Germline multigene panel testing in gynecological cancer
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Abstract
Multi-gene panel testing (MGPT) has become widely used in clinical practice. MGPT allows a set of genes to be tested simultaneously, making it a powerful, time- and cost-effective tool for detecting genetic variants. The purposes of identifying germline pathogenic variants is threefold: diagnosis, prevention, and treatment. Germline variants in certain genes cause hereditary tumor syndromes. Diagnosing hereditary tumors enable to predict the types of cancer that may develop in the future. The blood relatives of those diagnosed may also suffer from the same hereditary tumor syndrome. For such individuals, medical intervention tailored to their condition can reduce the incidence of cancer or help in cancer detection in the early stages. Diagnosis of hereditary tumors may also change the cancer treatment strategy for the diagnosed patient. To date, more than 100 genes have been found to have a predisposition to cancer. The type of cancer one develops, the risk of developing it, and the possible preventive strategy differs among genes. The association of some genes with cancer predisposition has not yet been fully confirmed. Nowadays, various types of MGPTs are available, and the genes included differ among the tests. In addition, no consensus has been reached on which genes to be included in MGPT. This review is aimed to summarize the advantages and limitations of MGPT along with some practical considerations while performing MGPT and the gynecological tumors associated with genes commonly included in MGPT.

Keywords
multi-gene panel testing (MGPT); hereditary gynecological cancer; cancer predisposition genes; Lynch syndrome; Peutz-Jeghers syndrome; PTEN hamartoma tumor syndrome; BRCA-related breast/ovarian cancer syndrome; DICER1 syndrome; rhabdoid tumor predisposition syndrome; Gorlin-Goltz syndrome

1. Introduction
The first human genome sequence was produced as a part of the Human Genome Project in 2003 with a timespan and effort of 13 years and a cost of more than $500 million. DNA sequencing technology has advanced so rapidly that it is now possible to sequence a human genome in almost a day at a cost of less than $1000. After sequencing, raw data are aligned to the reference sequence and the differences are identified, which are termed as variants. The variants, which are confirmed after filtration to remove artifacts, are further analyzed for their pathogenicity. Whole genome sequencing is not yet routinely used in clinical practice, while multi-gene panel testing (MGPT), which allows a series of genes to be tested simultaneously, has become widely used in clinical practice. MGPT offers significant advantages over testing for single genes one after another. It is more powerful, faster, and less expensive in detecting pathogenic variants.

Since the early 1990s, more than 100 genes have been identified as possible causative genes for hereditary cancers. Some genes are associated with hereditary gynecological cancers. For example, BRCA1 and BRCA2 (BRCA1/2) are associated with ovarian cancer and MLH1, MSH2, MSH6, and PMS2, which are generally referred to as mismatch repair (MMR) genes, are associated with endometrial cancers (ECs). Both BRCA1/2 and MMR genes play pivotal roles in DNA repair. Incorrectly or insufficiently repaired DNA leads to subsequent genome instability, resulting in malignant transformation of cells. Thus, loss of function variants in these genes increase the lifetime risk of developing cancer. Mechanisms underlying the increased risk of cancer vary by gene, as do the types of cancers that can be developed and accompanied physical findings.

Tumors caused by germline pathogenic variants (GPVs) in certain genes account for 5–10% of all cancers. With the widespread clinical use of DNA sequencing, several studies have reported the rate of patients detected with GPVs (Table 1). In the TCGA cohort, GPVs were detected in 8% of 10,389 adult cancer cases across 33 cancers [1]. GPVs were identified in 19.9% of 412 ovarian cancer cases, 6.8% of 543 EC cases and 6.6% of 305 cervical cancer cases. Some of the GPVs identified may not be the cause of the cancer that is currently developing. In another study including 11,947 patients, in
which 76% of the patients were suffering from a metastatic or recurrent disease, GPVs were identified in 17% of all patients, with the highest rate of 25.5% in 721 ovarian cancer patients [2]. The use of MGPT will contribute to improving the detection rate of GPVs in cancer predisposition genes.

Sequencing technology has become widely used in clinical practice to search not only GPVs but also somatic mutations. Germline sequencing is performed on blood sample to identify GPVs. Sequencing performed on tumor tissue or circulating tumor DNA (ctDNA) in the plasma is originally intended to detect somatic mutations in cancer cells. However, the variants detected through sequencing of tumor tissue or ctDNA are not limited to the somatic mutations, but can also reveal GPVs. In addition, a variety of test other than sequencing can also reveal the possibility of hereditary cancer syndrome. Homologous recombination repair deficiency (HRD) testing performed to determine the eligibility to Poly (ADP-ribose) polymerase (PARP) inhibitors may sometimes reveal the possibility of GPVs in genes involved in homologous recombination pathway including BRCA1/2. Positive results of microsatellite instability (MSI) testing, which are performed to determine the candidates for immune checkpoint inhibitors, may indicate the possible presence of GPVs in MMR genes. With these increasing opportunities to detect GPVs in the clinical setting, clinicians, particularly those involved in cancer treatment, are encouraged to become familiar with hereditary cancers caused by GPVs in certain genes.

This review summarizes the characteristics of MGPT and some points to consider in performing MGPT and describes the hereditary cancer syndromes associated with gynecological cancers or tumors.

### 2. Genetic Tests for Diagnosing Hereditary Tumors

The typical clinical features of hereditary cancers include younger age of onset, a history of specific cancers in families, the presence of multiple types of cancers in one person, and the occurrence of cancer in both organs in a pair of organs.

Historically, genetic testing for cancer patients has been conducted by first inferring the most likely hereditary cancer syndromes based on family and personal history of cancer and pathological subtypes and subsequently testing for the single genes associated with susceptible syndromes. The National Comprehensive Cancer Network (NCCN) hereditary cancer testing criteria is one of the current standards for identifying individuals at suspected risk of hereditary cancer. Previously, guidelines from the NCCN restricted recommendations for genetic testing to a few genes: BRCA1/2, PTEN, TP53, MMR genes, APC, and MUTYH. In addition, for each gene, there was a set of testing criteria, which consisted of personal or family history of cancer and pathological subtypes, and genetic testing was recommended to be offered to individuals who met those criteria.

However, next-generation sequencing technology has enabled a set of genes to be tested simultaneously at a low cost. The introduction of MGPT has increased the numbers of individuals diagnosed with GPVs in cancer predisposition genes, which could not be identified by conventional single-gene tests. Myriad had used patents for the BRCA1/2 for 15 years, blocking all other laboratories from offering clinical testing of these genes. In 2013, the U.S. Supreme Court ruled that human genes cannot be patented [3, 4]. Since then, several companies have entered the genetic testing market and now offer a variety of MGPTs.

Growing evidence has emerged that the genes other than BRCA1/2 confer an increased risk for ovarian and breast cancer predisposition. In case of breast cancer, GPVs in several genes including PTEN, TP53, PALB2, CHEK2, and ATM have been implicated with the elevated incidence. In fact, in 2014, MGPT replaced BRCA1/2-only tests in clinical practice, resulting in a two-fold higher detection rate of clinically relevant GPVs [5].

In 2020, the NCCN guidelines made a major paradigm shift by changing their description to consider MGPT first among genetic tests. As mentioned above, MGPT is a useful tool for diagnosing hereditary tumors; however, some points should be considered while performing MGPT.

### 3. Genetic Testing Criteria

Genetic testing criteria have been developed based on genetic risk assessment. Personal and family history of cancer plays a vital role in risk assessment. Several risk assessment tools and guidelines for genetic testing criteria based on the genetic risk assessment have been developed. Several professional organizations provide guidelines for genetic testing, including

<table>
<thead>
<tr>
<th>Study</th>
<th>No. Patients</th>
<th>Testing approach</th>
<th>Rate of patients detected with GPVs in cancer predisposition genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meric-Bernstam 2016</td>
<td>1000</td>
<td>Targeted exome sequencing of 201 genes</td>
<td>All cancer: 43/1000 (4.3%); Ovarian: 5/36 (13.9%); Endometrial: 0/9 (0%); Cervical: 0/5 (0%)</td>
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<tr>
<td>Mandelker 2017</td>
<td>1040</td>
<td>76-gene panel</td>
<td>182/1040 (17.5%); Ovarian: 6/19 (31.6%); Endometrial: 4/25 (16%); Cervical: 0/0 (0%)</td>
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<td>Huang 2018 [1]</td>
<td>10,389</td>
<td>Whole exome sequencing (WES)</td>
<td>818/10,389 (7.9%); Ovarian: 82/412 (19.9%); Endometrial: 37/543 (6.8%); Cervical: 20/305 (6.6%)</td>
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<td>Stadler 2021 [2]</td>
<td>11,947</td>
<td>76- or 88-gene panel</td>
<td>2037/11,947 (17.1%); Ovarian: 184/721 (25.5%); Endometrial: 121/871 (13.9%)</td>
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<tr>
<td>Cobain 2021 [102]</td>
<td>1015</td>
<td>WES or targeted exome sequencing of 1700 genes</td>
<td>160/1015 (15.8%); Ovarian: 5/15 (33.3%); Endometrial: 0/3 (0%); Cervical: 1/1 (100%)</td>
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<td>Samadder 2021 [103]</td>
<td>2984</td>
<td>83- or 84-gene panel</td>
<td>397/2984 (13.3%); Ovarian: 26/126 (20.6%); Endometrial: 13/98 (13.3%); Cervical: 0/2 (0%)</td>
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**TABLE 1. The rate of cancer patients detected with GPVs.**

No. Patients: the number of patients studied.
Testing approach: the type of testing used.
Rate of patients detected with GPVs: the percentage of patients identified with GPVs in each category.

**Note:** The data represents a summary of various studies and may not be exhaustive.
Society of Gynecologic Oncology (SGO), U.S. Preventive Service Task Force (UPSTF) and the American College of Obstetricians and Gynecologists (ACOG) [6–8]. American College of Medical Genetics (ACMG) and National Society of Genetic Counselors published a practice guideline on referral indications for cancer predisposition assessment in 2015 and later added a comment in 2019 stating that “it should not be considered comprehensive nor utilized to limit access to genetic professionals” [9, 10].

Multiple factors such as small family size, unknown family history, early deaths, de novo GPVs, and ethnic background may influence the accuracy of assessment; the conventional genetic testing criteria based on the risk assessment may miss individuals with GPVs. Indeed, several studies have reported that GPVs in cancer predisposition genes were identified not only in those who met the testing criteria based on personal and family history, but also in those who did not meet the criteria. A study which enrolled more than 1000 breast cancer patients showed that 9.39% (45/479) of those who met the NCCN criteria had GPVs and 7.9% (38/480) of those who did not meet the criteria had GPVs in 11 cancer predisposition genes (ATM, BRCA1/2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, STK11, and TP53). The difference was not statistically significant [11]. Another study which enrolled 3,907 breast cancer patients estimated the sensitivity of NCCN criteria to be at 87% for BRCA1/2 and 70% for 9 other predisposition genes (ATM, BRCA1/2, CDH1, CHEK2, NF1, PALB2, PTEN, and TP53) [12]. Another study examined the genetic testing criteria which was developed from the NCCN criteria with the slight modification showed that the rate of GPVs detected was 10.9% for those who met the criteria and 9% for those who did not, and the difference was not statistically significant [13]. Furthermore, a retrospective analysis showed that only 18.9% of positive results in genetic tests were consistent with the suspected syndromes and associated genes [14].

In recent years, the genetic testing criteria in the NCCN guidelines have been expanded. With respect to ovarian cancer, in the most recent NCCN guideline (Version 1. 2022, Genetic/Familial High-Risk Assessment, Breast, Ovarian, and Pancreatic), personal history of epithelial ovarian cancer alone will meet the testing criteria for multiple ovarian cancer susceptibility genes [15]. There is growing evidence that genes other than BRCA1/2 confer an increased risk of ovarian cancer predisposition. In a study including 721 ovarian cancer patients, most of the patients (94.9%) suffered from a metastatic disease, only 45% of the GPVs were detected in BRCA1/2, whereas 55% were detected in other genes [2]. Another study including 412 ovarian cancer patients identified 82 GPVs, in which 36 GPVs (44%) were in BRCA1, 27 (33%) in BRCA2, and 19 (23%) in other genes [1]. Considering the high GPV rates in genes other than BRCA1/2, it seems reasonable to perform MGPT in all patients with ovarian cancer. Recent ASCO annual meeting guideline recommend that all women diagnosed with epithelial ovarian cancer should have germline genetic testing for BRCA1/2 and other ovarian cancer susceptibility genes [16].

Patients with a personal history of ovarian cancer or EC with MMR deficiency will meet the Lynch syndrome (LS) testing criteria [17]. Furthermore, patients with EC who are diagnosed under the age of 50 years, or who have a synchronous or metachronous LS-related cancer, or who have a family history of LS related cancer also meet the testing criteria.

While these criteria are informative, it is worthwhile for clinicians to be aware that not a few individuals who met the criteria for BRCA1/2 testing have been reported to have GPVs in the MMR gene, and vice versa [14, 18]. In case of more than one testing criteria are fulfilled, MGPT would be more efficient and cost- and time-effective than phenotype-directed testing of single gene and would increase the ratio of detecting a GPV in a gene that will impact medical management for the individual or their at-risk family members. In addition, in cases where genetic test for a single syndrome is negative but personal and/or family history remains suggestive of an inherited susceptibility, MGPT may provide additional useful information. The spectra of GPVs detected by MGPT in patients with OC and EC are shown in Table 2.

Moreover, a small but not ignorable number of individuals have been reported to carry GPVs in more than one cancer susceptibility genes.

Consequently, clinicians involved in gynecological cancer treatment should carefully consider which patients would benefit from the MGPT.

### 4. Pitfalls of MGPT in Clinical Practice

Several issues other than genetic testing criteria should be considered when performing MGPT. First, commercially available MGPTs differ in genes to be analyzed. Genes commonly included in MGPT and the cancer types associated with each gene are shown in Table 3. Clinicians should choose appropriate MGPT according to the family and personal cancer history, cancer subtypes, pathological findings, and characteristic physical findings.

Second, MGPT increases the likelihood of detecting variants of uncertain significance (VUS) [5, 18]. Mersch et al. [19] reported that 7.7% of VUS were reclassified, of which, 91.2% were downgraded to benign or likely benign, while 8.7% were upgraded to pathogenic or likely pathogenic variants. In case of upgrading, these reclassifications can substantially affect patients’ options for cancer and health-related decision making. Therefore, genetic testing providers, physicians, and patients are encouraged to follow-up for new information regarding variants [20, 21].

Third, most MGPTs are based on conventional short-read next-generation sequencing (NGS) method. Some types of genetic alterations are difficult to detect with this method. These alterations include large insertions and deletions or CNVs. Clinicians to be aware that not a few individuals who met the criteria for BRCA1/2 testing have been reported to have GPVs in the MMR gene, and vice versa [14, 18]. In case of more than one testing criteria are fulfilled, MGPT would be more efficient and cost- and time-effective than phenotype-directed testing of single gene and would increase the ratio of detecting a GPV in a gene that will impact medical management for the individual or their at-risk family members. In addition, in cases where genetic test for a single syndrome is negative but personal and/or family history remains suggestive of an inherited susceptibility, MGPT may provide additional useful information. The spectra of GPVs detected by MGPT in patients with OC and EC are shown in Table 2.

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Third, most MGPTs are based on conventional short-read next-generation sequencing (NGS) method. Some types of genetic alterations are difficult to detect with this method. These alterations include large insertions and deletions which are larger than 15 base pair in size, copy-number variants (CNVs) such as exon deletion, variants in low-complexity or segmentally duplicated region, or putative mosaic. An analysis of 44,818 pathogenic or likely pathogenic (P/LP) variants in hereditary cancer revealed that 10.3% of P/LP variants might be difficult to be detected by NGS method [22]. The sensitivity to detect these P/LP variants would vary among the workflows used in each MGPT. Further investigation is occasionally needed to conclude a correct diagnosis. Multiplex Ligation-dependent Probe Amplification (MLPA) can detect large insertions and deletions or CNVs. Clinicians are encour-
TABLE 2. Spectrum of GPVs detected by MGPT in gynecological cancer patients by gene category.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cancer type</th>
<th>No. Patients</th>
<th>Testing approach</th>
<th>Rate of patients with GPVs</th>
<th>Spectrum of GPVs (GPVs in each gene/total GPVs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norquist 2016 [26]</td>
<td>Ovarian</td>
<td>1915</td>
<td>20-gene panel</td>
<td>347/1915 (18.1%)</td>
<td>BRCA1/2 280/352 (79.5%) MMR genes 8/352 (2.3%) Others 64/352 (18.2%)</td>
</tr>
<tr>
<td>Lilyquist 2017 [45]</td>
<td>Ovarian</td>
<td>7768</td>
<td>Multiple types of panel</td>
<td>992/7768 (12.8%)</td>
<td>BRCA1/2 551/1021 (54.0%) MMR genes 114/1021 (11.2%) Others 356/1021 (34.9%)</td>
</tr>
<tr>
<td>Carter 2018 [27]</td>
<td>Ovarian</td>
<td>4439</td>
<td>21- or 32-gene panel</td>
<td>588/4439 (13.2%)</td>
<td>BRCA1/2 332/609 (54.5%) MMR genes 52/609 (8.5%) Others 225/609 (36.9%)</td>
</tr>
<tr>
<td>Ring 2016 [72]</td>
<td>Endometrial</td>
<td>381</td>
<td>25-gene panel</td>
<td>35/381 (9.2%)</td>
<td>BRCA1/2 2/37 (5.4%) MMR genes 22/37 (59.5%) Others 11/37 (29.7%)</td>
</tr>
<tr>
<td>Levine 2021 [104]</td>
<td>Endometrial</td>
<td>961</td>
<td>47-gene panel</td>
<td>97/961 (10.1%)</td>
<td>BRCA1/2 10/100 (10.0%) MMR genes 29/100 (29.0%) Others 61/100 (61.0%)</td>
</tr>
</tbody>
</table>

Aged to have a clear understanding of the detection techniques used in each genetic testing and to inform their patients the limitations of the genetic testing.

Forth, the cancer risk associated with GPVs varies among genes in MGPTs. GPVs in genes such as BRCA1/2, MMR genes, PTEN, STK11, and TP53 strongly increase the risk of developing cancer; these genes are described as high-penetrance genes. The cancer risk associated with GPVs in other genes are lower than that reported for high-penetrance genes; these genes are described as low- or moderate-penetrance genes according to the cancer risk. MGPT often includes these low-to-moderate penetrance genes. For many of these genes, only limited data are available on the degree of cancer risk, and, currently, there may be no clear guidelines on risk management for carriers of GPVs. Therefore, the latest information on clinical management should be reviewed.

Finally, acquired somatic variants can be identified on germline testing. Blood cells carrying certain somatic variant may expand during hematopoiesis, which is referred to as clonal hematopoiesis (CH). The spectrum of genes commonly altered in CH has been reported [23, 24]. Some of these genes overlap with those available in genetic testing for hereditary cancer syndromes. Variants with CH have been detected most commonly in TP53, CHEK2 and ATM, and to a lesser extent in BRCA1/2 and MMR genes. The allele frequencies of variants associated with CH are often 30% or less. However, of the variants in TP53 with allele frequencies high enough to be considered heterozygous variants, 32.6% (15 of 46 tested) was not detected in fibroblasts of the variant carriers, indicating the variants detected in germline testing to be somatic variants [25]. Therefore, it should be noted that the results of genetic testing might lead to misdiagnosis of hereditary cancer.

5. Hereditary Epithelial Ovarian Cancers

Approximately 10–20% of epithelial ovarian cancer patients are estimated to have GPVs in ovarian cancer susceptibility genes [26–28]. A personal history of ovarian cancer is enough to meet the criteria for genetic testing according to the guidelines of NCCN and ACMG. Since several genes are responsible for hereditary ovarian cancer, once the testing criteria are met, the NCCN recommends to consider offering MGPT. The spectrum of GPVs in gynecological cancer patients and genes responsible for hereditary gynecological cancers or tumors and related syndromes are summarized in Tables 2, 4.

GPVs in BRCA1/2 are associated with a susceptibility to breast and ovarian cancer and have been referred to as Hereditary Breast and Ovarian Cancer (HBOC) Syndrome. However, there is now strong evidence that genes other than BRCA1/2 are also implicated in increasing the risk of breast and ovarian cancers. BRCA1 and BRCA2 are located on 17q21 and 13q12, respectively and both genes are referred to as tumor suppressors. BRCA1/2 encode for proteins involved in DNA repair via the homologous recombination repair (HRR) pathway.

Women with GPVs in BRCA1 have a cumulative risk of developing breast and ovarian cancer by the age of 80 years of 72% and 44% respectively; for women with GPVs in BRCA2, the risk is 69% and 17%, respectively [29]. GPVs in BRCA1/2 are responsible for at least 10% of epithelial ovarian cancers [30, 31]. Hirasawa et al. [28] reported that 8.3% and 3.5% of all patients with ovarian cancer in Japan had BRCA1 and BRCA2 GPVs, respectively. A study including 1915 patients with ovarian, fallopian tubes, and peritoneal cancer demonstrated that 9.5% and 5.1% of the patients had BRCA1 and BRCA2 GPVs, respectively [26]. The median age at diagnosis was 52 (range, 27–77 years) in patients with BRCA1 GPVs, 59 (range, 41–83 years) in those with BRCA2 GPVs, and 62 (range, 23–91 years) in those with no GPVs, indicating an earlier onset of ovarian cancer in BRCA1 GPV carriers.

The probability of detecting BRCA1/2 GPVs is also associated with the histological type of ovarian cancer. Serous adenocarcinoma, especially high-grade serous, has the highest probability for the presence of BRCA1/2 GPVs [26, 30, 31]. Endometrioid, clear cell, and other non-mucinous histology were associated with lower but substantial probabilities of detecting BRCA1/2 GPVs [26, 30, 31]. In mucinous ovarian cancer and ovarian low malignant potential tumors, BRCA1/2 GPVs were rarely present [30, 32].

Lynch syndrome (LS) is caused by GPVs in MMR genes; MLH1, MSH2, MSH6, and PMS2 [33]. In addition, being located immediately upstream of MSH2, deletion of the last exon of EPAC1 gene also causes LS through hypermethylation of the MSH2 promoter and subsequent MSH2 silencing [34]. As MMR system is responsible for maintaining genome stability, loss-of-function germline variants in genes involved in
TABLE 3. Genes commonly included in MGPT and related cancers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ovary</th>
<th>Endometrium</th>
<th>Cervix</th>
<th>Breast</th>
<th>Colon</th>
<th>Pancreas</th>
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The open circles indicate non-epithelial tumors.

this system may cause cancer predisposition. Women with LS are at a heightened risk for ovarian cancer, which varies based on the affected gene and age. An international, multicenter prospective observational study including 6350 participants with GPVs in MMR genes showed that the cumulative risk of developing ovarian cancers by the age of 75 years is 11.0% for MLH1, 17.4% for MSH2, 10.8% for MSH6, and 3.0% for PMS2 carriers [35].

Prevalence of LS in epithelial ovarian cancer patients has been reported to be 0.4–3% [36]. Most common histopathological subtypes in LS-associated ovarian cancer is endometrioid adenocarcinoma and clear cell carcinoma, although LS-associated ovarian cancers show diverse histopathology [37]. The age being diagnosed with ovarian cancer has been reported earlier in LS-associated ovarian cancer patients compared with sporadic ovarian cancer patients [38]. Synchronous EC was reported in LS-associated ovarian cancer patients [39].

Genes with susceptibility for hereditary ovarian cancers have recently been identified. These genes include ATM, BRIP1, PALB2, RAD51C, and RAD51D. Although the penetrance of these genes is lower compared with BRCA1/2 and MMR genes, these genes cannot be ignored. Among ovarian cancer patients, the prevalence of GPVs in these genes were higher than the frequency in healthy controls, with the ratio of 0.64–0.87% for ATM, 1% for BRIP1, 0.38–0.62% for PALB2, 0.5% for RAD51C and RAD51D, respectively [26, 27, 40].

The risk of developing ovarian cancer differs depending on the gene. GPVs in ATM were estimated to slightly increase the risk of developing ovarian cancer [41]. A large case-control study showed that GPVs in BRIP1 is associated with an increased risk for ovarian cancer, especially for high-grade serous ovarian cancer with a relative risk of 14.09 (95% CI, 4.04–45.02, p < 0.001). Individuals with BRIP1 GPVs have a cumulative risk of developing ovarian cancer by the age of 80 years of 5.8% [42]. Whether PALB2 GPVs increase ovarian cancer risk has been controversial. Three studies have shown no statistically significant association between PALB2 GPVs and ovarian cancer risk, although two other studies have demonstrated their association [26, 42–45]. Some case-control studies have identified association between RAD51C and RAD51D GPVs and increased ovarian cancer risk, with odds ratios of 3.4–5.2 and 4.78–12.0, respectively [26, 43, 46].

Identifying GPVs in genes described above can lead to determination of appropriate treatment strategies and preventive medical intervention or surveillance. PARP and BRCA1/2 are involved in DNA damage repair. When the function of BRCA1 or BRCA2 is lost due to GPVs in BRCA1/2, DNA repair relies on PARP1. Thus, inhibition of PARP1 in BRCA1/2 dysfunctional tumors leads to synthetic lethal damage to cancer cells, resulting in cell death. In this context, PARP inhibitors have gained usage in variety of tumors with BRCA1/2 GPVs and shown significant efficacy in these tumors. In addition, ATM, BRIP1, PALB2, RAD51C, and RAD51D are also involved in the HRR pathway as well as BRCA1/2, indicating that inhibition of PARP1 might also be a promising treatment strategy for tumors with GPVs in these genes.
### Table 4. Hereditary tumor syndromes related to gynecological cancers/tumors.

<table>
<thead>
<tr>
<th>Cancer/tumor type</th>
<th>Related syndrome</th>
<th>Common pathological subtypes</th>
<th>Responsible genes</th>
<th>Other non-gynecological tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ovarian cancer</strong></td>
<td><em>BRCA</em>-related breast/ovarian cancer syndrome</td>
<td>Serous / Non-mutinous</td>
<td><em>BRCA1, BRCA2</em></td>
<td>Breast cancer, Prostate cancer, Pancreatic cancer</td>
</tr>
<tr>
<td></td>
<td><strong>Lynch syndrome</strong></td>
<td>Endometrioid / Non-serous</td>
<td><em>MLH1, MSH2, MSH6, PMS2, EPCAM</em></td>
<td>colorectal cancer, Gastric cancer, Small bowel cancer, Urothelial cancer, Pancreatic cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>RAD51C, RAD51D</em></td>
<td>Breast cancer</td>
</tr>
<tr>
<td></td>
<td><strong>BRIP1</strong></td>
<td></td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td><strong>ATM, PALB2</strong></td>
<td></td>
<td></td>
<td>Breast cancer, Pancreatic cancer</td>
</tr>
<tr>
<td><strong>Non-epithelial ovarian tumor</strong></td>
<td><strong>Peutz-Jeghers syndrome</strong></td>
<td>Sex-cord tumor with annular tubules</td>
<td><em>STK11</em></td>
<td>Breast cancer, Pancreatic cancer, Colorectal cancer, Gastric cancer, Small bowel cancer</td>
</tr>
<tr>
<td></td>
<td><strong>Gorlin-Goltz syndrome</strong></td>
<td>Fibroma</td>
<td><em>PTCH1, SUFU</em></td>
<td>Basal cell carcinomas, Pediatric medulloblastomas</td>
</tr>
<tr>
<td></td>
<td><strong>DICER1 syndrome</strong></td>
<td>Sertoli-Leydig cell tumor</td>
<td><em>DICER1</em></td>
<td>Embryonal rhabdomyosarcoma</td>
</tr>
<tr>
<td></td>
<td><strong>Rhabdoid tumor predisposition syndrome</strong></td>
<td>Hypercalcemic type of small cell carcinomas</td>
<td><em>SMARCA4</em></td>
<td>Rhabdoid tumors of central nervous system, renal rhabdoid tumors</td>
</tr>
<tr>
<td><strong>Endometrial cancer</strong></td>
<td><strong>Lynch syndrome</strong></td>
<td>Endometrioid</td>
<td><em>MLH1, MSH2, MSH6, PMS2, EPCAM</em></td>
<td>colorectal cancer, Gastric cancer, Small bowel cancer, Urothelial cancer, Pancreatic cancer</td>
</tr>
<tr>
<td></td>
<td><strong>PTEN hamartoma tumor syndrome (Cowden syndrome)</strong></td>
<td></td>
<td><em>PTEN</em></td>
<td>Breast cancer, Epithelial thyroid cancer, Gastrointestinal hamartoma</td>
</tr>
<tr>
<td><strong>Cervical cancer</strong></td>
<td><strong>Peutz-Jeghers syndrome</strong></td>
<td>Gastric type mucinous carcinoma / LEGH</td>
<td><em>STK11</em></td>
<td>Breast cancer, Pancreatic cancer, Colorectal cancer, Gastric cancer, Small bowel cancer</td>
</tr>
<tr>
<td><strong>Non-epithelial cervical tumor</strong></td>
<td><strong>DICER1 syndrome</strong></td>
<td>Gastric type carcinoma / LEGH</td>
<td><em>DICER1</em></td>
<td>Sertoli-Leydig cell tumor of ovary</td>
</tr>
</tbody>
</table>
Risk-reducing salpingo-oophorectomy should be considered for GPV carriers in genes discussed in this section, according to the genes and personal and family history of ovarian cancer. Breast cancer screening using MRI is recommended in BRCA1/2 GPV carriers and should be considered in other genes involved in HRR pathways. Risk-reducing mastectomy could also be an option for them based on the personal and family history.

6. Hereditary Non-Epithelial Ovarian Tumors

Several genes have been identified to have an association with non-epithelial ovarian tumors. Since tumors implicated in GPVs in these genes often show characteristic pathology and are often accompanied with other characteristic clinical manifestations, the significance of MGPT may be less than in the case of epithelial ovarian cancer.

Peutz-Jeghers syndrome (PJS) is characterized by multiple hamartomatous polyps in the gastrointestinal tract and pigmentation of the skin mucosa. Patients with PJS also known to be susceptible to cancers of the gastrointestinal tract, uterine cervix, testes, ovary, and breast [47, 48]. The majority of cases occur due to GPVs in the STK11 (LKB1) gene [49, 50]. STK11, located on 19p13, encodes a serine-threonine kinase involved in cell polarity, metabolism, and growth. Ovarian tumors with PJS are mainly sex cord tumor with annular tubules (SCTAT). The lifetime risk of developing SCTAT is reported to be 21%, and the average age at diagnosis is 28 years [48]. Among all patients with ovarian SCTAT, approximately one-third have PJS [51].

Gorlin-Goltz syndrome, also referred to as Gorlin syndrome or nevoid basal cell carcinoma syndrome, is characterized by the classic triad, that is basal cell carcinoma, jaw cysts, and skeletal anomalies [52, 53]. Up to 80% of patients with this syndrome have GPVs in PTCH1 gene [54–56]. GPVs in SUFU gene are also associated with this syndrome [57]. PTCH1 and SUFU are members of the sonic hedgehog signaling pathway, which regulates cell differentiation and proliferation. One study reported that the risk of developing medulloblastoma in this syndrome depends on the underlying causative gene and is 5% for all individuals with this syndrome, 2% for individuals with GPVs in PTCH1, and 33% for those with GPVs in SUFU [57]. Ovarian tumors with this syndrome are mainly fibroma, which tend to be bilateral, multi-nodular, and highly calcified and are detected in 17% of females with this syndrome [54].

DICER1 syndrome is characterized by the pediatric pulmonary blastoma, nodular hyperplasia of the thyroid, cystic nephroma, Sertoli-Leydig cell tumors (SLCT) of the ovary, and other rare tumors [58, 59]. This syndrome is caused by GPVs in DICER1, located on 14q32, which encodes a RNase III endonuclease critical to the generation of mature microRNAs, which function through RNA interference to regulate gene expression [60, 61]. Most SLCTs in this syndrome are diagnosed from late childhood through early adulthood, with one study reporting 16.9 years as the median age at diagnosis [62]. Stewart et al. [63] reported that 21.2% (24 of 113) of female carriers of DICER1 GPVs developed SLCT. DICER1 GPVs were identified in 69% of patients with SLCT (18 of 26) [64].

SMARCA4, located on 19p13, encodes BRG1 which is involved in chromatin remodeling. Recently, biallelic inactivation of SMARCA4 and the consequent complete loss of BRG1 have been reported as a distinctive molecular event in small cell carcinoma of the ovary, hypercalcemic type (SCCOHT) [65–67]. SMARCA4 GPVs were identified in 26 of 60 SCCOHT patients (43%), who developed the disease at a significantly younger age than those without GPVs [68]. Individuals with SMARCA4 GPVs also develop rhabdoid tumors involving the central nervous system or kidney [69]. Since the prevalence of GPVs is high, the International SCCOHT Consortium recommends referral of all patients with SCCOHT to a clinical genetics service and offering genetic testing [70]. Although the risk of SCCOHT in women with SMARCA4 GPVs remains uncertain, International SCCOHT Consortium also recommend offering genetic counseling and predictive testing to all at-risk relatives of SCCOHT patients with SMARCA4 GPVs and considering risk-reducing bilateral salpingo-oophorectomy [70].

7. Hereditary Endometrial Cancers

In a series of 1170 unselected endometrial cancer (EC) cases, approximately 4.5% were reported to have GPVs in one of 12 cancer predisposition genes [71]. A total of 2.1% of cases were identified with GPVs in genes related to HRR pathway, 6.6% were in BRCA1/2, and 1.5% were in other HRR genes. The prevalence of GPVs in BRCA1/2 was similar to that in the general population. A total of 1.5% of cases were identified with GPVs in MMR genes, that is, LS. Another study of 381 unselected EC patients described that 9.2% of patients were identified with GPVs in cancer predisposition genes, and 63% of the detected GPVs were in MMR genes, 5.7% in BRCA1/2 and 2.9% in PTEN [73]. A study involving individuals who had received clinical genetic testing at a commercial laboratory demonstrated that 11.9% of 453 EC patients had GPVs, 60% of which were in MMR genes and 10.9% in BRCA1/2 [73].

An international, multicenter prospective observational study including 6350 LS patients showed that the cumulative risk of developing ECs by the age of 75 years varies depending on genes; 37.0% for MLH1, 48.9% for MSH2, 41.1% for MSH6, and 12.8% for PMS2 carriers [35]. A French multicenter study demonstrated that EC in LS is associated with younger age at diagnosis, with the mean age at 50 years [74]. In 81.4% of LS cases, EC was the first cancer in those individuals. The most common histology was endometrioid adenocarcinoma and the lower uterine segment was involved in 25% of cases. Synchronous ovarian cancer was present in 21.6% of patients.

PTEN hamartoma tumor syndrome (Cowden syndrome) is a multiple hamartoma syndrome frequently associated with GPVs in PTEN [75]. PTEN is located on 10q23 and encodes a phosphatase involved in cell signaling pathways affecting cell proliferation and survival. The lifetime risk of developing EC is estimated to be 28%. The risk begins to increase at age 25 and rises to 30% by age 60 [76]. However, GPVs are rare identified in EC patients, although somatic PTEN PVs are common in EC.
It remains unknown whether BRCA1/2 GPVs are associated with an increased risk of EC or not. A prospective cohort study showed a slightly increased risk of EC in the median follow-up of 5.7 years, with a standardized incidence ratios (SIR) of 1.91 (95% CI: 1.06–3.19) for BRCA1 GPV carriers and 1.75 (95% CI: 0.55–4.23) for BRCA2 GPV carriers. The difference of EC risk in BRCA2 GPV carriers was not statistically significant [77]. In this study, the most relevant risk factor for EC was tamoxifen use. Tamoxifen use significantly increased SIR in BRCA1 GPV carriers from 1.91 to 4.43 (95% CI: 1.94–8.76). In BRCA2 GPV carriers the association between EC risk and tamoxifen use was not statistically significant (SIR = 2.29, 95% CI: 0.38–7.59). Another study including 1083 BRCA1/2 GPV carriers who underwent risk-reducing salpingo-oophorectomy without hysterectomy, EC risk was not increased in the median follow-up of 5.1 years [78]. 8 incident uterine cancers were observed, 5 of 8 were serous/serous-like. 4 of the 5 serous/serous-like cancers were developed in BRCA1 GPV carriers, indicating increased risk for serous/serous-like EC in BRCA1 GPV carriers.

Most hereditary ECs are caused by GPVs in MMR genes, and rarely by GPVs in PTEN. However, a critical GPV could easily be missed if testing is limited to only those genes in a basic genetic panel or to a specific single gene associated with suspected syndrome.

We previously reported a case of 56-year-old woman suspected of having LS [79]. In that case, the patient was diagnosed with stage Ia EC at the age of 39 years and had a family history of colorectal cancer. However, MGPT revealed a BRCA2 GPV with no GPVs in MMR genes. Cancer prevention and treatment strategies have changed based on the result of the MGPT.

Most tumors with LS show microsatellite instability-high (MSI-H). MSI-H serves as a biomarker for response to immune checkpoint blockades, thus immune checkpoint inhibitors are widely used in a variety of cancers with MSI-H [80–82]. Individuals with LS are at a heightened risk for colorectal cancer; therefore, annual or semiannual colonoscopy is recommended [83–88]. No clear evidence has been found to support routine screening for other cancers in LS patients. Individuals with PTEN hamartoma tumor syndrome have the risk of developing different types of cancers such as breast cancer and thyroid cancers. Thus, screening for these cancers is recommended. As shown above, genetic information obtained from MGPT play pivotal roles both in treatment and prevention of cancer.

8. Hereditary Cervical Cancers

Cervical cancer is in most cases caused by the human papilloma virus, and is thus very unlikely to be hereditary. To date, two types of cervical cancer have been reported to be associated with hereditary tumors.

Cervical cancer with PJS is primarily cervical gastric type mucinous carcinoma of the endocervix (G-ECA) with an estimated lifetime risk of 10% and a mean age at diagnosis of 34–40 years, indicating a younger age of onset than sporadic cases [89]. G-ECAs in PJS are extremely well-differentiated forms of G-ECA, which are also known as adenoma malignum or minimal deviation adenocarcinoma (MDA). Approximately 11–17% of the patients with MDA have PJS [90, 91]. Lobular endocervical glandular hyperplasia (LEGH) is a basically benign gastric type mucinous lesion of cervix. LEGH. LEGH with atypia could be a precursor of MDA. Mikami et al. [92] reported that 7 of 20 cases of MDA were associated with LEGH, and six of these patients had atypical LEGH. The first case of LEGH in a PJS patient with a STK11 GPV was reported by Hirasawa et al. in 2012 [93]. Since then, a few case reports have shown a possible association of LEGH with PJS [94–96].

Emphyreal rhabdomyosarcoma of the cervix (cERMs) is rare tumor, which is observed in older children, adolescents, and young adults with a median age of 13–14 years [97]. The association of cERMs and SLCT was reported in a cohort of 14 cases [98]. Most of the cERMs (18 of 19 cases, 95%) were reported to have DICER1 mutations, 50% of which were of germine origin (6 of 12 cases tested) [99].

9. Conclusions

Recent advances in DNA sequencing technology have enabled the simultaneous analysis of multiple genes. It has now become a routine practice at many institutions to order MGPTs for cancer patients according to their personal and family history. The use of MGPT will contribute to minimizing missed hereditary cancer syndromes.

Germline genetic information will pass from parents to offspring and be shared within the family. Hereditary cancer syndromes mentioned in this review exhibit autosomal dominant inheritance. Thus, genetic information obtained from MGPT will influence not only those identified with GPVs themselves but also their family members. Physicians should be encouraged to discuss with their patients how to share the genetic information with other family members. Remote options, such as real-time two-way video conference involving the patients, their relatives and genetic experts, may help the relatives better understand the importance of the genetic information.

At present, MGPT is usually performed after the diagnosis of cancer, and then the diagnosis of hereditary cancer syndrome is made. However, in the near future, when whole exome and whole genome sequencing are clinically applied to the disease other than cancer, GPVs in cancer susceptible genes can be identified before cancer develops. Establishment of effective cancer prevention strategies not only for high-penetrance genes but also low-to-moderate-penetrance genes is urgently needed.

In addition, given the rapidly increasing number of approved therapies with germline indications, MGPT will be better performed as early as possible after the diagnosis of cancer. Information on GPVs in certain genes may also be useful in predicting the response to anti-cancer drugs. Thus, results of MGPT may contribute to the selection of better treatment strategies tailored to the patients.

AUTHOR CONTRIBUTIONS

SU: Conceptualization, Writing - Original Draft, Funding Acquisition. AH: Conceptualization, Writing - Reviewing and Editing.
CONFLICT OF INTEREST
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REFERENCES


