Submitted: 20 March, 2022 Accepted: 26 April, 2022 Published: 15 June, 2022 DOI:10.22514/ejgo.2022.008

REVIEW

European Journal of Gynaecological Oncology

Germline multigene panel testing in gynecological cancer

Sayaka Ueno[1,*], Akira Hirasawa[1]

[1]Department of Clinical Genomic Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Kita-ku, 700-8558 Okayama, Japan

***Correspondence**
sayaka-u@okayama-u.ac.jp
(Sayaka Ueno)

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

TABLE 1. The rate of cancer patients detected with GPVs.

Study	No. Patients	Testing approach	Rate of patients detected with GPVs in cancer predisposition genes			
			All cancer	Ovarian	Endometrial	Cervical
Meric-Bernstam 2016 [100]	1000	Targeted exome sequencing of 201 genes	43/1000 (4.3%)	5/36 (13.9%)	0/9 (0%)	0/5 (0%)
Mandelker 2017 [101]	1040	76-gene panel	182/1040 (17.5%)	6/19 (31.6%)	4/25 (16%)	0/0 (0%)
Huang 2018 [1]	10,389	Whole exome sequencing (WES)	818/10,389 (7.9%)	82/412 (19.9%)	37/543 (6.8%)	20/305 (6.6%)
Stadler 2021 [2]	11,947	76- or 88-gene panel	2037/11,947 (17.1%)	184/721 (25.5%)	121/871 (13.9%)	
Cobain 2021 [102]	1015	WES or targeted exome sequencing of 1700 genes	160/1015 (15.8%)	5/15 (33.3%)	0/3 (0%)	1/1 (100%)
Samadder 2021 [103]	2984	83- or 84-gene panel	397/2984 (13.3%)	26/126 (20.6%)	13/98 (13.3%)	0/2 (0%)

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

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 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.

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

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 associated 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 serve 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 *EPCAM* gene also causes LS through hypermethylation of the *MSH2* promoter and subsequent MSH2 silencing [34]. As MMR system is responsible for maintaining for genome stability, loss-of-function germline variants in genes involved in

TABLE 3. Genes commonly included in MGPT and related cancers.

	Ovary	Endometrium	Cervix	Breast	Colon	Pancreas	Others
ATM	●						
BRCA1	●			●			
BRCA2	●			●		●	
BRIP1	●						
CDH1				●			●
CHEK2				●	●		●
DICER1	○						●
EPCAM		●			●		
MLH1	●	●			●		●
MSH2	●	●			●		●
MSH6	●	●			●		●
PALB2	●			●		●	
PMS2		●			●		●
PTEN		●			●		
RAD51C	●			●			
RAD51D	●			●			
SMARCA4	○		○				
STK11	○		●				
TP53				●			●

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 depends 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 *BRIP*1 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.

Cancer/tumor type	Related syndrome	Common pathological subtypes	Responsible genes	Other non-gynecological tumors
Ovarian cancer	*BRCA*-related breast/ovarian cancer syndrome	Serous / Non-mutinous	*BRCA1*, *BRCA2*	Breast cancer, Prostate cancer, Pancreatic cancer
	Lynch syndrome	Endometrioid / Non-serous	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*	Colorectal cancer, Gastric cancer, Small bowel cancer, Urothelial cancer, Pancreatic cancer
			RAD51C, *RAD51D*	Breast cancer
			BRIP1	Unknown
			ATM, *PALB2*	Breast cancer, Pancreatic cancer
Non-epithelial ovarian tumor	Peutz-Jeghers syndrome	Sex-cord tumor with annular tubules	*STK11*	Breast cancer, Pancreatic cancer, Colorectal cancer, Gastric cancer, Small bowel cancer
	Gorlin-Goltz syndrome	Fibroma	*PTCH1*, *SUFU*	Basal cell carcinomas, Pediatric medulloblastomas
	DICER1 syndrome	Sertoli-Leydig cell tumor	*DICER1*	Embryonal rhabdomyosarcoma
	Rhabdoid tumor predisposition syndrome	Hypercalcemic type of small cell carcinomas	*SMARCA4*	Rhabdoid tumors of central nervous system, renal rhabdoid tumors
Endometrial cancer	Lynch syndrome	Endometrioid	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*	Colorectal cancer, Gastric cancer, Small bowel cancer, Urothelial cancer, Pancreatic cancer
	PTEN hamartoma tumor syndrome (Cowden syndrome)		*PTEN*	Breast cancer, Epithelial thyroid cancer, Gastrointestinal hamartoma
Cervical cancer	Peutz-Jeghers syndrome	Gastric type mucinous carcinoma / LEGH	*STK11*	Breast cancer, Pancreatic cancer, Colorectal cancer, Gastric cancer, Small bowel cancer
Non-epithelial cervical tumor	DICER1 syndrome	Gastric type carcinoma / LEGH	*DICER1*	Sertoli-Leydig cell tumor of ovary

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 pleuropulmonary 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, 0.6% 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].

Embryonal 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 germline 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.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by JSPS KAKENHI Grant Number 21K19667.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Huang KL, Mashl RJ, Wu Y, Ritter DI, Wang J, Oh C, *et al*. Pathogenic Germline Variants in 10,389 Adult Cancers. Cell. 2018; 173: 355–370.e314.

[2] Stadler ZK, Maio A, Chakravarty D, Kemel Y, Sheehan M, Salo-Mullen E, *et al*. Therapeutic Implications of Germline Testing in Patients with Advanced Cancers. Journal of Clinical Oncology. 2021; 39: 2698–2709.

[3] Conley JM, Cook-Deegan R, Lázaro-Muñoz G. MYRIAD AFTER MYRIAD: THE PROPRIETARY DATA DILEMMA. North Carolina Journal of Law & Technology. 2014; 15: 597–637.

[4] Cook-Deegan R, Niehaus A. After Myriad: Genetic Testing in the Wake of Recent Supreme Court Decisions about Gene Patents. Current Genetic Medicine Reports. 2014; 2: 223–241.

[5] Kurian AW, Ward KC, Hamilton AS, Deapen DM, Abrahamse P, Bondarenko I, *et al*. Uptake, Results, and Outcomes of Germline Multiple-Gene Sequencing after Diagnosis of Breast Cancer. JAMA Oncology. 2018; 4: 1066.

[6] Lancaster JM, Powell CB, Chen LM, Richardson DL. Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions. Gynecologic Oncology. 2015; 136: 3–7.

[7] Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, Caughey AB, *et al*. Risk Assessment, Genetic Counseling, and Genetic Testing for BRCA-Related Cancer: US Preventive Services Task Force Recommendation Statement. The Journal of the American Medical Association. 2019; 322: 652–665.

[8] Practice Bulletin No 182: Hereditary Breast and Ovarian Cancer Syndrome. Obstetrics & Gynecology. 2017; 130: e110–e126.

[9] Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL. A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. Genetics in Medicine. 2015; 17: 70–87.

[10] Bashford MT, Kohlman W, Everett J, Parrott A, Pollin TI. Addendum: a practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. Genetics in Medicine. 2019; 21: 2844.

[11] Beitsch PD, Whitworth PW, Hughes K, Patel R, Rosen B, Compagnoni G, *et al*. Underdiagnosis of Hereditary Breast Cancer: are Genetic Testing Guidelines a Tool or an Obstacle? Journal of Clinical Oncology. 2019; 37: 453–460.

[12] Yadav S, Hu C, Hart SN, Boddicker N, Polley EC, Na J, *et al*. Evaluation of Germline Genetic Testing Criteria in a Hospital-Based Series of Women with Breast Cancer. Journal of Clinical Oncology. 2020; 38: 1409–1418.

[13] Yang S, Axilbund JE, O'Leary E, Michalski ST, Evans R, Lincoln SE, *et al*. Underdiagnosis of Hereditary Breast and Ovarian Cancer in Medicare Patients: Genetic Testing Criteria Miss the Mark. Annals of Surgical Oncology. 2018; 25: 2925–2931.

[14] Ward, DNP, APRN, AGNP-BC M, Elder, PhD, RN B, Habtemariam, DNP, APRN M. Current Testing Guidelines: a Retrospective Analysis of a Community-Based Hereditary Cancer Program. Journal of the Advanced Practitioner in Oncology. 2021; 12: 693–701.

[15] Daly MB, Pal T, Berry MP, Buys SS, Dickson P, Domchek SM, *et al*. Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network. 2021; 19: 77–102.

[16] Konstantinopoulos PA, Norquist B, Lacchetti C, Armstrong D, Grisham RN, Goodfellow PJ, *et al*. Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. Journal of Clinical Oncology. 2020; 38: 1222–1245.

[17] Weiss JM, Gupta S, Burke CA, Axell L, Chen LM, Chung DC, *et al*. NCCN Guidelines® Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 1.2021. Journal of the National Comprehensive Cancer Network. 2021; 19: 1122–1132.

[18] LaDuca H, Polley EC, Yussuf A, Hoang L, Gutierrez S, Hart SN, *et al*. A clinical guide to hereditary cancer panel testing: evaluation of gene-specific cancer associations and sensitivity of genetic testing criteria in a cohort of 165,000 high-risk patients. Genetics in Medicine. 2020; 22: 407–415.

[19] Mersch J, Brown N, Pirzadeh-Miller S, Mundt E, Cox HC, Brown K, *et al*. Prevalence of Variant Reclassification Following Hereditary Cancer Genetic Testing. The Journal of the American Medical Association. 2018; 320: 1266.

[20] David KL, Best RG, Brenman LM, Bush L, Deignan JL, Flannery D, *et al*. Patient re-contact after revision of genomic test results: points to consider—a statement of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2019; 21: 769–771.

[21] Slavin TP, Manjarrez S, Pritchard CC, Gray S, Weitzel JN. The effects of genomic germline variant reclassification on clinical cancer care. Oncotarget. 2019; 10: 417–423.

[22] Lincoln SE, Hambuch T, Zook JM, Bristow SL, Hatchell K, Truty R, *et al*. One in seven pathogenic variants can be challenging to detect by NGS: an analysis of 450,000 patients with implications for clinical sensitivity and genetic test implementation. Genetics in Medicine. 2021; 23: 1673–1680.

[23] Slavin TP, Coffee B, Bernhisel R, Logan J, Cox HC, Marcucci G, *et al*. Prevalence and characteristics of likely-somatic variants in cancer susceptibility genes among individuals who had hereditary pan-cancer panel testing. Cancer Genetics. 2019; 235-236: 31–38.

[24] Chao EC, Astbury C, Deignan JL, Pronold M, Reddi HV, Weitzel JN. Incidental detection of acquired variants in germline genetic and genomic testing: a points to consider statement of the American College of Medical Genetics and Genomics (ACMG). Genetics in Medicine. 2021; 23: 1179–1184.

[25] Coffee B, Cox HC, Bernhisel R, Manley S, Bowles K, Roa BB, *et al*. A substantial proportion of apparently heterozygous TP53 pathogenic variants detected with a next-generation sequencing hereditary pan-cancer panel are acquired somatically. Human Mutation. 2020; 41: 203–211.

[26] Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, *et al*. Inherited Mutations in Women with Ovarian Carcinoma. JAMA Oncology. 2016; 2: 482.

[27] Carter NJ, Marshall ML, Susswein LR, Zorn KK, Hiraki S, Arvai KJ, *et al*. Germline pathogenic variants identified in women with ovarian tumors. Gynecologic Oncology. 2018; 151: 481–488.

[28] Hirasawa A, Imoto I, Naruto T, Akahane T, Yamagami W, Nomura H, *et al*. Prevalence of pathogenic germline variants detected by multigene sequencing in unselected Japanese patients with ovarian cancer. Oncotarget. 2017; 8: 112258–112267.

[29] Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, *et al*. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. The Journal of the American Medical Association. 2017; 317: 2402–2416.

[30] Zhang S, Royer R, Li S, McLaughlin JR, Rosen B, Risch HA, *et al*. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected

patients with invasive ovarian cancer. Gynecologic Oncology. 2011; 121: 353–357.

[31] Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, *et al*. BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. Journal of Clinical Oncology. 2012; 30: 2654–2663.

[32] Carter BA. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). Yearbook of Pathology and Laboratory Medicine. 2012; 2013: 34–37.

[33] Lynch HT, de la Chapelle A. Hereditary Colorectal Cancer. New England Journal of Medicine. 2003; 348: 919–932.

[34] Ligtenberg MJL, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, *et al*. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nature Genetics. 2009; 41: 112–117.

[35] Dominguez-Valentin M, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, *et al*. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genetics in Medicine. 2020; 22: 15–25.

[36] Pal T, Akbari MR, Sun P, Lee J, Fulp J, Thompson Z, *et al*. Frequency of mutations in mismatch repair genes in a population-based study of women with ovarian cancer. British Journal of Cancer. 2012; 107: 1783–1790.

[37] Helder-Woolderink JM, Blok EA, Vasen HFA, Hollema H, Mourits MJ, De Bock GH. Ovarian cancer in Lynch syndrome; a systematic review. European Journal of Cancer. 2016; 55: 65–73.

[38] Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W, *et al*. Risks of less Common Cancers in Proven Mutation Carriers with Lynch Syndrome. Journal of Clinical Oncology. 2012; 30: 4409–4415.

[39] Watson P, Bützow R, Lynch HT, Mecklin J, Järvinen HJ, Vasen HFA, *et al*. The Clinical Features of Ovarian Cancer in Hereditary Nonpolyposis Colorectal Cancer. Gynecologic Oncology. 2001; 82: 223–228.

[40] Kurian AW, Ward KC, Howlader N, Deapen D, Hamilton AS, Mariotto A, *et al*. Genetic Testing and Results in a Population-Based Cohort of Breast Cancer Patients and Ovarian Cancer Patients. Journal of Clinical Oncology. 2019; 37: 1305–1315.

[41] Suszynska M, Klonowska K, Jasinska AJ, Kozlowski P. Large-scale meta-analysis of mutations identified in panels of breast/ovarian cancer-related genes — Providing evidence of cancer predisposition genes. Gynecologic Oncology. 2019; 153: 452–462.

[42] Ramus SJ, Song H, Dicks E, Tyrer JP, Rosenthal AN, Intermaggio MP, *et al*. Germline Mutations in the BRIP1, BARD1, PALB2, and NBN Genes in Women With Ovarian Cancer. Journal of the National Cancer Institute. 2015; 107: djv214..

[43] Kurian AW, Hughes E, Handorf EA, Gutin A, Allen B, Hartman A, *et al*. Breast and Ovarian Cancer Penetrance Estimates Derived from Germline Multiple-Gene Sequencing Results in Women. JCO Precision Oncology. 2017; 10: 1–12.

[44] Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkäs K, Roberts J, *et al*. Breast-cancer risk in families with mutations in PALB2. The New England Journal of Medicine. 2014; 371: 497–506.

[45] Lilyquist J, LaDuca H, Polley E, Davis BT, Shimelis H, Hu C, *et al*. Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls. Gynecologic Oncology. 2017; 147: 375–380.

[46] Song H, Dicks E, Ramus SJ, Tyrer JP, Intermaggio MP, Hayward J, *et al*. Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. Journal of Clinical Oncology. 2015; 33: 2901–2907.

[47] Beggs AD, Latchford AR, Vasen HFA, Moslein G, Alonso A, Aretz S, *et al*. Peutz-Jeghers syndrome: a systematic review and recommendations for management. Gut. 2010; 59: 975–986.

[48] Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, *et al*. Very high risk of cancer in familial Peutz–Jeghers syndrome. Gastroenterology. 2000; 119: 1447–1453.

[49] Jenne DE, Reomann H, Nezu J, Friedel W, Loff. S, Jeschke R, *et al*. Peutz-Jeghers syndrome is caused by mutations in a novel serine threoninekinase. Nature Genetics. 1998; 18: 38–43.

[50] Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, *et al*. A serine/threonine kinase gene defective in Peutz–Jeghers syndrome. Nature. 1998; 391: 184–187.

[51] Young RH, Welch WR, Dickersin GR, Scully RE. Ovarian sex cord tumor with annular tubules. Review of 74 cases including 27 with Peutz-Jeghers syndrome and four with adenoma malignum of the cervix. Cancer. 1982; 50: 1384–1402.

[52] Gorlin RJ. Nevoid basal cell carcinoma (Gorlin) syndrome. Genetics in Medicine. 2004; 6: 530–539.

[53] Gorlin RJ, Goltz RW. Multiple Nevoid Basal-Cell Epithelioma, Jaw Cysts and Bifid Rib. New England Journal of Medicine. 1960; 262: 908–912.

[54] Kimonis VE, Mehta SG, Digiovanna JJ, Bale SJ, Pastakia B. Radiological features in 82 patients with nevoid basal cell carcinoma (NBCC or Gorlin) syndrome. Genetics in Medicine. 2004; 6: 495–502.

[55] Marsh A, Wicking C, Wainwright B, Chenevix-Trench G. DHPLC analysis of patients with Nevoid Basal Cell Carcinoma Syndrome reveals novel PTCH missense mutations in the sterol-sensing domain. Human Mutation. 2005; 26: 283.

[56] Soufir N, Gerard B, Portela M, Brice A, Liboutet M, Saiag P, *et al*. PTCH mutations and deletions in patients with typical nevoid basal cell carcinoma syndrome and in patients with a suspected genetic predisposition to basal cell carcinoma: a French study. British Journal of Cancer. 2006; 95: 548–553.

[57] Smith MJ, Beetz C, Williams SG, Bhaskar SS, O'Sullivan J, Anderson B, *et al*. Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations. Journal of Clinical Oncology. 2014; 32: 4155–4161.

[58] Slade I, Bacchelli C, Davies H, Murray A, Abbaszadeh F, Hanks S, *et al*. DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. Journal of Medical Genetics. 2011; 48: 273–278.

[59] Schultz KAP, Stewart DR, Kamihara J, Bauer AJ, Merideth MA, Stratton P, *et al*. DICER1 Tumor Predisposition. In Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, *et al*. (eds.) GeneReviews((R)): Seattle (WA).1993.

[60] Hill DA, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D, *et al*. DICER1 mutations in familial pleuropulmonary blastoma. Science. 2009; 325: 965.

[61] Foulkes WD, Priest JR, Duchaine TF. DICER1: mutations, microRNAs and mechanisms. Nature Reviews Cancer. 2014; 14: 662–672.

[62] Schultz KAP, Williams GM, Kamihara J, Stewart DR, Harris AK, Bauer AJ, *et al*. DICER1 and Associated Conditions: Identification of At-risk Individuals and Recommended Surveillance Strategies. Clinical Cancer Research. 2018; 24: 2251–2261.

[63] Stewart DR, Best AF, Williams GM, Harney LA, Carr AG, Harris AK, *et al*. Neoplasm Risk Among Individuals With a Pathogenic Germline Variant in DICER1. Journal of Clinical Oncology. 2019; 37: 668–676.

[64] de Kock L, Terzic T, McCluggage WG, Stewart CJR, Shaw P, Foulkes WD, *et al*. DICER1 Mutations are Consistently Present in Moderately and Poorly Differentiated Sertoli-Leydig Cell Tumors. American Journal of Surgical Pathology. 2017; 41: 1178–1187.

[65] Jelinic P, Mueller JJ, Olvera N, Dao F, Scott SN, Shah R, *et al*. Recurrent SMARCA4 mutations in small cell carcinoma of the ovary. Nature Genetics. 2014; 46: 424–426.

[66] Ramos P, Karnezis AN, Craig DW, Sekulic A, Russell ML, Hendricks WPD, *et al*. Small cell carcinoma of the ovary, hypercalcemic type, displays frequent inactivating germline and somatic mutations in SMARCA4. Nature Genetics. 2014; 46: 427–429.

[67] Witkowski L, Carrot-Zhang J, Albrecht S, Fahiminiya S, Hamel N, Tomiak E, *et al*. Germline and somatic SMARCA4 mutations characterize small cell carcinoma of the ovary, hypercalcemic type. Nature Genetics. 2014; 46: 438–443.

[68] Witkowski L, Goudie C, Ramos P, Boshari T, Brunet J, Karnezis AN, *et al*. The influence of clinical and genetic factors on patient outcome in small cell carcinoma of the ovary, hypercalcemic type. Gynecologic Oncology. 2016; 141: 454–460.

[69] Sredni ST, Tomita T. Rhabdoid Tumor Predisposition Syndrome. Pediatric and Developmental Pathology. 2015; 18: 49–58.

[70] Tischkowitz M, Huang S, Banerjee S, Hague J, Hendricks WPD, Huntsman DG, *et al*. Small-Cell Carcinoma of the Ovary, Hypercalcemic

Type–Genetics, New Treatment Targets, and Current Management Guidelines. Clinical Cancer Research. 2020; 26: 3908–3917.

[71] Long B, Lilyquist J, Weaver A, Hu C, Gnanaolivu R, Lee KY, *et al*. Cancer susceptibility gene mutations in type i and II endometrial cancer. Gynecologic Oncology. 2019; 152: 20–25.

[72] Ring KL, Bruegl AS, Allen BA, Elkin EP, Singh N, Hartman A, *et al*. Germline multi-gene hereditary cancer panel testing in an unselected endometrial cancer cohort. Modern Pathology. 2016; 29: 1381–1389.

[73] Susswein LR, Marshall ML, Nusbaum R, Vogel Postula KJ, Weissman SM, Yackowski L, *et al*. Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. Genetics in Medicine. 2016; 18: 823–832.

[74] Rossi L, Le Frere-Belda M, Laurent-Puig P, Buecher B, De Pauw A, Stoppa-Lyonnet D, *et al*. Clinicopathologic Characteristics of Endometrial Cancer in Lynch Syndrome: A French Multicenter Study. International Journal of Gynecological Cancer. 2017; 27: 953–960.

[75] Nelen MR, Padberg GW, Peeters EAJ, Lin AY, Helm BVD, Frants RR, *et al*. Localization of the gene for Cowden disease to chromosome 10q22–23. Nature Genetics. 1996; 13: 114–116.

[76] Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. Clinical Cancer Research. 2012; 18: 400–407.

[77] Segev Y, Iqbal J, Lubinski J, Gronwald J, Lynch HT, Moller P, *et al*. The incidence of endometrial cancer in women with BRCA1 and BRCA2 mutations: an international prospective cohort study. Gynecologic Oncology. 2013; 130: 127–131.

[78] Shu CA, Pike MC, Jotwani AR, Friebel TM, Soslow RA, Levine DA, *et al*. Uterine Cancer After Risk-Reducing Salpingo-oophorectomy Without Hysterectomy in Women With BRCA Mutations. JAMA Oncology. 2016; 2: 1434–1440.

[79] Yoshihama T, Hirasawa A, Sugano K, Yoshida T, Ushiama M, Ueki A, *et al*. Germline multigene panel testing revealed a BRCA2 pathogenic variant in a patient with suspected Lynch syndrome. International Cancer Conference Journal. 2021; 10: 6–10.

[80] Le DT, Kim TW, Van Cutsem E, Geva R, Jäger D, Hara H, *et al*. Phase II Open-Label Study of Pembrolizumab in Treatment-Refractory, Microsatellite Instability–High/Mismatch Repair–Deficient Metastatic Colorectal Cancer: KEYNOTE-164. Journal of Clinical Oncology. 2020; 38: 11–19.

[81] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, *et al*. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. The New England Journal of Medicine. 2015; 372: 2509–2520.

[82] Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, *et al*. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017; 357: 409–413.

[83] Balmaña J, Balaguer F, Cervantes A, Arnold D. Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines. Annals of Oncology. 2013; 24: vi73–vi80.

[84] Giardiello FM, Allen JI, Axilbund JE, Boland CR, Burke CA, Burt RW, *et al*. Guidelines on Genetic Evaluation and Management of Lynch Syndrome: a Consensus Statement by the us Multi-Society Task Force on Colorectal Cancer. Gastroenterology. 2014; 147: 502–526.

[85] Stoffel EM, Mangu PB, Gruber SB, Hamilton SR, Kalady MF, Lau MWY, *et al*. Hereditary Colorectal Cancer Syndromes: American Society of Clinical Oncology Clinical Practice Guideline Endorsement of the Familial Risk–Colorectal Cancer: European Society for Medical Oncology Clinical Practice Guidelines. Journal of Clinical Oncology. 2015; 33: 209–217.

[86] Rubenstein JH, Enns R, Heidelbaugh J, Barkun A, Adams MA, Dorn SD, *et al*. American Gastroenterological Association Institute Guideline on the Diagnosis and Management of Lynch Syndrome. Gastroenterology. 2015; 149: 777–782.

[87] Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, *et al*. Recommendations for the Care of Individuals with an Inherited Predisposition to Lynch Syndrome: A Systematic Review. The Journal of the American Medical Association. 2006; 296: 1507.

[88] Syngal S, Brand RE, Church JM, Giardiello FM, Hampel HL, Burt RW. ACG Clinical Guideline: Genetic Testing and Management of Hereditary Gastrointestinal Cancer Syndromes. American Journal of Gastroenterology. 2015; 110: 223–262.

[89] McGarrity TJ, Amos CI, Baker MJ. Peutz-Jeghers Syndrome. In Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, *et al*. (eds.) GeneReviews((R)): Seattle (WA). 1993.

[90] Gilks CB, Young RH, Aguirre P, DeLellis RA, Scully RE. Adenoma Malignum (Minimal Deviation Adenocarcinoma) of the Uterine Cervix. A clinicopathological and immunohistochemical analysis of 26 cases. The American Journal of Surgical Pathology. 1989; 13: 717–729.

[91] Chen KTK. Female genital tract tumors in Peutz-Jeghers syndrome. Human Pathology. 1986; 17: 858–861.

[92] Mikami Y, Kiyokawa T, Hata S, Fujiwara K, Moriya T, Sasano H, *et al*. Gastrointestinal immunophenotype in adenocarcinomas of the uterine cervix and related glandular lesions: a possible link between lobular endocervical glandular hyperplasia/pyloric gland metaplasia and 'adenoma malignum'. Modern Pathology. 2004; 17: 962–972.

[93] Hirasawa A, Akahane T, Tsuruta T, Kobayashi Y, Masuda K, Banno K, *et al*. Lobular endocervical glandular hyperplasia and peritoneal pigmentation associated with Peutz–Jeghers syndrome due to a germline mutation of STK11. Annals of Oncology. 2012; 23: 2990–2992.

[94] Ito M, Minamiguchi S, Mikami Y, Ueda Y, Sekiyama K, Yamamoto T, *et al*. Peutz–Jeghers syndrome-associated atypical mucinous proliferation of the uterine cervix: a case of minimal deviation adenocarcinoma ('adenoma malignum') in situ. Pathology - Research and Practice. 2012; 208: 623–627.

[95] Kobayashi Y, Masuda K, Kimura T, Nomura H, Hirasawa A, Banno K, *et al*. A tumor of the uterine cervix with a complex histology in a Peutz-Jeghers syndrome patient with genomic deletion of the STK11 exon 1 region. Future Oncology. 2014; 10: 171–177.

[96] Takei Y, Fujiwara H, Nagashima T, Takahashi Y, Takahashi S, Suzuki M. Successful pregnancy in a Peutz-Jeghers syndrome patient with lobular endocervical glandular hyperplasia. Journal of Obstetrics and Gynaecology Research. 2015; 41: 468–473.

[97] Minard-Colin V, Walterhouse D, Bisogno G, Martelli H, Anderson J, Rodeberg DA, *et al*. Localized vaginal/uterine rhabdomyosarcoma-results of a pooled analysis from four international cooperative groups. Pediatric Blood & Cancer. 2018; 65: e27096.

[98] Dehner LP, Jarzembowski JA, Hill DA. Embryonal rhabdomyosarcoma of the uterine cervix: a report of 14 cases and a discussion of its unusual clinicopathological associations. Modern Pathology. 2012; 25: 602–614.

[99] de Kock L, Yoon JY, Apellaniz-Ruiz M, Pelletier D, McCluggage WG, Stewart CJR, *et al*. Significantly greater prevalence of DICER1 alterations in uterine embryonal rhabdomyosarcoma compared to adenosarcoma. Modern Pathology. 2020; 33: 1207–1219.

[100] Meric-Bernstam F, Brusco L, Daniels M, Wathoo C, Bailey AM, Strong L, *et al*. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. Annals of Oncolog. 2016; 27: 795–800.

[101] Mandelker D, Zhang L, Kemel Y, Stadler ZK, Joseph V, Zehir A, *et al*. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. The Journal of the American Medical Association. 2017; 318: 825–835.

[102] Cobain EF, Wu YM, Vats P, Chugh R, Worden F, Smith DC, *et al*. Assessment of Clinical Benefit of Integrative Genomic Profiling in Advanced Solid Tumors. JAMA Oncology. 2021; 7: 525–533.

[103] Samadder NJ, Riegert-Johnson D, Boardman L, Rhodes D, Wick M, Okuno S, *et al*. Comparison of Universal Genetic Testing vs Guideline-Directed Targeted Testing for Patients With Hereditary Cancer Syndrome. JAMA Oncology. 2021; 7: 230–237.

[104] Levine MD, Pearlman R, Hampel H, Cosgrove C, Cohn D, Chassen A, *et al*. Up-Front Multigene Panel Testing for Cancer Susceptibility in Patients With Newly Diagnosed Endometrial Cancer: A Multicenter Prospective Study. JCO Precision Oncology. 2021; 5: 1588–1602.

How to cite this article: Ueno S, Hirasawa A. Germline Multigene Panel Testing in Gynecological Cancer. European Journal of Gynaecological Oncology. 2022; 43(3): 25-35. doi: 10.22514/ejgo.2022.008.