancer patients from two families.

However, because of the paucity of reports so far, and the fact that all studies to date have focused on ascertainment via probands with polyposis and/or colorectal cancer, the *POLD1*- and *POLE*-associated endometrial cancer risks are yet to be estimated. Similarly, little is known about prognosis for germline pathogenic variant carriers, but somatic *POLE* proofreading variants appear to predict favorable endometrial cancer prognosis.⁴⁶ It was initially suggested that, as an intermediate measure, the clinical management of Lynch syndrome and *MUTYH*-associated polyposis patients should be integrated for the management of these patients, with regular and frequent colonoscopic polypectomy and consideration of risk-reducing surgery.³⁹ Although the absolute cancer risks associated with (presumed) pathogenic variants in *POLD1* and *POLE* are not established, preliminary clinical management recommendations have since been published

as part of the comprehensive review by Bellido *et al*,⁴⁵ namely: colonoscopy every 1–2 years and gastroduodenoscopy every 3 years, starting at the age of 20–25 years (re-evaluate periodicity according to the findings), adding endometrial cancer screening beginning at the age of 40 years for *POLD1* female carriers.

This same review also published preliminary recommendations for genetic testing.⁴⁵ Given the overlapping phenotypes of *POLD1*/*POLE* with Lynch syndrome and attenuated adenomatous polyposis (*APC/MUTYH*), the clinical criteria for hereditary non-polyposis CRC (revised Bethesda) were adapted to the attenuated or oligopolyposis scenario, and additional specific *POLE*/*POLD1* characteristics were taken into consideration. It was suggested that *POLD1* clinical genetic testing of endometrial cancer patients may be considered in the context of a strong family history of colorectal or endometrial cancer (two or more relatives) or a single relative with colorectal cancer <60 years or endometrial cancer <60 years.⁴⁵ Extrapolating from these recommendations, *POLD1* and *POLE* testing of unselected endometrial cancer patients should be limited to the research setting, as should *POLE* testing of endometrial cancer patients identified in the familial cancer setting.

PTEN

The *PTEN* gene is a tumor suppressor that encodes a protein that has a crucial role in the control of the PI3K/AKT and MAPK pathways. Frequent somatic genetic inactivation of *PTEN* occurs in endometrial cancer, as is the case for many other tumor types. During tumor development, loss of the *PTEN* protein leads to increased cell proliferation and reduced cell death. Haploinsufficiency in mice (*pten*^{+/-}) causes the mice to develop a range of tumors, with endometrial hyperplasia and endometrial cancer being frequent.⁴⁷

Germline variants that cause loss of *PTEN* function cause an inherited predisposition to multiple cancers. Cowden syndrome is a *PTEN* hamartoma tumor syndrome characterized by multiple benign hamartomas—typically on the skin, mucous membranes, and the intestine—and a susceptibility to thyroid, breast, and endometrial cancer in particular.^{48–50} Increased risk of renal cancer, colorectal cancer, and melanoma have also been reported.^{48–50} The reported lifetime risk of endometrial cancer to age 70 years ranges from 19% (10–32%)⁵⁰ to 28% (17–39%).⁴⁸ The *PTEN*-related endometrial cancer risk is expected to significantly increase from age 25 years,⁴⁸ but reports of adolescent onset endometrial cancer in the context of a personal and/or family history of Cowden syndrome have been attributed to pathogenic germline variants in this gene.^{51–53} Endometrioid histology is reported to be the most prevalent histologic type in individuals who carry a *PTEN* pathogenic variant.⁵⁴

Genetic predisposition to endometrial cancer outside of a clinical Cowden syndrome context appears to be uncommon. Germline *PTEN* screening of a population-based cohort of 240 women with endometrial cancer did not identify any pathogenic variants, suggesting that they do not account for a significant proportion of endometrial carcinoma at the population level,⁵⁵ and it is notable that only a single *PTEN* pathogenic variant was identified across three different studies reporting results of multigene cancer panel testing of unselected⁵⁶ or familial^{57,58} endometrial cancer patients, and this patient carrying this variant reported a clinical history consistent with Cowden syndrome.⁵⁶ Nevertheless, identifying *PTEN*-related endometrial cancers is important because of the increased risks of other cancers and the implications for family members. Published clinical management guidelines⁵⁹ recommend endometrial screening starting at the age of 30 years by annual endometrial biopsy or transvaginal ultrasound, annual mammogram, annual examination of the thyroid, 2-yearly renal imaging, 2-yearly colonoscopy and annual dermatologic examination, and risk-reducing mastectomy may also be recommended for young women with dense breasts or indications for repeated biopsies.⁶⁰

Endometrial cancer is a major diagnostic criterion of Cowden syndrome. It is included in the strict international Cowden Consortium operational diagnostic criteria originally established to identify *PTEN* pathogenic variant carriers, and the more relaxed Cleveland Clinic Score (CC Score) system developed to identify individuals with clinical features reminiscent of Cowden syndrome (termed Cowden-like syndrome) and >3% probability of carrying a *PTEN* pathogenic variant.⁶¹ However, additional factors appear to have utility for identifying endometrial cancer patients with a germline *PTEN* pathogenic variant; analysis of 371 prospectively enrolled endometrial cancer patients showed that the clinical features predicting germline *PTEN*-related endometrial cancer are early age of onset (50 years or less), endometrioid histologic subtype, macrocephaly, a high *a priori* risk of Cowden syndrome (CC Score 29 or greater), synchronous renal cell carcinoma, and low *PTEN* tumor protein expression.⁵⁴ Clinical testing of *PTEN* is thus advisable for endometrial cancer patients with features or family history indicative of Cowden syndrome, and should be considered when endometrial cancer is diagnosed in adolescence. However, current evidence suggests that testing of ‘population-based’ endometrial cancer patients for germline pathogenic variant in *PTEN* should preferably be undertaken in the research setting only.

BRCA1* and *BRCA2

Pathogenic variants in *BRCA1/2* that lead to protein truncation or instability, or that disrupt the function

of important protein domains, predispose to breast, ovarian, and other cancers. In addition to several case-reports,^{62,63} multiple studies have been undertaken to investigate the risk of endometrial cancer attributed to *BRCA1/2* pathogenic variants, and others have screened endometrial cancer patients (purportedly familial or unselected) using multigene cancer panels that included *BRCA1* and *BRCA2*. The design of and evidence from these studies (23 total, 17 non-overlapping) is summarized in Supplementary Table 1. Unfortunately, despite active research on this topic, the published evidence remains conflicting; most studies report evidence based on a relatively small number of proven carriers with endometrial/uterine cancer, comparison with appropriate control/reference groups is lacking for panel gene studies, and assessment of the evidence is further complicated by the fact that tamoxifen use for treatment or prevention of breast cancer is known to lead to an increased risk of endometrial cancer.

Three non-overlapping studies have provided evidence that tamoxifen use is associated with increased endometrial cancer risk in *BRCA1* pathogenic variant carriers. Segev *et al*⁶⁴ prospectively followed 4456 women with a *BRCA1/2* pathogenic variant, with mean follow-up of 5.7 years. Endometrial cancer was reported for 17 women; 8 of these had received tamoxifen, translating to an RR of 4.1 (1.9–7.87; $P=0.001$) for this subgroup, compared with a modest nonsignificant increased risk for women who had not received tamoxifen (RR 1.7 (0.8–3.1); $P=0.1$). Similarly, analysis of 1203 high-risk breast/ovarian cancer families screened for pathogenic variants in *BRCA1* and *BRCA2*⁶⁵ showed that tamoxifen therapy was significantly associated with endometrial cancer risk in *BRCA1/2* families (HR 6.7 (3.1–15.2)), while endometrial cancer risk was marginally, but not significantly, greater than unity for pathogenic variant carriers compared with non-carrier family members (*BRCA1* HR 1.3 (0.7–2.4); *BRCA2* HR 1.1 (0.5–2.5)). Another recent study comparing observed and expected rates for serous/serous-like endometrial cancer in *BRCA* carriers⁶⁶ provides support for association of risk of aggressive endometrial cancer subtypes with tamoxifen exposure in *BRCA1/2* carriers (observed: expected (O:E) rate 24.4 (5.0–71.3), $P<0.001$). Further, a case–control study comparing risk factors among *BRCA1/2* carriers has reported endometrial cancer risk of 3.5-fold (1.5–8.1) associated with tamoxifen use.⁶⁷ These estimates of endometrial cancer risk with tamoxifen use among carriers are generally consistent with the consensus RR of 2.4 (1.5–4.0) reported from meta-analysis of five randomized breast cancer prevention trials,⁶⁸ although it should be noted that estimates from long-term follow-up of the IBIS-I trial alone indicate that risk of endometrial cancer is significantly increased during active treatment only, reducing from 3.8-fold risk in years 0–5 to 1.5 (0.8–2.7) after a median 16 years follow-up.⁶⁹

Three recent multigene panel studies did not specifically assess risk associated with *BRCA1/2* carrier status, but do provide measures of the frequency of *BRCA1/2* pathogenic variants in endometrial cancer patients, ranging from 0.5% in unselected cases⁵⁶ up to 1.4% in those referred for genetic testing by clinicians.^{57,58} None of these three studies provided information on tamoxifen use. Of the other 15 non-overlapping studies investigating association of carrier status with development of endometrial/uterine cancer, seven reported evidence supporting at least modest increased risk of endometrial cancer for carriers of a pathogenic variant in *BRCA1* or *BRCA2*. Tamoxifen use was recorded for only three of these, and it is possible that some endometrial cancer patients received adjuvant tamoxifen therapy for previous breast cancer. However, tamoxifen use is less likely to be confounding the interpretation of results for studies that included patients with first primary endometrial cancer diagnosed before 1990, the initiation of tamoxifen chemoprevention trials. Results reporting analysis that reached statistical significance were noted for only 3/15 studies. The largest study to date, analysis of 11 847 *BRCA1* pathogenic variant carriers from across Europe and North America, observed a statistically significant increased risk of endometrial cancer (RR 2.7 (1.7–4.2; $P<0.001$),⁷⁰ which remained similar when restricting analysis to women unaffected with breast cancer (RR=3.1 (1.7–5.7; $P<0.001$) to restrict the confounding effects of tamoxifen on endometrial cancer risk. Excess risk of endometrial cancer was also reported for relatives of 57 Ashkenazi ovarian cancer-affected *BRCA1* carriers (RR 9.4, $P=0.0003$), a result based on only four events.⁷¹ Most recently, a prospective study of 1083 *BRCA1/2* carriers after risk-reducing salpingo-oophorectomy⁶⁶ reported significantly increased risk of serous/serous-like endometrial cancers in *BRCA1/2* carriers overall (O:E ratio 14.8 (4.8–34.6), $P<0.001$), but no significant risk of endometrioid subtype cancer (O:E ratio 0.6 (0.1–2.0), $P=0.88$). Moreover, significantly increased risk of serous/like cancer was limited to *BRCA1* carriers specifically (O:E ratio 22.2 (6.1–56.9), $P<0.001$).⁶⁶ The same study reported evidence for increased risk of serous/serous-like cancer with and without tamoxifen exposure, presented only for *BRCA1/2* carriers combined. Another recent smaller study assessing occult and subsequent cancer incidence following risk-reducing salpingo-oophorectomy identified a single endometrioid subtype cancer in a *BRCA1* carrier at surgery, and authors concluded that they cannot endorse concurrent hysterectomy for cancer risk reduction.⁷² Another four studies (sample sizes 31–151) have investigated if *BRCA1/2* pathogenic variants may increase risk of endometrial cancer of serous subtype specifically, with three of these^{73–75} providing largely observational evidence that serous subtype endometrial cancer may be part of the *BRCA1/2* spectrum. However, it is also clear from

studies of unselected endometrial cancer patients where information on histological subtype was available that carriers of a *BRCA1* or *BRCA2* pathogenic variant mostly present with endometrioid subtype endometrial cancer, even in women exposed to tamoxifen.^{56,64,65,76–78}

Considering findings across all studies, results could be interpreted to suggest that endometrial cancer may be more likely to develop in *BRCA1* carriers compared with *BRCA2* carriers. The statistically significant associations noted above were observed for *BRCA1* carriers only, and in most of the more observational studies, endometrial cancer-affected patients appeared more often to carry a pathogenic variant in *BRCA1* compared with *BRCA2*.^{64,65,73–76,79} Only one study comparing risk by subtype has provided evidence that the significant increase in endometrial cancer risk for *BRCA1* carriers is for serous/serous-like cancers only,⁶⁶ and on this basis has advocated that advantages and risks of hysterectomy should be discussed with *BRCA1* carriers at the time of considering risk-reducing salpingo-oophorectomy.

All the evidence taken together indicates that *BRCA1/2* pathogenic variant carriers have an increased risk of endometrioid and non-endometrioid endometrial cancer after tamoxifen treatment that is at least comparable to that observed for non-carriers in the general population, and there is suggestive evidence for a modest increased endometrial cancer risk in the absence of tamoxifen exposure, in particular for *BRCA1* carriers. Larger-scale testing of these genes in endometrial cancer patients is necessary to generate information to clarify risk of endometrial cancer subtypes associated with carriage of *BRCA1* or *BRCA2* pathogenic variants. However, given that there are well-established clinical management protocols in place for *BRCA1* and *BRCA2* pathogenic variant carriers, it could be argued that there is value in including these genes on an endometrial cancer panel for both research purposes and report back of actionable incidental findings.

TP53

The *TP53* tumor suppressor gene encodes 12 different p53 isoform proteins,⁸⁰ which are involved in many anticancer processes, such as DNA repair, cell cycle control, apoptosis, and inhibition of angiogenesis. *TP53* inactivation is a common somatic event during endometrial cancer development, with *TP53* somatic variants being significantly more frequent in uterine serous tumors.⁴ Pathogenic germline variants in *TP53* cause Li–Fraumeni syndrome (LFS), a cancer predisposition syndrome characterized by sarcomas (including endometrial leiomyosarcoma), breast cancer, brain cancer, adrenocortical carcinoma, hematopoietic, and other malignancies.

Endometrial cancer (non-sarcomatous) is not a known manifestation of LFS,⁸¹ but it has been observed in *TP53* pathogenic variant carriers with a younger age of onset than what is expected in the population.^{82,83} Pennington *et al*⁷⁵ assessed 151 uterine serous papillary carcinoma patients and identified two patients with a germline *TP53* missense variant that were considered clinically significant since both had been reported as the causal variants in patients with LFS. Family history assessment was only possible for one variant carrier, but the cancer history was not suggestive of LFS (although family history may be lacking in LFS because of *de novo* germline alteration). Using a multigene panel test to simultaneously sequence 150 genes associated with hereditary cancer, Heitzer *et al*⁸⁴ tested a woman who presented with bilateral breast cancer, aged 38 and 44 years, and endometrial cancer aged 43 years with a significant family history of breast, brain, and gastric cancer. Pathogenic germline variants were identified in two high-penetrance genes, *TP53* and *CDH1*, explaining the diverse cancers in this patient and the family. The authors proposed that the endometrial, brain, and ductal breast cancer developed as a result of the *TP53* variant and the gastric and lobular breast cancer developed from the *CDH1* variant. Recently, Chao *et al*⁸⁵ describe a pathogenic *TP53* missense variant in a LFS-like patient with endometrial cancer coexisting with peritoneal malignant mesothelioma at the age of 54 years. However, no *TP53* pathogenic variants were identified in gene cancer panel testing of 381 unselected⁵⁶ or of a total of 745 clinician-referred familial^{57,58} endometrial cancer patients. In summary, there is limited information to suggest that *TP53* pathogenic variants may be associated with increased endometrial cancer risk.

Predisposition of *TP53* pathogenic variant carriers to multiorgan tumorigenesis presents a specific challenge for cancer risk management programs. There is some evidence from human studies and mouse models to suggest that use of radiotherapy may be contraindicated for cancer treatment (reviewed in McBride *et al*⁸⁶). Published reports indicate possible benefit of neonatal and annual whole-body magnetic resonance imaging (MRI) surveillance,^{87–89} and regular biochemical surveillance,⁸⁸ with detection of lesions at early stage. However, there is currently no international consensus on the components of a cancer screening program for *TP53* pathogenic variant carriers, and there is great variation in guidance for carriers across institutions, regions and countries—ranging from no action to comprehensive surveillance schedules being evaluated in a clinical trial setting at selected centers.⁸⁶

Given the complexities of counseling and clinical management of *TP53* variant carriers, even within the context of LFS, it is recommended that large-scale research studies be undertaken to establish the level of endometrial cancer risk, if any, associated with

pathogenic variants in *TP53*, before consideration of *TP53* in clinical testing for endometrial cancer diagnosis. Further, it should be acknowledged that most pathogenic *TP53* variants identified in the context of LFS are missense substitutions,⁹⁰ so assigning *TP53* variant pathogenicity for novel missense alterations will be particularly problematic for the analysis of a non-syndromic cancer. Thus, screening *TP53* in endometrial cancer patients identified both inside and outside of a LFS context will be useful for research purposes.

CHEK2

The *CHEK2* gene is a tumor suppressor that encodes a checkpoint kinase that participates in various cell processes, including cell cycle regulation, DNA repair, and apoptosis. Rare variants in this gene have been reported to be associated with moderately increased risks of cancer. The missense substitution c.470 T>C (p.Ile157Thr) has been reported to be associated with a 1.4-fold increased risk of breast cancer,^{91,92} and a 1.7-fold increased risk of colorectal cancer.⁹³ In addition, *CHEK2* c.349 A>G (p.Arg117-Gly), c.538C>T (p.Arg180Cys), and c.1036C>T (p.Arg346Cys) was reported to be associated with increased risk of breast cancer (ranging from 1.3- to 5.1-fold), whereas c.1312G>T (p.Asp438Tyr) and c.1343 T>G (p.Ile448Ser) were associated with a 2.2- to 3.0-fold risk of prostate cancer.⁹⁴ Most notably, *CHEK2* c.1100delC (p.Thr367Metfs*15), a founder variant in several European countries with carrier frequencies between 0.5 and 1%, has been convincingly shown to be associated with an ~2.3-fold increased risk of breast cancer for women.^{95–97} Consistent with these risk estimates from large-scale studies, segregation with disease is incomplete in families,⁹⁵ although it was observed at somewhat increased frequency of 5.1% in the initial study of breast cancer index cases with a family history of breast cancer,⁹⁵ and there is evidence for over-representation of the variant in cases enriched for family history.⁹⁷ Recently, a large-scale cohort study of 86 975 individuals from the Copenhagen region provided evidence that risk of multiple cancer types are associated with the c.1100delC variant;⁹⁸ the risks reported were 2.08-fold risk for breast cancer, and 1.45-fold for other cancer types combined. Considered individually, statistically significant associations were observed for cancers of the stomach, kidney, and prostate, although confidence intervals were wide. The risk estimate reported for uterine cancer in this study was 1.70 (0.70–4.14).

There have been a limited number of studies assessing the role of germline *CHEK2* variation in endometrial cancer specifically. A study supporting a link of endometrial cancer with *CHEK2* was a study of 629 colorectal cancer patients from the Netherlands which showed that c.1100delC

(p.Thr367Metfs*15) was more likely to be observed in those that reported a family history of colorectal or endometrial cancer than in sporadic endometrial cancer patients.⁹⁹ This observation was not replicated in a Swedish case–control study of 705 endometrial cancer cases (all subtypes) and 1565 controls, which found that *CHEK2* c.1100delC had no association with endometrial cancer risk.¹⁰⁰ Konstantinova *et al*¹⁰¹ showed that *CHEK2* c.470 T>C (p.Ile157Thr) was not associated with endometrial cancer when they compared 268 unselected endometrial cancer patients to 449 female controls from Bulgaria. In fact, the variant was observed more frequently in controls than in patients (2.45% vs 1.75% respectively) and showed no association with histological subtype, after being identified in individuals with endometrioid, mucinous, and clear-cell histologies. A Polish case–control study of four founder alleles in the *CHEK2* gene (c.1100delC (p.Thr367Metfs*15); c.470 T>C (p.Ile157Thr); c.909_1095del (p.Met304Leufs*16), reported as *del ex9–10*; c.444+1G>A) assessed colorectal and endometrial cancer risk from the analysis of 5496 unaffected controls, 1085 unselected colorectal cancer cases, 303 unselected endometrial cancer cases, and 463 colorectal or endometrial cancer probands reporting at least two cases of Lynch syndrome-associated cancer in first-degree relatives. Colorectal cancer risk was 2.5-fold increased for familial MMR-proficient cancer and unselected carriers of the c.470 T>C (p.Ile157Thr) variant, with no evidence for increased endometrial cancer risk in familial or unselected patients who tested negative for pathogenic variants in *MLH1*, *MSH2*, and *MSH6*.¹⁰² Assessment of 151 uterine serous carcinoma patients based in the USA⁷⁵ using a panel-based testing strategy identified two patients with mixed histology with either the germline *CHEK2* variant c.470 T>C (p.Ile157Thr) (0.7%) or c.1100delC (0.7%). Three other US-based multigene panel testing studies of endometrial cancer patients unselected for histological subtype have also identified *CHEK2* variants in probands, with frequencies of 0.3% (1/292, subtype unknown; variant nomenclature not specified) in patients with clinical features of Lynch syndrome⁵⁸, 1.0% (4/381, 3 endometrioid and 1 clear-cell subtype; all carrying c.1100delC) in unselected patients,⁵⁶ and 1.1% (5/453) in patients referred for genetic testing (subtype unknown).⁵⁷ The latter study,⁵⁷ reported 4/5 *CHEK2* variants as Pathogenic (c.433C>T (p.Arg145Trp), c.507delT (p.Phe169-LeufsX2), c.1100delC (p.Thr367MetfsX15), c.1283C>T (p.Ser428Phe)), and the other as Expected Pathogenic (c.470 T>C (p.Ile157Thr)).

In summary, evidence exists to support increased risk of cancers of the breast, colorectum, and possibly stomach, kidney, and prostate for the *CHEK2* c.1100delC loss-of-function variant, a finding that might be inferred to extend to other loss-of-function variants. However, the evidence based on limited data does not support association of *CHEK2*

variants with markedly increased risk of endometrial cancer, nor inclusion of this gene on an endometrial cancer-focused panel for clinical testing. It is relevant to note, although, that clinical testing of *CHEK2* c.1100delC variant has been introduced in the Netherlands for women with personal/family history of breast cancer referred for *BRCA1/2* testing and genetic counseling, and specific clinical management protocols including more intensive surveillance. For these and other clinical testing sites adopting such a stance, there may be an advantage to masked testing of *CHEK2* for endometrial cancer patients, with report back of the *CHEK2* c.1100delC variant as an actionable incidental finding only for those patients considered eligible for *CHEK2* testing due to their personal or family history of breast cancer.

MUTYH

The *MUTYH* gene product is part of the base excision repair system, which repairs oxidative damage to DNA. *MUTYH*-associated polyposis is an autosomal recessively inherited predisposition to adenomatous polyposis and colorectal cancer. From the most comprehensive study to date, the cumulative colorectal cancer risk to age 70 years for biallelic carriers is reported to be 75% (41–97%) for males and 72% (44–92%) for females, and for monoallelic carriers it is estimated to be 7% (5–11%) for males and 6% (4–9%) for females.¹⁰³ Data from 276 cases from 181 unrelated families provided evidence for significantly increased risk of cancers of the duodenum, ovary, bladder, and skin,¹⁰⁴ although confidence intervals on these estimates were very wide. The SIR reported for endometrial was 4.6 (0.6–16.5), based on only two reports in the data set.¹⁰⁴ Win *et al*¹⁰⁵ observed elevated cancer incidences in 2179 individuals estimated to be monoallelic *MUTYH* pathogenic variant carriers. The incidence of endometrial cancer in this cohort was 2.3 (1.2–5.1), giving a cumulative risk to age 70 years of 4% (2–8%). In an updated analysis of the same cohort, monoallelic carriers showed increased risk for gastric cancer (HR 9.3 (6.7–13)), hepatobiliary cancer (HR 4.5 (2.7–7.5)), endometrial cancer (HR 2.1 (1.1–3.9)), and breast cancer (HR 1.4 (1.0–2.0)).

Barnetson *et al*¹⁰⁶ assessed 225 unselected endometrial cancer patients for two common pathogenic *MUTYH* missense variants (reported in this study as Y165C and G382N; Human Genome Variation Society (HGVS) nomenclature to the common transcript NM_001128425.1: c.536A>G (p.Tyr179Cys), and c.1187G>A (p.Gly396Asp)) and analyzed the entire coding region if only one variant was identified. A single patient with endometrioid adenocarcinoma and a sebaceous carcinoma carried both pathogenic variants; five patients carried a single variant, a frequency was not significantly different to that found in unaffected controls from

the same population. Tricarico *et al*¹⁰⁷ have since reported two additional endometrial cancer patients with biallelic *MUTYH* pathogenic variants, as have Vogt *et al*¹⁰⁴ as noted above. In contrast, Ashton *et al*¹⁰⁸ genotyped 213 endometrial cancer patients and 226 controls for the two common missense variants and identified no biallelic carriers, and the genotype frequencies of the monoallelic carriers were not significantly different from the controls. One US-based multigene panel study did not identify any *MUTYH* variants in 381 unselected endometrial cancer patients⁵⁶ in patients, but two other US-based studies of 292 (ref. 58) and 453 (ref. 57) clinician-referred patients found 0.1–0.7% of endometrial cancer patients to be monoallelic carriers of *MUTYH* variants reported as Pathogenic (including c.536A>G (p.Tyr179Cys), c.545G>A (p.Arg182His), c.1145G>A (p.Gly382Asp), c.1185_1186dupGG (p.Glu396GlyfsX43)).

In summary, monoallelic carriers of *MUTYH* variant predisposing to colonic cancers may have a small increased risk of extracolonic cancers. Currently, evidence that monoallelic *MUTYH* carriers may be at a twofold risk of endometrial cancer is derived from essentially a single study, and confidence intervals on this estimate range from 1.1 to 3.9. Given the paucity of cases so far, it is not possible to ascertain an estimate of endometrial cancer risk for biallelic carriers. While some local diagnostic policies may recommend including genes on a panel if evidence supports a minimum of a twofold increased risk for the cancer being targeted, it is likely that the imprecision in risk estimates for endometrial cancer risks would prevent adoption of *MUTYH* on an endometrial cancer-focused clinical testing panel. However, as noted for *CHEK2* above, clinical management implications for other cancers may drive testing and selected reporting of this variant in endometrial cancer cases. Namely, current management recommendations for biallelic *MUTYH* carriers focus on the risk of colon cancer, and include colonoscopy every 2 years starting at the age of 18–20 years. Most patients are managed conservatively with polypectomy to remove adenomas, with surgery indicated if there are large numbers of adenomas.^{38,104} Usually no additional cancer screening is recommended in monoallelic *MUTYH* carriers pathogenic variant carriers; the National Comprehensive Cancer Network (<https://www.nccn.org>) guidelines recommendation for colonoscopy for mono-allelic *MUTYH* carriers is limited to only carrier individuals identified after presenting with personal history of adenomas or meeting guidelines for serrated polyposis syndrome i.e. management is defined by individual risk assessment. Thus, research testing with intent to report back results for the subset of endometrial cancer patients with personal or family history of polyposis or colorectal cancer may be considered appropriate by some test providers.

STK11

STK11 (also known as *LKB1*) is a tumor suppressor gene that is responsible for Peutz–Jeghers syndrome (PJS), which is a rare autosomal dominant disease that is characterized by gastrointestinal hamartomatous polyposis and mucocutaneous melanin spots. Individuals with PJS are at increased risk for a range of epithelial malignancies (colorectal, gastric, pancreatic, breast, and ovarian cancers), and rare cancers such as adenoma malignum of the cervix.¹⁰⁹ A meta-analysis of 104 women with PJS revealed that two PJS patients had developed endometrial cancer (RR = 16, 95% CI = 1.9–56), giving a cumulative risk from age 15 to 64 years of 9%.¹¹⁰ It would appear that *STK11* loss-of-function variants are very rare; only a single pathogenic *STK11* variant was identified across four different multigene panel testing studies of endometrial cancer patients^{56–58,75}—and it is notable that the variant occurred *in trans* with a deleterious *MSH6* variant in a 1/292 endometrial cancer patients with clinical features of Lynch syndrome.⁵⁸ Based on these observations, *STK11* testing may be appropriate for individuals from family with features of PJS syndrome, where there are implications for family management including extensive cancer surveillance recommendations¹¹¹ and family planning. However, there is no precise estimate of endometrial cancer risk, and inclusion in an endometrial cancer-focused panel should be considered for research purposes only.

Genes implicated in endometrial cancer for patients presenting as part of Cowden-like syndrome: *SDHB*, *SDHC*, *SDHD*, *AKT1*, *PIK3CA*, *KLLN*, and *SEC23B*

PTEN germline pathogenic variants are identified in only 5% of patients with features indicative of Cowden-like syndrome, which has led to investigation of other genetic causes of disease in these individuals. Germline variation in a number of genes has been reported as implicated in predisposition to cancer in Cowden-like families, namely the succinate dehydrogenase genes *SDHB*, *SDHC*, *SDHD*,^{54,112–114} and the *AKT1* and *PIK3CA* genes, which lie downstream of *PTEN* and act in the same molecular pathway.¹¹⁵

In initial studies, the standardized incidence rate of thyroid cancer was reported to be 63% (42–92%) for carriers of *SDHx* variants and 45% (26–73%) for *KLLN* ‘epimutations’,¹¹³ and inheritance of germline *SDHx* variants was implicated as causal for breast, renal and endometrial cancer in relatives of thyroid cancer patients.¹¹² However, more recent studies have shown that the frequency of *SDHB-D* variants is similar in cancer-affected probands with and without a germline pathogenic variant in *PTEN*. *SDHB-D* variants were identified in 8% of thyroid/breast/renal patients without *PTEN* pathogenic vari-

ants, vs 6% of those with a *PTEN* pathogenic variant ($P = 0.2$).⁶⁰ Similarly, germline missense alterations in *SDHB-D* were identified in 37/367 (10%) of endometrial cancer patients, two of whom also carried germline pathogenic variants in *PTEN*, and there was no difference in frequency of germline *SDHB-D* variants in *PTEN* carriers vs non-carriers ($P = 0.9$).⁵⁴ This has led to the suggestion^{54,60} that variants in *SDHB-D* may act as modifiers of cancer risk, that is, *SDHB-D* variants are likely not individually associated with a dominantly inherited high risk of cancer. The evidence for involvement of *AKT1* and *PIK3CA* germline variation in Cowden-like syndrome is from a single study of 91 individuals without germline *PTEN* pathogenic variants;¹¹⁵ variants considered to be deleterious to gene function, on the basis of molecular assays for all variants pooled, were identified in 10/91 individuals (11%) for *PIK3CA* (7 missense, 1 nonsense, 2 indels), and 2/91 (2%) for *AKT1* (both missense).

Further, germline promoter methylation of *KLLN*, which lies upstream of *PTEN*, has been proposed as a cause of inherited cancer in Cowden-like families.^{54,116} Germline *KLLN* methylation is reported to associate with cancer status in families,¹¹⁶ and with reduced expression of *KLLN* (but not *PTEN*). That is, the mechanism by which altered *KLLN* function would predispose to cancer is not directly related to altered function of *PTEN*. Endometrial cancer patients in Cowden-like families with *KLLN* germline methylation have been reported to have earlier age at onset, compared with those without methylation,⁵⁴ but as yet there is no evidence that genetic variation in *KLLN* is associated with methylation status, or directly with cancer risk.

Most recently, germline variants in *SEC23B* have been implicated as causal for cancer in Cowden-like families, by whole-exome sequencing of probands, follow-up of candidate pathogenic variants in multi-generational pedigrees, and subsequently in independent series of thyroid cancer patients with Cowden syndrome, and several TCGA cancer data sets.¹¹⁷ A *SEC23B* c.1781 T>G (p.Val594Gly) variant was identified in a single family, including a patient with endometrial cancer at the age of 35 years. Presumed pathogenic *SEC23B* germline variants were reported to be enriched in TCGA thyroid cancer patients with earlier age at onset (10 unique variants), four unique variants were identified in TCGA breast cancer cases, and three different variants were identified in TCGA endometrial cancer cases. Of the three variants identified in TCGA endometrial cancer cases (c.389 T>C (p.Ile130Thr), c.649C>T (p.Arg217*), c.490G>T (p.Val164Leu)), one overlapped with those reported for breast cancer cases and for thyroid cancer cases. Notably, 16/18 unique variations were missense alterations, predicted to be deleterious on the basis of bioinformatic prediction tools, and 8/18 variants were reported in the general population, albeit at low frequency (minor allele frequency ≤ 0.0075). A comparison of the *SEC23B* variant frequency in TCGA thyroid

cancer cases *vs* population controls, after selecting for the subset of variants with minor allele frequency ≤ 0.0004 , yielded an OR of 2.5 (1.3–4.7). While a similar analysis was not feasible for endometrial cancer (with only two cases eligible), these findings suggest that *SEC23B* variation is unlikely to be associated with a high risk of cancer.

In summary, there is still considerable uncertainty about the clinical significance of variants in the *SDHB-D* genes, and the relevance of *KLLN* methylation, with respect to endometrial cancer risk, and it has been publicly recommended that routine testing of these genes should not be conducted in the clinical setting.¹⁶ In addition, the current limited evidence suggesting a role for germline *AKT1* and *PIK3CA* variants in Cowden-like syndrome has not specifically addressed their relevance to endometrial cancer risk. Variation in *SEC23B* is unlikely to be associated with high levels of cancer risk. Taken together with some uncertainty about the functional and clinical significance of variants reported in these studies, it would seem premature to investigate variation in any of these proposed Cowden-like genes as causal for familial endometrial cancer outside the research setting. Germline mutations in the *SDH* genes (*SDHB/D* in particular) underlie predisposition to hereditary paraganglioma and pheochromocytoma, for which clinical management protocols have been developed,¹¹⁸ so report of variation in these genes might be considered appropriate only as incidental findings.

Additional genes reported as implicated in endometrial cancer risk predisposition from directed exome sequencing studies

Gene discovery approaches have identified three additional genes for which there is suggestive evidence for a role in hereditary endometrial cancer. For all of these genes, there is need to conduct large research studies to determine accurately the level and spectrum of cancer risk with variants considered deleterious to gene function, to inform inclusion of these genes in clinical testing and to define clinical management recommendations for variant carriers.

RINT1

Exome sequence analysis of early-onset breast cancer patients from multiple-case breast cancer families identified RAD50-interacting 1 (*RINT1*) as a putative cancer predisposition gene that is associated with breast and Lynch syndrome-spectrum cancers.¹¹⁹ One family was identified with a nonsense variant in *RINT1* (c.343C>T (p.Gln115*)) and included one individual diagnosed with both uterine cancer (age 30 years) and breast cancer (age 49 years) who was heterozygous for this variant. Additionally, comparisons of cancer incidences between the general

population and 23 families with rare *RINT1* variants (allele frequency < 0.5%) deemed to be potentially damaging by *in silico* prediction models showed an increased incidence of Lynch syndrome cancers in the families of *RINT1* variant carriers (SIR = 3.7 (1.4–7.7); $P = 0.009$). These rare variants consist of an in-frame deletion, a nonsense variant that truncates six amino acids from the end of the protein, and 21 missense variants. These findings suggested that *RINT1* variants that are deleterious to protein function may be associated with predisposition to multiple cancers, including endometrial cancer. However, a recent study evaluating *RINT1* in familial breast cancer risk has not provided support for this hypothesis, with no excess of rare truncating and evolutionarily conserved missense variants in 2024 cases *vs* 1886 controls, and no increased incidence of Lynch syndrome cancers in relatives of *RINT1* rare variant carriers.¹²⁰

NTLH1

The *NTLH1* base excision repair gene was recently discovered, also using a whole-exome sequencing approach, to be important in the development of adenomatous polyposis and colorectal cancer.¹²¹ Homozygous carriage of a germline nonsense alteration was identified in multiple polyposis-affected patients from three unrelated families, with progression to colorectal cancer in at least one family member. Of relevance to this review, all three polyposis-affected women developed an endometrial malignancy (at the age of 57 and 74 years) or premalignancy (at the age of 46 years). Numerous other cancers/tumors were reported in homozygous carriers, including breast cancer (at the age of 56 years), pancreatic cancer (at the age of 47 years), duodenal cancer (at the age of 52 years), and biliary tract hamartoma (at the age of 52 years). The frequency of homozygotes in the total 197 polyposis cases screened was significantly greater than expected ($P = 7 \times 10^{-8}$), based on the frequency of the risk allele in an in-house data set (0.0036), which was marginally greater than in the ExAC browser (0.0015). These findings suggest that individuals with homozygous loss of function of *NTLH1* are at increased risk of adenomatous polyposis, colorectal cancer, and possibly endometrial and other cancers.

FAN1

The FANCD2/FANCD1-associated nuclease 1 (*FAN1*) gene is a DNA interstrand crosslink repair gene. *FAN1* was recently linked to cancer predisposition by whole-exome sequencing of multiple colorectal cancer-affected relatives from a single family meeting the Amsterdam criteria, which identified the nonsense alteration c.141C>A (p.Cys47*) in all three patients tested.¹²² Follow-up testing of 176 additional colorectal cancer families identified three additional likely pathogenic *FAN1* variants in ~3% of families

with MMR-proficient colorectal cancer and Amsterdam positive family history: a truncating variant c.2854C>T (p.Arg952*), and two missense variants demonstrated to have functional effect in mitomycin assays, c.418G>T (p.Asp140Tyr) and c.1771C>T (p.Arg591Trp). An additional variant c.1018C>T (p.Pro340Ser), untested for function using mitomycin assays, was identified in additional families, with carrier status confirmed for two colorectal cancer patients (aged 40 and 70 years). Additional cancers were reported in these families, including breast cancer (aged 44 years, carrier status confirmed positive; aged 74 years, untested), pancreatic cancer (aged 63 years, untested), GI cancer (untested), and glioma (aged 50 years, untested). Of relevance to this review, early-onset endometrial cancer was reported in the mother and cousin (aged 46 and 42 years) of two brothers carrying the c.2854C>T (p.Arg952*) variant. However, these individuals (now deceased) were untested for the family variant. FAN1 deficiency, that is, homozygote loss of function, causes milder phenotypes compared with those observed for deficiency in other components of the FA pathway, namely the recessive disease karyomegalic interstitial nephritis,¹²³ characterized by slow progressive renal failure that leads to end-stage renal disease before the age of 50 years. So far, early-onset cancer has been reported for two families: rectal adenocarcinoma at the age of 30 years,¹²⁴ and hepatocellular carcinoma at the age of 22 years.¹²⁵ Taken together, these findings suggest that genetic variants deleterious to FAN1 function predispose to colorectal cancer, and possibly other cancers, that may include endometrial cancer.

Additional genes reported as implicated in endometrial cancer predisposition from multigene cancer panel screening studies

Variation in additional genes has been identified in endometrial cancer patients (unselected or clinician-referred for testing), by screening using commercial multigene cancer panels.^{56–58,126} Altogether, these include variants in *APC* (1 truncating), *ATM* (1 truncating, 2 splice site, 3 missense), *BRIP1* (5 truncating), *FANCC* (1 truncating), *NBN* (1 truncating), *PALB2* (1 truncating), and *RAD51C* (1 truncating, 1 large deletion). We consider these findings from two angles—as an explanation for development of endometrial cancer, or as clinically meaningful incidental findings.

Regarding a possible causal role in endometrial cancer, clinical information extracted from the studies above does not lend strong support for involvement of most of these gene variants in high-risk of endometrial cancer. Only one of these patients reported disease onset under the age of 50 years (*APC*, age 28 years),⁵⁶ and four patients reported endometrial cancer in addition to (prior) breast cancer (two *ATM* (truncating, splice site), *FANCC*, *PALB2*),⁵⁷ raising the question of possible endometrial cancer development due to

tamoxifen use. Second, there is limited evidence for inclusion of most of these genes in cancer panels, and/or for the level of cancer risk associated with variants reported as pathogenic (including loss-of-function and/or missense alterations in important protein functional domains). Indeed, although *BRIP1*-truncating variants were initially reported to be associated with a twofold risk of breast cancer from a study of ~1200 cases and 2000 controls,¹²⁷ the largest study to date (>16 000 breast cancer cases, 7300 controls) provides convincing evidence that there is no increased risk of breast cancer associated with truncating variants in *BRIP1*.¹²⁸ While *BRIP1* variation may be associated with risk of other cancers including ovarian cancer,¹²⁹ the findings reported so far are based on relatively small studies and should be interpreted with caution. Only *APC* would be considered a well-recognized high-risk cancer syndrome gene for which clinical practice guidelines have been established.³⁸ On the basis of convincing evidence for risk of breast cancer equivalent to that of truncating variants in *BRCA2* for *PALB2*-truncating variants,¹³⁰ and for a single *ATM* missense alteration (c.7271 T>G (p.Val2424Gly)),¹³¹ clinical management protocols for breast cancer prevention have been put forward for these specific gene alterations.^{132,133} Further, researchers from the Netherlands and United Kingdom have proposed intensified surveillance for breast cancer from 40 to 50 years of age for heterozygous carriers of any *ATM* loss-of-function variant, based on their meta-analysis of published data estimating RR 3.0 (2.1–4.5) for breast cancer.¹³³ However, while clinically meaningful risk association for other specific variants/variant types/genes is not to be discounted,¹³⁴ and a recent paper has presented a framework for counseling of moderate-penetrance cancer-susceptibility variants,¹³⁵ large-scale studies are essential to provide convincing evidence for wider (international) acceptance of their clinical value.

Given this background, apart from the exceptions noted above as relevant for report of incidental findings (*APC* loss-of-function, *PALB2*-truncating variants, *ATM*.7271 T>G (p.Val2424Gly), and possibly *ATM* loss-of-function variants), it would be inadvisable to interpret variation in the seven additional 'cancer panel' genes above as cause for altered clinical management in endometrial cancer patients. Testing of these genes for endometrial cancer risk should only be considered as a research exercise.

Evidence for proposed endometrial cancer risk genes from the analysis of germline variation in endometrial cancer patients from TCGA Project

Details of the sequencing analysis and classification methodology are summarized in the Supplementary Text. Table 1 summarizes the information for rare germline variants detected in TCGA endometrial

Table 1 Rare germline variants in confirmed/candidate hereditary endometrial cancer genes in TCGA endometrial cancer cases^a

Gene name	Transcript ID	Clinvar NM	Chromosome	Position-Hg-38	Reference allele	Alternate allele	Variant effect (SnPEff)	Sample ID(s)	Minor allele frequency (ExAC_nonTCGA)	rs ID	HGVS c. nomenclature	HGVS p. nomenclature	MaxEntScan (reference > alternate allele score)	Clinvar variant classification (submitter/s)	Classification assigned (this study) ^b	Reported by Lu et al ^c	Tumor subtype
MSH2	ENST00000233146	NM_000251.2	2	47 403 357	G	T	Stop_gained	TCGA-D1-A1O8	Null	Rs587779102	c.166G>T	p.(Glu56Ter)	NA	Pathogenic (InSIGHT)	Pathogenic: truncating	No—assessed in discovery phase only	EEC
MSH2	ENST00000233146	NM_000251.2	2	47 429 943	T	C	Splice_donor_variant	TCGA-D1-A175	Null	Rs267607953	c.1276+2T>C	p.?	8.92 > 1.17	Pathogenic (GeneDx); likely pathogenic (Mayo Clinic Genetic Testing Labs)	Likely pathogenic: likely to affect splicing, MSI-H	No—assessed in discovery phase only	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 800 546	AT	A	Frameshift_variant	TCGA-EC-A24G	Null	Null	c.2565delT	p.(Ile856LeufsTer12)	NA	Null	Pathogenic: truncating	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 806 605	AAAGC	A	Frameshift_variant	TCGA-A5-A0VP	Null	Null	c.3956_3959delAAGC	p.(Ala1320GlufsTer6)	NA	Null	Pathogenic: truncating	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 799 111	AAAGAG	A	Frameshift_variant	TCGA-AX-A1C5	Null	Rs267608077	c.1135_1139delAGAGA	p.(Arg379Ter)	NA	Pathogenic (InSIGHT/ Ambry Genetics/ GeneDx/University of Washington Department of Laboratory Medicine); likely pathogenic (Mayo Clinic Genetic Testing Labs)	Pathogenic: truncating	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 806 842	T	TTTGA	Frameshift_variant	TCGA-BS-A0TJ	2.43E-03	Rs55740729	c.4068_4071dupGATT	p.(Lys1358AspTer2)	NA	Benign (Invitae); likely benign (InSIGHT/ GeneDx/ Ambry Genetics/ Illumina Clinical Services Laboratory)	Likely benign (truncating at 3' end of gene)	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 799 750	TC	T	Frameshift_variant	TCGA-AJ-A3NE	Null	Null	c.1772delC	p.(Pro591GlnfsTer19)	NA	Pathogenic (Ambry Genetics)	Pathogenic: truncating	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 798 826	A	AAC	Frameshift_variant	TCGA-D1-A167	Null	Rs752540976	c.843_844insAC	p.(Val282ThrfsTer10)	NA	Null	Pathogenic: truncating	Yes	EEC
MSH6	ENST00000234420	NM_000179.2	2	47 806 652	GTAAC	G	Splice_donor_variant	TCGA-AP-A1DM; TCGA-BG-A0VZ; TCGA-BG-A187	1.27E-03	Rs267608132	c.4001+12_4001+15delACTA	p.?	6.25>6.25	Uncertain (InSIGHT/ Ambry Genetics/Mayo Clinic Genetic Testing Laboratories); benign (GeneDx/Invitae/Emory Genetics Laboratory); Likely Benign (Counsyl)	Uncertain: no predicted effect on splicing, 2 MSI-H and 1 MSI-L tumor	No	EEC; EEC; EEC
PMS2	ENST00000265849	NM_000535.6	7	5 986 883	G	A	Stop_gained	TCGA-DF-A2KY	2.83E-05	Rs63750451	c.1882C>T	p.(Arg628Ter)	NA	Pathogenic (InSIGHT/ Ambry Genetics/ GeneDx/OMIM)	Pathogenic: truncating	Yes	EEC
PMS2	ENST00000265849	NM_000535.6	7	5 987 078	G	A	Stop_gained	TCGA-B5-A11G; TCGA-B5-A3FC	Null	Rs587778618	c.1687C>T	p.(Arg563Ter)	NA	Pathogenic (Ambry Genetics/GeneDx)	Pathogenic: truncating	Yes	EEC; EEC
PTEN	ENST00000371953	NM_000314.6	10	87 957 961	CTG	C	Frameshift_variant	TCGA-QF-A5YT	Null	Rs780264945	c.750_751delTG	p.(Cys250TrpfsTer2)	NA	Null	Pathogenic: truncating	No—assessed in discovery phase only	EEC
BRCA1	ENST00000357654	NM_007294.3	17	43 124 027	ACT	A	Frameshift_variant	TCGA-BS-A0TI	2.17E-04	Rs386833395	c.68_69delAG	p.(Glu23ValfsTer17)	NA	Pathogenic (ENIGMA/ ColorGenomics/Quest Diagnostics/CIMBA/ Illumina Clinical Services Laboratory/ Counsyl/Emory Genetics Laboratory/ University of Washington Department of Laboratory Medicine/ Michigan Medical Genetics Laboratories/ Laboratory for Molecular Medicine, Partners HealthCare Personalized Medicine/ GeneDx/Invitae/ Ambry Genetics Pathway Genomics BIC/ GeneReviews/OMIM/ SCRP/Biesecker Lab/ Human Development Section, NIH-ClinSeq)	Pathogenic: truncating	Yes	EEC
BRCA1	ENST00000357654	NM_007294.3	17	43 082 517	TC	T	Frameshift_variant	TCGA-BG-A0M8	Null	Rs80357981	c.4243delG	p.(Glu1415LysfsTer4)	NA	Pathogenic (ENIGMA CIMBA/ Ambry Genetics/BIC/ SCRP)	Pathogenic: truncating	Yes	EEC

Table 1 (Continued)

Gene name	Transcript ID	Clinvar NM	Chromosome	Position-Hg-38	Reference allele	Alternate allele	Variant effect (SnPEff)	Sample ID(s)	Minor allele frequency (ExaC_nonTCGA)	rs ID	HGVS c. nomenclature	HGVS p. nomenclature	MaxEntScan (reference > alternate allele score)	Clinvar variant classification (submitter/s)	Classification assigned (this study) ^b	Reported by Lu <i>et al</i> ^c	Tumor subtype
<i>BRCA1</i>	ENST00000357654	NM_007294.3	17	43 091 783	C	A	Stop_gained	TCGA-A5-A0CJ	9.42E-06	Rs28897686	c.3748G>T	p.(Glu1250Ter)	NA	Pathogenic (ENIGMA/ CIMBA/Ambry Genetics/University of Washington Department of Laboratory Medicine, University of Washington/Quest Diagnostics Nichols Institute San Juan Capistrano/Invitae/ GeneDx/SCRIP/BIC/ OMIM)	Pathogenic: truncating	Yes	EEC
<i>TP53</i>	ENST00000269305	NM_000546.5	17	7 675 237	C	CT	Splice_acceptor_variant	TCGA-AP-A059	9.42E-06	Rs751253294	c.376-2dupA	p.?	10.63 > 5.09	Uncertain (Ambry Genetics/Invitae/ GeneDx)	Uncertain: predicted effect on splicing, but score change suggests partial effect	No—assessed in discovery phase only	EEC
<i>MUTYH</i>	ENST00000450313	NM_001128425.1	1	45 332 088	T	C	Splice_acceptor_variant	TCGA-BS-A0UT	1.09E-03	Rs77542170	c.934-2A>G	p.?	7.48 > -0.48	Likely pathogenic (GeneDx/Invitae/Lab for Molecular Medicine, Partners HealthCare Personalized Medicine-CSEB- Medseq/ Biesecker Lab/Human Development Section, NIH-ClinSeq); Uncertain (Ambry Genetics)	Likely pathogenic: likely to affect splicing	Yes	EEC
<i>NTHL1</i>	ENST00000219066	NM_002528.6	16	2 046 238	G	A	Stop_gained	TCGA-D1-A17F; TCGA-EO-A22X	1.59E-03	Rs150766139	c.268C>T	p.(Gln90Ter)	NA	Pathogenic (OMIM)	Pathogenic: evidence of functional effect in colorectal cancer	Not assessed	EEC; EEC
<i>FAN1</i>	ENST00000362065	NM_014967.4	15	3 0914 092	G	A	Splice_donor_variant	TCGA-BG-A220	9.42E-06	Rs766292876	c.1811+1G>A	p.?	10.8 > 2.58	Null	Likely pathogenic: predicted effect on splicing	Not assessed	EEC
<i>FAN1</i>	ENST00000362065	NM_014967.4	15	30 937 260	G	A	Splice_donor_variant	TCGA-A5-A0GR; TCGA-AP-A5FX; TCGA-B5-A11G; TCGA-B5-A11R; TCGA-BK-A6W3; TCGA-EY-A1GU; TCGA-EY-A3L3; TCGA-JU-AAVI	3.56E-03	Rs114680636	c.*3+1G>A	p.?	9.26 > 1.08	Null	Likely pathogenic: predicted effect on splicing	Not assessed	EEC; mixed; EEC; EEC; EEC; SEC; SEC
<i>BRIP1</i>	ENST00000259008	NM_032043.2	17	61 683 849	GA	G	Frameshift_variant	TCGA-AP-A052	2.83E-05	Rs730881645	c.3196delT	p.(Ser1066HisfsTer12)	NA	Uncertain (GeneDx/ Ambry Genetics)	Gene of uncertain significance for cancer risk	Yes	SEC
<i>BRIP1</i>	ENST00000259008	NM_032043.2	17	61 683 837	GA	G	Frameshift_variant	TCGA-EO-A3B0	Null	Null	c.3208delT	p.(Ser1070GlnfsTer8)	NA	Null	Gene of uncertain significance for cancer risk	Yes	EEC
<i>NBN</i>	ENST00000265433	NM_002485.4	8	89 971 173	CTGTT	C	Frameshift_variant	TCGA-B5-A11N	2.83E-05	Rs587780100	c.698_701delAACA	p.(Lys233SerfsTer5)	NA	Pathogenic (Ambry Genetics/GeneDx/ Invitae/Genetic Services Lab, University of Chicago/OMIM)	Pathogenic variant for NBS: truncating (moderate risk for breast cancer)	Yes	EEC

Abbreviations: ACMG, Annual Clinical Genetics Meeting; ENIGMA, Evidence-based Network for the Interpretation of Germline Mutant Alleles; HGVS, Human Genome Variation Society; MMR, mismatch repair; NA, not applicable; null, not present in database/s; TCGA, The Cancer Genome Atlas.

All patients are postmenopausal women except A0M8 (between pre- and postmenopausal) and A187 (information not available).

^aAlthough comprehensive structural variant analysis was not undertaken for the genes reviewed, the analysis pipeline identified a possible gene rearrangement in TCGA-AX-A063, confirmed as an *MLH1* exon 16 duplication following IGV review.

^bClassification assigned following interpretation criteria from InSiGHT (MMR genes), ENIGMA (*BRCA1/2*), or modification of ACMG criteria (others), considering moderate–high association with endometrial and/or other cancers.

^cLu *et al*¹³⁶ analyzed only a subset of the genes listed in the TCGA endometrial cancer patient data set. *MSH2*, *PTEN* and *TP53* were assessed in the discovery sample set only (258 endometrial cancer patients, IDs not provided).

Table 2 Evidence to support clinical vs research testing of genes implicated in hereditary endometrial cancer.

Syndrome	Gene(s)	Endometrial cancer risk associated with variants deleterious to gene/protein function	Other cancer types associated with variants deleterious to gene/protein function	Clinically actionable for prediction and prevention of non-endometrial cancer types?	Cancer risk management strategies ^a	References
Lynch syndrome	MLH1, MSH2, MSH6, PMS2, EPCAM (deletion)	High lifetime risk (18–71%)	Colorectal, ovarian, gastric, various other	Yes	<ul style="list-style-type: none"> •Colonoscopy/polypectomy •Gynecological screening •Risk-reducing hysterectomy and salpingo-oophorectomy •Endoscopy 	38,111
Polymerase proofreading-associated polyposis	POLD1, POLE	Unknown Limited data suggests moderate/high	Colorectal, gastric, various other	Uncertain	Currently, no consensus guidelines, but suggested management may include: <ul style="list-style-type: none"> •Colonoscopy/polypectomy •Endoscopy •Endometrial screening 	45
Cowden syndrome	PTEN	High lifetime risk (19–28%)	Thyroid, breast, colorectal, various other	Yes	<ul style="list-style-type: none"> •Endometrial screening •Risk-reducing hysterectomy •Mammography •Possibly risk-reducing mastectomy •Examination of the thyroid •Colonoscopy/polypectomy 	59,60
Hereditary breast–ovarian cancer syndrome	BRCA1, BRCA2	Uncertain, given possible confounding with tamoxifen use: if proven, likely modest (< 2-fold)	Breast, male breast, ovarian/fallopian tube/peritoneal, pancreatic, prostate	Yes	<ul style="list-style-type: none"> •Mammography, breast magnetic resonance imaging •Risk-reducing medication •Risk-reducing mastectomy •Risk-reducing salpingo-oophorectomy 	Various sources, summarized in Petrucelli <i>et al</i> ¹³⁷ and Easton <i>et al</i> ¹⁷
Li–Fraumeni syndrome	TP53	Unknown If proven, likely modest	Soft-tissue and bone sarcomas, breast, brain, adrenocortical, various other	Yes	Currently, no consensus guidelines, but may include: <ul style="list-style-type: none"> •Breast magnetic resonance imaging •Risk-reducing mastectomy •Whole-body magnetic resonance imaging •Biochemical surveillance •Radiotherapy may be contraindicated for cancer treatment 	86–89
CHEK2-associated cancer	CHEK2	Unknown Limited data suggests risk is likely modest < 2-fold	Breast	Yes	For patients with suspected familial breast cancer: <ul style="list-style-type: none"> •Mammography, breast magnetic resonance imaging 	138
MUTYH-associated polyposis	MUTYH	Monoallelic carriers—limited data suggests modest (~2-fold), ~4% lifetime risk Biallelic carriers—unknown	<i>Biallelic</i> : Colorectal, duodenal	Yes: <i>biallelic only</i>	<ul style="list-style-type: none"> •Colonoscopy/polypectomy •Endoscopy •Risk-reducing colectomy 	38,111,104
Peutz–Jeghers syndrome	STK11/LKB1	9% (based on limited data)	Colorectal, pancreatic, breast, ovarian, other	Yes	<ul style="list-style-type: none"> •Colonoscopy/polypectomy •Enteroscopy •Mammography, breast magnetic resonance imaging •Gynecological screening •Pancreatic screening 	111
Cowden-like syndrome	SDHB, SDHC, SDHD, AKT1, PIK3CA, KLLN, and SEC23B	Unknown Limited data suggests risk is modest—moderate (~2.5-fold).	SDH genes: paraganglioma, renal, gastrointestinal stromal sarcoma	Yes: <i>SDH</i> genes only	<ul style="list-style-type: none"> •Biochemical surveillance •Magnetic resonance imaging 	118

Table 2 (Continued)

Syndrome	Gene(s)	Endometrial cancer risk associated with variants deleterious to gene/protein function	Other cancer types associated with variants deleterious to gene/protein function	Clinically actionable for prediction and prevention of non-endometrial cancer types?	Cancer risk management strategies ^a	References
RINT1-associated cancer	<i>RINT1</i>	Unknown Association not replicated in a study with power to detect a 2-fold risk	Breast, colorectal, other	No	None yet	N/A
NTLH1-associated cancer	<i>NTLH1</i>	Unknown Limited data suggests risk for homozygote carriers only	Colorectal, various others	No	None yet	N/A
FAN1-associated cancer	<i>FAN1</i>	Unknown Limited data suggests moderate–high risk	Colorectal, various others	No ^b	None yet	N/A
Familial adenomatous polyposis	<i>APC</i>	High lifetime risk (close to 100% for colorectal cancer)	Colorectal, duodenal, thyroid, desmoid	Yes	<ul style="list-style-type: none"> •Colonoscopy/polypectomy •Endoscopy •Risk-reducing colectomy 	38,111
PALB2-associated cancer	<i>PALB2</i>	Unknown	Breast, pancreatic	Yes: Monoallelic for predicted truncating variants only for high risk of breast cancer	No formal recommendations, but suggestions include: <ul style="list-style-type: none"> •Increased surveillance for breast cancer 	132
				Biallelic for loss-of-function variants—for Fanconi anemia	Biallelic carriers of loss-of-function variants would be managed for multiple phenotypic manifestations of Fanconi anemia, in addition to cancer risk	139
Ataxia-Telangiectasia syndrome	<i>ATM</i>	Unknown	Breast, various other	Yes: Monoallelic for c.7271C>G—for high risk of breast cancer	Monoallelic carriers of c.7271C>G: <ul style="list-style-type: none"> •Breast cancer risk management as for <i>BRCA2</i> carriers 	131
				Monoallelic for other loss-of-function variants—for moderate risk of breast cancer	Monoallelic carriers of other loss-of-function variants (eg, truncating variants): <ul style="list-style-type: none"> •Increased surveillance for breast cancer 	133
				Biallelic for any loss-of-function variant for ataxia telangiectasia	Biallelic carriers of loss-of-function variants would be managed for multiple phenotypic manifestations of ataxia telangiectasia, in addition to cancer risk	140
Fanconi anemia	<i>BRIP1/FANCF</i> <i>FANCC</i> <i>RAD51C/FANCO</i>	Unknown	Uncertain (but <i>no breast</i> cancer risk for <i>BRIP1</i>)	Yes: <i>biallelic only</i> for Fanconi anemia	Biallelic carriers of loss-of-function variants would be managed for multiple phenotypic manifestations of Fanconi anemia, in addition to cancer risk	139
Nijmegen breakage syndrome	<i>NBN</i>	Unknown	Uncertain	Yes: <i>biallelic only</i> for Nijmegen breakage syndrome	Biallelic carriers of loss-of-function variants would be managed for multiple phenotypic manifestations of Nijmegen breakage syndrome, in addition to cancer risk	141

Abbreviation: N/A, not applicable.

For the subset of genes noted in bold, the level of evidence for association with *high* risk of endometrial cancer is considered sufficient to support clinical testing of endometrial cancer patients.

^aUnless otherwise noted, management strategies listed are those considered to be usual recommendations applied for patients ascertained in the familial cancer setting.

^bFAN1—biallelic loss of function is associated with early-onset kidney dysfunction, but there are no established guidelines for management of hereditary kidney dysfunction.

cancer patients,⁴ and for genes described in this review as known or potentially involved in endometrial cancer predisposition. The majority of presumed or likely pathogenic variants identified were in the MMR genes (2 *MSH2*, 5 *MSH6*, 2 *PMS2* (in 3 patients)), observed in 10/543 (1.8%) of patients. These results confirm the importance of MMR genes in endometrial cancer gene panel testing. Additional presumed or likely pathogenic variants were identified in *PTEN* ($n=1$), *BRCA1* ($n=3$, all endometrioid), *MUTYH* ($n=1$, heterozygote), *NTLH1* ($n=1$), *FAN1* ($n=2$), and *NBN* ($n=1$), providing additional evidence that pathogenic variants in these genes should be considered for inclusion in future population-based research studies estimating EC risk.

Conclusions

This review, combined with analysis of publicly available data for endometrial cancer patients, provides a comprehensive report of current evidence to guide the selection of genes for clinical and research gene testing of endometrial cancer patients (summarized in Table 2). Based on well-established association with endometrial cancer risk and agreed clinical management guidelines, only six genes are currently considered appropriate for clinical 'diagnostic' testing of an individual with endometrial cancer—the MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*), *EPCAM* deletion due to its effect on *MSH2*, and *PTEN*. Although *POLD1* and *POLE* have recently been suggested as familial endometrial cancer genes, absolute risks for endometrial and even other cancer types are very uncertain, so the clinical utility of testing is not yet firm. At present, there is insufficient evidence to support testing *BRCA1/2* in patients with isolated serous endometrial cancer in the absence of a relevant personal/family history of breast/ovarian cancer. *BRCA1/2* and other genes reported as implicated in inherited endometrial cancer are at best likely to be associated with moderate or low risk of endometrial cancer. It is recommended that testing of these additional genes in endometrial cancer cases should be considered a research exercise, with need for careful patient counseling to distinguish between test results relevant to hereditary endometrial cancer and those that are actionable incidental findings due to their association with risk of other (non-endometrial) cancers (outlined in Table 2). Research testing of both familial and unselected endometrial cancer cases will be necessary to re-evaluate the descriptions of known inherited cancer syndromes, and to estimate the contribution of specific genes and variant types to the etiology of endometrial cancer at the population level. Taken together, such information will drive meaningful clinical genetic testing and counseling of endometrial cancer patients in the future.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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