Hereditary Breast and Ovarian Cancer

Molecular Mechanism and Clinical Practice

Seigo Nakamura
Daisuke Aoki
Yoshio Miki
Editors

Springer

Hereditary Breast and Ovarian Cancer

Seigo Nakamura • Daisuke Aoki
Yoshio Miki
Editors

Hereditary Breast and Ovarian Cancer

Molecular Mechanism and Clinical Practice

Springer

Editors
Seigo Nakamura
Department of Breast Surgical Oncology
Showa University, School of Medicine
Tokyo
Japan

Daisuke Aoki
Department of Obstetrics and Gynecology
Keio University School of Medicine
Tokyo
Japan

Yoshio Miki
Department of Molecular Genetics, Medical Research Institute
Tokyo Medical and Dental University
Tokyo
Japan

ISBN 978-981-16-4520-4 ISBN 978-981-16-4521-1 (eBook)
https://doi.org/10.1007/978-981-16-4521-1

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

All cancers are caused by genetic alterations, but not all are hereditary. In the USA or in Europe, about 5–10% of breast cancer and 10–15% of ovarian cancer are thought to be hereditary. Nowadays, the incidence of breast cancer in Japan has rapidly increased up to 100000 cases per year. The proportion of HBOC cases is assumed to be almost the same in Japan. Therefore, we have established the Japanese Organization for Hereditary Breast and Ovarian Cancer (JOHBOC) since July 2016. The mission of the JOHBOC is (1) HBOC data registry, (2) education about HBOC to medical professionals, patients, and their families, and (3) clinical research related to the management of HBOC such as screening method including breast MRI, the significance of risk reducing mastectomy (RRM) or risk reducing salpingo-oophorectomy (RRSO), chemoprevention (tamoxifen or AI for prevention of breast cancer and oral contraceptive for ovarian cancer), and the positioning of new agents specific to BRCA mutations (PARP inhibitors). In the era of next-generation sequencing, we may encounter unexpected rare hereditary disease such as Li-Fraumeni syndrome (*P53* mutation) or Cowden disease (*PTEN* mutation), and we should not miss them and send them to the specialist of each rare disease. And one of the hot topics of basic research for HBOC is to detect new genetic alterations related to carcinogenesis.

This book was originally planned for medical professionals who are interested in HBOC practice from a variety of aspects. And it was recommended to distribute to not only Japan but also other countries. Because we anticipate this book will contribute to collaborative works worldwide.

Lastly, we deliver an address of thanks to editors Ms. Machi Sugimoto and Ms. Kripa Guruprasad of Springer Nature.

Tokyo, Japan — Seigo Nakamura
Tokyo, Japan — Daisuke Aoki
Tokyo, Japan — Yoshio Miki

Contents

History, Advancements, and Future Strategies

1

Reiko Yoshida

Abstract

Since the recognition of the genetic predisposition to breast and ovarian cancers, researchers have verified their genetic involvement and causative genes. Furthermore, treatment strategies and prevention care options to reduce the overall risk for hereditary cancers have been established based on rapid advancements in gene sequencing. Owing to the great efforts of our predecessors, the quality of life of patients diagnosed with hereditary tumors has been improved. This chapter introduces the history, advancements, and future strategies on hereditary breast and ovarian cancer (HBOC), which has a high prevalence of breast and ovarian cancer. In any field of medicine, first, clinical questions that foresee the truth arise; researchers then seek the truth, and clinicians deploy their knowledge in the medical field.

Management of hereditary breast and ovarian cancer (HBOC) is a typical model for other hereditary tumor syndromes.

Keywords

FBOC · HBOC · *BRCA1/BRCA2* · Genetic testing · Multi-gene panel · PARP inhibitor

R. Yoshida (✉)
Showa University, Advanced Cancer Translational Research Institute, Shinagawa-ku, Tokyo, Japan

Division of Cancer Genomics, Cancer Institute, Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan
e-mail: reiyosh@med.showa-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_1

1.1 History of Hereditary Breast Cancer (Fig. 1.1)

1.1.1 How It All Started

In 1866, Paul Broca, a French surgeon, was the first to describe a family with a high prevalence of breast cancer [1]. He tracked the causes of death of 38 people in his wife's family for 5 generations from 1788 to 1856 and identified that 10 of the 24 women died of breast cancer. He thus speculated that the predisposition to cancer is hereditary. In addition, he documented all other types of malignant neoplasms that included an excess of cancer of the gastrointestinal tract [2]. Jacobsen Oluf, who was one of the first investigators to question the inheritance of breast cancer as being solely site-specific, reported an increased frequency of cancer of all parts in the first-degree relatives in a series of 200 breast cancer patients [3]. In 1971, the autosomal dominant inheritance of a predisposition to both breast and ovarian cancers was first described by David E. Anderson [4]. Breast cancer patients with a family history have been reported to be associated with juvenile-onset, bilateral breast cancers and ovarian tumors compared with those without a family history. Since then, several clinical studies on familial breast and ovarian cancers have been conducted. In 1990, linkage analysis in 23 families of 146 early-onset familial breast cancers revealed an association with the D17S74 locus (CMM86) on the long arm of chromosome 17 [5]. This study used the positional cloning method to analyze DNA of multiple family members, and the authors used gene polymorphism markers, as well as the information from chromosomal recombination yielding a logarithm of the likelihood ratio for linkage during meiosis and germ-cell formation. They were thus able to limit the chromosomal region where the causative gene was located. Furthermore, in 1994, Yoshio Miki et al. succeeded in cloning *BRCA1* using reverse genetics to elucidate its function and determine its complete structure [6]. However, because there were few male breast cancer patients among *BRCA1* mutation carriers, another causative gene is implicated. As with *BRCA1*, using linkage analysis of multiple families with breast, ovarian, and male breast cancers,

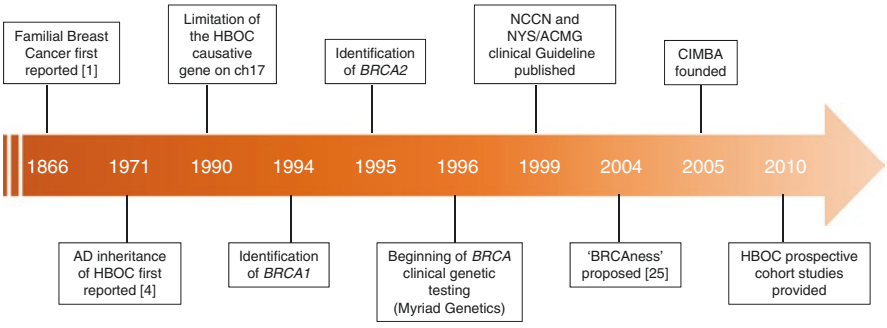


Fig. 1.1 Timeline of HBOC discovery and clinical setting. *AD* autosomal dominant, *NCCN* National Comprehensive Cancer Network, *NYS* New York State, *ACMG* American College of Medical Genetics, *CIMBA* Consortium of Investigators of Modifiers of BRCA1/2

Richard Wooster et al. identified *BRCA2* on the long arm of chromosome 13 [7, 8] in 1995. In 1994, Henry Lynch collectively referred to hereditary ovarian cancer (HOC), hereditary breast cancer (HBC), and hereditary syndrome that causes both breast and ovarian cancers as hereditary breast and ovarian cancers (HBOC) [9].

1.1.2 *BRCA1* and *BRCA2* in the Clinical Setting

After *BRCA1* and *BRCA2* were identified, they have been immediately applied in the clinical setting. In 1996, clinical genetic testing for *BRCA1* and *BRCA2* was patented by Myriad Genetics and made available worldwide. Various clinical studies were conducted by multiple research groups to increase the understanding of *BRCA1* and *BRCA2* cancers. The Breast Cancer Linkage Consortium, founded in 1989, reported that other cancers, such as prostate cancer, pancreatic cancer, and melanoma, are associated with BRCA1/BRCA2 mutations [10]. Meanwhile, the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA), a collaborative group of researchers in Europe, North and South Americas, Australia, Asia, and Africa founded in 2005, has described the clinicopathological features of *BRCA*-associated cancers [11], the genotype–phenotype correlations from a prospective study [12], the characteristics of *BRCA* male breast cancer [13], and analysis of the risk of multiple single nucleotide polymorphisms (SNPs) in *BRCA* mutation carriers [14]. Moreover, this group has phenotypic data of about 80,000 female and male BRCA1 and BRCA2 mutant carriers; so far about 43,000 have been genotyped in the CIMBA project, and an additional 25,000 will have been genotyped in 2020.

Currently, the ClinVar, a database provided by the National Center for Biotechnology Information, has recognized more than 3400 germline variants of *BRCA1* and 3900 of *BRCA2* as pathogenic or likely pathogenic (20/October/2020 assessed). The majority (80%) of which are truncating variants that form immature stop codons, such as frameshifts and nonsense, whereas missense mutations account for approximately 10%. Pathogenic missense variants tend to be concentrated in functionally essential sites, such as the really interesting new gene (RING) finger domain and BRCA1 C terminus (BRCT) domains of *BRCA1* or the regions spanning the oligosaccharide-binding folds and helical domains of *BRCA2* [15]. Abnormal copy number variants (CNVs) detected by deletion or duplication analysis account for approximately 10% and vary among populations [16].

A founder mutation is defined by the National Institutes of Health as follows: "A genetic alteration observed with high frequency in a group that is or was geographically or culturally isolated, in which one or more of the ancestors was a carrier of the altered gene." Particularly, in Ashkenazi Jews, three common mutations of *BRCA1*, namely, c.68_69delAG, c.5266dupC, and *BRCA2* c.5946delT, account for 98–99% of the pathogenic variants. Thus, targeted analysis of these three variants is recommended. However, in other ethnic groups, full-sequence analysis and CNV analysis are necessary [16]. Various cohort studies and clinical trials have also been conducted for the medical management for *BRCA1*and *BRCA2* carriers. Retrospective studies have reported the usefulness of prophylactic surgery and

breast magnetic resonance imaging (MRI) surveillance [17, 18]. Since 2010, the findings of prospective cohort studies have provided guidance on improving the quality of life of patients using these medical interventions [19–21].

With the increasing interest on clinical *BRCA* genetic testing and the accumulation of information about *BRCA1* and *BRCA2*-mutation carriers, HBOC clinical guidelines have been established and are widely used globally. Moreover, the American Society of Clinical Oncology (ASCO) published its own guidelines in 1996, and the National Comprehensive Cancer Network (NCCN) in 1999. In addition, the New York State and the American College of Medical Genetics (NYS/ACMG) guidelines were posted on the New York State website in 1999. These guidelines were developed under the close collaboration of numerous health professionals, including oncologists, geneticists, genetic counselors, primary-care physicians, and public health specialists, and contribute to the determination of a series of HBOC practices: genetic testing criteria, testing methods, interpretation of test results, and medical management [22].

The association between *BRCA* mutation location and breast and ovarian cancer risk has also been reported. In both *BRCA1* and *BRCA2* carriers, ovarian cancer cluster regions (OCCR) have been confirmed to be located within or adjacent to exon 11 [12]. Carriers with pathogenic variants in the OCCR possessed a higher risk of ovarian cancer, unlike those with pathogenic variants located elsewhere. Similarly, several breast cancer cluster regions (BCCRs) have been observed in *BRCA1* and *BRCA2*, respectively, and are associated with a relative increase in breast cancer risk but a relative decrease in ovarian cancer risk. However, in this previous study, each hazard ratio for cancer development owing to the difference in mutation locations was at most 2. Therefore, without additional information, it may be premature to use correlation between genotype location and cancer risk phenotype for individual risk assessment and management.

Immediately after cloning of *BRCA1* and *BRCA2*, new findings, including the role of *BRCA* in carcinogenesis and the genomic aberrations in *BRCA*-mutated cancers, have been reported. DNA repair mechanisms include DNA single-strand break (dsDNA) repair, double-strand break (dsDNA) repair, base mismatch repair (MMR), base excision repair (BER), and nucleotide repair (NER). *BRCA1* and *BRCA2* are cancer-suppressor genes that maintain genomic stability by repairing dsDNA via homologous recombination (HR) [23]. In addition to HR repair (HRR), *BRCA1* and *BRCA2* regulate centrosome dynamics, chromosome distribution, and cytokinesis and temporally and spatially stabilize the genome during cell cycle. Moreover, a hormone-dependent carcinogenic environment is speculated to contribute to genome instability via the disruption of these *BRCA* functions and the accelerated activation of survival signals and the mammary gland cells are converted to malignant traits [24]. In addition to its DNA-damage repair function, the involvement of *BRCA1* on normal embryogenesis, centrosome replication, spindle pole synthesis, heterochromatin-satellite RNA expression, estrogen metabolite synthesis, splicing, brain size regulation, and transcriptional co-activation have been reported [16]. Because the loss of *BRCA* function has been associated with HR defects, the concept “BRCAness” has been proposed in 2004 [25]. It refers to

sporadic breast cancers exhibiting similar clinicopathological features and characteristic genomic aberrations as *BRCA*-related cancers; thus, BRCAness can be considered as a therapeutic biomarker.

1.2 Current Developments (Fig. 1.2)

1.2.1 Multi-gene Panel Testing and Non-BRCA Genes

BRCA1/BRCA2 genetic testing achieved a major transformation in 2013. In the litigation of the *BRCA* gene patent against Myriad Genetics from 2009 to 2013, the US Supreme Court ruled in 2013 as follows: because separating that gene from its surrounding genetic material is not an act of invention, isolated human genes cannot be patented. Furthermore, owing to the development of next-generation sequence (NGS) technology in 2005 and its plummeting cost since 2007, genetic testing has become more powerful and generated tremendous data compared with the conventional Sanger sequence method. Subsequently, multiple genetic testing companies have started to provide multi-gene panel (MGP) testing, including *BRCA1*and *BRCA2*. In the United States, it has been reported that the number of MGP testing performed has exceeded that of *BRCA* alone testing since 2014 [26]. In addition, numerous MGP testings of large cohorts have been developed, resulting in the accumulation of information on genes that cause breast and ovarian cancer other than *BRCA*. Among all breast cancer patients without selection bias, the *BRCA1/BRCA2* mutation detection rate was 4–5%, whereas the total mutation detection rate of MGP was 6–9%, a 1.4- to 2-fold increase. Similarly, in ovarian cancer patients without selection bias, the *BRCA1/BRCA2* mutation detection rate was approximately 20%, whereas that of MGP was 26–31%, a 1.5-fold increase [27–31]. Because "nearly all known HBOC susceptibility genes encode tumor suppressors that participate in genome stability pathways—in particular HRR, and to some extent mismatch repair (MMR) and interstrand DNA crosslink repair via the Fanconi anemia pathway" as reported by Nielsen et al. [32], the detection of

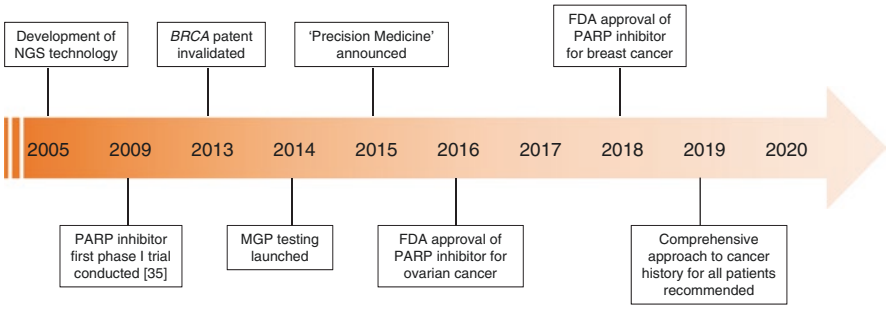


Fig. 1.2 Timeline of HBOC development. *NGS* next-generation sequence, *PARP* poly ADP ribose polymerase, *MGP* multi-gene panel, *FDA* US Food and Drug Administration

known susceptibility genes for breast and ovarian cancers other than HR-related genes is possible via MGP. In addition, hereditary cancer syndromes other than *BRCA1*- and *BRCA2*-related cancers, which could not be identified using one-panel testing, can be diagnosed using MGP testing. Thus, the utilization of MGP testing provides opportunities to discover genetic diseases that were not expected from family and individual medical histories and to take new measures for additional preventive medical management depending on the constitution of each genetic disorder. Figure 1.3 shows the results of MGP testing in a Japanese biobank cohort, including 11 breast cancer-susceptibility genes (*BRCA1*, *BRCA2*, *PALB2*, *TP53*, *PTEN*, *CHEK2*, *NF1*, *ATM*, *CDH1*, *NBN*, and *STK11*) [27]. Compared with MGP testing results in unaffected group with no familial cancer (the control group), breast cancer patient group without selection bias, and high-risk group comprising breast cancer patients meeting the *BRCA* testing criteria based on NCCN guideline, the overall mutation detection rate of MGP testing was the highest in the high-risk group; however, the highest frequency of pathogenic mutations other than *BRCA1* and *BRCA2* was identified in the control group. Thus, clients except high-risk group they were more likely to benefit from MGP testing than *BRCA* testing alone. It has been suggested that the benefits of MGP testing have been extended to all subjects

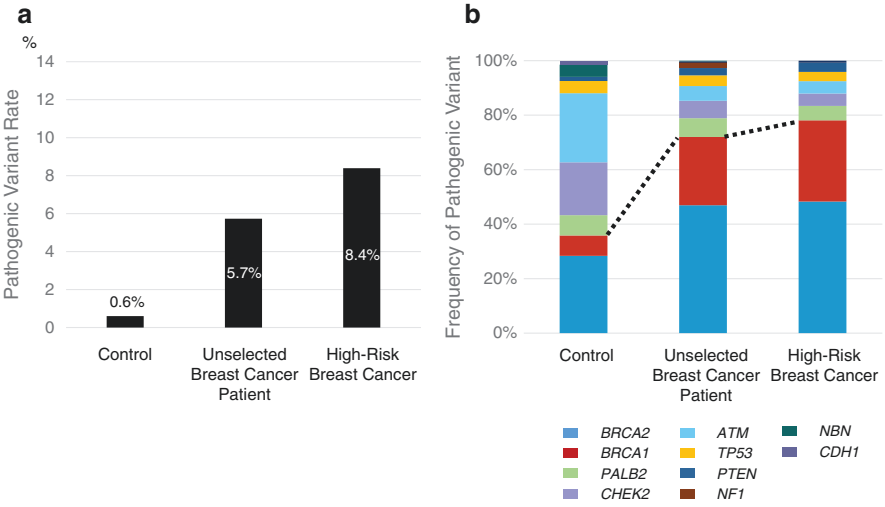


Fig. 1.3 The results of MGP testing in a Japanese biobank cohort. The data of the result of MGP testing including 11 breast cancer-susceptibility genes (*BRCA1*, *BRCA2*, *PALB2*, *TP53*, *PTEN*, *CHEK2*, *NF1*, *ATM*, *CDH1*, *NBN*, and *STK11*) using the Japanese biobank cohort was illustrated in bar plots. The overall mutation detection rate of MGP testing was the highest in the high-risk group; however, the highest frequency of pathogenic mutations other than *BRCA1* and *BRCA2* was identified in the control group. (**a**) The result of total pathogenic variant rate between three groups. (**b**) The result of frequency of pathogenic variants between three groups. Control group; unaffected group with no familial cancer, unselected breast cancer patients; breast cancer patient group without selection bias, high-risk breast cancer group; high-risk group comprising breast cancer patients meeting the *BRCA* testing criteria based on NCCN guideline

considering cancer preventive medicine, not just cancer patients with suspected HBOC.

1.2.2 Target Therapy

In 2005, tumor cells lacking *BRCA1* and *BRCA2* and key tumor-suppressor proteins involved in DSB repair via HR were found to be selectively sensitive to small-molecule inhibitors of the enzyme poly ADP ribose polymerase (PARP) family of DNA repair enzymes [33, 34]. Subsequently, a new cancer therapeutic strategy based on synthetic lethality was conducted in 2009 in the first phase I clinical trial of this PARP inhibitor in *BRCA1*- and *BRCA2*-positive individuals [35]. Then, clinical trials involving PARP inhibitors targeting ovarian and breast cancers with germline *BRCA* mutations showed good results [36, 37]. The PARP inhibitor olaparib has been approved by the FDA for treatment of metastatic ovarian cancer with germline *BRCA* mutations in 2016, and in 2018, it was approved to treat metastatic breast cancer. Currently, indications for prostate and pancreatic cancers are being expanded, and those for cancers using characteristic genomic aberrations representing the HR deficiency (HRD) of tumors as biomarkers, like "HRD score" [38], are being expanded.

As advances in NGS technology allow more precise analysis of changes in the cancer genome, it turns out that individual disease causes and cancer status are more complex than expected; in order to provide realistic medical practice, it is necessary to divide patients into subgroups, and in 2015, "Precision Medicine" was announced with the aim of establishing treatment methods and providing preventive medical care for each subgroup. Cancer medicine using genomic information is rapidly being developed for this subgrouping. Although the main purpose of these tumor tissue profiling tests is to select cancer drugs, at the same time, germline pathogenic variant in 4% to 12% was also found. The detection of pathogenic germline mutations is often a critical step in initiation of the cascade of genetic testing in relatives, which can clarify the patient's own cancer risk and translate into life-saving surveillance and risk reduction interventions for family members [39].

Owing to the abovementioned developments, MGP testing, companion diagnostics targeting PARP inhibitors, and cancer genomic medicine, the possibility of detecting genes other than germline *BRCA1* and *BRCA2* has increased rapidly in the clinical setting. In response, the 2013 version of the NCCN guidelines [40] had a new section titled "Additional genetic mutations associated with breast/ovarian cancer risk" in addition to the conventional HBOC syndrome, Li–Fraumeni syndrome, and Cowden syndrome. It listed 21 genes related to breast and ovarian cancers. In the 2014 version, a section on "Multi-gene Testing" was released. Particularly, it stated that when patients meeting the HBOC testing criteria are found negative for *BRCA1* and *BRCA2*, multi-gene testing should be considered. In its 2015 version, for 15 genes, including *BRCA1* and *BRCA2*, recommendations, considerations, and insufficient evidenced medical management for breast MRI surveillance and prophylactic surgery were presented. In 2017, risk and management options for patients

with breast cancer, ovarian cancer, and other cancers were described in detail, including high- and moderate-risk genes widely used in MGP testing. Furthermore, patients with *BRCA1* and *BRCA2* mutation detected via tumor profiling have been added to the *BRCA* testing criteria. Finally, the latest 2020 edition has radically shifted away from the *BRCA* gene toward a broad screening of other genes, consistent with current practice. Hereditary pancreatic cancer has also been added to this guideline, and the two-step approach, "Further Genetic Risk Evaluation" and "Testing Criteria," has been changed to "comprehensive approach to cancer history for all patients" (Fig. 1.4). Moreover, the "*BRCA* testing criteria" have been changed to the "High-penetrance Breast and/or Ovarian Cancer Susceptibility Genes Testing Criteria." When there are no known pathological gene mutations in relatives, comprehensive MGP testing should be performed from the beginning. It suggested that it is now time to handle and manage beyond *BRCA* genes in general practice.

However, the use of multiple genes with lower allele frequencies and lower penetrance than *BRCA1* and *BRCA2* has some challenges. First, this results in an increased number of variants of uncertain significance (VUSs). As the number of genes searched by MGP testing increases, the number of VUSs also increases. The VUS rates of other genes are generally higher than that of *BRCA* and higher than pathogenic mutation rates [41]. Second, the variant is examined based on the ACMG/AMP variant classification guideline [42]; however, because each genetic testing company makes the final judgment by its own method, some disagreement

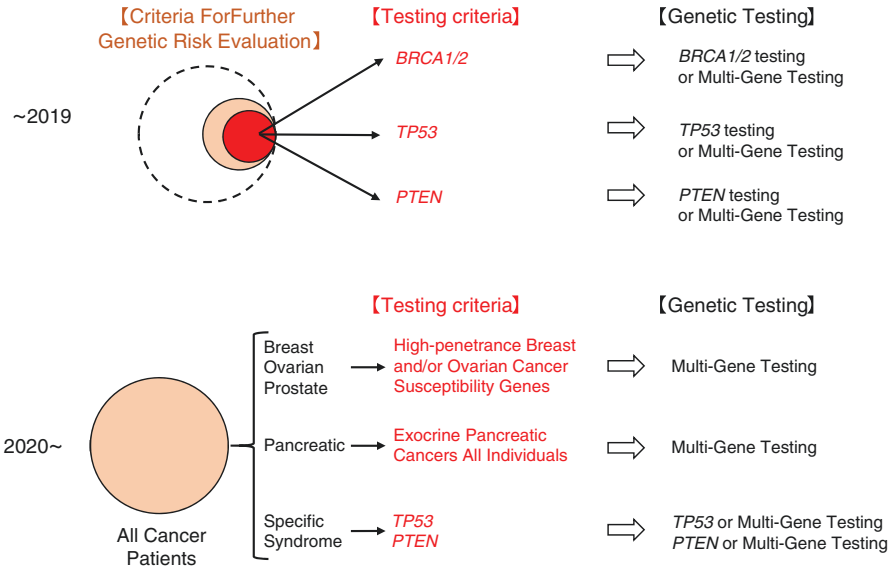


Fig. 1.4 Changes in genetic risk assessment and testing criteria in NCCN guideline. NCCN guideline 2020 edition has radically shifted away from *BRCA* genes toward a broad screening of other genes, consistent with current practice. Hereditary pancreatic cancer has also been added to this guideline, and the two-step approach, "Further Genetic Risk Evaluation" and "Testing Criteria," has been changed to "comprehensive approach to cancer history for all patients"

on the interpretation of certain variants may exist. To address this, the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) [43] and BRCA Share™ [44] have been established to provide a critical evaluation of the risk and assess the clinical importance of VUSs.

1.3 Future Strategies (Fig. 1.5)

Reverse genetics is an approach in molecular genetics that elucidates gene function by examining the changes in phenotypes via the suppression or enhancement of the gene expression. On the other hand, forward genetics is the technique of identifying genes from phenotypes, which existed before the concept of genes was reported. At the time

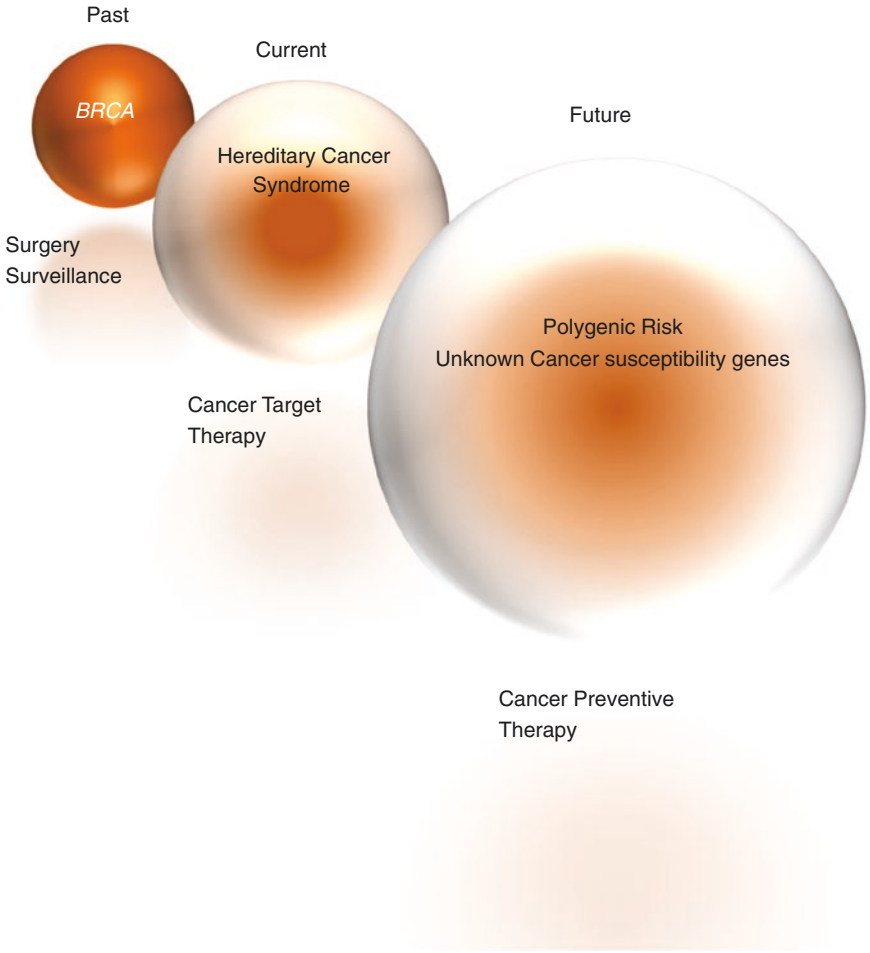


Fig. 1.5 HBOC strategy past, current, future. Targets for considering the risk of cancer susceptibility and effective medical practices are expanding

of the discovery of *BRCA1* and *BRCA2*, many causative genes for hereditary diseases, including hereditary cancer syndromes, were discovered using reverse genetics. However, since around 2000, reports of the discovery of the causative genes using reverse genetics have gradually decreased although there still exist hereditary diseases of unknown causative genes. Because gene identification by this method has reached its limit, with the innovative progress of genome sequence analysis technology, the following new methods have been used to report the discovery of multiple genes.

New methods, including whole-exome sequencing (WES) and whole-genome sequencing (WGS), can now be utilized at a relatively low cost and achieve fast results. Currently, WES is used to elucidate the causes of hereditary diseases for which the causative gene has not been identified. Both WES and WGS are already clinically available. However, the differences between these new sequencing technologies and MGP testing should be considered. The main difference is the amount of data generated. Whereas WGS yields sequence information of all regions in the genome, WES focuses on less than 2% of the genome. MGP testing selects and searches several to dozens of genes from more than 20,000 types of genes. WES and MGP testing read only the protein-coding regions and exclude the promoter or regulatory regions. For example, many commercially available MGP assays analyze the exon–intron border regions with a range of 2–5 bp. However, in case of a variant located in the deep intron region affecting the activity of the target gene, MGP testing and WES could not identify the said variant [45]. Notably, the required sample amount and cost for analysis do not tremendously differ among these methods. In addition, the VUS rate is expected to increase using new methods. Variants found using WES and WGS have indicated that validation using traditional Sanger sequencing is required [46].

While WES and WGS are expected to find a rare single causative gene that disrupts specific pathways and functions, cancer development is a multifactorial disease. More recently, polygenic risk score (PRS) that integrates the joint effects of common genetic variants on disease risk has been developed. PRS is a score that calculates the overall risk of developing a disease based on the dozens to thousands of single nucleotide polymorphisms (SNPs) suggested to be associated with each disease or trait derived from genome-wide association studies (GWAS). While common variants have small individual effects on disease risk, cumulatively, they can have large effects—in some individuals, risks equivalent to the strong monogenic variants such as *BRCA1* and *BRCA2* [14, 47]. Owing to monogenic mutations and PRS, the risk of breast cancer by age 75 ranges from 12.7% to 75.7% in *BRCA1* and *BRCA2* mutation carriers, whereas that in non-carriers ranges from only 3.3% to 29.6%. Higher PRS correlates with higher risk, whereas the risks of the carrier group with low PRS and that of the non-carrier group with high PRS are the same. Thus, the PRS-based approach to patient stratification based on cancer risk may further improve screening methods and prevention strategies compared with methods targeting a single gene.

In addition to the development of treatments for cancers following diagnosis, prophylactic surgery, and the early detection of cancer, evidence for chemoprevention of HBOC has been established. Although there are limited large prospective clinical studies involving only women with *BRCA* mutations who have not developed breast cancer, reports on breast cancer prevention using selective estrogen-receptor

modulators (SERMs) are available. The two largest studies (National Surgical Adjuvant Breast and Bowel Project-P1 (NSABP-P1) [48] and International Breast cancer Intervention Study-1 (IBIS-1) [49]) found that tamoxifen reduced the incidence of breast cancer by approximately 40%, and its protective effect extended beyond the treatment period. Among the 288 cases, there were 8 *BRCA1* and 11 *BRCA2* mutation carriers [48]. Although it was a result of a small sample size only for *BRCA* mutation carriers, a potential reduction in *BRCA2*- but not *BRCA1*-associated breast cancer was observed following tamoxifen use [50]. In addition, a meta-analysis report of four case–control studies have analyzed the risk reduction of CBC due to tamoxifen [51]. In the previous report, although tamoxifen did not exhibit protective effects in women with *BRCA1* mutations who had a high proportion of triple-negative breast cancer cells, it was protective for women with *BRCA2* mutations. In addition, tamoxifen reduced the risk of contralateral breast cancer in BRCA1 mutation carriers. However, these studies are not limited to BRCA and their sample sizes were small. Adverse effects (thrombosis, endometrial cancer, early menopause, etc.) due to SERMs have also been reported.

Chemoprevention is a promising preventive option for *BRCA* mutation carriers. A chemopreventive drug, i.e., a monoclonal antibody (denosumab) targeting RANKL, has been recently identified. Denosumab is used for the treatment of osteoporosis and bone metastasis. Various studies have demonstrated that the progesterone-mediated upregulation of the RANK/RANKL pathway plays a critical role in mammary epithelial proliferation, mammary stem cell expansion, and carcinogenesis [52]. In Brca1-mutant mice, the loss of RANKL reduced mammary tumors and suppressed tumor progression, and its inhibition prevented mammary tumor development [53]. Moreover, previous studies have reported that the circulating level of osteoprotegerin (OPG) is significantly lower correlated with higher progesterone levels in premenopausal *BRCA* mutation carriers than in non-carrier controls. This suggests a significant dysregulation of circulating OPG and sex hormone levels [54]. A chemopreventive clinical trial of denosumab involving unaffected *BRCA* mutation carriers is underway.

1.4 Conclusion

Through the collaboration among experts in many fields, such as basic science, bioinformatics, statistics, pharmacology, diagnostic imaging, surgery, clinical medicine, politics, genetics, and genetic clinical practice, increased understanding of HBOC can be achieved, thereby improving the quality of life of HBOC patients.

References

1. Lynch HT, Shaw TG, Lynch JF. Inherited predisposition to cancer: a historical overview. Am J Med Genet C. 2004;129C:5–22. https://doi.org/10.1002/ajmg.c.30026.
2. Broca PP. Traité des tumeurs, vol. 1. Paris: Asselin; 1866. p. 2

3. Jacobsen O. Heredity in breast cancer: a genetic and clinical study of two hundred probands. London: HK Lewis; 1946.
4. Anderson DE. Some characteristics of familial breast cancer. Cancer. 1971;28:1500–4. https://doi.org/10.1002/1097-0142(197112)28:6<1500::aid-cncr2820280623>3.0.co;2-d.
5. Hall JM, Lee MK, Newman B, Morrow JE, Anderson LA, Huey B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science. 1990;250:1684–9. https://doi.org/10.1126/science.2270482.
6. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science. 1994;266:66–71. https://doi.org/10.1126/science.7545954.
7. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene *BRCA2*. Nature. 1995;378:789–92. https://doi.org/10.1038/378789a0.
8. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, et al. Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome 13q12-13. Science. 1994;265:2088–90. https://doi.org/10.1126/science.8091231.
9. Lynch HT, Lynch J, Conway T, Watson P, Feunteum J, Lenoir G, et al. Hereditary breast cancer and family cancer syndromes. World J Surg. 1994;18:21–31. https://doi.org/10.1007/BF00348188.
10. Breast Cancer Linkage Consortium T. Cancer risks in *BRCA2* mutation carriers. J Natl Cancer Inst. 1999;91:1310–6. https://doi.org/10.1093/jnci/91.15.1310.
11. Mavaddat N, Barrowdale D, Andrulis IL, Domchek SM, Eccles D, Nevanlinna H, et al. Pathology of breast and ovarian cancers among *BRCA1* and *BRCA2* mutation carriers: results from the consortium of investigators of modifiers of *BRCA1/2* (CIMBA). Cancer Epidemiol Biomark Prev. 2012;21:134–47. https://doi.org/10.1158/1055-9965.EPI-11-0775.
12. Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, et al. Association of type and location of *BRCA1* and *BRCA2* mutations with risk of breast and ovarian cancer. JAMA. 2015;313:1347–61. https://doi.org/10.1001/jama.2014.5985.
13. Silvestri V, Leslie G, Barnes DR, Agnarsson BA, Aittomäki K, Alducci E, et al. Characterization of the cancer spectrum in men with germline *BRCA1* and *BRCA2* pathogenic variants: results from the consortium of investigators of modifiers of BRCA1/2 (CIMBA). JAMA Oncol. 2020;6:1218–30. https://doi.org/10.1001/jamaoncol.2020.2134.
14. Kuchenbaecker KB, McGuffog L, Barrowdale D, Lee A, Soucy P, Dennis J, et al. Evaluation of polygenic risk scores for breast and ovarian cancer risk prediction in *BRCA1* and *BRCA2* mutation carriers. J Natl Cancer Inst. 2017;109:djw302. https://doi.org/10.1093/jnci/djw302.
15. Venkitaraman AR. How do mutations affecting the breast cancer genes *BRCA1* and *BRCA2* cause cancer susceptibility? DNA Repair (Amst). 2019;81:102668. https://doi.org/10.1016/j.dnarep.2019.102668.
16. Yoshida R. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer. 2020;29:1–4. https://doi.org/10.1007/s12282-020-01148-2.
17. Rebbeck TR, Levin AM, Eisen A, Snyder C, Watson P, Cannon-Albright L, et al. Breast cancer risk after bilateral prophylactic oophorectomy in *BRCA1* mutation carriers. J Natl Cancer Inst. 1999;91:1475–9. https://doi.org/10.1093/jnci/91.17.1475.
18. Hartmann LC, Schaid DJ, Woods JE, Crotty TP, Myers JL, Arnold PG, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med. 1999;340:77–84. https://doi.org/10.1056/NEJM199901143400201.
19. Heemskerk-Gerritsen BA, Rookus MA, Aalfs CM, Ausems MG, Collée JM, Jansen L, et al. Improved overall survival after contralateral risk-reducing mastectomy in *BRCA1/2* mutation carriers with a history of unilateral breast cancer: a prospective analysis. Int J Cancer. 2015;136:668–77. https://doi.org/10.1002/ijc.29032.
20. Finch AP, Lubinski J, Møller P, Singer CF, Karlan B, Senter L, et al. Impact of oophorectomy on cancer incidence and mortality in women with a *BRCA1* or *BRCA2* mutation. J Clin Oncol. 2014;32:1547–53. https://doi.org/10.1200/JCO.2013.53.2820.

21. Evans DG, Kesavan N, Lim Y, Gadde S, Hurley E, Massat NJ, et al. MRI breast screening in high-risk women: cancer detection and survival analysis. Breast Cancer Res Treat. 2014;145:663–72. https://doi.org/10.1007/s10549-014-2931-9.
22. Levy DE, Garber JE, Shields AE. Guidelines for genetic risk assessment of hereditary breast and ovarian cancer: early disagreements and low utilization. J Gen Intern Med. 2009;24:822–8. https://doi.org/10.1007/s11606-009-1009-6.
23. Venkitaraman AR. Cancer suppression by the chromosome custodians, *BRCA1* and *BRCA2*. Science. 2014;343:1470–5. https://doi.org/10.1126/science.1252230.
24. Sasanuma H, Tsuda M, Morimoto S, Saha LK, Rahman MM, Kiyooka Y, et al. *BRCA1* ensures genome integrity by eliminating estrogen-induced pathological topoisomerase II-DNA complexes. Proc Natl Acad Sci U S A. 2018;115:E10642–51. https://doi.org/10.1073/pnas.1803177115.
25. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer. 2004;4:814–9. https://doi.org/10.1038/nrc1457.
26. 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 Oncol. 2018;4:1066–72. https://doi.org/10.1001/jamaoncol.2018.0644.
27. Momozawa Y, Iwasaki Y, Parsons MT, Kamatani Y, Takahashi A, Tamura C, et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun. 2018;9:4083. https://doi.org/10.1038/s41467-018-06581-8.
28. Buys SS, Sandbach JF, Gammon A, Patel G, Kidd J, Brown KL, et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer. 2017;123:1721–30. https://doi.org/10.1002/cncr.30498.
29. Sun J, Meng H, Yao L, Lv M, Bai J, Zhang J, et al. Germline mutations in cancer susceptibility genes in a large series of unselected breast cancer patients. Clin Cancer Res. 2017;23:6113–9. https://doi.org/10.1158/1078-0432.CCR-16-3227.
30. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20:764–75. https://doi.org/10.1158/1078-0432.CCR-13-2287.
31. Li W, Shao D, Li L, Wu M, Ma S, Tan X, et al. Germline and somatic mutations of multi-gene panel in Chinese patients with epithelial ovarian cancer: a prospective cohort study. J Ovarian Res. 2019;12:80. https://doi.org/10.1186/s13048-019-0560-y.
32. Nielsen FC, van Overeem HT, Sørensen CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer. 2016;16:599–612. https://doi.org/10.1038/nrc.2016.72.
33. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. Nature. 2005;434:917–21. https://doi.org/10.1038/nature03445.
34. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of *BRCA2*-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:913–7. https://doi.org/10.1038/nature03443.
35. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. N Engl J Med. 2009;361:123–34. https://doi.org/10.1056/NEJMoa0900212.
36. Franzese E, Centonze S, Diana A, Carlino F, Guerrera LP, Di Napoli M, et al. PARP inhibitors in ovarian cancer. Cancer Treat Rev. 2019;73:1–9. https://doi.org/10.1016/j.ctrv.2018.12.002.
37. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline *BRCA* mutation. N Engl J Med. 2017;377:523–33. https://doi.org/10.1056/NEJMoa1706450.
38. Timms KM, Abkevich V, Hughes E, et al. Association of BRCA1/2defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. Br Cancer Res. 2014;16:475. https://doi.org/10.1186/s13058-014-0475-x.
39. DeLeonardis K, Hogan L, Cannistra SA, Rangachari D, Tung N. When should tumor genomic profiling prompt consideration of germline testing? J Oncol Pract. 2019;15:465–73. https://doi.org/10.1200/JOP.19.00201.

40. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) genetic/familial high-risk assessment: breast and ovarian.
41. van Marcke C, Collard A, Vikkula M, Duhoux FP. Prevalence of pathogenic variants and variants of unknown significance in patients at high risk of breast cancer: a systematic review and meta-analysis of gene-panel data. Crit Rev Oncol Hematol. 2018;132:138–44. https://doi.org/10.1016/j.critrevonc.2018.09.009.
42. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24. https://doi.org/10.1038/gim.2015.30.
43. Spurdle AB, Healey S, Devereau A, Hogervorst FB, Monteiro AN, Nathanson KL, et al. ENIGMA--evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in *BRCA1* and *BRCA2* genes. Hum Mutat. 2012;33:2–7. https://doi.org/10.1002/humu.21628.
44. Béroud C, Letovsky SI, Braastad CD, Caputo SM, Beaudoux O, Bignon YJ, et al. BRCA share: a collection of clinical *BRCA* gene variants. Hum Mutat. 2016;37:1318–28. https://doi.org/10.1002/humu.23113.
45. Evans DGR, van Veen EM, Byers HJ, Wallace AJ, Ellingford JM, Beaman G, et al. A dominantly inherited 5′ UTR variant causing methylation-associated silencing of *BRCA1* as a cause of breast and ovarian cancer. Am J Hum Genet. 2018;103:213–20. https://doi.org/10.1016/j.ajhg.2018.07.002.
46. O'Daniel JM, Lee K. Whole-genome and whole-exome sequencing in hereditary cancer: impact on genetic testing and counseling. Cancer J. 2012;18:287–92. https://doi.org/10.1097/PPO.0b013e318262467e.
47. Fahed AC, Wang M, Homburger JR, Patel AP, Bick AG, Neben CL, et al. Polygenic background modifies penetrance of monogenic variants for tier 1 genomic conditions. Nat Commun. 2020;11:3635. https://doi.org/10.1038/s41467-020-17374-3.
48. Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and bowel project P-1 study. J Natl Cancer Inst. 2005;97:1652–62. https://doi.org/10.1093/jnci/dji372.
49. Cuzick J, Forbes JF, Sestak I, Cawthorn S, Hamed H, Holli K, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst. 2007;99:272–82. https://doi.org/10.1093/jnci/djk049.
50. King MC, Wieand S, Hale K, Lee M, Walsh T, Owens K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in *BRCA1* and *BRCA2*: National Surgical Adjuvant Breast and bowel project (NSABP-P1) breast cancer prevention trial. JAMA. 2001;286:2251–6. https://doi.org/10.1001/jama.286.18.2251.
51. Xu L, Zhao Y, Chen Z, Wang Y, Chen L, Wang S. Tamoxifen and risk of contralateral breast cancer among women with inherited mutations in *BRCA1* and *BRCA2*: a meta-analysis. Breast Cancer. 2015;22:327–34. https://doi.org/10.1007/s12282-015-0619-6.
52. Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, et al. Progesterone induces adult mammary stem cell expansion. Nature. 2010;465:803–7. https://doi.org/10.1038/nature09091.
53. Sigl V, Owusu-Boaitey K, Joshi PA, Kavirayani A, Wirnsberger G, Novatchkova M, et al. RANKL/RANK control *BRCA1* mutation-driven. Cell Res. 2016;26:761–74. https://doi.org/10.1038/cr.2016.69.
54. Odén L, Akbari M, Zaman T, Singer CF, Sun P, Narod SA, et al. Plasma osteoprotegerin and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. Oncotarget. 2016;7:86687–94. https://doi.org/10.18632/oncotarget.13417.

2 Molecular Basis of BRCA1 and BRCA2: Homologous Recombination Deficiency and Tissue-Specific Carcinogenesis

Tomohiko Ohta and Wenwen Wu

Abstract

Mutations in BRCA1 and BRCA2 cause hereditary breast and ovarian cancer (HBOC) syndrome, and these genes play multiple critical roles in maintaining genomic stability. One particularly important function of these genes is the homologous recombination (HR) repair of DNA. HR repair is an essential error-free repair mechanism for DNA double-strand breaks that utilizes an intact sister chromatid as a template. In addition to its role in HBOC oncogenesis, HR dysfunction is a target for treatment with poly (ADP-ribose) polymerase (PARP) inhibitors. Germline mutations of *BRCA1/BRCA2* cause breast, ovarian, fallopian tube, and peritoneal cancers with high rates of genomic alterations accompanied by poor prognoses. The mechanism underlying this tissue specificity has not yet clearly been explained, but several studies have examined its possible association with estrogen signaling. In this review, we first introduced the molecular mechanisms of HR mediated by BRCA1 and BRCA2 in the context of PARP inhibitor sensitivity. We also discussed several hypotheses describing estrogen- and HR deficiency-dependent genomic instability. Understanding these mechanisms is crucial for the adequate treatment and prevention of HBOC-related cancers.

Keywords

BRCA1 · BRCA2 · PARP inhibitor · Homologous recombination · Non-homologous end-joining · Alternative end-joining · Tissue specificity · Estrogen

T. Ohta (✉) · W. Wu
Department of Translational Oncology, St. Marianna University Graduate School of Medicine, Kawasaki, Japan
e-mail: to@marianna-u.ac.jp; wuwenwen@marianna-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_2

2.1 Introduction

Hereditary breast and ovarian cancer (HBOC) syndrome is attributable to germline mutations in genes encoding DNA repair proteins and cell cycle checkpoints, the two most prominent being *BRCA1* and *BRCA2* [1, 2]. The primary function of the BRCA1 and BRCA2 is maintaining genomic stability. In this role, BRCA1 and BRCA2 critically control a broad range of cellular processes including DNA repair, cell cycle checkpoints, apoptosis induction, chromatin modification, and centrosome duplication. In addition to *BRCA1* and *BRCA2*, germline mutations in *PALB2* (also called *FANCN*), *RAD51C*, *ATM*, *CHEK2*, and *TP53*, which participate in homologous recombination (HR) repair and cell cycle checkpoint regulation, cause HBOC [3, 4]. This indicates that HR deficiency (HRD) is a crucial cause of HBOC. In addition to its importance in oncogenesis, HRD is also critical for the treatment of HBOC-related cancers using poly (ADP-ribose) polymerase (PARP) inhibitors, which induce synthetic lethality in cancers with HRD [5, 6].

HBOC is one of the most well-studied inheritable gene mutation-derived and tissue-specific cancers. Other inheritable tissue-specific cancers include gastrointestinal cancers and skin cancers caused by mutations in mismatch repair genes and xeroderma pigmentosum (XP) genes, respectively [7, 8]. This tissue specificity is likely the consequence of the combination of the vulnerability of specific DNA repair pathways caused by the mutations and the tissues that particularly require the pathways for their genetic stability. For example, each XP gene is required for the nucleotide excision repair of DNA adducts generated by ultraviolet light exposure [9]. However, the mechanisms underlying the tissue specificity of gastrointestinal cancers caused by mismatch repair gene mutations and breast and ovarian cancer caused by *BRCA1/BRCA 2* mutations have not been clearly explained. Oxidative stresses induced by bacterial exposure could be implicated in gastrointestinal carcinogenesis caused by mismatch repair gene mutations [10]. Concerning HBOC, several studies attempted to reveal the mechanisms underlying its tissue specificity. HRD is involved in the process, and estrogen signaling likely plays important roles.

In this review, we first introduced the fundamental mechanism of HR mediated by BRCA1 and BRCA2 to illustrate the mechanism by which PARP inhibitors induce synthetic lethality. We also discussed several hypotheses describing estrogen- and HRD-dependent genomic instability including tissue-specific DNA damage induced by estrogen receptor α (ERα)-mediated transcription, the tissue-specific paracrine effect of receptor activator of nuclear factor kappa-B ligand (RANKL) secreted from mammary glands, and tissue-specific cancer cell survival associated with the stress-responsive transcription factor NRF2 activated by estrogen.

2.2 Structure and Binding Partners of BRCA1/BRCA2 Proteins

BRCA1 comprises functional domains including an N-terminal RING finger, exon 11, a coiled-coil domain, and C-terminal tandem BRCT repeats, whereas BRCA2

possesses BRC repeats and single-stranded DNA (ssDNA) binding domains (Fig. 2.1). BRCA1 constitutes a RING heterodimer ubiquitin E3 ligase with another structurally similar RING finger protein BARD1 [11–13] that participates in heterochromatin formation by ubiquitinating histone H2A [14–16]. The BRCT repeats of BRCA1 interact with the phosphorylated forms of CtIP, FANCJ (also called BRIP1 or BACH1), or Abraxas (also called ABRA1) [17–23]. FANCJ is a DNA helicase that is critical for the repair of DNA damage including DNA crosslinking, and homozygous mutation of this gene causes Fanconi anemia [24]. Abraxas is an adopter protein connecting BRCA1 with RAP80, which interacts with ubiquitin chains generated at DNA double-strand breaks (DSBs) [21–23]. Whereas BRCA1 complexed with CtIP and FANCJ performs HR through DNA end resection, BRCA1 complexed with Abraxas antagonizes and fine-tunes HR [25–27]. The coiled-coil domain of BRCA1 recruits BRCA2 to sites of DNA damage. This domain interacts with PALB2, which bridges BRCA1 and BRCA2 [28, 29]. BRCA2 interacts with ssDNA through the ssDNA binding domain in its C-terminus, and it comprises eight repeats of the BRC domain, each of which is capable of interacting with one RAD51 molecule [30–33]. *BRCA1*, *BRCA2*, *PALB2*, and the *RAD51* homolog *RAD51C* are also known as the Fanconi anemia genes *FANCS*,

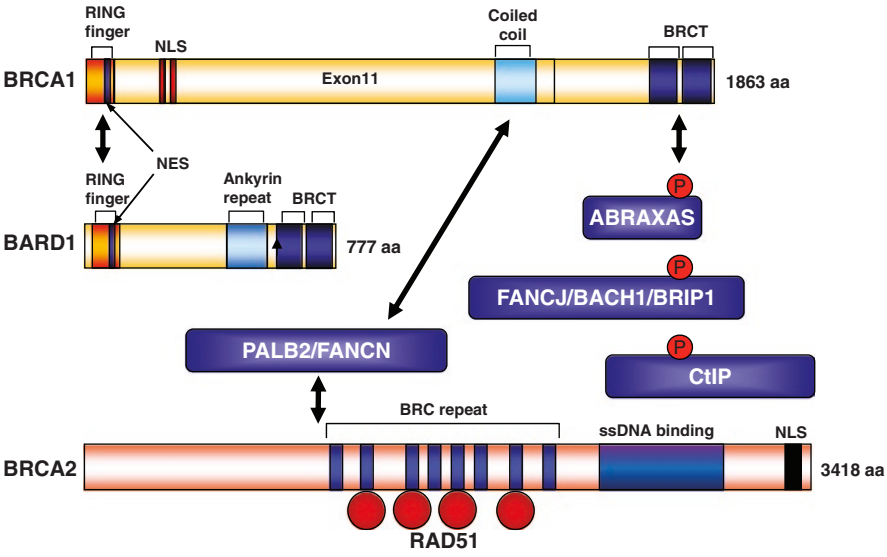


Fig. 2.1 Molecular structures of BRCA1, BRCA2, and their binding partners. BRCA1 comprises an N-terminal RING finger domain, exon 11, a coiled-coil domain, and C-terminal tandem BRCT domains. BRCA2 comprises eight repeats of the BRC domain and a single-stranded DNA binding domain. The RING domain interacts with the structurally similar protein BARD1, whereas BRCT repeats interact with the phosphorylated forms of CtIP, FANCJ, or Abraxas. The coiled-coil domain interacts with BRCA2 via PALB2. The BRC domains in BRCA2 interact with RAD51. The nuclear localization signal (NLS) and nuclear export signal (NES) are also presented. *P* phosphorylation

FANCD1, *FANCN*, and *FANCR*, respectively, and homozygous mutations in these genes cause Fanconi anemia [34–38].

2.3 BRCA1/BRCA2 Functions in HR and the Synthetic Lethality of PARP Inhibitors

2.3.1 HR Mediated by BRCA1/BRCA2 and a Backup Pathway Mediated by PARP1/PARP2

DNA damage can be broadly classified as two types: single-strand breaks and more cytotoxic DSBs. There are at least four mechanisms of DSB repair: non-homologous end-joining (NHEJ), single-strand annealing (SSA), alternative end-joining (Alt-EJ, also called Alt-NHEJ or microhomology-mediated EJ), and HR (Fig. 2.2). Most DSBs are repaired by NHEJ. NHEJ is available throughout all cell cycle phases, but it is most important during G1 phase when HR is not available [39]. NHEJ simply joins the blunt ends of DSBs via a process mediated by the heterodimer Ku70/Ku80 complexed with DNA-dependent protein kinase catalytic subunit (DNA-PKcs), XRCC4, and DNA ligase 4 (Lig4) (Fig. 2.2a) [40–41]. NHEJ is therefore relatively error-prone, and despite the deletion of residues at the broken ends in some instances, such deletions in most genetic regions do not affect cellular viability.

Contrarily, HR is an error-free process that is ideal for genetic stability, but it is only available during S and G2 phases when sister chromatids are accessible as

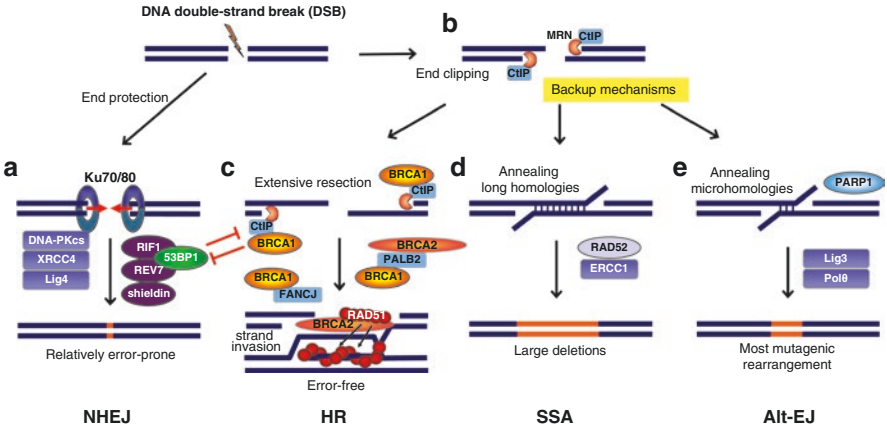


Fig. 2.2 Double-strand break (DSB) repair machineries. Four distinct mechanisms of DSB repair, namely, (**a**) non-homologous end-joining (NHEJ), (**c**) homologous recombination (HR), (**d**) single-strand annealing (SSA), and (**e**) alternative end-joining (Alt-EJ), and their representative repair proteins are presented. End-clipping by CtIP-MRN (**b**) is also presented. NHEJ simply joins the blunt ends of DSBs in a relatively error-prone process, whereas HR is an error-free pathway mediated by BRCA1, BRCA2, and RAD51. SSA and Alt-EJ are backup pathways for end-clipped DSBs in cells with HR deficiency. SSA and Alt-EJ lead to significant genetic alterations. Poly (ADP-ribose) polymerase 1/2 (PARP1/PARP2) is required for Alt-EJ

templates for recombination [39]. Directed by CtIP, a protein capable of interacting with the C-terminal BRCT domain of BRCA1, DSBs are first processed by the MRN complex consisting of MRE11, RAD50, and NBS1 for endonucleolytic clipping, in which the nuclease activity of MRE11 removes small amounts (~20 bp) of DNA at the broken ends (Fig. 2.2b) [42–44]. Once the DNA end is clipped, DNA is no longer a substrate for NHEJ, and it should be repaired by HR. One of most critical functions of BRCA1 in HR is extending DNA resection by supporting CtIP, which is phosphorylated in S phase and is therefore capable of binding to BRCA1, to generate ssDNA of sufficient length for strand invasion of the sister chromatid (Fig. 2.2c) [45, 46]. Another critical role of BRCA1 is the recruitment of BRCA2 to DSB sites via PALB2 [28, 29, 47]. While BRCA2 directed to DSBs interacts with ssDNA, multiple RAD51 molecules on BRCA2 are transferred onto ssDNA to create ssDNA-RAD51 filaments that invade the sister chromatid and execute homology searching via the recombinase activity of RAD51 [30–33].

The end-clipped DSBs in cells with HRD caused by BRCA1/BRCA2 dysfunction cannot be repaired by NHEJ, and they are cytotoxic if they are left unprocessed. Therefore, such lesions are repaired by the alternative backup pathways SSA and Alt-EJ (Fig. 2.2d and e). The majority of such DNA lesions are repaired by Alt-EJ, the most mutagenic repair pathway that anneals the broken ends with minimal homologous sequences (called microhomology), whereas SSA anneals DNA regions with longer homologous repeated sequences [48, 49]. Hence, SSA and Alt-EJ are beneficial for cancer cell survival but disadvantageous for individuals because they lead to genetic alterations and cancer. The genetic alteration created in this process can be detected as signature 3 genetic scars by next-generation sequencers, which are often used to assess HRD in cancer specimens for therapeutic purposes [50]. Importantly, PARP1/PARP2 is required for Alt-EJ in addition to DNA single-strand break repair (SSBR) [48, 51]. Therefore, PARP inhibitors inhibit the backup pathway required for the survival of cells with HRD.

HR and NHEJ are antagonistically regulated by BRCA1 and 53BP1 in response to DSBs. Whereas 53BP1 protects the DSB ends from CtIP-MRN-mediated end-clipping and consequently directs DSB repair toward the NHEJ pathway, BRCA1 blocks the action of 53BP1 by protecting CtIP [52, 53]. Interestingly, suppression of 53BP1 or its functional partners RIF1, Rev7, and Shieldin dramatically restores HR in BRCA1-/BRCA2-deficient cells and therefore causes PARP inhibitor resistance [54–62]. As described previously, the two major functions of BRCA1, namely, ssDNA elongation and BRCA2-RAD51 recruitment, are mediated through the CtIP-MRN complex and PALB2, which interact with BRCT and the coiled-coil region of BRCA1, respectively. In addition, a recent analysis revealed that exon 11 of BRCA1 is required for the suppression of 53BP1 [63]. Deletion of this exon coupled with *TP53* knockout leads to breast cancer in mice [64]. Interestingly, whereas homozygous deletion of the coiled-coil domain ($BRCA1^{CC/CC}$) or exon 11 ($BRCA1^{\Delta 11/\Delta 11}$) induces a Fanconi anemia-like phenotype with a low birth frequency or embryonic lethality, respectively, compound heterozygous mice possessing a combination of each deletion ($BRCA1^{CC/\Delta 11}$) were born at Mendelian frequencies indistinguishable from those of wild-type mice [63]. Hence, the 53BP1 counteraction

and BRCA2 recruitment, namely, DNA end resection and RAD51 loading, are individually essential functions for BRCA1.

2.3.2 Essential Role of BRCA1/BRCA2 in DNA Replication with PARP Trapping

DSBs are generated by exogenous insults, such as ionizing irradiation and topoisomerase II inhibitors, and endogenous insults accompanied by DNA replication (Fig. 2.3). DNA adducts represent a common cause of stalled replication. DNA adducts are generally removed by base excision repair followed by SSBR performed by PARP1 and to a lesser extent by PARP2 (Fig. 2.3a) [65–67]. When this pathway fails to function, for example, in the presence of PARP inhibitors, replication is maintained by either translesion synthesis (Fig. 2.3b), template switching (Fig. 2.3c), or HR (Fig. 2.3d). There are at least four pathways to continue replication, and therefore, HRD does not greatly affect cell viability in this situation. However, the situation differs if fork stalling is prolonged. In cases of prolonged stalling, forks are cleaved by the MUS81-EME1 nuclease complex, generating one-ended DSBs that absolutely require HR to restart break-induced replication (Fig. 2.3e) [68–70]. Prolonged fork stalling is caused by DNA secondary structures such as R-loops and G-quadruplexes, as well as PARP trapping, a phenomenon in which PARP

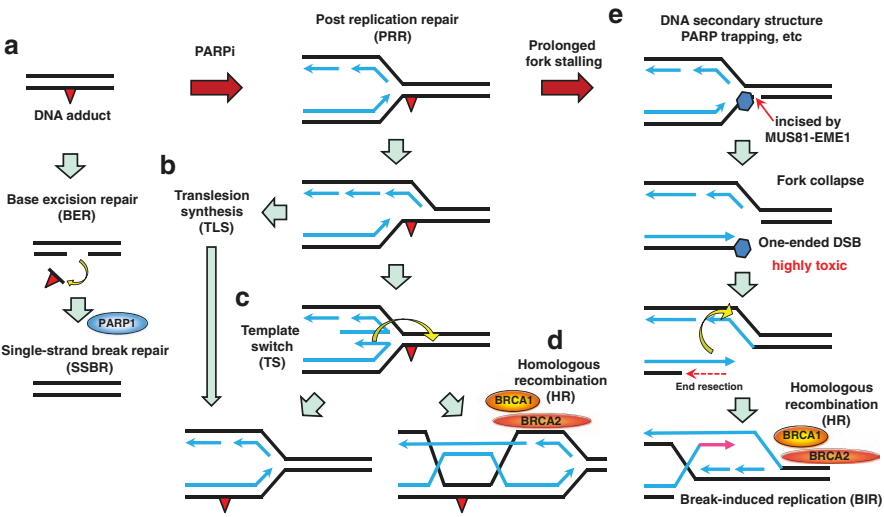


Fig. 2.3 BRCA1/BRCA2 function in DNA replication. DNA adducts are generally removed by base excision repair (BER) followed by single-strand break repair (SSBR) conducted by poly (ADP-ribose) polymerase 1/2 (PARP1/PARP2) (**a**). Translesion synthesis (**b**), template switching (**c**), and homologous recombination (HR) (**d**) function as backup mechanisms for BER. Prolonged stalled forks caused by DNA secondary structures or PARP trapping are cleaved by the MUS81-EME1 nuclease complex, resulting in one-ended double-strand breaks, which are highly toxic and require HR for cell survival (**e**)

persistently interacts with damaged DNA sites while its enzymatic activity is suppressed by PARP inhibitors [71]. Because one-ended DSBs are highly toxic, the ability of PARP inhibitors to induce PARP trapping is important for the induction of synthetic lethality in *BRCA1*- or *BRCA2*-mutated cancers with HRD. In addition to HR, BRCA1 and BRCA2 play additional essential roles in this process. BRCA1 and BRCA2 protect stalled replication forks against nucleolytic degradation by nucleases including MRE11, EXO1, and DNA2, thereby preventing fork collapse [72–78]. BRCA1 and BRCA2 are also required for preventing DNA damage driven by R-loops [79, 80], which are DNA-RNA hybrids that often accumulate at sites of DNA secondary structures including G-quadruplexes [81]. Collisions between the DNA replication machinery and R-loops result in fork collapse and subsequent DSBs. BRCA1 and BRCA2 associate with the DNA-RNA hybrid helicase SETX and mRNA export factor TREX-2, respectively, to resolve R-loops [79, 80]. G-quadruplexes are stacked structures built in guanine-rich DNA regions, such as rDNA, telomeres, and promoter sequences, with DNA motifs containing four stretches of three or more consecutive guanines [82–85]. Importantly, G-quadruplex-interacting compounds that stabilize G-quadruplex formation and therefore cause stalled replication forks sensitize cells to PARP inhibitors in PARP inhibitor-resistant BRCA1-/BRCA2-deficient tumors [81, 86, 87]. The G-quadruplex stabilizer CX-5461 is currently in a clinical trial of patients with BRCA1-/BRCA2-deficient tumors [87].

2.4 Hypotheses for Tissue-Specific Carcinogenesis

Germline mutation of BRCA1 or BRCA2 causes breast and ovarian cancers including ovarian cancer-related fallopian tube and peritoneal cancers. Although such mutations also cause other cancers such as prostate and pancreatic cancers, the incidence of cancer is much higher in the breasts and ovaries. The mechanism by which this tissue specificity occurs is not completely understood at present. However, accumulated evidence indicates that estrogen signaling is an important factor contributing to tissue specificity.

Clinically, the incidence of *BRCA1/BRCA2* mutation-derived breast cancer is significantly reduced by the suppression of estrogen signaling by treatment with the anti-estrogen tamoxifen or risk-reducing salpingo-oophorectomy [88–90]. *BRCA1* mutation-derived breast cancer was also prevented by oophorectomy in a mouse model [91]. Interestingly, complementation of estrogen, but not progesterone, in these mice resulted in breast cancer development. These estrogen-dependent phenotypes support the hypothesis that the tissue specificity of HBOC is ascribed to its estrogen dependency. However, the mechanism by which HRD or other functional deficiencies attributed to germline mutation of *BRCA1/BRCA2* contribute to estrogen-dependent carcinogenesis is not completely understood. HBOC carriers possess heterozygous germline mutations of *BRCA1/BRCA2*, and a second hit in the intact allele, such as loss of heterozygosity, triggers carcinogenesis [92–94]. Because the total loss of function caused by homozygous *BRCA1/BRCA2* mutation

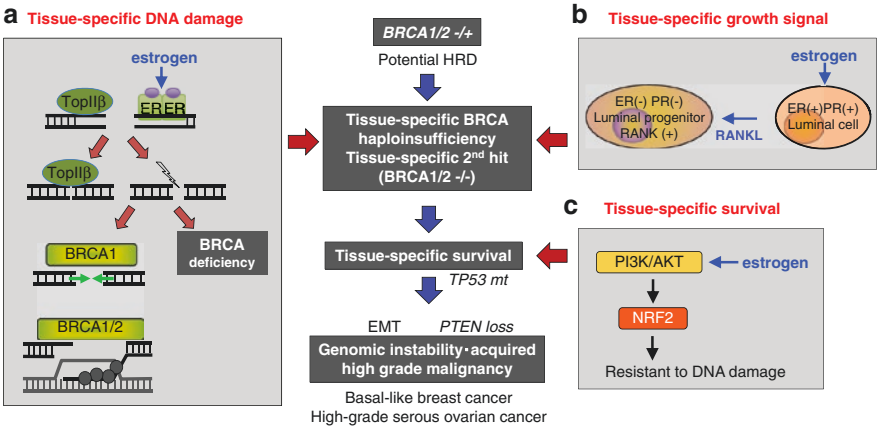


Fig. 2.4 Models of tissue-specific carcinogenesis induced by *BRCA1/BRCA2* mutation. Tissue-specific carcinogenesis caused by estrogen receptor α (ER)- and topoisomerase IIβ (TopIIβ)-dependent DNA damage (**a**), estrogen-dependent growth escalation induced by paracrine receptor activator of nuclear factor kappa-B ligand (RANKL) (**b**), and estrogen-dependent cell survival induced by NRF2 (**c**) are presented

leads to apoptosis induced by p53 activation, inactivation of the apoptosis pathway via the simultaneous mutation of *TP53* or by other conditions is required for cells to survive and develop into cancer [64, 95, 96]. Hence, tissue-specific carcinogenesis may be caused by a tissue-specific second hit, tissue-specific survival, or both (Fig. 2.4). In the next sections, representative evidence supporting these mechanisms induced by estrogen signaling is introduced.

2.4.1 Tissue-Specific DNA Damage Induced by ERα-Mediated Transcription

Estrogen-bound ERα translocates to the nucleus and functions as a transcription factor. The ERα-conducted transcription process requires topoisomerase IIβ (TopIIβ)-mediated transient truncation and rejoining of double-stranded DNA (dsDNA) to relax dsDNA distortion [97]. ERα and androgen receptor (AR) both control this process. In the case of AR, DSBs are generated via incomplete rejoining of the truncated ends of dsDNA, leading to the fusion gene *TMPRSS2-ERG*, the most common driver mutation of prostate cancer [98, 99]. Importantly, HRD, including that induced by BRCA1 depletion, accelerates the production of *TMPRSS2-ERG* [98]. This strongly suggests that AR-mediated transcription causes prostate cancer in *BRCA* mutation carriers. It is possible that a similar mechanism underlies the development of estrogen- and HRD-generated breast and ovarian cancers (Fig. 2.4a). In addition, it has been reported that TopIIβ frequently fails to rejoin truncated dsDNA ends in the presence of estrogen and remains attached to the 5′ ends of DNA [100]. BRCA1 complements this process by removing TopIIβ

adducts from the DSB ends and completing the rejoining. BRCA depletion leads to the remarkable accumulation of TopIIβ-DNA cleavage complex intermediates upon estrogen treatment. These findings suggest that BRCA1 dysfunction or HRD specifically exacerbates genomic instability in tissues expressing ERα or AR, thereby promoting carcinogenesis.

One possible contradiction for this scenario is that ovaries produce, but are not affected by, estrogen, and the ovarian epithelium does not express ERα. However, it has been suggested that the origin of *BRCA1/BRCA2* mutation-derived ovarian cancer could be the fallopian tubes opposed to the ovaries. The typical subtype of ovarian cancers attributed to *BRCA1/BRCA2* mutations is high-grade serous ovarian cancer (HGSC), which is the most aggressive phenotype of ovarian cancers and is normally detected in its advanced stage, making it impossible to distinguish its origin. However, as risk-reducing salpingo-oophorectomy has become widely performed in *BRCA1/BRCA2* mutation carriers, cancers are more likely to be detected in the early stage. Interestingly, fallopian tube cancer is more common than ovarian cancer in such early-stage cancers [101–104], suggesting that the majority of advanced cancers that were previously recognized as ovarian cancer could actually have been fallopian tube cancer. In addition ovarian cancer detected in the early stage could have originated in the fallopian tube because the fimbria cells of the fallopian tube physiologically move to the ovaries during ovulation. In a mouse model, Cre-mediated conditional inactivation of *BRCA1* or *BRCA2*, together with *TP53* and *PTEN*, which are frequently altered in ovarian cancers, in the fallopian tube led to the development of HGSC and peritoneal metastases, in addition to serous tubal intraepithelial carcinomas [105]. Importantly, inactivation of these genes in the ovarian epithelium did not promote the development of such cancers. Thus, the fimbriae of the fallopian tubes have been recognized as principal sites for HGSC in the pelvis. Because the fimbriae strongly express ERα, the DNA damage induced by ERα-mediated transcription is compatible for the tissue specificity of *BRCA1/BRCA2* mutation-induced carcinogenesis.

2.4.2 Tissue-Specific Growth Signaling by Estrogen in BRCA1-Defective Progenitor Cells

Breast cancers caused by germline *BRCA1* mutation are commonly the triple-negative subtype, which lacks ERα, progesterone receptor (PR), and human epidermal growth factor receptor 2 expression, and most of them are classified as basal-like cancer, which exhibits a similar gene expression profile as mammary basal stem cells. However, basal-like breast cancer caused by germline *BRCA1* mutation originates in ERα- and PR-negative luminal progenitor cells but not in basal stem cells. *BRCA1* inactivation in basal stem cells leads to adenomyoepithelioma, but not to basal-like breast cancer, whereas that in the luminal progenitor cells leads to basal-like breast cancer [106, 107]. Interestingly, BRCA1-defective luminal progenitor cells are hypersensitive to estrogen and progesterone despite being negative for ERα and PR. The mechanism underlying the contradiction is the paracrine effect of

neighboring luminal cells. ERα- and PR-positive mature luminal cells secrete RANKL in response to estrogen signaling, thereby promoting the growth of BRCA1-defective and ERα- and PR-negative luminal progenitor cells and resulting in the development of basal-like cancer [108–111] (Fig. 2.4b). Of note, it has been reported that the RANK inhibitor denosumab prevented *BRCA1* mutation-derived breast cancer in a mouse model [111].

2.4.3 Tissue-Specific Survival Mediated by Estrogen

In addition to a tissue-specific second hit, it has been proposed that estrogen-mediated survival in BRCA1-deficient cells may be the basis for tissue-specific carcinogenesis [112]. In support of this hypothesis, estrogen-induced NRF2 reactivation in BRCA1-defective cells has been revealed to generate tissue specificity (Fig. 2.4c). NRF2 is a master transcription factor of antioxidant pathways that protects cells against oxidative stress-induced DNA damage [113, 114]. BRCA1 physically interacts with NRF2 and promotes its stability and activation by blocking its interaction with the ubiquitin ligase KEAP1 [115]. Thus, BRCA1 deficiency suppresses NRF2 responses and leads to cell death with reactive oxygen species accumulation. Notably, estrogen increases NRF2 protein expression and activity through activation of the PI3K-AKT-mTOR pathway [116]. In vivo, the survival defect of BRCA1-deficient mammary epithelial cells is rescued by pregnancy, and estrogen administration stimulates the growth of BRCA1-deficient mammary tumors in male mice.

2.5 Perspectives

The mechanisms underlying the biology of carcinogenesis induced by *BRCA1/BRCA2* deficiency have intensively been studied for more than two decades. Although the initial questions have not been entirely clarified, tremendous progress has been achieved in the basic understanding of the responsible mechanisms, leading to many benefits in the treatment and prevention of HBOC. This includes synthetic lethality mediated by PARP inhibitors. Future works may focus on the mechanisms of acquired resistance and strategies to overcome this resistance. In addition, surgical and medical prevention strategies should also be improved on the basis of the mechanisms revealed by those efforts.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266:66–71.
2. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378:789–92.

3. Lu H-M, Li S, Black MH, et al. Association of Breast and Ovarian Cancers with predisposition genes identified by large-scale sequencing. JAMA Oncol. 2019;5:51–7.
4. Couch FJ, Shimelis H, Hu C, et al. Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol. 2017;3:1190–6.
5. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:913–7.
6. Farmer H, McCabe H, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434:917–21.
7. Liu B, Parsons R, Papadopoulos N, et al. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med. 1996;2:169–74.
8. Masutani C, Kusumoto R, Yamada A, et al. The XPV (xeroderma pigmentosum variant) gene encodes human DNA polymerase η. Nature. 1999;399:700–4.
9. Nakane H, Takeuchi S, Yuba S. High incidence of ultraviolet-B-or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group a gene. Nature. 1995;377:165–8.
10. Hardbower DM, de Sablet T, Chaturvedi R, et al. Chronic inflammation and oxidative stress: the smoking gun for helicobacter pylori-induced gastric cancer? Gut Microbes. 2013;4:475–81.
11. Wu LC, Wang ZW, Tsan JT, et al. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nat Genet. 1996;14:430–40.
12. Hashizume R, Fukuda M, Maeda I, et al. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem. 2001;276:14537–40.
13. Brzovic PS, Keeffe JR, Nishikawa H, et al. Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci U S A. 2003;100:646–5651.
14. Zhu Q, Pao GM, Huynh AM, et al. BRCA1 tumour suppression occurs via heterochromatin-mediated silencing. Nature. 2011;477:179–84.
15. Kalb R, Mallery DL, Larkin C, et al. BRCA1 is a histone-H2A-specific ubiquitin ligase. Cell Rep. 2014;8:999–1005.
16. Densham RM, Garvin AJ, Stone HR, et al. Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. Nat Struct Mol Biol. 2016;23:647–55.
17. Yu X, Wu LC, Bowcock AM, et al. The C-terminal (BRCT) domains of BRCA1 interact in vivo with CtIP, a protein implicated in the CtBP pathway of transcriptional repression. J Biol Chem. 1998;273:25388–92.
18. Cantor SB, Bell DW, Ganesan S, et al. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell. 2001;105:149–60.
19. Yu X, Chini CCS, He M, et al. The BRCT domain is a Phospho-protein binding domain. Science. 2003;302:639–42.
20. Manke IA, Lowery DM, Nguyen A, et al. BRCT repeats as Phosphopeptide-binding modules involved in protein targeting. Science. 2003;302:636–9.
21. Wang B, Matsuoka S, Ballif BA, et al. Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. Science. 2007;316:1194–8.
22. Sobhian B, Shao G, Lilli DR, et al. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. Science. 2007;316:1198–202.
23. Kim H, Chen J, Yu X. Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science. 2007;316:1202–5.
24. Litman R, Peng M, Jin Z, et al. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell. 2005;8:255–65.
25. Hu Y, Scully R, Sobhian B, et al. RAP80-directed tuning of BRCA1 homologous recombination function at ionizing radiation-induced nuclear foci. Genes Dev. 2011;25:685–700.
26. Coleman KA, Greenberg RA. The BRCA1-RAP80 complex regulates DNA repair mechanism utilization by restricting end resection. J Biol Chem. 2011;286:13669–80.
27. Vohhodina J, Toomire KJ, Petit SA, et al. RAP80 and BRCA1 PARsylation protect chromosome integrity by preventing retention of BRCA1-B/C complexes in DNA repair foci. Proc Natl Acad Sci U S A. 2020;117:2084–91.

28. Zhang F, Ma J, Wu J, et al. PALB2 links BRCA1 and BRCA2 in the DNA-damage response. Curr Biol. 2009;19:524–9.
29. Sy SMH, Huen MSY, Chen J. PALB2 is an integral component of the BRCA complex required for homologous recombination repair. Proc Natl Acad Sci U S A. 2009;106:7155–60.
30. Yang H, Jeffrey P, Miller J, et al. BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. Science. 2002;297:1837–48.
31. Sharan SK, Morimatsu M, Albrecht U, et al. Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking Brca2. Nature. 1997;386:804–10.
32. Wong AKC, Pero R, Ormonde PA, et al. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. J Biol Chem. 1997;272:31941–4.
33. Pellegrini L, Yu DS, Lo T, et al. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex. Nature. 2002;420:287–93.
34. Sawyer SL, Tian L, Kähkönen M, et al. Biallelic mutations in BRCA1 cause a new Fanconi anemia subtype. Cancer Discov. 2015;5:135–42.
35. Howlett NG, Taniguchi T, Olson S, et al. Biallelic inactivation of BRCA2 in Fanconi anemia. Science. 2002;297:606–9.
36. Reid S, Schindler D, Hanenberg H, et al. Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet. 2007;39:162–4.
37. Meindl A, Hellebrand H, Wiek C, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. Nat Genet. 2010;42:410–4.
38. Vaz F, Hanenberg H, Schuster B, et al. Mutation of the RAD51C gene in a Fanconi anemia-like disorder. Nat Genet. 2010;42:406–9.
39. Karanam K, Kafri R, Loewer A, et al. Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Mol Cell. 2012;47:320–9.
40. Mari PO, Florea BI, Persengiev SP, et al. Dynamic assembly of end-joining complexes requires interaction between Ku70/80 and XRCC4. Proc Natl Acad Sci U S A. 2006;103:18597–602.
41. Ahnesorg P, Smith P, Jackson SP. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell. 2006;124:301–13.
42. Lengsfeld BM, Rattray AJ, Bhaskara V, et al. Sae2 is an endonuclease that processes hairpin DNA cooperatively with the Mre11/Rad50/Xrs2 complex. Mol Cell. 2007;28:638–51.
43. Sartori AA, Lukas C, Coates J, et al. Human CtIP promotes DNA end resection. Nature. 2007;450:509–14.
44. Truong LN, Li Y, Shi LZ, et al. Microhomology-mediated end joining and homologous recombination share the initial end resection step to repair DNA double-strand breaks in mammalian cells. Proc Natl Acad Sci U S A. 2013;110:7720–5.
45. Bunting SF, Callen E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;141:243–54.
46. Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature. 2009;459:460–3.
47. Oliver AW, Swift S, Lord CJ. Structural basis for recruitment of BRCA2 by PALB2. EMBO Rep. 2009;10:990–6.
48. Wang M, Wu W, Wu W, et al. PARP-1 and Ku compete for repair of DNA double strand breaks by distinct NHEJ pathways. Nucleic Acids Res. 2006;34:6170–82.
49. Wu Y, Kantake N, Sugiyama T, et al. Rad51 protein controls Rad52-mediated DNA annealing. J Biol Chem. 2008;283:14883–92.
50. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016;534:47–54.
51. Audebert M, Salles B, Calsou P. Involvement of poly(ADP-ribose) Polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. J Biol Chem. 2004;279:55117–26.

52. Cao L, Xu X, Bunting SF, et al. A selective requirement for 53BP1 in the biological response to genomic instability induced by Brca1 deficiency. Mol Cell. 2009;35:534–41.
53. Bouwman P, Aly A, Escandell JM, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol. 2010;17:688–95.
54. Escribano-Diaz C, Orthwein A, Fradet-Turcotte A, et al. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. Mol Cell. 2013;49:872–83.
55. Chapman JP, Barral P, Vannier JB, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Mol Cell. 2013;49:858–71.
56. Zimmermann M, Lottersberger F, Buonomo SB, et al. 53BP1 regulates DSB repair using Rif1 to control 5′ end resection. Science. 2013;339:700–4.
57. Xu G, Chapman JR, Brandsma I. REV7 counteracts DNA double-strand break resection and affects PARP inhibition. Nature. 2015;521:541–4.
58. Noordermeer SM, Adam S, Setiaputra D, et al. The shieldin complex mediates 53BP1-dependent DNA repair. Nature. 2018;560:117–21.
59. Ghezraoui H, Oliveira C, Becker JR, et al. 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. Nature. 2018;560:122–7.
60. Mirman Z, Lottersberger F, Takai H, et al. 53BP1–RIF1–shieldin counteracts DSB resection through CST- and Polα-dependent fill-in. Nature. 2018;560:112–6.
61. Dev H, Chiang TWW, Lescale C, et al. Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. Nat Cell Biol. 2018;20:954–65.
62. Findlay S, Heath J, Luo VM, et al. SHLD2/FAM35A co-operates with REV7 to coordinate DNA double-strand break repair pathway choice. EMBO J. 2018:e100158.
63. Nacson J, Marcantonio DD, Wang Y, et al. BRCA1 mutational complementation induces synthetic viability. Mol Cell. 2020;78:951–9.
64. Xu X, Qiao W, Linke SP, et al. Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. Nat Genet. 2001;28:266–71.
65. Strom CE, Johansson F, Uhlen M, et al. Poly (ADP-ribose) polymerase (PARP) is not involved in base excision repair but PARP inhibition traps a single-strand intermediate. Nucleic Acids Res. 2011;39:3166–75.
66. Reynolds P, Cooper S, Lomax M, et al. Disruption of PARP1 function inhibits base excision repair of a sub-set of DNA lesions. Nucleic Acids Res. 2015;43:4028–38.
67. Ame JC, Rolli V, Schreiber V, et al. PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. J Biol Chem. 1999;274:17860–8.
68. Regairaz M, Zhang YW, Fu H, et al. Mus81-mediated DNA cleavage resolves replication forks stalled by topoisomerase I-DNA complexes. J Cell Biol. 2011;195:739–49.
69. Pepe A, West SC. MUS81-EME2 promotes replication fork restart. Cell Rep. 2014;7:1048–55.
70. Hanada K, Budzowska M, Davies SL, et al. The structure-specific endonuclease Mus81 contributes to replication restart by generating double-strand DNA breaks. Nat Struct Mol Biol. 2007;14:1096–104.
71. Murai J, Huang SN, Das BB, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 2012;72:5588–99.
72. Ray Chaudhuri A, Callen E, Ding X, et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature. 2016;535:382–7.
73. Schlacher K, et al. Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. Cell. 2011;145:529–42.
74. Schlacher K, Wu H, Jasin M. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51-BRCA1/2. Cancer Cell. 2012;22:106–16.
75. Lemacon D, Jackson J, Quinet A, et al. MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells. Nat Commun. 2017;8:860.

76. Thangavel S, Berti M, Levikova M, et al. DNA2 drives processing and restart of reversed replication forks in human cells. J Cell Biol. 2015;208:545–62.
77. Iannascoli C, Palermo V, Murfuni I, et al. The WRN exonuclease domain protects nascent strands from pathological MRE11/EXO1-dependent degradation. Nucleic Acids Res. 2015;43:9788–803.
78. Higgs MR, Reynolds JJ, Winczura A, et al. BOD1L is required to suppress deleterious resection of stressed replication forks. Mol Cell. 2015;59:462–77.
79. Hatchi E, Skourti-Stathaki K, Ventz S, et al. BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair. Mol Cell. 2015;57:636–47.
80. Bhatia V, Barroso SI, García-Rubio ML, et al. BRCA2 prevents R-loop accumulation and associates with TREX-2 mRNA export factor PCID2. Nature. 2014;511:362–5.
81. De Magis A, Manzo SG, Russo M, et al. DNA damage and genome instability by G-quadruplex ligands are mediated by R loops in human cancer cells. Proc Natl Acad Sci U S A. 2019;116:816–25.
82. Huppert JL, Balasubramanian S. Prevalence of quadruplexes in the human genome. Nucleic Acids Res. 2005;33:2908–16.
83. Huppert JL, Balasubramanian S. G-quadruplexes in promoters throughout the human genome. Nucleic Acids Res. 2007;35:406–13.
84. Bochman ML, Paeschke K, Zakian VA. DNA secondary structures: stability and function of G-quadruplex structures. Nat Rev Genet. 2012;13:770–80.
85. Rhodes D, Lipps HJ. G-quadruplexes and their regulatory roles in biology. Nucleic Acids Res. 2015;43:8627–37.
86. Zimmer J, Tacconi EMC, Folio C, et al. Targeting BRCA1 and BRCA2 deficiencies with G-Quadruplex-interacting compounds. Mol Cell. 2016;61:449–60.
87. Xu H, Antonio MD, McKinney S. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. Nat Commun. 2017;8:14432.
88. King MC, Wieand S, Hale K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2. JAMA. 2001;286:2251–6.
89. Phillipa KA, Milne RL, Rookus MA, et al. Tamoxifen and risk of contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. J Clin Oncol. 2013;31:3091–9.
90. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing Salpingooophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst. 2009;101:80–7.
91. van de Ven M, Liu X, van der Burg E, et al. BRCA1-associated mammary tumorigenesis is dependent on estrogen rather than progesterone signaling. J Pathol. 2018;246:41–53.
92. Neuhausen SL, Marshall CJ. Loss of heterozygosity in familial tumors from three BRCA1-linked kindreds. Cancer Res. 1994;54:6069–72.
93. Collins N, McManus R, Wooster R, et al. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12–13. Oncogene. 1995;10:1673–5.
94. Gudmundsson J, Johannesdottir G, Bergthorsson JT, et al. Different tumor types from BRCA2 carriers show wild-type chromosome deletions on 13q12–q13. Cancer Res. 1995;55:4830–2.
95. Hakem R, de la Pompa JL, Elia A, et al. Partial rescue of Brca1$^{5-6}$ early embryonic lethality by p53 or p21 null mutation. Nat Genet. 1997;16:298–302.
96. Ludwig T, Chapman DL, Papaioannou VE, et al. Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. Genes Dev. 1997;11:1226–41.
97. Ju BG, Lunyak VV, Perissi V, et al. A topoisomerase IIβ–mediated dsDNA break required for regulated transcription. Science. 2006;312:1798–802.
98. Lin C, Yang L, Tanasa B, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell. 2009;139:1069–83.
99. Haffner MC, Aryee MJ, Toubaji A, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet. 2010;42:668–75.

100. Sasanuma H, Tsuda M, Morimoto S, et al. BRCA1 ensures genome integrity by eliminating estrogen-induced pathological topoisomerase II–DNA complexes. Proc Natl Acad Sci U S A. 2018;115:E10642–51.
101. Zweemer RP, van Diest PJ, Verheijen RH, et al. Molecular evidence linking primary cancer of the fallopian tube to BRCA1 germline mutations. Gynecol Oncol. 2000;76:45–50.
102. Medeiros F, Muto MG, Lee Y, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol. 2006;30:230–6.
103. Finch A, Shaw P, Rosen B, et al. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. Gynecol Oncol. 2006;100:58–64.
104. Reade CR, McVey RM, Tone AA, et al. The fallopian tube as the origin of high grade serous ovarian cancer: review of a paradigm shift. J Obstet Gynaecol Can. 2014;36:133–40.
105. Perets R, Wyant GA, Muto KW, et al. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca;Tp53;Pten models. Cancer Cell. 2013;24:751–65.
106. Molyneux G, Geyer FC, Magnay FA, et al. BRCA1 basal-like breast cancers originate from luminal epithelial progenitors and not from basal stem cells. Cell Stem Cell. 2010;7:403–17.
107. Lim E, Vaillant F, Wu D, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med. 2009;15:907–13.
108. Asselin-Labat ML, Vaillant F, Sheridan JM, et al. Control of mammary stem cell function by steroid hormone Signalling. Nature. 2010;465:798–802.
109. Gonzalez-Suarez E, Jacob AP, Jones J, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature. 2010;468:103–7.
110. Schramek D, Leibbrandt A, Sigl V, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature. 2010;468:98–102.
111. Nolan E, Vaillant F, Branstetter D, et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med. 2016;22:933–9.
112. Elledge SJ, Amon A. The BRCA1 suppressor hypothesis: an explanation for the tissue-specific tumor development in BRCA1 patients. Cancer Cell. 2002;1(2):129–32.
113. Itoh K, Chiba T, Takahashi S, et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997;236:313–22.
114. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem. 2009;284:13291–5.
115. Gorrini C, Baniasadi PS, Harris IS, et al. BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival. J Exp Med. 2013;210:1529–44.
116. Gorrini C, Ganga BP, Bassi C, et al. Estrogen controls the survival of BRCA1-deficient cells via a PI3K–NRF2-regulated pathway. Proc Natl Acad Sci U S A. 2014;111:4472–7.

Genetic Testing

3

Sana Yokoi

Abstract

Genetic testing for HBOC can be a life-determining event for clients. Therefore, it is very important when, to whom, and by which method the genetic testing is performed. Genetic testing has a variety of purposes, including HBOC diagnosis, companion diagnosis, relative diagnosis, carrier diagnosis, and confirmation of secondary findings. Who is the best test candidate depends on the purpose of the test. Various sizes of BRCA1/BRCA2 variants have been reported, from single nucleotide substitutions and small indels to large-sized structural abnormalities. The locus of variants is distributed not only in exons but also in splice sites and deep introns. There are various tests depending on the variant size, from the specific variant detection by Sanger sequencing to multi-gene panel using next-generation sequencing, and there are also several companion diagnostics to determine the indications for molecular targeted drugs. It also introduces the accuracy control required for clinical diagnosis and the limitations of interpretation of results. After reading this chapter, you will be able to choose the genetic testing that best suits your purpose.

Keywords

BRCA1/BRCA2 · Germline variant · Genetic testing · Companion diagnostics Testing criteria · In vitro diagnostics · HRD · Quality control

S. Yokoi (✉)
Division of Genetic Diagnostics, Chiba Cancer Center, Chiba, Japan
e-mail: syokoi@chiba-cc.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_3

3.1 Introduction

Genetic testing includes various types of analysis:

- Testing for exogenous pathogens (e.g., microorganisms such as viruses and bacteria) that cause infectious diseases in humans
- Testing for somatic variants in human cancers
- Testing for germline variants in human genome

Testing for germline variants includes a test that reveals the lifelong genetic information that an individual inherently possesses in her genome and mitochondria. Testing involves the diagnosis of monogenic diseases, risk assessment of multifactorial diseases, drug effects/side effects/metabolism, and personal identification.

This section refers to genetic testing for germline *BRCA1/BRCA2*, which are associated with hereditary breast and ovarian cancer syndrome (HBOC).

Based on linkage analysis of familial breast cancer, the BRCA1 and BRCA2 genes were cloned in 1994 and 1995, respectively [1, 2]. Myriad Genetics started clinical BRCA1/BRCA2 germline testing on November 27, 1996, and had conducted more than 1.5 million tests worldwide in 20 years. Around 2000, Lynx developed the technology of massively parallel signature sequencing, which is the basis of next-generation sequencing (NGS) [3]. The decoding of the human genome was completed on April 14, 2003 [4]. In 2005, 454 Life Sciences launched the next-generation sequencer GS20 [5]. NGS analysis has expanded its use from genomic research to clinical testing. On June 13, 2013, the US Supreme Court ruled that the patent rights for the human BRCA1/BRCA2 genes owned by Myriad et al. were invalid [6]. It was determined that the DNA fragment was a product of nature and was ineligible for protection because it was isolated. Hours after the Supreme Court's ruling, a biotech company has announced that they will test BRCA1/BRCA2 for about one-third the price of Myriad. Due to lower prices for NGS analysis and differentiation from Myriad, BRCA1/BRCA2 genetic testing has been increasingly used with multi-gene panel (MGP) since this ruling.

3.2 Aim of the Genetic Testing

The main purposes of genetic testing performed in clinical practice are as follows:

- Diagnosis of hereditary cancer and companion diagnostics for certain drugs in cancer patients
- Carrier diagnosis to relatives
- Confirmation of germline findings in examinations using cancer tissues for detecting somatic mutations in cancer patients

3.2.1 Diagnosis of Hereditary Cancer and Companion Diagnostics for Certain Drugs in Cancer Patients

For three main purposes, cancer patients take genetic testing for HBOC diagnosis.

3.2.1.1 Appropriate Medical Care for Cancer

1. Diagnosis of HBOC is useful for selecting the operative procedure.
   For patients with preoperative breast cancer, choosing breast-conserving surgery for HBOC has been reported to increase the risk of developing the second breast cancer in the preserved breast [7, 8].
2. Long-term and precise follow-up is provided even for early-stage cancer.
   In the case of breast cancer, it is recommended to continue follow-up with contrast-enhanced MRI of the preserved and contralateral breasts until at least 75 years of age [8–10].
3. Companion diagnostics for PARP inhibitors.

In breast cancer, ovarian cancer, pancreatic cancer, and prostate cancer, PARP inhibitors are effective in patients with pathogenic/likely pathogenic variants (P/LPVs) of BRCA1/BRCA2 [11].

3.2.1.2 Prevention Against Cancer Risk of Other Organs That Are Not Currently Affected

Surveillance for HBOC includes annual contrast breast MRI for breast cancer, transvaginal ultrasound and serum CA-125 for ovarian cancer, serum PSA for prostate cancer, and MRCP for pancreatic cancer. To prevent cancer, risk-reducing mastectomy and risk-reducing salpingo-oophorectomy can be selected [12–15].

3.2.1.3 Clarify the Possibility of Inheritance to Relatives

Many cancer patients say, “I already have cancer, so I get medical care, whether genetic or not. I do not want my daughter to have the same experience as me. The purpose of taking a genetic test is to know if my daughter is HBOC and to allow her to have an early screening for her cancer risk” [16, 17].

3.2.2 Diagnosis of Relatives

The pathogenic variant information of the proband allows relatives to diagnose HBOC by analyzing only their variant site. However, if the relative has a cancer to which a PARP inhibitor is applied (i.e., breast cancer, ovarian cancer, prostate cancer, pancreatic cancer) and is expected to be treated with a PARP inhibitor in the future, it is better to choose a genetic testing that is accepted as a companion diagnostic. For example, in a country like Japan where BRACAnalysis is the only germline genetic test accepted as a companion diagnostic for olaparib, their relatives can use PARP inhibitors only by the BRACAnalysis test, which analyzes the full-length BRCA1/BRCA2 sequence.

In the case of a client without cancer, the age and gender of the client should be taken into consideration. If the client is a minor, the test is postponed until adulthood. HBOC rarely develops cancer in childhood, so there is no benefit to having the test done in childhood. After the client has grown up, the healthcare professional will assist the client in deciding whether or not to undergo the genetic testing at his or her will [18].

3.2.3 Secondary Findings (SF) (Germline Findings)

In tumor-only MGP, the detection rate of SF is reported to be about 4–8% [19, 20]. Of these, BRCA1/BRCA2 was the most common, accounting for about 60%. Additionally, about 80% of the P/LPVs detected in BRCA1/BRCA2 by cancer MGP are reported to be of germline origin. Therefore, if a P/LPV of BRCA1/BRCA2 is detected in tumor-only cancer MGP, it is highly recommended to suspect that the P/LPV is a germline origin and to confirm by blood test.

3.3 Best Test Candidate

In a family that is diagnosed with HBOC for the first time, the person who is suitable for the first test is called "the best test candidate." Examples are young breast cancer patients and ovarian cancer patients. See "Genetic/familial high-risk assessment: Breast, ovarian, and pancreatic" in the NCCN guidelines (Fig. 3.1) [16]. The NCCN guidelines are frequently updated, so download the latest from the website. The NCCN guidelines were significantly revised in 2019. Because the application of PARP inhibitors has been greatly expanded from breast cancer and ovarian

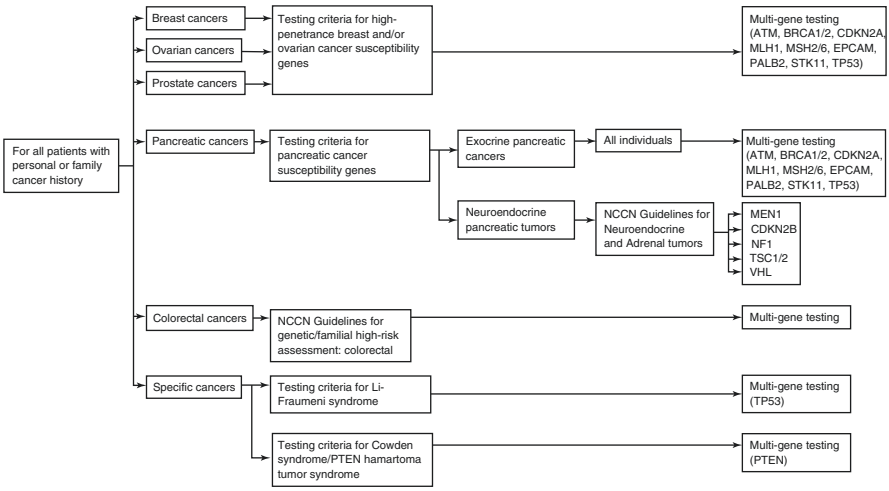


Fig. 3.1 Cancer risk assessment

cancer to prostate cancer and pancreatic cancer, germline MGP has become widespread as a clinical genetic testing. The main points of the revision are the following:

- Pancreatic cancer had been added to the title.
- The assessment candidates had been expanded to all patients with a personal or family cancer history.
- The germline MGP was recommended as the first choice in most genetic testing.

As shown in Fig. 3.1, candidates are for all patients, and testing criteria are indicated for each cancer type in the patient's medical history or family history. Germline MGP is the first choice when met to testing criteria.

Even if none of the testing criteria are met, genetic testing is also recommended or considered if the probability of BRCA1/BRCA2 pathogenic variants exceeds 5.0% or 2.5%–5.0%, respectively.

Probability models of BRCA1/BRCA2 pathogenic variants come in many varieties. There are two ways to create a probability model. One is an empirical model, such as Myriad II [21], which mainly consists of Western data, and KOHCal [22], which mainly consists of East Asian data. The other is a statistical model for calculating genetic risk using the Bayesian method, such as BRCAPRO [23] and CanRisk [24]. CanRisk uses a polygenic risk model, formerly BOADICEA.

3.4 Variants of BRCA1/BRCA2 Genes

The functions of BRCA1/BRCA2 are homologous recombination repairs for DNA damage and act as tumor suppressor genes (TSGs) in carcinogenesis. Since BRCA1 and BRCA2 are TSGs, there are no hot spots of variants, and genotype-phenotype correlation is unclear, however breast and ovarian cancer cluster regions have been reported [25].

BRCA1 is located at 17q21.31 and consists of 24 exons and 1863 amino acids. The coding region starts with exon 2, and the main transcript skips exon 4. The breast cancer cluster regions (BCCRs) of BRCA1 are reported within c.179–505, c.4328–4945, and c.5261–5563, and ovarian cancer cluster region (OCCR) is within c.1380–4062.

BRCA2 is located at 13q13.1 and consists of 27 exons and 3418 amino acids. The coding region starts with exon 2. BCCRs of BRCA2 are reported within c.1–596, c.772–1806, and c.7394–8904, and OCCRs are within c.2831–6401 and c.6645–7471.

The frequency of BRCA1/BRCA2 variants in the non-Ashkenazi Caucasian population in the United Kingdom and the United States is estimated to be approximately 1 in 400 [26, 27]. According to Myriad Genetics, there are about 19,000 BRCA1/BRCA2 variants; however, the database is not publicly available. Approximately 80% of BRCA1/BRCA2 variants are detected by whole gene sequencing and approximately 10% are detected by copy number analysis [28–31].

The size of variants detected by whole gene sequencing ranges from 1 to several bases and includes:

- Nonsense variant
- Missense variant
- Small indel
- Splice site variant
- Deep intronic variant

Large genome rearrangements that are difficult to detect by whole gene sequencing include loss, amplification, translocation, and inversion.

Nonsense variants are pathogenic because they create unplanned stop codons by single nucleotide substitutions. However, in the case of the flanking carboxyterminal variant, deletion of the last few amino acids may not affect function. If the frameshift caused by the small indel is not a multiple of 3, it is pathogenic because stop codons are created early.

In missense variants and small indels that are multiples of 3, mRNA is translated to the 3′ end. In this case, it is not easy to predict the functional changes of the mutant protein. Refer to various public databases and interpret variants based on the ACMG guideline [32]. Amino acid changes at special sites, such as the first methionine, are pathogenic, even if they are missense variants or one amino acid deletions. A splice site is a consensus sequence at both ends of an intron near the exon-intron junction. The 5′ end of the intron is called the donor site, the 3′ end is called the acceptor site, and GT and AG are the respective consensus sequences. When this sequence is mutated, intron removal becomes incomplete, exon skipping occurs, and the splice pattern changes, so it is pathogenic. Myriad tests 20 bp from the 5′ end and 10 bp from the 3′ end, and Ambry tests 5 bp from both ends, as variants can affect splicing even if they are more than 2 bases away from the exon-intron junction.

A mechanism called secondary epimutation has also been reported in which the promoter is methylated by the deep intron variant c.-107A > T of BRCA1 and the expression of BRCA1 is reduced [33].

Large-sized genomic structural abnormalities, copy number loss, translocation, etc. result in loss of function.

3.5 Analytical Methods

Table 3.1 shows a list of US FDA-approved in vitro diagnostics (IVD) including BRCA1/BRCA2.

3.5.1 Sanger Sequencing

Suitable variant size for detection is about 1–500 bases. We can read exon and exon-intron boundaries base substitutions or small indels. The ddNTP labeled with the

Table 3.1 FDA-approved human genetic tests including BRCA1/BRCA2

Type of test	Trade name	Manufacturer	Tumor tissue	Normal sample	Plasma
Germline Sanger sequencing and fragment analysis	BRACAnalysis CDx	Myriad Genetic Laboratories, Inc.		○	
Germline DNA chip	23andMe PGS genetic health risk report for BRCA1/BRCA2 (selected variants)	23andMe		○	
Germline whole-exome sequencing	Helix laboratory platform	Helix OpCo, LLC		○	
Matched-pair MGP	MSK-IMPACT	Memorial Sloan-Kettering Cancer Center	○	○	
	Omics Core	NantHealth, Inc.	○	○	
Tumor-only MGP	FoundationFocus CDxBRCA	Foundation Medicine, Inc.	○		
	FoundationOne CDx	Foundation Medicine, Inc.	○		
	Myriad myChoice CDx	Myriad Genetic Laboratories, Inc.	○		
	PGDx elio tissue complete	Personal Genome Diagnostics	○		
Liquid biopsy	FoundationOne® Liquid CDx	Foundation Medicine, Inc.			○
	Guardant360® CDx	Guardant Health, Inc.			○

four-color fluorescent dye is mixed with the dNTP to amplify the target DNA region, and the fluorescent signal is read by capillary electrophoresis.

3.5.2 Fragment Analysis

The PCR product size suitable for detection is about 200–500 bases, but by tiling several PCR products, the analysis length becomes 10 M base. We can analyze the number of repeats in the repeat sequence and copy number aberrations. In this method, the DNA region of interest is PCR amplified and capillary-electrophoresed along with a size standard to identify differences in the size of PCR products. Fragment analysis can be used to analyze the loss of heterozygosity (LOH), multiplex ligation-dependent probe amplification (MLPA) [34], and microsatellite instability (MSI). LOH detects allelic imbalance by comparing the number of repeat

sequences in the paternal and maternal alleles. MLPA detects genomic rearrangements such as exonic deletions and duplications. MSI compares the number of repetitions of the microsatellite region between tumor DNA and normal DNA to detect genomic stability.

3.5.3 Array Comparative Genomic Hybridization (Array-CGH)

A method for detecting DNA copy number aberrations from about 1000 bases to the chromosome level in the entire genomic region at once [35]. Patient DNA and control DNA are labeled with different fluorescence and hybridized competitively on the chip to detect the ratio of the two-color fluorescence signals. We can detect copy number variations from exon size to chromosomal size for the entire genomic region.

3.5.4 Next-Generation Sequencing (NGS)

NGS is a massively parallel sequencing method that analyzes 10G base or more. There are short-read sequencers (SRSs) with a read length of 100–200 bases and long-read sequencers (LRSs) with a read length of 10 k base or more. LRS developed by Oxford Nanopore and Pacbio can be expected to analyze complex genomic structural abnormalities, but is currently for research use only. MGP using SRSs has become widespread as a clinical test. Illumina's NGS detects a fluorescence signal by incorporating fluorescently labeled dNTPs one base at a time by DNA polymerase on the flow cell. Thermo's NGS does not use fluorescence and detects the potential change of H^+ during the elongation reaction. In genetic testing, the number of MGPs exceeded the number of Sanger sequencing in 2014. Currently, various MGPs containing the high-risk and moderate-risk genes are being produced [36]. Using MGP, it is possible to analyze not only BRCA1/BRCA2 but also many genes at the same time in one analysis. This improves the diagnostic rate of genetic testing, but increases the frequency of a variant of uncertain significance (VUS) per test. MGP analysis of 42 genes reported that an average of 2.1 VUS per person was observed (Kurian AW, 2014). Additionally, unexpected hereditary diseases may be diagnosed. MGP has already been widely used in clinical tests as a tumor-only panel and liquid biopsy. These tests require an evaluation of germline findings.

3.6 Companion Diagnostics (CDx)

3.6.1 CDx

Regulatory approvals for CDx vary from country to country. Alternatively, the number of international clinical trials is increasing today, and the same CDx as clinical trials is being adopted increasingly. Table 3.2 shows the US FDA-approved

Table 3.2 FDA-approved companion diagnostic devices including BRCA1/BRCA2

Diagnostic name	Diagnostic manufacturer	Methods	Indication(s)	Lynparza (olaparib)	Rubraca (rucaparib)	Zejula® (niraparib)	Talzenna (talazoparib)
BRACAnalysis CDx	Myriad Genetic Laboratories, Inc.	Sanger sequencing and fragment analysis	Breast cancer	Approved			Approved
			Ovarian cancer	Approved	Approved		
			Pancreatic cancer	Approved			
			Metastatic castrate-resistant prostate cancer (mCRPC)	Approved			
FoundationOne CDx	Foundation Medicine, Inc.	MGP	Ovarian cancer	Approved	Approved		
			Metastatic castrate-resistant prostate cancer (mCRPC)	Approved			
FoundationFocus CDx*BRCA* assay	Foundation Medicine, Inc.	MGP	Ovarian cancer		Approved		
Myriad myChoice® CDx	Myriad Genetic Laboratories, Inc.	MGP	Ovarian cancer	Approved		Approved	
FoundationOne® Liquid CDx	Foundation Medicine, Inc.	MGP	Metastatic castrate-resistant prostate cancer (mCRPC) (plasma)	Approved	Approved		
			Ovarian cancer (plasma)		Approved		

companion diagnostics, including BRCA1/BRCA2. Five types of CDx have been adopted as four types of PARP inhibitors for four types of cancer:

- BRACAnalysis analyzes BRCA1/BRCA2 of blood genomic DNA by the Sanger method and fragment analysis. The Sanger sequencing primer is designed on the basis of GenBank data, avoiding easily variable regions, and has an M13 tail added. The BRACAnalysis large rearrangement test detects large rearrangements by fragment analysis. If PV is detected, it is confirmed to be HBOC.
- Myriad myChoice HRD CDx analyzes cancer tissue by NGS, sequences total BRCA1/BRCA2, and determines HRD based on the genomic instability score (GIS) calculated from LOH, telomeric allelic imbalance (TAI), and large-scale state transitions (LST).
- FoundationFocus CDxBRCA sequences total BRCA1/BRCA2 in cancerous tissue using NGS.
- FoundationOne CDx sequences 324 cancer-related genes, including BRCA1/BRCA2, and calculates MSI, TMB, and LOH for cancer tissues using NGS.
- FoundationOne® Liquid CDx sequences 324 cancer-related genes using NGS for circulating tumor DNA (ctDNA) from the blood.

If PV is detected on BRCA1/BRCA2 in this testing, inform the patient that PV may be germline findings.

3.6.2 HRD

PARP inhibitors are effective in HRD-positive tumors [37]. HRD is determined from the cause and result. The cause of HRD is loss of function variants in DDR and HRR genes, and the result of HRD is that genomic scar occurs due to decreased homologous recombination repair activity (Fig. 3.2). The variants are detected by whole gene sequencing. However, there are various methods for detecting genomic scars, and neither the detected phenomenon nor the quantification algorithm is the same (Table 3.3). Figure 3.2 shows where and how the four different HRD tests detect the process from genetic mutations to genomic scars. FoundationOne CDx and myChoice HRD assay determine HRD based on both sides of the cause and result.

3.7 Quality Control

The main process of quality control of genetic testing is shown by taking NGS analysis using peripheral blood as an example.

3.7.1 Preanalytic Phase

In the sample collection, patient misidentification, insufficient blood collection volume, incorrect blood collection tube, hemolysis, infusion solution contamination,

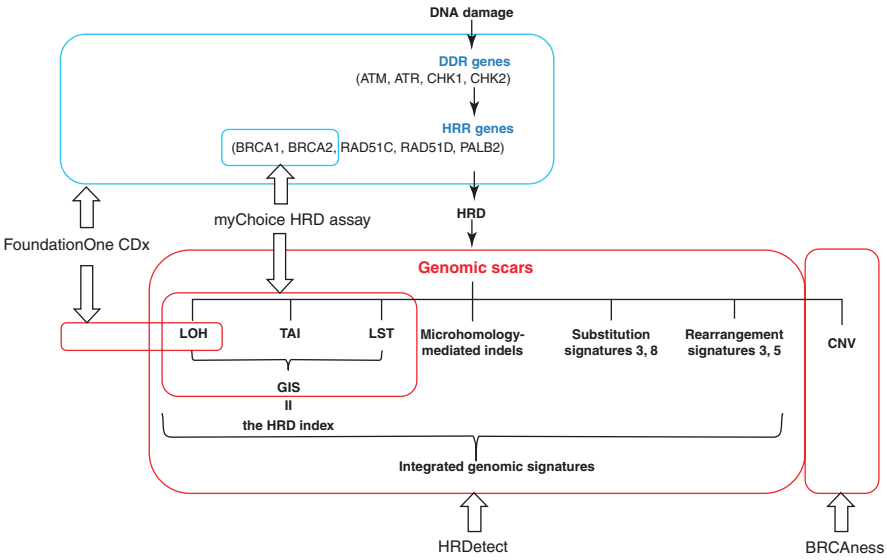


Fig. 3.2 HRD assay. *DDR* DNA damage response, *HRR* homologous recombination repair, *HRD* homologous recombination deficiency, *LOH* loss of heterozygosity, *TAI* telomeric allelic imbalance, *GIS* genomic instability status, *LST* large-scale state transition, *CNV* copy number variant

Table 3.3 HRD assay

	BRCAness	myChoice	FoundationOne CDx	HRDetect
Index	Copy number abberation	BRCA1/BRCA2 sequencing and genomic instability score	DDR and HRR gene sequencing and LOH score	Integrated genomic signatures
Analysis	MLPA	MGP	MGP	WGS
Purpose	Predicting the BRCA1-deficient breast cancer	Predicting the effects of PARP inhibitors	Detect pathogenic variants of genes and LOH	Predicting the BRCA1 and BRCA2 deficiency

insufficient inversion and miscibility, improper transportation/storage temperature, etc. may occur. In nucleic acid extraction, quantity deficiency, fragmentation, contamination, etc. may occur.

3.7.2 Analytic Phase

NGS wet-lab process. In library creation, check steps such as DNA fragmentation, barcode addition, target enrichment, etc. In sequencing, check the read depth and uniformity.

3.7.3 Postanalytic Phase

NGS dry-lab process. For mapping, variant call, and annotation, which are bioinformatics pipelines, also check the type and version of the database used. In preparing the report, describe the interpretation of the variant and match the anonymization code.

3.7.4 Validation

Evaluate accuracy, repeatability, reproducibility, sensitivity, specificity, robustness, reportable range, and reference interval for the test. If you use IDV, you can omit it because it has already been done.

3.7.5 Internal Quality Control

Regularly measure quality control materials that mimic patient samples. Use artificially created reference materials by introducing known variants.

3.7.6 External Quality Assessment

Participate in an interlaboratory comparison program and undergo proficiency testing. The US College of American Pathologists (CAP) survey is conducting NGS proficiency testing to detect SNVs and indels in 28 genes.

3.7.7 Certification Programs

3.7.7.1 Clinical Laboratory Improvement Amendments (CLIA)

It is a US law that sets out quality assurance standards for clinical laboratories. In the United States, all clinical laboratories that handle human specimens are required to be certified.

3.7.7.2 Laboratory Accreditation Program (LAP)

It is a clinical laboratory accreditation program by CAP. Laboratories outside the United States can also be certified.

3.7.7.3 ISO15189

The International Organization for Standardization/Technical Committee 212 (ISO/TC212) is a nongovernment organization founded in 1947 and promotes international standardization of products and services. ISO15189 is an international standard for quality and competence in the medical laboratory. Laboratories around the world can be certified.

3.8 Interpretation

Variant pathogenicity is classified into the following five categories according to the ACMG variant classification guideline 2016 v2 [32].

- Pathogenic
- Likely pathogenic
- Variant of uncertain significance (VUS)
- Likely benign
- Benign

Of these, pathogenic and likely pathogenic have pathological significance, and the diagnosis of HBOC is confirmed, and PARP inhibitors are applied. In the case of benign and likely benign, HBOC can be almost denied and PARP inhibitors are not applicable. In the case of VUS, PARP inhibitors are not applicable, but HBOC cannot be denied.

These five main databases are useful for interpretation of germline BRCA1/BRCA2 variants.

- ClinVar
- HGMD (the Human Genome Mutation Database)
- BRCA Share (UMD)
- BRCA Exchange
- Leiden Open Variation Database (LOVD)

The interpretations of variants in these databases do not always match. Disagreement rate between five databases (ClinVar, HGMD, UMD, BIC, LOVD) is 3–14% [38].

3.9 VUS

The VUS rate in Myriad is as high as 2% for all patients, but 5% for Asian and Middle Eastern.

The NCCN guideline recommends that high-risk individuals with a lifetime cancer risk of 20% or higher should undergo surveillance. The lifetime cancer risk model is a model that predicts the risk of developing cancer based on epidemiological data, pregnancy and childbirth, cancer history, gene mutation information, family history, and the like. BRCAPRO, CanRisk, and Tyrer-Cuzick [39] are also probability models of BRCA1/BRCA2 pathogenic variants. The Gail model [40] and Claus model [41] are models that predict the development of breast cancer in average or high-risk women, respectively, and the probability of BRC1/BRCA2 pathological variant is not possible. Additionally, there are two main things that healthcare professionals can clarify VUS interpretation. One is segregation analysis.

The presence of genotype-phenotype correlation in the history of associated cancers provides strong evidence for pathogenicity. This information cannot be obtained without a trusting relationship between the patient/family and the healthcare professionals. The other is the functional analysis of the variant. Since both BRCA1/BRCA2 have a homologous recombination repair function, analysis for detecting homologous recombination activity is useful. The cells are treated with X-ray, ultraviolet light, PARP inhibitors, mitomycin C, cisplatin, topoisomerase inhibitor, alkylating agent, etc. to induce DNA recombination, and the following phenomena are detected:

- DNA damage sensitivity assay.
- Measure cell survival for dose.
- γH2AX foci.
- Phosphorylated histone H2AX (γH2AX) accumulates around double-strand breaks.
- HRR protein foci.
- HRR proteins such as RAD51 accumulate at the recombination site.
- BrdU incorporation: Detects DNA repair synthesis.
- Sister chromatid exchange (SCE) assay.
- Sister chromatid exchange in metaphase spread.
- Chromosome breakage assay.
- Chromosomal breakage in metaphase spread.
- Homologous recombination (HR) assay.

After the induction of artificial double-strand breaks with endonuclease I-SceI, the percentage of cells repaired by HR is measured using GFP.

3.10 Summary

Genetic testing is expanding to MGP for all patients. The purpose of the test has been extended to companion diagnostics of PARP inhibitors in addition to the diagnosis of HBOC. Medical professionals who perform the test understand the analysis method, control the quality, interpret the results, and respond to germline findings.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994 Oct 7;266(5182):66–71. https://doi.org/10.1126/science.7545954.
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995 Dec 21-28;378(6559):789–92. https://doi.org/10.1038/378789a0.
3. Brenner S, et al. Nat Biotechnol. 2000;18:630.

4. International Human Genome Sequencing Consortium. Nature. 2001;409:860.
5. Ronaghi M, et al. Science. 1998;281:5375.
6. Association for Medical Pathology v. Myriad Genetics, Inc., 569 U.S.___. 133 S.Ct.2107 (2013).
7. Seynaeve C, Verhoog LC, van de Bosch LM, van Geel AN, Menke-Pluymers M, Meijers-Heijboer EJ, van den Ouweland AM, Wagner A, Creutzberg CL, Niermeijer MF, Klijn JG, Brekelmans CT. Ipsilateral breast tumour recurrence in hereditary breast cancer following breast-conserving therapy. Eur J Cancer. 2004;40:1150–8.
8. Valachis A, Nearchou AD, Lind P. Surgical management of breast cancer in BRCA–mutation carriers: a systematic review and meta–analysis. Breast Cancer Res Treat. 2014;144(3):443–55.
9. Gao X, Fisher SG, Emami B. Risk of second primary cancer in the contralateral breast in women treated for early-stage breast cancer: a population-based study. Int J Radiat Oncol Biol Phys. 2003;56:1038–45.
10. Waener E, et al. J Clin Oncol. 2011;29:1664–9.
11. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer. 2016 Feb;16(2):110–20. https://doi.org/10.1038/nrc.2015.21.
12. Li X, You R, Wang X, Liu C, Xu Z, Zhou J, et al. Effectiveness of prophylactic surgeries in BRCA1 or BRCA2 mutation carriers: meta–analysis and systematic review. Clin Cancer Res. 2016;22(15):3971–81.
13. Rebbeck TR, Kauff ND, Domchek SM. Meta–analysis of risk reduction estimates associated with risk–reducing salpingo–oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst. 2009;101(2):80–7.
14. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk–reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967–75.
15. Ingham SL, Sperrin M, Baildam A, Ross GL, Clayton R, Lalloo F, et al. Risk–reducing surgery increases survival in BRCA1/2 mutation carriers unaffected at time of family referral. Breast Cancer Res Treat. 2013;142(3):611–8.
16. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Genetic/familial high-risk assessment: Breast and ovarian, and pancreatic. ver1. 2020.
17. https://www.nytimes.com/2013/05/14/opinion/my-medical-choice.html?_r=2&
18. https://www.nsgc.org/Policy-Research-and-Publications/Position-Statements/Position-Statements/Post/genetic-testing-of-minors-for-adult-onset-conditions
19. Bryce A. Seifert et al. germline analysis from tumor-germline sequencing dyads to identify clinically actionable secondary findings. Clin Cancer Res. 2016 Aug 15;22(16):4087–94. https://doi.org/10.1158/1078-0432.CCR-16-0015.
20. Mandelker D, Donoghue M, Talukdar S, Bandlamudi C, Srinivasan P, Vivek M, Jezdic S, Hanson H, Snape K, Kulkarni A, Hawkes L, Douillard JY, Wallace SE, Rial-Sebbag E, Meric-Bersntam F, George A, Chubb D, Loveday C, Ladanyi M, Berger MF, Taylor BS, Turnbull C. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO precision medicine working group. Ann Oncol. 2019 Aug 1;30(8):1221–31. https://doi.org/10.1093/annonc/mdz136.
21. Frank TS, Manley SA, Olopade OI, Cummings S, Garber JE, Bernhardt B, Antman K, Russo D, Wood ME, Mullineau L, Isaacs C, Peshkin B, Buys S, Venne V, Rowley PT, Loader S, Offit K, Robson M, Hampel H, Brener D, Winer EP, Clark S, Weber B, Strong LC, Thomas A. Sequence analysis of BRCA1 and BRCA2: correlation of mutations with family history and ovarian cancer risk. J Clin Oncol. 1998;16:2417–25.
22. Kang E, Park SK, Lee JW, Kim Z, Noh WC, Jung Y, Yang JH, Jung SH, Kim SW. KOHBRA BRCA risk calculator (KOHCal): a model for predicting BRCA1 and BRCA2 mutations in Korean breast cancer patients. J Hum Genet. 2016 May;61(5):365–71. https://doi.org/10.1038/jhg.2015.164.
23. Parmigiani G, Berry D, Aguilar O. Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. Am J Hum Genet. 1998;62:145–58.
24. Lee A, Yang X, Tyrer J, Gentry-Maharaj A, Ryan A, Mavaddat N, Cunningham AP, Carver T, Archer S, Leslie G, Kalsi J, Gaba F, Manchanda R, Gayther S, Ramus S, Walter F, Tischkowitz

M, Jacobs I, Menon U, Easton D, Pharoah P, Antonio A. A comprehensive epithelial Tubo-ovarian cancer risk prediction model incorporating genetic and epidemiological risk factors. medRxiv. 2020;12(04):20244046. https://doi.org/10.1101/2020.12.04.20244046.
25. Timothy R. Rebbeck, et al. association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. JAMA. 2015 Apr 7;313(13):1347–61. https://doi.org/10.1001/jama.2014.5985.
26. Whittemore AS, Gong G, John EM, et al. Prevalence of BRCA1 mutation carriers among U.S. non-Hispanic whites. Cancer Epidemiol Biomark Prev. 2004;13(12):2078–83.
27. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer. 2000;83(10):1301–8.
28. Palma MD, Domchek SM, Stopfer J, Erlichman J, Siegfried JD, Tigges-Cardwell J, Mason BA, Rebbeck TR, Nathanson KL. The relative contribution of point mutations and genomic rearrangements in BRCA1 and BRCA2 in high-risk breast cancer families. Cancer Res. 2008;68:7006–14.
29. Ewald IP, Ribeiro PL, Palmero EI, Cossio SL, Giugliani R, Ashton-Prolla P. Genomic rearrangements in BRCA1 and BRCA2: a literature review. Genet Mol Biol. 2009;32:437–46.
30. Kang P, Mariapun S, Phuah SY, Lim LS, Liu J, Yoon SY, Thong MK, Mohd Taib NA, Yip CH, Teo SH. Large BRCA1 and BRCA2 genomic rearrangements in Malaysian high risk breast-ovarian cancer families. Breast Cancer Res Treat. 2010;124:579–84.
31. Judkins T, Rosenthal E, Arnell C, Burbidge LA, Geary W, Barrus T, Schoenberger J, Trost J, Wenstrup RJ, Roa BB. Clinical significance of large rearrangements in BRCA1 and BRCA2. Cancer. 2012;118:5210–6.
32. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL. ACMG laboratory quality assurance committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the association for molecular pathology. Genet Med. 2015 May;17(5):405–24. https://doi.org/10.1038/gim.2015.30.
33. Evans DGR, van Veen EM, Byers HJ, Wallace AJ, Ellingford JM, Beaman G, Santoyo-Lopez J, Aitman TJ, Eccles DM, Lalloo FI, Smith MJ, Newman WG. A. Dominantly inherited 5' UTR variant causing methylation-associated silencing of BRCA1 as a cause of breast and ovarian cancer. Am J Hum Genet. 2018 Aug 2;103(2):213–20. https://doi.org/10.1016/j.ajhg.2018.07.002.
34. Hömig-Hölzel C, Savola S. Diagn. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. Mol Pathol. 2012 Dec;21(4):189–206. https://doi.org/10.1097/PDM.0b013e3182595516.
35. Inazawa J, Inoue J, Imoto I. Comparative genomic hybridization (CGH)-arrays pave the way for identification of novel cancer-related genes. Cancer Sci. 2004 Jul;95(7):559–63. https://doi.org/10.1111/j.1349-7006.2004.tb02486.x.
36. Harris TJ, McCormick F. The molecular pathology of cancer. Nat Rev Clin Oncol. 2010;7(5):251–65. https://doi.org/10.1038/nrclinonc.2010.41. Epub 2010 Mar 30
37. Santana Dos Santos E, Lallemand F, Petitalot A, Caputo SM, Rouleau E. *HRness* in breast and ovarian cancers. Int J Mol Sci. 2020 May 28;21(11):3850. https://doi.org/10.3390/ijms21113850.
38. Vail PJ, Morris B, van Kan A, Burdett BC, Moyes K, Theisen A, Kerr ID, Wenstrup RJ, Eggington JM. Comparison of locus-specific databases for BRCA1 and BRCA2 variants reveals disparity in variant classification within and among databases. J Community Genet. 2015 Oct;6(4):351–9. https://doi.org/10.1007/s12687-015-0220-x.
39. Yrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med. 2004;23(7):1111–30.
40. Bondy ML, Newman LA. Breast cancer risk assessment models: applicability to African-American women. Cancer. 2003;97(1 Suppl):230–5.
41. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. Cancer. 1994;73(3):643–51.

Variants of Uncertain Significances in Hereditary Breast and Ovarian Cancer

4

Yuki Yoshino and Natsuko Chiba

Abstract

Hereditary breast and ovarian cancer syndrome is a *BRCA1*- or *BRCA2*-linked genetic disorder associated with a high risk of developing breast, ovarian, and other cancers. Detection of a *BRCA1* or *BRCA2* pathogenic variants by genetic testing triggers several clinical management approaches, such as surveillance and prophylactic surgery for healthy carriers, and chemotherapy using poly (ADP-ribose) polymerase (PARP) inhibitors for patients with cancer. Therefore, accurate diagnoses are critical for clinical decision-making and improvement of prognosis.

BRCA1 and *BRCA2* variants, whose pathogenicity can be inferred from the genetic code, are classified as pathogenic, likely pathogenic, variants of uncertain significance (VUS), likely benign, or benign. Most variants established to be pathogenic are premature truncation variants, including nonsense or frameshift alterations. VUS are primarily missense and splicing variants and are sequence changes whose impact on function cannot be inferred. Recently, next-generation sequencing has been broadly applied in research and clinical diagnostics to aid both basic research and clinical patient management, where it has led to identification of a vast number of VUS that require interpretation. The pathogenicity of VUS can be evaluated by multifactorial likelihood models that

Y. Yoshino · N. Chiba (✉)
Department of Cancer Biology, Institute of Development, Aging and Cancer (IDAC), Tohoku University, Sendai, Japan

Laboratory of Cancer Biology, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Department of Cancer Biology, Tohoku University Graduate School of Medicine, Sendai, Japan
e-mail: natsuko.chiba.c7@tohoku.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_4

use personal and family history of cancer, segregation data, functional assays, and in silico prediction tools.

Here, we focus on classification of variants in *BRCA1* and *BRCA2*, and the use of functional assays in attempts to classify VUS, with the aim of improving the clinical management and prognosis of carriers and patients.

Keywords

Variants of uncertain significance (VUS) · Hereditary breast and ovarian cancer (HBOC) · *BRCA1* · *BRCA 2* · Germline mutation · Variant classification · Functional assay

4.1 Introduction

Inherited disorders have historically been diagnosed based on direct clinical evidence, such as patient phenotype, segregation of genomic markers or variants with the disorder, or personal and family history. The advent of gene sequencing has clarified the roles of genetic variants in inherited disorders, including hereditary cancer syndromes. Inherited variation in the *BRCA1* and *BRCA2* genes can indicate genetic predisposition to breast, ovarian, and other cancers [1, 2]. Hereditary breast and ovarian cancer (HBOC) syndrome is a *BRCA1*- or *BRCA2*-linked genetic disorder, diagnosis of which is by genetic testing for germline mutations of *BRCA1* or *BRCA2*. Identification of a pathogenic *BRCA1* or *BRCA2* variant is critical for medical management of individuals, which can include intensified screening programs for early detection of cancer, prophylactic surgery, and preventive medication. In addition, genetic testing can identify individuals in HBOC families who are not carriers of the pathogenic variant and therefore not at elevated risk of cancer. Such individuals can be discharged from intensive follow-up and avoid unnecessary surgery to reduce cancer risk.

Genetic testing of *BRCA1 or BRCA2* is also becoming relevant for the cancer chemotherapy. Homologous recombination (HR) is a major pathway for repair of DNA double-strand breaks. Because both BRCA1 and BRCA2 are essential for HR, *BRCA1*- or *BRCA2*-linked cancers result in HR deficiency [3]. Cancer cells with HR deficiency are sensitive to DNA-damaging agents and poly (ADP-ribose) polymerase (PARP) inhibitors, which cause synthetic lethality in HR-deficient cells [4]. Since carriers of pathogenic variants in *BRCA1* or *BRCA2* will benefit from treatment with DNA-damaging agents and PARP inhibitors, identification of pathogenic *BRCA1* or *BRCA2* variants is also critical for stratification of cancer patients for chemotherapy [5]. Thus, accurate variant classification is especially important for actionable genes, such as *BRCA1* and *BRCA2*, which are important in clinical practice [6].

Currently, the pathogenicity of *BRCA1* and *BRCA2* variants are classified using multifactorial likelihood models, as benign, likely benign, variants of uncertain significance (VUS), likely pathogenic, or pathogenic. Most variants established as

pathogenic are premature truncation variants, including nonsense or frameshift variants. VUS are primarily missense and splicing variants, whose impact on function cannot be inferred from the genetic code.

Next-generation sequencing (NGS) is massively parallel sequencing technology that allows simultaneous sequencing of hundreds of DNA fragments. NGS analyses have created opportunities to collect information about variants derived from sporadic cancers and allowed deep molecular characterization of various tumors. Simultaneously, NGS of tumor tissues is driving a paradigm shift in genetic testing and identification of potential germline mutations, leading to an exponential expansion of VUS.

Established pathogenic variants are considered in standard clinical processes; however, when genetic testing reveals VUS, this creates confusion for clinicians and patients. Counseling patients with VUS results is challenging, because the test results cannot be used to quantify risk and guide management. Thus, it is important to decrease the number of VUS and differences in interpretation of variants among laboratories. Here, we describe VUS, with a particular focus on the HBOC-related genes, *BRCA1* and *BRCA2*, and functional assays to classify VUS for better clinical management.

4.2 Guidelines for Variant Classification

Variability of gene sequences among individuals is common within the general population and between those of different ethnic backgrounds. These variabilities can lead to difficulties in interpretation of VUS. Guidelines to define the pathogenicity of variants in inherited disorders and classification of VUS have been established by the American College of Medical Genetics (ACMG) and the Association for Molecular Pathology (AMP) [7]. The guidelines provide a five-tier nomenclature for assertions about gene variants with respect to Mendelian disorders: pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign (LB), and benign (B). Moreover, the recommendations describe a process for classifying variants based on various types of evidence, such as population, computational predictive, functional, and co-segregation data.

The International Agency for Research on Cancer (IARC) classification also subdivides gene variants into five classes (Table 4.1) [8, 9]. Classes 5 and 4 include pathogenic and likely pathogenic variants, respectively; Class 3 comprises VUS; and Classes 2 and 1 represent likely benign and benign variants, respectively. Although the interpretation of genetic findings from investigation of inherited cancer susceptibility genes is frequently conflicting, Class 3 (VUS) is the most numerous, comprising approximately 40% of all variants in inherited cancer susceptibility genes [6].

To classify *BRCA1* and *BRCA2* VUS, the IARC Unclassified Genetic Variants Working Group developed a multifactorial likelihood classification model, together with other groups, such as the Breast Cancer Information Core (BIC) [10, 11]. The clinical inference of the IARC classification is based on variants that are associated

Table 4.1 The IARC classification system for genetic variants

The five-tier classification system recommended by IARC and endorsed by ENIGMA			
Class	Description	Probability of being pathogenic	Surveillance recommendation
1	Benign	<0.001	Consider as if no mutation detected
2	Likely benign	0.001–0.049	Consider as if no mutation detected
3	Uncertain	0.05–0.949	Survey depending on family history and other risk factors
4	Likely pathogenic	0.95–0.99	Full high-risk surveillance
5	Pathogenic	>0.99	Full high-risk surveillance

The five-tier classification system recommended by IARC and endorsed by ENIGMA

with high risk, comparable to that associated with a truncating variant in *BRCA1* or *BRCA2* [8]. They present variant frequency in cases and controls, co-occurrence with a known pathogenic variant in the same gene, co-segregation with disease in pedigrees, personal and family history, species conservation, functional studies, loss of heterozygosity, and pathological classification, as potentially useful evidence for classification of variants. Furthermore, based on these pieces of evidence, IARC provides standards for classification of VUS, with the addition of in silico assessments of sequence and structure variation, based on evolutionary conservation and assessment of the potential for a variant to influence splicing [8, 12, 13].

The development of these models and guidelines required the formation of curated databases containing integrated information about variants. The Clinical Genome (ClinGen) Resource is a National Institutes of Health (NIH)-supported program dedicated to producing a publicly available database that assesses the clinical association of variants with specific diseases (Table 4.2) [16]. The gene–disease relationships for genes commonly found on hereditary breast and ovarian cancer panels were analyzed using the ClinGen clinical validity framework. *BRCA1* and *BRCA2* were the only genes that could be definitively linked to predisposition to both breast and ovarian cancers, while *ATM*, *BARD1*, *CDH1*, *CHEK2*, and *PALB2* were only definitively associated with breast cancer, and *BRIP1*, *RAD51C*, and *RAD51D* only with ovarian cancer [22].

A major clinically oriented database currently used by the global scientific and clinical community is ClinVar (Table 4.2) [14, 15]. ClinVar is a key partner of ClinGen and an international, submission-driven archive of variant-condition interpretations hosted by the National Center for Biotechnology Information (NCBI), based on query-search engine technology. ClinVar represents a wide genetic data collection, allowing exploration, interpretation, and sharing of single variants. Furthermore, to better understand the impact of variants and achieve a wider perspective, a working group from Utah University developed the ClinVar Miner website [17].

Table 4.2 Disease and function databases

Tools	Description	Website	References
ClinVar	ClinVar is a freely available public archive of reports of the relationships between human variations and phenotypes presented with supporting evidence and an indication of likely clinical significance. Interpretations of the clinical significance of variants are submitted by clinical testing and research laboratories. The database includes germline and somatic variants of any size, type, or genomic location	https://www.ncbi.nlm.nih.gov/clinvar/	[14, 15]
ClinGen	The Clinical Genome, ClinGen, resource is supported by NIH and intended to be an authoritative central resource that defines the clinical relevance of genomic variants for use in precision medicine and research. ClinGen is developing several resources for the community, such as ClinVar	https://clinicalgenome.org/	[16]
ClinVar Miner	ClinVar Miner is an interface for viewing ClinVar data. It complements the existing ClinVar database by enabling exploration of the data at different levels of granularity and from different perspectives. Statistics for current and historical data can be viewed relative to all submissions, submitters, conflicting submissions, and genes	https://clinvarminer.genetics.utah.edu/	[17]
LOVD	Leiden Open (source) Variation Database. This is a flexible, freely available tool for gene-centered collection and display of DNA variants. LOVD allows both patient-centered and gene-centered views	http://www.lovd.nl/3.0/home	[18]
HGMD	The public version of the Human Gene Mutation Database (HGMD) is a freely available, comprehensive collection of germline mutations in all known genes underlying human inherited disease together with disease-associated/functional polymorphisms published in the peer-reviewed literature	http://www.hgmd.cf.ac.uk/ac/index.php	[19]
BRCA Exchange	BRCA Exchange is a freely available tool for displaying BRCA1 and BRCA2 variants drawn from global sources and to enable BRCA1 and BRCA2 variants to be expertly reviewed, interpreted, classified, and aggregated in an integrated data system. The publicly accessible display of these classifications, with supporting evidence, advances accurate understanding of the clinical relevance of BRCA1 and BRCA2 variants. The website is a product of the BRCA Challenge project, a driver project of the Global Alliance for Genomics and Health (GA4GH)	https://brcaexchange.org/	[20]

(continued)

Table 4.2 (continued)

Tools	Description	Website	References
BIC	The Breast Cancer Information Core. A database that acted for *BRCA1* and *BRCA2* variants deposited by submitters from research and clinical sites internationally. A copy of all BIC data has been shared with several other variation databases, such as ClinVar and BRCA Exchange. BIC database is no longer actively curated	https://research.nhgri.nih.gov/projects/bic	
BRCA1 Circos	BRCA1 Circos is a visualization resource that compiles and displays functional data on all documented *BRCA1* missense variants. BRCA1 Circos consists of data derived from functional assays and bioinformatic predictions for *BRCA1* missense variants present in the BIC database. This resource provides an interactive display of data from published BRCA1 functional studies that will aid researchers in interpreting the functional consequences of *BRCA1* variants	https://research.nhgri.nih.gov/bic/circos/	[21]

In response to the increase in *BRCA1* and *BRCA2* VUS, the need for interpretation of test results became apparent, since identification of VUS complicates genetic test reporting and counseling. The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) international consortium was established to address this issue (https://enigmaconsortium.org/) [23]. ENIGMA is a research-based international collaborative group, recognized as an expert panel by ClinVar, that aims to encourage and improve research efforts and methods for the classification of variants in *BRCA1* and *BRCA2* and other breast–ovarian cancer susceptibility genes. The consortium has developed variant classification criteria that incorporate both quantitative (statistical) and qualitative (rules-based) methods to assess the clinical significance of variants in *BRCA1* and *BRCA2*. The quantitative classification of variants is derived from the multifactorial likelihood models that include population and clinical evaluation, as well as bioinformatic predictions, and the consortium promotes data sharing from large-scale projects with variant annotations [10, 11, 24].

Furthermore, the BRCA1 Challenge is a data-sharing project initiated within the Global Alliance for Genomics and Health (GA4GH) to aggregate *BRCA1* and *BRCA2* data for collaborative research activities and created the BRCA Exchange database (Table 4.2) [20]. The goal of the BRCA1 Challenge is to improve understanding of the impact of variants in *BRCA1* and *BRCA2* and provide updated variant classifications and revised reports of variant interpretations. The data set is based on shared information from existing clinical databases, including BIC, ClinVar, and Leiden Open Variation Database (LOVD) (Table 4.2) [18], as well as population databases. The BRCA1 Challenge brought together the existing international ENIGMA consortium expert panel with expert clinicians, diagnosticians, researchers, and data providers [20]. The ENIGMA approach has been exemplary for *BRCA1* and *BRCA2* classification for clinical utility and is used

as a model for the implementation of genetic testing and variant classification in other tumor-related genes [3, 25].

4.3 VUS in *BRCA1* and *BRCA2*

Generally, classification of VUS is difficult, due to insufficient population-based statistical evidence. When VUS are novel or rare, clinical information for correlation is scarce. Further, variants are often identified in different pathological conditions and populations, interfering with statistical evaluation. Moreover, differences in evaluation of variants by clinicians and researchers can confuse their classification.

Genes implicated in HBOC, including *BRCA1* and *BRCA2*, are tumor suppressor genes; therefore, variants leading to functional deficiency are considered pathogenic. Genetic alterations that cause loss of function include frameshift and nonsense variants leading to protein truncation in functionally critical regions of the protein, alteration of donor and acceptor splice sites, and large genomic rearrangements that alter the coding region. Synonymous changes that do not influence mRNA splicing are considered non-pathogenic.

VUS are mainly missense variants or in-frame insertions/deletions. They may be found in non-coding regions, at less conserved residues, at splicing boundaries, or in less functionally relevant domains, relative to pathological variants. The impact of these VUS on protein function and expression is difficult to evaluate, compared with nonsense or frameshift mutations that cause protein truncations. Therefore, there is a critical need to classify variants according to their pathogenicity.

In genetic testing for familial breast and ovarian cancers, up to 20% of *BRCA1* and *BRCA 2* variants are classified as VUS [26–28]; however, the frequency of VUS is generally lower in well-characterized ethnic populations. For example, in Japan, the frequency of VUS in *BRCA1* and *BRCA2* is 6%–7% [29, 30]. In ClinVar, as of July 2020, 2853 and 5132 variants of *BRCA1* and *BRCA2*, respectively, are recorded as VUS, comprising 32.4% and 40.2% of *BRCA1* and *BRCA2* variants. The most frequently reported class of VUS is single-nucleotide variants, with missense alteration representing the majority, comprising 79.9% and 86.1% of all *BRCA1* and *BRCA2* VUS, respectively.

Distinct pathogenic variants in the same gene may confer significantly different levels of risk. Indeed, there are *BRCA1* and *BRCA2* variants that cause a relatively moderate risk of cancer, compared with deleterious variants. Recently, it was reported that some pathogenic missense variants of *BRCA1* (R1699Q and V1736A) and *BRCA2* (G2508S and Y3035S) confer only moderate breast cancer risk [23, 31–33]. Of these variants, V1736A has been reported as a biallelic pathogenic variant in *BRCA1* [31]. Further, the *BRCA2* variant, K3326X, which was initially classified as non-pathogenic, is associated with a mild increase in risk of breast and ovarian cancer [34, 35]. There are currently no consensus guidelines regarding clinical management of patients with these variants, except R1699Q, for which the EGNIMA consortium has made recommendations for clinical management [33]. Interestingly, the association of the *BRCA2* G2508S variant with a moderate risk of

breast cancer was observed in Asian women, but a comparable risk was not found in the Caucasian population [32]. Genetic and environmental modifiers in the Asian population might affect the influence of this variant on breast cancer risk.

Some functional assays may contribute to estimation of risk level by evaluating specific biological activity. Recent advances in computational technology enable evaluation of large numbers of variants using computational variant-effect prediction algorithms; however, these are not sufficiently accurate for routine clinical use. By contrast, functional assays are considered strong evidence, according to the ACMG guidelines and multifactorial likelihood models to evaluate variant pathogenicity [7]. Therefore, the role of functional data is important in supporting VUS classification.

4.4 Functional Assays to Evaluate the Effects of VUS

4.4.1 Functional Assays of BRCA1

BRCA1 has a RING domain (amino acids (aa) 8–98) at its amino (N)-terminal region that binds to BARD1, and the BRCA1/BARD1 heterodimer shows E3 ubiquitin ligase activity (Fig. 4.1). Further the BRCA1 coiled-coil motif (aa 1367–1437) binds to PALB2 and two BRCT domains (aa 1646–1859) at its carboxy (C)-terminal region bind to BRIP1, CtIP, and ABRAXAS1. *BARD1*, *PALB2*, *BRIP1*, and *ABRAXAS1* are also breast cancer susceptibility genes [3, 36]. Most pathogenic missense variants of BRCA1 in ClinVar are located in the RING and BRCT domains.

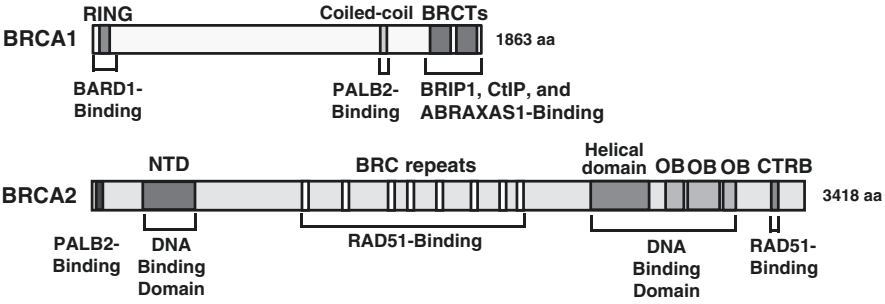


Fig. 4.1 Structures of BRCA1 and BRCA2. BRCA1 has a RING domain in the N-terminal region, and a coiled-coil domain and two BRCT domains in the C-terminal region. BRCA1 forms a heterodimer with BARD1 via the RING domain. A coiled-coil domain of BRCA1 mediates complex with PALB2. Two BRCT domains bind to BRIP1, CtIP, and ABRAXAS1. BRCA2 has PALB2 binding domain in the N-terminal region, eight BRC repeats in the middle portion, and a helical domain, three oligonucleotide/oligosaccharide binding (OB) folds, and a RAD51-binding domain, (C-terminal RAD51-binding; CTRB) in the C-terminal region. The middle portion, which includes eight BRC repeats, also functions in binding to RAD51. The DNA-binding domain in the C-terminal domain is composed of a helical domain and three OB folds. There is also another DNA-binding domain, the N-terminal DNA-binding domain (NTD)

BRCA1 functions in multiple cellular processes, and functional assays have been proposed to evaluate the impact of BRCA1 variants on its biological and biochemical roles, such as HR, transcriptional activation, restoration of radiation resistance, centrosome amplification, cell cycle control, subcellular localization, E3 ubiquitin ligase activity, protease sensitivity, and phosphopeptide binding [21, 37]. The results of various functional assays for BRCA1 variants are catalogued on the BRCA1 Circos Web tool, which aggregates data from published functional studies of BRCA1 missense variants to facilitate visualization of complex data [21].

Monteiro et al. established a transcriptional activation assay based on fusion of the GAL4 DNA-binding domain to the C-terminal region of BRCA1 (aa 1396–1863) [38–40] and used it to assess 347 missense variants in the BRCA1 C-terminal region, where transcriptional activation activity is located. Furthermore, they combined the results of this transcriptional activation assay with VarCall, a Bayesian integrative statistical model, to determine the likelihood of pathogenicity, given the functional data.

By contrast, assays to evaluate HR and cellular resistance to DNA damage have the potential to assess variants across the entire *BRCA1* gene. The direct-repeat GFP (DR-GFP) assay has been used to measure HR activity in cells [41]. The RING domain, coiled-coil motif, and BRCT domains are all important for HR and cellular resistance to DNA damage [42–44]. The RING domain is important for binding to BARD1 [45] and recruitment of BRCA1 to DNA double-strand breaks [46]. The coiled-coil motif is required for recruitment of PALB2/BRCA2/RAD51 to initiate strand invasion, and the BRCT domains are required for binding to CtIP nuclease, to promote end resection of DNA double-strand breaks [36]. In addition to these domains, deletions of the middle portion of BRCA1 decrease the HR activity; hence, these middle portions are also involved in the repair of DNA double-strand breaks [42].

Recently, two saturation mutagenesis-based high-throughput approaches succeeded in evaluating thousands of BRCA1 missense variants using distinct functions of BRCA1. Findlay et al. assayed the effects of BRCA1 variants on viability of the haploid human cell line, HAP1, in which HR factors are essential for cell viability [47], while Starita et al. evaluated the role of BRCA1 in HR [48]. Interestingly, the results of the assays were consistent with the data in the ClinVar data base, and catalogues of these functional data will provide valuable information for clinical annotation.

4.4.2 Functional Assays of BRCA2

BRCA2 also binds to PALB2 via its N-terminal region (aa 10–40), while eight BRC motifs (aa 1008–2082) in its middle portion and C-terminal region (aa 3270–3305) bind to the RAD51 recombinase (Fig. 4.1). A helical domain (aa 2402–2668) and three oligonucleotide/oligosaccharide binding (OB) folds (aa 2670–3102) in the C-terminal region bind to single-strand DNA, and the N-terminal DNA-binding domain (NTD) also has DNA-binding activity.

BRCA2 also has multiple functions, such as HR, protection of stalled replication forks, G2 checkpoint arrest, restoration of DNA damage resistance, cytokinesis, centrosome amplification, and transcriptional activation, and functional assays of BRCA2 have been developed to analyze these functions [49]. BRCA2 plays critical roles in HR by recruitment of RAD51 to sites of DNA damage and oligomerization of RAD51 with single-strand DNA. As these activities are considered critical for the role of BRCA2 as tumor suppressor, functional assays for this protein are mainly performed by evaluation of HR activity, RAD51 foci formation after DNA damage, and cellular resistance to DNA damage. Similar to BRCA1, these functions of BRCA2 require entire regions of BRCA2. Furthermore, binding of the N-terminal region to PALB2 and of the C-terminal region DNA-binding domain is used to help evaluate missense variants in these regions.

Guidugli et al. performed a comprehensive functional assay for BRCA2 variants in the DNA-binding domain (aa 2460–3170) by evaluating HR activity [50]. They compared the results of this assay with predictions of probability of pathogenicity from the Align-GVGD protein-sequence-based prediction algorithm [51] and combined functional and Align-GVGD prediction results in VarCall to determine the overall probability of pathogenicity for *BRCA2* VUS. Furthermore, they used the endophenotype-Optimized Sequence Ensemble (ePOSE) algorithm to train classifiers for *BRCA2* variants according to HR activity.

Recently, Ikegami et al. developed a high-throughput functional assay to evaluate HR activity of BRCA2 variants via sensitivity to PARP inhibitors, using *BRCA2*-deficient cells [52]. They classified the functional impact of 186 *BRCA2* VUS and the results showed high concordance with IARC and ACMG classifications. In addition, they presented a simplified, on-demand annotation system that can be used as a companion diagnostic tool for application of PARP inhibitors for patients with *BRCA2* VUS.

4.4.3 Perspective on Functional Assays of BRCA1 and BRCA2

Because both BRCA1 and BRCA2 have critical domains for their functions at or near their C-terminal regions, almost all nonsense and frameshift mutations causing protein truncations are classified as pathogenic. Functional assays have been developed to assess their likelihood of pathogenicity of VUS of missense variants. Understanding of how the multiple functions of the BRCA1 and BRCA2 proteins relate to cancer predisposition is limited. Therefore, it remains unclear which assays are most biologically appropriate for assessing variant pathogenicity.

To maximize the possibility of identification of pathogenic variants, researchers have focused on domain and motifs important for their tumor suppressor functions. Therefore, most assays have focused on variants of the RING and BRCT domains of BRCA1 and the DNA-binding domain of BRCA2.

Many functional assays have been reported for BRCA1 and BRCA2, and most of these have classified variant using a binary system (functional or nonfunctional), whereas some assays have revealed variants with intermediate function. Several

missense variants exhibit intermediate effects in functional assays. Indeed, the BRCA1 variants, R1699Q and V1736A, and the BRCA2 variants, G2508S and Y3035, which are associated with moderate cancer risk (as described above), have intermediate effects on protein function [23, 31, 32, 44]; however, it is difficult to choose a specific activity threshold that separates pathogenic from non-pathogenic variants. Several functional assays use arbitrary thresholds. Integration of well-established functional data can also be achieved using the ACMG/AMP classification model.

Furthermore, evaluation in rodent cells may have limited reliability or capacity to assess variants, because of the relatively low conservation of certain BRCA1 and BRCA2 domains between humans and rodents. Moreover, a relatively neglected issue in the development of functional assays for BRCA1 and BRCA2 is tissue specificity. BRCA1 and BRCA2 are important for DNA repair across tissue types; however, carriers of these mutations exhibit specificity in the organs and cells at highest risk for developing tumors. Therefore, tissue-specific factors are likely to modulate the context in which these mutations influence cancer development. Hence, it may be important to perform assays in human cell lines relevant to particular tumor types.

4.4.4 Utility of BRCA1 and BRCA2 Functional Assays

The introduction of PARP inhibitors in cancer therapy has led to major changes in the framework for genetic testing of patients with cancer. PARP inhibitors induce synthetic lethality of cells with HR deficiency caused by *BRCA1* or *BRCA2* dysfunction [4, 53–55]. In general, *BRCA1* and *BRCA2* mutation carriers are heterozygous for the pathogenic allele, whereas tumor cells frequently also lose the wild-type allele; therefore, tumor cells are not viable in the presence of PARP inhibitors, whereas normal cells survive. PARP inhibitors improve progression-free survival in several types of BRCA-related cancer such as ovary, breast, pancreas, and prostate and have been approved in the USA, Europe, and Japan for treatment of advanced and metastatic breast and ovarian cancers [56]. At present, the results of genetic testing of *BRCA1* and *BRCA2* are used clinically as surrogate markers to detect HR deficiency in cancer cells. Therefore, in addition to the classification of germline variants to estimate cancer risk, the classification of germline and somatic variants, to predict the response of cancer cells that express these variants to PARP inhibitors, has become useful and required in the clinic.

As described above, the DR-GFP assay has been used to measure HR activity in cells and evaluate the functions of *BRCA1* and *BRCA2* variants. Indeed, BRCA1 variants resulting in HR deficiency result in elevated sensitivity to PARP inhibitors and the DNA-damaging agent, cisplatin [43, 44]. Recently, we developed a novel assay to evaluate HR activity in cells [57], the Assay for Site-specific HR Activity (ASHRA), in which cells are transiently transfected with a CRISPR/Cas9 expression vector and an HR donor sequence containing a marker gene. DSBs are created by Cas9 and then repaired by HR, using donor vector sequences homologous to the

target gene. The level of genomic integration of the marker gene is then quantified by quantitative PCR. We found that ASHRA could predict the sensitivity of cells that express *BRCA1* variants to PARP inhibitors more precisely than the DR-GFP assay (unpublished data).

Nevertheless, it remains unclear whether HR activity measured by functional assay can reliably predict the sensitivity of tumor cells that express a BRCA1 or BRCA2 variant to PARP inhibitors and DNA-damaging agents. The C61G variant in the BRCA1 RING domain is one of the most frequently reported pathogenic variants in HBOC. This variant abolishes binding to BARD1 and abrogates BRCA1/BARD1 heterodimer E3 ubiquitin ligase activity [58–60]. Furthermore, we and other groups have reported that C61G variant also lose HR activity [42, 43]. Genetically engineered mice carrying the BRCA1 C61G variant developed tumors, whereas the variant does not render tumors sensitive to PARP inhibitors or platinum agents [61].

Furthermore, HR is not only impaired by alteration of not only *BRCA1* and *BRCA2* but also by dysfunction of other HR factors. Indeed, as much as half of the HR deficiency in ovarian cancers is caused by alteration of factors other than *BRCA1* and *BRCA2* [62]. Cells with HR deficiency are also sensitive to other DNA-damaging agents. Therefore, evaluation of HR activity itself is useful to predict sensitivities to platinum agents and other DNA-damaging agents, in addition to PARP inhibitors. Several approaches for estimating cellular HR activity in cancer have been developed. One example is the HRD score, which is calculated from the number of genetic alterations caused by HR deficiency [63]. High HRD scores are associated with sensitivity of breast cancers to platinum agents [64].

4.5 In Silico Prediction Tools

The effects of rare VUS can be predicted by a wide variety of publicly available in silico tools with variable performance [65, 66] (Table 4.3). The most commonly used prediction tools, such as SIFT [67], Polyphen-2 [68, 70], Align-GVGD [51], and MutationTaster2 [69] (Table 4.3), were developed using large-scale databases such as the Human Gene Mutation Database (HGMD) [19] or ClinVar (Table 4.2). Although they have some limitations, the algorithms are constantly improving and the data resulting from analyses with these tools are sufficiently consistent with those from functional assays [71]; however, functional data is still necessary for clinical annotation of rare variants lacking sufficient clinical information.

Recent studies suggest that a combination of functional assays with sequence-based predictors can contribute to the clinical classification of VUS in the absence of the family information currently used in multifactorial probability models of pathogenicity. VarCall is a Bayesian hierarchical model to estimate the likelihood of pathogenicity using direct functional measurements. Established in silico prediction methods for missense variants were recalibrated using results from BRCA1 and BRCA2 functional assays, and these classifications outperformed individual in silico models [38, 39, 50, 66].

Table 4.3 In silico prediction tools

Tools	Description	Website	References
SIFT	Sorting Intolerant From Tolerant (SIFT) is used to predict whether an amino acid substitution affects protein function, based on sequence homology and the physical properties of amino acids, and classifies substitutions as tolerated or deleterious	https://sift.bii.a-star.edu.sg/	[67]
PolyPhen-2	Polymorphism Phenotyping v2 (PolyPhen-2) is available as software and via a web server from Harvard University. It predicts the possible impact of amino acid substitutions on the stability and function of human proteins using structural and comparative evolutionary considerations	http://genetics.bwh.harvard.edu/pph2/	[67, 68]
Align-GVGD	Align Grantham Variation and Grantham Deviation (Align-GVCG) is a web-based program that combines the biophysical characteristics of amino acids and protein multiple sequence alignments to predict where missense substitutions in genes of interest fall in a spectrum from enriched deleterious to enriched neutral. Align-GVGD is an extension of the original Grantham difference to multiple sequence alignments and true simultaneous multiple comparisons	http://agvgd.hci.utah.edu/agvgd_input.php	[51]
MutationTaster2	MutationTaster2 predicts the functional consequences of amino acid substitutions, intronic and synonymous alterations, short insertion and/or deletion (indel) mutations, and variants spanning intron-exon borders. It includes all publicly available single-nucleotide polymorphisms and indels from the 1000 genomes project, as well as known disease variants from ClinVar and HGMD public	http://www.mutationtaster.org/	[69]

Recently, Hart et al. trained and evaluated hundreds of machine learning (ML) algorithms, based on data from validated functional assays of BRCA1 and BRCA2. They developed an optimal BRCA-ML model that can accurately predict the pathogenicity of missense variants in these factors [72].

4.6 Multi-gene Panel Testing

The development of NGS has enhanced the ability to test many genes concurrently and significantly lowered the cost of genetic testing. We have gained greater insights into hereditary cancer, with the identification of additional genes found to confer

significant risk for either breast or ovarian cancer. Including genes listed in ClinGen whose variants are associated with cancer predisposition [22], there are nine genes with established associations between protein-truncating variants and breast cancer risk (*BRCA1*, *BRCA2*, *PALB2*, *ATM*, *CHK2*, *CDH1*, *PTEN*, *TP53*, and *STK11*) and several more (*BARD1*, *RAD51D*, *MSH2*, and *MSH6*) where associations are suggested, but not yet firmly established [71, 73, 74]. Twelve genes are implicated in ovarian cancer risk (*BRCA1*, *BRCA2*, *BRIP1*, *MSH6*, *MSH2*, *RAD51C*, *ATM*, *RAD51D*, *PALB2*, *MLH1*, and *PMS2*) [75, 76]. These genes are listed in multi-gene panels used to diagnose these hereditary cancers.

Multi-gene panel testing is clinically beneficial for patients suspected to have hereditary cancer; however, the frequency of VUS results from such tests is considerable, because the association of several genes with disease is not established and the clinical impact of rare variants cannot be determined.

4.7 Conclusions

Advances in DNA sequencing have enabled rapid, accurate, and cost-effective genetic testing of cancer-related genes, including *BRCA1* and *BRCA2*, and improved cancer patient management by informing diagnosis and treatment decisions for patients with HBOC; however, VUS in *BRCA1* and *BRCA2* identified because of these improvements in sequencing continue to pose a challenge for the management of patients and their relatives. Most VUS will not be associated with a high risk of cancer; however, misinterpretation of VUS has the potential to lead to mismanagement of both patients and their relatives. Thus, accurate interpretation of these variants is critical for appropriate clinical decision-making.

Due to recent progress in computational science, in silico prediction tools may facilitate classification by predicting the potential impact of variants on protein function; however, to develop a mature in silico system which can accurately predict the effects of diverse variants requires integrated functional data related to the variants. Thus, development of accurate functional assays will provide additional data that could lead to reclassification of some variants, resulting in different recommendations for surveillance and therapy in clinical practice.

It has become evident that the use of integrated databases including clinical, in silico prediction, and functional data is preferred; however, due to insufficient availability of functional assays at present, variant classification is still influenced more by clinical information than by the effects of gene alteration on protein function. In addition, optimization of genome data management will improve reference clinical databases and help to reclassify VUS into specific pathological classes.

NGS and multi-gene panel tests improve our knowledge of diseases, while also providing new challenges related to the increased frequency of VUS. These advances have revealed that many other genes implicated in HBOC are involved in DNA damage responses, DNA repair, and the DNA damage checkpoint. Thus, basic

research in these fields will contribute to development of better functional assays for accurate classification of VUS to improve patient outcomes.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266(5182):66–71.
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378(6559):789–92.
3. Nielsen FC, van Overeem HT, Sorensen CS. Hereditary breast and ovarian cancer: new genes in confined pathways. Nat Rev Cancer. 2016;16(9):599–612.
4. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res. 2006;66(16):8109–15.
5. Pilie PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol. 2019;16(2):81–104.
6. Balmana J, Digiovanni L, Gaddam P, Walsh MF, Joseph V, Stadler ZK, et al. Conflicting interpretation of genetic variants and cancer risk by commercial laboratories as assessed by the prospective registry of multiplex testing. J Clin Oncol. 2016;34(34):4071–8.
7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.
8. Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat. 2008;29(11):1282–91.
9. Lindor NM, Guidugli L, Wang X, Vallee MP, Monteiro AN, Tavtigian S, et al. A review of a multifactorial probability-based model for classification of BRCA1 and BRCA2 variants of uncertain significance (VUS). Hum Mutat. 2012;33(1):8–21.
10. Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tavtigian SV, Couch FJ, et al. Integrated evaluation of DNA sequence variants of unknown clinical significance: application to BRCA1 and BRCA2. Am J Hum Genet. 2004;75(4):535–44.
11. Goldgar DE, Easton DF, Byrnes GB, Spurdle AB, Iversen ES, Greenblatt MS, et al. Genetic evidence and integration of various data sources for classifying uncertain variants into a single model. Hum Mutat. 2008;29(11):1265–72.
12. Tavtigian SV, Greenblatt MS, Goldgar DE, Boffetta P, Group IUGVW. Assessing pathogenicity: overview of results from the IARC unclassified genetic variants working group. Hum Mutat. 2008;29(11):1261–4.
13. Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB, Group IUGVW. In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat. 2008;29(11):1327–36.
14. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42(Database issue):D980–5.
15. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 2016;44(D1):D862–8.
16. Rehm HL, Berg JS, Brooks LD, Bustamante CD, Evans JP, Landrum MJ, et al. ClinGen--the clinical genome resource. N Engl J Med. 2015;372(23):2235–42.

17. Henrie A, Hemphill SE, Ruiz-Schultz N, Cushman B, DiStefano MT, Azzariti D, et al. ClinVar miner: demonstrating utility of a web-based tool for viewing and filtering ClinVar data. Hum Mutat. 2018;39(8):1051–60.
18. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v.2.0: the next generation in gene variant databases. Hum Mutat. 2011;32(5):557–63.
19. Stenson PD, Ball EV, Mort M, Phillips AD, Shaw K, Cooper DN. The human gene mutation database (HGMD) and its exploitation in the fields of personalized genomics and molecular evolution. Curr Protoc Bioinformatics. 2012;Chapter 1:Unit1 13.
20. Cline MS, Liao RG, Parsons MT, Paten B, Alquaddoomi F, Antoniou A, et al. BRCA challenge: BRCA exchange as a global resource for variants in BRCA1 and BRCA2. PLoS Genet. 2018;14(12):e1007752.
21. Jhuraney A, Velkova A, Johnson RC, Kessing B, Carvalho RS, Whiley P, et al. BRCA1 Circos: a visualisation resource for functional analysis of missense variants. J Med Genet. 2015;52(4):224–30.
22. Lee K, Seifert BA, Shimelis H, Ghosh R, Crowley SB, Carter NJ, et al. Clinical validity assessment of genes frequently tested on hereditary breast and ovarian cancer susceptibility sequencing panels. Genet Med. 2019;21(7):1497–506.
23. Spurdle AB, Whiley PJ, Thompson B, Feng B, Healey S, Brown MA, et al. BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. J Med Genet. 2012;49(8):525–32.
24. Parsons MT, Tudini E, Li H, Hahnen E, Wappenschmidt B, Feliubadalo L, et al. Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: an ENIGMA resource to support clinical variant classification. Hum Mutat. 2019;40(9):1557–78.
25. Spurdle AB, Healey S, Devereau A, Hogervorst FB, Monteiro AN, Nathanson KL, et al. ENIGMA--evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. Hum Mutat. 2012;33(1):2–7.
26. Lee JS, Oh S, Park SK, Lee MH, Lee JW, Kim SW, et al. Reclassification of BRCA1 and BRCA2 variants of uncertain significance: a multifactorial analysis of multicentre prospective cohort. J Med Genet. 2018;55(12):794–802.
27. Mesman RLS, Calleja F, Hendriks G, Morolli B, Misovic B, Devilee P, et al. The functional impact of variants of uncertain significance in BRCA2. Genet Med. 2019;21(2):293–302.
28. Eccles DM, Mitchell G, Monteiro AN, Schmutzler R, Couch FJ, Spurdle AB, et al. BRCA1 and BRCA2 genetic testing-pitfalls and recommendations for managing variants of uncertain clinical significance. Ann Oncol. 2015;26(10):2057–65.
29. Nakamura S, Takahashi M, Tozaki M, Nakayama T, Nomizu T, Miki Y, et al. Prevalence and differentiation of hereditary breast and ovarian cancers in Japan. Breast Cancer. 2015;22(5):462–8.
30. Arai M, Yokoyama S, Watanabe C, Yoshida R, Kita M, Okawa M, et al. Genetic and clinical characteristics in Japanese hereditary breast and ovarian cancer: first report after establishment of HBOC registration system in Japan. J Hum Genet. 2018;63(4):447–57.
31. Domchek SM, Tang J, Stopfer J, Lilli DR, Hamel N, Tischkowitz M, et al. Biallelic deleterious BRCA1 mutations in a woman with early-onset ovarian cancer. Cancer Discov. 2013;3(4):399–405.
32. Shimelis H, Mesman RLS, Von Nicolai C, Ehlen A, Guidugli L, Martin C, et al. BRCA2 Hypomorphic missense variants confer moderate risks of breast cancer. Cancer Res. 2017;77(11):2789–99.
33. Moghadasi S, Meeks HD, Vreeswijk MP, Janssen LA, Borg A, Ehrencrona H, et al. The BRCA1 c. 5096G>a p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. J Med Genet. 2018;55(1):15–20.
34. Meeks HD, Song H, Michailidou K, Bolla MK, Dennis J, Wang Q, et al. BRCA2 polymorphic stop codon K3326X and the risk of breast, prostate, and ovarian cancers. J Natl Cancer Inst. 2016;108(2):djv315.

35. Arbustini E, Sgarella A, Ferrari A, Grasso D, Cassani C, Lucioni M, et al. RE: BRCA2 polymorphic stop codon K3326X and the risk of breast, prostate, and ovarian cancers. J Natl Cancer Inst. 2016;108(12):djw172.
36. Takaoka M, Miki Y. BRCA1 gene: function and deficiency. Int J Clin Oncol. 2018;23(1):36–44.
37. Millot GA, Carvalho MA, Caputo SM, Vreeswijk MP, Brown MA, Webb M, et al. A guide for functional analysis of BRCA1 variants of uncertain significance. Hum Mutat. 2012;33(11):1526–37.
38. Iversen ES Jr, Couch FJ, Goldgar DE, Tavtigian SV, Monteiro AN. A computational method to classify variants of uncertain significance using functional assay data with application to BRCA1. Cancer Epidemiol Biomark Prev. 2011;20(6):1078–88.
39. Woods NT, Baskin R, Golubeva V, Jhuraney A, De-Gregoriis G, Vaclova T, et al. Functional assays provide a robust tool for the clinical annotation of genetic variants of uncertain significance. NPJ Genom Med. 2016;1
40. Fernandes VC, Golubeva VA, Di Pietro G, Shields C, Amankwah K, Nepomuceno TC, et al. Impact of amino acid substitutions at secondary structures in the BRCT domains of the tumor suppressor BRCA1: implications for clinical annotation. J Biol Chem. 2019;294(15):5980–92.
41. Pierce AJ, Hu P, Han M, Ellis N, Jasin M. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. Genes Dev. 2001;15(24):3237–42.
42. Ransburgh DJ, Chiba N, Ishioka C, Toland AE, Parvin JD. Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. Cancer Res. 2010;70(3):988–95.
43. Anantha RW, Simhadri S, Foo TK, Miao S, Liu J, Shen Z, et al. Functional and mutational landscapes of BRCA1 for homology-directed repair and therapy resistance. elife. 2017;6
44. Bouwman P, van der Gulden H, van der Heijden I, Drost R, Klijn CN, Prasetyanti P, et al. A high-throughput functional complementation assay for classification of BRCA1 missense variants. Cancer Discov. 2013;3(10):1142–55.
45. Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, et al. Identification of a RING protein that can interact in vivo with the BRCA1 gene product. Nat Genet. 1996;14(4):430–40.
46. Wei L, Lan L, Hong Z, Yasui A, Ishioka C, Chiba N. Rapid recruitment of BRCA1 to DNA double-strand breaks is dependent on its association with Ku80. Mol Cell Biol. 2008;28(24):7380–93.
47. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, et al. Accurate classification of BRCA1 variants with saturation genome editing. Nature. 2018;562(7726):217–22.
48. Starita LM, Islam MM, Banerjee T, Adamovich AI, Gullingsrud J, Fields S, et al. A multiplex homology-directed DNA repair assay reveals the impact of more than 1,000 BRCA1 missense substitution variants on protein function. Am J Hum Genet. 2018;103(4):498–508.
49. Guidugli L, Carreira A, Caputo SM, Ehlen A, Galli A, Monteiro AN, et al. Functional assays for analysis of variants of uncertain significance in BRCA2. Hum Mutat. 2014;35(2):151–64.
50. Guidugli L, Shimelis H, Masica DL, Pankratz VS, Lipton GB, Singh N, et al. Assessment of the clinical relevance of BRCA2 missense variants by functional and computational approaches. Am J Hum Genet. 2018;102(2):233–48.
51. Tavtigian SV, Samollow PB, de Silva D, Thomas A. An analysis of unclassified missense substitutions in human BRCA1. Familial Cancer. 2006;5(1):77–88.
52. Ikegami M, Kohsaka S, Ueno T, Momozawa Y, Inoue S, Tamura K, et al. High-throughput functional evaluation of BRCA2 variants of unknown significance. Nat Commun. 2020;11(1):2573.
53. Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, et al. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A. 2008;105(44):17079–84.
54. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21.
55. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913–7.

56. Klinakis A, Karagiannis D, Rampias T. Targeting DNA repair in cancer: current state and novel approaches. Cell Mol Life Sci. 2020;77(4):677–703.
57. Yoshino Y, Endo S, Chen Z, Qi H, Watanabe G, Chiba N. Evaluation of site-specific homologous recombination activity of BRCA1 by direct quantitation of gene editing efficiency. Sci Rep. 2019;9(1):1644.
58. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, et al. The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem. 2001;276(18):14537–40.
59. Ruffner H, Joazeiro CA, Hemmati D, Hunter T, Verma IM. Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proc Natl Acad Sci U S A. 2001;98(9):5134–9.
60. Mallery DL, Vandenberg CJ, Hiom K. Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. EMBO J. 2002;21(24):6755–62.
61. Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, et al. BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. Cancer Cell. 2011;20(6):797–809.
62. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer. Cancer Discov. 2015;5(11):1137–54.
63. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. Clin Cancer Res. 2016;22(15):3764–73.
64. Jin J, Zhang W, Ji W, Yang F, Guan X. Predictive biomarkers for triple negative breast cancer treated with platinum-based chemotherapy. Cancer Biol Ther. 2017;18(6):369–78.
65. Ernst C, Hahnen E, Engel C, Nothnagel M, Weber J, Schmutzler RK, et al. Performance of in silico prediction tools for the classification of rare BRCA1/2 missense variants in clinical diagnostics. BMC Med Genet. 2018;11(1):35.
66. Hart SN, Hoskin T, Shimelis H, Moore RM, Feng B, Thomas A, et al. Comprehensive annotation of BRCA1 and BRCA2 missense variants by functionally validated sequence-based computational prediction models. Genet Med. 2019;21(1):71–80.
67. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. Genome Res. 2001;11(5):863–74.
68. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7(4):248–9.
69. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11(4):361–2.
70. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7 20.
71. Monteiro AN, Bouwman P, Kousholt AN, Eccles DM, Millot GA, Masson JY, et al. Variants of uncertain clinical significance in hereditary breast and ovarian cancer genes: best practices in functional analysis for clinical annotation. J Med Genet. 2020;
72. Hart SN, Polley EC, Shimelis H, Yadav S, Couch FJ. Prediction of the functional impact of missense variants in BRCA1 and BRCA2 with BRCA-ML. NPJ Breast Cancer. 2020;6:13.
73. Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, Nathanson KL, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med. 2015;372(23):2243–57.
74. Couch FJ, Shimelis H, Hu C, Hart SN, Polley EC, Na J, et al. Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol. 2017;3(9):1190–6.
75. Jones MR, Kamara D, Karlan BY, Pharoah PDP, Gayther SA. Genetic epidemiology of ovarian cancer and prospects for polygenic risk prediction. Gynecol Oncol. 2017;147(3):705–13.
76. 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. Gynecol Oncol. 2017;147(2):375–80.

Genetic Counseling in Hereditary Breast and Ovarian Cancer

5

Mayuko Inuzuka

Abstract

Those with mutations in *BRCA1* or *BRCA2* germlines are diagnosed with hereditary breast and ovarian cancer (HBOC). *BRCA1/BRCA2* genetic testing could facilitate the selection of a surgical method, early detection of related lesions, early treatment, or preventive intervention; thus, its clinical utility is high. In recent years, it has become even more important to identify patients with *BRCA1/BRCA2* mutations because of the potential of chemotherapy with platinum formulations and poly ADP ribose polymerase (PARP) inhibitors. In contrast, genetic information obtained through such a test could cause unexpected confusion and mental conflict; therefore, the role of genetic counseling before and after genetic testing is significant. In this chapter, we will discuss an outline of genetic counseling associated with HBOC and important points that providers of genetic counseling need to know.

Keywords

Genetic counseling · Hereditary breast and ovarian cancer · *BRCA* · Genetic testing · Risk communication · Psychosocial · Decision making

5.1 Introduction

Those with mutations in *BRCA1* or *BRCA2* germline are diagnosed with hereditary breast and ovarian cancer (HBOC). HBOC patients are known to have a higher risk

M. Inuzuka (✉)
Department of Genetic Counseling, Showa University Graduate School of Health Sciences, Tokyo, Japan
e-mail: inuzuka-m@cnt.showa-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_5

of breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer compared to the general population. *BRCA1/BRCA2* genetic testing could facilitate the selection of a surgical method, early detection of related lesions, early treatment, or preventive intervention; as a result, the clinical utility of this test is high. HBOC is of autosomal dominant inheritance, where a carrier of a genetic mutation passes the mutation to the next generation with a probability of 50%. *BRCA2* gene is known to cause Fanconi anemia, which is a genetic disease of autosomal recessive inheritance. Genetic information obtained from *BRCA1/BRCA2* genetic testing is crucial because it is associated with health management of not only patients themselves but also their relatives. However, a genetic testing also has a chance of causing unexpected confusion and mental conflict; thus, it is important for the patient to decide whether to have a genetic testing or not. After a genetic testing, the patient must make various decisions associated with the genetic testing, such as whether to have risk-reducing surgeries and share the result with their family. Medical staff must gather and evaluate appropriate information, offer information, and provide psychological support so that the patient can make the right decision.

The National Society of Genetic Counselors (NSGC) defines genetic counseling as follows: "Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease" [1]. Genetic counseling for HBOC identifies potential patients and their families, appropriately assesses the risk, and discusses about genetic testing, medical management, and psychological challenges. In addition, it is important to discuss ethical issues and legal/social issues. In any case, the latest information must be provided. Even for the same disease, each client's choice will vary based on their own values, social status, situation at the time, and family relationship; thus, the goal is to aim for the ideal choice for the client based on scientific evidences. For *BRCA1/BRCA2* genetic testing and medical management, the timing of choice including an option of not making a choice must take therapeutic policy of the cancer that the patient has, the patient's age, their financial burden, and their family planning into consideration, but there is no right answer that works for everyone. It should not be forced by healthcare providers, and clients' wishes must be respected. For continuous follow-up, not only their medical history and family history but also their psychological and social changes must be understood.

5.2 Subjects of Genetic Counseling and *BRCA1/BRCA2* Genetic Testing

Subjects of genetic counseling for HBOC likely have various backgrounds and reasons, such as worries over inheriting breast cancer or ovarian cancer for themselves or for their family, or interest in genetic testing. There is no limit in motivation for taking genetic counseling, and anyone interested in undergoing genetic counseling can become the client; however, from the perspective of a healthcare provider, a common point of discussion is which type of patients should be referred

to genetic counseling and *BRCA1/BRCA2* genetic testing. Because there is no simple screening for HBOC, there are various criteria and guidelines for genetic counseling and testing for subjects who are considered at risk of HBOC based on clinical information. According to the guideline jointly prepared by the American College of Medical Genetics and Genomics (ACMG) and NSGC and published in 2015 [2], criteria for referral of HBOC genetic counseling are summarized in Table 5.1. Some characteristics of medical and family histories of patients are closely associated with the possibility of hereditary tumors; therefore, various risk assessment models have been developed. With the National Institute for Health and Care Excellence (NICE) [3], in cases when the pre-test probability with Manchester Scoring System or BOADICEA is 10% or higher, subjects are indicated for genetic testing and counseling where pre-test probability is assumed. With the US Preventive Services Task Force (USPSTF) [4], women with personal or family history of breast cancer, ovarian cancer, fallopian tube cancer, and peritoneal cancer, or women with families with *BRCA1/BRCA2* mutation, should be assessed. Women with a positive result from an appropriate familial risk assessment tool, such as Ontario Family History Assessment Tool, Manchester Scoring System, Tyrer–Cuzick model, and BRCAPRO, should receive genetic counseling, as well as a genetic testing, if indicated after the counseling [4]. As such, many guidelines focus on identification of high-risk patients.

However, in recent years, limitations of basing on clinical criteria/family history have been noted [5]. With the potential efficacy of chemotherapy with platinum formulations [6] and poly ADP ribose polymerase inhibitors [7], identification of patients with *BRCA1/BRCA2* mutations has become even more important. A study pointed out the possibility that patients with *BRCA1/BRCA2* mutations may be overlooked if it is only based on clinical criteria and family history [8]; thus, how wide the range of subjects needs to be is a subject of discussion. The American

Table 5.1 The criteria for HBOC genetic consultation referral

Referral should be considered for:
• Any individual with a personal history of or first-degree relative with:
– Breast cancer diagnosed at or before age 50
– Triple-negative breast cancer diagnosed at or before age 60
– Two or more primary breast cancers in the same person
– Ovarian, fallopian tube, or primary peritoneal cancer
– Ashkenazi Jewish ancestry and breast or pancreatic cancer at any age
– Male breast cancer
• Individuals with a family history of three or more cases of breast, ovarian, pancreatic, and/or aggressive prostate cancer (Gleason score ≥7) should also be referred. Note that this should not include families in which all three cases are aggressive prostate cancer

Adapted from Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL, Guideline Development Group, American College of Medical Genetics and Genomics Professional Practice and Guidelines Committee and National Society of Genetic Counselors Practice Guidelines Committee. 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. Genet Med. 2015 Jan;17(1):70–87

Society of Breast Surgeons stated in the 2019 Consensus Guidelines on Genetic testing for Hereditary Breast Cancer [9] that for patients who are newly diagnosed with breast cancer, identification of genetic mutation could impact localized treatment. They recommend the following: "genetic testing should be made available to all patients with a personal history of breast cancer" [9]. Globally, genetic risk assessment criteria by the National Comprehensive Cancer Network (NCCN) Guidelines [10] are well known; however, these guidelines can be revised up to several times a year and the latest changes should thus be confirmed at all times. In recent years, it is recommended that genetic counseling and testing should also be considered if a *BRCA1/BRCA2* pathogenic/likely pathogenic variant is detected through tumor profiling [10]. The criteria for subjects of genetic counseling and testing for HBOC have changed over time due to factors such as relationship to treatment and cost-effectiveness. However, in any case, a report shows that, in a systematic review of international guidelines on screening and management of *BRCA1/BRCA2* mutation breast cancer patients, the 15 related guidelines that touched on recommendations for genetic counseling had a consensus with regard to the importance of genetic counseling before and after *BRCA1/BRCA2* genetic testing [11].

5.3 Contents in Genetic Counseling

The Practice Guideline published by the NSGC in 2013 describes the steps that must be included in cancer risk assessment and HBOC genetic counseling, as shown in Table 5.2 [12]. However, because contents of genetic counseling change based on the chief complaints and conditions of the client, all the contents may not necessarily be covered in one session of counseling. When a provider of genetic counseling prepares a case for genetic counseling, it is important to check the information to be provided, points to be emphasized, manner of providing information to promote understanding by patients, and available counseling aids that support this process [13]. Below, we will discuss several points that genetic counselors should be mindful of when providing genetic counseling.

Table 5.2 The process of cancer risk assessment and genetic counseling for HBOC

1. Gathering personal medical and family history data
2. Psychosocial assessment
3. Discussion of cancer and mutation risk and how personalized risk estimates are derived
4. Facilitation of the informed consent process through discussion of the risks, benefits, limitations, and likelihood of identifying a mutation with genetic susceptibility testing
5. Result disclosure (if applicable)
6. Discussion of medical management options
7. Review of issues related to genetic discrimination

Adapted from Berliner JL, Fay AM, Cummings SA, Burnett B, & Tillmanns T. NSGC practice guideline: risk assessment and genetic counseling for hereditary breast and ovarian cancer. J Genet Couns. 2013;22:155–163

5.3.1 Family History and Risk Assessment

When assessing the risk in genetic counseling, the patient's pedigree is evaluated, genetic etiology of the condition is determined, and the risks for the patient and other relevant family members are calculated [13]. Accounting for the patient's family history in genetic counseling is useful in making an accurate diagnosis and treatment. Taking accurate family history into account is, therefore, an important element in HBOC genetic counseling. It can help in accurately assessing the amount of risk for the client and can provide insight with regard to important decisions such as whether there is a need undergo a genetic testing or the identifying the specific type of test required by the client. When performing risk assessment assuming HBOC, the possibility of other hereditary tumors may arise. Assuming onset and prognosis for not only the affected patient but also their family members may allow for a diagnosis before onset and prevention of onset. Some beneficial byproducts can be expected from taking a family history including the following: a trust relationship can be built between providers of genetic counseling and clients, family relationship other than medical and treatment history can be understood, and those who need information and psychological support can be identified. However, it should be noted that medical and family history may not be accurate as clients' memory may be vague. According to a study, only 87% could accurately report the site of cancer in first-degree relatives, which decreased to 67% for second-degree relatives and 60% for third-degree relatives [14]. As such, when taking a family history of clients presenting with a hereditary tumor, the importance of family history should be explained to clients first to gain their understanding. In addition, it is important to note that family history may change with time. With new information, contents of risk assessment and genetic counseling of the patient will change; thus, information in changes to family history should be updated regularly.

When taking family history into consideration during genetic counseling for hereditary tumors, specialized questions on hereditary tumors are asked in addition to questions on general family history. In cases of hereditary tumors, useful information is as follows: within the patient's family, multiple members have related cancers, a member has cancer that developed earlier than the typical age of onset, and a member has multiple cancers. In HBOC genetic counseling, the counselor must pay attention to the timing and number of breast cancers; subtype of breast cancer; treatment details of breast cancer; surgical method for breast cancer; pathology result for ovarian cancer; treatment history for benign conditions; pathology result for prostate cancer; presence or absence of family or medical history of male breast cancer, peritoneal cancer, pancreatic cancer, or melanoma; racial background with high incidence of mutations (e.g., Ashkenazi Jews); and presence or absence of wish to become pregnant in the case of young people. Furthermore, providers of genetic counseling must consider characteristics of hereditary diseases where differentiation is necessary, such as mode of inheritance, penetrance, and mutation, during assessment. Moreover, history of radiation treatment, pregnancies and deliveries, hormone use, and breast biopsy needs to be included. To examine the possibility of founder mutation and autosomal recessive inheritance, consanguineous

marriages should be confirmed. Appropriate questions should be asked with attention to these issues, so that necessary information would not be missed.

5.3.2 First Person in the Family to Be Tested

In genetic counseling, it may be necessary to discuss who should have the test first in a family. When considering a genetic testing for a family with a suspected hereditary tumor, those with cancers related to the hereditary tumor become the first candidate for the screening test. When there are multiple patients who should be tested, those presenting with conditions in which there is a relatively higher probability of detecting mutations, such as younger age at the time of diagnosis, multiple primary cancers, and being more closely related to the proband, should be prioritized [10]. In cases where all family members with related cancers have died, or cancer patients do not wish to undergo genetic testing, subjects without any onset of cancer may receive a screening test. Generally, someone who is genetically closest to the person with related cancer would be chosen for such screening. When someone without an onset of cancer has a screening test, interpretation of the result has limitations. For example, if someone without an onset of cancer in a family suspected of HBOC has a negative result in a screening test, it cannot be determined if the person did not inherit the mutations in a HBOC family (true negative) or if the family did not have HBOC to begin with. Thus, this point needs to be adequately explained before a test.

5.3.3 Interpretation of *BRCA1/BRCA2* Genetic Testing Results

Providing information regarding interpretation of the outcomes of *BRCA1/BRCA2* genetic testing is extremely important for genetic counseling. The ACMG [15] assessed genetic variations and presented rules for the classification into five types: pathogenic, likely pathogenic, benign, likely benign, and uncertain significance. Basically, variants are interpreted according to this classification. Medical management proposed based on the test result and its limitations, including cases of variant of uncertain significance (VUS), must be discussed thoroughly before and after the test.

Pathogenic/likely pathogenic result is a basis for providing definitive diagnosis of HBOC and appropriate medical management, such as planned surveillance, chemoprevention, risk-reducing salpingo-oophorectomy, and risk-reducing mastectomy. It also allows for a survey to find out if any family member has the same pathogenic variant. When a pathogenic variant is confirmed, subsequent specific medical management and provision of information to family members must be discussed during genetic counseling sessions before and after the test. During the discussion, providers of genetic counseling must sufficiently understand the difficulty of interpreting penetrance and provide adequate explanations. At present, accurate determination of penetrance per family is not possible, and the accurate relationship between genotype and phenotype is also unclear [16]. Due to the limitations,

providers of genetic counseling should remember that it is important to present the range of cancer onset risks to patients and to explain that their risk probably falls somewhere within the range [16]. Benign/likely benign result indicates that there is no pathogenic variant or VUS in the *BRCA1/BRCA2* gene, the causal gene of HBOC. When conducting *BRCA1/BRCA2* genetic testing for a proband or for the first person in a family to have a test, it is recommended to inform the patient that the possibility of the cancer being hereditary cannot be ruled out and propose a surveillance plan that is individualized based on the patient's medical and family history [10, 17]. Patients undergoing genetic counseling should also be well explained and understand that there may be variants that may not be detected with the implemented method and that other tests may be added. VUS merely indicates that the significance of the detected variant is unclear. Because a possibility of pathogenic variant cannot be denied, careful surveillance is required. Thus, existence of VUS is a major limitation of genetic testing. Though the rate of VUS varies between ethnicities of patients, it is observed in approximately 7% of patients who undergo *BRCA1/BRCA2* genetic testing [18]. When diagnosed as a VUS carrier, the genetic information cannot be used to determine medical management strategies such as surveillance; thus, similar to before genetic testing, it is recommended that an individualized surveillance plan should be proposed based on medical and family history of each patient [10, 17]. Information of VUS is difficult to understand accurately without appropriate genetic counseling; thus, its possibility must be discussed before the test and even after the test. Adequate information must be provided while confirming the level of patient's understanding of the significance of VUS diagnosis.

5.3.4 Sharing Genetic Information with Relatives

With hereditary tumors, if a mutation is identified in the proband during genetic testing, relatives who may have the same variant may also need to undergo. This is primarily done to identify relatives at risk and use the information for their health management. Possibility of sharing genetic conditions with relatives, significance of sharing that with relatives, and explaining the benefits to relatives are important elements of genetic counseling. Inadequate understanding by subjects could prevent transmission of the information to relatives; thus, it is important to evaluate if subjects accurately understand the result and its significance and are capable of conveying it accurately to relatives. In addition, disclosure of information to others including relatives requires the consent of the subject in principle. Information obtained from genetic testing may be useful for onset prevention and treatment for the subject and their relatives; however, if the subject does not consent to disclosure of the information to relatives, it could create various ethical dilemmas. The importance of encouraging subjects to share the genetic information with relatives as their moral duty is emphasized [19]. In genetic counseling before and after the test, the counselor discusses the meaning of genetic information for the client and their family, anxieties and troubles, concerns for the family, and future directions, while respecting the wishes of the client. The process of carefully supporting clients'

emotional changes is important. Underage patients with HBOC hardly ever have an onset of HBOC-related cancer before adulthood; thus, it is desirable that they undergo genetic testing once they are of age where they can make their own decisions and have a test by their own free will. ACMG states the following: "if the medical or psychosocial benefits of a genetic test will not accrue until adulthood, as in the case of carrier status or adult-onset diseases, genetic testing generally should be deferred" [20].

5.3.5 Risk of Discrimination

In genetic counseling, it is essential to discuss the risk of discrimination in employment and insurance because of the genetic information. In 2003, the United Nations Educational, Scientific and Cultural Organization, in its International Declaration on Human Genetic Data, mentions that human genetic data have a special status for the following four reasons: "(1) they can be predictive of genetic predispositions concerning individuals; (2) they may have a significant impact on the family, including offspring, extending over generations, and in some instances, on the whole group to which the person concerned belongs; (3) they may contain information the significance of which is not necessarily known at the time of the collection of the biological samples; (4) they may have cultural significance for persons or groups" [21]. In the USA, to broadly protect genetic information of individuals, discrimination in employment and health insurance based on genetic information has been prohibited through the Genetic Information Nondiscrimination Act (GINA, 2008) [22]. Such laws vary between countries and the risk of discrimination in life insurance and accident insurance exists; thus, patients must be informed of such risks before genetic testing.

5.3.6 Presenting an Option of Not Undergoing Genetic Testing

In genetic counseling before the test, it is important to inform the patients that their free will is respected, there is an option of not undergoing a genetic testing, and they can take a genetic testing at any time if they change their minds. In a report, reasons for which individuals did not receive HBOC genetic counseling and/or *BRCA1/BRCA2* genetic testing included having no children of their own and fear of knowing the test result [23]. Clients who initially decided against *BRCA1/BRCA2* genetic testing may change their minds several years later when situations change, such as onset of cancer that occurred to themselves or a family member, or through life events such as new job, marriage, and childbirth. Clients who are strongly suspected of HBOC even without genetic testing should have a long-term surveillance that suits HBOC. The NCCN Guidelines [10] state that if mutation had already confirmed for the family of a subject who does not wish for a genetic testing, surveillance for HBOC should be proposed and adequate follow-up should be continued. If mutation had not been confirmed in the family, depending on the subject and

family history, along with individually appropriate surveillance, continuous survey of the family should be proposed [10].

5.3.7 Psychosocial Assessment

Eijzenga W et al. [24] surveyed specific psychosocial issues experienced by subjects who had genetic counseling for cancer and aimed to determine a unifying theme underlying these issues. In this review, they presented the following specific issues within six themes: "(a) coping with cancer risk, (b) practical problems, (c) family-related problems, (d) children-related problems, (e) living with cancer, and (f) emotions." In genetic counseling, clients provide personal information in various ways, and a provider of genetic counseling carefully reads into clients' attitudes and comments. In this manner, the counselor assesses the relationship with the family; psychological impact such as anxiety, stress, and guilt; and whether clients are in any condition to make decisions calmly. Psychosocial assessments should specifically include the following: "(1) anticipated reaction to results and coping strategies, (2) timing and readiness for testing, (3) family dynamics and relationships, and (4) preparing for result disclosure" [25]. Considering that the client's state of mind may change over time owing to changes in life stages and life events, continuous psychological evaluation should be performed while paying attention to at-risk family members. Support should also be provided in a way that does not impose an excessive psychological burden. Peer support groups and their activities also play a part in psychosocial support.

5.3.8 Reproductive Issues

There are various views on whether consider HBOC with adult onset and appropriate methods of response as a disease that requires prenatal diagnosis or preimplantation genetic diagnosis; however, in an American survey on women with *BRCA1/BRCA2* mutation, 41% experienced an impact on the decision about childbearing [26]. In genetic counseling, the counselor must understand such anxieties of clients and explain risks and limitations of options. In addition, when *BRCA2* mutation carriers decide to bear a child, the risk of Fanconi anemia must be sufficiently discussed. It is important to assess if the spouse also has *BRCA2* mutations.

5.4 Risk Communication

Risk communication in genetic counseling refers to the discussion between the provider of genetic counseling and patients/family regarding hereditary diseases [27]. When presenting risks, three concrete and basic methods are the presentation of numerical format, the explanation with verbal terms, and graphical presentations with figures and tables [28]. When verbal terms (e.g., "high risk") are used to

express risks, messages could be vague as interpretation of verbal terms varies notably between people [29]. By graphically or visually presenting risk information, risk communication can be improved especially for people with poor literacy and numeracy skills [30]. Lautenbach DM et al. [31] listed the following as evidence-based strategies to present various types of risk information: "(a) presenting risk information in multiple formats while avoiding qualitative modifiers, (b) being conscious of framing biases and framing risk in multiple ways, (c) using carefully chosen graphics, (d) using a small denominator (from 50 to 100) when possible, (e) remembering that less is more, (f) paying attention to emotions that may influence perception and adoption of risk figures, and (g) engaging recipients in communication." Providers of genetic counseling must understand the effects and limitations of each method and pay careful attention to how risks are conveyed.

For appropriate risk communication within genetic counseling, clients' recognition of risks related to genetics must be understood. Each client recognizes the same risk information differently. There is a close relationship between risk recognition and decision making, but clients' choices of genetic testing and treatment that reduces risk are influenced more by subjective risk recognition and emotions than by the actual degree of risk [32]. Factors that influence clients' risk recognition are personality factors, such as age, family history with cancer, previous prophylactic tests and treatments, cognitive/emotional traits, and numerical information processing skills [31, 33]. Women at high risk of HBOC may experience emotional stress such as experiences of cancer in the family, multiple experiences of previous bereavement, and the fear of their own disease onset [34]. For these reasons, cancer-related suffering and individual characteristics could also prevent adequate understanding of individualized genetic risk information [34]. In genetic counseling, it is quite important to understand that there is individual risk perception of each client at the same time as objective risks. It is important to confirm the ideas of clients by first confirming their understanding of genetics before genetic counseling and interviewing about their experiences with the disease and personal significance of the disease.

5.5 Future Outlook for Genetic Counseling

With the arrival of multigene panel testing, genetic counseling has notably changed. In 2013, the US Supreme Court determined that "a naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated" [35]. Hooker GW et al. [36] reported that after the court's decision against Myriad and introduction of multigene panel testing, genetic counselors felt that they experienced a notable change in the contents of pre-test counseling. Genetic counselors not only incorporated discussion on possible use of multigene panel testing in a session but also had less time to talk about implications of each gene/syndrome and had more time to discuss a range of information obtained from genetic testing, such as management and unknown penetrance of "newer" genes on the panel and increased possibility of VUS [36]. There is a report of performing a multigene panel

testing on cases without detection of mutation in *BRCA1/BRCA2* genetic testing, and the result showed that a pathogenic variant was detected in 11% [37]. For women with a 10% or higher probability of *BRCA1/BRCA2*, it is more cost-effective to perform a multigene panel testing instead of performing *BRCA1/BRCA2* genetic testing alone [38]. In a case where hereditary tumor syndrome other than HBOC, including hereditary breast cancer and ovarian cancer, is suspected based on medical and family history, multigene panel testing that simultaneously analyzes a series of genes related to a single or multiple phenotypes increases the effectiveness. However, there is also a report that VUS was detected in 30–40% of cases where multigene panel testing was performed [39]. In addition, if there is a change in moderate risk genes, data on penetration has limitations, and there are cases where there is no clear guideline for medical management. Therefore, when performing multigene panel testing, the NCCN Guidelines [10] recommend that the multigene testing should be performed within the context of professional expertise and pre- and post-counseling. As such, multigene panel testing is expected to fulfill a certain role, but its advantages and issues should be explained during genetic counseling, and the patients should be treated with utmost caution.

In recent years, identification of genetic mutation in high- and moderate-penetrance breast and/or ovarian cancer susceptibility genes other than *BRCA1/BRCA2* has also been considered to have a potentially important role in cancer treatment [40]. As the options available to patients in genetic testing increase, it can be considered that the importance of genetic counseling will continue to increase. Furthermore, it will be increasingly important for providers of genetic counseling to be knowledgeable about cancer treatment. However, in any case, building mutual trust with patients is a fundamental of genetic counseling. Each client has different beliefs, levels of sensitivity, prior knowledge, understanding, degree of anxiety, and trust in medicine; thus, it is important to understand that no single type of counseling fits all even in counseling patients with the same disease.

References

1. National Society of Genetic Counselors' Definition Task Force, Resta R, Biesecker BB, Bennett RL, Blum S, Hahn SE, et al. A new definition of genetic counseling: National Society of Genetic Counselors' Task Force report. J Genet Couns. 2006;15:77–83.
2. Hampel H, Bennett RL, Buchanan A, Pearlman R, Wiesner GL, Guideline Development Group, American College of Medical Genetics and Genomics Professional Practice and Guidelines Committee and National Society of Genetic Counselors Practice Guidelines Committee. 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. Genet Med. 2015;17:70–87.
3. National Institute for Health and Care Excellence. Clinical guidelines. Familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. https://www.nice.org.uk/guidance/CG164. Accessed 13 Oct 2020.
4. US Preventive Services Task Force. Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer US preventive services task force recommendation statement. JAMA. 2019;322:652–65.

5. González-Santiago S, Ramón y Cajal T, Aguirre E, Alés-Martínez JE, Andrés R, Balmaña J, et al. SEOM clinical guidelines in hereditary breast and ovarian cancer (2019). Clin Transl Oncol. 2020;22:193–200.
6. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT trial. Nat Med. 2018;24:628–37.
7. Keung MYT, Wu Y, Vadgama JV. PARP inhibitors as a therapeutic agent for homologous recombination deficiency in breast cancers. J Clin Med. 2019;8:435.
8. 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? J Clin Oncol. 2019;37:453–60.
9. Manahan ER, Kuerer HM, Sebastian M, Hughes KS, Boughey JC, Euhus DM, et al. Consensus guidelines on genetic' testing for hereditary breast cancer from the American Society of Breast Surgeons. Ann Surg Oncol. 2019;26:3025–31.
10. The National Comprehensive Cancer Network. Genetic/familial high-risk assessment: breast and ovarian. Ver1. 2021. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf. Accessed 13 Oct 2020.
11. Forbes C, Fayter D, Kock S, Quek RG. A systematic review of international guidelines and recommendations for the genetic screening, diagnosis, genetic counseling, and treatment of BRCA-mutated breast cancer. Cancer Manag Res. 2019;11:2321–37.
12. Berliner JL, Fay AM, Cummings SA, Burnett B, Tillmanns T. NSGC practice guideline: risk assessment and genetic counseling for hereditary breast and ovarian cancer. J Genet Couns. 2013;22:155–63.
13. Uhlmann WR. In: Uhlmann WR, Schuette JL, Yashar B, editors. A guide to genetic counseling. 2nd ed. Hoboken, NJ: Wiley-Blackwell; 2009. p. 93–131.
14. Love RR, Evans AM, Josten DM. The accuracy of patient reports of a family history of cancer. J Chronic Dis. 1985;38:289–93.
15. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
16. Matloff ET, Bonadies DC. In: VT DV, Lawrence TS, Rosenberg SA, editors. Cancer: principles & practice of oncology: primer of the molecular biology of cancer. Philadelphia, PA: Lippincott Williams & Wilkins; 2015. p. 405–15.
17. Euhus D. Genetic testing today. Ann Surg Oncol. 2014;21:3209–15.
18. Ready K, Gutierrez-Barrera AM, Amos C, Meric-Bernstam F, Lu K, Hortobagyi G, Arun B. Cancer risk management decisions of women with BRCA1 or BRCA2 variants of uncertain significance. Breast J. 2011;17:210–2.
19. Nycum G, Knoppers BM, Avard D. Intra-familial obligations to communicate genetic risk information: what foundations? What forms? McGill J Law Health. 2009;3:21–48.
20. American Society of Human Genetics Board of Directors and American College of Medical Genetics Board of Directors. Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. Am J Hum Genet. 1995;57:1233–41.
21. United Nations Educational, Scientific and Cultural Organization. International declaration on human genetic data. http://portal.unesco.org/en/ev.php-URL_ID=17720&URL_DO=DO_TOPIC&URL_SECTION=201.html. Accessed 13 Oct 2020.
22. The Genetic Information Nondiscrimination Act of 2008 (H.R.493). https://www.congress.gov/bill/110th-congress/house-bill/493. Accessed 13 Oct 2020.
23. Schlich-Bakker KJ, Kroode HFJ, Wárlám-Rodenhuis CC, Bout J, Ausems MGEM. Barriers to participating in genetic counseling and BRCA testing during primary treatment for breast cancer. Genet Med. 2007;9:766–77.
24. Eijzenga W, Hahn DEE, Aaronson NK, Kluijt I, Bleiker EMA. Specific psychosocial issues of individuals undergoing genetic counseling for cancer - a literature review. J Genet Couns. 2014;23:133–46.

25. Lewis KM. Identifying hereditary cancer: genetic counseling and cancer risk assessment. Curr Probl Cancer. 2014;38:216–25.
26. Chan JL, Johnson LN, Sammel MD, DiGiovanni L, Voong C, Domchek SM, Gracia CR. Reproductive decision-making in women with BRCA1/2 mutations. J Genet Couns. 2017;26:594–603.
27. Baty BJ. In: Uhlmann WR, Schuette JL, Yashar B, editors. A guide to genetic counseling, second edition. Hoboken, NJ: Wiley-Blackwell; 2009. p. 207–50.
28. Henneman L, Marteau TM, Timmermans DRM. Clinical geneticists' and genetic counselors' views on the communication of genetic risks: a qualitative study. Patient Educ Couns. 2008;73:42–9.
29. Timmermans D. The roles of experience and domain of expertise in using numerical and verbal probability terms in medical decisions. Med Decis Mak. 1994;14:146–56.
30. Lipkus IM, Samsa G, Rimer BK. General performance on a numeracy scale among highly educated samples. Med Decis Mak. 2001;21:37–44.
31. Lautenbach DM, Christensen KD, Sparks JA, Green RC. Communicating genetic risk information for common disorders in the era of genomic medicine. Annu Rev Genomics Hum Genet. 2013;14:491–513.
32. Lerman C, Lustbader E, Rimer B, Daly M, Miller S, Sands C, Balshem A. Effects of individualized breast cancer risk counseling: a randomized trial. J Natl Cancer Inst. 1995;87:286–92.
33. Tilburt JC, James KM, Sinicrope PS, Eton DT, Costello BA, Carey J, et al. Factors influencing Cancer risk perception in high risk populations: a systematic review. Hered Cancer Clin Pract. 2011;9:2.
34. Foster C, Evans DGR, Eeles R, Eccles D, Ashley S, Brooks L, et al. Predictive testing for BRCA1/2: attributes, risk perception and management in a multi-centre clinical cohort. Br J Cancer. 2002;86:1209–16.
35. Association for Molecular Pathology v. Myriad Genetics. 133 U.S. Supreme Court 2107 C.F.R.; 2013.
36. Hooker GW, Clemens KR, Quillin J, Postula KJV, Summerour P, Nagy R, et al. Cancer genetic counseling and testing in an era of rapid change. J Genet Couns. 2017;26:1244–53.
37. Kurian AW, Hare EE, Mills MA, Kingham KE, McPherson L, Whittemore AS, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol. 2014;32:2001–9.
38. Manchanda R, Patel S, Gordeev VS, Antoniou AC, Smith S, Lee A, et al. Cost-effectiveness of population-based BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, PALB2 mutation testing in unselected general population women. J Natl Cancer Inst. 2018;110:714–25.
39. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015;121:25–33.
40. Lima ZS, Ghadamzadeh M, Arashloo FT, Amjad G, Ebadi MR, Younesi L. Recent advances of therapeutic targets based on the molecular signature in breast cancer: genetic mutations and implications for current treatment paradigms. J Hematol Oncol. 2019;12:38.

Hereditary Breast Cancer

6

Minoru Miyashita and Takanori Ishida

Abstracts

In hereditary breast cancer, the different strategies from sporadic cancer might be required due to its vulnerable feature. We reviewed the published data of breast cancers with germline BRCA1/BRCA2, TP53, PTEN, CDH1, PALB2, CHEK2, ATM, and STK11 focusing on the treatment. The standard of locoregional treatment including surgery and radiation therapy (RT) should be considered in hereditary breast cancer except for TP53-related breast cancer as in sporadic breast cancer. Mastectomy is recommended without RT for germline TP53 mutation carriers. Because there is a lack of reliable data about treatment of hereditary breast cancer, the discussion about the risk of both recurrence and new breast cancer is encouraged. Chemotherapy including platinum is recommended for metastatic breast cancer with BRCA1/BRCA2 mutation. However, there is no data supporting the use of platinum in (neo)adjuvant settings for early breast cancer with BRCA1/BRCA2 mutation. More researches about treatment for hereditary breast cancer are considered indispensable.

Keywords

Hereditary breast cancer · BRCA1/BRCA2 · TP53 · PTEN · CDH1 · PALB2 · CHEK2 · ATM · STK11

M. Miyashita (✉) · T. Ishida
Departments of Breast and Endocrine Surgical Oncology, Tohoku University Graduate School of Medicine, Sendai, Japan
e-mail: atihsayim8m8@med.tohoku.ac.jp; takanori@med.tohoku.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_6

6.1 Introduction

Breast cancer is one of the most important health problems for women throughout the world, reporting woman's lifetime risk of developing breast cancer at 1 in 8. About 5% to 10% of breast cancer cases are thought to be hereditary so that the multidisciplinary approach is demanded. Because of the vulnerable feature of hereditary breast cancer, the different strategies from sporadic cancer might be required. Here, we reviewed the published data of breast cancers with germline BRCA1/BRCA2, TP53, PTEN, CDH1, PALB2, CHEK2, ATM, and STK11 focusing on the treatment.

6.2 BRCA1-/BRCA2-Related Breast Cancer

The rate of germline BRCA1/BRCA2 mutations in all patients with breast cancer is around 4% [1, 2], and the incidences are particularly higher in patients with triple-negative breast cancer and Jewish women with breast cancer which are around 15% in both populations [3–5]. The prognostic risk is reported to vary based on the objective population [6, 7]. According to the study which included Japanese female breast cancer having strong family history of breast cancer based on the NCCN guidelines, the rates of germline BRCA1 and BRCA2 mutations among 260 breast cancer are 17.7% and 13.5%, respectively [8] (Fig. 6.1).

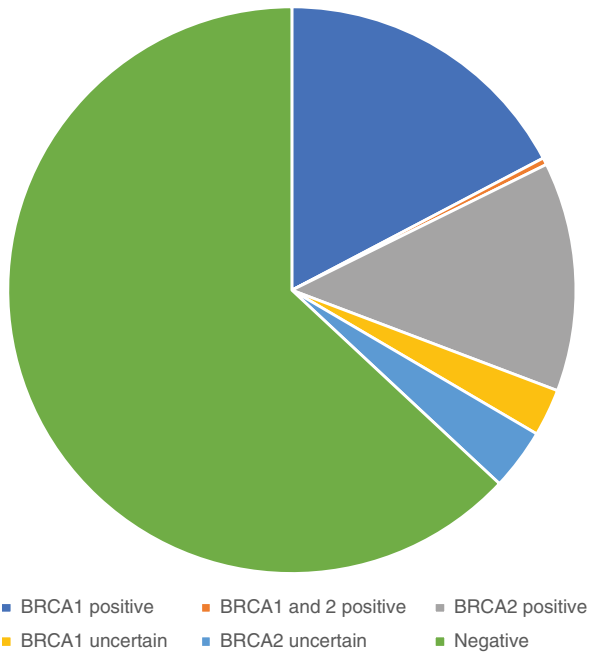


Fig. 6.1 Result of BRCA1/BRCA2 genetic testing of 260 Japanese female breast cancer having strong family history of breast cancer based on the NCCN guidelines. Modified from [8]

Treatment decisions for BRCA1-/BRCA2-related breast cancer might be influenced by the genetic instability. Here, we comprehensively reviewed the treatments and prognosis of breast cancer with BRCA1/BRCA2 mutation carriers. We refrained from describing about therapeutic endocrine therapy because of the lack of evidence.

6.3 What Is the Appropriate Surgical Management for BRCA1-/BRCA2-Related Breast Cancer?

6.3.1 Breast Conserving Surgery or Mastectomy

One of the clinical questions we need to address is whether or not breast conserving surgery (BCS) is safe for BRCA1/BRCA2 mutation carriers as a part of treatment because of the higher incidence of breast cancer. Van den Broek AJ, et al. evaluated the effects comparing among BCS by radiotherapy, mastectomy without radiotherapy, and mastectomy followed by radiotherapy in terms of overall and breast cancer-specific survival as well as local recurrence rates and ipsilateral new primary breast cancer [9]. After adjusting the confounders affecting the treatment choice, both BRCA1 mutation carriers ($N = 191$) and non-carriers ($N = 5820$) had a similar overall survival regardless of the type pf local treatment, BCS or mastectomy. In their study, numbers for BRCA2 mutation carriers ($N = 70$) were insufficient to reach conclusions. Interestingly, the 10-year risk of local recurrence rates after BCS did not differ between BRCA1 mutation carriers and non-carriers (7.3% and 7.9%, respectively). In contrast, Nilsson MP, et al. reported the increment of local recurrence rates in BRCA1/BRCA2 mutation carriers receiving BCS. They investigated local recurrence and overall survival of BRCA1/BRCA2 mutation carriers in the comparison between BCS ($N = 45$) and mastectomy ($N = 118$) [10]. The cumulative local recurrence risk in 5, 10, and 15 years was 15%, 25%, and 32% in patients with BCS although it was 9% throughout 15 years in patients with mastectomy. No significant difference of distant recurrence or overall survival between the groups was observed. As the largest study, Pierce LJ, et al. examined long-term outcome of 655 breast cancer patients with BRCA1/BRCA2 mutation. Cumulative local recurrence rate in 15 years was significantly higher in patients who underwent BCS compared to mastectomy, 23.5 vs. 5.5%, respectively [11]. There were no differences in both distant recurrences and overall survival between two groups.

Throughout the literature review of this clinical question, BCS is considered to be feasible in breast cancer with BRCA1/BRCA2 mutation carriers based on the survival data. However, discussion about the increasing risk of local recurrence is mandatory between patients and physicians. No randomized control trial exists so that we have to make a clinical decision following the observational studies having the selection bias.

6.3.2 Nipple-Sparing Mastectomy

Nowadays, nipple-sparing mastectomy (NSM) is widely performed with breast reconstruction. A few studies examined the option of NSM for breast cancer patients with BRCA1/BRCA2 mutation carriers. Yao K, et al. retrospectively analyzed the clinical data of BRCA1/BRCA2 mutation carriers ($N = 51$) who underwent NSM for newly diagnosed breast cancer [12]. Three patients experienced the cancer events including one patient with local and distant recurrence and two patients with axillary recurrences. There was no patient with BRCA1/BRCA2 mutation who had a recurrence at the nipple-areolar complex.

Manning AT, et al. identified 26 breast cancer patients with BRCA1/BRCA2 mutation who underwent NSM, while analyzing details of patient demographics, surgical procedures, complications, and relevant disease stage and follow-up [13]. There was no event of local recurrence and two cancer-related deaths were observed; one patient had distant metastases after NSM and another patient had ovarian cancer after NSM for DCIS.

In the field of therapeutic NSM in BRCA1/BRCA2 mutation carrier, limited reports are available to the best of our knowledge. Although the mentioned studies suggested the acceptable rates of local recurrence after NSM, the median follow-up period was not enough: 32.6 months in the study of Yao K, et al. and 28 months in the study of Manning AT, et al. The safety of therapeutic NSM in BRCA1/BRCA2 mutation carrier remains unclear due to the unavailability of the reliable data with larger sample size and longer follow-up time. Shared decision making with clinicopathological factors and patients' preference should be thoroughly done for the optional surgical procedure.

6.3.3 Contralateral Risk-Reducing Mastectomy

High risk of contralateral event is well known among breast cancer patients with BRCA1/BRCA2 mutation. For these women, contralateral risk-reducing mastectomy (CRRM) decreases the newly diagnosed contralateral breast cancer, whereas whether CRRM improves overall survival needs to be clarified. Heemskerk-Gerritsen BAM, et al. evaluated the role of CRRM on survival in BRCA1/BRCA2 mutation carriers with a history of primary breast cancer [14]. Out of patients receiving CRRM ($N = 242$), 4 patients developed contralateral breast cancer (2%) with the median follow-up period of 11.4 years after primary breast cancer, which was fewer than 64 patients (19%) out of the surveillance group ($N = 341$). The mortality was also lower in the CRRM group than in the surveillance group (9.6 and 21.6 per 1000 person-years of observation, respectively).

Metcafe K, et al. studied 390 BRCA1/BRCA2 mutations carriers with stage I or II breast cancer including 181 patients who had CRRM [15]. In the median follow-up time of 14.3 years, 18 women died in the CRRM group and 61 in the unilateral mastectomy group.

The survival rates at 20 years were 88% and 66% in the CRRM and the unilateral mastectomy group, respectively. In a multivariable analysis, CRRM was significantly associated with a 48% reduction in breast cancer death. Soenderstrup IM, et al. analyzed 237 breast cancer patients with BRCA1/BRCA2 mutation according to the types of surgery, treatments, and characteristics [16]. The results showed that CRRM was associated with reduced risk of death, but not with disease-free survival. Evans DG, et al. investigated the impact of CRRM on survival in unilateral breast cancer with BRCA1/BRCA2 mutations [17]. In a matched case–control analysis designed to control for potential confounding factors (BPO, stage, and tumor characteristics), overall survival in the 105 CRRM cases was significantly higher, which was 89% versus 73% in 105 controls who did not have CRRM.

Contrary, Van Sprundel TC et al., reported the opposite result that CRRM for 79 breast cancer patients with BRCA1/BRCA2 mutation reduced the risk of contralateral breast cancer by 91%. At 5-year follow-up, overall survival was 94% for the CRRM group against with 77% for the surveillance group. After adjustment for bilateral prophylactic oophorectomy (BPO) in a multivariate analysis, however, CRRM was not significantly prognostic for overall survival.

Overall, CRRM clearly decreases the incidence of contralateral breast cancer in BRCA1/BRCA2 mutation carriers, whereas the benefit of CRRM for survival differs among the studies and the analytic methods. There is insufficient evidence we can utilize whether CRRM improves survival so that various factors around patients should be taken into consideration to decide the indication of CRRM for BRCA1/BRCA2 mutation carriers having a history of unilateral breast cancer.

6.4 Can RT Be Recommended for BRCA1-/BRCA2-Related Breast Cancer?

6.4.1 Breast Radiation After BCS

To plan a series of treatment for women with BRCA1-/BRCA2-related breast cancer, revealing the benefit and the risk of RT is necessary. The meta-analysis including ten studies which investigated the safety of RT after BCS in breast cancer patients with BRCA1/BRCA2 mutation was conducted by Valachis A, et al. [18]. The results suggested no significant difference between carriers and controls in terms of ipsilateral breast recurrence, which was 17.3% in BRCA1/BRCA2 mutation carriers and 11.0% in non-carriers (RR 1.45, 95% CI 0.98–2.14). Additionally, use of adjuvant chemotherapy and oophorectomy decreased the incidence of ipsilateral breast recurrence for BRCA mutation carriers. However, a significant higher risk for IBR in BRCA mutation carriers was observed when only studies with a median follow-up of 7 years were analyzed (RR 1.51, 95% CI 1.15–1.98). Therefore, further follow-up time is required. RT after BCS can be considered as a reasonable option and should not be withheld only due to mutation status based on the currently available evidence.

6.4.2 Postmastectomy Radiation Therapy

Limited studies reported the data about the efficacy of postmastectomy radiation therapy (PMRT) in BRCA1/BRCA2 mutation carriers. Pierce LJ, et al. compared the local recurrence rates of patients with mastectomy and PMRT ($N = 103$) with that of patients with mastectomy only ($N = 250$) among BRCA1/BRCA2 mutation carriers [11]. Despite higher stage among with PMRT, the local recurrence rates were similar between two groups. Median time to local failure was 9.4 years for patients with mastectomy. Drooger JC, et al. performed multivariate analysis of the subgroups under 40 ages as a part of entire cohort of BRCA1/BRCA2 mutation carriers [19]. The risk of contralateral breast cancer did not differ among groups, RT after BCS, RT after mastectomy, and mastectomy alone. In this study, ipsilateral local recurrence after mastectomy was not evaluated. The decision regarding PMRT should not be based predominantly on BRCA1/BRCA2 mutation status.

6.4.3 RT-Related Toxicity

By the time we searched, three studies were reported about their investigation about RT-related toxicity in breast cancer patients with BRCA1/BRCA2 mutations. Pierce LJ, et al. reported no differences about the incidence rates of chronic skin, subcutaneous tissue, lung, or bone complications between BRCA1/BRCA2 mutation carriers ($N = 71$) and sporadic cohorts ($N = 213$) [20]. Park H, et al. also reported no increased risk in acute skin toxicity in BRCA1/BRCA2 mutation carriers ($N = 46$) receiving BCS and RT compared with women with sporadic breast cancer [21]. Shanley S, et al. reported the similar finding about acute and late radiation effects between BRCA1/BRCA2 mutation carriers ($N = 55$) and sporadic breast cancer ($N = 55$) in a matched case–control study of patients treated with RT [22]. Although further studies are required to identify genetic effects to normal tissue responses after RT, there is no evidence of a significant increase of RT-related toxicity among breast cancer patients with BRCA1/BRCA2 mutation.

6.5 What Is the Role of Chemotherapy (Platinum) for BRCA1-/BRCA2-Related Breast Cancer?

6.5.1 Early Breast Cancer

Although several studies investigated the efficacy of platinum for early breast cancer (EBC) with BRCA1/BRCA2 mutation in (neo)adjuvant settings, there are only two randomized controlled trials. The exploratory analysis of 50 BRCA1/BRCA2 mutation carriers from GeparSixto trial was reported by Hahnen E, et al. [23]. The pathological complete response (pCR) rate was 66.7% (16 of 24) for BRCA1/

BRCA2 mutation carriers and 36.4% (44 of 121) for non-carrier patients (OR, 3.50; 95% CI, 1.39–8.84; $P = 0.008$) without carboplatin. However, the addition of carboplatin to the neoadjuvant chemotherapy regimen did not increase the pCR rate of BRCA1/BRCA2 mutation carriers (17 of 26 [65.4%]). Disease-free survival of patients with BRCA1/BRCA2 mutation carriers did not differ between the treatment regimens with and without carboplatin. Loibl S, et al. performed the subgroup analysis of 92 BRCA1/BRCA2 mutation carriers from BrighTNess trial [24]. Overall, the pCR rate was 51% (47 of 92 patients) with BRCA1/BRCA2 mutation carriers and similar with that of non-carrier patients, 48% (262 of 542). The pCR rates of each regimen in BRCA1/BRCA2 mutation carriers were 57% (26 of 46), 50% (12 of 24), and 41% (9 of 22) in paclitaxel + carboplatin + veliparib group, paclitaxel + carboplatin group, and paclitaxel group, respectively. Although adding carboplatin increased the pCR rate to some degree, the stratified analysis showed that additive benefit of carboplatin was observed for non-carrier patients rather than BRCA1/BRCA2 mutation carriers.

The meta-analysis including non-randomized controlled trial indicated that 93 of 159 (58.4%) patients with BRCA1/BRCA2 mutation achieved pCR, while 410 of 808 (50.7%) with non-carrier patients by the platinum-containing regimens [25]. The result did not show statistical significance (OR 1.459 CI 95% [0.953–2.34] $P = 0.082$). As shown, platinum to current standard regimens of anthracycline and taxane is not recommended as the routine addition for breast cancer patients with germline BRCA mutation.

6.5.2 Metastatic Breast Cancer

Two prospective studies addressed the efficacy of platinum in metastatic breast cancer patients who have BRCA1/BRCA2 mutation. Tutt A, et al. reported the result of TNT trial which evaluated the efficacy of two single-agent chemotherapies, carboplatin or docetaxel, in metastatic TNBC [26]. In the preplanned subject with 43 germline BRCA1/BRCA2 mutated patients from entire cohort, carboplatin had more than double the objective response rate of docetaxel (68% vs. 33%, respectively). Progression-free survival of patients with BRCA1/BRCA2 mutation was longer than that of non-carrier patients (6.8 months vs. 4.4 months, $P = 0.002$). Zhang J, et al. reported the result of CBCSG006 trial which included 14 BRCA1/BRCA2 mutation carriers [27]. Patients with germline BRCA1/BRCA2 mutation had suggestively higher objective response rate by cisplatin-containing regimen (83.3% in cisplatin plus gemcitabine group vs. 37.5% in paclitaxel plus gemcitabine group, $P = 0.086$). Cisplatin plus gemcitabine regimen also prolonged progression-free survival compared to paclitaxel plus gemcitabine regimen (8.9 months vs. 3.2 months, $P = 0.459$). Although there is no randomized controlled trial focusing on only BRCA mutation carriers, platinum could be an optional regimen for metastatic breast cancer patients with BRCA1/BRCA2 mutation.

6.6 BRCA1-/BRCA2-Related Breast Cancer Has Worse Prognosis?

According to the reports from retrospective studies which investigated the prognosis of breast cancer patients with BRCA1/BRCA2 mutation, there are conflicting results of the contribution by the germline mutation. However, Templetion AJ, et al. reported that BRCA mutation of 1325 patients was not associated with worse prognosis by the systematic review which consists of 16 studies comprising 10,180 patients [28].

Two large-scale prospective studies found no clear evidence that germline BRCA1/BRCA2 mutations significantly affect overall survival. Goodwin PJ, et al. conducted an international population-based cohort study of 3220 women with incident breast cancer observed prospectively, which included 93 BRCA1 mutations and 71 BRCA2 mutations: 1, both mutations [29]. With mean follow-up of 7.9 years, distant disease recurrence survival and overall survival did not differ between BRCA1 mutation carriers and non-carriers. Although distant disease recurrence survival and overall survival was worse in BRCA2 mutation carriers compared with non-carriers in univariable analysis, no difference was observed in both endpoints after adjustment for age, tumor stage and grade, nodal status, hormone receptors, and year of diagnosis. Copson ER, et al. performed a prospective cohort study of 2733 breast cancer patients aged 40 years or younger at histological diagnosis of invasive breast cancer [30]. Survival of 338 breast cancer patients with BRCA mutation (201 with BRCA1, 137 with BRCA2) was compared to that of sporadic breast cancer patients within a median follow-up of 8·2 years. The results showed no significant difference in overall survival between BRCA mutation carriers and non-carrier patients in multivariable analysis at any follow-up timepoint. Conversely, triple-negative breast cancer with BRCA mutation had better overall survival than non-carriers at 2 years. However, this better outcome was not observed at 5 and 10 years. Following the high-evidence studies, there was no data showing the worse prognosis of breast cancer with BRCA1/BRCA2 mutation.

6.7 TP53-Related Breast Cancer

TP53 gene is one of the most common tumor suppressors among cancers, providing its function to suppress tumor growth through making a protein p53. Li–Fraumeni syndrome (LFS) related to germline alterations of TP53 causes the early-onset of cancers among adolescent and young adult, especially soft-tissue sarcomas, breast cancers, central nervous system tumors, and so on. Currently, breast cancer with germline TP53 variants is more identified due to the more availability of multigene tests. At the review about germline TP53 variants in breast cancer patients outside the strict clinical criteria for LFS testing, the incidence rate of TP53 carriers was from 0% to 7.7% among the 59 studies [31]. TP53 carrier rate outside LFS was from 3.8% and 7.7% when the tests were performed for selected patients based on early-onset but not family history.

When offering treatments for breast cancer patients with germline TP53 mutation, RT particularly should be paid attention. Because of the function of TP53 gene to repair DNA damage, RT to breast tissue, chest wall, and other region would cause unfavorable effects in breast cancer patients. Heymann S. et al. studied 8 breast cancer patients diagnosed as the first tumor event of LFS among 47 documented Li–Fraumeni families [32]. Median age at the diagnosis was 30 years and six patients had received RT (three for conserving breast and three for chest wall). With median follow-up of 6 years, three ipsilateral breast recurrences, three contralateral breast cancers, two radio-induced cancers, and three new primaries (one of which was an in-field thyroid cancer with atypical histology) were diagnosed among six patients receiving RT. Other case reports suggested the unfavorable outcomes of TP53-related breast cancers as well [33–35]. Based on the current available data, BCS and RT for breast tissue should not be indicated for breast cancer patients with germline TP53 mutation. Although an alternative option does not exist except mastectomy, PMRT should be considered only in patients with higher risk of recurrence.

6.8 PTEN-Related Breast Cancer

PTEN gene is known as a tumor suppressor which produces the enzyme regulating cancer cells in various ways. Among hereditary breast cancer, Cowden syndrome (CS) is well known as a germline PTEN mutation causing multi-system disorder including malignant tumors of the breast, endometrium, thyroid, and so on. The lifetime risk of breast cancer associated with a mutation in PTEN is estimated from 77% to 85% for women [36, 37]. Unfortunately, there are few reports about what treatment is recommended for women with PTEN-related breast cancer. The only thing we could mention is that breast cancer patients with germline PTEN mutation are at increased risk of not only second breast cancer but endometrial, thyroid, renal, and colorectal cancers. Therefore, the active screening and prophylactic surgery could be considered. Even if breast cancer patients without germline PTEN mutation meet the CS diagnostic criteria, a comprehensive approach to those women is necessary as well as mutation carriers [38].

6.9 CDH1-Related Breast Cancer

CDH1 gene provides a protein E-cadherin which functions as an adhesion factor in the cell membrane and characterizes especially the morphological feature of lobular breast cancer (LBC). Hereditary invasive lobular breast-diffuse gastric cancer related to germline CDH1 mutations is one of the genetically high-penetration breast cancers. The International Gastric Cancer Linkage Consortium reported that the estimated risk for diffuse gastric cancer was from 67% to 83% [39, 40]. On the other hand, the estimated risk for LBC was around 40% by age 80 years. Corso G, et al. reported the results of their literature review which included 483 IBCs from 9 studies outside the pedigrees of diffuse gastric cancer [41]. Mean age at the

diagnosis of LBC was 46 years. Out of 483 patients, 14 novel deleterious alterations (2.9%) have been reported. Apart from prophylactic surgery, appropriate management of surgery and RT remains unclear. The clinical decision should be made taking into account the various factors, like the extent of tumor, the quality of imaging, the preference of patient, and so on.

6.10 PALB2-Related Breast Cancer

PALB2 gene encodes a protein which helps genome maintenance, especially double-strand break repair of BRCA2. While biallelic germline mutation (loss-of-function) in PALB2 is related to the onset of Fanconi's anemia, monoallelic mutations (loss-of-function) increase the risk of breast cancer and pancreatic cancer [42]. Antoniou AC, et al. analyzed the information of 362 members in 154 families who had deleterious PALB2 mutations [43, 44]. The estimated absolute risks of breast cancer for PALB2 mutation carriers were 33% and 58% for those without and with family history of breast cancer. Cybulski C, et al. reported the result of their retrospective study to evaluate the incidence rate of mutation and prognosis [45]. Out of 12,529 women with breast cancer, 116 patients (0.93%) were detected as the PALB2 mutation carriers. As controls, 10 participants were positive of PALB2 mutation in 4730 women who were free from cancer. The authors suggested that breast cancer patients with PALB2 mutations had worse prognosis than non-carrier patients. However, the adjustment of variable seems not to be done thoroughly. In this study, the 5-year cumulative incidence of contralateral breast cancer was reported to be 10% in PALB2 mutation carriers. Although the appropriate therapeutic approach for breast cancer patients with PALB2 mutation is unclear, the standard management should not be withheld for the reason of germline mutation in PALB2.

6.11 CHEK2-Related Breast Cancer

CHEK2 gene is one of tumor suppressors among cancers, providing its function to induce cell death through producing a protein CHK2. CHEK2 (1100delC) is generally classified into moderate risk category and the lifetime risk is estimated from 25% to 30% [46]. Lee A, et al. newly proposed the risk prediction model of hereditary breast cancer using both genetic and non-genetic risk factors [47]. Based on their risk mode, the cumulative incidence of breast cancer among CHEK2 mutation carriers varied from 20% to 35% depending on the questionnaire-based risk factors, mammographic density, and polygenic risk scores. Several studies reported the increasing risk of second breast cancer in breast cancer patients with CHEK2 mutation [48–51]. This information of CHEK2 1100delC about the risk of second breast cancer, especially contralateral breast cancer, should be shared when discussing the therapeutic options.

6.12 ATM-Related Breast Cancer

ATM gene codes a protein which is a key regulator of cellular pathways protecting cells from DNA double-strand break. The lifetime risk of breast cancer related to germline ATM mutation is approximately 30% which changes due to the other non-genetic risks [46, 47]. When considering BCS and RT for breast cancer patients with ATM mutation, ipsilateral cancer recurrence and the toxicity of RT need to be taken into account. Meyer A, et al. studied 135 breast cancer patients treated with RT after BCS including 20 ATM mutation carriers [52]. The results showed no significant difference between carriers and non-carriers in terms of local recurrence and metastatic-free survival by multivariate analysis. Regarding the toxicity of RT especially ones related to skin and subcutaneous tissues, the conflict results exist [53–55]. Some studies reported the data about the incidence of CBC after RT to breast tissue in breast cancer patients with ATM mutation.

Bernstein JL, et al. suggested that RT was significantly associated the risk of CBC in breast cancer patients with ATM deleterious missense variant compared to non-carriers [56]. In contrast, the other two studies reported no increase of CBC among breast cancer patients with ATM mutation who received RT after BCS [57, 58]. The evidence on hand is limited so that more research is required. The current practice including BCS and RT should be offered for breast cancer patients with ATM mutation if indicated. The physician also needs to discuss about the toxicity of RT and the potential CBC.

6.13 STK11-Related Breast Cancer

STK11 gene which is sometimes called LKB1 suppresses cell growth by producing the enzyme. The gene is also known to lead to Peutz–Jeghers syndrome (PJS) composing a wide spectrum of cancers, gastrointestinal cancers, breast cancers, ovary cancers, and so on. The cumulative risks of breast cancer in PJS patients are 8%, 13%, 31%, and 45% at the age of 40, 50, 60, and 70 years, respectively [59]. Unfortunately, there was no available data to decide what treatment is indicated for breast cancer patients with STK11 mutation. The standard of care should be offered for the population while discussing the risk caused by STK11 mutation.

6.14 Conclusion

In this field, there are few reliable evidences about treatment of hereditary breast cancer so that we need to discuss about the balance between benefit and risk of treatment, adapting to each patient. More researches about treatment for hereditary breast cancer are considered indispensable.

References

1. Institute. NC. Genetics of breast and gynecologic cancers (PDQ®) - health professional version. 2019. https://www.cancer.gov/types/breast/hp/breast-ovarian-genetics-pdq.
2. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61–70.
3. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302:643–6.
4. Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res. 2011;17:1082–9.
5. Rummel S, Varner E, Shriver CD, Ellsworth RE. Evaluation of BRCA1 mutations in an unselected patient population with triple-negative breast cancer. Breast Cancer Res Treat. 2013;137:119–25.
6. Friebel TM, Andrulis IL, Balmaña J, et al. BRCA1 and BRCA2 pathogenic sequence variants in women of African origin or ancestry. Hum Mutat. 2019;40:1781–96.
7. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295:2492–502.
8. Nakamura S, Takahashi M, Tozaki M, et al. Prevalence and differentiation of hereditary breast and ovarian cancers in Japan. Breast Cancer. 2015;22:462–8.
9. van den Broek AJ, Schmidt MK, van 't Veer LJ, et al. Prognostic impact of breast-conserving therapy versus mastectomy of BRCA1/2 mutation carriers compared with noncarriers in a consecutive series of young breast cancer patients. Ann Surg. 2019;270:364–72.
10. Nilsson MP, Hartman L, Kristoffersson U, et al. High risk of in-breast tumor recurrence after BRCA1/2-associated breast cancer. Breast Cancer Res Treat. 2014;147:571–8.
11. Pierce LJ, Phillips KA, Griffith KA, et al. Local therapy in BRCA1 and BRCA2 mutation carriers with operable breast cancer: comparison of breast conservation and mastectomy. Breast Cancer Res Treat. 2010;121:389–98.
12. Yao K, Liederbach E, Tang R, et al. Nipple-sparing mastectomy in BRCA1/2 mutation carriers: an interim analysis and review of the literature. Ann Surg Oncol. 2015;22:370–6.
13. Manning AT, Wood C, Eaton A, et al. Nipple-sparing mastectomy in patients with BRCA1/2 mutations and variants of uncertain significance. Br J Surg. 2015;102:1354–9.
14. Heemskerk-Gerritsen BA, Rookus MA, Aalfs CM, et al. Improved overall survival after contralateral risk-reducing mastectomy in BRCA1/2 mutation carriers with a history of unilateral breast cancer: a prospective analysis. Int J Cancer. 2015;136:668–77.
15. Metcalfe K, Gershman S, Ghadirian P, et al. Contralateral mastectomy and survival after breast cancer in carriers of BRCA1 and BRCA2 mutations: retrospective analysis. BMJ. 2014;348:g226.
16. Soenderstrup IMH, Laenkholm AV, Jensen MB, et al. Clinical and molecular characterization of BRCA-associated breast cancer: results from the DBCG. Acta Oncol. 2018;57:95–101.
17. Evans DG, Ingham SL, Baildam A, et al. Contralateral mastectomy improves survival in women with BRCA1/2-associated breast cancer. Breast Cancer Res Treat. 2013;140:135–42.
18. Valachis A, Nearchou AD, Lind P. Surgical management of breast cancer in BRCA-mutation carriers: a systematic review and meta-analysis. Breast Cancer Res Treat. 2014;144:443–55.
19. Drooger J, Akdeniz D, Pignol JP, et al. Adjuvant radiotherapy for primary breast cancer in BRCA1 and BRCA2 mutation carriers and risk of contralateral breast cancer with special attention to patients irradiated at younger age. Breast Cancer Res Treat. 2015;154:171–80.
20. Pierce LJ, Strawderman M, Narod SA, et al. Effect of radiotherapy after breast-conserving treatment in women with breast cancer and germline BRCA1/2 mutations. J Clin Oncol. 2000;18:3360–9.
21. Park H, Choi DH, Noh JM, et al. Acute skin toxicity in Korean breast cancer patients carrying BRCA mutations. Int J Radiat Biol. 2014;90:90–4.

22. Shanley S, McReynolds K, Ardern-Jones A, et al. Late toxicity is not increased in BRCA1/BRCA2 mutation carriers undergoing breast radiotherapy in the United Kingdom. Clin Cancer Res. 2006;12:7025–32.
23. Hahnen E, Lederer B, Hauke J, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: secondary analysis of the GeparSixto randomized clinical trial. JAMA Oncol. 2017;3:1378–85.
24. Loibl S, O'Shaughnessy J, Untch M, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol. 2018;19:497–509.
25. Caramelo O, Silva C, Caramelo F, et al. The effect of neoadjuvant platinum-based chemotherapy in BRCA mutated triple negative breast cancers -systematic review and meta-analysis. Hered Cancer Clin Pract. 2019;17:11.
26. Tutt A, Tovey H, Cheang MCU, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT trial. Nat Med. 2018;24:628–37.
27. Zhang J, Lin Y, Sun XJ, et al. Biomarker assessment of the CBCSG006 trial: a randomized phase III trial of cisplatin plus gemcitabine compared with paclitaxel plus gemcitabine as first-line therapy for patients with metastatic triple-negative breast cancer. Ann Oncol. 2018;29:1741–7.
28. Templeton AJ, Gonzalez LD, Vera-Badillo FE, et al. Interaction between hormonal receptor status, age and survival in patients with BRCA1/2 germline mutations: a systematic review and meta-regression. PLoS One. 2016;11:e0154789.
29. Goodwin PJ, Phillips KA, West DW, et al. Breast cancer prognosis in BRCA1 and BRCA2 mutation carriers: an international prospective breast cancer family registry population-based cohort study. J Clin Oncol. 2012;30:19–26.
30. Copson ER, Maishman TC, Tapper WJ, et al. Germline BRCA mutation and outcome in young-onset breast cancer (POSH): a prospective cohort study. Lancet Oncol. 2018;19:169–80.
31. Fortuno C, James PA, Spurdle AB. Current review of TP53 pathogenic germline variants in breast cancer patients outside Li-Fraumeni syndrome. Hum Mutat. 2018;39:1764–73.
32. Heymann S, Delaloge S, Rahal A, et al. Radio-induced malignancies after breast cancer post-operative radiotherapy in patients with Li-Fraumeni syndrome. Radiat Oncol. 2010;5:104.
33. Henry E, Villalobos V, Million L, et al. Chest wall leiomyosarcoma after breast-conservative therapy for early-stage breast cancer in a young woman with Li-Fraumeni syndrome. J Natl Compr Cancer Netw. 2012;10:939–42.
34. Ferrarini A, Auteri-Kaczmarek A, Pica A, et al. Early occurrence of lung adenocarcinoma and breast cancer after radiotherapy of a chest wall sarcoma in a patient with a de novo germline mutation in TP53. Familial Cancer. 2011;10:187–92.
35. Limacher JM, Frebourg T, Natarajan-Ame S, Bergerat JP. Two metachronous tumors in the radiotherapy fields of a patient with Li-Fraumeni syndrome. Int J Cancer. 2001;96:238–42.
36. Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. J Med Genet. 2013;50:255–63.
37. Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res. 2012;18:400–7.
38. Genetic/familial high-risk assessment: breast and ovarian. http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf.
39. Dossus L, Benusiglio PR. Lobular breast cancer: incidence and genetic and non-genetic risk factors. Breast Cancer Res. 2015;17:37.
40. van der Post RS, Vogelaar IP, Carneiro F, et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet. 2015;52:361–74.
41. Corso G, Intra M, Trentin C, et al. CDH1 germline mutations and hereditary lobular breast cancer. Familial Cancer. 2016;15:215–9.
42. Tischkowitz M, Xia B. PALB2/FANCN: recombining cancer and Fanconi anemia. Cancer Res. 2010;70:7353–9.

43. Antoniou AC, Foulkes WD, Tischkowitz M. Breast-cancer risk in families with mutations in PALB2. N Engl J Med. 2014;371:1651–2.
44. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. N Engl J Med. 2014;371:497–506.
45. Cybulski C, Kluźniak W, Huzarski T, et al. Clinical outcomes in women with breast cancer and a PALB2 mutation: a prospective cohort analysis. Lancet Oncol. 2015;16:638–44.
46. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med. 2015;372:2243–57.
47. Lee A, Mavaddat N, Wilcox AN, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. Genet Med. 2019;21:1708–18.
48. Kriege M, Hollestelle A, Jager A, et al. Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy. Br J Cancer. 2014;111:1004–13.
49. Weischer M, Nordestgaard BG, Pharoah P, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. J Clin Oncol. 2012;30:4308–16.
50. Fletcher O, Johnson N, Dos Santos SI, et al. Family history, genetic testing, and clinical risk prediction: pooled analysis of CHEK2 1100delC in 1,828 bilateral breast cancers and 7,030 controls. Cancer Epidemiol Biomark Prev. 2009;18:230–4.
51. Schmidt MK, Tollenaar RA, de Kemp SR, et al. Breast cancer survival and tumor characteristics in premenopausal women carrying the CHEK2*1100delC germline mutation. J Clin Oncol. 2007;25:64–9.
52. Meyer A, John E, Dörk T, et al. Breast cancer in female carriers of ATM gene alterations: outcome of adjuvant radiotherapy. Radiother Oncol. 2004;72:319–23.
53. Bremer M, Klöpper K, Yamini P, et al. Clinical radiosensitivity in breast cancer patients carrying pathogenic ATM gene mutations: no observation of increased radiation-induced acute or late effects. Radiother Oncol. 2003;69:155–60.
54. Iannuzzi CM, Atencio DP, Green S, et al. ATM mutations in female breast cancer patients predict for an increase in radiation-induced late effects. Int J Radiat Oncol Biol Phys. 2002;52:606–13.
55. Andreassen CN, Overgaard J, Alsner J, et al. ATM sequence variants and risk of radiation-induced subcutaneous fibrosis after postmastectomy radiotherapy. Int J Radiat Oncol Biol Phys. 2006;64:776–83.
56. Bernstein JL, Concannon P. ATM, radiation, and the risk of second primary breast cancer. Int J Radiat Biol. 2017;93:1121–7.
57. Broeks A, Braaf LM, Huseinovic A, et al. Identification of women with an increased risk of developing radiation-induced breast cancer: a case only study. Breast Cancer Res. 2007;9:R26.
58. Su Y, Swift M. Outcomes of adjuvant radiation therapy for breast cancer in women with ataxia-telangiectasia mutations. JAMA. 2001;286:2233–4.
59. Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res. 2006;12:3209–15.

Hereditary Ovarian Cancer

7

Kenta Masuda, Megumi Satake, and Daisuke Aoki

Abstract

The treatment of ovarian cancer has changed significantly over the past few years, particularly in the case of hereditary breast and ovarian cancer (HBOC) syndrome. Genetic testing for *BRCA1* and *BRCA2* is used not only for a diagnosis for HBOC but also a biomarker for PARP inhibitors, which is of great importance in the treatment of ovarian cancer. The characteristics of ovarian cancer in HBOC have been reported of the highest prevalence in high-grade serous carcinoma subtype, high sensitivity to platinum salt chemotherapies and PARP inhibitors, and a better prognosis compared to *BRCA*-negative ovarian cancer. It is important to note that ovarian cancer with a family history is also associated with Lynch syndrome, although less frequently than HBOC. In addition, recent multipanel genetic analysis has led to the identification of genes other than HBOC that are involved in the development of ovarian cancer, which may require further clinical practice.

Keywords

HBOC · Histology · Prevalence · Penetrance · Chemotherapy · Surveillance · Lynch syndrome · Moderate-risk genes

K. Masuda (✉) · M. Satake · D. Aoki
Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan
e-mail: ma-su-ken.a2@keio.jp; mgm.stk@keio.jp; aoki@z7.keio.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_7

7.1 Overview of Ovarian Cancer

Ovarian cancer is the most lethal gynecologic malignancy with more than 200,000 cases every year [1]. Due to the lack of effective screening methods, most patients are diagnosed at advanced stages. Less than 40% of women with ovarian cancer can be cured.

7.1.1 Symptoms

Symptoms associated with ovarian cancer were reported as pelvic/abdominal pain, urinary urgency/frequency, increased abdominal size/bloating, and difficulty eating/feeling full when they were frequently present for <1 year [2]. However, the screening by these symptoms, especially in patients with early-stage ovarian cancer, did not show enough sensitivity or specificity [3, 4]. Thus, ovarian cancer is still called as "a silent killer."

7.1.2 Histologic Subtypes

Epithelial ovarian cancer has four main histologic subtypes, including serous, endometrioid, mucinous, and clear cell. High-grade serous carcinoma (HGSC) characterized by *TP53* mutations is the most common and aggressive subtype. This subtype is related to hereditary breast and ovarian cancer (HBOC) syndrome, and its origin is said to be the fallopian tube or ovarian epithelium. Low-grade serous, mucinous, clear cell, and endometrioid tumors are believed to have developed from inclusion cysts or implants in the ovarian surface epithelium. They also have *KRAS, BRAF*, or *PTEN* mutations [5, 6]. Clear cell carcinoma has characteristics of being resistant to anticancer drugs, contrary to its slow growth, and is more common in Japan [7].

7.1.3 Risk Factors

The risk factors of developing ovarian cancer are age, nulliparity, and age (>35 years) at first pregnancy or first birth. Thirty percent to sixty percent decreased risk for cancer, in contrast, is associated with younger age at first pregnancy or first birth (≤25 years), the use of oral contraceptives, and history of breastfeeding [5]. As we will discuss later, having a family cancer syndrome is the most relevant risk of developing ovarian cancer.

7.1.4 Screening

Screening for ovarian cancer did not reduce mortality in two large screening trials. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial by

annual screening with serum CA125 and ultrasound showed no reduction in mortality [8]. The other result from the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) based on serum CA125-based screening seemed encouraging, but the mortality reduction was not significant [9].

7.1.5 Treatment

For epithelial ovarian cancer, primary treatment consists of appropriate surgical staging and debulking surgery, followed by systemic chemotherapy in most, but not all, patients. For most patients, initial surgery includes total abdominal hysterectomy and bilateral salpingo-oophorectomy with comprehensive staging with omentectomy, pelvic and para-aortic lymphadenectomy, and peritoneal biopsy [5, 10]. Debulking surgery is recommended for patients at stage II, III, or IV, because the maximal cytoreduction improves survival [11, 12]. Neoadjuvant chemotherapy followed by interval debulking surgery is recommended for patients diagnosed as advanced disease and the optimal surgery is difficult [10]. Regarding systematic lymphadenectomy, one recent RCT has shown that systematic pelvic and paraaortic lymphadenectomy after maximal cytoreduction did not improve survival and might cause postoperative complications when lymph nodes have no suspicious findings [13].

Most patients with epithelial ovarian cancer receive postoperative systemic chemotherapy. The combination of platinum and taxane agents is typically administered as a first-line chemotherapy for ovarian cancer. The effect of bevacizumab, anti-angiogenesis agent, was assessed by two RCTs, ICON7 and GOG218. These trials showed that the addition of bevacizumab to upfront chemotherapy with carboplatin/paclitaxel followed by bevacizumab as maintenance therapy improved PFS (hazard ratio[HR] 0.72, 0.81) and is recommended for patients at stage III or IV [14, 15].

The effect of poly(ADP-ribose) polymerase (PARP) inhibitors as maintenance therapy was assessed by several RCTs. The SOLO1 study demonstrated that PFS was prolonged substantially by using olaparib as a maintenance therapy in patients with a germline or somatic *BRCA1/BRCA*2 variant (HR 0.30) [16]. In Japan, olaparib is currently available for advanced ovarian cancer patients with *BRCA1/BRCA2* variants as maintenance therapy. Furthermore, the three RCTs of PARP inhibitors—veliparib, niraparib, and olaparib plus bevacizumab—have recently been shown to improve PFS [HR 0.68, 0.62, 0.59] when used after primary treatment regardless of *BRCA1/BRCA2* variant, but the better outcome was seen in homologous recombination deficiency (HRD)-positive patients [17–19].

The recurrent disease is categorized by platinum-sensitive disease (if the patients have the disease ≥6 months after completion prior platinum-based therapy) or platinum-resistant disease (if the patients have the disease <6 months after completion prior platinum-based therapy). For platinum-sensitive disease, six cycles of platinum-based chemotherapy are preferred. The addition of bevacizumab to standard chemotherapy and maintenance therapy until progression improved PFS for

platinum-sensitive recurrent ovarian cancer. OS was also improved in GOG213 [20, 21]. PARP inhibitors as maintenance therapy improved PFS of platinum-sensitive disease with germline *BRCA1/BRCA*2 variant, platinum-sensitive recurrent HGSC, and HRD positive [22–25]. For platinum-resistant disease, non-platinum-based agents or regimens are preferred. The prognosis is poor, but adding bevacizumab to chemotherapy improved PFS [26].

7.2 Hereditary Ovarian Cancer

It is now known that at least 10% of epithelial ovarian cancer have germline pathogenic variant in ovarian cancer-susceptibility genes, commonly *BRCA1/BRCA2* and DNA mismatch repair (MMR) genes. Of 1915 ovarian cancer patients, 347 (18%) carried a germline mutation in a gene associated with ovarian cancer risk [27]. In Japan, of 230 unselected women with ovarian cancer, 17.8% women had pathogenic germline variant. The variants include genes associated with *BRCA1* (prevalence; 8.3%), *BRCA2* (3.5%), and mismatch repair genes (2.6%) [28]. Patients with HGSC may have germline variants in other genes involved in HR, including *BRIP1*, *BARD1*, *RAD51C*, and *RAD51D*, but the frequency is less compared to *BRCA1* and *BRCA*2 [29]. In addition, some large studies using multiple-gene, next-generation sequencing panels and whole-exome sequencing were conducted, and gene-phenotype associations were examined. Table 7.1 shows the list of genes related to hereditary ovarian cancer and their risks of ovarian cancer reported in NCCN guidelines, and these studies [30, 31, 37]. Detailed personal and family history of cancer is important for cancer risk assessment and choice of gene testing. Comprehensive testing with multigene panel for *BRCA1-/BRCA2*-negative patients and individuals without a known familial pathogenic variant should be considered. In gynecological clinics, a self-administered questionnaire would be a useful tool for screening patient's medical history and familial cancer history [38].

7.2.1 Ovarian Cancer in HBOC

7.2.1.1 Frequency of HBOC in Ovarian Cancer

The frequency of germline *BRCA1/BRCA2* variants in ovarian cancer patients was reported to be 13–15% in some large studies [39, 40]. In Japan, one multicenter analysis reported that the overall prevalence of germline *BRCA1/BRCA2* variants was 14.7% (93/634), where germline *BRCA1* mutations (9.9%) were more common than germline *BRCA2* mutations (4.7%) [41]. In another report, of 230 unselected Japanese women with ovarian cancer, 11.7% women had pathogenic germline variants of *BRCA1/BRCA2* [28].

7.2.1.2 Germline Testing for Ovarian Cancer Patients

Based on personal and familial cancer history, germline *BRCA1/BRCA2* testing should be considered for individuals from a family without a known *BRCA1/BRCA2*

Table 7.1 The list of genes related to hereditary ovarian cancer and their risks of ovarian cancer. In NCCN guidelines, the risk is categorized as increase, potential increase, no increase, or unknown. The risk ratio of ovarian cancer for women with pathogenic variant of each gene is shown here according to each literature

Gene	Ovarian cancer risk (NCCN guidelines)	Odds Ratio (95% CI) [30]	Standardized risk ratio (95% CI) [31]	Relative Risk (95% CI) [32–36]	Odds Ratio (95% CI) [37]
BRCA1	increase	11.8 (9.99–14.0)	11.78 (10.42–13.28)		
BRCA2	increase	5.26 (4.38–6.31)	7.96 (7.00–9.01)		
RAD51C	increase	4.98 (3.09–8.04)	5.12 (3.72–6.88)	5.88 (2.91–11.88) 5.2 (1.1–24)	
RAD51D	increase	4.78 (3.09–8.04)	6.34 (3.16-11.34)	6.30 (2.86–13.85) 12.0 (1.5–90)	ND
BRIP1	increase	2.62 (1.72–3.98)	4.99 (3.79–6.45)	11.22 (3.22–34.10) 8.13 (4.74–13.95)	
NBN	potential increase	1.85 (1.05–3.24)	2.03 (1.27–3.08)		
ATM	potential increase	1.69 (1.19–2.40)	2.25 (1.69–2.94)	0.88 (0.02–5.84)	
PALB2	potential increase	1.60 (0.98–2.60)	3.08 (1.93–4.67)		
CHEK2	no increase	0.86 (0.56–1.33)	0.98 (0.75–1.27)		
TP53	no increase	0.66 (0.05–8.68)			28.96 (2.32–1506.64)
BARD1	unknown	0.59 (0.21–1.68)	1.28 (0.55–2.51)		
MMR genes	increase				
MLH1		3.11 (1.47–6.59)	2.20 (0.81–4.78)		
MSH2		2.04 (1.08–3.84)	13.91 (8.82–20.87)		
MSH6		1.92 (1.19–3.10)	5.04 (3.70–6.70)		6.73 (2.34–18.43)
PMS2		1.57 (0.94–2.60)	1.48 (0.81–2.48)		
STK11	increase of non-epithelial ovarian tumors	41.9 (5.55–315) (No of patients = 5)			

variant. NCCN guidelines recommend testing should be considered for patients at any age with a personal history of ovarian cancer (including fallopian tube cancer or peritoneal cancer) and those with a first- or second-degree blood relative of ovarian cancer [42]. Since 2020, Japanese public health insurance has covered 70% of germline *BRCA1/BRCA2* testing costs for cancer patients suspected of HBOC, and this includes ovarian cancer patients, too.

Detailed personal and family history of cancer is important for cancer risk assessment and choice of gene testing. For those without a known familial pathogenic variant or *BRCA1-/BRCA2*-negative patients, comprehensive testing with multigene panel should be considered. BRCA-related ovarian cancers are associated with epithelial, non-mucinous histology as discussed below; however, bear in mind that Lynch syndrome or other syndromes could be associated with both non-mucinous and mucinous histology.

7.2.1.3 Penetrance of *BRCA1/BRCA2* Variant Carriers in Ovarian Cancer

Women with a *BRCA1/BRCA2* pathogenic variant are at increased risk of ovarian cancers (including fallopian tube cancer and primary peritoneal cancer). The reliable prediction of developing ovarian cancer (the penetrance) is critical in genetic counseling and a gynecological practice for *BRCA1/BRCA2* variant carriers. A meta-analysis showed the mean cumulative ovarian cancer risks for *BRCA1/BRCA2* variant carriers at age 70 years were 40% (95% CI, 35% to 46%) for *BRCA1* and 18% (95% CI, 13% to 23%) for *BRCA2* variant carriers [43]. A large prospective cohort study of 6036 *BRCA1* and 3820 *BRCA2* female variant carriers showed that the cumulative ovarian cancer risks to age 80 years were 44% (95% CI, 36%–53%) for *BRCA1* and 17% (95% CI, 11%–25%) for *BRCA2* carriers [44]. The risk of ovarian cancer is not the same for all *BRCA1/BRCA2* mutations. A large observational study from data collected by the Consortium of Investigators of Modifiers of BRCA (CIMBA) initiative revealed that women with a variant in the central part of *BRCA1/BRCA2*, especially in exon11 where ovarian cancer cluster regions (OCCRs) were identified, will have a higher lifetime risk of ovarian cancer [45]. The estimated penetrance of ovarian cancer can be influenced by allelic heterogeneity, modifier genes, and environmental and hormonal cofactors, such as oral-contraceptive use or parity and nationality [46].

7.2.1.4 Histology of Ovarian Cancer in HBOC

There are four main histologic subtypes of epithelial ovarian cancer: serous carcinoma (low-grade and high-grade), mucinous carcinoma, endometrioid carcinoma, and clear cell carcinoma. Germline *BRCA1/BRCA2* variants were reported in all histologic subtypes except mucinous carcinoma (Table 7.2). In several large studies, high-grade serous carcinoma had the highest prevalence of *BRCA1/BRCA2* variant [27, 39]. In Japan, a multicenter analysis reported that 28.5% of high-grade serous carcinoma has germline *BRCA* variants [41]. Another multivariate analysis showed that the high-grade serous carcinoma subtype is an independent predictive factor for pathogenic germline *BRCA1/BRCA2* variants [28]. It should be noted that the

Table 7.2 Prevalence of germline *BRCA1/BRCA2* variants in each histological subtype of ovarian cancer

	Prevalence of germline *BRCA1/2* variants			
	Enomoto et al. [41] (Japan)	Hirasawa et al. [28] (Japan)	Norquist et al. [27]	Alsop et al. [39]
Overall	14.7%(93/634)	11.7%(27/230)	15%(280/1915)	14.1%(141/1001)
HGSC	28.5%(78/274)	29.7%(22/74)	16.1%(241/1498)	22.6%(98/433)
LGSC	20%(1/5)	0%(0/3)	5.7%(4/70)	NA
endometrioid	6.7%(8/120)	3.4%(2/58)	not shown	8.4%(10/119)
clear	2.1%(4/187)	2.8%(2/71)	6.9%(4/58)	6.3%(4/63)
mucinous	0%(0/19)	0%(0/18)	0%(0/16)	NA

prevalence of germline *BRCA1/BRCA2* variants differs between studies, especially in clear cell carcinoma. This is because the frequency of germline *BRCA1/BRCA2* variants has not been clarified, due to the low incidence of clear cell carcinoma in Western countries. Large-scale studies will be necessary in the future.

7.2.1.5 Ovarian Cancer Initiation in HBOC

After risk-reducing salpingo-oophorectomy (RRSO), a precursor lesion called tubal intraepithelial carcinoma (TIC) was detected in 5–10% of cases in women with *BRCA* variants [47–50]. The distal fallopian tube is suspected to be the dominant origin of early malignancies found in RRSO samples [47, 50, 51]. TICs and their associated ovarian carcinomas share identical mutations of *TP53* [52]. Although the idea of fallopian tube to be the origin of many serous carcinomas of ovary for *BRCA1/BRCA2* variant carriers is now generally accepted, there is a subset of HGSC with no apparent precursor lesion in the fallopian tube, so further study is needed to understand how these cancers develop [29]. It is not clear whether surgical staging and/or adjuvant chemotherapy is beneficial for women with STIC.

7.2.1.6 Prognosis of *BRCA1/BRCA2* Variant Carriers with Ovarian Cancer

Recently, meta-analysis of women's survival with ovarian cancer was done. This study was based on 26 reports including data from 1213 epithelial ovarian cancer patients with germline *BRCA1/BRCA2* variants and 2666 noncarriers. Germline variants in *BRCA1* or *BRCA2* are associated with higher 5-year overall survival among patients with ovarian cancer. After adjusting the methods of studies and years of diagnosis, *BRCA1/BRCA2* variant carriers showed better survival than noncarriers (for *BRCA*1, hazard ratio [HR], 0.78; 95% CI, 0.68–0.89, and for *BRCA2*, HR, 0.61; 95% CI, 0.50–0.76) [53]. However, other reports suggested a positive effect of germline *BRCA1/BRCA2* variant, where mortality in patients with ovarian cancer decreased to 10 years [54].

7.2.1.7 Chemosensitivity and HRD of Ovarian Cancer in HBOC

Both *BRCA1* and *BRCA2* take part in DNA repair such as homologous recombination (HR) and the maintenance of genomic integrity. Cells with defective *BRCA1* or

BRCA2 are hypersensitive to agents that crosslink DNA strands. These are also sensitive to agents that produce breaks in double-stranded DNA, such as platinum salt chemotherapies [46]. Multiple case-control studies compared the effect of primary therapy between ovarian cancer patients with and without *BRCA1/BRCA2* variants. These studies revealed that *BRCA*-related ovarian cancer showed better survival outcomes and platinum sensitivity [39, 55, 56]. However, one study showed that, among women with high-grade serous ovarian cancer, *BRCA2* mutation, but not *BRCA1* deficiency, was associated with improved survival and chemotherapy response [57]. Not only germline *BRCA1/BRCA2* variant but also germline variants of other cancer-associated genes such as *BRIP1*, *RAD51C*, *RAD51D*, *PALB*2, and *BARD1* were more frequent in patients with ovarian cancer than in the general population. There wasn't a significant difference in survival rate between women with mutations in *BRCA1* and other ovarian cancer-associated genes [27]. In addition to germline variant, ovarian cancer with somatic *BRCA1/BRCA2* variants or somatic variants in other homologous recombination DNA repair genes, such as *ATM*, *BARD1*, *BRIP1*, *CHEK1*, *CHEK2*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*, had higher primary platinum sensitivity and improved overall survival than those without variants [39, 58].

Almost 50% of epithelial ovarian cancers exhibit defects within the homologous recombination DNA repair (HRR) pathway. As cells with double-strand break repair deficiency have synthetic lethality to PARP inhibitors (PARPi), ovarian cancer with homologous recombination repair deficiency (HRD) exhibits a sensitivity to PARPi and platinum salt chemotherapies [58]. HRD is often caused by loss of function mutations in HRR genes, such as *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, or *PALB*2, promoter hypermethylation of the *BRCA1* and *RAD51C* gene promoter (leading to reduced expression), or unknown mechanisms. HRD testing is hoped to be a predictive biomarker for PARPi sensitivity. A wide range of assays, referred as "HRD tests," have been developed to define which cancers have HRD. These HRD tests fall into three main categories: (1) HRR pathway, which is related to genes that identify specific causes of HRD, (2) genomic "scars" or mutational signatures which identify the patterns of somatic mutations that accumulate in HRD cancers irrespective of the underlying defect, and (3) functional assays that have the potential to provide a real-time readout of HRD or homologous recombination proficiency (HRP) [59]. A commercially available assay, Myriad MyChoice®, can now be used as a biomarker for PARPi in Japan. This test is the combination of *BRCA1/BRCA2* variant and genomic instability scores (GIS). GIS includes loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST), which is categorized by the genomic scar assay. Although there are several clinical benefits of HRD testing on PARPi response in ovarian cancer, HRD testing is not completely overlapped to PARPi sensitivity, where HRP ovarian cancer has sensitivity to PARPi. Better biomarkers are needed for HGSC management [59] (Fig. 7.1). When analyzing cancer genome by next-generation sequencing like HRD testing, we should bear in mind that mutations in DNA of a tumor may reveal germline variants with clinical significance [60]. Further detail about significance of PARPi for ovarian cancer is described in another section.

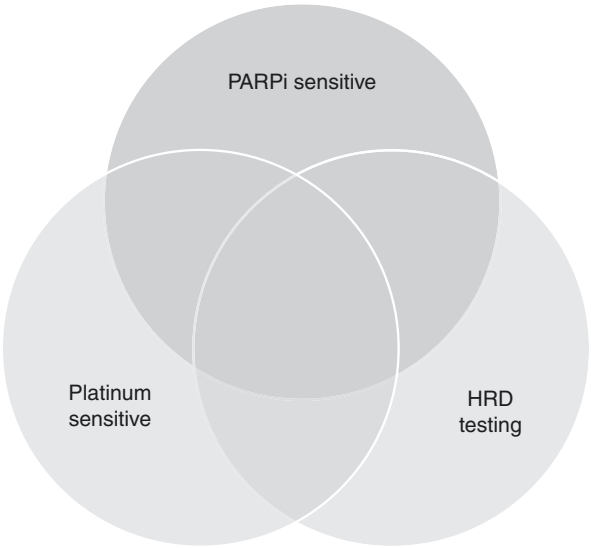


Fig. 7.1 Venn diagram showing the relation between HRD testing, platinum sensitivity, and PARPi sensitivity. Although HRD testing is approved as a clinical biomarker for PARPi sensitivity, patients with positive for HRD testing are not completely overlapped to responses of PARPi

7.2.1.8 Surveillance for Ovarian Cancer

Women with a *BRCA1/BRCA2* pathogenic variant are at increased risk of having ovarian cancers. Several studies on significance of ovarian cancer screening had been conducted. Phase II study of the UK Familial Ovarian Cancer Screening Study (UK FOCSS) included 4348 women with an estimated lifetime ovarian cancer risk of ≥10% and did not choose risk-reducing salpingo-oophorectomy (RRSO). They were assessed by serum CA-125 tests (every 3 months, with using the risk of ovarian cancer algorithm [ROCA]) and TVUS (annually or within 2 months of an abnormal ROCA result). Thirteen ovarian cancer patients were screen-detected, and 5 (38.5%) of the 13 patients were diagnosed at an early stage (stages I to II). Sensitivity, positive predictive value, and negative predictive value for detecting ovarian cancer within 1 year were 94.7%, 10.8%, and 100%, respectively [61].

In another study, 3692 women with a strong family history of breast/ovarian cancer or *BRCA1/BRCA2* variant were assessed by serum CA125 (every 3 months, with using the risk of ovarian cancer algorithm [ROCA]) and transvaginal ultrasound (TVUS) (if ROCA increased above a baseline). Three (50%) of six incidental ovarian cancers were at early stage. ROCA flagged 50% of incidental cases. This method had better early-stage sensitivity at high specificity, but low PPV compared with CA125 every 6 months or annually [62].

Given its high sensitivity and significance in stage shift, these surveillance methods could be an option for *BRCA1/BRCA2* variant carriers who did not choose RRSO. However, significance of these strategies to improve survival rate in screened *BRCA1/BRCA2* variant carriers remains unknown. In NCCN guidelines, RRSO is

the standard method of ovarian cancer risk management in *BRCA1/BRCA2* carriers. For those patients who did not select RRSO, regular checkup by transvaginal ultrasound and serum CA-125 for ovarian cancer screening may be considered from the age of 30 to 35, although its benefit is not certain [42]. Further details about RRSO and chemoprevention for ovarian cancer are described in other sections.

7.2.2 Ovarian Cancer in Lynch Syndrome

Lynch syndrome is a hereditary syndrome associated with familial cancers, including colorectal cancer and Lynch syndrome-related cancers, such as endometrial cancer. The cause of the disease is the germline variant of DNA mismatch repair (MMR) genes, such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*, characterized by autosomal dominant inheritance. Women with Lynch syndrome are also at increased risk of ovarian cancer.

The histological types of ovarian cancer were mixed type (mucinous/endometrioid/clear cell carcinomas) 33%, endometrioid carcinoma 25%, serous carcinoma 22%, clear cell carcinoma 12%, and mucinous carcinoma 4%. Most tumors (65%) were diagnosed at an early stage [63].

Microsatellites are short DNA repeat sequences that increase or decrease in number when MMR is dysfunctional. An MSI test is recommended before examining germline mutation when a patient is suspected of suspected Lynch syndrome. Screening of ovarian cancer specimens by MSI may be an efficient way to diagnose Lynch syndrome [64].

There is no definite evidence to support routine screening for ovarian cancers in Lynch syndrome. Total abdominal hysterectomy and bilateral salpingo-oophorectomy are options that may be considered for risk reduction in women with Lynch syndrome who have completed childbearing [5].

7.2.3 Other Germline Variants Associated with Ovarian Cancer

7.2.3.1 *RAD51C, RAD51D, BRIP1*

DNA recombinase RAD51 protein is a central player in homologous recombination and DNA repair. *BRIP1* encodes the BRCA1-interacting protein C-terminal helicase 1 protein, which is required for the normal double-strand break repair function of *BRCA1*. *RAD51C* and *RAD51D*, genes in the RAD51 protein family, and *BRIP1* have been shown to be associated with increased risk for ovarian cancer [32–36].

The frequency of germline *RAD51C/RAD51D* variants and *BRIP1* variants in ovarian cancer patients was reported to be about 1% [30, 31].

In carriers of a *RAD51D* variant or *BRIP1* variant, the cumulative risk of ovarian cancer approaches 2.6% around 50 to 54 years of age, which is the expected lifetime risk for a woman with a BRCA-negative family history of ovarian cancer [65]. The NCCN guidelines recommend that RRSO in carriers of *RAD51C*, *RAD51D*, or *BRIP1* pathogenic or likely pathogenic variants be considered beginning at 45 to

50 years of age. In women with variants in these genes who also have a family history of ovarian cancer in a first-degree relative, the risk threshold might cross earlier and the timing for RRSO should be considered [42].

7.2.3.2 *NBN, ATM, PALB2*

Some studies suggest that there may be a moderately increased risk for ovarian cancer in carriers of an *NBN*, *ATM*, or *PALB2* variant, but there is currently insufficient evidence to recommend RRSO in these carriers [27, 30, 31].

References

1. Bray F, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424. https://doi.org/10.3322/caac.21492.
2. Goff BA, et al. Development of an ovarian cancer symptom index: possibilities for earlier detection. Cancer. 2007;109(2):221–7. https://doi.org/10.1002/cncr.22371.
3. Gilbert L, et al. Assessment of symptomatic women for early diagnosis of ovarian cancer: results from the prospective DOvE pilot project. Lancet Oncol. 2012;13(3):285–91. https://doi.org/10.1016/S1470-2045(11)70333-3.
4. Lim AWW, et al. Predictive value of symptoms for ovarian cancer: comparison of symptoms reported by questionnaire, interview, and general practitioner notes. J Natl Cancer Inst. 2012;104:114–24. https://doi.org/10.1093/jnci/djr486.
5. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Ovarian cancer including fallopian tube cancer and primary peritoneal cancer version 1. 2020.
6. Wentzensen N, et al. Ovarian cancer risk factors by histologic subtype: an analysis from the ovarian cancer cohort consortium. J Clin Oncol. 2016;34(24):2888–98. https://doi.org/10.1200/JCO.2016.66.8178.
7. Takano M, Tsuda H, Sugiyama T. Clear cell carcinoma of the ovary: is there a role of histology-specific treatment? J Exp Clin Cancer Res. 2012; https://doi.org/10.1186/1756-9966-31-53.
8. Buys SS, et al. Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. JAMA. 2011;305(22):2295–302. https://doi.org/10.1001/jama.2011.766.
9. Jacobs IJ, et al. Ovarian cancer screening and mortality in the UK collaborative trial of ovarian Cancer screening (UKCTOCS): a randomised controlled trial. Lancet. 2016;387(10022):945–56. https://doi.org/10.1016/S0140-6736(15)01224-6.
10. JSGO Guidelines. Guidelines for treatment of ovarian cancer, fallopian tube cancer and primary peritoneal cancer. 2020 edition. Japan Society of Gynecologic Oncology (JSGO); 2020.
11. Bristow RE, et al. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. J Clin Oncol. 2002;20(5):1248–59. https://doi.org/10.1200/jco.2002.20.5.1248.
12. Panici PB, et al. Systematic aortic and pelvic lymphadenectomy versus resection of bulky nodes only in optimally debulked advanced ovarian cancer: a randomized clinical trial. J Natl Cancer Inst. 2005;97(8):560–6. https://doi.org/10.1093/jnci/dji102.
13. Harter P, et al. A randomized trial of lymphadenectomy in patients with advanced ovarian neoplasms. N Engl J Med. 2019;380(9):822–32. https://doi.org/10.1056/nejmoa1808424.
14. Burger RA, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26):2473–83. https://doi.org/10.1056/nejmoa1104390.
15. Perren TJ, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26):2484–96. https://doi.org/10.1056/nejmoa1103799.

16. Moore K, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379(26):2495–505. https://doi.org/10.1056/nejmoa1810858.
17. Coleman RL, et al. Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med. 2019;381(25):2403–15. https://doi.org/10.1056/nejmoa1909707.
18. González-Martín A, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2019;381(25):2391–402. https://doi.org/10.1056/nejmoa1910962.
19. Ray-Coquard I, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381(25):2416–28. https://doi.org/10.1056/nejmoa1911361.
20. Aghajanian C, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17):2039–45. https://doi.org/10.1200/JCO.2012.42.0505.
21. Coleman RL, Brady MF, et al. Bevacizumab and paclitaxel–carboplatin chemotherapy and secondary cytoreduction in recurrent, platinum-sensitive ovarian cancer (NRG oncology/gynecologic oncology group study GOG-0213): a multicentre, open-label, randomised, phase 3 trial. Lancet Oncol. 2017;18(6):779–91. https://doi.org/10.1016/S1470-2045(17)30279-6.
22. Coleman RL, Oza AM, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949–61. https://doi.org/10.1016/S0140-6736(17)32440-6.
23. Ledermann J, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92. https://doi.org/10.1056/nejmoa1105535.
24. Ledermann J, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15(8):852–61. https://doi.org/10.1016/S1470-2045(14)70228-1.
25. Mirza MR, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154–64. https://doi.org/10.1056/nejmoa1611310.
26. Pujade-Lauraine E, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: the AURELIA open-label randomized phase III trial. J Clin Oncol. 2014;32(13):1302–8. https://doi.org/10.1200/JCO.2013.51.4489.
27. Norquist BM, et al. Inherited mutations in women with ovarian carcinoma. JAMA Oncol. 2016;2(4):482. https://doi.org/10.1001/jamaoncol.2015.5495.
28. Hirasawa A, et al. Prevalence of pathogenic germline variants detected by multigene sequencing in unselected Japanese patients with ovarian cancer. Oncotarget. 2017;8(68):112258–67. https://doi.org/10.18632/oncotarget.22733.
29. Bowtell DD, et al. Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. Nat Rev Cancer. 2015;15(11):668–79. https://doi.org/10.1038/nrc4019.
30. Kurian AW, et al. Breast and ovarian cancer penetrance estimates derived from germline multiple-gene sequencing results in women. JCO Precis Oncol. 2017;1:1–12. https://doi.org/10.1200/po.16.00066.
31. Lilyquist J, et al. Frequency of mutations in a large series of clinically ascertained ovarian cancer cases tested on multi-gene panels compared to reference controls. Gynecol Oncol. 2017;147(2):375–80. https://doi.org/10.1016/j.ygyno.2017.08.030.
32. Loveday C, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet. 2011;43(9):879–82. https://doi.org/10.1038/ng.893.
33. Loveday C, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet. 2012;44(5):475–6. https://doi.org/10.1038/ng.2224.
34. Rafnar T, et al. Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet. 2011;43(11):1104–7. https://doi.org/10.1038/ng.955.
35. Ramus SJ, et al. Germline mutations in the BRIP1, BARD1, PALB2, and NBN genes in women with ovarian cancer. J Natl Cancer Inst. 2015;107(11) https://doi.org/10.1093/jnci/djv214.

36. Song H, et al. Contribution of germline mutations in the RAD51B, RAD51C, and RAD51D genes to ovarian cancer in the population. J Clin Oncol. 2015;33(26):2901–7. https://doi.org/10.1200/JCO.2015.61.2408.
37. Lu HM, et al. Association of Breast and Ovarian Cancers with predisposition genes identified by large-scale sequencing. JAMA Oncol. 2019;5(1):51–7. https://doi.org/10.1001/jamaoncol.2018.2956.
38. Masuda K, et al. Clinical utility of a self-administered questionnaire for assessment of hereditary gynecologic cancer. Jpn J Clin Oncol. 2017;47(5):401–6. https://doi.org/10.1093/jjco/hyx019.
39. Alsop K, 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. J Clin Oncol. 2012;30(21):2654–63. https://doi.org/10.1200/JCO.2011.39.8545.
40. Risch HA, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario, Canada. J Natl Cancer Inst. 2006;98(23):1694–706. https://doi.org/10.1093/jnci/djj465.
41. Enomoto T, et al. The first Japanese nationwide multicenter study of BRCA mutation testing in ovarian cancer: CHARacterizing the cross-sectionaL approach to ovarian cancer geneTic TEsting of BRCA (CHARLOTTE). Int J Gynecol Cancer. 2019;29(6):1043–9. https://doi.org/10.1136/ijgc-2019-000384.
42. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/familial high-risk assessment: breast, ovarian, and pancreatic. 2020.
43. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol. 2007;25(11):1329–33. https://doi.org/10.1200/JCO.2006.09.1066.
44. Kuchenbaecker KB, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA. 2017;317(23):2402–16. https://doi.org/10.1001/jama.2017.7112.
45. Rebbeck TR, et al. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. JAMA. 2015;313(13):1347–61. https://doi.org/10.1001/jama.2014.5985.
46. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer. 2004;4(9):665–76. https://doi.org/10.1038/nrc1431.
47. Callahan MJ, et al. Primary fallopian tube malignancies in BRCA-positive women undergoing surgery for ovarian cancer risk reduction. J Clin Oncol. 2007;25(25):3985–90. https://doi.org/10.1200/JCO.2007.12.2622.
48. Powell BC, et al. Risk-reducing salpingo-oophorectomy in BRCA mutation carriers: role of serial sectioning in the detection of occult malignancy. J Clin Oncol. 2005;23(1):127–32. https://doi.org/10.1200/JCO.2005.04.109.
49. Powell CB, et al. Risk-reducing salpingo-oophorectomy (RRSO) in BRCA mutation carriers experience with a consecutive series of 111 patients using a standardized surgical-pathological protocol. Int J Gynecol Cancer. 2011;21(5):846–51. https://doi.org/10.1097/IGC.0b013e31821bc7e3.
50. Shaw PA, et al. Candidate serous cancer precursors in fallopian tube epithelium of BRCA1/2 mutation carriers. Mod Pathol. 2009;22(9):1133–8. https://doi.org/10.1038/modpathol.2009.89.
51. Medeiros F, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol. 2006;30(2):230–6. https://doi.org/10.1097/01.pas.0000180854.28831.77.
52. Lee Y, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol. 2007;211(1):26–35. https://doi.org/10.1002/path.2091.
53. Bolton KL, et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA. 2012;307(4):382–90. https://doi.org/10.1001/jama.2012.20.

54. Candido-dos-Reis FJ, et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. Clin Cancer Res. 2015;21(3):652–7. https://doi.org/10.1158/1078-0432.CCR-14-2497.
55. Gallagher DJ, et al. Survival in epithelial ovarian cancer: a multivariate analysis incorporating BRCA mutation status and platinum sensitivity. Ann Oncol. 2011;22(5):1127–32. https://doi.org/10.1093/annonc/mdq577.
56. Vencken PMLH, et al. Chemosensitivity and outcome of BRCA1- and BRCA2-associated ovarian cancer patients after first-line chemotherapy compared with sporadic ovarian cancer patients. Ann Oncol. 2011;22(6):1346–52. https://doi.org/10.1093/annonc/mdq628.
57. Yang D, et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA. 2011;306(14):1557–65. https://doi.org/10.1001/jama.2011.1456.
58. Pennington KP, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20(3):764–75. https://doi.org/10.1158/1078-0432.CCR-13-2287.
59. Miller RE, et al. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. Ann Oncol. 2020; https://doi.org/10.1016/j.annonc.2020.08.2102.
60. Meric-Bernstam F, et al. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. Ann Oncol. 2016;27(5):795–800. https://doi.org/10.1093/annonc/mdw018.
61. Rosenthal AN, et al. Evidence of stage shift in women diagnosed with ovarian cancer during phase II of the United Kingdom familial ovarian cancer screening study. J Clin Oncol. 2017;35(13):1411–20. https://doi.org/10.1200/JCO.2016.69.9330.
62. Skates SJ, et al. Early detection of ovarian cancer using the risk of ovarian cancer algorithm with frequent CA125 testing in women at increased familial risk – combined results from two screening trials. Clin Cancer Res. 2017;23(14):3628–37. https://doi.org/10.1158/1078-0432.CCR-15-2750.
63. Helder-Woolderink JM, et al. Ovarian cancer in lynch syndrome; a systematic review. Eur J Cancer. 2016;55:65–73. https://doi.org/10.1016/j.ejca.2015.12.005.
64. Akbari MR, et al. Correlation between germline mutations in MMR genes and microsatellite instability in ovarian cancer specimens. Familial Cancer. 2017;16(3):351–5. https://doi.org/10.1007/s10689-017-9973-1.
65. Tung N, et al. Counselling framework for moderate-penetrance cancer-susceptibility mutations. Nat Rev Clin Oncol. 2016;13:581–8. https://doi.org/10.1038/nrclinonc.2016.90.

Risk-Based Breast Cancer Screening

8

Wakana Murakami, Mitsuhiro Tozaki, Kelly E. McCann, and Stephanie Lee-Felker

Abstract

In Japan, the current definition of women at high risk for breast cancer is limited to those with hereditary breast and ovarian cancer (HBOC) syndrome. In contrast, many other countries have a broader definition of "high risk" and have screening guidelines tailored to such high-risk groups of women, as prevention of breast cancer development is the goal. In Japan, national healthcare insurance was instituted in April 2020 for clinical management of women with established breast or ovarian cancer who are later determined to have HBOC; this approach is not focused on prevention.

W. Murakami (✉)
Department of Radiological Sciences, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA

Department of Radiology, Division of Radiology, Showa University Graduate School of Medicine, Shinagawa-ku, Tokyo, Japan
e-mail: WMurakami@mednet.ucla.edu

M. Tozaki
Department of Radiology, Division of Radiology, Showa University Graduate School of Medicine, Shinagawa-ku, Tokyo, Japan

Department of Radiology, Sagara Hospital, Kagoshima City, Kagoshima, Japan
e-mail: tozaki@sagara.or.jp

K. E. McCann
Division of Hematology/Oncology, Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA
e-mail: KMccann@mednet.ucla.edu

S. Lee-Felker
Department of Radiological Sciences, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA
e-mail: StLee@mednet.ucla.edu

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_8

In this chapter, we discuss issues to be considered in Japan based on studies of risk-based imaging screening in other countries. We believe there are three major issues for high-risk breast cancer screening in Japan: (1) the need to establish a breast cancer risk model based on the data of Japanese women, (2) the preparation of a screening system suitable for each risk-stratified group, and (3) the need to create breast radiologist positions and team-based care for high-risk women and breast cancer patients. We hope that this chapter will contribute to the creation of such guidelines for high-risk groups in Japan in the near future.

Keywords

BRCA1/BRCA2 mutation carrier · Family history · Lifetime risk · Breast cancer screening · High-risk screening · Risk-based screening · Mammography · Ultrasound · Magnetic resonance imaging · Japanese women

Abbreviations

ADH	Atypical ductal hyperplasia
ALH	Atypical lobular hyperplasia
BI-RADS	Breast Imaging Reporting and Data System
BPE	Background parenchymal enhancement
CESM	Contrast-enhanced spectral mammography
DCIS	Ductal carcinoma in situ
LCIS	Lobular carcinoma in situ
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network

8.1 Introduction

In April 2020, the approval of national healthcare insurance coverage for risk-reducing mastectomy, breast reconstruction, and risk-reducing salpingo-oophorectomy (RRSO) for patients with hereditary breast and ovarian cancer (HBOC) syndrome was an important first step in cancer prevention in women at high risk for breast and ovarian cancers in Japan. It is expected that treatment needs will increase for those who have already developed breast cancer as well as for those who have not. At present, even university hospitals and major hospitals cannot provide comprehensive clinical follow-up to asymptomatic HBOC patients without cancer because of out-of-pocket health expenses and the absence of organized teams of specialists from relevant departments to provide care. Therefore, data about these women are still lacking due to an immature clinical management system and non-mandatory data registry. In addition, it should be noted that these HBOC patients are only one part of the high-risk population for breast cancer.

Table 8.1 Breast cancer risk factors

Hereditary	Personal/hormonal	Breast disease/ histopathological
• Family history of breast cancer • Family history of ovarian cancer • Male breast cancer • Mutation carrier • Ethnicity	• Age at menarche • Age at menopause • Parity • Age at first live birth • Obesity • Alcohol use • Exercise • Hormone replacement therapy with years used • Tumor makers • Breast density on mammography • Chest radiation therapy	• Lobular carcinoma in situ (LCIS) • Atypical lobular hyperplasia (ALH) • Atypical ductal hyperplasia (ADH) • Number of biopsies

In this chapter, we would like to propose risk assessment, risk-based imaging screening, and management guidelines for women at high risk of breast cancer for the Japanese population based on previous breast cancer risk studies in Japan and modeled after the screening system in the USA.

8.2 Comparison Between Japan and the USA

8.2.1 Definition of "High Risk"

Many risk factors for breast cancer have been identified, loosely classified into the following three categories: (1) hereditary, (2) personal/hormonal, and (3) breast disease/histopathological predisposition (Table 8.1).

Table 8.2 delineates which subjects are considered "high risk" for breast cancer per the Japanese Association of Breast Cancer Screening, the American Cancer Society, and the American College of Radiology. "Lifetime risk" per the American Cancer Society is calculated by validated risk assessment models, such as the Tyrer-Cuzick model,[1] BRCAPRO,[2] and Gail model.[3] Among these, the Tyrer-Cuzick model is a cross-sectional coverage of the above three categories and is used in many American facilities. The Tyrer-Cuzick model includes age, BMI, age at menarche, age at menopause, age of first childbirth (if any), use of hormone replacement therapy, *BRCA1/BRCA2* testing results, history of breast biopsy, history of lobular carcinoma in situ (LCIS), atypical ductal hyperplasia (ADH), or atypical lobular

[1] International Breast Cancer Intervention Study (IBIS) Breast Cancer Risk Evaluation Tool version 8. Centre for Cancer Prevention, London. www.ems-trials.org/riskevaluator/

[2] CancerGene with BRCAPRO, MMRpro, PancPRO, and MelaPRO. www4.utsouthwestern.edu/breasthealth/cagene/

[3] Breast Cancer Risk Assessment Tool. National Cancer Institute and National Surgical Adjuvant Breast and Bowel Project. www.cancer.gov/bcrisktool/

Table 8.2 Increased-risk group of major guidelines in Japan and the USA

Japan	USA	USA
Japan Association of Breast Cancer Screening 2013	American Cancer Society 2007 [1]	American College of Radiology (ACR) 2018 [2]
• *BRCA1/BRCA2* germline mutation carriers • Women with two or more first- or second-degree relatives with breast cancer satisfying any of the following criteria: (1) One relative with early-onset breast cancer (under 40 years old) (2) One relative with bilateral breast cancer (3) One relative diagnosed with both breast and ovarian cancer (4) One relative with male breast cancer (5) At least one relative with breast cancer and at least one relative with ovarian cancer	Recommendations for breast MRI screening as an adjunct to mammography 【Based on evidence】[a] • *BRCA1/BRCA2* mutation • First-degree relative of *BRCA1/BRCA2* carrier, but untested • Lifetime risk ~20–25% or greater, as defined by BRCAPRO or other models that are largely dependent on family history 【Based on expert consensus opinion】[b] • Li-Fraumeni syndrome in first-degree relatives • Cowden and Bannayan-Riley-Ruvalcaba syndromes in first-degree relatives • High-dose radiation to chest between age 10 and 30 years 【Insufficient evidence to recommend for or against MRI screening】[c] • Lifetime risk 15–20%, as defined by BRCAPRO or other models that are largely dependent on family history • LCIS/ADH/ALH • Heterogeneously or extremely dense breast on mammography • Women with a personal history of breast cancer, including ductal carcinoma in situ (DCIS)	Population subgroups at higher risk • Women with genetics-based increased risk (and their untested first-degree relatives) • Women with a calculated lifetime risk of 20% or more • Women with histories of chest radiation (cumulative dose of ≥10 Gy before age 30) • Women with personal histories of breast cancer and dense breast tissue • Women with breast cancer diagnosed before age 50 • Women with personal histories of breast cancer not included in the above or with ADH, ALH, or LCIS, MRI should be considered, especially if other risk factors are present • All women, especially African American women and those of Ashkenazi Jewish descent, should be evaluated for breast cancer risk no later than age 30, so that those at higher risk can be identified and can benefit from supplemental screening

[a]Evidence from nonrandomized screening trials and observational studies
[b]Based on evidence of lifetime risk for breast cancer
[c]Payment should not be a barrier. Screening decisions should be made on a case-by-case basis, as there may be particular factors to support MRI. More data on these groups is expected to be published soon

hyperplasia (ALH), breast density on mammography, and family history (including history of breast or ovarian cancer with age at diagnosis, Ashkenazi Jewish ancestry, and presence of deleterious mutations in *BRCA1/BRCA2*) as risk factors for assessment of 10-year risk and lifetime risk. None of these risk assessment tools is perfect, but they are useful for reference when teaching women about breast cancer risk factors and for designing a treatment plan.

The National Comprehensive Cancer Network (NCCN) Guidelines Version 1.2021, which is used as a clinical practice guide worldwide, includes many breast cancer-associated genes in addition to deleterious mutations in *BRCA1/BRCA2*. The lifetime risk of developing breast cancer in female carriers of deleterious germline mutations in *BRCA1* and *BRCA2* is approximately 40–87% and 18–88%, respectively [3–6]. Regarding other genes, it was reported that those with deleterious germline mutations in *ATM* are at lifetime risk of approximately 27%, *CHEK2* 29%, *NBN* 23%, *NF1* 26%, *PALB2* 45%, *PTEN* 85%, *STK11* 32%, *TP53* 95%, and *CDH1* 53% [7, 8] (Table 8.3).

In terms of histopathological risks, atypical epithelial hyperplasias (including LCIS, ADH, and ALH) are considered high-risk lesions. Women with lobular neoplasias (LCIS and ALH) have the risk for subsequent invasive breast cancer over 15–20 years of 10–20% [9]. For women diagnosed with LCIS, both breasts are at risk for invasive carcinoma, with more than 50% of subsequent cancers occurring more than 15 years after the original diagnosis of LCIS [9]. Women with ADH are also at risk for invasive breast cancer. During follow-up for 17 years, the relative risk for invasive cancer is six- to tenfold for women with a history of LCIS, and four- to fivefold for those with a history of ADH. The American College of Radiology and NCCN Guidelines recommend that supplemental MRI screening be considered after taking into account other risk factors (family history, past medical history, dense breasts, etc.). Those with a personal history of chest radiation therapy (cumulative dose of 10 Gy or greater) before age 30 are also considered to be at high risk of breast cancer about 8 years after treatment [10, 11].

The 2010 the Japan Breast Cancer Society Group Study published "Research on Countermeasures for Hereditary Breast Cancer/Ovarian Cancer Patients and Preclinical Patients in Japan" (Nakamura Group). In 2015, the clinicopathologic differences in malignancies in *BRCA1* versus *BRCA2* mutation carriers in Japan were reported [12]. Among 260 Japanese proband cases with strong family history of breast cancer according to the NCCN guidelines, 17.7% were positive for *BRCA1* and 13.5% were *BRCA2*. The most prevalent relevant site of mutation on *BRCA1* was L63X, which might be a founder mutation unique to the Japanese population.

Table 8.3 Gene associated with breast cancer per NCCN Guidelines

Risk	Gene
Increased risk of breast cancer	*BRCA1*, *BRCA2*
Increased risk of female breast cancer	*ATM*, *CHEK2*, *NBN*, *NF1*, *PALB2*, *PTEN*, *SKT11*, *TP53*
Increased risk of female lobular breast cancer	*CDH1*

62.2% of *BRCA1* mutation carriers with breast cancer were triple negative, while 82.9% among *BRCA2* mutations developed luminal, hormone-driven cancers. This research led to the establishment of the Japanese HBOC Consortium in 2012, with its main task being the transfer and registration of individual data of the HBOC families to the Japanese Organization of Hereditary Breast and Ovarian Cancer (JOHBOC), established in 2016. Based on non-Japanese guidelines, "Guidebook for Diagnosis and Treatment of Hereditary Breast and Ovarian Cancer Syndrome 2017" was published in Japan. At present, there is a tendency for only *BRCA1/BRCA2* mutation carriers to be treated as "high risk" in Japan. Since genetic testing for *BRCA1/BRCA2* has become more commonplace in Japan, assessment criteria to select subjects who should be tested have been created. However, the criteria and risk prediction tools are quite different from "lifetime risk" in worldwide guidelines and are insufficient for Japanese women because comprehensive evaluation has been rarely discussed in Japan.

8.2.2 Role of Breast Imaging Radiologists in the USA

In Japan, there are very few radiology facilities with sub-specialized, dedicated breast imaging radiologists, and even fewer with sub-specialized breast radiologists engaged in mammography, ultrasound, MRI interpretation, and image-guided biopsies.

In the USA and Europe, breast imaging is a subspecialty within radiology, complete with its own Society of Breast Imaging (SBI) in the USA and the European Society of Breast Imaging (EUSOBI) in Europe. In the USA, academic radiology departments at most hospitals are frequently subdivided by organ system into specialties such as neuroradiology, breast imaging, and musculoskeletal radiology. Breast radiologists often staff dedicated breast imaging centers as outpatient clinics. Reading rooms for breast imaging are located near imaging examination rooms at hospitals with breast imaging centers. The roles required of a breast radiologist are as follows (although there are minor differences depending on institution):

- Oversee imaging technologists and provide input as needed.
- Interpret mammograms, breast ultrasounds, and breast MRIs according to American College of Radiology Breast Imaging and Data Reporting System (BI-RADS).
- Supervise and discuss results of diagnostic imaging with patients to provide immediate communication and follow-up recommendations and to answer questions directly.
- Report BI-RADS assessment category with a management recommendation (e.g., annual screening by mammogram, second-look diagnostic ultrasound, or MRI-guided biopsy).
- Report breast density, which is required by law in many states.
- Send summary letters or reports to patients in simple language with their breast cancer risk noted.

- Act as expert consultants to referring clinical physicians, including aiding in the choice of the proper imaging and presenting imaging for patients at tumor board.
- Conduct diagnostic image-guided procedures such as stereotactic, ultrasound-guided, or MRI-guided core biopsies, wire localization for excisional biopsy, or surgery using all imaging modalities.
- Report concordance/discordance with pathology result to clinical physicians.
- Ensure national quality standards are met for all equipment.
- Perform yearly audit of radiologist's screening mammogram callback rate (<10%), recall, and biopsy recommendation to ensure that relevant positive predictive values are within society guidelines.
- Train residents and dedicated breast imaging fellows in the art of breast imaging.

8.2.3 High-Risk Program Clinic in the USA

The high-risk program based in an outpatient clinic platform is still unfamiliar in Japan, but it is expected that care will be provided to all high-risk women who have not already developed cancer once national reimbursement for patients with cancer is available.

We introduce an American outpatient high-risk program as a model below.

Clinic Goals

- Promote preventive care.
- Provide genetic counseling and education by experts.
- Evaluate individual risk.
- Stratify risk and tailor management according to risk level.
- Establish research collaborations.

Team Management

High-risk breast clinic management is supported by a multidisciplinary team with patient-centered care focusing on the needs of patients. The multidisciplinary team includes surgical breast oncologists, medical oncologists, radiation oncologists, radiologists, pathologists, nurse practitioners, genetic counselors, psychologists, and social workers. These experts regularly conduct multidisciplinary conferences multiple times per week.

Services Provided

In the USA, women usually meet their primary care physicians (PCPs) or gynecologists first. These providers determine which specialists to consult. The high-risk breast clinic is managed mainly by medical oncologists, surgical oncologists, nurse practitioners, and genetic counselors. The service is as follows:

1. Risk assessment and guidelines for high-risk women in the USA.

   Lifetime risk is usually first assessed according to the Tyrer-Cuzick model and/or the Gail model.

   Many expert groups have offered their recommendations for breast cancer screening, and much of the controversy lies in the fact that there is not a consensus about when to begin and end screening, how often to screen, and by which modality. These groups include the following:
   - American Cancer Society (ACS)
   - American College of Obstetricians and Gynecologists (ACOG)
   - American College of Physicians (ACP)
   - American College of Radiology (ACR)
   - American Academy of Family Physicians (AAFP)
   - American Medical Association (AMA)
   - American Society of Breast Surgeons (ASBrS)
   - National Cancer Institute (NCI)
   - National Comprehensive Cancer Network (NCCN)
   - National Consortium of Breast Centers (NCBC)
   - Society of Breast Imaging (SBI)
   - United States Preventive Services Task Force (USPSTF)

   Breast radiologists follow ACR, SBI, or NCCN Guidelines.
2. Genetic counseling and education.

   If a patient has a lifetime risk of breast cancer of 20% or more or if the patient wishes, physicians may recommend individual assessment by genetic counselors. By the end of the visit, each patient gains a thorough understanding of what it means to be high risk through personalized discussions of the genetic and lifestyle variables that affect breast cancer risk and the emotional impact of a high-risk designation. Counselors listen to patients' concerns and help them weigh their options and develop plans.
3. Clinical breast exam.

   Routine breast physical exams are typically provided by PCPs, gynecologists, and any of the providers in the high-risk clinic. They perform breast exams on asymptomatic women every 6 months.
4. Imaging-based screening.

   According to lifetime risk, the above experts may advise additional screening imaging for patients. In particular, imaging management is supported by breast imaging radiologists, who are familiar with all modalities related to breast imaging and appropriate clinical management for women at increased risk of breast cancer. At the breast imaging center, radiologists provide explanations and discuss the result of imaging examinations or the clinical indication for next steps with patients at increased breast cancer risk in addition to interpreting imaging and performing diagnostic procedures.
5. Counseling in lifestyle modification.

   Physicians or nurse practitioners work with women at increased risk of breast cancer to develop personalized risk-management plans. Plans might include additional screening or close surveillance. These include options to reduce risk, including prophylactic mastectomy and oophorectomy. Preventive chemother-

apy by tamoxifen could be also considered for such patients to reduce the risk of invasive breast cancer whether or not they've gone through menopause.

6. Personalized surveillance plans.

   A common high-risk screening protocol includes annual screening mammography and annual supplemental MRI, staggered at 6-month intervals so the woman undergoes imaging every 6 months.

8.3 Appropriate Clinical Management for High-Risk Women in Japan

8.3.1 The New Era of Personalized, Risk-Based Screening

Why do we need to use risk models in the general population? First, it is to identify women that may be at high risk, second to educate women about their own risk, and third to provide appropriate risk-based screening. For women at high risk of breast cancer, early and more intensive screening should be pursued compared with the average-risk group. From the perspective of preventive medicine, we must eventually discuss intensive surveillance versus chemoprevention versus prophylactic surgery based on scientific data. Personalized cancer screening and clinical management will be in high demand in the future. In Japan, in addition to genetic predisposition, personal and histopathological predispositions should be taken into consideration with outcomes data to develop stratified risk models for Japanese women. With such tools, formal risk assessment, genetic counseling, risk-based imaging management, and risk reduction strategies should be applied.

8.3.2 Risk-Based Imaging Management

8.3.2.1 Clinical and Imaging Features of Women at High Risk for Breast Cancer

Features of *BRCA1/BRCA2* Germline Mutation Carriers

Imaging features of cancers sometimes differ among risk categories [13]. The image feature comparison of *BRCA1* and *BRCA2* mutation carriers is listed below with reference to past reports [12–15].

- BRCA1
  - Absence of microcalcifications [13, 15]
  - Lower sensitivity of mammography [14]
  - Lower proportion of ducal carcinoma in situ (DCIS) [13–15]
  - High prevalence of tumors with benign morphologic features, fibroadenomas, or even cysts [13]
  - Higher proportion of triple-negative breast cancer [12]
  - Younger age at diagnosis [14]

  - Larger incidence of interval cancer (cancers that are diagnosed within a year after a negative screening mammogram) [14]
  - Larger tumor size at diagnosis [14]
- BRCA2
  - Approximately half of cases have microcalcifications [13, 15].
  - Larger proportion of luminal, hormone receptor-positive cancers [12].
- BRCA1 and BRCA2
  - Tendency to occur toward a posterior portion (prepectoral region) [13, 15]

Cancers in women with *BRCA* may have benign features such as round shape and the radiologist must be wary.

In addition, radiologists must carefully assess when reporting BI-RADS [16] category 3 for such high-risk populations. The previous study by Chikarmane et al. found that the cancer rate in patients with a BI-RADS category 3 lesion and with a genetic mutation or personal history of breast cancer was 3.8% (11/288), whereas no malignancies were found in those without family history or personal history [0% (0/147)] [17]. Caution is advised when assigning BI-RADS 3 category assessment to women with *BRCA1* or *BRCA2* due to higher likelihood of cancer.

Breast Density

Breast density is a known risk factor for developing breast cancer. As of July 17, 2020, 38 out of 50 states in the USA had state patient notification laws mandating that radiologists inform women of their breast density. Reasons for this include the masking effect associated with increased breast density as well as the increased risk of developing breast cancer. Dense breast tissue does not carry the same degree of cancer as predisposing genetic mutations or a strong family history, but breast density is included as a risk factor in the Tyrer-Cuzick model. The BCRAT model, BCSC model, and Rosner and Colditz model risk assessment tools include breast density as one of the risk evaluation items [18]. Previous reports indicate that those with heterogeneously dense breasts have a 3.39-fold increased risk of developing breast cancer [19] and those with extremely dense breasts have a 4.7-fold [20] compared to those with almost entirely fatty breasts. In addition, in women with heterogeneously dense or extremely dense breasts, an overall 16% increase in diagnosis of breast cancer was demonstrated, a 40% increase in interval cancers was noted, and a 12% increase in screen-detected cancer was observed [20]. In this study, it is expected the reason for the increase in the risk of breast cancer is because of the masking effect rather than the rapid growth of tumors and that a more personalized schedule of screening could improve this issue.

In Japan, there has been a strong correlation between dense breasts and the risk of developing breast cancer as reported by a multicenter population-based case-control study by Nishiyama et al. in 2019 [21] in postmenopausal women and obese women. The odds ratio for development of breast cancer in those with extremely dense breasts compared to almost entirely fatty breasts was 2.85 (Table 8.4) in postmenopausal women and 11.89 in obese women [21].

Table 8.4 Odds ratio of mammographic density for breast cancer adjusted for age, body mass index, parity and breast feeding for all patients, and according to menopausal status; study from Japan [21]

Mammographic density[a]	All (n = 1572)		Premenopausal (n = 534)	Postmenopausal (n = 1038)
C1	1.00	(Ref)	1.00 (Ref)	1.00 (ref)
C2	1.54	(1.15–2.06)	0.96 (0.53–1.72)	1.90 (1.34–2.70)
C3	1.08	(0.77–1.53)	0.65 (0.36–1.17)	1.31 (0.82–2.06)
C4	2.05	(1.17–3.58)	1.06 (0.49–2.31)	2.85 (1.10–7.16)
P for trend	0.17		0.37	0.03

[a]Each mammogram was assessed for breast density according to the BI-RADS breast density categories: C1, almost fatty (<25% glandular); C2, scattered fibroglandular densities (25–50% glandular); C3, heterogeneously dense (51–75% glandular); C4, extremely dense (>75% glandular)

Asian women have a significantly higher prevalence of increased breast density compared to other ethnicities [22]. Physicians in future high-risk clinics should comprehensively evaluate risk, including breast density. It is also important for women to be informed of their breast density and cancer risks. In this process, it is essential to have a shared decision-making conversation taking into account the health literacy of the patient. Some of the report notification letters in the USA are working on improving readability for their patients [23, 24]. Supplemental screening modalities should be reviewed based on Japanese data. Kuhl et al. state that the MRI is useful as supplemental screening even for women at average risk with dense breasts in terms of higher detection rate and greater sensitivity compared with ultrasound and the interval cancer rate of zero in MRI screening [25].

Background Parenchymal Enhancement (BPE)

BPE is the enhancement degree of normal fibroglandular tissue on breast MRI, which has been included in the BI-RADS lexicon since the latest version. The most typical pattern of normal BPE is bilateral, fairly symmetric, and diffuse, with slow minimal or mild early enhancement and persistent delayed enhancement [26]. BPE could affect MRI interpretations with both false-positive and false-negative results. The level of BPE is hormone-sensitive, particularly to serum estrogen concentrations [27], and affected by menopausal status, hormonal treatment, breast density, and prior breast radiation therapy [28]. Efforts should be made to schedule premenopausal patients for breast MRIs during the optimal time period in their menstruation cycles. Tamoxifen, aromatase inhibitors, and a personal history of risk-reducing salpingo-oophorectomy (RRSO) reduce the level of BPE [28–31]. Recently, BPE has been reported as one of the independent risk factors associated with breast cancer development [32–34]. In high-risk women, the odds ratio of high BPE to low BPE is 2.1–9 [33, 35–37]. This is in accord with the correlation of RRSO with a reduction in the incidence of breast cancer [38]. We should be cautious in reading MR images of women with a high level of BPE, understanding that small malignant lesions might be masked. A disclaimer should be added to the

reports for women with marked BPE as is done on mammogram reports in women with dense breast tissue.

8.3.2.2 Imaging Modality Choice

Mammography: Limitations of Mammography

The NCCN Guidelines recommend starting mammographic screening at an age younger than 40 years for many high-risk women. The ACR indicates that while *BRCA2* carriers are more frequently detected only by mammographic calcification, *BRCA1* carriers may have less benefit from mammography before age 40 [2]. In the Netherlands, it has been suggested that *BRCA1* mutation carriers should primarily be screened with breast MRI with mammogram added at 40 years old [39]. The same has been suggested for all women at high risk due to the limited added value of cancer detection with mammography [40]. A study by Schrading and Kuhl proposed different management plans for *BRCA1* and *BRCA2* mutation carriers. For young *BRCA1* mutation carriers, they proposed MRI (with or without ultrasound) as opposed to mammography due to lack of calcifications seen in *BRCA1*-associated cancers. For *BRCA2* mutation carriers and other women at increased risk for breast cancer, they proposed mammography to evaluate for the microcalcifications typically seen in *BRCA2*-associated cancers [13].

In Japan, mammography once every 2 years is generally recommended for all women aged 40 and over as population-based screening by the Ministry of Health, Labour and Welfare. Surveillance guidelines for high-risk women have not yet been developed. Compared to Europe and the USA, there is a higher proportion of dense breasts among Japanese women [41], and considering that the subjects of high-risk groups are younger, the proportion of dense breasts inevitably become higher. Thus, the question remains whether mammography is essential systemically for the whole high-risk group in Japanese women. It should be noticed that mammography may be beneficial in Japanese females with *BRCA2* mutations as in other countries [15]. We need more scientific evidence based on data in Japanese women with and without breast cancer [42].

Since its approval by the Food and Drug Administration (FDA) in 2011, tomosynthesis has been gaining rapid popularity and is set to replace mammography [43, 44]. Per an ACR statement issued in November 2014:

> The ACR position on digital breast tomosynthesis (DBT) is that it is no longer investigational. Tomosynthesis has been shown to improve key screening parameters compared to digital mammography. The College has assembled information to assist members in working with private payers to secure coverage of this important technology.

Tomosynthesis is not yet a common screening modality due to lack of national health insurance coverage in Japan and clinician preference for ultrasound. It may be essential for Japanese women to learn the pros and cons of tomosynthesis [45].

Contrast-enhanced spectral mammography (CESM), which was approved by the FDA in 2011 and has been gradually expanding into clinical practices, is expected to be a valuable alternative method for high-risk screening [46]. Since it reflects

increased blood supply from pathologic neoangiogenesis similarly to contrast-enhanced MRI, diagnostic performance of CESM provides comparable results to MRI [47–50] although specificity for lesion detection is also limited due to BPE [51]. It is not widely available due to non-coverage of CESM by health insurance in both Japan and the USA. In addition, there are concerns about increased ionizing radiation dose of 20–80% above a standard mammogram [46] and the requirement for iodinated contrast, with the potential for nephrotoxicity and contrast reactions.

Ultrasound: Differences Between Japan and the USA

In the USA, ultrasound is rarely used as a primary breast cancer screening modality, but rather for diagnostic purposes or supplemental screening with mammography. The superiority of mammogram screening over ultrasound screening was demonstrated in seven large, randomized, controlled studies showing an average of 30% reduced mortality using mammography for screening. The ACR announced that ultrasound is not suitable as an independent screening tool based on the ACRIN 6666 study [52, 53] in women in the USA with heterogeneously or extremely dense breasts and at least one other risk factor (such as family history, history of high risk lesions, history of chest radiation therapy, etc.) [2]. Compared to mammography, reasons include higher false-positive rates (8.1% with ultrasound vs. 4.4% with mammography), lower positive predictive value (baseline prevalence round of 8.9% in ultrasound vs. 22.6% in mammography and subsequent incidence rate of 11.7% for ultrasound vs. 38.1% in mammography), high dependency on the operator, and the burden of time and labor.

Regarding automated whole-breast ultrasonography (ABUS) approved by the FDA in 2012, it seems that the limitations include the high false-positive rates, the need to obtain additional handheld ultrasounds for indeterminate results, the large volume of imaging data, the interpretive time by radiologists, and artifacts [54]. Further improvement of artifact issues is necessary [55, 56].

On the other hand, ultrasound could be of great significance to Japanese women, as shown by the results of J-START in which the combination of ultrasound with mammography increased the cancer detection rate by 1.5 times that of mammography alone [57]. The proportion of women with dense breasts in Japan is higher than in the USA and Europe [41]. In addition, considering that the size of the breasts of the average Japanese woman is smaller than that of US women [41] and that ultrasound is a cost-effective and convenient examination compared to MRI, it is reasonable to add ultrasound to mammography to provide a comprehensive assessment. However, in Japan, some clinicians believe that ultrasound is sufficient without MRI even in high-risk populations. MRI screening for high-risk women without cancer is still a big hurdle due to out-of-pocket expenses. We should not deviate from worldwide guideline standards without collecting data about high-risk Japanese women. Until scientific data is collected to prove otherwise, it would be appropriate to provide MRI for such patients until Japanese data is available.

Pursuit of Quality-Assured MRI

Until the 1990s, MRI screening was not popular for breast cancer screening. In 1994 and 1995, *BRCA1* and *BRCA2* mutations were identified [58, 59].

Mammography was considered inadequate for *BRCA* carriers, who not only have high lifetime risk but also are diagnosed at a younger age. Kuhl et al. began studying MRI screening for women with *BRCA1/BRCA2* mutations shortly thereafter in Germany [60]. As a result of studies accumulated in a large number of facilities after the publication of the group's research in 2000, MRI screening was rapidly introduced for women with *BRCA1/BRCA2* mutations [61–63]. In the USA, annual MRI screening is recommended by the American Cancer Society guidelines not only for *BRCA* mutation carriers but also for women with a lifetime risk of breast cancer of 20% or more [64]. Other international guidelines such as ACR, ASBrS, EUSOBI [65], National Institute for Health and Care Excellence (www.nice.org.uk/guidance/cg164), and Cancer Australia (gov/au/clinical-best-practice/breast-cancer/screening-and-early-detection/mri-high-risk-women) have also recommended supplemental annual screening breast MRIs in addition to mammography for women at high risk for breast cancer. However, screening guidelines such as the recommended starting age, supplemental imaging modality(ies), schedule of screening, and the definition of "high risk" vary based on country [66]. Kuhl et al.'s EVA trial screened 687 women with a lifetime breast cancer risk of >20% with clinical breast exam, mammography, ultrasound, and/or MRI in various combinations. Of the 27 women diagnosed with breast cancer, 11 were diagnosed with ductal carcinoma in situ and 16 developed invasive breast cancers, including 3 with node-positive disease. The "cancer yield" of mammogram was determined to be 5.4/1000, 6.0/1000 for ultrasound, 7.7/1000 with mammogram plus ultrasound, and 14.9/1000 for MRI [63]. The frequency of breast cancer identified by MRI alone in *BRCA* mutation carriers was 3.0% (7/236) [67] in a Canadian female study and 9.1% (2/22) [67] in a Japanese female study.

High-quality MRI screening is a pre-requisite for diagnosing women at high risk for breast cancer. Per NCCN Guidelines, "the criteria for high-quality breast MRI includes a dedicated breast coil, the ability to perform biopsy under MRI guidance, radiologists experienced in breast MRI, and regional availability. Breast MRI is preferably performed on days 7–15 of a menstrual cycle for premenopausal women." The EVA trial for women at elevated familial risk of breast cancer pursued quality-assured MRIs by requiring (1) radiologist experience with at least 200 breast MRI studies per year, (2) verifiable radiologist experience with MR-guided biopsies (wire localization and/or MR-guided vacuum biopsy), and (3) a preliminary version of the MR-BIRADS lexicon to organize interpretation and reporting, including training in the proper application of MR-BIRADS terminology, including for DCIS [63]. In order to carry out high-risk screening in Japan, it is essential to make efforts to increase the number of facilities nationwide that meet these criteria for high-quality breast MRI. In particular, MRI-guided biopsy, which tends to be sparingly used in Japan, plays a very important role for high-risk women in other countries, and it will be necessary to popularize and train practitioners in Japan [68].

Kuhl et al. proposed an abbreviated MRI in 2014 to compensate for issues such as high cost and long imaging times associated with MRI screening. They reported achieving a negative predictive value of 99.8% with their updated screening technique, which costs an MRI acquisition time of 3 min and an expert radiologist

maximum-intensity projection (MIP) image reading time of 3 s [69]. For high-risk women, Kuhl proposes that the conventional full diagnostic protocol be applied for the first (prevalent) MRI, with a possible switch to abbreviated protocols during later screening, when a comparison with previous examination findings could assist interpretation [70].

8.3.2.3 Suggestions for Risk-Based Screening Management

In a particularly informative clinical correlation study performed by Vreemann et al., MRI and mammography screening were retrospectively analyzed for evidence of malignancy after prophylactic bilateral mastectomy in women with *BRCA1* and *BRCA2* mutations, a significant family history, or a personal history of breast cancer [71]. Imaging sensitivity for cancers found on pathology after prophylactic mastectomy was 81.3% for women with *BRCA1* mutations, 92% for women with *BRCA2* mutations, 95% for women at high risk for breast cancer due to family history, and 91% for women with a history of breast cancer. More intensive screening and prophylactic surgery are recommended for *BRCA1* carriers due to this relative lack of imaging sensitivity. Rijnsburger et al. have suggested MRI screening twice a year as one of possible options for *BRCA1* mutation carriers [14]. At some US medical institutions, screening mammogram is omitted from the screening regimen for women with *BRCA1* mutation, and MRI is the main screening modality for these women. In women with germline *BRCA2* mutations, annual MRI and mammography alternating every 6 months with or without the addition of prophylactic therapy (e.g., bilateral salpingo-oophorectomy or tamoxifen) might be more appropriate [72]. When to perform imaging and which type should be reviewed based on the most recent available scientific literature and guidelines. For Japanese high-risk women, we propose a baseline MRI (unabbreviated) be performed with supplemental diagnostic ultrasound if necessary, followed by ultrasound screening 6 months later with mammogram if indicated, followed by abbreviated MRI screening alternating with ultrasound every 6 months (Fig. 8.1).

For screening in an intermediate-risk group (lifetime risk between 13% and 19%), we consider a study of MRI screening performed on 2181 average-risk women (40–70 years old, lifetime risk 6–12% in the Gail model) [25]. This study demonstrated that (1) 60 out of 61 cancers were detected only by MRI (all 48 lesions identified in the initial screening could only be detected by MRI), (2) no cancer was detected by mammography or ultrasound alone, (3) no interval cancers were observed, (4) the average time until occurrence of incident cancer was 34.9 months, (5) no one was diagnosed with cancer during the 2-year follow-up period, (6) additional cancer yield by supplemental MRI screening was 15.5 per 1000, which was

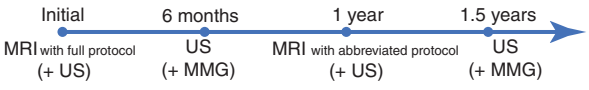


Fig. 8.1 A proposed screening algorithm for high-risk Japanese women. *US* Ultrasound, *MMG* Mammography

substantially higher than tomosynthesis (1.2/1000) and ultrasound (3.5–4.4/1000), (7) MRI-detected lesions were very small with a median of 8 mm, and 93.4% were lymph node negative. For the average-risk group, it was implied that MRI examination every 3 years might be sufficient. Considering the abovementioned high-risk group that requires annual MRI screening, MRI screening every 1–3 years may be sufficient for the intermediate-risk group.

We also would like to discuss high-risk lesions (LCIS, ADH, and ALH). These lesions are included as a risk factor in the Tyrer-Cuzick model, and they often contribute to a lifetime breast cancer risk of >20%. NCCN Guidelines recommend that MRI screening be considered. In a study by Sung et al. evaluating MRI follow-up in women with a history of LCIS [73], the cancer detection rate was 2.0% (17 cancers/840 screening rounds). Two reports of MRI screening for women with ADH or ALH showed a cancer detection rate of 0–1.5% [74, 75]. Since the cancer detection rate with supplemental MRI screening in women with average risk [25] was 1.6% (61 cancers/3861 screening MRI studies), annual supplemental MRI screening for women with a history of atypia hyperplasia lesions may not be useful. However, since these two reports were performed between 1999–2005 and 2005–2011, respectively, the low detection rate might depend on the quality of MRI devices at that time. In addition, according to our literature review, there were no studies shown about specific morphologic kinetic and other imaging features of high-risk lesions to predict malignancy upgrade [76–79]. We believe that there is room for further consideration.

Lastly, we would like to discuss screening for patients with a history of therapeutic radiation therapy to the chest (e.g., previous treatment for Hodgkin's lymphoma in the mediastinum of a 30-year-old woman) [80]. In a total of 247 screenings of 91 radiation-treated women, 10 cancerous lesions were identified (of which 4 were identified on MRI, 3 on mammogram, and the remaining 3 on both). The addition of MRI to mammography increased the cancer detection rate by 4.4%, which is estimated to be in line with the cancer detection rate of the first prevalence round in other high-risk groups [60, 61, 81]. Considering the overall risk of each individual would be helpful for women with elevated risk in terms of the tailored screening system for them.

8.4 Training of Breast Imaging Experts in Japan

8.4.1 Necessity of Breast Imaging Specialists

Since insurance coverage of MRI surveillance began in April 2020 in Japan, radiologists in Japan are under higher demand. Recommended follow-up timing and biopsy protocols should be written, as well as assessment of BI-RADS categorizations. Radiologists should be able to give appropriate advice to each patient after understanding and weighing the imaging modalities described above and familiarizing themselves with the patient's clinical status and breast pathology, if any. Training of breast imaging radiologists is a pressing task, especially in facilities

with high-risk breast cancer program clinics. The authors think the Japan Radiological Society should support facilities nationwide that provide breast cancer screening and clinics for high-risk patients. It is an important responsibility of the radiologist to make efforts for comprehensive and appropriate image management. Surgeons, oncologists, and genetic counselors depend on radiologists to provide ideal high-risk screening.

8.4.2 Communication with Patients via Outpatient Clinics and Reporting

Although it is very common for breast imaging radiologists in the USA to communicate directly with patients, how many Japanese radiologists communicate with patients at outpatient clinics? The most comprehensive outpatient management should be done by clinicians, but the radiologist should be responsible for detailed explanation of the diagnostic imaging results and decisions such as short-term follow-up and biopsy recommendations to high-risk patients, particularly if dealing with sensitive genetic information. In reporting, radiologists are also required to have interpretation skills to take into account the risks of each individual. Context about risk in reports is also very important. It is essential for patients to be aware of their own risk of breast cancer in order to make educated decisions regarding screening and to follow an appropriate screening program.

8.4.3 Team-Based Care for Women at High Risk for Breast Cancer

One of the most important factors in patient care is a team-based care system. Management of patients at high risk for breast cancer needs professional support not only in medical care but also in meeting psychosocial needs. In Japan, the number of facilities that have introduced the multidisciplinary tumor board seems to be gradually increasing. Multidisciplinary care is also important in the prevention of breast cancer in women at high risk.

8.5 Conclusion

Japan lags behind Europe and the USA in screening women at high risk for breast cancer. It is important to keep in mind that the Western system does not fully apply to Japan, and so we must construct our own high-risk screening system. First, we need to include other women at high risk for breast cancer in addition to those with deleterious germline mutations in *BRCA*. Management strategies for all women at increased risk of breast cancer may have to be tailored in accordance with their stratified risk category. In addition, advances in diagnostic imaging and care management are expected to contribute greatly to a future of personalized screening and

preventive medicine. The authors urge radiologists in Japan to seriously discuss these pressing issues for the future of our patients.

References

1. Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin. 2007;57(2):75–89. http://www.ncbi.nlm.nih.gov/pubmed/17392385
2. Monticciolo DL, Newell MS, Moy L, Niell B, Monsees B, Sickles EA. Breast cancer screening in women at higher-than-average risk: recommendations from the ACR. J Am Coll Radiol. 2018;15(3):408–14.
3. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips K-A, Mooij TM, Roos-Blom M-J, et al. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. JAMA. 2017;317(23):2402–16. http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2017.7112
4. Engel C, Fischer C. Breast cancer risks and risk prediction models. Breast Care (Basel). 2015;10(1):7–12. http://www.ncbi.nlm.nih.gov/pubmed/25960719
5. Mavaddat N, Peock S, Frost D, Ellis S, Platte R, Fineberg E, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. J Natl Cancer Inst. 2013;105(11):812–22. http://www.ncbi.nlm.nih.gov/pubmed/23628597
6. Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S, McGuffog L, et al. Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. JAMA. 2015;313(13):1347–61. http://www.ncbi.nlm.nih.gov/pubmed/25849179
7. Easton DF, Pharoah PDP, Antoniou AC, Tischkowitz M, Tavtigian SV, Nathanson KL, et al. Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med. 2015;372(23):2243–57. http://www.ncbi.nlm.nih.gov/pubmed/21900617
8. Shiovitz S, Korde LA. Genetics of breast cancer: a topic in evolution. Ann Oncol. 2015;26(7):1291–9. https://doi.org/10.1093/annonc/mdv022.
9. Arpino G, Laucirica R, Elledge RM. Premalignant and in situ breast disease: biology and clinical implications. Ann Intern Med. 2005;143(6):446–57. http://www.ncbi.nlm.nih.gov/pubmed/16172443
10. Bhatia S, Yasui Y, Robison LL, Birch JM, Bogue MK, Diller L, et al. High risk of subsequent neoplasms continues with extended follow-up of childhood Hodgkin's disease: report from the late effects study group. J Clin Oncol. 2003;21(23):4386–94. http://www.ncbi.nlm.nih.gov/pubmed/14645429
11. Kenney LB, Yasui Y, Inskip PD, Hammond S, Neglia JP, Mertens AC, et al. Breast cancer after childhood cancer: a report from the childhood cancer survivor study. Ann Intern Med. 2004;141(8):590–7. https://linkinghub.elsevier.com/retrieve/pii/S0084395407702683
12. Nakamura S, Takahashi M, Tozaki M, Nakayama T, Nomizu T, Miki Y, et al. Prevalence and differentiation of hereditary breast and ovarian cancers in Japan. Breast Cancer. 2015;22(5):462–8. https://doi.org/10.1007/s12282-013-0503-1.
13. Schrading S, Kuhl CK. Mammographic, US, and MR imaging phenotypes of familial breast cancer. Radiology. 2008;246(1):58–70. https://doi.org/10.1148/radiol.2461062173.
14. Rijnsburger AJ, Obdeijn IM, Kaas R, Tilanus-Linthorst MMA, Boetes C, Loo CE, et al. BRCA1-associated breast cancers present differently from BRCA2-associated and familial cases: long-term follow-up of the Dutch MRISC screening study. J Clin Oncol. 2010;28(36):5265–73.
15. Murakami W, Tozaki M, Nakamura S, Ide Y, Inuzuka M, Hirota Y, et al. The clinical impact of MRI screening for BRCA mutation carriers: the first report in Japan. Breast Cancer. 2019;26(5):552–61. https://doi.org/10.1007/s12282-019-00955-6.

16. Ishibashi H, Miyamoto M, Shinnmoto H, Murakami W, Soyama H, Nakatsuka M, et al. Cervical varicosities may predict placenta accreta in posterior placenta previa: a magnetic resonance imaging study. Arch Gynecol Obstet. 2017;296(4):731–6.
17. Chikarmane SA, Birdwell RL, Poole PS, Sippo DA, Giess CS. Characteristics, malignancy rate, and follow-up of BI-RADS category 3 lesions identified at breast MR imaging: implications for MR image interpretation and management. Radiology. 2016;280(3):707–15. http://www.ncbi.nlm.nih.gov/pubmed/17392385
18. Louro J, Posso M, Hilton Boon M, Román M, Domingo L, Castells X, et al. A systematic review and quality assessment of individualised breast cancer risk prediction models. Br J Cancer. 2019;121(1):76–85. https://doi.org/10.1038/s41416-019-0476-8.
19. Yaghjyan L, Colditz GA, Collins LC, Schnitt SJ, Rosner B, Vachon C, et al. Mammographic breast density and subsequent risk of breast cancer in postmenopausal women according to tumor characteristics. J Natl Cancer Inst. 2011;103(15):1179–89. https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/djr225
20. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med. 2007;356(3):227–36. www.nejm.org
21. Nishiyama K, Taira N, Mizoo T, Kochi M, Ikeda H, Iwamoto T, et al. Influence of breast density on breast cancer risk: a case control study in Japanese women. Breast Cancer. 2020;27(2):277–83.
22. Tice JA, Cummings SR, Smith-Bindman R, Ichikawa L, Barlow WE, Kerlikowske K. Using clinical factors and mammographic breast density to estimate breast cancer risk: development and validation of a new predictive model. Ann Intern Med. 2008;148(5):337–47. http://www.ncbi.nlm.nih.gov/pubmed/18316752
23. Nguyen DL, Ambinder EB, Jones MK, Mullen LA, Harvey SC. Improving state-mandated breast density notifications. J Am Coll Radiol. 2020;17(3):384–90. https://doi.org/10.1016/j.jacr.2019.08.023.
24. Saraiya A, Baird GL, Lourenco AP. Breast density notification letters and websites: are they too "dense"? J Am Coll Radiol. 2019;16(5):717–23. https://doi.org/10.1016/j.jacr.2018.11.007.
25. Kuhl CK, Strobel K, Bieling H, Leutner C, Schild HH, Schrading S. Supplemental breast MR imaging screening of women with average risk of breast cancer. Radiology. 2017;283(2):361–70. http://www.ncbi.nlm.nih.gov/pubmed/28221097
26. Giess CS, Yeh ED, Raza S, Birdwell RL. Background parenchymal enhancement at breast MR imaging: normal patterns, diagnostic challenges, and potential for false-positive and false-negative interpretation. Radiographics. 2014;34(1):234–47. http://www.ncbi.nlm.nih.gov/pubmed/24428293
27. Brooks JD, Sung JS, Pike MC, Orlow I, Stanczyk FZ, Bernstein JL, et al. MRI background parenchymal enhancement, breast density and serum hormones in postmenopausal women. Int J Cancer. 2018;143(4):823–30. http://www.ncbi.nlm.nih.gov/pubmed/29524207
28. Sogani J, Morris EA, Kaplan JB, D'Alessio D, Goldman D, Moskowitz CS, et al. Comparison of background parenchymal enhancement at contrast-enhanced spectral mammography and breast MR imaging. Radiology. 2017;282(1):63–73.
29. DeLeo MJ, Domchek SM, Kontos D, Conant E, Chen J, Weinstein S. Breast MRI fibroglandular volume and parenchymal enhancement in BRCA1 and BRCA2 mutation carriers before and immediately after risk-reducing salpingo-oophorectomy. AJR Am J Roentgenol. 2015;204(3):669–73. http://www.ncbi.nlm.nih.gov/pubmed/25714301
30. Price ER, Brooks JD, Watson EJ, Brennan SB, Comen EA, Morris EA. The impact of bilateral salpingo-oophorectomy on breast MRI background parenchymal enhancement and fibroglandular tissue. Eur Radiol. 2014;24(1):162–8. http://link.springer.com/10.1007/s00330-013-2993-9
31. Schrading S, Schild H, Kühr M, Kuhl C. Effects of tamoxifen and aromatase inhibitors on breast tissue enhancement in dynamic contrast-enhanced breast MR imaging: a longitudinal intraindividual cohort study. Radiology. 2014;271(1):45–55. http://www.ncbi.nlm.nih.gov/pubmed/24475835

32. King V, Brooks JD, Bernstein JL, Reiner AS, Pike MC, Morris EA. Background parenchymal enhancement at breast MR imaging and breast cancer risk. Radiology. 2011;260(1):50–60.
33. Dontchos BN, Rahbar H, Partridge SC, Korde LA, Lam DL, Scheel JR, et al. Are qualitative assessments of background parenchymal enhancement, amount of fibroglandular tissue on MR images, and mammographic density associated with breast cancer risk? Radiology. 2015;276(2):371–80. http://www.ncbi.nlm.nih.gov/pubmed/25965809
34. Telegrafo M, Rella L, Stabile Ianora AA, Angelelli G, Moschetta M. Breast MRI background parenchymal enhancement (BPE) correlates with the risk of breast cancer. Magn Reson Imaging. 2016;34(2):173–6. https://linkinghub.elsevier.com/retrieve/pii/S0730725X15002519
35. Grimm LJ, Saha A, Ghate SV, Kim C, Soo MS, Yoon SC, et al. Relationship between background parenchymal enhancement on high-risk screening MRI and future breast cancer risk. Acad Radiol. 2019;26(1):69–75. https://linkinghub.elsevier.com/retrieve/pii/S1076633218301260
36. Sippo DA, Rutledge GM, Burk KS, Mercaldo SF, Dontchos BN, Edmonds CE, et al. Effect of background parenchymal enhancement on cancer risk across different high-risk patient populations undergoing screening breast MRI. Am J Roentgenol. 2019;212(6):1412–8. https://www.ajronline.org/doi/10.2214/AJR.18.20566
37. Bermot C, Saint-Martin C, Malhaire C, Sebbag-Sfez D, Mouret-Fourme E, Carton M, et al. Background parenchymal enhancement and fibroglandular tissue on breast MRI in women with high genetic risk: are changes before and after risk-reducing salpingo-oophorectomy associated with breast cancer risk? Eur J Radiol. 2018;109(September):171–7. https://doi.org/10.1016/j.ejrad.2018.10.030.
38. Xiao Y-L, Wang K, Liu Q, Li J, Zhang X, Li H-Y. Risk reduction and survival benefit of risk-reducing salpingo-oophorectomy in hereditary breast cancer: meta-analysis and systematic review. Clin Breast Cancer. 2019;19(1):e48–65. https://doi.org/10.1016/j.clbc.2018.09.011.
39. Obdeijn IM, Winter-Warnars GAO, Mann RM, Hooning MJ, Hunink MGM, Tilanus-Linthorst MMA. Should we screen BRCA1 mutation carriers only with MRI? A multicenter study. Breast Cancer Res Treat. 2014;144(3):577–82.
40. Vreemann S, van Zelst JCM, Schlooz-Vries M, Bult P, Hoogerbrugge N, Karssemeijer N, et al. The added value of mammography in different age-groups of women with and without BRCA mutation screened with breast MRI. Breast Cancer Res. 2018;20(1):84. http://www.ncbi.nlm.nih.gov/pubmed/30075794
41. Maskarinec G, Meng L, Ursin G. Ethnic differences in mammographic densities. Int J Epidemiol. 2001;30(5):959–65. http://www.ncbi.nlm.nih.gov/pubmed/11689504
42. Tozaki M, Nakamura S. Current status of breast cancer screening in high-risk women in Japan. Breast Cancer. 2020; https://doi.org/10.1007/s12282-020-01103-1.
43. Friedewald SM, Rafferty EA, Rose SL, Durand MA, Plecha DM, Greenberg JS, et al. Breast cancer screening using tomosynthesis in combination with digital mammography. JAMA. 2014;311(24):2499–507. http://www.ncbi.nlm.nih.gov/pubmed/25058084
44. Lee CI, Cevik M, Alagoz O, Sprague BL, Tosteson ANA, Miglioretti DL, et al. Comparative effectiveness of combined digital mammography and tomosynthesis screening for women with dense breasts. Radiology. 2015;274(3):772–80. http://www.ncbi.nlm.nih.gov/pubmed/25350548
45. Rafferty EA, Durand MA, Conant EF, Copit DS, Friedewald SM, Plecha DM, et al. Breast cancer screening using tomosynthesis and digital mammography in dense and nondense breasts. JAMA. 2016;315(16):1784–6. http://www.ncbi.nlm.nih.gov/pubmed/27115381
46. Phillips J, Miller MM, Mehta TS, Fein-Zachary V, Nathanson A, Hori W, et al. Contrast-enhanced spectral mammography (CESM) versus MRI in the high-risk screening setting: patient preferences and attitudes. Clin Imaging. 2017;42:193–7. https://doi.org/10.1016/j.clinimag.2016.12.011.
47. Lee-Felker SA, Tekchandani L, Thomas M, Gupta E, Andrews-Tang D, Roth A, et al. Newly diagnosed breast cancer: comparison of contrast-enhanced spectral mammography and breast MR imaging in the evaluation of extent of disease. Radiology. 2017;285(2):389–400. http://www.ncbi.nlm.nih.gov/pubmed/28654337

48. Wang Q, Li K, Wang L, Zhang J, Zhou Z, Feng Y. Preclinical study of diagnostic performances of contrast-enhanced spectral mammography versus MRI for breast diseases in China. Springerplus. 2016;5(1):763. http://www.ncbi.nlm.nih.gov/pubmed/27386249
49. Jochelson MS, Pinker K, Dershaw DD, Hughes M, Gibbons GF, Rahbar K, et al. Comparison of screening CEDM and MRI for women at increased risk for breast cancer: a pilot study. Eur J Radiol. 2017;97(August):37–43. https://doi.org/10.1016/j.ejrad.2017.10.001.
50. Ghaderi KF, Phillips J, Perry H, Lotfi P, Mehta TS. Contrast-enhanced mammography: current applications and future directions. Radiographics. 2019;39(7):1907–20. http://www.ncbi.nlm.nih.gov/pubmed/31697627
51. Patel BK, Naylor ME, Kosiorek HE, Lopez-Alvarez YM, Miller AM, Pizzitola VJ, et al. Clinical utility of contrast-enhanced spectral mammography as an adjunct for tomosynthesis-detected architectural distortion. Clin Imaging. 2017;46:44–52. https://doi.org/10.1016/j.clinimag.2017.07.003.
52. Berg WA, Zhang Z, Lehrer D, Jong RA, Pisano ED, Barr RG, et al. Detection of breast cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. JAMA. 2012;307(13):1394–404.
53. Berg WA, Bandos AI, Mendelson EB, Lehrer D, Jong RA, Pisano ED. Ultrasound as the primary screening test for breast cancer: analysis from ACRIN 6666. J Natl Cancer Inst. 2016;108(4):djv367. https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/djv367
54. Lee CI, Chen LE, Elmore JG. Risk-based breast cancer screening: implications of breast density. Med Clin North Am. 2017;101(4):725–41.
55. Vourtsis A. Three-dimensional automated breast ultrasound: technical aspects and first results. Diagn Interv Imaging. 2019;100(10):579–92. https://doi.org/10.1016/j.diii.2019.03.012.
56. Kim SH. Image quality and artifacts in automated breast ultrasonography. Ultrasonography. 2019;38(1):83–91. http://e-ultrasonography.org/journal/view.php?doi=10.14366/usg.18016
57. Ohuchi N, Suzuki A, Sobue T, Kawai M, Yamamoto S, Zheng Y-F, et al. Sensitivity and specificity of mammography and adjunctive ultrasonography to screen for breast cancer in the Japan strategic anti-cancer randomized trial (J-START): a randomised controlled trial. Lancet. 2016;387(10016):341–8. https://doi.org/10.1016/S0140-6736(15)00774-6.
58. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266(5182):66–71. http://www.ncbi.nlm.nih.gov/pubmed/7545954
59. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378(6559):789–92.
60. Kuhl CK, Schmutzler RK, Leutner CC, Kempe A, Wardelmann E, Hocke A, et al. Breast MR imaging screening in 192 women proved or suspected to be carriers of a breast cancer susceptibility gene: preliminary results. Radiology. 2000;215(1):267–79. http://www.ncbi.nlm.nih.gov/pubmed/10751498
61. Morris EA, Liberman L, Ballon DJ, Robson M, Abramson AF, Heerdt A, et al. MRI of occult breast carcinoma in a high-risk population. AJR Am J Roentgenol. 2003;181(3):619–26. http://www.ncbi.nlm.nih.gov/pubmed/12933450
62. Kuhl CK, Schrading S, Leutner CC, Morakkabati-Spitz N, Wardelmann E, Fimmers R, et al. Mammography, breast ultrasound, and magnetic resonance imaging for surveillance of women at high familial risk for breast cancer. J Clin Oncol. 2005;23(33):8469–76.
63. Kuhl C, Weigel S, Schrading S, Arand B, Bieling H, König R, et al. Prospective multicenter cohort study to refine management recommendations for women at elevated familial risk of breast cancer: the EVA trial. J Clin Oncol. 2010;28(9):1450–7. http://www.ncbi.nlm.nih.gov/pubmed/20177029
64. Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. Obstet Gynecol Surv. 2007;62(7):458–60. http://journals.lww.com/00006254-200707000-00021
65. Mann RM, Balleyguier C, Baltzer PA, Bick U, Colin C, Cornford E, et al. Breast MRI: EUSOBI recommendations for women's information. Eur Radiol. 2015;25(12):3669–78.

66. Heller SL, Moy L. MRI breast screening revisited. J Magn Reson Imaging. 2019;49(5):1212–21. http://www.ncbi.nlm.nih.gov/pubmed/30693603
67. Warner E, Plewes DB, Hill KA, Causer PA, Zubovits JT, Jong RA, et al. Surveillance of BRCA1 and BRCA2 mutation carriers with magnetic resonance imaging, ultrasound, mammography, and clinical breast examination. JAMA. 2004;292(11):1317–25. http://www.ncbi.nlm.nih.gov/pubmed/15367553
68. Takahama N, Tozaki M, Ohgiya Y. Current status of MRI-guided vacuum-assisted breast biopsy in Japan. Breast Cancer. 2020;0123456789:1–5.
69. Kuhl CK, Schrading S, Strobel K, Schild HH, Hilgers RD, Bieling HB. Abbreviated breast magnetic resonance imaging (MRI): first postcontrast subtracted images and maximum-intensity projection - a novel approach to breast cancer screening with MRI. J Clin Oncol. 2014;32(22):2304–10.
70. Kuhl CK. Abbreviated magnetic resonance imaging (MRI) for breast cancer screening: rationale, concept, and transfer to clinical practice. Annu Rev Med. 2019;70:501–19. http://www.ncbi.nlm.nih.gov/pubmed/30691370
71. Vreemann S, Gubern-Mérida A, Schlooz-Vries MS, Bult P, van Gils CH, Hoogerbrugge N, et al. Influence of risk category and screening round on the performance of an MR imaging and mammography screening program in carriers of the *BRCA* mutation and other women at increased risk. Radiology. 2018;286(2):443–51. http://pubs.rsna.org/doi/10.1148/radiol.2017170458
72. King MC, Wieand S, Hale K, Lee M, Walsh T, Owens K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: national surgical adjuvant breast and bowel project (NSABP-P1) breast cancer prevention trial. JAMA. 2001;286(18):2251–6. http://www.ncbi.nlm.nih.gov/pubmed/11710890
73. Sung JS, Malak SF, Bajaj P, Alis R, Dershaw DD, Morris EA. Screening breast MR imaging in women with a history of lobular carcinoma in situ. Radiology. 2011;261(2):414–20. http://www.ncbi.nlm.nih.gov/pubmed/21900617
74. Port ER, Park A, Borgen PI, Morris E, Montgomery LL. Results of MRI screening for breast cancer in high-risk patients with LCIS and atypical hyperplasia. Ann Surg Oncol. 2007;14(3):1051–7. http://link.springer.com/10.1245/s10434-006-9195-5
75. Schwartz T, Cyr A, Margenthaler J. Screening breast magnetic resonance imaging in women with atypia or lobular carcinoma in situ. J Surg Res. 2015;193(2):519–22. https://doi.org/10.1016/j.jss.2014.08.059.
76. Malhaire C, El Khoury C, Thibault F, Athanasiou A, Petrow P, Ollivier L, et al. Vacuum-assisted biopsies under MR guidance: results of 72 procedures. Eur Radiol. 2010;20(7):1554–62. http://www.ncbi.nlm.nih.gov/pubmed/20119729
77. Crystal P, Sadaf A, Bukhanov K, McCready D, O'Malley F, Helbich TH. High-risk lesions diagnosed at MRI-guided vacuum-assisted breast biopsy: can underestimation be predicted? Eur Radiol. 2011;21(3):582–9. http://www.ncbi.nlm.nih.gov/pubmed/20839000
78. Heller SL, Moy L. Imaging features and management of high-risk lesions on contrast-enhanced dynamic breast MRI. AJR Am J Roentgenol. 2012;198(2):249–55. http://www.ncbi.nlm.nih.gov/pubmed/22268165
79. Okamoto S, Chen ST, Covelli JD, DeMartini WB, Daniel BL, Ikeda DM. High-risk lesions diagnosed at MRI-guided vacuum-assisted breast biopsy: imaging characteristics, outcome of surgical excision or imaging follow-up. Breast Cancer. 2020;27(3):405–14.
80. Sung JS, Lee CH, Morris EA, Oeffinger KC, Dershaw DD. Screening breast MR imaging in women with a history of chest irradiation. Radiology. 2011;259(1):65–71. http://www.ncbi.nlm.nih.gov/pubmed/21325032
81. Warner E, Plewes DB, Shumak RS, Catzavelos GC, Di Prospero LS, Yaffe MJ, et al. Comparison of breast magnetic resonance imaging, mammography, and ultrasound for surveillance of women at high risk for hereditary breast cancer. J Clin Oncol. 2001;19(15):3524–31. http://www.ncbi.nlm.nih.gov/pubmed/11481359

Chemoprevention for Breast Cancer

9

Rurina Watanuki, Aiko Nagayama, Tetsu Hayashida, and Yuko Kitagawa

Abstract

Cancer chemoprevention is defined as the use of natural, synthetic, or biochemical agents to reverse, suppress, or prevent carcinogenic processes in neoplastic diseases. Although the precise mechanisms that promote breast cancer are not fully understood, several recent clinical trials suggest that chemoprevention is a rational and attractive strategy for selected high-risk populations in a prophylactic setting. Conventionally, endocrine interventions using selective estrogen receptor modulators and aromatase inhibitors have already been applied clinically in high-risk populations. In particular, the chemoprevention approach for *BRCA* germline mutation carriers is drawing attention as an alternative option to invasive prophylactic mastectomy. Although the evidence from prospective clinical studies was limited, this review aims to provide an up-to-date overview of the biological mechanisms and the efficacy of various chemopreventive agents, including new promising candidates that target *BRCA* deficiency, and discuss future challenges and prospects for breast cancer chemoprevention.

Keywords

Breast cancer · Chemoprevention · *BRCA* mutation carriers · Selective estrogen receptor modulators · Denosumab · Poly ADP-ribose polymerase (PARP) inhibitors

R. Watanuki · A. Nagayama · T. Hayashida (✉) · Y. Kitagawa
Department of Surgery, Keio University School of Medicine, Shinjuku, Tokyo, Japan
e-mail: rurina.w.629@keio.jp; anagayama@keio.jp; tetsu@keio.jp; kitagawa@a3.keio.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_9

9.1 Introduction

Given the increasing incidence and mortality of cancer worldwide as well as the rising cost of medical treatment, there is a growing interest in developing strategies for disease prevention. One of the approaches with enormous potential is chemoprevention. In 1976, Sporn defined the term "chemoprevention" as the use of natural, synthetic, or biological agents to reverse, inhibit, or prevent either the initial phases of carcinogenesis or the progression of premalignant cells to invasive disease [1]. The process of breast carcinogenesis begins with the accumulation of an unspecified number of genetic events, followed by the emergence of progressive dysplastic cells with genotypic and phenotypic alterations that lead to deregulated cell growth. Chemoprevention aims to reduce the incidence of disease by arresting or modifying these mechanisms.

Those at increased risk for developing breast cancer could benefit from preventive therapy, as it is the most prevalent malignancy in women. The risk factors for breast cancer are described in various available risk calculation models, including the Tyrer-Cuzick and Gail models, to provide a numeric risk that can be used to help quantify the level of individual risk. Other individual risk factors for the selection of candidates for preventive therapy include the presence of premalignant diseases, such as lobular carcinoma in situ (LCIS), atypical ductal hyperplasia (ADH), and atypical lobular hyperplasia (ALH); high mammographic density; use of hormone replacement therapy; and presence of either high-risk penetrant genes, including *BRCA1/BRCA2* mutation carriers or less penetrant genes, but higher-frequency polygenic risk score SNPs [2, 3]. The National Comprehensive Cancer Network (NCCN) guidelines and the United States Preventive Services Task Force (USPSTF) have stated and recommended the use of breast cancer risk-reducing agents in high-risk populations. However, there is insufficient evidence showing the efficacy of chemopreventive agents in women who are carriers of pathogenetic *BRCA1/BRCA2* mutations. Hence, herein, we reviewed the current risk-reducing agents for breast cancer and pathogenetic *BRCA1/BRCA2* mutation carriers suitable for chemopreventive therapy.

9.2 Chemopreventive Drugs for Breast Cancer

9.2.1 Selective Estrogen Receptor Modulators

Hormones play a significant role in almost 70% of breast cancer cases [4], and current chemopreventive strategies have targeted hormonally responsive breast cancers. The two major classes of antiestrogenic drugs, selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs), have been recently used for breast cancer prevention. A list of prospective trials regarding the use of SERMs and AIs as primary preventive treatments for breast cancer is provided in Table 9.1 [5–14].

Estrogen is the main factor that stimulates the development and growth of breast cancer. Deprivation of estrogenic signaling has been the primary form of hormonal

Table 9.1 Prospective trials for the primary prevention of breast cancer using selective estrogen receptor modulators and aromatase inhibitors

Trial	Study design	Participants	Interventions	End point	Major results
Royal Marsden trial [5]	Placebo-controlled, double-blind, randomized trial (n = 2,471)	• High-risk women • Family history of breast cancer • Age: 30–70 years	TAM 20 mg/day vs. placebo (for 8 years)	• Occurrence of invasive breast cancer • Occurrence of ER-positive invasive breast cancer	• Median follow-up: 13 years • Invasive breast cancer HR: 0.78, 95% CI: 0.58–1.04, P = 0.1 • ER-positive invasive breast cancer HR:0.61, 95% CI: 0.43–0.86, P = 0.05
NSABP-P1 trial [6]	Placebo-controlled, double-blind, randomized trial (unblinded after 5 years) (n = 13,388)	• High-risk women • Age: ≥60 years • Age: 35–59 years with a Gail model 5-year breast cancer risk of ≥1.66% • Age 35–59 years with a history of LCIS	TAM 20 mg/day vs. placebo (for 5 years)	• Cumulative rate of invasive breast cancer • Cumulative rate of noninvasive breast cancer	• Median follow-up 7 years • Invasive breast cancer RR: 0.57, 95% CI: 0.46–0.70, P < 0.001 • Noninvasive breast cancer RR: 0.63, 95% CI: 0.45–0.89, P < 0.008
IBIS-1 trial [7]	Placebo-controlled, double-blind, randomized trial (n = 7,154)	• High-risk women • Age: 35–70 years • ≥2-fold relative risk of breast cancer	TAM 20 mg/day vs. placebo (for 5 years)	• Incidence of breast cancer including DCIS • Side effects	• Median follow-up: 8 years • All breast cancer RR: 0.73, 95% CI: 0.58–0.91, P = 0.004 • The risk-reducing effect persisted for at least 10 years • Most side effects do not continue after a 5-year treatment period
Italian trial [8]	Placebo-controlled, double-blind, randomized trial (n = 5,408)	• Normal-risk women • Age: 35–70 years • Total hysterectomy	TAM 20 mg/day vs. placebo (for 5 years)	• Incidence of breast cancer including DCIS • Side effects	• Median follow-up: 11 years • The incidence rates of breast cancer were similar in both groups of women with low risk • Much lower in the tamoxifen group among women at high risk

(continued)

Table 9.1 (continued)

Trial	Study design	Participants	Interventions	End point	Major results
MORE trial [9]	Placebo-controlled, double-blind, randomized trial (n = 7,705)	• Postmenopausal women with osteoporosis (aged up to 80 years)	RAL 60 mg/day or 120 mg/day vs. placebo (for 4 years)	• Incidence of invasive breast cancer • Incidence of ER-positive breast cancer	• Median follow-up: 4 years • Invasive breast cancer 72% risk reduction with RAL (RR: 0.28, 95% CI: 0.17–0.46) • ER-positive breast cancer 84% risk reduction with RAL (RR = 0.16, 95% CI: 0.09–0.30)
CORE trial [10]	Placebo-controlled, double-blind trial (n = 4,011)	• Postmenopausal women with osteoporosis (aged up to 80 years)	RAL 60 mg/day or 120 mg/day vs. placebo (for 8 years)	• Incidence of invasive breast cancer • Incidence of ER-positive breast cancer	• Four-year incidence rates of invasive breast cancer 59% risk reduction with RAL (HR: 0.41, 95% CI: 0.24–0.71) • Four-year incidence rates of ER-positive breast cancer 66% risk reduction with RAL (HR: 0.34, 95% CI: 0.18–0.66) • Over the 8 years of MORE and CORE trial Invasive breast cancer: 66% risk reduction (HR = 0.34, 95% CI: 0.22–0.50) ER-positive breast cancer: 76% risk reduction (HR: 0.24, 95% CI: 0.15–0.40)
RUTH trial [11]	Placebo-controlled, double-blind, randomized trial (n = 10,101)	• Postmenopausal women (≥55 years old) with CHD or multiple risk factors for CHD	RAL 60 mg/day vs. placebo (for 5 years)	• Incidence of coronary events • Incidence of invasive breast cancer	• Median follow-up: 5.6 years • RAL had no significant effect on the risk of primary coronary events (HR: 0.95, 95% CI: 0.84–1.07) • RAL reduced the risk of invasive breast cancer (HR: 0.56, 95% CI: 0.38–0.83)

STAR trial [12]	Double-blind, randomized trial (n = 19,747)	• Postmenopausal women (≥35 years old) • 5-year risk of breast cancer ≥1.66%	RAL 60 mg vs. TAM 20 mg (for 5 years)	• Incidence of invasive breast cancer • Incidence of noninvasive breast cancer • Toxicity	• Median follow-up: 6.8 years • Invasive breast cancer RR (RAL: TAM): 1.24, 95% CI: 1.05–1.47 • Noninvasive breast cancer RR (RAL: TAM): 1.22, 95% CI: 0.95–1.59 • RAL is far less toxicity (highly significantly less endometrial cancer)
MAP.III trial [13]	Placebo-controlled, double-blind, randomized trial (n = 4,560)	• Postmenopausal women (≥35 years old) • Gail 5-year risk of breast cancer ≥1.66%	EXE 25 mg/day vs. placebo (for 5 years)	• Incidence of invasive breast cancer • Toxicity	• Median follow-up: 3 years • Invasive breast cancer: 65% risk reduction with EXE (HR: 0.35, 95% CI: 0.18–0.70, P = 0.002) • EXE was associated with no serious toxic effects
IBIS-II trial [14]	Placebo-controlled, double-blind, randomized trial (n = 3,864)	• Postmenopausal women • Age: 40–70 years • High risk of breast cancer	ANA 1 mg/day vs. placebo (for 5 years)	• Incidence of all breast cancer • Incidence of invasive breast cancer	• Median follow-up: 5 years • All breast cancer HR: 0.47, 95% CI: 0.32–0.68, $P < 0.0001$ • Invasive breast cancer HR: 0.50, 95% CI: 0.32–0.76, P = 0.001

TAM tamoxifen, *ER* estrogen receptor, *HR* hazard ratio, *CI* confidence interval, *LCIS* lobular carcinoma in situ, *RR* risk ratio, *DCIS* ductal carcinoma in situ, *RAL* raloxifene, *CHD* coronary heart disease, *EXE* exemestane, *ANA* anastrozole

therapy for patients with estrogen receptor (ER)-positive and/or progesterone (PgR)-positive disease. Over the past three decades, tamoxifen, a type of SERM, is an antiestrogen drug that inhibits the binding of estrogen to its receptors and has become the mainstay of hormone therapy [15]. Figure 9.1 illustrates the mechanism of estrogen deprivation [15].

Four large historical studies [5–8] evaluating the efficacy of tamoxifen as a primary chemopreventive drug have been conducted, and long-term follow-up data are available. An integrated analysis of tamoxifen primary prevention trials, including these studies, showed a 38% (95% confidence interval [CI] = 28–46; $P < 0.0001$) reduction in breast cancer incidence [16]. However, this drug was not effective in patients with ER-negative breast cancers (hazard ratio [HR] = 1.22, 95% CI = 0.89–1.67; $P = 0.21$); nonetheless, tamoxifen prevention trials reported that the incidence of ER-positive cancers decreased by 48% (95% CI = 36–58; $P < 0{\cdot}0001$) [16]. The data from these studies, particularly the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention trial (P-1 trial), led to the US Food and Drug Administration (FDA) approval of tamoxifen in 1998 for breast cancer risk reduction in high-risk women. A large-scale study on tamoxifen and raloxifene (STAR) trial, which directly compared tamoxifen with raloxifene, found that tamoxifen was more effective in reducing the breast cancer risk than raloxifene after a long-term follow-up [17]. Data from the STAR trial and the other raloxifene/placebo trial (MORE-CORE and RUTH) resulted in the approval of raloxifene by the US FDA for risk reduction of invasive breast cancer in postmenopausal women

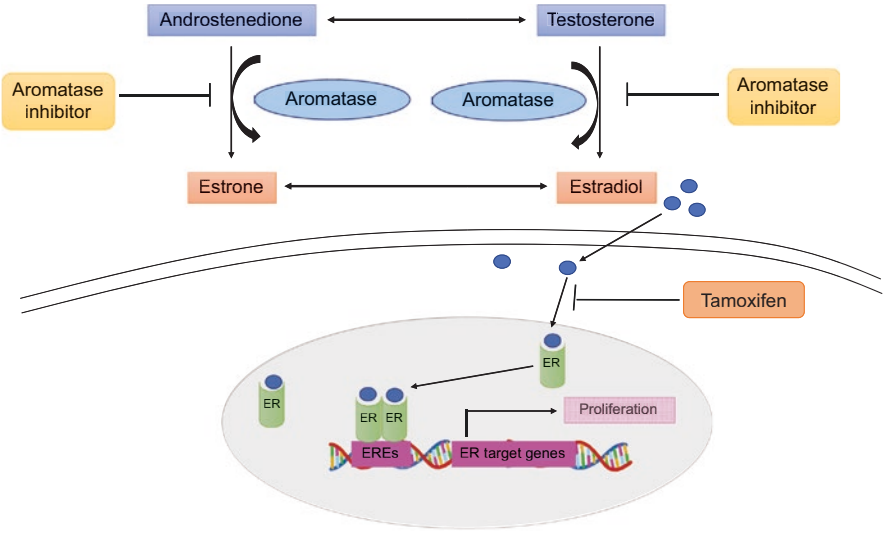


Fig. 9.1 Mechanism of estrogen deprivation by tamoxifen and aromatase inhibitor
Estradiol binds to estrogen receptors (ERs), causing receptor dimerization and subsequent binding to estrogen-responsive elements upstream of estrogen-responsive genes, including genes involved in proliferation. Tamoxifen competes with estradiol for binding to the ER, and aromatase inhibitors decrease the synthesis of estrogen from androgen precursors

with osteoporosis as well as for risk reduction of invasive breast cancer in postmenopausal women at high risk of invasive breast cancer. Cuzick et al. performed a meta-analysis using individual data from nine randomized double-blind trials comparing the efficacy of four SERMs with placebo or another drug in women with no history of breast cancer. They showed a 38% reduction in the overall breast cancer incidence, including that of ductal carcinoma in situ (DCIS) [18]. Interestingly, the impact of reduction was larger in the first 5 years of follow-up than in the 5–10 years of follow-up (42% vs. 25%). Treatment with all types of SERMs increased the incidence of venous thromboembolic events, whereas treatment with tamoxifen alone resulted in an increase in the incidence of endometrial cancers. Despite a 10–20% reduction in LDL cholesterol after treatment with SERMs, no reduction in cardiovascular disease was noted. Moreover, a significant reduction of 34% in the incidence of vertebral fractures was reported in this analysis.

Only a subgroup analysis of the NSABP P-1trial evaluated the effect of tamoxifen on breast cancer risk in women with *BRCA1/BRCA2* pathogenic variants [19]. Tamoxifen reduced the breast cancer risk by 62% in *BRCA2* carriers (relative risk [RR]: 0.38, 95% CI: 0.06–1.56), but not in *BRCA1* carriers (RR: 1.67, 95% CI: 0.32–10.07). However, this analysis is limited by the small number of participants carrying pathogenic variants; among 288 women with breast cancer, only 8 had *BRCA1* pathogenic variants and 11 had *BRCA2* pathogenic variants. To date, no primary prevention trials using tamoxifen or raloxifene have been conducted among women with *BRCA1/BRCA2* mutations. Although not validated as a chemopreventive agent for primary breast cancer in *BRCA1/BRCA2* mutation carriers, tamoxifen prevents contralateral breast cancer by up to 50% [20–22]. In a recent meta-analysis, tamoxifen was significantly associated with a reduced risk of contralateral breast cancer among *BRCA1/BRCA2* mutation carriers (summary RR, 0.56; 95% CI, 0.41–0.76) [23]. Similar findings were observed in *BRCA1* mutation carriers (summary RR, 0.47; 95% CI, 0.37–0.60) and *BRCA2* mutation carriers (summary RR, 0.39; 95% CI, 0.28–0.54), respectively [23]. Gronwald et al. demonstrated that the use of tamoxifen for 1 year was associated with a 63% reduction in the risk of contralateral breast cancer (95% CI, 0.37–0.75; $P = 0.003$) [22]. They suggested that short-term use of tamoxifen for chemoprevention in *BRCA1/BRCA2* mutation carriers may be as effective as a conventional 5-year course of treatment.

Previous data suggest a role for tamoxifen in estrogen receptor blockade and the prevention of contralateral breast cancer, even among *BRCA1* mutation carriers who have a tendency to develop hormone receptor-negative disease. Although the underlying mechanisms mediating the protective role of tamoxifen in contralateral breast cancer remain unclear, a reduction in mammary cell proliferation [24], the number of mammary stem cells, and mammographic density [25] have been proposed. Premenopausal carriers of *BRCA1/BRCA2* mutations usually exhibit higher titers of estradiol and progesterone [26], which is one of the reasons for developing cancer prevention strategies in premenopausal women.

De Censi et al. conducted a multicenter randomized phase III trial evaluating the effectiveness of 5 mg/day tamoxifen or placebo administered for 3 years in women with breast intraepithelial neoplasia, including those with ADH, DCIS, and LCIS

[27]. Low-dose tamoxifen reduced the risk of breast cancer development by 52%, and the incidence of side effects in the tamoxifen arm was not higher than that in the placebo arm [27]. This study indicated that low-dose tamoxifen may be an effective chemopreventive method with good tolerability.

9.2.2 Aromatase Inhibitors

In premenopausal women, aromatase and estrogen are produced by the granulosa cells in the functional ovaries and are also present in other normal tissues, including the mesenchymal cells of subcutaneous fat, breast, and bone [15, 28]. After menopause, estrogen is no longer produced in the ovaries, but aromatase activity and production of estrogen persists in all the other sites [15].

Tamoxifen competes with estradiol for ER binding, whereas AIs reduce the synthesis of estrogens from androgenic precursors (Fig. 9.1). A significant association exists between breast cancer risk and plasma levels of the common circulating estrogens in postmenopausal women [29], and AIs achieve almost complete inhibition of aromatase in vivo and suppression of plasma estrogen levels. The significant reduction in contralateral breast cancer in adjuvant AI clinical trials [30] has led to the increased interest in the use of these agents for primary prevention, especially due to the less incidence of toxicities, such as thrombotic events and endometrial cancer compared with SERMs. Two landmark studies were conducted to evaluate the efficacy of AI for the primary prevention of breast cancer (Table 9.1).

In the National Cancer Institute of Canada Mammary Prevention 3 (MAP.3) trial, after 35 months of follow-up, treatment with exemestane reduced the breast cancer risk by 65% in high-risk postmenopausal women [13]. Similarly, the European IBIS-II trial reported a 53% reduction in the breast cancer risk in women at increased risk of breast cancer after treatment with anastrozole [14]. Neither exemestane nor anastrozole was associated with an increased risk of thromboembolic or cardiovascular events or other cancer types. The MAP.3 trial showed that short-term use of exemestane exacerbated the age-related bone loss despite calcium and vitamin D supplementation, but long-term follow-up is needed to assess its impact on the risk of fracture in the prevention population [31]. The side effects of exemestane, including vasomotor, sexual, and musculoskeletal symptoms, had limited impact on patients' quality of life [32]. In addition to vasomotor symptoms, musculoskeletal events were more common in the anastrozole arm [14]. In the NCCN guidelines and the USPSTF, AI is recommended as a risk-reducing agent for breast cancer. However, it remains unclear whether SERMs or AIs are preferred agents for the prevention of breast cancer because of the absence of head-to-head comparisons and differences in patient characteristics between studies.

Retrospective data suggested that AIs could reduce the risk of ER-positive contralateral breast cancer in *BRCA1/BRCA2* mutation carriers who are receiving AIs as adjuvant therapy [33]; however, data on the effectiveness of AIs as well as tamoxifen for primary prevention in *BRCA* mutation carriers are insufficient.

9.2.3 Denosumab

The receptor activator of nuclear factor κB (RANK), its cytokine ligand (RANKL), and the soluble receptor osteoprotegerin (OPG) form a functional triad in the tumor necrosis factor (TNF) and TNF receptor superfamily [34, 35]. RANK and RANKL are known for their involvement in bone metabolism [34]. The binding of RANKL to RANK on osteoclast precursors induces osteoclast maturation and activation, thereby promoting bone resorption, whereas the binding of RANKL by OPG inhibits its RANKL-mediated signaling pathways, resulting in the inhibition of bone resorption and maintenance of bone density (Fig. 9.2) [34, 36]. Denosumab, a human anti-RANKL monoclonal antibody, is approved for the treatment of osteoporosis and for the prevention of skeletal damage due to bone metastases in patients with breast cancer and other types of solid tumors [37]. Various experimental data have demonstrated that progesterone-mediated upregulation of RANK/RANKL may also play a critical role in mammary gland epithelial cell proliferation, mammary stem cell expansion, and carcinogenesis, particularly in *BRCA1* mutation carriers [38–42].

A precancerous *BRCA1*$^{mut/+}$ tissue harbors an aberrant population of luminal progenitor cells [43], and deregulated progesterone signaling has been implicated in *BRCA1*-associated oncogenesis [44–46]. Nolan et al. showed that a highly proliferative subset of luminal progenitor cells that gives rise to basal-like breast cancer, constitutively expresses RANK and is hyper-responsive to RANKL (Fig. 9.3) [47]. They proposed that this finding suggests an exciting opportunity for the precision

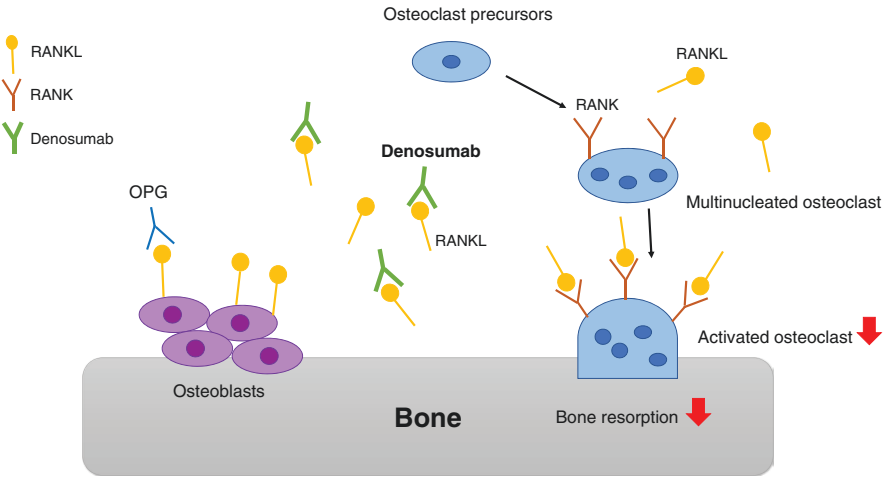


Fig. 9.2 Mechanism of action of denosumab
Binding of RANKL to RANK on osteoclast precursors induces osteoclast maturation and activation, thereby promoting bone resorption. Conversely, the binding of RANKL by osteoprotegerin inhibits the RANKL-mediated signaling pathway, thereby inhibiting bone resorption. Denosumab binds to RANKL and reduces osteoclasts by directly inhibiting the RANK-RANKL signaling pathway

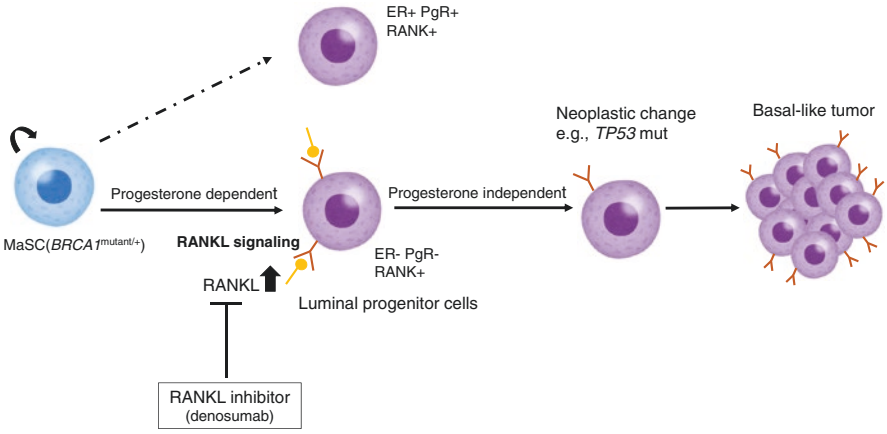


Fig. 9.3 Progression of *BRCA1*$^{mut/+}$ RANK+ luminal progenitor cells to basal-like tumors *BRCA1*$^{mut/+}$ RANK+ subset of mammary luminal progenitor cells give rise to basal-like tumors. Progesterone-dependent RANK signaling in luminal progenitor cells is responsive to RANKL inhibition. Denosumab abrogates progesterone-dependent signaling of RANK+ *BRCA1*$^{mut/+}$ luminal progenitor cells to prevent the progression to basal-like tumors. *MaSC* mammary stem cell, *ER* estrogen receptor, *PgR* progesterone receptor

cancer prevention in *BRCA1* mutation carriers [47, 48]. Important preclinical studies relevant to women with *BRCA1* mutations demonstrated that genetic or pharmacological inhibition of RANKL significantly suppressed mammary tumorigenesis in *BRCA1*-deficient mice [47, 49]. In *BRCA1*-deficient mice, the loss of RANKL reduced the progression of mammary tumors, and the inhibition of RANKL suppressed the development of mammary tumor [47]. Furthermore, the proliferation of mammary progenitor cells in *BRCA1*-mutant mice was suppressed by inhibiting RANK, supporting the paracrine activity of RANKL on RANK expression in ER-negative and PR-negative cells [50, 51]. Evidence from studies using human breast cells of *BRCA1* mutation carriers consistent with the data of animal trials supports the inhibition of the RANK pathway as a new target for prevention. Among the mammary progenitor cells of *BRCA1* mutation carriers, RANK-positive cells had significantly higher clonogenic potential than RANK-negative cells [47]. In a three-dimensional organoid model constructed using *BRCA1* mutant breast cancer cells, exposure to progesterone increased the expression of Ki67, but treatment with denosumab inhibited this progesterone-induced increased expression of Ki67 [47]. A pilot window study was conducted in three women within this research, and biopsies taken before and after denosumab treatment showed a significant decrease in Ki67 expression after treatment [47].

OPG is an endogenous decoy receptor of RANKL that antagonizes RANK/RANKL-mediated signaling [34]. Interestingly, women with *BRCA1* mutations may have inherently lower circulating OPG levels than those with baseline risk. Widschwendter et al. reported significantly lower free serum OPG levels among premenopausal *BRCA* mutation carriers compared with non-carrier controls

throughout the menstrual cycle [52]. In addition, the difference was more pronounced in *BRCA1* mutation carriers than in *BRCA2* mutation carriers. Oden et al. conducted a prospective study in 206 *BRCA* mutation carriers with an average follow-up period of 6.5 years [53]. They found a significant inverse relationship between circulating OPG levels and breast cancer risk among women with either a *BRCA1* or *BRCA2* mutation. Women with high plasma OPG levels had a significantly decreased risk of developing breast cancer compared with women with low OPG levels (HR: 0.25; 95% CI: 0.08–0.78; $P = 0.02$) [53]. These data suggest that OPG may be a promising biomarker to help identify women who are at a higher risk of developing breast cancer and who would be ideal candidates for RANKL-based chemopreventive therapy.

As a clinical trial, the ABCSG 18 study provided important results supporting that targeting the RANKL pathway improves the outcomes for breast cancer patients. In this prospective, double-blind, placebo-controlled phase III trial, 3420 postmenopausal breast cancer patients with early hormone receptor-positive disease were treated with an aromatase inhibitor and randomized to receive denosumab 60 mg or placebo biannually [54]. The study reported a reduction in clinical fractures in the denosumab group compared with the placebo group, with no additional toxicities [54]. Moreover, a follow-up analysis showed improved disease-free survival in women who received adjuvant denosumab with an acceptable safety profile [55]. Following the preclinical study that revealed the role of the progesterone/RANK/RANKL pathway in mammary carcinogenesis, which is thought to be particularly relevant in women with *BRCA1* mutations, a randomized, double-blind, placebo-controlled, multicenter, international phase III trial (BRCA-P trial) is now underway to determine the primary preventive effect of denosumab on breast cancer in healthy women with mutations in the *BRCA1* gene. Osteonecrosis of the jaw is one of the adverse events of denosumab treatment, although it is less frequent. In the ABCSG 18 trial, none of the participants reported osteonecrosis of the jaw. If the safety of denosumab can be demonstrated in the BRCA-P trial, in which denosumab is administered to healthy *BRCA1* mutation carriers, it could be used for RAKL-based chemoprevention, which represents a plausible, non-surgical prevention of breast cancer in *BRCA* mutation carriers.

9.2.4 Poly ADP-Ribose Polymerase Inhibitors

Poly ADP-ribose polymerases (PARPs) are a family of enzymes that play a key role in the repair of DNA damage [56]. In particular, PARP-1 and PARP-2 are the most important enzymes used in the treatment for *BRCA1* or *BRCA2* mutation carriers [57, 58]. An important role of PARP-1 and PARP-2 is to maintain genomic integrity, particularly through base excision repair of single-stranded DNA damage [59]. The inhibition of these enzymes leads to the accumulation of DNA single-strand breaks, which can result in the occurrence of DNA double-strand breaks at replication forks [60]. In *BRCA* mutant cells, the function of BRCA protein, which is required for homologous recombination repair against

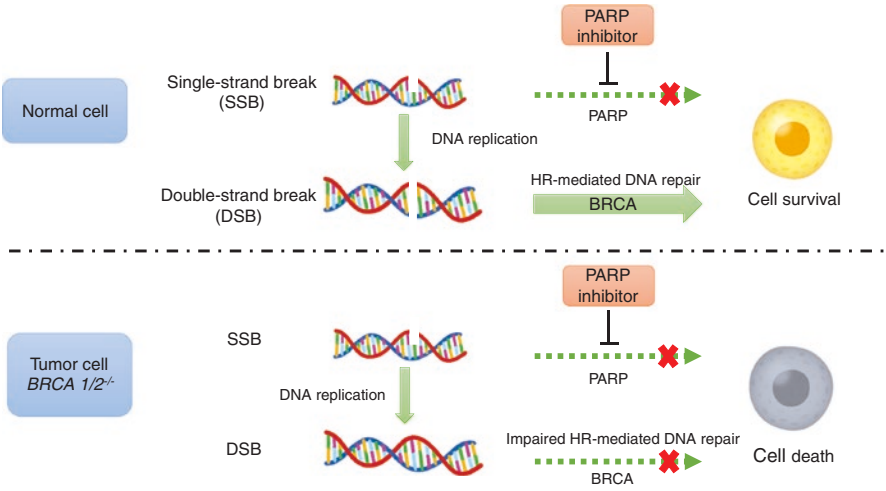


Fig. 9.4 Mechanism of synthetic lethality in *BRCA1/BRCA2*-deficient tumor cell
PARP inhibitors selectively induce cell death in *BRCA1/BRCA2*-deficient cells through the mechanism of synthetic lethality, where cancer cells cannot tolerate the loss of both single-strand break and double-strand break repair machinery. HR; homologous recombination

double-strand breaks, is lost. Therefore, when PARPs are inhibited in *BRCA* mutant cells, the DNA repair mechanism is disrupted and cell death is selectively induced (Fig. 9.4), resulting in an antitumor effect [61, 62]. The concept of synthetic lethality has paved the way for the development of PARP inhibitors for cancer patients with defects in homologous recombination repair, particularly those with *BRCA1* and *BRCA2* bi-allelic loss [63, 64]. This new strategy has led to major advances in the treatment of patients with ovarian cancer and, subsequently, in those with pancreatic, prostate, and breast cancers. Currently, there are two PARP inhibitors approved for treatment in HER2-negative metastatic breast cancer patients with *BRCA*1/*BRCA*2 mutations: olaparib and talazoparib. Both have demonstrated improvements in progression-free survival compared with chemotherapy, overall better tolerability, and low discontinuation rates documented in the trials that led to the approval of these agents [65, 66]. The results of the OlympiA trial, a double-blind, randomized controlled, phase III trial that aimed to evaluate the efficacy of olaparib as adjuvant therapy in patients with high-risk HER2-negative breast cancer and germline *BRCA* mutations, are underway.

Thus, PARP inhibitors have emerged as promising agents for the treatment of cancer patients with *BRCA* mutations via synthetic lethality, but their role in chemoprevention has not been elucidated. Although preclinical data showed that veliparib and olaparib are effective in delaying mammary tumor development and extending the lifespan of *BRCA1*-deficient mice [67], the possible long-term effect of PARP inhibitor treatment on normal tissues in a patient without any cancer or even a high-risk individual needs further clinical evaluation [68].

9.2.5 Nonsteroidal Anti-inflammatory Drugs

In experimental animal models, nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit tumor growth [69, 70]. Aspirin may influence the cancer risk primarily through its effect on the cyclooxygenase (COX) activity. Like other NSAIDs, aspirin inhibits the COX enzyme that converts arachidonic acid into prostaglandins [71]. Aspirin is presumed to have an effect on the tumor growth due to the inhibition of the cyclooxygenase-2 (COX-2) enzyme, which is associated with inflammation, apoptosis, cell migration, and angiogenesis [72]. Aspirin is also thought to be an effective antioxidant [71] and helpful in modulating estrogen biosynthesis [73].

Aspirin and NSAIDs are reported to be effective in preventing colon cancer [74, 75]. Epidemiological studies showed accumulating evidence regarding the cancer-preventive effects of these agents, and the long-term use of aspirin could also reduce the risk of breast cancer by approximately 14% [76, 77]. However, the efficacy of aspirin in the primary prevention of cancer remains controversial, because results from a large-scale, randomized control study suggested that alternate-day use of low-dose aspirin (100 mg) within a period of 10 years did not lower the risk of total, breast, colorectal, or other site-specific cancers [78]. Recently, a prospective study examined the association between regular NSAID use and breast cancer risk in a large cohort of women with a family history of breast cancer, including 1054 *BRCA1* or *BRCA2* mutation carriers [79]. This study found that regular use of aspirin and COX-2 inhibitors was associated with a reduced risk of breast cancer (39% and 61%, respectively) in women with either familial or genetic risk [79]. However, in a series of subgroup analyses, the strength of these associations did not differ by family risk profile or mutation status; although not nominally significant, negative associations were found for both *BRCA1* and *BRCA2* mutation carriers [79]. Similarly, the association was not modified by ER status [79].

The use of aspirin and other NSAIDs for primary breast cancer prevention can be an attractive strategy because they are inexpensive and widely available, but the benefits of NSAIDs need to be weighed against the potential harm of long-term use. Secondary prevention trials in women affected by breast cancer, such as the Aspirin for Breast Cancer (ABC) trial and the Add-Aspirin trial [80, 81], are ongoing and the results are awaited.

9.2.6 Retinoids

Retinoids have been studied as chemopreventive agents due to their role in regulating cell growth, differentiation, and apoptosis in preclinical models [82]. Fenretinide (N-(4-hydroxyphenyl) retinamide), a synthetic derivative of all-trans-retinoic acid, has been the most studied retinoid in clinical trials of breast cancer chemoprevention owing to its selective accumulation in breast tissue and its unique ability to inhibit cell growth proliferation through the induction of apoptosis rather than differentiation [83, 84]. A multicentric phase III randomized trial evaluating the efficacy of fenretinide was initiated in 1987. The participants were stage I breast cancer

patients aged 33–70 years who had undergone surgery for breast cancer within the previous 10 years. Women were randomly assigned to receive either no treatment or 200 mg/day of fenretinide orally for 5 years. The main outcome measure was the occurrence of contralateral breast cancer as the first malignant event. A statistically significant beneficial trend was observed in premenopausal women with contralateral and those with ipsilateral breast cancer (HR: 0.66 and HR: 0.65, respectively), compared with an opposite trend in postmenopausal women (contralateral breast cancer HR: 1.32; ipsilateral breast cancer HR: 1.19), when the analysis was stratified by menopausal status [85]. This result was confirmed after a 15-year follow-up. Fenretinide has demonstrated a favorable toxicological profile, which mainly includes reversible skin dryness and rashes and dark adaptation difficulties, often overcome by a regular 3-day/month suspension of the drug. However, teratogenicity remains a major issue, and contraception is required [86].

This agent has shown antitumor activity in ovarian cancer animal models [87]. In the phase III breast cancer prevention trial, the incidence of ovarian cancer during the 5-year intervention period was significantly lower in the fenretinide group (no cases vs. six in the control group) [85, 88], although no significant difference was shown in the long-term follow-up [89]. Moreover, fenretinide was highly effective in inhibiting the growth of *BRCA1* mutant breast cancer cell lines [90]. Considering the protective effect of fenretinide in young women with second breast cancer and a similar trend in ovarian cancer, it can be used for chemoprevention in women with *BRCA1* or *BRCA2* mutations [83].

9.3 Future Challenges

We reviewed the current candidate drugs for the chemoprevention of breast cancer. Among them, endocrine intervention is considered as the standard of care for breast cancer with relatively few side effects; thus, it is most likely considered as a starting point for chemoprevention in high-risk breast cancer populations. However, despite the recommendation of chemopreventive therapy for breast cancer in some guidelines, many women do not prefer to take chemopreventive agents, and chemoprevention strategies are not widely used in clinical practice. In terms of primary prevention for breast cancer, the most important consideration is the balance between adverse events and their effects. A recent retrospective study in the United States indicated that the use of chemoprevention among women at increased risk for breast cancer remains low, especially among those aged below 50 years, largely because of the fear of adverse events [91]. In particular, teratogenic drugs, such as tamoxifen, may not be a good option for young women of childbearing age who are well aware of the possibility of chemoprevention. Given the low chemoprevention uptake among high-risk populations, healthcare providers must be encouraged to provide appropriate counseling to women who are eligible for chemoprevention, which includes further education about the adverse effects and recruitment of women to participate in a trial regarding chemoprevention when appropriate [91].

Given the aforementioned limitations, chemoprevention options should be offered to women who have a significantly higher risk of breast cancer with germline *BRCA* mutations. This is because prophylactic mastectomy is still the gold standard risk reduction method for women with *BRCA* mutations, but it is an invasive procedure and requires psychological considerations because of its impact on cosmetic appearance. Therefore, further evidence regarding the specific chemoprevention options for these women should be obtained. A particularly promising approach is to focus on the differences in the mechanisms of carcinogenesis and phenotypes between *BRCA1*-deficient and *BRCA2*-deficient breast cancers, and to develop strategies for chemoprevention in *BRCA1* and *BRCA2* mutation carriers. Considering these differences, subtype-based approaches are expected, such as endocrine therapy for *BRCA2* mutation carriers and denosumab for *BRCA1* mutation carriers. In addition, PARP inhibitors may be suitable agents for both *BRCA1* and *BRA2* mutation carriers.

There is insufficient evidence to confer an optimal duration of administration in chemoprevention. However, the administration of chemopreventive treatment may require the suspension of prophylactic mastectomy, thus avoiding the potential harm from surgery in healthy women with *BRCA* mutations.

Although several steps must be overcome to ensure the feasibility of chemoprevention in the clinical setting, an individualized treatment using the recently developed molecularly targeted drugs will help improve the efficacy of chemopreventive strategies in both research and clinical settings. The development of rational, effective, and minimally toxic prophylactic drugs with the ability to modify carcinogenesis at an early stage is needed to improve the clinical outcome of chemoprevention.

References

1. Sporn MB. Approaches to prevention of epithelial cancer during the preneoplastic period. Cancer Res. 1976;36(7 PT 2):2699–702.
2. Cummings SR, Tice JA, Bauer S, Browner WS, Cuzick J, Ziv E, et al. Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. J Natl Cancer Inst. 2009;101(6):384–98.
3. Mavaddat N, Pharoah PD, Michailidou K, Tyrer J, Brook MN, Bolla MK, et al. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015;107(5):djv036.
4. Althuis MD, Fergenbaum JH, Garcia-Closas M, Brinton LA, Madigan MP, Sherman ME. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomark Prev. 2004;13(10):1558–68.
5. Powles TJ, Ashley S, Tidy A, Smith IE, Dowsett M. Twenty-year follow-up of the Royal Marsden randomized, double-blinded tamoxifen breast cancer prevention trial. J Natl Cancer Inst. 2007;99(4):283–90.
6. Fisher B, Costantino JP, Wickerham DL, Cecchini RS, Cronin WM, Robidoux A, et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and bowel project P-1 study. J Natl Cancer Inst. 2005;97(22):1652–62.
7. Cuzick J, Forbes JF, Sestak I, Cawthorn S, Hamed H, Holli K, et al. Long-term results of tamoxifen prophylaxis for breast cancer--96-month follow-up of the randomized IBIS-I trial. J Natl Cancer Inst. 2007;99(4):272–82.

8. Veronesi U, Maisonneuve P, Rotmensz N, Bonanni B, Boyle P, Viale G, et al. Tamoxifen for the prevention of breast cancer: late results of the Italian randomized tamoxifen prevention trial among women with hysterectomy. J Natl Cancer Inst. 2007;99(9):727–37.
9. Cauley JA, Norton L, Lippman ME, Eckert S, Krueger KA, Purdie DW, et al. Continued breast cancer risk reduction in postmenopausal women treated with raloxifene: 4-year results from the MORE trial. Multiple outcomes of raloxifene evaluation. Breast Cancer Res Treat. 2001;65(2):125–34.
10. Martino S, Cauley JA, Barrett-Connor E, Powles TJ, Mershon J, Disch D, et al. Continuing outcomes relevant to Evista: breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst. 2004;96(23):1751–61.
11. Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355(2):125–37.
12. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Update of the national surgical adjuvant breast and bowel project study of tamoxifen and raloxifene (STAR) P-2 trial: preventing breast cancer. Cancer Prev Res (Phila). 2010;3(6):696–706.
13. Goss PE, Ingle JN, Ales-Martinez JE, Cheung AM, Chlebowski RT, Wactawski-Wende J, et al. Exemestane for breast-cancer prevention in postmenopausal women. N Engl J Med. 2011;364(25):2381–91.
14. Cuzick J, Sestak I, Forbes JF, Dowsett M, Knox J, Cawthorn S, et al. Anastrozole for prevention of breast cancer in high-risk postmenopausal women (IBIS-II): an international, double-blind, randomised placebo-controlled trial. Lancet. 2014;383(9922):1041–8.
15. Johnston SR, Dowsett M. Aromatase inhibitors for breast cancer: lessons from the laboratory. Nat Rev Cancer. 2003;3(11):821–31.
16. Cuzick J, Powles T, Veronesi U, Forbes J, Edwards R, Ashley S, et al. Overview of the main outcomes in breast-cancer prevention trials. Lancet. 2003;361(9354):296–300.
17. Nelson HD, Fu R, Zakher B, Pappas M, McDonagh M. Medication use for the risk reduction of primary breast cancer in women: updated evidence report and systematic review for the US preventive services task force. JAMA. 2019;322(9):868–86.
18. Cuzick J, Sestak I, Bonanni B, Costantino JP, Cummings S, DeCensi A, et al. Selective oestrogen receptor modulators in prevention of breast cancer: an updated meta-analysis of individual participant data. Lancet. 2013;381(9880):1827–34.
19. King MC, Wieand S, Hale K, Lee M, Walsh T, Owens K, et al. Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2 - national surgical adjuvant breast and bowel project (NSABP-P1) breast cancer prevention trial. JAMA. 2001;286(18):2251–6.
20. Reding KW, Bernstein JL, Langholz BM, Bernstein L, Haile RW, Begg CB, et al. Adjuvant systemic therapy for breast cancer in BRCA1/BRCA2 mutation carriers in a population-based study of risk of contralateral breast cancer. Breast Cancer Res Treat. 2010;123(2):491–8.
21. Phillips KA, Milne RL, Rookus MA, Daly MB, Antoniou AC, Peock S, et al. Tamoxifen and risk of contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. J Clin Oncol. 2013;31(25):3091–9.
22. Gronwald J, Robidoux A, Kim-Sing C, Tung N, Lynch HT, Foulkes WD, et al. Duration of tamoxifen use and the risk of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat. 2014;146(2):421–7.
23. Xu L, Zhao Y, Chen Z, Wang Y, Chen L, Wang S. Tamoxifen and risk of contralateral breast cancer among women with inherited mutations in BRCA1 and BRCA2: a meta-analysis. Breast Cancer. 2015;22(4):327–34.
24. Bramley M, Clarke RB, Howell A, Evans DGR, Armer T, Baildam AD, et al. Effects of oestrogens and anti-oestrogens on normal breast tissue from women bearing BRCA1 and BRCA2 mutations. Br J Cancer. 2006;94(7):1021–8.
25. Cuzick J, Warwick J, Pinney E, Duffy SW, Cawthorn S, Howell A, et al. Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case–control study. J Natl Cancer Inst. 2011;103(9):744–52.

26. Widschwendter M, Rosenthal AN, Philpott S, Rizzuto I, Fraser L, Hayward J, et al. The sex hormone system in carriers of BRCA1/2 mutations: a case-control study. Lancet Oncol. 2013;14(12):1226–32.
27. DeCensi A, Puntoni M, Guerrieri-Gonzaga A, Caviglia S, Avino F, Cortesi L, et al. Randomized placebo controlled trial of low-dose tamoxifen to prevent local and contralateral recurrence in breast intraepithelial neoplasia. J Clin Oncol. 2019;37(19):1629–37.
28. Simpson ER, Dowsett M. Aromatase and its inhibitors: significance for breast cancer therapy. Recent Prog Horm Res. 2002;57:317–38.
29. Key T, Appleby P, Barnes I, Reeves G, Endogenous H, Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst. 2002;94(8):606–16.
30. Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol. 2010;28(3):509–18.
31. Cheung AM, Tile L, Cardew S, Pruthi S, Robbins J, Tomlinson G, et al. Bone density and structure in healthy postmenopausal women treated with exemestane for the primary prevention of breast cancer: a nested substudy of the MAP.3 randomised controlled trial. Lancet Oncol. 2012;13(3):275–84.
32. Maunsell E, Goss PE, Chlebowski RT, Ingle JN, Ales-Martinez JE, Sarto GE, et al. Quality of life in MAP.3 (mammary prevention 3): a randomized, placebo-controlled trial evaluating exemestane for prevention of breast cancer. J Clin Oncol. 2014;32(14):1427–36.
33. Nemati Shafaee M, Gutierrez-Barrera AM, Lin HY, Arun B. Aromatase inhibitors and the risk of contralateral breast cancer in BRCA mutation carriers. J Clin Oncol. 2015;33(28_suppl):3.
34. Nagy V, Penninger JM. The RANKL-RANK story. Gerontology. 2015;61(6):534–42.
35. Hanada R, Hanada T, Sigl V, Schramek D, Penninger JM. RANKL/RANK—beyond bones. J Mol Med. 2011;89(7):647–56.
36. Kiesel L, Kohl A. Role of the RANK/RANKL pathway in breast cancer. Maturitas. 2016;86:10–6.
37. Hanley DA, Adachi JD, Bell A, Brown V. Denosumab: mechanism of action and clinical outcomes. Int J Clin Pract. 2012;66(12):1139–46.
38. Fata JE, Kong YY, Li J, Sasaki T, Irie-Sasaki J, Moorehead RA, et al. The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development. Cell. 2000;103(1):41–50.
39. Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature. 2010;468(7320):98–102.
40. Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature. 2010;468(7320):103–7.
41. Asselin-Labat ML, Vaillant F, Sheridan JM, Pal B, Wu D, Simpson ER, et al. Control of mammary stem cell function by steroid hormone signalling. Nature. 2010;465(7299):798–802.
42. Joshi PA, Jackson HW, Beristain AG, Di Grappa MA, Mote PA, Clarke CL, et al. Progesterone induces adult mammary stem cell expansion. Nature. 2010;465(7299):803–7.
43. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med. 2009;15(8):907–13.
44. King TA, Gemignani ML, Li W, Giri DD, Panageas KS, Bogomolniy F, et al. Increased progesterone receptor expression in benign epithelium of BRCA1-related breast cancers. Cancer Res. 2004;64(15):5051–3.
45. Ma Y, Katiyar P, Jones LP, Fan S, Zhang Y, Furth PA, et al. The breast cancer susceptibility gene BRCA1 regulates progesterone receptor signaling in mammary epithelial cells. Mol Endocrinol. 2006;20(1):14–34.
46. Poole AJ, Li Y, Kim Y, Lin SC, Lee WH, Lee EY. Prevention of Brca1-mediated mammary tumorigenesis in mice by a progesterone antagonist. Science. 2006;314(5804):1467–70.

47. Nolan E, Vaillant F, Branstetter D, Pal B, Giner G, Whitehead L, et al. RANK ligand as a potential target for breast cancer prevention in BRCA1-mutation carriers. Nat Med. 2016;22(8):933–9.
48. Yurgelun MB, Chenevix-Trench G, Lippman SM. Translating germline cancer risk into precision prevention. Cell. 2017;168(4):566–70.
49. Sigl V, Owusu-Boaitey K, Joshi PA, Kavirayani A, Wirnsberger G, Novatchkova M, et al. RANKL/RANK control Brca1 mutation-driven mammary tumors. Cell Res. 2016;26(7):761–74.
50. Obr AE, Grimm SL, Bishop KA, Pike JW, Lydon JP, Edwards DP. Progesterone receptor and Stat5 signaling cross talk through RANKL in mammary epithelial cells. Mol Endocrinol. 2013;27(11):1808–24.
51. Lee HJ, Gallego-Ortega D, Ledger A, Schramek D, Joshi P, Szwarc MM, et al. Progesterone drives mammary secretory differentiation via RankL-mediated induction of Elf5 in luminal progenitor cells. Development. 2013;140(7):1397.
52. Widschwendter M, Burnell M, Fraser L, Rosenthal AN, Philpott S, Reisel D, et al. Osteoprotegerin (OPG), the Endogenous inhibitor of receptor activator of NF-kappaB ligand (RANKL), is dysregulated in BRCA mutation carriers. EBioMedicine. 2015;2(10):1331–9.
53. Oden L, Akbari M, Zaman T, Singer CF, Sun P, Narod SA, et al. Plasma osteoprotegerin and breast cancer risk in BRCA1 and BRCA2 mutation carriers. Oncotarget. 2016;7(52):86687–94.
54. Gnant M, Pfeiler G, Dubsky PC, Hubalek M, Greil R, Jakesz R, et al. Adjuvant denosumab in breast cancer (ABCSG-18): a multicentre, randomised, double-blind, placebo-controlled trial. Lancet. 2015;386(9992):433–43.
55. Gnant M, Pfeiler G, Steger GG, Egle D, Greil R, Fitzal F, et al. Adjuvant denosumab in postmenopausal patients with hormone receptor-positive breast cancer (ABCSG-18): disease-free survival results from a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2019;20(3):339–51.
56. Ame JC, Spenlehauer C, de Murcia G. The PARP superfamily. BioEssays. 2004;26(8):882–93.
57. Dantzer F, Ame JC, Schreiber V, Nakamura J, Menissier-de Murcia J, de Murcia G. Poly(ADP-ribose) polymerase-1 activation during DNA damage and repair. Methods Enzymol. 2006;409:493–510.
58. Ame JC, Rolli V, Schreiber V, Niedergang C, Apiou F, Decker P, et al. PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. J Biol Chem. 1999;274(25):17860–8.
59. Ratnam K, Low JA. Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. Clin Cancer Res. 2007;13(5):1383–8.
60. Calvert H, Azzariti A. The clinical development of inhibitors of poly(ADP-ribose) polymerase. Ann Oncol. 2011;22(Suppl 1):i53–9.
61. Rehman FL, Lord CJ, Ashworth A. Synthetic lethal approaches to breast cancer therapy. Nat Rev Clin Oncol. 2010;7(12):718–24.
62. Iglehart JD, Silver DP. Synthetic lethality--a new direction in cancer-drug development. N Engl J Med. 2009;361(2):189–91.
63. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913–7.
64. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21.
65. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017;377(6):523–33.
66. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Goncalves A, Lee KH, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med. 2018;379(8):753–63.
67. To C, Kim EH, Royce DB, Williams CR, Collins RM, Risingsong R, et al. The PARP inhibitors, veliparib and olaparib, are effective chemopreventive agents for delaying mammary tumor development in BRCA1-deficient mice. Cancer Prev Res (Phila). 2014;7(7):698–707.

68. Chand SN, Blanco FF, Jimbo M, Tsangaris TN, Cristofanilli M, Yeo CJ, et al. PARP inhibitors for chemoprevention--letter. Cancer Prev Res (Phila). 2014;7(11):1170–1.
69. Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J Natl Cancer Inst. 1998;90(20):1529–36.
70. Taketo MM. Cyclooxygenase-2 inhibitors in tumorigenesis (part II). J Natl Cancer Inst. 1998;90(21):1609–20.
71. Awtry EH, Loscalzo J. Aspirin. Circulation. 2000;101(10):1206–18.
72. Thun MJ. Beyond willow bark: aspirin in the prevention of chronic disease. Epidemiology. 2000;11(4):371–4.
73. Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, et al. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. JAMA. 2004;291(20):2433–40.
74. Rostom A, Dube C, Lewin G, Tsertsvadze A, Barrowman N, Code C, et al. Nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors for primary prevention of colorectal cancer: a systematic review prepared for the U.S. preventive services task force. Ann Intern Med. 2007;146(5):376–89.
75. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010;376(9754):1741–50.
76. Luo T, Yan H-M, He P, Luo Y, Yang Y-F, Zheng H. Aspirin use and breast cancer risk: a meta-analysis. Breast Cancer Res Treat. 2012;131(2):581–7.
77. Lu L, Shi L, Zeng J, Wen Z. Aspirin as a potential modality for the chemoprevention of breast cancer: a dose-response meta-analysis of cohort studies from 857,831 participants. Oncotarget. 2017;8(25):40389–401.
78. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Low-dose aspirin in the primary prevention of cancer: the women's health study: a randomized controlled trial. JAMA. 2005;294(1):47–55.
79. Kehm RD, Hopper JL, John EM, Phillips K-A, MacInnis RJ, Dite GS, et al. Regular use of aspirin and other non-steroidal anti-inflammatory drugs and breast cancer risk for women at familial or genetic risk: a cohort study. Breast Cancer Res. 2019;21(1):52.
80. Chen WY, Winer EP, Ballman KV, Partridge AH, Carey LA, Carvan M, et al. ABC trial (A011502): a randomized phase III double-blinded placebo controlled trial of aspirin as adjuvant therapy breast cancer. J Clin Oncol. 2019;37(15_suppl):TPS599-TPS.
81. Coyle C, Cafferty FH, Rowley S, MacKenzie M, Berkman L, Gupta S, et al. ADD-ASPIRIN: a phase III, double-blind, placebo controlled, randomised trial assessing the effects of aspirin on disease recurrence and survival after primary therapy in common non-metastatic solid tumours. Contemp Clin Trials. 2016;51:56–64.
82. Decensi A, Serrano D, Bonanni B, Cazzaniga M, Guerrieri-Gonzaga A. Breast cancer prevention trials using retinoids. J Mammary Gland Biol Neoplasia. 2003;8(1):19–30.
83. Bonanni B, Lazzeroni M, Veronesi U. Synthetic retinoid fenretinide in breast cancer chemoprevention. Expert Rev Anticancer Ther. 2007;7(4):423–32.
84. Mehta RG, Moon RC, Hawthorne M, Formelli F, Costa A. Distribution of fenretinide in the mammary gland of breast cancer patients. Eur J Cancer Clin Oncol. 1991;27(2):138–41.
85. Veronesi U, Mariani L, Decensi A, Formelli F, Camerini T, Miceli R, et al. Fifteen-year results of a randomized phase III trial of fenretinide to prevent second breast cancer. Ann Oncol. 2006;17(7):1065–71.
86. Veronesi U, De Palo G, Marubini E, Costa A, Formelli F, Mariani L, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst. 1999;91(21):1847–56.
87. Formelli F, Cleris L. Synthetic retinoid fenretinide is effective against a human ovarian carcinoma xenograft and potentiates cisplatin activity. Cancer Res. 1993;53(22):5374.
88. De Palo G, Veronesi U, Camerini T, Formelli F, Mascotti G, Boni C, et al. Can fenretinide protect women against ovarian cancer? J Natl Cancer Inst. 1995;87(2):146–7.

89. De Palo G, Mariani L, Camerini T, Marubini E, Formelli F, Pasini B, et al. Effect of fenretinide on ovarian carcinoma occurrence. Gynecol Oncol. 2002;86(1):24–7.
90. Simeone A-M, Deng C-X, Kelloff GJ, Steele VE, Johnson MM, Tari AM. N-(4-Hydroxyphenyl)retinamide is more potent than other phenylretinamides in inhibiting the growth of BRCA1-mutated breast cancer cells. Carcinogenesis. 2005;26(5):1000–7.
91. Flanagan MR, Zabor EC, Stempel M, Mangino DA, Morrow M, Pilewskie ML. Chemoprevention uptake for breast cancer risk reduction varies by risk factor. Ann Surg Oncol. 2019;26(7):2127–35.

Chemoprevention for Ovarian Cancer

10

Hiroshi Kobayashi

Abstract

Objectives: This chapter describes the effects and precautions of chemoprevention of ovarian cancer.

Methods: We have collected literature searches reported in English from PubMed and Embase databases between 1990 and 2002.

Results: One of the important risk factors for ovarian cancer is a family history of ovarian and breast cancer. There is no clear evidence that screening tests with transvaginal ultrasonography or serum CA125 alone or in combination can reduce ovarian cancer incidence and mortality. Prophylactic risk-reduction salpingo-oophorectomy is an effective risk management option for ovarian cancer in high-risk populations. Chemoprevention is another option. We investigated the chemopreventive strategies by dividing the subjects into the general population and BRCA1/BRCA2 gene mutation carriers. OC significantly reduces the risk of ovarian cancer in the general population because there is an inverse relationship between the duration of OC use and risk reduction of ovarian cancer, with long-lasting protective effects even after OC is discontinued. In BRCA mutation carriers, several studies, including systematic review, meta-analysis of case-control studies, and case-control studies and review articles, have shown that OC reduces the risk of ovarian cancer, and that the longer OC is used, the lower the risk of ovarian cancer. Relative risk is expected to decrease by over 20% with every 5 years of use. OC significantly reduces ovarian cancer risk in both the general population and BRCA mutation carriers. On the other hand, OC has a modest but significant increased risk of breast cancer in certain BRCA mutation carriers when taking OC from under the age of 20 (or 30) or when using

H. Kobayashi (✉)
Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, Japan

Department of Gynecology, Ms.Clinic MayOne, Kashihara, Japan
e-mail: hirokoba@naramed-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_10

long-term OC before delivery. Use of natural progesterone or dydrogesterone is preferred to avoid increased breast cancer risk. The impact of OC on breast cancer risk is still controversial, so it is essential to consider some details: patient age, duration of use, and progesterone components.

Conclusion: In conclusion, OC shows a clear chemopreventive effect on ovarian cancer, but slightly increases the risk of breast cancer.

Keywords

Chemoprevention · Oral contraceptives · Ovarian cancer · Breast cancer · BRCA1/BRCA2 mutation carriers

10.1 Introduction

Epithelial ovarian cancer is one of the most common gynecologic cancers. Ovarian cancer is typically diagnosed at a late stage, mainly due to limitations in effective screening strategy and early diagnosis. 5-year overall survival rate in stage IIIC is as low as 44.4% in Japan [1]. The overall incidence and mortality rate from ovarian cancer are declining (29% and 33% in 30 years, respectively) in the United States, while the number of ovarian cancer patients is increasing in Japan (approximately doubled in 30 years) [2]. Ovarian cancer comprises a heterogeneous group of tumors [3] and is known to have distinct clinical and biological behaviors, including epidemiology; different identifiable risk factors; cellular origins; morphologic, immunophenotypic, and molecular features; genetic complexity; and driver mutations [4]. The common histological subtypes of epithelial ovarian cancer are serous, endometrioid, clear cell, and mucinous carcinoma, with the presence of specific driver mutations allowing further risk stratification [3]. Serous cancer is the most common in Western countries, but Asians have a higher incidence of clear cell and endometrioid carcinomas [2]. Ovarian cancer, especially high-grade serous cancer, has unique biological features that reflect the effects of germline BRCA1/BRCA2 mutations or somatic TP53 variants [5]. In particular, BRCA genetic mutation carriers are prone to breast cancer and ovarian cancer so it is necessary to take preventive measures against carcinogenesis throughout life. This chapter describes the effects and problems of chemoprevention of ovarian cancer.

10.2 Methods

10.2.1 Literature Search

We have collected literature searches reported in English from PubMed and Embase databases between January 1990 and March 2002, combining the keywords “chemoprevention” and “oral contraceptives” combined with “ovarian cancer,” “breast cancer,” “estrogen,” “progesterone,” “progestin,” “risk,” “general population,” and

"BRCA mutation carriers." A variety of combinations of these terms were used. Furthermore, the references of each article were searched to identify potentially relevant studies. Publications of original studies, review papers, and some guidelines were included, while those documenting opinions, points of view, or anecdotes were excluded.

10.3 Results

10.3.1 Risk Management Options for Ovarian Cancer

Epidemiological studies to date have identified risk factors for ovarian cancer. Several reproductive and hormonal factors may lower risk, including parity, lactation (breastfeeding), oral contraceptive (OC) use, the use of an intrauterine device (IUD), a hysterectomy, tubal ligation, or prophylactic bilateral salpingo-oophorectomy, while others, such as early age at menarche, older age at menopause, infertility, obesity, hormone replacement therapy (HRT), and a personal history of endometriosis, polycystic ovarian syndrome, or pelvic inflammatory disease, confer increased risks [6]. In clinical practice, it is necessary to identify the subgroups that are most effective in chemoprevention. The most important risk factor for ovarian cancer is a family history of ovarian and breast cancer [7]. Hereditary breast and ovarian cancer (HBOC) syndrome has pathogenic BRCA1 and BRCA2 gene mutations and accounts for 5–15% [8] and 15–20% [9] of all breast cancers and ovarian cancers, respectively. The rates of BRCA1/BRCA2 variants and variants of uncertain significance (VUS) were 19.7% and 6.5%, respectively, in 830 Japanese families who underwent BRCA1/BRCA2 genetic testing [10]. HBOC is frequently diagnosed in Japan as in the West [5]. Pathogenic variants in the BRCA genes greatly increase lifetime risk of breast cancer (40–80%, up to 85%) and ovarian cancer (11–50%, up to 60%) [11].

In general, three strategies are promising to reduce the incidence of cancer: prevention of carcinogenesis in gene mutation carriers, effective treatment after the onset of cancer, and reduction of future cancer risk for survivors. First, we outline the primary (lifestyle improvement), secondary (early detection and early treatment), and tertiary prevention (prevention of recurrence after cancer treatment) of cancer [12]. Primary prevention efforts include the lifestyle improvement, promotion of physical activity, and elimination of factors that increase the risk of cancer. Smoking cessation, a balanced diet, and moderate exercise are effective preventative measures, but they do not completely prevent the development of cancer. Secondary prevention includes early detection and early treatment of cancer. For example, cervical cancer has primary prevention with HPV vaccination and secondary prevention with cervical cancer screening. Combining primary and secondary prevention can reduce the incidence of cervical cancer. Finally, tertiary prevention is comprised of follow-up to prevent and delay recurrence and improve prognosis. Prevention efforts and their effectiveness in reducing risk factors have been demonstrated in breast, prostate, and colon cancers [13–15]. Selective estrogen receptor

modulators (SERMs) have been proposed for the primary prevention of estrogen receptor-positive breast cancer of postmenopausal women [14]. The prognosis of patients with colorectal cancer can be improved by tertiary prevention efforts with low-dose aspirin [13]. Green tea catechins may reduce the incidence of prostate cancer from precancerous lesions [15]. Epidemiological studies have revealed that the risk of ovarian cancer is reduced by childbirth, lactation, and fallopian tube ligation in both the general population and BRCA1 mutation carriers [16].

Next, we discuss management options to reduce ovarian cancer risk. For breast cancer, there are three options for risk-reducing management: close surveillance with clinical examinations and imaging studies, chemoprevention with drugs, and risk-reducing mastectomy (RRM) [5]. However, there is no evidence that screening tests, such as transvaginal ultrasonography, serum CA125 measurements, or their combination, can reduce the incidence and mortality of ovarian cancer [17]. Risk management options for ovarian cancer are limited, and prophylactic risk-reducing salpingo-oophorectomy is an effective strategy in high-risk populations [5]. NCCN and other guidelines recommend prophylactic surgery at ages 35–40. In actual practice, some patients at risk are not willing to undergo prophylactic surgery, so they choose surveillance. Chemoprevention is another option [5].

10.3.2 Impact of Chemoprevention on Ovarian Cancer Risk

The ideal chemopreventive agent is a natural or synthetic molecule that prophylactically and safely reduces the incidence or recurrence of malignancy. One of the most widely used chemopreventive agents for ovarian cancer is combined oral contraceptive (OC). Recently, OC combined with low-dose estrogen (ethinylestradiol <50 μg) and progestins is often used. Naturally synthesized in the body is called progesterone, and artificially synthesized is called progestin or progestogen. Medical care in Japan is divided into self-pay and insurance, and medical insurance is provided under the universal health insurance system. The terms “OC” and “low-dose estrogen and progestin (LEP)” in the narrow sense are used for contraception (self-payment) and treatment of endometriosis and dysmenorrhea (paid by insurance), respectively.

We investigated the chemopreventive strategies by dividing the subjects into the general population and BRCA1/BRCA2 gene mutation carriers. The results of each study are summarized in Table 10.1. In the general population, OC markedly reduces ovarian cancer risk [18–25]. There is a consistent inverse relation between duration of OC use and ovarian cancer risk [23]. The protective effect of OC against ovarian cancer persists for many years after stopping OC use [24]. Furthermore, OC use was shown to significantly prolong progression-free survival among patients with ovarian cancer [22]. However, a lack of randomized controlled trials limits the strength of evidence [21].

In addition, a literature search was conducted to determine whether OC use is recommended to reduce the risk of developing ovarian cancer in BRCA mutation carriers. In BRCA mutation carriers, several studies [26, 28, 30, 31, 34, 35],

Table 10.1 Impact of OC on ovarian cancer risk

Drugs	Targeted patients	First author	Publication year	Type of analysis	Results	Ref.
OC	General population	Bernstein L	1992	Review article	Oral contraceptives are effective as chemoprevention for ovarian cancer	[18]
OC	General population	Bosetti C	2002	Case-control study	A reduced risk of ovarian cancer: ever users compared to never users (OR = 0.66, 95% confidence interval (CI), 0.56–0.79); and women who had used OCs for ≥5 years compared to those who had used them for <5 years (OR = 0.50, 95% CI, 0.33–0.76). The OC protection persists for a long time after stopping use (more than 20 years)	[19]
OC	General population	Beral V	2008	Collaborative reanalysis of data	Data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Overall 7308 (31%) cases and 32,717 (37%) controls had ever used OC. The longer women used OC, the greater the reduction in ovarian cancer risk ($p < 0.0001$). Risk reduction continues for more than 30 years after discontinuing OC	[20]
OC	General population	Havrilesky LJ	2013	Meta-analysis	OC use reduces the risk of ovarian cancer (OR = 0.73; 95% CI, 0.66–0.81). Ovarian cancer risk is inversely correlated with duration of OC use	[21]
OC	General population	Jatoi A	2015	Retrospective cohort study	OC use reduces the risk of ovarian cancer by over 20% for every 5 years. Ovarian cancer patients who have previously used OC (ever users) have a significant longer progression-free survival than never users (HR = 0.78; 95% CI, 0.64–0.96)	[22]
OC	General population	La Vecchia C	2017	Case-control study	There are consistent inverse relations between parity, OC use, and the risk of ovarian cancer	[23]
OC	General population	Momenimovahed Z	2019	Observational study	Pregnancy, lactation, and OC use play a role in reducing the risk of ovarian cancer	[24]
OC	General population	Michels KA	2018	A population-based study	In the general population, risk reduction of ovarian cancer was correlated with the duration of OC use (long-term OC use [≥10 years] HR = 0.60; 95% CI, 0.47–0.76)	[25]

(continued)

Table 10.1 (continued)

Drugs	Targeted patients	First author	Publication year	Type of analysis	Results	Ref.
OC	BRCA mutation carriers	Narod SA	1998	Case-control study	207 women with hereditary ovarian cancer (179 cases with BRCA1 mutations and 28 cases with BRCA2 mutations). The adjusted odds ratio for ovarian cancer in ever OC users is 0.5 (95% CI, 0.3–0.8) [BRCA1 mutation (OR = 0.5; 95% CI, 0.3–0.9) and BRCA2 mutation (OR = 0.4; 95% CI, 0.2–1.1)]. OC use is associated with a statistically significant, time-dependent reduction in the risk of ovarian cancer, with 20% risk reduction for up to 3 years of use and up to 60% for 6 or more years of use	[26]
OC	General population and BRCA mutation carriers	Modan B	2001	A population-based case-control study	OC is less effective in preventing ovarian cancer in BRCA mutation carriers than in the general population	[27]
OC	BRCA mutation carriers	Cibula D	2010	Cohort and case-control studies	OC has a significant protective effect on the risk of ovarian cancer for BRCA1/BRCA2 mutation carriers. Protection increases with duration of use, with a 20% reduction in relative risk every 5 years of use	[28]
OC	BRCA mutation carriers	Rice LW	2010	Review article	OC significantly reduces the incidence of ovarian cancer in both the general population and BRCA1/BRCA2 mutation carriers. The risk-reduction effect is enhanced by long-term use. Its effect persists for more than 30 years after discontinuation of OC, but diminishes over time	[29]
OC	BRCA mutation carriers	Iodice S	2010	Meta-analysis of 18 studies	1503 ovarian cancer patients with BRCA1/BRCA2 mutations are included in the meta-analysis based on a total of 18 studies. OC significantly reduces the risk of ovarian cancer in BRCA mutation carriers to 50% (summary relative risk (SRR) = 0.50; 95% CI, 0.33–0.75) [BRCA1 mutation carriers (SRR = 0.51; 95% CI, 0.40–0.65) and BRCA2 mutation carriers (SRR = 0.52; 95% CI, 0.31–0.87)]. A significant 36% risk reduction for each additional 10 years of OC use (SRR = 0.64; 95% CI, 0.53–0.78)	[30]

OC	BRCA mutation carriers	Cibula D	2011	Meta-analysis of three case-control studies	Among BRCA1/BRCA2 mutation carriers, ever OC users result in a significantly lower risk of ovarian cancer than never users (OR = 0.57; 95% CI, 0.47–0.70). Ever OC users carrying BRCA1/BRCA2 mutations have a lower risk of ovarian cancer with longer use (OR = 0.95; 95% CI: 0.93–0.97; $p < 0.001$). The reduction of ovarian cancer risk in OC users is similar in BRCA1/BRCA2 mutation carriers and the general population	[31]
OC	Genetic high-risk patients	Davidson BA	2014	Review article	OC suppresses the development of ovarian cancer in women with strong family history	[32]
OC	BRCA mutation carriers	Perri T	2015	Historical cohort study	OC significantly reduces the risk of ovarian cancer in BRCA1/BRCA2 mutation carriers (OR; 0.19, 95% CI, 0.13–0.28) [33]	[34]
OC	BRCA mutation carriers	Huber D	2020	Systematic review including four meta-analyses, one review, one case-control study, and one retrospective cohort study	All papers report that OC use significantly reduces the risk of ovarian cancer in BRCA mutation carriers. In addition, several papers report an inverse association between the duration of OC use and ovarian cancer risk	[35]

including systematic review, meta-analysis of case-control studies, and case-control studies and review articles [36–40], have shown that OC reduces the risk of ovarian cancer, and that the longer OC is used, the lower the risk of ovarian cancer. Recently, Kathawala et al. published an elegant paper on current status and future directions on chemopreventive strategies for ovarian cancer [36]. The relative risk is expected to decrease by more than 20% for each 5 years of OC use. The latest 2020 systematic review also revealed that OC clearly reduces ovarian cancer risk in BRCA mutation carriers [35]. As a chemoprevention of ovarian cancer in BRCA mutation carriers, OC may be an alternative strategy for young women who do not accept prophylactic risk-reducing salpingo-oophorectomy [28, 31]. OC is recommended for reducing the risk of developing ovarian cancer, but has not been extensively and prospectively tested [22, 41]. Taken together, in all studies, OC significantly reduces ovarian cancer risk in both the general population and BRCA mutation carriers.

Besides OC, there are several reports on chemopreventive drugs for ovarian cancer. Chemoprevention with SERM, tamoxifen and raloxifene, not only reduces cancer risk [42] but also delays the onset of cancer in BRCA mutation carriers [43]. Meanwhile, long-term HRT may increase the risk of ovarian cancer [43].

First, we summarize the impact of OC on breast cancer risk. A later age at menarche, one full-term pregnancy, breastfeeding, and oophorectomy might be protective for BRCA1-associated breast cancer [44–46]. Nulliparous BRCA mutation carriers developed breast cancer about 5 years earlier than those who have given birth (36.4 vs. 40.9; $p = 0.001$) [47]. In a case-control study of BRCA1 mutation carriers, ever OC users were reported to have a 40% (odds ratio [OR] = 1.40; 0.95% CI 1.14–1.70) increased risk of early-onset breast cancer diagnosed before age 40 compared to never users [48]. The increased risk was greatest for women who started taking OC prior to age 20 (OR ever vs. never = 1.45; 95% CI 1.20–1.75) [48]. As shown in Table 10.2, several papers [21, 28, 49–54] reported that OC showed a modest but significant increase in breast cancer risk not only in the general population but also in BRCA mutation carriers, while others [16, 30, 31, 55–58] stated that OC did not increase the risk. According to the ACOG statement, “OC is not contraindicated in patients with a family history of breast cancer or in those with BRCA mutations” [62]. OC clearly reduces the risk of ovarian cancer, but taking OC for more than 5 years before age 30 can increase breast cancer risk [62]. Regarding OC use and breast cancer risk, various biases make it difficult to compare results between studies. The following factors are relevant: differences in study design used in case-control studies, differences in criteria for family history of breast cancer or ovarian cancer, and differences in criteria defining the control population of the study, such as BRCA mutation carriers who are not currently diagnosed with cancer, age of use, age distribution of population, age at onset of ovarian cancer or breast cancer, duration of use, OC dosage used, moderate- or low-dose ethinylestradiol, questionnaire survey method, the nationality, region, and ethnicity of the examined group. In summary, OC has a modest but significant increased risk of breast cancer in certain BRCA mutation carriers when taking OC from under the age of 20 (or 30) or when using long-term OC before delivery [47]. Thus, OC shows

Table 10.2 Impact of chemopreventive agents on breast cancer risk

Drugs	Breast cancer risk	Targeted patients	First author	Publication year	Type of analysis	Results	Ref.
OC	Increased[a]	General population	Santen RJ	2003		Prolonged administration of progestin to postmenopausal women can promote mammary epithelial cell proliferation	[49]
OC	Increased	General population	Cibula D	2010	All cohort and case-control studies published up to 2008	The impact of breast cancer risk disappears 5–10 years after stopping OC use	[28]
OC	Increased	General population	Havrilesky LJ	2013	Review article	Breast cancer incidence is slightly but significantly increased in current users of OC (OR = 1.08; 95% CI, 1.00–1.17), and the risk is significantly reduced over time from discontinuation	[21, 50]
OC	Increased	General population	Gierisch JM	2013	A systematic review		[50]
OC	Increased	BRCA mutation carriers	Bhothisuwan K	2004	Review article	Women who start OC before age 20 are at a higher risk of breast cancer than women who take OC at an older age	[51]
OC	Increased	BRCA mutation carriers	Jernström H	2005	Matched case-control study	OC use before the age of 20 significantly increases the risk of breast cancer. Results are similar with and without BRCA1/BRCA2 mutations	[52]
OC	Increased	BRCA mutation carriers	Brohet RM	2007	A cohort study	Women who took OC before age 20 have an increased risk of breast cancer. The impact on breast cancer risk disappears 5 to 10 years after the end of OC use. Given the high incidence of breast cancer in women taking moderate dose of OC before 1975, risk may depend on estrogen content	[53]

(continued)

Table 10.2 (continued)

Drugs	Breast cancer risk	Targeted patients	First author	Publication year	Type of analysis	Results	Ref.
OC	Increased	BRCA mutation carriers	Gadducci A	2010	Review article	Breast cancer risk is greater in women who took OC for more than 5 years, those who took OC before age 30, and those who had a long period of use until first full-term pregnancy	[54]
OC	Not increased	BRCA mutation carriers	Heimdal K	2002	A matched case-control study	No significant increase in breast cancer risk is associated with OC use in BRCA1 mutation carriers (HR = 2.00; 95% CI, 0.36–10.9)	[55]
OC	Inconsistent	General population	Fournier A	2008	A cohort study	Breast cancer risk is dependent on estrogen-only use and progesterone type. Progestins other than dydrogesterone may be associated with breast cancer risk	[56]
OC	Not increased/ inconsistent[b]	BRCA mutation carriers	Iodice S	2010	A meta-analysis	OC formulations used before 1975 are associated with a significantly increased risk of breast cancer in BRCA mutation carriers possibly due to their higher estrogen and progestin content than those available today (SRR = 1.47; 95% CI, 1.06–2.04). There is little evidence that recent OC formulations increase breast cancer risk	[30]
OC	Not increased/ inconsistent	BRCA mutation carriers	Cibula D	2011	A meta-analysis	No significant increase in breast cancer risk is associated with OC use in BRCA mutation carriers [BRCA1 (OR = 1.08; $p = 0.250$), in BRCA2 (OR = 1.03; $p = 0.788$)]. Since data on OC use and breast cancer risk are heterogeneous in BRCA mutation carriers, results are inconsistent across studies	[31]

OC	Not increased	BRCA mutation carriers	Moorman PG	2013	A meta-analysis	Meta-analysis showed a trend toward increased risk of breast cancer in ever OC users, but it was not statistically significant. The association between OC use and breast cancer risk in BRCA1/BRCA2 mutation carriers is similar to that reported in the general population	[57]
OC	Not increased	BRCA mutation carriers	Friebel TM	2014	Systematic review and meta-analysis	OC does not increase breast cancer risk in BRCA mutation carriers	[16]
OC	Not increased/ inconsistent	BRCA mutation carriers	Textbook	2015	Medical eligibility criteria for contraceptive use recommended by the WHO	The textbook published by the WHO (medical eligibility criteria for contraceptive use. Fifth edition, 2015, states that OC does not increase breast cancer risk for patients with a family history of breast cancer or for BRCA mutation carriers	[58]
Tamoxifen, raloxifene, aromatase inhibitors	Decreased	High-risk women	Reimers LL	2015	Self-administered questionnaires and medical chart abstraction	Chemoprevention with anti-estrogens, such as tamoxifen, raloxifene, and aromatase inhibitors, reduce breast cancer incidence in high-risk women. Selective estrogen receptor modulators (SERMs) are thought to reduce breast cancer risk. Tamoxifen has been reported to reduce breast cancer risk in BRCA2 mutation carriers and not in BRCA1 mutation carriers	[42]

(continued)

Table 10.2 (continued)

Drugs	Breast cancer risk	Targeted patients	First author	Publication year	Type of analysis	Results	Ref.
Levonorgestrel	Increased	General population	Soini T	2016	A nationwide cohort study	Intrauterine administration of levonorgestrel is associated with increased risk of lobular breast cancer (SIR = 1.33; 95% CI, 1.20–1.46) as well as ductal breast cancer (SIR = 1.20; 95% CI, 1.14–1.25)	[59]
HRT	Increased	General population	Azam S	2018	A cohort study	Compared to never users, current HRT use was statistically significantly associated with higher risk of breast cancer (HR = 1.87; 95% CI, 1.40–2.48)	[60]
HRT	Increased/ inconsistent	General population/ BRCA mutation carriers	Gordhandas S	2019	A systematic review	Prolonged HRT increased the risk of breast cancer moderately in the general population. However, HRT following RRSO does not adversely affect the reduction of breast cancer risk in BRCA mutation carriers	[61]

[a]Chemopreventive agents increased the risk of breast cancer

[b]Chemopreventive agents do not increase the risk of breast cancer. Results are inconsistent across studies

a clear chemopreventive effect on ovarian cancer, but slightly increases the risk of breast cancer.

Next, we investigate the chemopreventive effect of OC on cancers other than breast cancer. OC reduces risk of colorectal cancer (OR = 0.86; 95% CI, 0.79–0.95) [21, 50] and endometrial cancer (OR = 0.57; 95%CI, 0.43–0.76) [21, 28, 50], while it increases the risk of cervical cancer [21, 28, 50] and liver cancer [26]. A significant increase in the risk of both cervical intraepithelial neoplasia grade 3 (CIN3)/carcinoma in situ (CIS) and invasive cervical cancer was correlated with the duration of OC use (HR = 1.6 and HR = 1.8, respectively, for ≥15 years) versus never use [63]. OC use was associated with a significantly increased risk for hepatocellular carcinoma (relative risk 3.8, 95% CI, 1.0 to 14.6), with use over 8 years showing a marked increased risk (RR 20.1, [2.3 to 175.7]) [64].

Finally, we review the impact of chemopreventive agents other than OC on breast cancer risk. There are interesting reports regarding the risk of breast cancer caused by HRT [56]. Some papers have reported that HRT increases breast cancer risk [60], while others do not [61]. In the general population, current HRT use was significantly associated with higher risk of breast cancer compared to never users (hazard ratio 1.87, 95% CI 1.40–2.48) [60]. BRCA1/BRCA2 mutation carriers who undergo HRT after RRSO did not have an increased risk of breast cancer [65]. Intrauterine administration of levonorgestrel significantly increased risk of lobular breast cancer (standardized incidence ratio (SIR) 1.33, 95% CI 1.20–1.46) and ductal breast cancer (SIR 1.20, 95% CI, 1.14–1.25) [59]. On the other hand, chemoprevention with tamoxifen and raloxifene significantly reduced the risk of breast cancer in high-risk women [42].

10.3.3 Biological Diversity of OC

The mechanism by which OC reduces the risk of developing ovarian cancer is mainly due to three factors: inhibition of ovulation, suppression of excessive gonadotropin levels, and elimination of precancerous cells by progesterone [34]. First, the development of ovarian cancer may result from persistent ovulation-induced inflammation in fallopian tube epithelial cells or ovarian surface epithelial cells [33]. In vitro study has shown that the addition of follicular fluid to fallopian tube epithelial cells causes overexpression of the inflammatory cytokine interleukin-8, DNA double-strand breaks, induction of DNA repair pathways, and TP53 accumulation [66]. The accumulation of DNA damage through misrepair or incomplete repair during ovulation may lead to mutagenesis and consequently ovarian carcinogenesis. Thus, suppression of ovulation by OC may explain the chemopreventive effect on ovarian cancer [33, 67].

Second is the gonadotropin hypothesis that excessive gonadotropin exposure may induce ovarian carcinogenesis [68]. In vitro experiments clearly showed that FSH promotes cell proliferation, invasion, and angiogenesis via upregulation of vascular endothelial growth factor (VEGF) expression [69]. Also, FSH inhibits cell apoptosis via overexpression of the organic cation/carnitine transporter4 (OCT4)/

Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway [70]. However, serum FSH levels did not differ between women with ovarian cancer (median, 44.0 mIU/mL; range, 13.8–101.2) and controls (median, 43.4 mIU/mL; range, 13.5–109.5; $p = 0.17$) [71]. Furthermore, a nested case-control study revealed that women with high serum FSH levels were less likely to develop future ovarian cancer ($p = 0.005$) [72]. Serum gonadotropin levels are unlikely to reflect local concentrations in the ovary, but these clinical findings do not appear to actively support the gonadotropin hypothesis for ovarian carcinogenesis.

Finally, there are two issues to solve: one is to elucidate the mechanism by which progesterone suppresses or promotes carcinogenesis depending on the type of cancer, and the other is to investigate the effect of different types of progesterone (synthetic or natural) on cell proliferation. Progesterone can exert different hormonal effects on the ovary and breast. Progesterone induces apoptosis in normal and malignant human ovarian epithelial cells via upregulation of tumor suppressor gene p53 expression, resulting in inhibition of cell proliferation [73, 74]. Therefore, progesterone acts as a growth inhibitor on the fallopian tube and ovarian epithelial cells. Brisken et al. reported that progesterone is a key regulator of cell proliferation and stem cell activation in adult mammary gland [75]. Natural progesterone may either promote or inhibit the growth of mammary gland epithelial cells [49]. Synthetic progestins (particularly the combination of conjugated equine estrogens and medroxyprogesterone acetate) are always growth promoting [49]. In postmenopausal women, long-term medical treatment options including estrogen-progestin oral contraceptives may promote the growth of the terminal duct lobular units and subsequently enhance breast density [49].

Some paper reported that OC significantly increases the risk of breast cancer, while others do not. Why are the results of clinical trials different from study to study? The reason is probably because the risk of breast cancer depends on the type of progestin used [56]. The relative risk of breast cancer was 1.00 (95% CI, 0.83–1.22) for estrogen-natural progesterone, 1.16 (0.94–1.43) for estrogen-dydrogesterone, and 1.69 (1.50–1.91) for estrogen combined with other progestins [56]. The fact that synthetic progesterone has a higher carcinogenic potential than natural progesterone suggests that the progestin component may affect breast cancer risk. Use of natural progesterone or dydrogesterone is preferred to avoid increased breast cancer risk [49, 56].

10.4 Discussion

This chapter summarizes the effects and problems of chemoprevention of ovarian cancer in the general population and BRCA mutation carriers. There was no difference in chemopreventive effect on ovarian cancer between the two groups. The most frequently used chemopreventive agent is OC, and there was an inverse relationship between the duration of OC use and the risk reduction of ovarian cancer, and the protective effect persisted for many years after stopping OC use (Tables 10.1 and 10.2). On the other hand, taking OC from under the age of 20 or using long-term OC

before delivery gives a small but significant risk of breast cancer [47]. Use of natural progesterone or dydrogesterone does not increase breast cancer risk [49, 56]. The impact of OC on breast cancer risk is still controversial, so it is necessary to consider patient age, duration of use, and type of progesterone.

Compliance with Ethical Standards

Author Contributions: Hiroshi Kobayashi performed the literature search, collected data using the Web database, and contributed to the interpretation of included research studies.
Funding: No fund.
Ethical Approval: This article does not contain any studies with human participants or animals.
Conflict of Interest: Hiroshi Kobayashi declares that he has no conflict of interest.
Informed Consent (Optional): For this type of study, formal consent is not required.

References

1. Yamagami W, Nagase S, Takahashi F, Ino K, Hachisuga T, Mikami M, Enomoto T, Katabuchi H, Aoki D. A retrospective study for investigating the relationship between old and new staging systems with prognosis in ovarian cancer using gynecologic cancer registry of Japan Society of Obstetrics and Gynecology (JSOG): disparity between serous carcinoma and clear cell carcinoma. J Gynecol Oncol. 2020; https://doi.org/10.3802/jgo.2020.31.e45.
2. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A, Siegel RL. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68(4):284–96. https://doi.org/10.3322/caac.21456.
3. Ramalingam P. Morphologic, immunophenotypic, and molecular features of epithelial ovarian cancer. Oncology (Williston Park). 2016;30(2):166–76.
4. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers. 2016;2:16061. https://doi.org/10.1038/nrdp.2016.61.
5. Yamauchi H, Takei J. Management of hereditary breast and ovarian cancer. Int J Clin Oncol. 2018;23(1):45–51. https://doi.org/10.1007/s10147-017-1208-9.
6. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017;14(1):9–32. https://doi.org/10.20892/j.issn.2095-3941.2016.0084.
7. Nguyen HN, Averette HE, Janicek M. Ovarian carcinoma. A review of the significance of familial risk factors and the role of prophylactic oophorectomy in cancer prevention. Cancer. 1994;74(2):545–55.
8. Buys SS, Sandbach JF, Gammon A, Patel G, Kidd J, Brown KL, Sharma L, Saam J, Lancaster J, Daly MB. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer. 2017;123(10):1721–30. https://doi.org/10.1002/cncr.30498.
9. Shao D, Cheng S, Guo F, Zhu C, Yuan Y, Hu K, Wang Z, Meng X, Jin X, Xiong Y, Chai X, Li H, Zhang Y, Zhang H, Liu J, Ye M. Prevalence of hereditary breast and ovarian cancer (HBOC) predisposition gene mutations among 882 HBOC high-risk Chinese individuals. Cancer Sci. 2020;111(2):647–57. https://doi.org/10.1111/cas.14242.
10. Arai M, Yokoyama S, Watanabe C, Yoshida R, Kita M, Okawa M, Sakurai A, Sekine M, Yotsumoto J, Nomura H, Akama Y, Inuzuka M, Nomizu T, Enomoto T, Nakamura S. Genetic and clinical characteristics in Japanese hereditary breast and ovarian cancer: first report after

establishment of HBOC registration system in Japan. J Hum Genet. 2018;63(4):447–57. https://doi.org/10.1038/s10038-017-0355-1.
11. Singer CF, Tea MK, Pristauz G, Hubalek M, Rappaport C, Riedl CC, Helbich TH. Clinical practice guideline for the prevention and early detection of breast and ovarian cancer in women from HBOC (hereditary breast and ovarian cancer) families. Wien Klin Wochenschr. 2015;127(23–24):981–6. https://doi.org/10.1007/s00508-015-0880-x.
12. Goldberg JI, Borgen PI. Breast cancer susceptibility testing: past, present and future. Expert Rev Anticancer Ther. 2006;6(8):1205–14.
13. Brenner H, Chen C. The colorectal cancer epidemic: challenges and opportunities for primary, secondary and tertiary prevention. Br J Cancer. 2018;119(7):785–92. https://doi.org/10.1038/s41416-018-0264-x.
14. Mocellin S, Pilati P, Briarava M, Nitti D. Breast cancer chemoprevention: a network meta-analysis of randomized controlled trials. J Natl Cancer Inst. 2015;108(2):pii: djv318. https://doi.org/10.1093/jnci/djv318.
15. Cui K, Li X, Du Y, Tang X, Arai S, Geng Y, Xi Y, Xu H, Zhou Y, Ma W, Zhang T. Chemoprevention of prostate cancer in men with high-grade prostatic intraepithelial neoplasia (HGPIN): a systematic review and adjusted indirect treatment comparison. Oncotarget. 2017;8(22):36674–84. https://doi.org/10.18632/oncotarget.16230.
16. Friebel TM, Domchek SM, Rebbeck TR. Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. J Natl Cancer Inst. 2014;106(6):dju091. https://doi.org/10.1093/jnci/dju091.
17. Henderson JT, Webber EM, Sawaya GF. Screening for ovarian cancer: updated evidence report and systematic review for the US preventive services task force. JAMA. 2018;319(6):595–606. https://doi.org/10.1001/jama.2017.21421.
18. Bernstein L, Ross RK, Henderson BE. Relationship of hormone use to cancer risk. J Natl Cancer Inst Monogr. 1992;12:137–47.
19. Bosetti C, Negri E, Trichopoulos D, Franceschi S, Beral V, Tzonou A, Parazzini F, Greggi S, La Vecchia C. Long-term effects of oral contraceptives on ovarian cancer risk. Int J Cancer. 2002;102(3):262–5.
20. Collaborative Group on Epidemiological Studies of Ovarian Cancer, Beral V, Doll R, Hermon C, Peto R, Reeves G. Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls. Lancet. 2008;371(9609):303–14. https://doi.org/10.1016/S0140-6736(08)60167-1.
21. Havrilesky LJ, Gierisch JM, Moorman PG, Coeytaux RR, Urrutia RP, Lowery WJ, Dinan M, McBroom AJ, Wing L, Musty MD, Lallinger KR, Hasselblad V, Sanders GD, Myers ER. Oral contraceptive use for the primary prevention of ovarian cancer. Evid Rep Technol Assess (Full Rep). 2013;212:1–514.
22. Jatoi A, Foster NR, Kalli KR, Vierkant RA, Zhang Z, Larson MC, Fridley B, Goode EL. Prior oral contraceptive use in ovarian cancer patients: assessing associations with overall and progression-free survival. BMC Cancer. 2015;15:711. https://doi.org/10.1186/s12885-015-1774-z.
23. La Vecchia C. Ovarian cancer: epidemiology and risk factors. Eur J Cancer Prev. 2017;26(1):55–62.
24. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Women's Health. 2019;11:287–99. https://doi.org/10.2147/IJWH.S197604.
25. Michels KA, Pfeiffer RM, Brinton LA, Trabert B. Modification of the associations between duration of oral contraceptive use and ovarian, endometrial, breast, and colorectal cancers. JAMA Oncol. 2018;4(4):516–21. https://doi.org/10.1001/jamaoncol.2017.4942.
26. Narod SA, Risch H, Moslehi R, Dørum A, Neuhausen S, Olsson H, Provencher D, Radice P, Evans G, Bishop S, Brunet JS, Ponder BA. Oral contraceptives and the risk of hereditary ovarian cancer. Hereditary Ovarian Cancer Clinical Study Group. N Engl J Med. 1998;339(7):424–8.
27. Modan B, Hartge P, Hirsh-Yechezkel G, Chetrit A, Lubin F, Beller U, Ben-Baruch G, Fishman A, Menczer J, Struewing JP, Tucker MA, Wacholder S, National Israel Ovarian Cancer Study

Group. Parity, oral contraceptives, and the risk of ovarian cancer among carriers and noncarriers of a BRCA1 or BRCA2 mutation. N Engl J Med. 2001;345(4):235–40.
28. Cibula D, Gompel A, Mueck AO, La Vecchia C, Hannaford PC, Skouby SO, Zikan M, Dusek L. Hormonal contraception and risk of cancer. Hum Reprod Update. 2010;16(6):631–50. https://doi.org/10.1093/humupd/dmq022.
29. Rice LW. Hormone prevention strategies for breast, endometrial and ovarian cancers. Gynecol Oncol. 2010;118(2):202–7. https://doi.org/10.1016/j.ygyno.2010.03.014.
30. Iodice S, Barile M, Rotmensz N, Feroce I, Bonanni B, Radice P, Bernard L, Maisonneuve P, Gandini S. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. Eur J Cancer. 2010;46(12):2275–84. https://doi.org/10.1016/j.ejca.2010.04.018.
31. Cibula D, Zikan M, Dusek L, Majek O. Oral contraceptives and risk of ovarian and breast cancers in BRCA mutation carriers: a meta-analysis. Expert Rev Anticancer Ther. 2011;11(8):1197–207. https://doi.org/10.1586/era.11.38.
32. Davidson BA, Moorman PG. Risk-benefit assessment of the combined oral contraceptive pill in women with a family history of female cancer. Expert Opin Drug Saf. 2014 Oct;13(10):1375–82. https://doi.org/10.1517/14740338.2014.951327.
33. Sapoznik S, Bahar-Shany K, Brand H, Pinto Y, Gabay O, Glick-Saar E, Dor C, Zadok O, Barshack I, Zundelevich A, Gal-Yam EN, Yung Y, Hourvitz A, Korach J, Beiner M, Jacob J, Levanon EY, Barak M, Aviel-Ronen S, Levanon K. Activation-induced cytidine deaminase links ovulation-induced inflammation and serous carcinogenesis. Neoplasia. 2016;18(2):90–9. https://doi.org/10.1016/j.neo.2015.12.003.
34. Perri T, Lifshitz D, Sadetzki S, Oberman B, Meirow D, Ben-Baruch G, Friedman E, Korach J. Fertility treatments and invasive epithelial ovarian cancer risk in Jewish Israeli BRCA1 or BRCA2 mutation carriers. Fertil Steril. 2015;103(5):1305–12. https://doi.org/10.1016/j.fertnstert.2015.02.011.
35. Huber D, Seitz S, Kast K, Emons G, Ortmann O. Use of oral contraceptives in BRCA mutation carriers and risk for ovarian and breast cancer: a systematic review. Arch Gynecol Obstet. 2020;301(4):875–84. https://doi.org/10.1007/s00404-020-05458-w.
36. Kathawala RJ, Kudelka A, Rigas B. The chemoprevention of ovarian cancer: the need and the options. Curr Pharmacol Rep. 2018;4(3):250–60. https://doi.org/10.1007/s40495-018-0133-6.
37. Powell CB. Clinical management of patients at inherited risk for gynecologic cancer. Curr Opin Obstet Gynecol. 2015;27(1):14–22. https://doi.org/10.1097/GCO.0000000000000143.
38. Friedman LC, Kramer RM. Reproductive issues for women with BRCA mutations. J Natl Cancer Inst Monogr. 2005;34:83–6.
39. Brekelmans CT. Risk factors and risk reduction of breast and ovarian cancer. Curr Opin Obstet Gynecol. 2003;15(1):63–8.
40. Grenader T, Peretz T, Lifchitz M, Shavit L. BRCA1 and BRCA2 germ-line mutations and oral contraceptives: to use or not to use. Breast. 2005;14(4):264–8.
41. Drew DA, Cao Y, Chan AT. Aspirin and colorectal cancer: the promise of precision chemoprevention. Nat Rev Cancer. 2016;16(3):173–86. https://doi.org/10.1038/nrc.2016.4.
42. Reimers LL, Sivasubramanian PS, Hershman D, Terry MB, Greenlee H, Campbell J, Kalinsky K, Maurer M, Jayasena R, Sandoval R, Alvarez M, Crew KD. Breast Cancer chemoprevention among high-risk women and those with ductal carcinoma in situ. Breast J. 2015;21(4):377–86. https://doi.org/10.1111/tbj.12418.
43. Grann VR, Jacobson JS, Whang W, Hershman D, Heitjan DF, Antman KH, Neugut AI. Prevention with tamoxifen or other hormones versus prophylactic surgery in BRCA1/2-positive women: a decision analysis. Cancer J Sci Am. 2000;6(1):13–20.
44. Toss A, Grandi G, Cagnacci A, Marcheselli L, Pavesi S, De Matteis E, Razzaboni E, Tomasello C, Cascinu S, Cortesi L. The impact of reproductive life on breast cancer risk in women with family history or BRCA mutation. Oncotarget. 2017;8(6):9144–54. https://doi.org/10.18632/oncotarget.13423.
45. Narod SA. Hormonal prevention of hereditary breast cancer. Ann N Y Acad Sci. 2001;952:36–43.
46. Kotsopoulos J, Lubinski J, Salmena L, Lynch HT, Kim-Sing C, Foulkes WD, Ghadirian P, Neuhausen SL, Demsky R, Tung N, Ainsworth P, Senter L, Eisen A, Eng C, Singer C,

Ginsburg O, Blum J, Huzarski T, Poll A, Sun P, Narod SA. Hereditary breast Cancer clinical study group. Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res. 2012;14(2):R42. https://doi.org/10.1186/bcr3138.
47. Rieder V, Salama M, Glöckner L, Muhr D, Berger A, Tea MK, Pfeiler G, Rappaport-Fuerhauser C, Gschwantler-Kaulich D, Weingartshofer S, Singer CF. Effect of lifestyle and reproductive factors on the onset of breast cancer in female BRCA 1 and 2 mutation carriers. Mol Genet Genomic Med. 2015;4(2):172–7. https://doi.org/10.1002/mgg3.191.
48. Kotsopoulos J, Lubinski J, Moller P, Lynch HT, Singer CF, Eng C, Neuhausen SL, Karlan B, Kim-Sing C, Huzarski T, Gronwald J, McCuaig J, Senter L, Tung N, Ghadirian P, Eisen A, Gilchrist D, Blum JL, Zakalik D, Pal T, Sun P, Narod SA, Hereditary Breast Cancer Clinical Study Group. Timing of oral contraceptive use and the risk of breast cancer in BRCA1 mutation carriers. Breast Cancer Res Treat. 2014;143(3):579–86. https://doi.org/10.1007/s10549-013-2823-4.
49. Santen RJ. Risk of breast cancer with progestins: critical assessment of current data. Steroids. 2003;68(10–13):953–64.
50. Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, Dinan M, McBroom AJ, Hasselblad V, Sanders GD, Myers ER. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomark Prev. 2013;22(11):1931–43. https://doi.org/10.1158/1055-9965.EPI-13-0298.
51. Bhothisuwan K. Contraception and breast cancer. J Med Assoc Thail. 2004;87(Suppl 3):S167–8.
52. Jernström H, Loman N, Johannsson OT, Borg A, Olsson H. Impact of teenage oral contraceptive use in a population-based series of early-onset breast cancer cases who have undergone BRCA mutation testing. Eur J Cancer. 2005 Oct;41(15):2312–20.
53. Brohet RM, Goldgar DE, Easton DF, Antoniou AC, Andrieu N, Chang-Claude J, Peock S, Eeles RA, Cook M, Chu C, Noguès C, Lasset C, Berthet P, Meijers-Heijboer H, Gerdes AM, Olsson H, Caldes T, van Leeuwen FE, Rookus MA. Oral contraceptives and breast cancer risk in the international BRCA1/2 carrier cohort study: a report from EMBRACE, GENEPSO, GEO-HEBON, and the IBCCS Collaborating Group. J Clin Oncol. 2007;25(25):3831–6.
54. Gadducci A, Biglia N, Cosio S, Sismondi P, Genazzani AR. Gynaecologic challenging issues in the management of BRCA mutation carriers: oral contraceptives, prophylactic salpingo-oophorectomy and hormone replacement therapy. Gynecol Endocrinol. 2010;26(8):568–77. https://doi.org/10.3109/09513590.2010.487609.
55. Heimdal K, Skovlund E, Moller P. Oral contraceptives and risk of familial breast cancer. Cancer Detect Prev. 2002;26(1):23–7.
56. Fournier A, Berrino F, Clavel-Chapelon F. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. Breast Cancer Res Treat. 2008;107(1):103–11.
57. Moorman PG, Havrilesky LJ, Gierisch JM, Coeytaux RR, Lowery WJ, Peragallo Urrutia R, Dinan M, McBroom AJ, Hasselblad V, Sanders GD, Myers ER. Oral contraceptives and risk of ovarian cancer and breast cancer among high-risk women: a systematic review and meta-analysis. J Clin Oncol. 2013;31(33):4188–98. https://doi.org/10.1200/JCO.2013.48.9021.
58. World Health Organization. Medical eligibility criteria for contraceptive use. 5th ed. WHO; 2015. https://apps.who.int/iris/handle/10665/181468
59. Soini T, Hurskainen R, Grénman S, Mäenpää J, Paavonen J, Joensuu H, Pukkala E. Levonorgestrel-releasing intrauterine system and the risk of breast cancer: a nationwide cohort study. Acta Oncol. 2016;55(2):188–92. https://doi.org/10.3109/0284186X.2015.1062538.
60. Azam S, Lange T, Huynh S, Aro AR, von Euler-Chelpin M, Vejborg I, Tjønneland A, Lynge E, Andersen ZJ. Hormone replacement therapy, mammographic density, and breast cancer risk: a cohort study. Cancer Causes Control. 2018;29(6):495–505. https://doi.org/10.1007/s10552-018-1033-0.
61. Gordhandas S, Norquist BM, Pennington KP, Yung RL, Laya MB, Swisher EM. Hormone replacement therapy after risk reducing salpingo-oophorectomy in patients with BRCA1

or BRCA2 mutations; a systematic review of risks and benefits. Gynecol Oncol. 2019;153(1):192–200. https://doi.org/10.1016/j.ygyno.2018.12.014.
62. ACOG Committee on Practice Bulletins-Gynecology. ACOG practice bulletin. No. 73: use of hormonal contraception in women with coexisting medical conditions. Obstet Gynecol. 2006;107(6):1453–72.
63. Roura E, Travier N, Waterboer T, de Sanjosé S, Bosch FX, Pawlita M, Pala V, Weiderpass E, Margall N, Dillner J, Gram IT, Tjønneland A, Munk C, Palli D, Khaw KT, Overvad K, Clavel-Chapelon F, Mesrine S, Fournier A, Fortner RT, Ose J, Steffen A, Trichopoulou A, Lagiou P, Orfanos P, Masala G, Tumino R, Sacerdote C, Polidoro S, Mattiello A, Lund E, Peeters PH, Bueno-de-Mesquita HB, Quirós JR, Sánchez MJ, Navarro C, Barricarte A, Larrañaga N, Ekström J, Lindquist D, Idahl A, Travis RC, Merritt MA, Gunter MJ, Rinaldi S, Tommasino M, Franceschi S, Riboli E, Castellsagué X. The influence of hormonal factors on the risk of developing cervical cancer and pre-Cancer: results from the EPIC cohort. PLoS One. 2016;11(1):e0147029. https://doi.org/10.1371/journal.pone.0147029.
64. Forman D, Vincent TJ, Doll R. Cancer of the liver and the use of oral contraceptives. Br Med J (Clin Res Ed). 1986;292(6532):1357–61. https://doi.org/10.1136/bmj.292.6532.1357.
65. Marchetti C, De Felice F, Boccia S, Sassu C, Di Donato V, Perniola G, Palaia I, Monti M, Muzii L, Tombolini V, Benedetti Panici P. Hormone replacement therapy after prophylactic risk-reducing salpingo-oophorectomy and breast cancer risk in BRCA1 and BRCA2 mutation carriers: a meta-analysis. Crit Rev Oncol Hematol. 2018;132:111–5. https://doi.org/10.1016/j.critrevonc.2018.09.018.
66. Bahar-Shany K, Brand H, Sapoznik S, Jacob-Hirsch J, Yung Y, Korach J, Perri T, Cohen Y, Hourvitz A, Levanon K. Exposure of fallopian tube epithelium to follicular fluid mimics carcinogenic changes in precursor lesions of serous papillary carcinoma. Gynecol Oncol. 2014;132(2):322–7. https://doi.org/10.1016/j.ygyno.2013.12.015.
67. Fathalla MF. Incessant ovulation and ovarian cancer - a hypothesis re-visited. Facts Views Vis Obgyn. 2013;5(4):292–7.
68. Spona J, Elstein M, Feichtinger W, Sullivan H, Lüdicke F, Müller U, Düsterberg B. Shorter pill-free interval in combined oral contraceptives decreases follicular development. Contraception. 1996;54(2):71–7.
69. Huang Y, Hua K, Zhou X, Jin H, Chen X, Lu X, Yu Y, Zha X, Feng Y. Activation of the PI3K/AKT pathway mediates FSH-stimulated VEGF expression in ovarian serous cystadenocarcinoma. Cell Res. 2008;18(7):780–91. https://doi.org/10.1038/cr.2008.70.
70. Zhang Z, Zhu Y, Lai Y, Wu X, Feng Z, Yu Y, Bast RC Jr, Wan X, Xi X, Feng Y. Follicle-stimulating hormone inhibits apoptosis in ovarian cancer cells by regulating the OCT4 stem cell signaling pathway. Int J Oncol. 2013;43(4):1194–204. https://doi.org/10.3892/ijo.2013.2054.
71. Arslan AA, Zeleniuch-Jacquotte A, Lundin E, Micheli A, Lukanova A, Afanasyeva Y, Lenner P, Krogh V, Muti P, Rinaldi S, Kaaks R, Berrino F, Hallmans G, Toniolo P. Serum follicle-stimulating hormone and risk of epithelial ovarian cancer in postmenopausal women. Cancer Epidemiol Biomark Prev. 2003;12(12):1531–5.
72. McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, Kaaks R, Rinaldi S, Helzlsouer KJ. Prediagnostic circulating follicle stimulating hormone concentrations and ovarian cancer risk. Int J Cancer. 2009;125(3):674–9. https://doi.org/10.1002/ijc.24406.
73. Ivarsson K, Sundfeldt K, Brännström M, Janson PO. Production of steroids by human ovarian surface epithelial cells in culture: possible role of progesterone as growth inhibitor. Gynecol Oncol. 2001;82(1):116–21.
74. Murdoch WJ, Van Kirk EA. Steroid hormonal regulation of proliferative, p53 tumor suppressor, and apoptotic responses of sheep ovarian surface epithelial cells. Mol Cell Endocrinol. 2002;186(1):61–7.
75. Brisken C, Hess K, Jeitziner R. Progesterone and overlooked endocrine pathways in breast cancer pathogenesis. Endocrinology. 2015;156(10):3442–50. https://doi.org/10.1210/en.2015-1392.

Risk-Reducing Mastectomy (RRM)

11

Rina Kotake, Yasue Tsuchida, Kumiko Kida, and Hideko Yamauchi

Abstract

Hereditary breast and ovarian cancer (HBOC) syndrome comprises breast and ovarian cancer at exceptionally high rates in patients who have genetic mutations in *BRCA 1* and/or *BRCA 2* genes. Risk-reducing mastectomy (RRM) and bilateral salpingo-oophorectomy are effective preemptive strategies for women with BRCA mutations. Bilateral RRM (BRRM) decreases the incidence of breast cancer but is a radical surgical procedure; thus, it should be considered only for individuals at high risk and should not be routinely considered for those with low to average risk of breast cancer. Contralateral RRM (CRRM) can also reduce the incidence of contralateral breast cancer, but its effect on survival is difficult to determine owing to the concurrent risk-reducing salpingo-oophorectomy (RRSO) performed; thus, further studies that control for possible confounding variables are recommended.

RRM may reduce anxiety in high-risk individuals who think that developing breast cancer is inevitable, but understanding its true risk through genetic counseling may also reduce anxiety and perception of inevitability. Physical morbidity, lifestyle choices, and postoperative surgical complications are factors that should be considered when planning RRM since satisfaction with one's decision is generally high among individuals who underwent the procedure, although not with the cosmetic outcome brought about by surgical complications or reconstruction. Psychiatric morbidity owing to a negative body image and lack of sexual feelings are observed in individuals who undergo RRM. Psychological

R. Kotake · Y. Tsuchida · K. Kida · H. Yamauchi (✉)
Department of Breast Surgical Oncology, St. Luke's International Hospital, Tokyo, Japan
e-mail: yasuchi@luke.ac.jp; kidakumi@luke.ac.jp; hideyama@luke.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_11

support should thus also be a part of the process from decision-making to post-operative follow-up.

The decision to undergo RRM is highly personal. High-risk individuals must be provided with all available information on the merits and demerits of the procedure as well as alternatives and other risk management strategies such as RRSO, chemoprevention, and breast screening.

Keywords

Breast cancer · BRCA · Hereditary · Genetic · Prophylactic surgery

11.1 Introduction

Owing to advancements in genomic medicine, cancer care has shifted from curative medicine to preemptive medicine. We can now offer genetic testing to estimate an individual's cancer risk and consider preemptive interventions. One such preemptive strategy is risk-reducing surgery. Patients eligible for the procedure are well informed and provided with the choice of undergoing the procedure after weighing all possible options.

Hereditary breast and ovarian cancer syndrome (HBOC) comprises breast and ovarian cancer at exceptionally high rates in patients who have genetic mutations in *BRCA 1* and/or *BRCA 2* genes. To protect this population from developing the disease, it is critical to identify and encourage them to undergo genetic counseling and intensive screening. While other preventive strategies for women with HBOC are chemoprevention with tamoxifen and oral contraceptives, risk-reducing mastectomy (RRM) and bilateral salpingo-oophorectomy are effective strategies for women with BRCA mutations. Since RRM is an invasive and irreversible procedure that results in physical and emotional morbidity, women who are contemplating this procedure should be able to make informed decisions based on currently available evidence and weigh the benefits and limitations of the procedure with the benefits and limitations of other alternatives. Guiding and supporting a woman's decision entails the physician and other healthcare professionals to have the appropriate knowledge of the available evidence and establish a consensus to design a sustainable support system for risk-reducing procedures in the Asian community.

11.2 History of RRM

A study conducted by Hartmann et al. in 1999 described the efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. The subjects of the study were classified as high risk and moderate risk based on family

history. Prophylactic mastectomy was subsequently performed, and the incidence of breast cancer in respective groups was compared with that of their sisters as the control group. At the time the study was conducted, specific indications for prophylactic mastectomy included a family or personal history of breast cancer, multiple previous breast biopsies, unreliable results on physical examination of nodular breasts, findings of dense breast tissue on mammography, mastodynia, and cancer phobia. Genetic testing was not necessarily performed to opt for bilateral RRM (BRRM). This study concluded that in women with a high risk of breast cancer based on family history, prophylactic mastectomy can significantly reduce the incidence of breast cancer.

Tuttle et al. [1] reported in 2007 that their study on patient data from the Surveillance, Epidemiology, and End Results database in the United States showed an increasing trend of contralateral prophylactic mastectomy among patients with unilateral breast cancer during a 6-year period from 1998 to 2003. Increased rates were observed in all cancer stages. The authors concluded that there was a trend toward more aggressive surgical treatment for these patients.

In 2010, Domchek et al. [2] published a study on the association of risk-reducing surgery in BRCA1/BRCA2 mutation carriers with cancer risk and mortality. This study showed that among a cohort of women with BRCA1/BRCA2 mutations, the use of RRM was associated with a lower risk of breast cancer and RRSO was associated with a lower risk of ovarian cancer, breast cancer, and overall breast- and ovarian-cancer-specific mortality. The results of this study emphasized the reduced incidence and potential survival benefit of performing prophylactic surgery in patients with BRCA1/BRCA2 mutations.

11.3 Risk Reduction of Developing Breast Cancer

The following sections will be discussed in two parts: bilateral RRM (BRRM) for individuals with no personal history of breast cancer and contralateral RRM (CRRM) for those with a history of unilateral breast cancer.

11.3.1 BRRM

High-risk women who do not have a personal history of breast cancer may be provided the option of undergoing BRRM as a primary prevention strategy for the disease. A woman's decision to opt for BRRM is strongly correlated with her BRCA1 or BRCA2 mutation test results, as well as the recommendation of her physician.

The latest update of the Cochrane review by Carbine et al. [3] on RRM for the prevention of primary breast cancer revealed that 21 BRRM studies had consistent results showing a reduced incidence of breast cancer, reduced cancer mortality, or both. These studies showed a risk reduction ranging from 94% to 100%. One study showed that the incidence of breast cancer was 0.8% in the BRRM group in contrast

to 1.7% in the non-BRRM group; this indicated a protective effect, although not significant.

Risk-reducing surgery is not perfect, and complete removal of the breast tissue is not technically possible. However, the data from the studies in the Cochrane review add biological plausibility to the theory that reducing the amount of breast tissue reduces the risk of breast cancer.

11.3.2 CRRM

Women who have a personal history of unilateral breast cancer and are at a higher risk of developing a second primary cancer in the contralateral breast may consider RRM of the unaffected breast as a preventive strategy.

The Cochrane reviewed 26 CRRM studies and consistently reported a reduction in the incidence of contralateral breast cancer. However, there is a continuing risk of recurrence or metastasis from primary cancers. Healthcare professionals must consider other options to reduce breast cancer risk in addition to CRRM, such as bilateral risk-reducing salpingo-oophorectomy (BRRSO) and chemoprevention, for high-risk individuals.

11.4 Survival Benefit

11.4.1 BRRM

Studies have shown that BRRM not only decreases the incidence of breast cancer but also decreases the mortality rate due to breast cancer. Table 11.1 shows a summary of selected studies that examined the effect of BRRM on overall survival measured as hazard ratios.

However, Ingham et al. showed that when BRCA1/BRCA2 carriers who underwent BRRM were compared with those who did not, a borderline significant result was obtained: BRRM with BRRSO showed a significant survival advantage, and only BRRSO alone was significantly associated with improved survival. Therefore, the survival advantage could be attributed to BRRSO and not BRRM.

Arguments on the ethical aspect of removing the breast without disease were raised by Rookus et al., emphasizing that even BRCA 1/BRCA2 mutations have incomplete penetrance estimated at 70%; thus, 30% of BRRMs in carriers may be non-therapeutic and unnecessary. However, the authors further argued that the ineffectiveness of surveillance and the high lethality of late diagnosis of breast cancer make the recommendation of risk-reducing surgery a reasonable strategy.

Table 11.1 Literature on overall survival among subjects who underwent BRRM

Reference	Study design	Intervention	Outcome	Subjects #	Event#	Hazard ratio	Confidence interval
Heemskerk-Gerritsen et al. [4]	Cohort study	BRRM	Overall survival	BRRM: $n = 1128$ (BRCA1 $n = 722$, BRCA2 $n = 406$) Surveillance: $n = 1729$ (BRCA1 $n = 990$, BRCA2 $n = 739$)	BRRM: $n = 14$ (BRCA1 $n = 10$, BRCA2 $n = 4$) Surveillance: $n = 81$ (BRCA1 $n = 52$, BRCA2 $n = 29$)	BRCA1; 0.4, BRCA; 0.45	BRCA1; 95% CI 0.20–0.90, BRCA2; 95% CI 0.15–1.36
Li et al. [5]	Meta-analysis and systemic review	BRRM	Overall survival	NA	NA	0.23	95% CI 0.05–1.02, $p = 0.885$
Ingham et al. [6]	Cohort study	BRRM	Overall survival	BRRM: $n = 126$ (of which $n = 58$ had BRRM only, $n = 68$ had BRRM+RRSO) No risk-reducing surgery: $n = 457$	BRRM: $n = 2$ (not specified) No risk-reducing surgery: $n = 71$ (not specified)	0.25	95% CI 0.03–1.81, $p = 0.14$
Heemskerk-Gerritsen et al. [7]	Cohort study	BRRM	Overall survival	BRRM: $n = 212$ (BRCA1 $n = 156$, BRCA2 $n = 56$) Surveillance: $n = 358$ (BRCA1 $n = 249$, BRCA2 $n = 109$)	BRRM: $n = 1$ Surveillance: $n = 6$	0.22	95% CI 0.02–1.68

Table 11.2 Literature on overall survival among subjects who underwent CRRM

Reference	Study design	Intervention	Outcome	Subjects #	Event#	Hazard ratio	Confidence interval
Heemskerk-Gerritsen et al. [8]	Cohort study	CRRM	Overall survival	CRRM: $n = 242$ (BRCA1 $n = 193$, BRCA2 $n = 49$) Surveillance: $n = 341$ (BRCA1 $n = 261$, BRCA2 $n = 80$)	CRRM: $n = 19$ (not specified) Surveillance: $n = 65$ (not specified)	0.49	95% CI 0.29–0.82
Metcalfe et al. [9]	Cohort study	CRRM	Breast cancer-specific survival	CRRM[a]: $n = 181$ (BRCA1 $n = 103$, BRCA2 $n = 76$[b]) No CRRM[c]: $n = 209$ (BRCA1 $n = 123$, BRCA2 $n = 82$[d])	CRRM: $n = 18$ (not specified) No CRRM: $n = 61$ (not specified)	0.55	95% CI 0.27–1.13 ($p = 0.10$)
van Sprundel et al. [10]	Cohort study	CRRM	Overall survival	CRRM: $n = 79$ (not specified) Surveillance: $n = 69$ (not specified)	CRRM: $n = 3$ (not specified) Surveillance: $n = 11$ (not specified)	0.35	95% CI 0.09–1.39 ($p = 0.14$)
Li et al. [5]	Meta-analysis and systematic review	CRRM	Overall survival	CRRM: $n = 545$ Surveillance: $n = 1127$	NA	0.512	95% CI 0.368–0.714
Soenderstrup et al. [11]	Cohort study	CRRM	Overall survival	CRRM: $n = 147$ (BRCA1 $n = 95$, BRCA2 $n = 52$) No CRRM: $n = 90$ (BRCA1 $n = 46$, BRCA2 $n = 44$)	NA	0.42	95% CI 0.21–0.84 ($p < 0.01$)

[a]The term used in the text is "bilateral mastectomy"
[b]Those who did not undergo the examination are included; thus, the total number is less than 181
[c]The term used in the text is "unilateral mastectomy"
[d]Those who did not undergo the examination are included; thus, the total number is less than 209

11.4.2 CRRM

Improving survival for women who have already been diagnosed with breast cancer is the most important point to be considered in CRRM since the procedure itself does not alter the primary breast cancer outcome. However, conducting research studies on this group of individuals is difficult owing to various confounding factors such as selection bias, including age and/or other concurrent treatments undertaken.

Table 11.2 shows a summary of the selected studies, which examined the effect of CRRM on overall survival measured as hazard ratios.

Three studies [12–14] found evidence from analyzing survival data that the survival advantage may be due to selection bias with healthier, younger women selecting CRRM. These women may have had fewer comorbidities. Therefore, it is possible that the observed survival benefits may be a result of healthier people choosing or being recommended for CRRM rather than the actual benefit of CRRM over unilateral mastectomy of the affected breast.

There were three other studies [15–17], which assessed at the impact of tumor size and breast cancer stage on survival results. One study [15] found that CRRM was associated with improved disease-specific survival among participants with stage I to III breast cancer and that survival declined with age. The group of participants older than 60 years had no risk reduction from the procedure, showing that the risk of mortality due to contralateral disease should be weighed against the risk of mortality due to primary tumor metastases. A study by Peralta et al. controlled for prognostic factors, such as features of the primary tumor, when assessing whether CRRM improves survival. The study found no overall survival benefit at 15 years, but when they assessed breast cancer (disease-specific) survival, there was a significant benefit for the subgroup of participants with early stages of the disease (stages 0, I, and II). A study by Zeichner et al. had major differences in tumor size and follow-up period between the CRRM and no CRRM groups; thus, the results showed a detection bias.

We cannot overemphasize the fact that it is difficult for studies to be conducted to determine whether CRRM improves survival because in a real clinical setting RRSO is concurrently performed in individuals undergoing CRRM. It would be unethical to design a study in which RRSO is deliberately omitted in the treatment of an individual. Therefore, we cannot determine the effect of CRRM alone on survival since RRSO is a confounding factor that cannot be controlled for in the statistical analysis.

11.5 Occult Cancer

A study conducted by [18, 19] showed that among the 53 prophylactic mastectomy specimens examined, 6 (11.3%) were found to have occult cancer despite extensive preoperative imaging assessment studies, which included mammography, ultrasound, and magnetic resonance imaging. This was a higher percentage than that in previous studies, as summarized by the authors (Table 11.3). They reviewed the

Table 11.3 Literature reporting on occult cancer

References	Subjects#	% of BRCA	# of BPM	# of total PM	Occult cancer rate by total PM#	Pre-PM exam	Pathological method
Hartmann [20]	645	NA	645	1290	6/1290 (0.5%)	NA	NA
Meijers-Heijboer [21]	76	100	76	152	1/152 (0.7%) LCIS: 1, no DCIS or IDC	PE, MMG, or MRI	3 random blocks/quadrant
Yao [22]	150	100	148	298	4/298 (1.3%) IDC: 1, DCIS: 3	PE, MMG, or US, all MRI	NA
Burger [23]	71	8.5	12	83	4/83 (4.8%) ILC (3.5 mm): 1, LCIS: 3	NA	NA
Boughey [24]	409	5.6	27	436	22/436 (5.0%) IDC: 2, ILC: 6 (IDC and ILC: 2–9 mm) DCIS: 14	PE, MMG	2 sections/each quadrant and nipple
Van Sprundel [10]	79	100	0	79	4/79 (5.1%) IDC (32 mm): 1, DCIS: 3	PE, radiological	NA
McLaughlin [25]	529	9.3	84	613	33/613 (5.4%) IDC: 10, DCIS: 23	PE, MMG (US and/or MRI) (235/529 pts.: MRI)	2 sections/each quadrant and nipple
Evans [26]	105	100	0	105	6/105 (5.7%) IDC: 4, DCIS: 2	NA	NA
Hoogerbrugge [27]	67	66	41	108	10/108 (9.3%) IDC (4 mm):1DCIS (2–40 mm): 9 (17/67 pts.: LCIS)[a]	PE, MMG, 4/10 pts. MRI (27/67 pts.: MRI)	5-mm slices and radiological examinations, then on appearance of suspicious lesions, they randomly selected each quadrant and nipple (average: 19 slides)
Kauff [28]	24	100	7	31	3/31 (9.7%) DCIS (7–20 mm): 3 (LCIS: 1)[a]	MMG	2–4 sections/each quadrant and nipple
Black [29]	173	17	19	192	19/192 (9.9%) IDC (1.5–10 mm): 5, DCIS: 14	59/173 pts MRI	NA
Yamauchi [19]	51	92	2	53	6/53 (11.3%) IDC (5 mm): 1, DCIS: 5	PE, MMG, US, and MRI	Approximately 1-cm slices

BPM, bilateral prophylactic mastectomy; *DCIS*, ductal carcinoma in situ; *IDC*, invasive ductal carcinoma; *ILC*, invasive lobular carcinoma; *LCIS*, lobular carcinoma in situ; *MMG*, mammography; *MRI*, magnetic resonance imaging; *NA*, not available; *PE*, physical examination; *PM*, prophylactic mastectomy; *US*, ultrasound

[a]LCIS was detected but not included as occult cancer cases as it may co-exist with DCIS

*Table used with permission from Yamauchi et al.

possible factors that were thought to influence occult cancer occurrence, including rates of bilateral prophylactic mastectomy, preoperative examination methods, BRCA1/BRCA2 mutation rates among subjects, and pathological methods. However, the study design and methods of the previous studies varied; therefore, a conclusive factor was not identified. It is notable, however, that occult cancers were diagnosed despite extensive preoperative examinations; thus, surveillance with similar examinations would not have been sufficient for these individuals.

11.6 Psychological Relief

Anxiety and fear brought about by the results of genetic testing or the diagnosis of unilateral breast cancer is considered to be addressed by risk-reducing surgeries. Informed decision-making by high-risk individuals can be made through proper and thorough education about the risks and nature of the disease, as well as the merits and demerits of each possible option.

Twenty studies assessed psychological measures. Most studies reported high levels of satisfaction with the decision to undergo RRM but had variation in satisfaction with cosmetic outcomes. Cancer anxiety or worry over breast cancer was significantly lower after BRRM than before BRRM and for the groups who opted for surveillance rather than BRRM. However, satisfaction with body image and sexual feelings diminished.

The healthcare team can facilitate or guide the individual in the decision-making process, but each individual is unique in their values, priorities, and expectations. What may be considered as the "best option" clinically by healthcare professionals may not be the "best" for the individual. Therefore, it is inevitable to cater to the needs of the individual and pay attention to what can provide them satisfaction.

11.7 Cost Effectivity

A systematic review by Petelin et al. [30] showed that RRSO with bilateral RRM was associated with the greatest increase in life expectancy and, therefore, the dominant strategy in terms of cost-effectiveness. In this study, combined RRSO/BRRM was less effective after adjusting for quality of life; therefore, RRSO alone may be a cost-effective alternative. In addition, this systematic review showed that following a primary breast cancer diagnosis, CRRM with or without RRSO was the more effective for managing secondary breast cancer risk and cost savings than breast cancer screening. Lastly, for breast cancer risk management after a diagnosis of ovarian cancer, BRRM was only cost-effective in younger BRCA1 carriers between 40 and 50 years of age and needed to be performed at least 5 years after the original ovarian cancer diagnosis. The authors expressed that the major limiting factor in all studies included in this systematic review was the lack of direct mortality data owing to the absence of conclusive longitudinal studies on BRCA risk management strategies. In addition, the studies were based on a small selection of high-income

Organisation for Economic Co-operation and Development countries, and these countries have a range of different healthcare systems, leading to potential differences in access and service delivery.

A study conducted by Yamauchi et al. in Japan showed that preventive strategies (RRM, RRSO, RRM, and RRSO) were more cost-effective than surveillance, with RRSO, RRM, and RRSO being preferable for BRCA1 mutation carriers and RRM, RRM, and RRSO for BRCA2 mutation carriers; quality adjustment was based on preference ratings. Analysis of the four strategies, including surveillance, using preference ratings, identified RRM and RRSO as the most cost-effective strategy for BRCA1 mutation carriers and RRM for BRCA2 mutation carriers. They also found that RRM was the optimal strategy for BRCA2 mutation carriers with quality-adjusted life years based on preference ratings, while RRM and RRSO were optimal when analyzed by life years. This suggests that RRM would be preferable if quality of life was emphasized, while RRM and RRSO would be preferred for survival.

Zendejas et al. [31] conducted a study on the cost-effectiveness of CRRM compared with routine surveillance in patients with a personal history of unilateral breast cancer in the USA. This study showed that CRRM was more cost-effective than surveillance for patients with breast cancer who were younger than 70 years of age. The results were sensitive to the BRCA-positive status and assumptions of quality of life differences between CRRM and surveillance patients, emphasizing the importance of tailoring the treatment for individual patients.

11.8 Disadvantages

11.8.1 Surgical Procedure Risk

Risk-reducing surgery, similar to any other surgical procedure, has procedural risks. Surgical site infection, hemorrhage, and postoperative pain are among the most common surgical complications. Additional procedures, such as breast reconstruction after mastectomy, may also add procedural risks. Seventeen case series reporting on adverse events due to RRM with or without reconstruction were reviewed by the Cochrane, and the reported rates of unanticipated reoperations ranged from 4% in those without reconstruction to 64% in participants who underwent reconstruction.

11.8.2 Morbidity and Physical Consequences

The physical condition at the time of RRM may affect morbidity. One study [32] found that women with a BMI of 25 to 30 kg/m^2 had a higher proportion of infections after RRM than women with a BMI < 25 kg/m^2, and the proportion of implant loss increased with increasing weight. Arver et al. also found that wound necrosis or epidermolysis was more common among smokers than nonsmokers, making smoking history one of the factors associated with postoperative morbidity.

A review of the literature by Alaofi et al. [33] stated that though RRMs have low morbidity, decrease cancer-specific distress, and improve symmetry, women still experience long-term effects in cosmetic, psychological, and social domains. Despite seemingly high satisfaction rates after undergoing the procedure, body image issues were significantly affected, especially with bilateral mastectomies. Many factors contribute to this, such as self-consciousness, feeling less sexually attractive, and dissatisfaction with scars. In addition, reconstruction may be associated with less satisfaction in the long term owing to more frequent surgical complications and concerns regarding the implants.

11.8.3 Psychological Consequences

A study by Van Dijk et al. [34] showed a significant decrease in perceived risk of breast cancer after genetic counseling, especially for women at relatively low risk as opposed to very high-risk women. RRM may help women feel more in control of their health and reassure themselves that they did everything possible to reduce their risk of developing breast cancer.

According to the Cochrane review, women generally reported satisfaction with their decision to undergo BRRM but were less consistent in satisfaction with cosmetic outcomes. Diminished satisfaction was often due to surgical complications. Two studies in the review showed that dissatisfaction with the decision to undergo BRRM correlated with either the discussion being initiated by the physician or the physician's advice to undergo BRRM being the primary deciding factor for high-risk individuals. This correlation between regret and the physician's role was not found to be true in the CRRM study by Montgomery et al., which investigated regret. A CRRM study by Nekhlyudov et al., who investigated satisfaction, showed that women who made the decision alone with or without their physician's opinions were twice more likely to be satisfied with their CRRM 6 months postoperatively than those who shared decision-making with their physician.

A study by Unukovych et al. [35] reported that more than 50% of women from families with a history of breast cancer who underwent CRRM had problems with appearance and scars and felt less attractive and feminine 2 years after the procedure. Den Heijer et al. [36] found that among women at a high risk of breast or ovarian cancer or both, who had undergone RRM, their general and breast cancer-specific stress levels were significantly diminished; however, their breast body image was diminished as well when preoperative and postoperative responses were compared.

11.9 Conclusion

RRM is an effective preemptive strategy for reducing the risk of breast cancer among women with BRCA mutations. BRRM decreases the incidence of breast cancer but is a radical surgical procedure that may be considered only for

high-risk individuals. CRRM can also reduce the incidence of contralateral breast cancer, but its effect on survival is difficult to determine because of the concurrent RRSO performed. Physical morbidity, lifestyle choices, and postoperative surgical complications should be considered when deciding on RRM. Psychological support should also be a part of the process from decision-making to postoperative follow-up. The healthcare team must evaluate and understand the true risk for each individual before recommending BRRM/CRRM to avoid over-treatment. High-risk individuals must be provided all the available information on the merits and disadvantages of the procedure, as well as alternatives and other risk management strategies such as RRSO, chemoprevention, and breast screening.

References

1. Tuttle TM, Habermann EB, Grund EH, Morris TJ, Virnig BA. Increasing use of contralateral prophylactic mastectomy for breast cancer patients: a trend toward more aggressive surgical treatment. J Clin Oncol Off J Am Soc Clin Oncol. 2007;25(33):5203–9. https://doi.org/10.1200/JCO.2007.12.3141.
2. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967–75. https://doi.org/10.1001/jama.2010.1237.
3. Carbine NE, Lostumbo L, Wallace J, Ko H. Risk-reducing mastectomy for the prevention of primary breast cancer. Cochrane Database Syst Rev. 2018;4(4):CD002748. https://doi.org/10.1002/14651858.CD002748.pub4.
4. Heemskerk-Gerritsen BAM, Jager A, Koppert LB, et al. Survival after bilateral risk-reducing mastectomy in healthy BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat. 2019;177(3):723–33. https://doi.org/10.1007/s10549-019-05345-2.
5. Li X, You R, Wang X, et al. Effectiveness of prophylactic surgeries in BRCA1 or BRCA2 mutation carriers: a meta-analysis and systematic review. Clin Cancer Res. 2016;22(15):3971–81. https://doi.org/10.1158/1078-0432.CCR-15-1465.
6. Ingham SL, Sperrin M, Baildam A, et al. Risk-reducing surgery increases survival in BRCA1/2 mutation carriers unaffected at time of family referral. Breast Cancer Res Treat. 2013;142(3):611–8.
7. Heemskerk-Gerritsen BAM, Menke-Pluijmers MBE, Jager A, et al. Substantial breast cancer risk reduction and potential survival benefit after bilateral mastectomy when compared with surveillance in healthy BRCA1 and BRCA2 mutation carriers: a prospective analysis. Ann Oncol. 2013;24(8):2029–35. https://doi.org/10.1093/annonc/mdt134.
8. Heemskerk-Gerritsen BAM, Rookus M, Aalfs CM, et al. Improved overall survival after contralateral risk-reducing mastectomy in brca1/2 mutation carriers with a history of unilateral breast cancer: a prospective analysis. Int J Cancer. 2015;136:668–77. https://doi.org/10.1002/ijc.29032.
9. Metcalfe K, Gershman S, Ghadirian P, et al. Contralateral mastectomy and survival after breast cancer in carriers of BRCA1 and BRCA2 mutations: retrospective analysis. BMJ. 2014;348:g226.
10. van Sprundel TC, Schmidt MK, Rookus MA, et al. Risk reduction of contralateral breast cancer and survival after contralateral prophylactic mastectomy in BRCA1 or BRCA2 mutation carriers. Br J Cancer. 2005;93(3):287–92. https://doi.org/10.1038/sj.bjc.6602703.
11. Soenderstrup IMH, Laenkholm AV, Jensen MB, et al. Clinical and molecular characterization of BRCA-associated breast cancer: results from the DBCG. Acta Oncol. 2018;57(1):95–101. https://doi.org/10.1080/0284186X.2017.1398415.

12. Herrinton LJ, Barlow WE, Yu O, et al. Efficacy of prophylactic mastectomy in women with unilateral breast cancer: a cancer research network project. J Clin Oncol Off J Am Soc Clin Oncol. 2005;23(19):4275–86.
13. Jatoi I, Parsons HM. Contralateral prophylactic mastectomy and its association with reduced mortality: evidence for selection bias. Breast Cancer Res Treat. 2014;148(2):389–96.
14. Kruper L, Kauffmann RM, Smith DD, Nelson RA. Survival analysis of contralateral prophylactic mastectomy: a question of selection bias. Ann Surg Oncol. 2014;21(11):3448–56.
15. Bedrosian I, Hu CY, Chang GJ. Population-based study of contralateral prophylactic mastectomy and survival outcomes of breast cancer patients. J Natl Cancer Inst. 2010;102(6):401–9.
16. Peralta EA, Ellenhorn JD, Wagman LD, Dagis A, Andersen JS, Chu DZ. Contralateral prophylactic mastectomy improves the outcome of selected patients undergoing mastectomy for breast cancer. Am J Surg. 2000;180(6):439–45.
17. Zeichner SB, Zeichner SB, Ruiz AL, Markward NJ, Rodriguez E. Improved long-term survival with contralateral prophylactic mastectomy among young women. Asian Pacific J Cancer Prevention: APJCP. 2014;15(3):1155–62.
18. Yamauchi H, Nakagawa C, Kobayashi M, Kobayashi Y, Mano T, Nakamura S, Arai M. Cost-effectiveness of surveillance and prevention strategies in BRCA1/2 mutation carriers. Breast Cancer (Tokyo, Japan). 2018a;25(2):141–50. https://doi.org/10.1007/s12282-017-0803-y.
19. Yamauchi H, Okawa M, Yokoyama S, et al. High rate of occult cancer found in prophylactic mastectomy specimens despite thorough presurgical assessment with MRI and ultrasound: findings from the hereditary breast and ovarian cancer registration 2016 in Japan. Breast Cancer Res Treat. 2018b;172(3):679–87. https://doi.org/10.1007/s10549-018-4953-1.
20. Hartmann LC, Schaid DJ, Woods JE, et al. Efficacy of bilateral prophylactic mastectomy in women with a family history of breast cancer. N Engl J Med. 1999;340(2):77–84.
21. Meijers-Heijboer H, van Geel B, van Putten WLJ, et al. Breast cancer after prophylactic bilateral mastectomy in women with a BRCA1 or BRCA2 mutation. N Engl J Med. 2001;345(3):159–64. https://doi.org/10.1056/NEJM200107193450301001071934503 01.
22. Yao K, Liederbach E, Tang R, et al. Nipple-sparing mastectomy in BRCA1/2 mutation carriers: an interim analysis and review of the literature. Ann Surg Oncol. 2014;22(2):370–6. 4-014-3883-3. https://doi.org/10.1245/s1043.
23. Burger A, Thurtle D, Owen S, Mannu G, Pilgrim S, Vinayagam R, Pain S. Sentinel lymph node biopsy for risk-reducing mastectomy. Breast Journal. 2013;19(5):529–32. https://doi.org/10.1111/tbj.12157.
24. Boughey JC, Khakpour N, Meric-Bernstam F, et al. Selective use of sentinel lymph node surgery during prophylactic mastectomy. Cancer. 2006;107(7):1440–7. https://doi.org/10.1002/cncr.22176.
25. McLaughlin SA, Stempel M, Morris EA, Liberman L, King TA. Can magnetic resonance imaging be used to select patients for sentinel lymph node biopsy in prophylactic mastectomy? Cancer. 2008;112(6):1214–21. https://doi.org/10.1002/cncr.23298.
26. Evans DG, Ingham SL, Baildam A, et al. Contralateral mastectomy improves survival in women with BRCA1/2-associated breast cancer. Breast Cancer Res Treat. 2013;140(1):135–42. https://doi.org/10.1007/s1054.
27. Hoogerbrugge N, Bult P, de Widt-Levert LM, et al. High prevalence of premalignant lesions in prophylactically removed breasts from women at hereditary risk for breast cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2003;21(1):41–5. https://doi.org/10.1200/JCO.2003.02.137.
28. Kauff ND, Brogi E, Scheuer L, et al. Epithelial lesions in prophylactic mastectomy specimens from women with BRCA mutations. Cancer. 2003;97(7):1601–8. https://doi.org/10.1002/cncr.11225.
29. Black D, Specht M, Lee JM, et al. Detecting occult malignancy in prophylactic mastectomy: preoperative MRI versus sentinel lymph node biopsy. Ann Surg Oncol. 2007;14(9):2477–84. https://doi.org/10.1245/s1043.
30. Petelin L, Trainer AH, Mitchell G, Liew D, James PA. Cost-effectiveness and comparative effectiveness of cancer risk management strategies in BRCA1/2 mutation carriers: a systematic review. Genetic Med. 2018;20(10, Oct):1145–56. https://doi.org/10.1038/gim.2017.255.

31. Zendejas B, Moriarty JP, O'Byrne J, Degnim AC, Farley DR, Boughey JC. Cost-effectiveness of contralateral prophylactic mastectomy versus routine surveillance in patients with unilateral breast cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29(22):2993–3000. https://doi.org/10.1200/JCO.2011.35.6956.
32. Arver B, Isaksson K, Atterhem H, et al. Bilateral prophylactic mastectomy in Swedish women at high risk of breast cancer: a national survey. Ann Surg. 2011;253(6):1147–54.
33. Alaofi RK, Nassif MO, Al-Hajeili MR. Prophylactic mastectomy for the prevention of breast cancer: review of the literature. Avicenna Journal of Medicine. 2018;8(3):67–77.
34. Van Dijk S, Otten W, Zoeteweij MW, et al. Genetic counselling and the intention to undergo prophylactic mastectomy: effects of a breast cancer risk assessment. Br J Cancer. 2003;88(11):1675–81.
35. Unukovych D, Sandelin K, Liljegren A, Arver B, Wickman M, Johansson H, Brandberg Y. Contralateral prophylactic mastectomy in breast cancer patients with a family history: a prospective 2-years follow-up study of health related quality of life, sexuality and body image. Eur J Cancer. 2012 Nov;48(17):3150–6. https://doi.org/10.1016/j.ejca.2012.04.023.
36. Den Heijer M, Seynaeve C, Timman R, Duivenvoorden H, Vanheusden K, Tilanus-Linthorst M, Menke-Pluijmers M, Tibben A. Body image and psychological distress after prophylactic mastectomy and breast reconstruction in genetically predisposed women: a prospective long-term follow-up study. Eur J Cancer. 2012;48(9):1263–8.

Risk-Reducing Salpingo-oophorectomy (RRSO)

12

Yusuke Kobayashi and Daisuke Aoki

Abstract

Risk-reducing salpingo-oophorectomy (RRSO) is performed for the primary prevention of ovarian cancer in patients with hereditary breast–ovarian cancer (HBOC) syndrome. When HBOC is diagnosed without ovarian cancer, surveillance is performed using transvaginal ultrasound and serum CA125 assessment, and chemoprophylaxis is administered using oral contraceptives (OCs) or low-dose estrogen–progestin (LEP); however, RRSO is the most reliable treatment for ovarian cancer prevention. While RRSO is expected to gain popularity, due attention must be paid to the fact that this procedure is not easy to perform. Performing RRSO requires a deep understanding of the biological and anatomical characteristics of the structures surrounding ovarian cancer, paying attention to important points while performing surgical procedures, and taking precautions to facilitate pathology examination; moreover, a thorough understanding of gynecologic oncology and female reproductive medicine, such as treatment for surgical menopause, is required. Furthermore, following RRSO, minute ovarian cancers, which cannot be identified on preoperative evaluation, and occult cancers, which are serous tubal intraepithelial carcinoma (STIC) lesions of the fallopian tubes, can become apparent. To detect occult cancer, pathological

Y. Kobayashi (✉)
Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan

Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan
e-mail: kobax@a2.keio.jp

D. Aoki
Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan
e-mail: aoki@z7.keio.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_12

examination is inadequate in cases of benign disease, and it is important to proceed with the sectioning and extensively examining the fimbriated end (SEE-FIM) protocol in collaboration with pathologists. Moreover, for RRSO to perform its original role, which is primary prevention, it should be kept in mind to introduce the procedure at the end of childbirth between the age of 35 and 40 years, as recommended in the guidelines, and at an appropriate time based on the earliest age of ovarian cancer onset among individuals in the patient's family. To provide the maximum benefit to patients with HBOC, individuals involved in the care of such patients must deepen their knowledge not only in their own field of expertise but also in genetic medicine and incorporate this knowledge into routine medical care.

Keywords

Risk-reducing salpingo-oophorectomy (RRSO) · Hereditary breast–ovarian cancer (HBOC) syndrome · Ovarian cancer · Breast cancer · Peritoneal cancer Concurrent hysterectomy · Two-stage surgery · Sectioning and extensively examining the fimbriated end (SEE-FIM) protocol · Health care · Hormonal replacement therapy

12.1 Significance of RRSO

Among patients with HBOC, the risk of ovarian cancer onset before the age of 80 years is high at 44% among those harboring *BRCA1* gene variant and 17% among those harboring *BRCA2* gene variant [1]. Prophylactic treatments against this risk include RRSO and chemoprophylaxis using low-dose oral contraceptives (OCs) or low-dose estrogen–progestin (LEP). When RRSO cannot be performed, surveillance is performed using transvaginal ultrasound and serum CA125 assessment. Reportedly, the use of OC/LEP significantly reduces the risk of ovarian cancer by approximately 50% even in patients harboring *BRCA1/BRCA2* gene variants [2]. However, regarding the relationship between OC/LEP usage and breast cancer onset, some reports indicate an increased risk [3–5], whereas others indicate no relationship [6, 7]; thus, these issues should be fully explained when prescribing prophylactic agents. Regarding surveillance, it has been reported that screening using transvaginal ultrasound and serum CA125 assessment does not contribute to reducing mortality due to ovarian cancer [8, 9]. Nevertheless, the National Comprehensive Cancer Network (NCCN) guidelines recommend the following for patients who opt not to undergo RRSO and patients for whom the period until RRSO is long: commence surveillance via transvaginal ultrasound and serum CA 125 assessment from 35 years of age or 5–10 years before the earliest age of ovarian cancer diagnosis within the patient's family. However, there is no clear evidence to support these recommendations, and under such circumstances, attention has been recently drawn to RRSO, which actually helps prevent ovarian cancer onset. Many reports have described the effect of RRSO on lowering the risk of ovarian cancer onset in patients harboring *BRCA* gene variant; in a meta-analysis of 2840 individuals harboring *BRCA* gene variants, it was found that the risk of ovarian cancer onset (including fallopian tube cancer and

peritoneal carcinoma) was reduced by 79% following RRSO [10]. Furthermore, RRSO reportedly increases the overall survival rate and reduces the risk of onset of breast cancer and high-grade serous carcinoma (HGSC) [11]; thus, prophylactic treatment with RRSO is the most effective option for patients with HBOC. However, there are contradictory reports negating the effects of RRSO in reducing the risk of breast cancer onset [12]. Conversely, it has been reported that the probability of peritoneal carcinoma development following RRSO is 0.3% [13]; thus, surveillance for peritoneal carcinoma is necessary even after undergoing RRSO.

12.2 Standard RRSO Procedure

From the perspective of healthy organ resection, it is preferable to perform minimally invasive surgery. Reportedly, epithelial ovarian cancer, fallopian tube cancer, and peritoneal carcinoma are detected from RRSO specimens in 2.2–4.6% cases [10, 14–16], and if peritoneal findings are observed while performing RRSO, thorough observation of the peritoneal cavity, peritoneal lavage cytology, and biopsy must be performed. Although there are no reports of ovarian cancer developing from the residual ovary following oophorectomy among individuals harboring *BRCA* variants, there are reports of benign illness [17]; therefore, care must be taken to achieve total removal of the ovary or ovaries. In a prospective study of 20 patients whose uterine specimens were pathologically examined upon the excision of the fallopian tubes at the uterine horns using a procedure resembling RRSO, residual fallopian tubes with a median length of 6 mm and median surface area of 14 mm^2 were found at 29 out of 40 uterine horn sites (73%) [18]. Considering these reports, the following recommendations are suggested for performing RRSO [19–21]:

- Perform minimally invasive surgery (laparoscopy).
- Observe upper abdomen, bowel surface, greater momentum, appendix, and pelvic organs, and if peritoneal findings are present, perform a biopsy.
- Obtain pelvic washing for cytology.
- Surgically remove 2 cm of proximal ovarian the suspensory ligament, the entire fallopian tube up to cornua, the entire peritoneum covering the ovary and fallopian tube, and particularly, the peritoneum beneath adhesions between the fallopian tube and ovary and the pelvic wall.
- To avoid cell loss caused by operative manipulations, minimize manipulations of the fallopian tube and ovary.
- Collect resected specimens from within the peritoneal cavity using an endobag.

12.3 Surgical Options when Performing RRSO: Concurrent Hysterectomy and Two-Stage Surgery

The suitability of concurrent hysterectomy during RRSO has long been an ongoing debate [19, 22] and remains controversial. It is thought that concurrent hysterectomy is advantageous during hormone therapy for breast cancer and hormone replacement therapy (HRT) such as that for ovarian deficiency syndrome [23, 24].

In a prospective cohort study of 1083 individuals harboring *BRCA* variants who underwent RRSO only, uterine cancer developed in eight individuals during the median follow-up period of 5.1 years, with there being no clear increase in the observed risk following RRSO [25]. Conversely, in individuals harboring *BRCA1* gene variants, the risk of uterine body serous cancer increased (0.18 expected [O:E ratio, 22.2; 95% CI, 6.1–56.9; $P < 0.001$]). Reportedly, in women aged 40 years, longer overall survival (4.9 months) with higher cost-effectiveness was observed among those who underwent RRSO and total hysterectomy than among those who underwent RRSO alone [26]. Therefore, the advantages and disadvantages of performing concurrent hysterectomy should be fully explained preoperatively.

RRSO is recommended at the end of childbirth between the age of 35 and 40 years or at the earliest age of ovarian cancer onset among individuals in the patient's family [27]. It is considered to delay RRSO until age 40–45 years in patients with BRCA2 variant [27]; however, it has been reported that performing RRSO in women of reproductive age results in surgical menopause and increases the risk of osteoporosis, coronary heart disease, and cognitive dysfunction, which shortens the survival period compared with that among women who experience natural menopause [28]. Therefore, we examined two-stage prophylactic salpingectomy with delayed oophorectomy, whereby risk-reducing salpingectomy alone is performed while ovarian function is still present and risk-reducing oophorectomy is performed at menopause. Based on the report indicating that HGSC originates from the fallopian tube epithelium [29], HGSC occurrence in the fallopian tube is prevented by surgically removing the fallopian tubes, thereby preserving the ovaries and avoiding surgical menopause. While the aim is to prevent HGSC originating in the fallopian tubes and avoid premature menopause, outcomes such as the remaining risk of ovarian cancer and the impact on breast cancer have not been fully elucidated; thus, two-stage surgery is not recommended at this stage, and the results of ongoing current clinical trials are awaited.

12.4 Pathological Examination of RRSO Samples

During postoperative pathological examination, due caution must be exercised to detect minute ovarian cancers, which cannot be detected on preoperative evaluation, and occult cancers, which are STIC lesions of the fallopian tubes [16, 30, 31]. Regarding the histopathological diagnosis of RRSO-resected specimens, pathological examination is inadequate to detect occult cancer in cases of benign illness. Therefore, it is preferable to perform diagnosis after preparing specimens in accordance with the SEE-FIM protocol, whereby the fimbriae of the fallopian tubes are sectioned longitudinally and slices of the ovaries and fallopian tubes are prepared at 2–3-mm intervals and evaluated as serial sections [32]. At institutions that provide genetic counseling and have a collaboration system with pathologists, it is recommended that gynecologic oncologists perform RRSO in cooperation with clinical genetic specialists [16].

12.5 Post-RRSO Health Care

Care should be paid to various conditions that might arise following RRSO, such as climacteric disturbance, dyslipidemia, and osteoporosis (seen after natural menopause) as well as severe urogenital symptoms and psychological and/or somatovegetative symptoms seen in young women who undergo RRSO prior to natural menopause [33]. In particular, in many cases, ovarian dysfunction symptoms following RRSO such as palpitations, constipation, and shoulder stiffness are observed to be more severe compared with those observed after natural menopause [34]; moreover, sexuality can be disturbed [35]. Furthermore, regarding lipid profile and cardiovascular illness, high total cholesterol levels and metabolic syndrome are more common in women who have undergone RRSO [36, 37]. Regarding bone mass, it is unlikely that RRSO decreases bone mass and increases the incidence of bone fractures compared with natural menopause [34]. However, it has been reported that following RRSO, women experience reduced bone mass postoperatively from an earlier age while they are still young and bone mass decreases more rapidly after surgical menopause than after natural menopause [38]. For these reasons, regular assessment of patient's bone mass is important during postoperative follow-up. Health care following RRSO includes traditional Chinese medicine for ovarian insufficiency, statin therapy for dyslipidemia, and administration of calcium and bisphosphonate preparations for osteoporosis; however, HRT is considered for women with no history of breast cancer. HRT has been found to improve sexuality in women after RRSO [39, 40] and is useful for maintaining cognitive function for up to 45 years after RRSO [41]. Conversely, women harboring *BRCA* variant are at a high risk for breast cancer onset; therefore, risk elevation owing to HRT is a cause for concern among women in general. However, in a recent meta-analysis, HRT following RRSO was not found to increase the risk of breast cancer [42], and it is considered that HRT does not increase this risk in a short period [43].

12.6 Current State of RRSO and Future Prospects

The state of implementation of risk-reducing surgery differs between the Western and Asian countries. Reportedly, the proportion of individuals harboring a *BRCA1/BRCA 2* gene variant who have undergone RRSO ranges from 10% to 78% in the Western countries and an overwhelming majority of women (86.4%–97%) were satisfied with the decision to undergo surgery [44]. The results of this study showed that RRSO has gained popularity as an option among Western patients. Conversely, the number of studies including Asian patients is limited, and in a recent Japanese study including 488 individuals diagnosed with HBOC, it was reported that of all the participants, 153 (31.4%) underwent RRSO; however, the RRSO implementation rate is lower in Japan than in the Western countries, and its use is less widespread in Japan [45]. This is due to the fact that, in Japan, a limited number of institutions perform genetic screening for *BRCA1/BRCA2* and that RRSO has not gained popularity as it is not covered by public health insurance; moreover, medical

staff still have insufficient knowledge and experience for performing genetic diagnosis [46]. There have been several events that drew public attention to HBOC in Korea. First, the Korean Hereditary Breast Cancer Study was started in 2007 with support from the Ministry of Health and Welfare of Korea and the Korean Breast Cancer Society [47]. Second, the strategy of *BRCA* testing coverage by the National Health Insurance system was promoted in May 2012. Later, American actress Angelina Jolie announced that she harbored the *BRCA1* gene variant and had undergone bilateral risk-reducing mastectomy, which subsequently drew increased attention to risk-reducing surgery. This has been called the "Angelina effect." Following her disclosure, the rate of risk-reducing surgery increased from RRSO performed in 27 patients at 25 institutions in 2009 to 75 patients at 27 institutions in 2015 [48]. In Japan, RRSO has been covered under insurance for HBOC patients with a history of breast cancer since April 2020 and is expected to gain popularity in future. Conversely, the peak age for undergoing RRSO has been delayed to the late 40s or older in Japan [31, 45]; thus, some individuals develop occult cancer by the time of surgery, and consequently, the original role of RRSO, i.e., primary prevention, cannot be achieved. We must reconfirm the role of RRSO and firmly bear in mind that RRSO should be performed at the recommended appropriate time. Furthermore, more number of individuals are expected to undergo RRSO in future. Apart from *BRCA*, there are genes that cause HBOC, and because *BRIP1* [49], *RAD51C* [50], and *RAD51D* [51] increase the risk of ovarian cancer onset, RRSO should be considered at 45–50 years of age [27]. Other genes that might increase the risk of ovarian cancer onset include *ATM* [52] and *PALB2* [53]; however, further study is needed to determine whether RRSO should be performed in patients harboring these genes [27].

References

1. 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. JAMA. 2017;317(23):2402–16.
2. Iodice S, Barile M, Rotmensz N, Feroce I, Bonanni B, Radice P, et al. Oral contraceptive use and breast or ovarian cancer risk in BRCA1/2 carriers: a meta-analysis. Eur J Cancer. 2010;46(12):2275–84.
3. Anothaisintawee T, Wiratkapun C, Lerdsitthichai P, Kasamesup V, Wongwaisayawan S, Srinakarin J, et al. Risk factors of breast cancer: a systematic review and meta-analysis. Asia Pac J Public Health. 2013;25(5):368–87.
4. Gierisch JM, Coeytaux RR, Urrutia RP, Havrilesky LJ, Moorman PG, Lowery WJ, et al. Oral contraceptive use and risk of breast, cervical, colorectal, and endometrial cancers: a systematic review. Cancer Epidemiol Biomark Prev. 2013;22(11):1931–43.
5. Mørch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard Ø. Contemporary hormonal contraception and the risk of breast cancer. N Engl J Med. 2017;377(23):2228–39.
6. Ichida M, Kataoka A, Tsushima R, Taguchi T. No increase in breast cancer risk in Japanese women taking oral contraceptives: a case-control study investigating reproductive, menstrual and familial risk factors for breast cancer. Asian Pac J Cancer Prev. 2015;16(9):3685–90.
7. Kawai M, Minami Y, Kuriyama S, Kakizaki M, Kakugawa Y, Nishino Y, et al. Reproductive factors, exogenous female hormone use and breast cancer risk in Japanese: the Miyagi cohort study. Cancer Causes Control. 2010;21(1):135–45.

8. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. JAMA. 2011;305(22):2295–303.
9. Evans DG, Gaarenstroom KN, Stirling D, Shenton A, Maehle L, Dørum A, et al. Screening for familial ovarian cancer: poor survival of BRCA1/2 related cancers. J Med Genet. 2009;46(9):593–7.
10. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst. 2009;101(2):80–7.
11. Eleje GU, Eke AC, Ezebialu IU, Ikechebelu JI, Ugwu EO, Okonkwo OO. Risk-reducing bilateral salpingo-oophorectomy in women with BRCA1 or BRCA2 mutations. Cochrane Database Syst Rev. 2018;8(8):Cd012464.
12. Heemskerk-Gerritsen BA, Seynaeve C, van Asperen CJ, Ausems MG, Collée JM, van Doorn HC, et al. Breast cancer risk after salpingo-oophorectomy in healthy BRCA1/2 mutation carriers: revisiting the evidence for risk reduction. J Natl Cancer Inst. 2015;107(5)
13. Domchek SM, Friebel TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010;304(9):967–75.
14. Rebbeck TR, Lynch HT, Neuhausen SL, Narod SA, Van't Veer L, Garber JE, et al. Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. N Engl J Med. 2002;346(21):1616–22.
15. Finch A, Beiner M, Lubinski J, Lynch HT, Moller P, Rosen B, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 mutation. JAMA. 2006;296(2):185–92.
16. Sherman ME, Piedmonte M, Mai PL, Ioffe OB, Ronnett BM, Van Le L, et al. Pathologic findings at risk-reducing salpingo-oophorectomy: primary results from gynecologic oncology group trial GOG-0199. J Clin Oncol. 2014;32(29):3275–83.
17. Donnez O, Squiffflet J, Marbaix E, Jadoul P, Donnez J. Primary ovarian adenocarcinoma developing in ovarian remnant tissue ten years after laparoscopic hysterectomy and bilateral salpingo-oophorectomy for endometriosis. J Minim Invasive Gynecol. 2007;14(6):752–7.
18. Cass I, Walts A, Karlan BY. Does risk-reducing bilateral salpingo-oophorectomy leave behind residual tube? Gynecol Oncol. 2010;117(1):27–31.
19. Kauff ND, Barakat RR. Risk-reducing salpingo-oophorectomy in patients with germline mutations in BRCA1 or BRCA2. J Clin Oncol. 2007;25(20):2921–7.
20. Powell CB, Chen LM, McLennan J, Crawford B, Zaloudek C, Rabban JT, et al. Risk-reducing salpingo-oophorectomy (RRSO) in BRCA mutation carriers: experience with a consecutive series of 111 patients using a standardized surgical-pathological protocol. Int J Gynecol Cancer. 2011;21(5):846–51.
21. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Ovarian cancer including fallopian tube cancer and primary peritoneal cancer, ver1. 2020. Accessed Sep 30, 2020.
22. Practice Bulletin No ACOG. 103: hereditary breast and ovarian cancer syndrome. Obstet Gynecol. 2009;113(4):957–66.
23. 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. Gynecol Oncol. 2013;130(1):127–31.
24. Segev Y, Rosen B, Lubinski J, Gronwald J, Lynch HT, Moller P, et al. Risk factors for endometrial cancer among women with a BRCA1 or BRCA2 mutation: a case control study. Familial Cancer. 2015;14(3):383–91.
25. 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 Oncol. 2016;2(11):1434–40.
26. Havrilesky LJ, Moss HA, Chino J, Myers ER, Kauff ND. Mortality reduction and cost-effectiveness of performing hysterectomy at the time of risk-reducing salpingo-oophorectomy

for prophylaxis against serous/serous-like uterine cancers in BRCA1 mutation carriers. Gynecol Oncol. 2017;145(3):549–54.
27. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology. Genetic/Familial high-risk assessment: breast, Ovarian, and Pancreatic, ver1. 2020. Accessed Sep 30, 2020.
28. Gaba F, Manchanda R. Systematic review of acceptability, cardiovascular, neurological, bone health and HRT outcomes following risk reducing surgery in BRCA carriers. Best Pract Res Clin Obstet Gynaecol. 2020;65:46–65.
29. Reitsma W, de Bock GH, Oosterwijk JC, Bart J, Hollema H, Mourits MJ. Support of the 'fallopian tube hypothesis' in a prospective series of risk-reducing salpingo-oophorectomy specimens. Eur J Cancer. 2013;49(1):132–41.
30. Zakhour M, Danovitch Y, Lester J, Rimel BJ, Walsh CS, Li AJ, et al. Occult and subsequent cancer incidence following risk-reducing surgery in BRCA mutation carriers. Gynecol Oncol. 2016;143(2):231–5.
31. Kobayashi Y, Hirasawa A, Chiyoda T, Ueki A, Masuda K, Misu K, et al. Retrospective evaluation of risk-reducing salpingo-oophorectomy for BRCA1/2 pathogenic variant carriers among a cohort study in a single institution. Jpn J Clin Oncol 2020.
32. Cheng A, Li L, Wu M, Lang J. Pathological findings following risk-reducing salpingo-oophorectomy in BRCA mutation carriers: a systematic review and meta-analysis. Eur J Surg Oncol. 2020;46(1):139–47.
33. Stuursma A, van Driel CMG, Wessels NJ, de Bock GH, Mourits MJE. Severity and duration of menopausal symptoms after risk-reducing salpingo-oophorectomy. Maturitas. 2018;111:69–76.
34. Vermeulen RFM, Beurden MV, Korse CM, Kenter GG. Impact of risk-reducing salpingo-oophorectomy in premenopausal women. Climacteric. 2017;20(3):212–21.
35. Tucker PE, Cohen PA. Review article: sexuality and risk-reducing Salpingo-oophorectomy. Int J Gynecol Cancer. 2017;27(4):847–52.
36. Michelsen TM, Tonstad S, Pripp AH, Tropé CG, Dørum A. Coronary heart disease risk profile in women who underwent salpingo-oophorectomy to prevent hereditary breast ovarian cancer. Int J Gynecol Cancer. 2010;20(2):233–9.
37. Michelsen TM, Pripp AH, Tonstad S, Tropé CG, Dørum A. Metabolic syndrome after risk-reducing salpingo-oophorectomy in women at high risk for hereditary breast ovarian cancer: a controlled observational study. Eur J Cancer. 2009;45(1):82–9.
38. Ozdemir S, Celik C, Görkemli H, Kiyici A, Kaya B. Compared effects of surgical and natural menopause on climacteric symptoms, osteoporosis, and metabolic syndrome. Int J Gynaecol Obstet. 2009;106(1):57–61.
39. Johansen N, Liavaag AH, Tanbo TG, Dahl AA, Pripp AH, Michelsen TM. Sexual activity and functioning after risk-reducing salpingo-oophorectomy: impact of hormone replacement therapy. Gynecol Oncol. 2016;140(1):101–6.
40. Vermeulen RFM, Beurden MV, Kieffer JM, Bleiker EMA, Valdimarsdottir HB, Massuger L, et al. Hormone replacement therapy after risk-reducing salpingo-oophorectomy minimises endocrine and sexual problems: a prospective study. Eur J Cancer. 2017;84:159–67.
41. Rocca WA, Bower JH, Maraganore DM, Ahlskog JE, Grossardt BR, de Andrade M, et al. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. Neurology. 2007;69(11):1074–83.
42. Marchetti C, De Felice F, Boccia S, Sassu C, Di Donato V, Perniola G, et al. Hormone replacement therapy after prophylactic risk-reducing salpingo-oophorectomy and breast cancer risk in BRCA1 and BRCA2 mutation carriers: a meta-analysis. Crit Rev Oncol Hematol. 2018;132:111–5.
43. Vermeulen RFM, Korse CM, Kenter GG, Brood-van Zanten MMA, Beurden MV. Safety of hormone replacement therapy following risk-reducing salpingo-oophorectomy: systematic review of literature and guidelines. Climacteric. 2019;22(4):352–60.

44. Miller SM, Roussi P, Daly MB, Scarpato J. New strategies in ovarian cancer: uptake and experience of women at high risk of ovarian cancer who are considering risk-reducing salpingo-oophorectomy. Clin Cancer Res. 2010;16(21):5094–106.
45. Nomura H, Sekine M, Yokoyama S, Arai M, Enomoto T, Takeshima N, et al. Clinical background and outcomes of risk-reducing salpingo-oophorectomy for hereditary breast and ovarian cancers in Japan. Int J Clin Oncol. 2019;24(9):1105–10.
46. Hirasawa A, Masuda K, Akahane T, Tsuruta T, Banno K, Makita K, et al. Experience of risk-reducing salpingo-oophorectomy for a BRCA1 mutation carrier and establishment of a system performing a preventive surgery for hereditary breast and ovarian cancer syndrome in Japan: our challenges for the future. Jpn J Clin Oncol. 2013;43(5):515–9.
47. Han SA, Park SK, Ahn SH, Lee MH, Noh DY, Kim LS, et al. The Korean hereditary breast cancer (KOHBRA) study: protocols and interim report. Clin Oncol (R Coll Radiol). 2011;23(7):434–41.
48. Lee J, Kim S, Kang E, Park S, Kim Z, Lee MH. Influence of the Angelina Jolie announcement and insurance reimbursement on Practice patterns for hereditary breast cancer. J Breast Cancer. 2017;20(2):203–7.
49. 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. J Natl Cancer Inst. 2015;107(11).
50. Loveday C, Turnbull C, Ruark E, Xicola RM, Ramsay E, Hughes D, et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet. 2012;44(5):475–6. author reply 6
51. Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet. 2011;43(9):879–82.
52. 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. Gynecol Oncol. 2017;147(2):375–80.
53. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, et al. Inherited mutations in women with ovarian carcinoma. JAMA Oncol. 2016;2(4):482–90.

Panel Testing

13

Yasuyuki Kojima

Abstract

In order to provide optimal treatment options for each patient, genetic panel tests with genomic profiling, which can detect diverse genetic abnormalities found in tumors and provide medical interpretation, have been implemented in routine clinical practice. For standard treatment-resistant solid tumors, two panel tests were authorized in Japan. Although the primary outcome of these panel tests is to find a way for possible treatment options, in some cases secondary findings for germline mutation could be suggested or pointed out, in which percentage has been reported in range from 3.3 to 10.7%. In clinical practice, it is essential to refer cases that are considered to require genetic counseling based on the results of the expert panel to genetic specialists and genetic counselors as appropriate.

There are no insured genetic multigene panel tests for germline mutations yet in Japan. However, tests for germline mutations including less common syndromes will be used more frequently in clinical practice for cases of potential hereditary diseases. Alongside the process of developing management for BRCA1/2 pathogenic variant, the most frequent gene mutation associated with breast and ovarian cancer, the development of high-quality guidelines for comprehensive germline mutations is warranted in the near future.

Keywords

Cancer genome medicine · Multigene panel test · Secondary findings

Y. Kojima (✉)
Division of Breast and Endocrine Surgery, Department of Surgery, St. Marianna University School of Medicine, Kawasaki, Japan
e-mail: kojiyasu@marianna-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_13

13.1 Introduction

In order to provide optimal treatment options for each patient, genetic panel tests with genomic profiling, which can detect diverse genetic abnormalities found in tumors in a multiplex fashion and provide medical interpretation and meaningfulness, have been implemented in routine clinical practice. For standard treatment-resistant solid tumors, two panel tests were authorized in June 2019, in Japan. Although the primary outcome of these panel tests is to find a way for possible treatment options, in some cases secondary findings for germline mutation could be suggested or pointed out.

This article describes the system cancer genome panel test, the results obtained, and how they are interpreted, including secondary findings, and also mentions briefly about panel tests for germline mutations.

13.2 Cancer Genome Panel Tests

Two gene panel tests covered by Japanese national health insurance are FoundationOne Companion Diagnostics (hereinafter, "F1CDx") and OncoGuide™ NCC Oncopanel System (hereinafter, "NCC Oncopanel Test").

These cancer genome profiling tests were offered at specific institutions that have a system to conduct the test, such as "core center hospitals," "center hospitals," and "collaborative hospitals."

The "Guidelines for the Development of Core Cancer Genome Center Hospitals" issued by the Health Bureau of the Ministry of Health, Labor and Welfare lists the following seven requirements for core center hospitals: (1) the hospital must have a specimen laboratory and pathology laboratory certified by a third party and must be able to properly conduct gene panel tests in accordance with the procedures, and must be able to medically interpret the results, and a panel of experts to meet at least once a month to interpret the results; (2) having a system to ensure that the treatment of secondary findings is clearly documented and that genetic counseling can be conducted appropriately; (3) having a system that can appropriately collect and manage genomic information and register it with the Center for Cancer Genomics and Advanced Therapeutics (C-CAT); (4) having a system that can appropriately store the biological materials; (5) having a department that oversees cancer genome medicine; (6) having a system that can provide information on cancer genome medicine to patients and their families; and (7) having a system as a core clinical research hospital or equivalent to it.

In order to implement genomic medicine, pathologists, specialists in molecular genetics, and genetic counselors are required to work for and have a proven track record in the field.

As of April 2020, 12 core center hospitals, 33 center hospitals, and 161 collaborating hospitals, altogether 206 facilities, have been designated.

Currently, only about 10–15% of patients who undergo cancer genome profiling are actually found to have the recommended treatment [1, 2].

13.3 Types of Panel Tests

With the development of genetic analysis equipment, we are now able to examine a large number of genes in a single test. The generic term for the genes to be searched is called panel. There are several types of panel tests, and the specimens used for each test are different depending on the purpose. Panel tests for detecting genetic abnormalities in cancer include those for tumor cells only, those for both tumor cells, and peripheral blood. The panel test to detect germline mutation is intended for peripheral blood only. In the current situation, there are many kinds of panel tests are available on the market for different purposes.

The F1CDx is provided by Foundation Medicine Inc. (Massachusetts, USA) and is the first FDA-approved tissue-based broad companion diagnostic for all solid tumors. The NCC Oncopanel Test is a gene panel testing designed specifically for Japanese solid tumor genome mutations, including childhood cancers, which was developed jointly by the National Cancer Center Japan and Sysmex Corporation (Kobe, Japan) [3, 4].

Next-generation sequencers are used in both gene panel tests: the F1CDx uses Illumina's HiSeq4000 targeting in 324 genes and selects gene rearrangements (Table 13.1), as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB); on the other hand, the NCC Oncopanel uses Illumina's NextSeq 550Dx to test 114 genes where Japanese people are prone to express cancer mutations (Table 13.2). Both target all exons and are examined using sequential synthetic sequencing on next-generation sequencers after library preparation using hybrid capture methods.

With the advent of next-generation sequencers, the number of nucleotide sequences and regions that can be analyzed at a time has been dramatically increased, enabling the analysis of multiple genes at a time and enabling cancer gene panel testing. Extraction of nucleic acids used for panel testing is obtained from formalin-fixed paraffin-embedded (FFPE) samples.

The percentage of tumor content on the section is important, and a tumor content of 20% or more is recommended for both testing. If the tumor content in the specimen is low, genetic analysis cannot be performed adequately, and the opportunity to obtain useful information from the expensive tests performed may be lost.

Specimens provided for panel testing include not only the tumor but also surrounding normal cells. The range of the targeted sequence region and the detection accuracy, that is, the read depth, is set in each test. Unlike whole genome sequencing, which reads a large amount of sequence information, the cancer gene panel test is designed to read only specific genes related to cancer with high accuracy. Therefore, the types of gene abnormalities, base substitutions, insertions, deletions, amplifications, gene fusions, etc. that can be analyzed differ depending on the type of test.

Based on the data detected by the sequencer, sequenced data are generated as FASTQ files. Based on this data, mapping is performed by referring to reference sequences of human genes in open public databases, and BAM files are generated.

Table 13.1 A list of 324 target genes in the FoundationOne CDx [3]

F1CDx gene list								
ABL1	ACVR1B	AKT1	AKT2	AKT3	ALK	ALOX12B	AMER1 (FAM123B)	APC
AR	ARAF	ARFRP1	ARID1A	ASXL1	ATM	ATR	ATRX	AURKA
AURKB	AXIN1	AXL	BAP1	BARD1	BCL2	BCL2L1	BCL2L2	BCL6
BCOR	BCORL1	BRAF	BRCA1	BRCA2	BRD4	BRIP1	BTG1	BTG2
BTK	C11orf30	CALR	CARD11	CASP8	CBFB	CBL	CCND1	CCND2
CCND3	CCNE1	CD22	CD274(PD-L1)	CD70	CD79A	CD79B	CDC73	CDH1
CDK12	CDK4	CDK6	CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN2C
CEBPA	CHEK1	CHEK2	CIC	CREBBP	CRKL	CSF1R	CSF3R	CTCF
CTNNA1	CTNNB1	CUL3	CUL4A	CXCR4	CYP17A1	DAXX	DDR1	DDR2
DIS3	DNMT3A	DOT1L	EED	EGFR	EP300	EPHA3	EPHB1	EPHB4
ERBB2	ERBB3	ERBB4	ERCC4	ERG	ERRFI1	ESR1	EZH2	FAM46C
FANCA	FANCC	FANCG	FANCL	FAS	FBXW7	FGF10	FGF12	FGF14
FGF19	FGF23	FGF3	FGF4	FGF6	FGFR1	FGFR2	FGFR3	FGFR4
FH	FLCN	FLT1	FLT3	FOXL2	FUBP1	GABRA6	GATA3	GATA4
GATA6	GID4 (C17orf39)	GNA11	GNA13	GNAQ	GNAS	GRM3	GSK3B	H3F3A
HDAC1	HGF	HNF1A	HRAS	HSD3B1	ID3	IDH1	IDH2	IGF1R
IKBKE	IKZF1	INPP4B	IRF2	IRF4	IRS2	JAK1	JAK2	JAK3
JUN	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KEL	KIT	KLHL6
KMT2A (MLL)	KMT2D (MLL2)	FRAS	LTK	LYN	MAF	MAP2K1 (MEK1)	MAP2K2 (MEK2)	MAP2K4
MAP3K1	MAP3K13	MAPK1	MCL1	MDM2	MDM4	MED12	MEF2B	MEN1
MERTK	MET	MITF	MKNK1	MLH1	MPL	MRE11A	MSH2	MSH3
MSH6	MST1R	MTAP	MTOR	MUTYH	MYC	MYCL (MYCL1)	MYCN	MYD88
NBN	NF1	NF2	NFE2L2	NFKBIA	NKX2-1	NOTCH1	NOTCH2	NOTCH3

F1CDx gene list								
NPM1	NRAS	NT5C2	NTRK1	NTRK2	NTRK3	P2RY8	PALB2	PARK2
PARP1	PARP2	PARP3	PAX5	PBRM1	PDCD1(PD-1)	PDCD1LG2 (PD-L2)		PDGFRA
PDGFRB	PDK1	PIK3C2B	PIK3C2G	PIK3CA	PIK3CB	PIK3R1	PIM1	PMS2
POLD1	POLE	PPARG	PPP2R1A	PPP2R2A	PRDM1	PRKAR1A	PRKCI	PTCH1
PTEN	PTPN11	PTPRO	QKI	RAC1	RAD21	RAD51	RAD51B	RAD51C
RAD51D	RAD52	RAD54L	RAF1	RARA	RB1	RBM10	REL	RET
RICTOR	RNF43	ROS1	RPTOR	SDHA	SDHB	SDHC	SDHD	SETD2
SF3B1	SGK1	SMAD2	SMAD4	SMARCA4	SMARCB1	SMO	SNCAIP	SOCS1
SOX2	SOX9	SPEN	SPOP	SRC	STAG2	STAT3	STK11	SUFU
SYK	TBX3	TEK	TET2	TGFBR2	TIPARP	TNFAIP3	TNFRSF14	TP53
TSC1	TSC2	TYRO3	U2AF1	VEGFA	VHL	WHSC1 (MMSET)	WHSC1L1	WT1
XPO1	XRCC2	ZNF217	ZNF703					
Gene with full coding exonic regions included in F1CDx for the detection of substitutions, insertions/deletions, and copy number alterations								
Selected rearrangements								
ALK	BCL2	BCR	BRAF	BRCA1	BRCA2	CD74	EGFR	ETV4
ETV5	ETV6	EWSR1	EZR	FGFR1	FGFR2	FGFR3	KIT	KMT2A
MSH2	MYB	MYC	NOTCH2	NTRK1	NTRK2	NUTM1	PDGFRA	RAF1
RARA	RET	ROS1	RSPO2	SDC4	SLC34A2	TERC (noncoding RNA gene)		
TERT (promoter only)		TMPRSS2						
Genes with select intronic regions for the detection of gene rearrangements								

Table 13.2 A list of 114 target genes in the NCC Oncopanel Test [4]

Gene list studied for mutation and amplification								
ABL1	ACTN4	AKT1	AKT2	AKT3	ALK	APC	ARAF	ARID1A
ARID2	ATM	AXIN1	AXL	BAP1	BARD1	BCL2L11	BRAF	BRCA1
BRCA2	CCND1	CD274/PD-L1	CDK4	CDKN2A	CHEK2	CRKL	CREBBP	CTNNB
CUL3	DDR2	EGFR	ENO1	EP300	ERBB2	ERBB3	ERBB4	ESR1/ER
EZH2	FBXW7	FGFR1	FGFR2	FGFR3	FGFR4	FLT3	GNA11	GNAQ
GNAS	HRAS	IDH1	IDH2	IGF1R	IGF2	IL7R	JAK1	JAK2
JAK3	KDM6A/UTX	KEAP1	KIT	KRAS	MAP2K1/MEK1	MAP2K2/MEK2	MAP2K4	MAP3K1
MAP3K4	MDM2	MDM4	MET	MLH1	MTOR	MSH2	MYC	MYCN
NF1	NFE2L2	NOTCH1	NOTCH2	NOTCH3	NRAS	NRG1	NTRK1	NTRK2
NTRK3	NT5C2	PALB2	PBRM1	PDGFRA	PDGFRB	PIK3CA	PIK3R1	PIK3R2
POLD1	POLE	PRKCI	PTCH1	PTEN	RAC1	RAC2	RAD51C	RAF1
RB1	RET	RHOA	ROS1	SETBP1	SETD2	SMAD4	SMARCA4	SMARCB1
SMO	STAT3	STK11/LKB1	TP53	TSC1	VHL			
Genes studied for fusion								
ALK	AKT2	BRAF	ERBB4	FGFR2	FGFR3	NRG1 NTRK1	NTRK2	PDGFRA
RET	ROS1							

Then, based on the BAM files, each gene mutation location was mapped on the chromosomes, and VCF files are generated to show the results.

The F1CDx targets only tissue sample for genetic analysis, while the NCC Oncopanel Test also collects peripheral blood sample for the analysis to detect genetic changes in tumors. Thus, there are two types of panel tests used in cancer genome medicine, and the specimens handled by the panel tests are different from test to test. From normal cells obtained from blood samples, information on patient-specific genetic polymorphisms can be obtained, which can be used as a control for higher test accuracy. Both panel tests can determine or detect the possibility of germline genetic variants, and especially in cases with germline genetic variants, clinical genetic considerations are required.

13.4 Purpose of the Gene Panel Test

The purpose of an oncogene panel test is to detect cancer-derived somatic mutations and to realize personalized medicine that can be used to select more specific effective cancer drugs based on the genetic mutation information (Fig. 13.1). F1CDx has also been used as a companion diagnostic for some genes (lung cancer, EGFR mutation, ALK fusion, ROS1 fusion; malignant melanoma, BRAF mutation; breast cancer, ERBB2 amplification; colorectal cancer, KRAS, NRAS wild type; solid tumors, NTRK1/2/3 fusion; ovarian cancer, BRCA1/2 mutation).

The European Society for Medical Oncology (ESMO) Precision Medicine Working Group published recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers. Based on the current evidence, ESMO recommends routine use of NGS on tumor samples in advanced

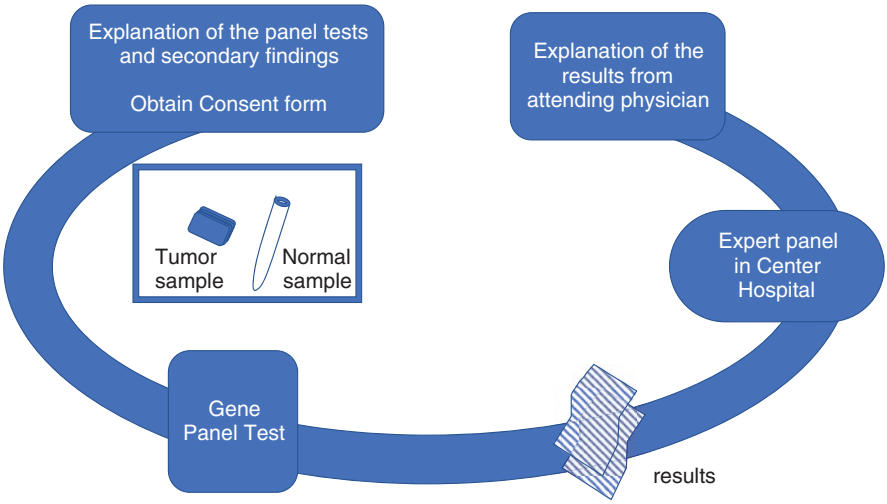


Fig. 13.1 Flow of genomic medicine

non-squamous non-small-cell lung cancer (NSCLC), prostate cancers, ovarian cancers, and cholangiocarcinoma. In these tumors, large multigene panels could be used if they add acceptable extra cost compared with small panels. In colon cancers, NGS could be an alternative to PCR. In addition, based on the KN158 trial and considering that patients with endometrial and small-cell lung cancers should have broad access to anti-programmed cell death 1 (anti-PD1) antibodies, it is recommended to test tumor mutational burden (TMB) in cervical cancers, well- and moderately differentiated neuroendocrine tumors, salivary cancers, thyroid cancers, and vulvar cancers, as TMB-high predicted response to pembrolizumab in these cancers [5].

There is no drug approved in Japan for selective treatment of breast cancer for PIK3CA hotspot mutation, the relatively frequent detection of HER2 can be determined by immunohistochemical staining, and no other genetic mutation information is routinely useful for treatment selection for breast cancer. According to this guideline, it is not recommended that cancer gene panel testing be performed in routine clinical practice for breast cancer and limited to circumstances where patients can find opportunity to participate in clinical trials targeting some genetic mutations, such as AKT1E17K, PTEN, ERBB2, ESR1, and NF1, at certain hospitals, such as the aforementioned center hospitals.

13.5 Report Structure of the F1CDx

F1CDx consists of two sets of report for a result. First, CDx Associated Findings and Other Alterations & Biomarkers Identified report includes detected genetic variants and corresponding drug names related to the companion diagnosis and detected genetic variants which are not related to the companion diagnosis, as well as other biomarkers such as MSI status and TMB score. Japanese version of this report also includes “Approved Therapeutic Options in Japan.”

On the other hand, *Professional Services* report includes information of detected biomarkers and genetic mutations, as well as information of the corresponding therapeutic agents and ongoing clinical trials, including indications, recommendations based on NCCN guideline, and drug resistance with some available reference information. The report has not been approved by the FDA or the Ministry of Health, Labor and Welfare and is a supporting document.

13.6 Report Structure of the NCC Oncopanel Test

The NCC Oncopanel Test consists of three reports (summary report, sequencing report, and QC report). The summary report contains (1) gene abnormality information, (2) somatic mutation numbers and mutation occurrence rates, and (3) annotation information. First, the mutated genes and mutant allele frequencies, amplified genes, and fusion genes detected will be listed in the gene abnormality information section. Mutations suspected to be pathogenic variants

among the germline mutation information will also be described here. Second, the number and frequency of single nucleotide variants (SNVs) and insertion/deletion mutations (InDel), as well as the total number and frequency of each mutation for the exon and non-exon regions, will be described for each somatic mutation. The total mutation rate is used to determine the tumor mutation burden (TMB). Third, annotation information obtained by referring to databases such as Expert Panel Data Base (EPDB), COSMIC, and ClinVar will be described.

The sequencing report contains detailed sequencing analysis information of tumor and non-tumor cells. The types of mutation detected and their locations will be described, and variant of unknown significance (VUS) will also be included here. Details of the detected germline gene mutations are also described in the column of germline mutation information.

The NCC Oncopanel Test includes 13 of the genes recommended by the American College of Medical Genetics and Genomics (ACMG) guidance to be informed to their personnel. If pathogenic variants are detected for APC, BRCA1/2, MLH1, MSH2, PTEN, RB1, RET, STK11, SMAD4, TP53, TSC1, and VHL, the results will be reported. Currently, results from other germlines obtained from peripheral blood samples are not returned; results other than the ACMG59 gene are reported as a difference between tumor sample and peripheral blood sample, which means that the germline variant may be present but masked and in most cases not be able to even be suspected. However, the format of the report may change as necessary in the future. The expert panel then should fully consider how and whether to disclose the information.

13.7 C-CAT Report Structure

C-CAT is a new center for cancer genome medicine established in accordance with the Cancer Control Act Law and provides a mechanism for collecting and storing information on cancer genome medicine from all over Japan. C-CAT supports cancer genome medicine in Japan not only by returning the "C-CAT reports" with the annotated genetic alterations to the expert panels at the Cancer Genome Center Hospitals but also by understanding or using the genome and medical information of cancer patients for secondary purpose such as the development of policies for cancer control. For patients who have not consented to secondary use, C-CAT will accept the information if they agree to provide it to C-CAT and will not delete the patient's information after the patient's death and will retain the patient's information [6].

Not only NCC Oncopanel Test but also for F1CDx, if consent is obtained from the patient, the test data are sent to C-CAT, and the C-CAT report is generated. The report format for the Japanese version of the CDx Associated Findings and Other Alterations & Biomarkers Identified report, "Approved Therapeutic Options in Japan," is based on the information on approved drugs at the time of F1CDx approval in Japan.

The C-CAT report is based on the Cancer Knowledge Data Base (CKDB), which was developed by C-CAT, and consists of survey results, candidate drugs and clinical trials, detailed information on mutated genes and references, and evidence levels. In the matched pair test, the following items are also reported for germline mutations: the names of mutant genes and mutation information, allele frequency, evidence type (predictive, predisposing), clinical significance and disease name, evidence level, and corresponding drug and availability.

13.8 Expert Panel Configuration

The reports need to be reviewed by a multidisciplinary panel of experts in order to provide patients with the appropriate treatment, which is the purpose of the panel tests. To this end, an expert panel meets to discuss and finalize all of the test reports before returning the results to the attending physician. The expert panel consists of the following eight items:

1. Include several full-time physicians in different fields of practice who have specialized knowledge and skills in anticancer drug treatment.
2. Include at least one physician with specialized knowledge and skills in genetic medicine.
3. Include at least one person with specialized genetic counseling skills in genetic medicine.
4. Include more than one physician with specialized knowledge and skills in pathology.
5. One or more experts with sufficient knowledge of molecular genetics and genomic medicine should be included. The expert should have written a peer-reviewed paper in English on cancer genome medicine or cancer genome research within the past 3 years prior to the time of application.
6. If the sequencing will be carried out at the institution, at least one expert with sufficient knowledge of bioinformatics for electronic analysis using next-generation sequencers should be included. The expert should have written a peer-reviewed English-language paper on cancer genome medicine or genome research within 3 years prior to the time of application.
7. In an institution that handles pediatric oncology cases, at least one pediatric oncology physician with some experience of participating in expert panels must be included.
8. Include the attending physician or alternate physician of the subject patient to be reviewed by the expert panel.

The final report consists of not only the reviewed summary of the presence and content of recommended treatments, or other treatment options, but also the presence and content of secondary findings that are recommended to be explained to patients. A secondary finding is defined as a genetic mutation in the germline that can be confirmed as a pathogenic variant in the cancer gene panel test.

The term "secondary findings" is used because they are considered important incidental findings for patients and their families, even though they are not the primary purpose of the test. Recently, it has been proposed that these findings be called germline findings. A pathogenic variant of a germline mutation may not be considered an incidental finding when a patient undergoes a paired-specimen cancer gene panel test, bearing in mind that a patient's medical history and family history indicate that he or she may have a hereditary tumor. Furthermore, it is possible that a genetic variant such as BRCA1 or BRCA2, for example, that is called by the cancer gene panel test, may be a genetic variant that also defines a treatment approach.

13.9 Handling of Germline Findings in Panel Testing

The evaluation of germline findings is based on five levels of ACMG/AMP criteria: pathogenic, likely pathogenic, variant of uncertain significance, likely benign, and benign. The classification is based on information such as variant frequency information in the general population; functional prediction; functional analysis data; databases such as ClinVar, HGMD, and MGeND; and reported articles.

The possibility of germline findings should be explained to the patient during the explanation of the panel test, and consent should be obtained before having the test. Among the germline findings, genetic mutation findings will only be disclosed to patients for those variants that are determined to be pathogenic or likely pathogenic and that are related to hereditary tumors for which management methods have been established. Disclosing findings related to a disease for which there is no established treatment or surveillance would only cause anxiety and confusion for patients and families. It should be confirmed that the gene is an eligible gene for disclosure in the guidelines developed by each society. In Japan, a proposal on the information transfer process in genomic medicine has been published, and which describes which secondary findings should be picked up and how they should be disclosed [7].

Among the target genes included in the NCC Oncopanel Test, the following 13 genes (APC, BRCA1/2, MLH1, MSH2, PTEN, RB1, RET, STK11, SMAD4, TP53, TSC1, and VHL) correspond to the gene groups to be reported by ACMG.

Although the actual test results will be somewhat scattered with results that would be assessed as mutations of unknown significance, we should be cautious in disclosing such results. Therefore, a genetic medicine specialist or a genetic counselor is required for the expert panel. Cases that should be carefully evaluated for pathogenicity based on a detailed family history and other information should be handled in an outpatient genetic counseling clinic.

The frequency of detection of somatic or germline pathogenic variants using paired specimens varies by gene [8]. For instance, TP53 somatic variants were much more common: 337 patients had somatic and 10 had germline TP53 variants. The same trend was observed for RB1 and PTEN. In TSC2 and MSH2, approximately 80% of the patient had somatic variants. In comparison, BRCA1/2 was commonly seen as germline variants: there were 3 patients with somatic and 11 with germline BRCA1 variants and 3 with somatic and 10 with germline

BRCA2 variants. In PALB2 variants, the frequency of germline and somatic variants was even, and in MSH6 variants, less than 60% of the patients had germline variants.

Among several panel testing, the percentage of secondary findings found in the ACMG gene has been reported to range from 3.3 to 10.7% [8–11].

Pathogenic variants of germline mutation may be suspected in panel tests such as F1CDx, which only targets tumor tissue. For example, BRCA1/2 is most likely pathogenic variant of the germline origin, as mentioned above, and germline confirmation testing should be performed regardless of its allele frequency. When dealing with genes other than BRCA1/2, it is important to consider the primary organ, duplicated or multiple cancers, family history, as well as tumor content of the specimen, copy number alterations, and variant allele frequency (VAF). It is known that VAF is often high when the detected gene mutation is a germline mutation and VAF is often low when the mutation is of tumor origin for genes such as CHEK2, ATM, and PALB2. ESMO Precision Medicine Working Group addressed a guideline for management of tumor-detected pathogenic variants of potential germline origin [12]. They found that crude "pan-tumor" VAF thresholds (20% for small insertions/deletions, 30% for SNVs) enabled reduction by 54% (9222/17075) the number of tumor-detected variants requiring follow-up while losing only 3.5% (52/1494) proportion of true germline variants. After excluding variants from germline-focused tumor analysis of gene/context/age scenarios in which the germline conversion rate is <10%, 27 genes remained. As a result, these 27 genes (at any age, any tumor type, BRCA1, BRCA2, BRIP1, MLH1, MSH2, MSH6, PALB2, PMS2, VHL, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TSC2, MUTYH; at any age with associated tumor type only, FLCN, FH, BAP1, POLE; tumor arising age < 30 only with any tumor type, RB1, APC; tumor arising age < 30 with associated tumor type only, TP53, NF1) are recommended to be included for germline-focused analysis and triggering of germline sample laboratory confirmation.

The similar operational guideline is also used in Japan (Fig. 13.2, Table 13.3) [7, 13]. Within the guideline, BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, APC, MEN1, RET, RB1, and VHL are the genes recommended to be disclosed to patients. The criteria for this are the existence of Japanese guidelines for surveillance of unaffected patients, the ability to outsource single-site tests to a registered laboratory from any center or affiliated hospital, and variants that are included in several gene panel tests. However, it is possible that other genes may be found in addition to these genes that are associated with hereditary tumors and should be thoroughly examined by expert panels.

If the possibility of pathogenic variants is considered, it is necessary to confirm the results with another single-site genetic test using normal tissues, such as blood, and it is recommended to disclose this fact in the report [14]. In clinical practice, it is essential to refer cases that are considered to require genetic counseling by the expert panel to a clinical geneticist and certifiedgenetic counselors as appropriate.

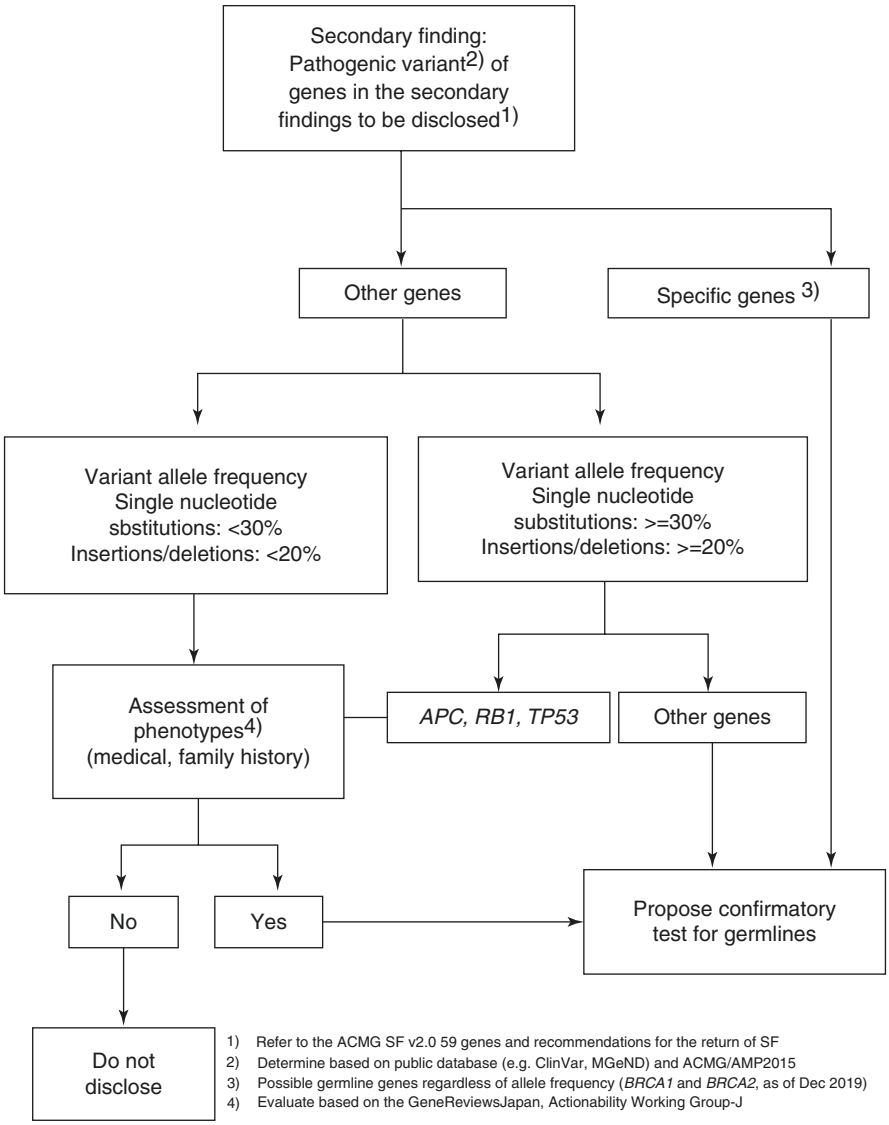


Fig. 13.2 Operational guidelines for germline tests to confirm secondary findings from tumor profiling test of tumor cells [7]

13.10 Gene Panel Testing for Germline Variants

In Japan, there are no insured genetic panel tests for the detection of germline mutations, so we must pay for them at our own expense. There are already many multigene panel tests for cancer predisposition mutations available overseas, for example, those provided by Ambry Genetics such as "CancerNext, BreastNext, and

Table 13.3 Disclosure recommendations list for secondary findings of cancer genetic panel test [13]

Potentially actionable secondary findings gene list			NCC Oncopanel Test		F1CDx		Necessity of germline testing for T-only panel**
Gene	Major phenotype		Tumor	Germline	Tumor	Disclosure recommendation*	
APC	FAP		O	O	O	AAA	D
ATM	Breast ca		O		O	A	A
BAP1	Malignant mesothelioma, etc.		O		O	B	C
BMPR1A	Juvenile polyposis					AA	C
BRCA1	HBOC		O	O	O	AAA	A
BRCA2	HBOC		O	O	O	AAA	A
BRIP1	Ovarian ca				O	A	A
CDH1	Diffuse gastric ca				O	AA	B
CDK4	Melanoma		O		O	B	B
CDKN2A	Melanoma/pancreatic ca		O		O	A	B
CHEK2	Breast ca		O		O	A	B
EPCAM	Lynch	Deletion				AA	C
FH	Hereditary leiomyomatosis and renal cell ca				O	B	B
FLCN	Birt-Hogg-Dube syndrome				O	B	B
MAX	HPPS					B	C
MEN1	MEN1				O	AAA	B
MET	GIST		O		O	B	C
MLH1	Lynch		O	O	O	AAA	A
MSH2	Lynch		O	O	O	AAA	A
MSH6	Lynch				O	AAA	A
MUTYH	MAP	Biallelic			O	AA	A
NBN	Breast ca				O	A	C
NF1	NF1		O		O	A	D
NF2	NF2				O	AA	B
PALB2	Breast ca		O		O	AA	A
PMS2	Lynch				O	AAA	A
POLD1	Colon ca		O		O	B	C
POLE	Colon ca		O		O	B	B
POT1	Malignant melanoma					B	C
PTEN	PTEN hamartoma		O	O	O	AA	D
RAD51C	Ovarian ca		O		O	A	A
RAD51D	Ovarian ca				O	A	A
RB1	Retinoblastoma		O	O	O	AAA	D
RET	MEN2		O	O	O	AAA	A
SDHA	HPPS				O	A	C

Table 13.3 (continued)

Potentially actionable secondary findings gene list			NCC Oncopanel Test		F1CDx		Necessity of germline testing for T-only panel**
Gene	Major phenotype		Tumor	Germline	Tumor	Disclosure recommendation*	
SDHAF2	HPPS				O	AA	A
SDHB					O	AA	A
SDHC	HPPS				O	AA	A
SDHD	HPPS				O	AA	A
SMAD3	Loeys-Dietz	Non-tumor				A	C
SMAD4	Juvenile polyposis		O	O	O	AA	B
STK11	Peutz-Jeghers		O	O	O	AA	D
TERF2IP						B	C
TERT	Acute myeloid leukemia					B	C
TGFBR1	Loeys-Dietz	Non-tumor				A	C
TGFBR2	Loeys-Dietz	Non-tumor			O	A	C
TMEM127	Pheochromocytoma					B	C
TP53	Li-Fraumeni		O	O	O	AA	D
TSC1	Tuberous sclerosis		O	O	O	AA	B
TSC2	Tuberous sclerosis				O	AA	A
VHL	VHL		O	O	O	AAA	A
WT1	WT1-related Wilms				O	AA	B

*Grades of disclosure recommendation

AAA	Guidelines exist in our country for medical policies for mutation carriers
AA	Hereditary tumor-causing gene included in ACMG 59 genes (ACMG SF v2)Genes listed in the NCCN guidelines with consistent disclosure recommendation in major papers
A	Genes listed in the NCCN guidelines with inconsistent disclosure recommendation in major papersNon-hereditary tumor-causing gene included in ACMG SF v2
B	Genes with disclosure recommendations in only one paper

***Grades of necessity of germline testing for T-only panel*

A	Recommended in either major paper and must be tested
B	Conditionally recommended in either major paper and test as much as possible
C	Test if possible
D	Not proactively tested, but only if there is clinical suspicion

BRCAplus"; "Comprehensive Cancer Panel, Breast Cancer High/Moderate Risk, and BreastOvarian Cancer" from GeneDx; and "MyRisk, Breast and Ovarian, Breast Cancer" from Myriad, which are developed by commercial companies or by medical centers such as MSK-IMPACT, Color, Counsyl Reliant Cancer Screen, and University of Washington BROCA Cancer. The number of genes included in a test is so varied that it is difficult to decide which test to choose, even for breast and ovarian cancer. As a pretest probability model, Myriad II [15], BRCAPRO [16], IBIS [17], and others have been used, but these models only cover BRCA1/2; predictive model for other less frequent pathogenic mutations is limited to BOADACEA [18], now including ATM, CHEK2, and PALB2; all of these panel tests were developed on the basis of a small number of data or a biased cohort. Recently, a model has been developed by the Mayo Clinic in collaboration with Ambry Genetics that can predict larger numbers of genetic variations based on a larger number of data [19].

NGS can detect many genetic variants at one time, and the challenge that always accompanies such tests is that some variants of unknown clinical significance may be detected which must be treated with caution. In a study of 1085 BRCA1/2-negative breast cancer patients, O'Leary et al. [20] found that the higher the number of genes included in a panel test, the more the frequency of finding genetic mutations which are considered to be pathogenic or likely pathogenic would increase. Buys et al. [21] reported that pathogenic variants were found in about 10% of the total and about half were BRCA1/2. In addition, one or more VUS was detected in about 37% of all cases.

In light of this current situation, guidelines have been released. For example, American Society of Breast Surgeons (ASBrS) presented consensus guideline on genetic testing for hereditary breast cancer. Although BRCA1/2 is the most frequent pathogenic variant associated with the development of breast and ovarian cancer, more comprehensive panel tests, including other less common syndromes, have become widely available. The most frequently reported variants other than BRCA1/2 are PALB2, CHEK2, and ATM [22–24]. The addition of MRI with contrast to annual surveillance is supported when there is a lifetime breast cancer risk of 20% or more, including these genetic variants. In this way, panel testing can contribute to more efficient and cost-effective risk assessment and recommended management of patients for whom hereditary breast cancer testing is recommended than conventional sequential gene testing. There is a report examining the performance of the NCCN genetic testing criteria for BRCA-related breast and/or ovarian cancer syndrome and Lynch syndrome (version 1.2018) in 165,000 patients who underwent hereditary cancer predisposition testing [25]. Within the report, among the female BRCA1/2 pathogenic variant carriers not meeting BRCA1/2 testing criteria, 59.1% (143/242) had a personal history of breast cancer. Meanwhile, of patients with PVs in Lynch syndrome genes failing to meet Lynch criteria, 41.5% had a personal history of a Lynch syndrome-related cancer. Therefore, genetic testing is recommended that "should be made available to all patients with a personal history of breast cancer."

Furthermore, for VUS, the ASBrS states in its recommendations that variants of uncertain significance are DNA sequences that are *not* clinically actionable in its recommendations. It is said that a VUS take several years to reclassify its uncertainty [26]. Until then, the variant should be considered as inconclusive and should be managed based on the patient's own risk factors.

Although there are issues that need to be resolved, it is clear that multigene panel tests will be used more frequently in clinical practice for cases of potential hereditary diseases, and by accumulating evidences flexibly, the development of high-quality international guidelines is warranted in the near future.

References

1. Kou T, Kanai M, Yamamoto Y, Kamada M, Nakatsui M, Sakuma T, et al. Clinical sequencing using a next-generation sequencing-based multiplex gene assay in patients with advanced solid tumors. Cancer Sci. 2017;108:1440–6. https://doi.org/10.1111/cas.13265. Epub 2017 May 22
2. Sunami K, Ichikawa H, Kubo T, Kato M, Fujiwara Y, Shimomura A, et al. Feasibility and utility of a panel testing for 114 cancer-associated genes in a clinical setting: a hospital-based study. Cancer Sci. 2019;110:1480–9.0. https://doi.org/10.1111/cas.13969. Epub 2019 Apr 2
3. Gene list of F1CDx. In: Technical specifications, foundation medicine https://assets.ctfassets.net/w98cd481qyp0/YqqKHaqQmFeqc5ueQk48w/0a34fcdaa3a71dbe460cdcb01cebe8ad/F1CDx_Technical_Specifications_072020.pdf. Accessed on 10 October 2020.
4. Table: Genes studied in the NCC Oncopanel Test. In: Cancer gene panel test OncoGuideTM ncc oncopanel system added to health insurance coverage list. National Cancer Center. https://www.ncc.go.jp/en/information/press_release/20190717/20190717152024.html. Accessed on 10 October 2020.
5. Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO precision medicine working group. Ann Oncol 2020 Aug 24: S0923–7534(20)39971-3. doi: https://doi.org/10.1016/j.annonc.2020.07.014.
6. Guidance for cancer treatment based on gene panel testing using next-generation sequencers (in Japanese). https://www.jsmo.or.jp/about/doc/20200310.pdf
7. Proposal concerning the information transmission process in genomic medicine Part 1: Focusing on comprehensive tumor genomic profiling analysis [Revised 2nd edition] https://www.amed.go.jp/content/000064662.pdf
8. 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. Ann Oncol. 2016;27(5):795–800. https://doi.org/10.1093/annonc/mdw018.
9. Schrader KA, Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, et al. Germline variants in targeted tumor sequencing using matched Normal DNA. JAMA Oncol. 2016;2(1):104–11. https://doi.org/10.1001/jamaoncol.2015.5208.
10. 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. JAMA. 2017 Sep 5;318(9):825–35. https://doi.org/10.1001/jama.2017.11137.
11. Jones S, Anagnostou V, Lytle K, Parpart-Li S, Nesselbush M, Riley DR, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med. 2015 Apr 15;7(283):283ra53. https://doi.org/10.1126/scitranslmed.aaa7161.
12. Mandelker D, Donoghue M, Talukdar S, Bandlamudi C, Srinivasan P, Vivek M, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO

precision medicine working group. Ann Oncol. 2019;30:1221–31. https://doi.org/10.1093/annonc/mdz136.
13. Disclosure Recommendations List for Secondary Findings of Cancer Genetic Panel Test. Japan agency for medical research and development. https://www.amed.go.jp/content/000056448.pdf. Accessed on 10 October 2020.
14. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®)Genetic/Familial high-risk assessment: breast, ovarian, and pancreatic Version 1.2021—September 8, 2020 https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf
15. Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, Lingenfelter B, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol. 2002 Mar 15;20(6):1480–90. https://doi.org/10.1200/JCO.2002.20.6.1480.
16. Biswas S, Atienza P, Chipman J, Hughes K, Barrera AM, Amos CI, et al. Simplifying clinical use of the genetic risk prediction model BRCAPRO. Breast Cancer Res Treat. 2013 Jun;139(2):571–9. https://doi.org/10.1007/s10549-013-2564-4.
17. Tyrer J, Duffy SW, Cuzick J. A breast cancer prediction model incorporating familial and personal risk factors. Stat Med. 2004 Apr 15;23(7):1111–30. https://doi.org/10.1002/sim.1668.
18. Lee A, Mavaddat N, Wilcox AN, Cunningham AP, Carver T, Hartley S, et al. BOADICEA: a comprehensive breast cancer risk prediction model incorporating genetic and nongenetic risk factors. Genet Med. 2019 Aug;21(8):1708–18. https://doi.org/10.1038/s41436-018-0406-9.
19. Hart SN, Polley EC, Yussuf A, Yadav S, Goldgar DE, Hu C, et al. Mutation prevalence tables for hereditary cancer derived from multigene panel testing. Hum Mutat. 2020 Aug;41(8):e1–6. https://doi.org/10.1002/humu.24053.
20. O'Leary E, Iacoboni D, Holle J, Michalski ST, Esplin ED, Yang S, et al. Expanded gene panel use for women with breast cancer: identification and intervention beyond breast cancer risk. Ann Surg Oncol. 2017 Oct;24(10):3060–6. https://doi.org/10.1245/s10434-017-5963-7.
21. Buys SS, Sandbach JF, Gammon A, Patel G, Kidd J, Brown KL, et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer. 2017 May 15;123(10):1721–30. https://doi.org/10.1002/cncr.30498.
22. Castéra L, Krieger S, Rousselin A, Legros A, Baumann JJ, Bruet O, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur J Hum Genet. 2014 Nov;22(11):1305–13. https://doi.org/10.1038/ejhg.2014.16.
23. Kapoor NS, Curcio LD, Blakemore CA, Bremner AK, McFarland RE, West JG, et al. Multigene panel testing detects equal rates of pathogenic BRCA1/2 mutations and has a higher diagnostic yield compared to limited BRCA1/2 analysis alone in patients at risk for hereditary breast cancer. Ann Surg Oncol. 2015 Oct;22(10):3282–8. https://doi.org/10.1245/s10434-015-4754-2.
24. Tung N, Battelli C, Allen B, Kaldate R, Bhatnagar S, Bowles K, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015 Jan 1;121(1):25–33. https://doi.org/10.1002/cncr.29010.
25. 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. Genet Med. 2020 Feb;22(2):407–15. https://doi.org/10.1038/s41436-019-0633-8.
26. King MC, Levy-Lahad E, Lahad A. Population-based screening for BRCA1 and BRCA2: 2014 Lasker Award. JAMA. 2014 Sep 17;312(11):1091–2. https://doi.org/10.1001/jama.2014.12483.

Germline Findings Through Precision Oncology for Ovarian Cancer

14

Mika Okazawa-Sakai and Akira Hirasawa

Abstract

Precision oncology has the potential to identify germline pathogenic variants in genes known to be associated with hereditary diseases; these data are called "germline findings." They could have implications in the assessment and management of future primary cancer risk, family risk assessment and guidance, and personalized treatment determination. Approximately 25% of all ovarian cancers are caused by an inherited genetic condition, and medical societies recommend germline genetic testing for all women diagnosed with ovarian cancer. Tumor genomic profiling and germline findings could allow the use of more personalized diagnostic, predictive, prognostic, and therapeutic strategies for patients with ovarian cancer. Additionally, this information could have clinical implications for the family members of the patients.

Keywords

Precision oncology · Tumor genomic profiling · Germline findings · Presumed germline pathogenic variant(s) · Ovarian cancer · Genetic testing · *BRCA1* · *BRCA2*

14.1 Introduction

In the early 1990s, scientists began to reveal the molecular basis of hereditary breast and ovarian cancer (HBOC) through advanced molecular biological technologies and the genetic linkage approach [1, 2]. Genes involved in

M. Okazawa-Sakai · A. Hirasawa (✉)
Department of Clinical Genomic Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
e-mail: hir-aki45@okayama-u.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_14

predisposition to cancer possess their own biological functions, such as DNA repair, cell proliferation, cell death, or signaling pathways [3]. Although the mechanisms underlying cancer predisposition are not fully understood, accumulated data indicate that cancer susceptibility genes could serve as prognostic or predictive response biomarkers during treatment. In addition, advances in precision medicine have led to new approaches for care in patients with cancer, such as genomic profiling of an individual's tumor [4]. With the evolution of next-generation sequencing (NGS) technology, which permits the characterization of large amounts of DNA sequence, tumor genomic profiling is being integrated into oncology practice.

14.2 Germline Findings when Targeting the Tumor Genome

Nussbaum et al. have described the idea that "Cancer is fundamentally a genetic disease," which means that the malignant phenotype is affected by the alteration of genetic pathways in the tumor [3]. Sequence variants detected in the tumor include both somatic variants acquired during cancer development and germline sequence variants. Tumor genomic profiling can potentially detect germline pathogenic variants in genes known to be associated with hereditary diseases; these data are called "germline findings"; however, the major goal of tumor genomic profiling is identification of tumor-specific variants with potential therapeutic implications [5].

Recent studies have revealed that 3–17% of tumor genomic profiling tests carry germline pathogenic variants (Table 14.1) [6–12]. The American Society of Clinical Oncology (ASCO) guidelines state that oncology providers should explain the potential of germline findings to patients and carefully ascertain patient preferences regarding the receipt of germline findings before conducting tumor genomic profiling [13]. The increased detection of potential clinically significant germline pathogenic variants has given rise to important medical issues, which indicate the need for an optimal approach for obtaining germline findings [4].

Table 14.1 Frequency of germline pathogenic variants detected in tumor genomic profiling tests

No. of samples	No. of genes included in the panel	Frequency of germline pathogenic variants (%)	Ref.
815	111	3.3	[6]
1566	341	15.7	[7]
1000	202	4.3	[8]
439	247	4.3	[9]
1040	410	17.5	[10]
17,152	410	8.7	[11]
1000	202	8.7	[12]

14.3 Why Are Germline Findings Important?

Germline findings potentially affect the treatment of the current cancer. Some germline variants are highly predictive of response to specific cancer-directed therapies. *BRCA1* and *BRCA2* (*BRCA1/2*) are part of the BRCA-Fanconi anemia DNA repair pathway and play key roles in homologous recombination [14]. Patients with pathogenic variants in *BRCA1/2* or homologous recombination deficiency (HRD) who develop ovarian, breast, pancreatic, or prostate cancers showed higher response rates and longer survival when treated with poly(ADP-ribose) polymerase (PARP) inhibitors. The US Food and Drug Administration (FDA) approved olaparib, niraparib, rucaparib, and talazoparib for these cancers (Table 14.2) [15–30]. Patients with germline pathogenic variants in DNA mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*), which cause Lynch syndrome, respond to immune checkpoint blockade with antibodies to programmed death 1 (PD-1), regardless of the tumor origin [31]. Pembrolizumab, an anti-PD-1 inhibitor, induced a high response rate in patients with high-level microsatellite instability (MSI-H) or mismatch-repair-deficient (dMMR) solid tumors and was approved by the FDA for the treatment of unresectable or metastatic cancer in these patients [32–35].

Additionally, germline findings have critical implications for the assessment and management of the risk of future cancers. They can provide information regarding the risk of second primary cancer. Furthermore, the detection of germline pathogenic variants is often a critical step in initiating a cascade of genetic testing in relatives for determining their cancer risk. For at-risk individuals, lifesaving surveillance and risk-reduction interventions can be instituted [36, 37]. For example, women harboring pathogenic variants in genes implicated in the inherited risk of ovarian cancer, such as *BRCA1/2*, *BRIP1*, *RAD51C*, and *RAD51D*, are recommended to consider risk-reducing salpingo-oophorectomy [36]; women harboring pathogenic variants in mismatch repair genes are recommended to undergo surveillance for colorectal, gastric, urothelial, endometrial, and ovarian cancer [37].

14.4 Candidates for Germline Genetic Testing

Personal and family history is essential for the identification of an individual with a risk of inherited predisposition to malignancy or other diseases. The US Preventive Services Task Force (USPSTF) recommends that primary care clinicians assess women with a personal or family history of breast or ovarian cancer or those who have an ancestry associated with *BRCA1/2* pathogenic variants using an appropriate brief familial risk assessment tool [38]. Moreover, ASCO recommends that the family history of patients with cancer should be assessed at the initial visit and reassessed periodically [39]. Any patient whose personal and/or family histories meet the criteria of germline genetic testing is recommended for referral to genetic specialists, regardless of the patient's likelihood of undergoing tumor genomic profiling [39]. Oncology providers play an important role in the identification of individuals at risk of hereditary cancer syndromes.

Table 14.2 FDA approvals for PARP inhibitors (as of October 7, 2020)

Tumor type	PARP inhibitor	Biomarker	Treatment setting	Pivotal trials supporting the approval
Ovarian cancer	Olaparib	Germline or somatic *BRCA*m	Maintenance treatment of patients with advanced OC who are in CR/PR to first-line platinum-based CT	SOLO-1 [15]
		Tumor *BRCA*m or genomic instability	Combination with bevacizumab for first-line maintenance treatment of patients with advanced OC who are in CR/PR to first-line platinum-based CT	PAOLA-1 [16]
		N/A	Maintenance treatment of patients with recurrent OC who are in CR/PR to platinum-based CT	Study19 [17] SOLO-2 [18]
		Germline *BRCA*m	Patients who have been treated with three or more prior CT regimens	Study42 [19]
	Niraparib	N/A	Maintenance treatment of patients with advanced OC who are in CR/PR to first-line platinum-based CT	PRIMA [20]
		N/A	Maintenance treatment of patients with recurrent OC who are in CR/PR to platinum-based CT	NOVA [21]
		Tumor *BRCA*m or genomic instability	Patients who have been treated with three or more prior CT regimens and with disease progression greater than 6 months after response to the last platinum-based CT	QUADRA [22]
	Rucaparib	Tumor *BRCA*m or high LOH	Maintenance treatment of patients with recurrent epithelial OC who are in CR/PR to platinum-based CT	ARIEL3 [23]
		Germline or somatic *BRCA*m	Patients who have been treated with two or more prior CT regimens	ARIEL2 [24] Study10 [25]
Breast cancer	Olaparib	Germline *BRCA*m	Patients with HER2-negative metastatic BC who have been treated with CT either in the neoadjuvant, adjuvant, or metastatic setting	OlympiAD [26]
	Talazoparib	Germline *BRCA*m	Patients with HER2-negative locally advanced or metastatic BC	EMBRACA [27]
Pancreatic cancer	Olaparib	Germline *BRCA*m	Maintenance treatment of patients with metastatic pancreatic adenocarcinoma whose disease has not progressed with at least 16 weeks of a first-line platinum-based CT	POLO [28]

Table 14.2 (continued)

Tumor type	PARP inhibitor	Biomarker	Treatment setting	Pivotal trials supporting the approval
Prostate cancer	Olaparib	Germline or somatic HRR gene-mutated	Patients with mCRPC whose disease progressed following prior treatment with enzalutamide or abiraterone	PROfound [29]
	Rucaparib	Germline or somatic *BRCA*m	Patients with mCRPC who have been treated with androgen receptor-directed therapy and a taxane-based CT	TRITON2 [30]

*BRCA*m *BRCA1/2* mutation; *OC* ovarian cancer; *CR* complete response; *PR* partial response; *CT* chemotherapy; *LOH* loss of heterozygosity; *BC* breast cancer; *HRR* homologous recombination repair; *mCRPC* metastatic castration-resistant prostate cancer; *N/A* not applicable. HRR genes include *ATM, BRCA1, BRCA2, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D,* and *RAD54L*

Hereditary cancer predisposition is considered if the patient displays an early age of cancer onset, multiple affected relatives with cancer on the same side of the family, or multiple primary tumors. In addition, patients with specific tumor types are considered for germline genetic testing, regardless of family history. Recent guidelines and statements demonstrate that germline genetic testing for *BRCA1/2* should be conducted for all patients with epithelial ovarian cancer at initial diagnosis (Table 14.3) [36, 38, 40–44]. Importantly, the most recent ASCO guideline for patients with ovarian cancer recommended that germline sequencing of *BRCA1/2* be performed as part of a multigene panel that includes genes associated with inherited risk of ovarian cancer, including at least *RAD51C*, *RAD51D*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *PALB2* [44].

14.5 Report of Germline Findings

Recent advances in NGS have expanded tumor sequencing modalities to whole-genome sequencing (WGS) and whole-exome sequencing (WES) [5]. Recognizing the potential for the integration of WGS and WES into clinical practice, in 2012, the American College of Medical Genetics and Genomics (ACMG) published a policy statement on genomic sequencing that highlighted the importance of germline findings [45]. The ACMG additionally proposed a minimum list of genes for which germline variants should be reported by clinical laboratories, regardless of the indication for which the sequencing test was ordered [46]. In 2015, the list was updated to include 59 genes, of which 25 were cancer susceptibility genes [47]. Recently, the ACMG guideline broadened the scope to targeted analysis of multiple genes of interest simultaneously using NGS [48]. Moreover, the European Society for Medical Oncology (ESMO) and the French Society of Predictive and Personalized

Table 14.3 Recent guidelines and statements on risk assessment, genetic counseling, and genetic testing for patients with ovarian cancer

Year	Stakeholder	Recommendations	Ref.
2017	ACOG and SGO	Genetic counseling is recommended for all women with ovarian epithelial cancer and for individuals who have a personal or family history of breast cancer or ovarian cancer	[40]
2017	SGO	All women with epithelial ovarian cancer should be offered and strongly encouraged to undergo genetic testing for hereditary ovarian cancer risk	[41]
2019	ESGO and ESMO	Testing for *BRCA1/2* mutations is recommended for all patients with non-mucinous ovarian cancer	[42]
2019	NCCN	Patients with ovarian cancer should undergo genetic risk evaluation and germline and somatic testing	[43]
2019	USPSTF	Primary care clinicians assess women with a personal or family history of breast and ovarian cancer or those who have an ancestry associated with *BRCA1/2* mutations using an appropriate brief familial risk assessment tool. Women with a positive result on the risk assessment tool should receive genetic counseling and, if indicated after counseling, genetic testing	[38]
2020	ASCO	All women diagnosed with epithelial ovarian cancer should undergo germline genetic testing for *BRCA1/2* and other ovarian cancer susceptibility genes. In women who do not carry a germline pathogenic BRCA1/2 variant, somatic tumor testing for *BRCA1/2* pathogenic variants should be performed. Women diagnosed with clear cell, endometrioid, or mucinous ovarian cancer should be offered somatic tumor testing for mismatch repair deficiency	[44]
2020	NCCN	Testing criteria for high-penetrance breast and/or ovarian cancer susceptibility genes: epithelial ovarian cancer at any age	[36]

ACOG American College of Obstetricians and Gynecologists; *SGO* Society of Gynecologic Oncology; *ESGO* European Society of Gynaecological Oncology; *ESMO* European Society for Medical Oncology; *NCCN* National Comprehensive Cancer Network; *USPSTF* US Preventive Services Task Force; *ASCO* American Society of Clinical Oncology

Medicine (SFMPP) have recommended lists of genes for inclusion in reports for germline findings [11, 49]. In addition, the NCCN guidelines specify genes for which the presence of germline pathogenic variants requires specific management [36, 37]. The lists of genes recommended in the ACMG, ESMO, SFMPP, and NCCN guidelines are compared in Fig. 14.1.

14.6 Testing Methods for Germline Pathogenic Variants and Presumed Germline Pathogenic Variants

Currently, there are three types of testing approaches for tumor genomic profiling: tumor-normal paired testing with germline variant subtraction, tumor-normal paired testing with established analyses of genes associated with germline cancer predisposition, and tumor-only testing (Table 14.4) [48].

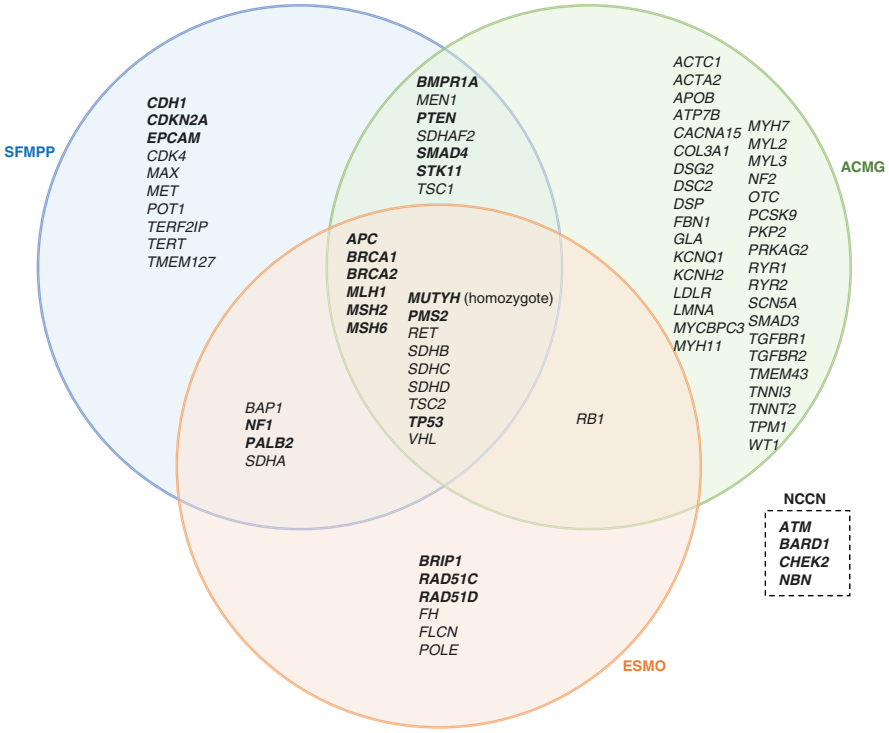


Fig. 14.1 Genes recommended for return of results**.** The lists of genes that are recommended for the return of results in ACMG [47], ESMO [11], SFMPP [49], and NCCN guidelines [36, 37] are compared. The genes shown in the SFMPP part of this figure are "class 1 genes," which are defined as those for which information given to patients is recommended by SFMPP [49]. Genes for which germline pathogenic variants have specific management by NCCN are shown in bold in the parts of ACMG, ESMO, SFMPP, and NCCN. SFMPP, French Society of Predictive and Personalized Medicine; ACMG, American College of Medical Genetics and Genomics; ESMO, European Society for Medical Oncology; NCCN, National Comprehensive Cancer Network

14.6.1 Tumor-Normal Paired Testing with Germline Variant Subtraction

In this method, tumor and normal DNA are analyzed simultaneously, and germline variants detected in the normal DNA are subtracted from the variants detected in the tumor DNA. Although this approach can detect somatic variants specific to the tumor, it renders germline pathogenic variants invisible [48]. Furthermore, because there is a lack of information on the comparison between the germline sequence and a reference, this approach cannot identify germline variants without additional dedicated analysis [48].

Table 14.4 Testing methods and germline findings

Testing method	Germline findings
Tumor-normal paired testing	
- With germline variant subtraction	Any germline pathogenic variants may be invisible
- With established analyses of genes associated with germline cancer predisposition	Germline pathogenic variants are detected based ontest design
Tumor-only testing	Germline pathogenic variants can be inferred as PGPVs

PGPV presumed germline pathogenic variant

14.6.2 Tumor-Normal Paired Testing with Established Analyses of Genes Associated with Germline Cancer Predisposition

In this approach, additional germline testing is not needed for the patient but is needed as a positive control when the family members undergo germline genetic testing for the same variant [48].

14.6.3 Tumor-Only Testing

Germline pathogenic variants can be inferred from tumor sequencing results without direct analysis of germline DNA. Tumor-detected pathogenic variants of potential germline origin are called presumed germline pathogenic variants (PGPVs) [11, 48]. The germline status of variants can be deduced but must be confirmed with additional germline testing in the tumor-only genomic profiling. Clinicians should carefully evaluate all variants detected through the test for PGPV recognition [50].

14.7 Recognition of PGPVs Through Tumor-Only Testing

The first step in the recognition of PGPVs is the determination of the pathogenicity of variants detected within genes associated with inherited predisposition to malignancy or other diseases [50]. The ACMG and the Association for Molecular Pathology have established practice guidelines for the interpretation of genetic variants using a five-tier classification system: benign, likely benign, variant of uncertain significance, likely pathogenic, and pathogenic [51]. Only pathogenic or likely pathogenic variants are actionable. Several public databases catalog the clinical impact of previously reported somatic or germline variants: Catalog of Somatic Mutations in Cancer (COSMIC) [52], cBioPortal [53], and Clinical Interpretations of Variants in Cancer (CIViC) [54] for somatic variants and ClinVar [55] for the relationship between germline variants and diseases.

Second, a reassessment of the patient's clinical presentation and family history is needed when a pathogenic/likely pathogenic variant is identified in a gene associated with inherited predisposition to malignancy or other diseases [50]. Generally,

somatic pathogenic variants are commonly detected in *APC*, *NF1*, *PTEN*, *RB1*, *STK11*, and *TP53* but less frequently detected in *BRCA1/2*, *PALB2*, *MSH2*, and *MSH6* through tumor genomic profiling [8, 11]. If the gene is commonly mutated in cancer, the reassessment of personal and family history is crucial for referral to a genetic specialist [50]. Conversely, when *BRCA1/2* pathogenic/likely pathogenic variants are identified through tumor-only testing, a genetic specialist should be considered regardless of the tumor type and the presence of personal and family history that meets the criteria for germline genetic testing because almost 80% of these variants are of germline origin [8, 11].

Finally, the variant allele frequency (VAF) has been considered as an important factor in the detection of variants of true germline origin through tumor-only testing [11]. The VAF of heterozygous PGPVs is generally proposed to range from 30 to 70% [5, 11, 48]. However, because the VAF depends on the tumor purity, the tumor ploidy, and the local copy number [56], it is not always within this range. There are no established cutoffs of the VAF, and the exclusion or confirmation of germline origin using the VAF alone is not recommended for tumor-only testing [48].

Currently, there is no established guideline for clinicians on determining which somatic findings may be PGPVs and on the optimal clinical practice for referral to genetic specialists [48]. Figure 14.2 summarizes the algorithm described in this section, based on personal and family history and results of tumor-only testing.

14.8 Clinical Utility of Tumor Genomic Profiling for Ovarian Cancer

Currently, genetic testing for germline and/or somatic *BRCA1/2* is an essential part of the care for patients with ovarian cancer [36, 43, 44, 57]. *BRCA1/2* germline pathogenic variants and somatic *BRCA1/2* mutations are present in approximately 10–18% and 7% of unselected ovarian cancer [58–65]. For first-line therapy in epithelial ovarian cancer, randomized phase III clinical trials demonstrated the benefit of maintenance therapy with PARP inhibitors or a combination of a PARP inhibitor and bevacizumab among patients with germline or somatic *BRCA1/2* pathogenic variants (Table 14.2) [15, 16, 18, 22–25]. These results endorse the testing for germline and/or somatic *BRCA1/2* status in all patients with ovarian cancer at initial diagnosis [44].

Germline genetic testing has revealed a high frequency of heritable genetic conditions—approximately 18–24% of all ovarian cancers [59–62]. A large number of germline pathogenic variants were detected in genes associated with homologous recombination repair, such as *BRCA1/2*, *ATM*, *BARD1*, *BRIP1, CHEK1*, *CHEK22*, *FAM175A*, *MRE11A*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*, and DNA mismatch repair (Fig. 14.3) [59]. Beyond *BRCA*-mutated tumors, patients with ovarian cancer harboring HRD exhibit high sensitivity to platinum, PARP inhibitors, or experimental agents targeting DNA repair or cell-cycle pathways [14, 60, 66]. Meanwhile, the frequency of MSI-H or dMMR in ovarian cancer is not very high, ranging from 3 to 12% [67–70]. A meta-analysis revealed

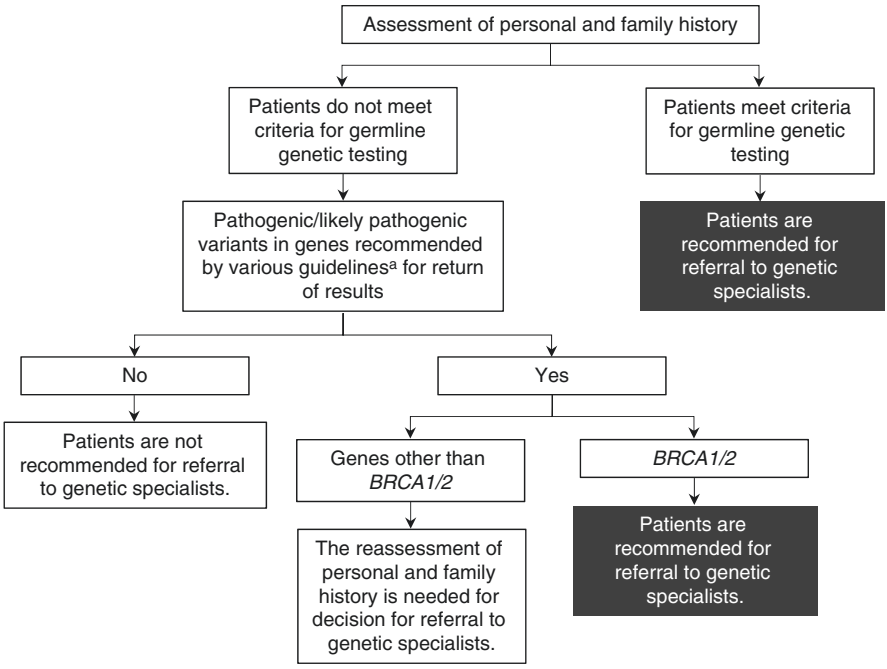


Fig. 14.2 Algorithm for referral to genetic specialists based on personal and family history and results of tumor-only testing**.** First, any patients for whom personal and/or family history meets the criteria of germline genetic testing are recommended for referral to genetic specialists. Second, when *BRCA1/2* pathogenic/likely pathogenic variants are detected, genetic specialists should be considered regardless of the tumor type and the variant allele frequency (VAF). Lastly, when pathogenic/likely pathogenic variants in genes on the lists of guidelines for return of results except for *BRCA1/2*, the reassessment of personal and family history is needed for decision for referral to genetic specialists, especially *APC*, *NF1*, *PTEN*, *RB1*, *STK11*, and *TP53*. The exclusion or confirmation of germline origin by using the VAF alone is not recommended. [a] indicates ACMG [47], ESMO [11], SFMPP [49], and NCCN guidelines [36, 37]

an overrepresentation of non-serous histology in ovarian cancer harboring dMMR [67]. These data support the testing for the status of microsatellite instability or mismatch repair determined from tumor tissue in patients with ovarian cancer, especially for those with non-serous histology [44]. The presence of MSI-H or dMMR in ovarian cancer provides an opportunity for treatment with pembrolizumab and also supports a referral to genetic specialists to confirm germline testing for mismatch repair genes [37]. Considering these clinical benefits of tumor genomic alterations, ESMO recommends the routine use of tumor genomic profiling in daily practice for ovarian cancer [71].

Finally, it should be noted that a normal/negative result for tumor sequencing is not equivalent to a normal/negative germline result [11]. Sequencing of germline DNA is the most sensitive approach, and sequencing of tissue DNA possibly misses almost 5% of germline pathogenic variants [15]. Germline genetic testing is still recommended for patients with ovarian cancer, even if tumor-only testing shows no

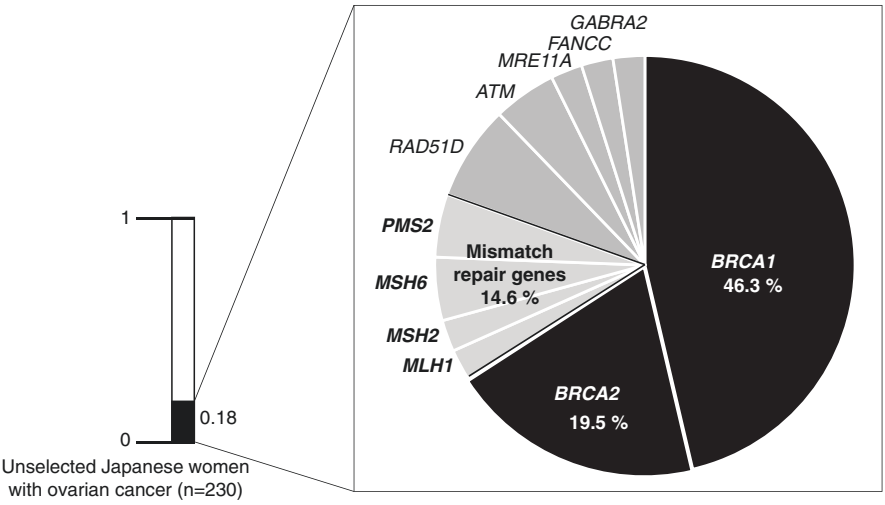


Fig. 14.3 Proportion of patients with germline pathogenic variants in cancer susceptibility genes in ovarian cancer. Two hundred and thirty unselected Japanese women with ovarian cancer were screened for pathogenic germline variants in 75 or 79 cancer susceptibility genes. Pathogenic variants of 11 genes were identified in 41 (17.8%) women, including 19 (46.3%) in *BRCA1*, 8 (19.5%) in *BRCA2*, and 6 (14.6%) in mismatch repair genes. Source: Oncotarget. 2017 Nov 28;8(68):112258–112267

BRCA1/2 pathogenic variant [44]. Multigene panel testing for germline sequencing that includes *BRCA1/2*, other homologous recombination repair genes, and mismatch repair genes will serve as a standard tool for ovarian cancer [36, 44].

14.9 Summary

Germline findings identified through tumor genomic profiling could have implications in the assessment and management of future primary cancer risk, family risk assessment and guidance, and personalized treatment determination. Oncology providers must understand that somatic DNA analysis may reveal PGPVs that could have important implications not only for the patient but also for the patient's family members.

Till date, a systematic approach has not been established for the assessment of PGPVs when tumor-only testing is performed [48, 50]. Somatic genetic findings through tumor genomic profiling must be interpreted carefully, especially in patients with ovarian cancer. Determination of germline findings using tumor genomic information is an urgent task in the era of precision oncology.

In conclusion, HBOC is the most common cause of genetic predisposition to cancer; it has become one of the best characterized hereditary syndromes in terms of diagnosis, treatment, and prevention [72], which indicates that HBOC is the most suitable model for precision oncology.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266(5182):66–71. https://doi.org/10.1126/science.7545954.
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378(6559):789–92. https://doi.org/10.1038/378789a0.
3. Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson Genetics in medicine. 8th ed. Elsevier Inc; 2016.
4. Bombard Y, Robson M, Offit K. Revealing the incidentalome when targeting the tumor genome. JAMA. 2013;310(8):795–6. https://doi.org/10.1001/jama.2013.276573.
5. Raymond VM, Gray SW, Roychowdhury S, Joffe S, Chinnaiyan AM, Parsons DW, et al. Germline findings in tumor-only sequencing: points to consider for clinicians and laboratories. J Natl Cancer Inst. 2015;108(4):djv351. https://doi.org/10.1093/jnci/djv351.
6. Jones S, Anagnostou V, Lytle K, Parpart-Li S, Nesselbush M, Riley DR, et al. Personalized genomic analyses for cancer mutation discovery and interpretation. Sci Transl Med. 2015;7(283):283ra53. https://doi.org/10.1126/scitranslmed.aaa7161.
7. Schrader KA, Cheng DT, Joseph V, Prasad M, Walsh M, Zehir A, et al. Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncol. 2016;2(1):104–11. https://doi.org/10.1001/jamaoncol.2015.5208.
8. 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. Ann Oncol. 2016;27(5):795–800. https://doi.org/10.1093/annonc/mdw018.
9. Seifert BA, O'Daniel JM, Amin K, Marchuk DS, Patel NM, Parker JS, et al. Germline analysis from tumor-germline sequencing dyads to identify clinically actionable secondary findings. Clin Cancer Res. 2016;22(16):4087–94. https://doi.org/10.1158/1078-0432.CCR-16-0015.
10. 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. JAMA. 2017;318(9):825–35. https://doi.org/10.1001/jama.2017.11137.
11. Mandelker D, Donoghue M, Talukdar S, Bandlamudi C, Srinivasan P, Vivek M, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO precision medicine working group. Ann Oncol. 2019;30(8):1221–31. https://doi.org/10.1093/annonc/mdz136.
12. Dumbrava EI, Brusco L, Daniels M, Wathoo C, Shaw K, Lu K, et al. Expanded analysis of secondary germline findings from matched tumor/normal sequencing identifies additional clinically significant mutations. JCO Precis Oncol. 2019;3:PO.18.00143. https://doi.org/10.1200/PO.18.00143.
13. Robson ME, Bradbury AR, Arun B, Domchek SM, Ford JM, Hampel HL, et al. American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility. J Clin Oncol. 2015;33(31):3660–7. https://doi.org/10.1200/JCO.2015.63.0996.
14. Pilie PG, Tang C, Mills GB, Yap TA. State-of-the-art strategies for targeting the DNA damage response in cancer. Nat Rev Clin Oncol. 2019;16(2):81–104. https://doi.org/10.1038/s41571-018-0114-z.
15. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379(26):2495–505. https://doi.org/10.1056/NEJMoa1810858.
16. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzalez-Martin A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381(25):2416–28. https://doi.org/10.1056/NEJMoa1911361.

17. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92. https://doi.org/10.1056/NEJMoa1105535.
18. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(9):1274–84. https://doi.org/10.1016/S1470-2045(17)30469-2.
19. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmana J, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol. 2015;33(3):244–50. https://doi.org/10.1200/JCO.2014.56.2728.
20. Gonzalez-Martin A, Pothuri B, Vergote I, DePont CR, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2019;381(25):2391–402. https://doi.org/10.1056/NEJMoa1910962.
21. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154–64. https://doi.org/10.1056/NEJMoa1611310.
22. Moore KN, Secord AA, Geller MA, Miller DS, Cloven N, Fleming GF, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol. 2019;20(5):636–48. https://doi.org/10.1016/S1470-2045(19)30029-4.
23. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949–61. https://doi.org/10.1016/S0140-6736(17)32440-6.
24. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol. 2017;18(1):75–87. https://doi.org/10.1016/S1470-2045(16)30559-9.
25. Kristeleit R, Shapiro GI, Burris HA, Oza AM, LoRusso P, Patel MR, et al. A phase I-II study of the oral PARP inhibitor rucaparib in patients with germline BRCA1/2-mutated ovarian carcinoma or other solid tumors. Clin Cancer Res. 2017;23(15):4095–106. https://doi.org/10.1158/1078-0432.CCR-16-2796.
26. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017;377(6):523–33. https://doi.org/10.1056/NEJMoa1706450.
27. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Goncalves A, Lee KH, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med. 2018;379(8):753–63. https://doi.org/10.1056/NEJMoa1802905.
28. Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. N Engl J Med. 2019;381(4):317–27. https://doi.org/10.1056/NEJMoa1903387.
29. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for metastatic castration-resistant prostate cancer. N Engl J Med. 2020;382(22):2091–102. https://doi.org/10.1056/NEJMoa1911440.
30. Abida W, Patnaik A, Campbell D, Shapiro J, Bryce AH, McDermott R, et al. Rucaparib in men with metastatic castration-resistant prostate cancer harboring a BRCA1 or BRCA2 gene alteration. J Clin Oncol. 2020:JCO2001035. https://doi.org/10.1200/JCO.20.01035.
31. 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(6349):409–13. https://doi.org/10.1126/science.aan6733.
32. Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mis-

match repair-deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38(1):1–10. https://doi.org/10.1200/JCO.19.02105.
33. Varga A, Piha-Paul S, Ott PA, Mehnert JM, Berton-Rigaud D, Morosky A, et al. Pembrolizumab in patients with programmed death ligand 1-positive advanced ovarian cancer: analysis of KEYNOTE-028. Gynecol Oncol. 2019;152(2):243–50. https://doi.org/10.1016/j.ygyno.2018.11.017.
34. Le DT, Kim TW, Van Cutsem E, Geva R, Jager 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. J Clin Oncol. 2020;38(1):11–9. https://doi.org/10.1200/JCO.19.02107.
35. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509–20. https://doi.org/10.1056/NEJMoa1500596.
36. NCCN Clinical Practice Guidelines in Oncology. Genetic/familial high-risk assessment: breast, ovarian, and pancreatic version 1.2021.. https://www.nccn.org/professionals/physician_gls. Accessed 17 Sept 2020.
37. NCCN Clinical Practice Guidelines in Oncology. Genetic/familial high-risk assessment: colorectal version 1.2020.. https://www.nccn.org/professionals/physician_gls. Accessed 25 Sept 2020.
38. US Preventive Services Task Force, Owens DK, Davidson KW, Krist AH, Barry MJ, Cabana M, et al. Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer: US Preventive Services Task Force Recommendation Statement. JAMA. 2019;322(7):652–65. https://doi.org/10.1001/jama.2019.10987.
39. Lu KH, Wood ME, Daniels M, Burke C, Ford J, Kauff ND, et al. American Society of Clinical Oncology expert statement: collection and use of a cancer family history for oncology providers. J Clin Oncol. 2014;32(8):833–40. https://doi.org/10.1200/JCO.2013.50.9257.
40. Committee on Practice Bulletins—Gynecology, Committee on Genetics, Society of Gynecologic Oncology. Practice bulletin no 182: hereditary breast and ovarian cancer syndrome. Obstet Gynecol. 2017;130(3):e110–26. https://doi.org/10.1097/AOG.0000000000002296.
41. Randall LM, Pothuri B, Swisher EM, Diaz JP, Buchanan A, Witkop CT, et al. Multi-disciplinary summit on genetics services for women with gynecologic cancers: a Society of Gynecologic Oncology White Paper. Gynecol Oncol. 2017;146(2):217–24. https://doi.org/10.1016/j.ygyno.2017.06.002.
42. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease dagger. Ann Oncol. 2019;30(5):672–705. https://doi.org/10.1093/annonc/mdz062.
43. NCCN Clinical Practice Guidelines in Oncology. Ovarian cancer including fallopian tube cancer and primary peritoneal cancer version 1.2020.. https://www.nccn.org/professionals/physician_gls. Accessed 8 Aug 2020.
44. 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. J Clin Oncol. 2020;38(11):1222–45. https://doi.org/10.1200/JCO.19.02960.
45. ACMG Board of Directors. Points to consider in the clinical application of genomic sequencing. Genet Med. 2012;14(8):759–61. https://doi.org/10.1038/gim.2012.74.
46. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15(7):565–74. https://doi.org/10.1038/gim.2013.73.
47. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19(2):249–55. https://doi.org/10.1038/gim.2016.190.
48. Li MM, Chao E, Esplin ED, Miller DT, Nathanson KL, Plon SE, et al. Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American

College of Medical Genetics and Genomics (ACMG). Genet Med. 2020;22(7):1142–8. https://doi.org/10.1038/s41436-020-0783-8.
49. Pujol P, Vande Perre P, Faivre L, Sanlaville D, Corsini C, Baertschi B, et al. Guidelines for reporting secondary findings of genome sequencing in cancer genes: the SFMPP recommendations. Eur J Hum Genet. 2018;26(12):1732–42. https://doi.org/10.1038/s41431-018-0224-1.
50. DeLeonardis K, Hogan L, Cannistra SA, Rangachari D, Tung N. When should tumor genomic profiling prompt consideration of germline testing? J Oncol Pract. 2019;15(9):465–73. https://doi.org/10.1200/JOP.19.00201.
51. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24. https://doi.org/10.1038/gim.2015.30.
52. The Catalogue Of Somatic Mutations In Cancer (COSMIC). 2020. https://cancer.sanger.ac.uk/cosmic. Accessed 18 Sept 2020.
53. cBioPortal. https://www.cbioportal.org/. Accessed 18 Sept 2020.
54. Clinical Interpretations of Variants in Cancer (CIViC). https://civicdb.org/home. Accessed 18 Sept 2020.
55. ClinVar. https://www.ncbi.nlm.nih.gov/clinvar/. Accessed 18 Sept 2020.
56. Sun JX, He Y, Sanford E, Montesion M, Frampton GM, Vignot S, et al. A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. PLoS Comput Biol. 2018;14(2):e1005965. https://doi.org/10.1371/journal.pcbi.1005965.
57. Hereditary Cancer Syndromes and Risk Assessment. ACOG COMMITTEE OPINION, Number 793. Obstet Gynecol. 2019;134(6):e143–9. https://doi.org/10.1097/AOG.0000000000003562.
58. Enomoto T, Aoki D, Hattori K, Jinushi M, Kigawa J, Takeshima N, et al. The first Japanese nationwide multicenter study of BRCA mutation testing in ovarian cancer: CHARacterizing the cross-sectionaL approach to Ovarian cancer geneTic TEsting of BRCA (CHARLOTTE). Int J Gynecol Cancer. 2019;29(6):1043–9. https://doi.org/10.1136/ijgc-2019-000384.
59. 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(68):112258–67. https://doi.org/10.18632/oncotarget.22733.
60. Pennington KP, Walsh T, Harrell MI, Lee MK, Pennil CC, Rendi MH, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res. 2014;20(3):764–75. https://doi.org/10.1158/1078-0432.CCR-13-2287.
61. Walsh T, Casadei S, Lee MK, Pennil CC, Nord AS, Thornton AM, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A. 2011;108(44):18032–7. https://doi.org/10.1073/pnas.1115052108.
62. Norquist BM, Harrell MI, Brady MF, Walsh T, Lee MK, Gulsuner S, et al. Inherited mutations in women with ovarian carcinoma. JAMA Oncol. 2016;2(4):482–90. https://doi.org/10.1001/jamaoncol.2015.5495.
63. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609–15. https://doi.org/10.1038/nature10166.
64. 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. J Clin Oncol. 2012;30(21):2654–63. https://doi.org/10.1200/JCO.2011.39.8545.
65. Jorge S, McFaddin AS, Doll KM, Pennington KP, Norquist BM, Bennett RL, et al. Simultaneous germline and somatic sequencing in ovarian carcinoma: mutation rate and impact on clinical decision-making. Gynecol Oncol. 2020;156(3):517–22. https://doi.org/10.1016/j.ygyno.2019.12.010.

66. Norquist BM, Brady MF, Harrell MI, Walsh T, Lee MK, Gulsuner S, et al. Mutations in homologous recombination genes and outcomes in ovarian carcinoma patients in GOG 218: an NRG oncology/gynecologic oncology group study. Clin Cancer Res. 2018;24(4):777–83. https://doi.org/10.1158/1078-0432.CCR-17-1327.
67. Pal T, Permuth-Wey J, Kumar A, Sellers TA. Systematic review and meta-analysis of ovarian cancers: estimation of microsatellite-high frequency and characterization of mismatch repair deficient tumor histology. Clin Cancer Res. 2008;14(21):6847–54. https://doi.org/10.1158/1078-0432.CCR-08-1387.
68. Murphy MA, Wentzensen N. Frequency of mismatch repair deficiency in ovarian cancer: a systematic review. Int J Cancer. 2011;129(8):1914–22. https://doi.org/10.1002/ijc.25835.
69. Kelderman S, Schumacher TN, Kvistborg P. Mismatch repair-deficient cancers are targets for anti-PD-1 therapy. Cancer Cell. 2015;28(1):11–3. https://doi.org/10.1016/j.ccell.2015.06.012.
70. Cortes-Ciriano I, Lee S, Park WY, Kim TM, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. Nat Commun. 2017;8:15180. https://doi.org/10.1038/ncomms15180.
71. Mosele F, Remon J, Mateo J, Westphalen CB, Barlesi F, Lolkema MP, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO precision medicine working group. Ann Oncol. 2020; https://doi.org/10.1016/j.annonc.2020.07.014.
72. Lu KH. Hereditary gynecologic cancer: risk, prevention and management. CRC Press; 2008.

Germline Findings from Genetic Testing for Breast Cancer

15

Akihiro Sakurai

Abstract

Five to 10 percent of breast cancers are hereditary tumors caused by pathogenic germline mutations in a responsible gene. Hereditary breast and ovarian cancer (HBOC), caused by loss-of-function germline mutation of tumor suppressor genes *BRCA1* and *BRCA2 (BRCA1/2)*, is the best known, but many other causative genes of hereditary breast cancer are also recognized. Accurate diagnosis of hereditary cancer not only provides patients with more appropriate surveillance and treatment options but also allows for early intervention in relatives at the same risk.

In Japan, genetic tests, surveillance, and risk reduction surgery for HBOC patients have finally been covered by public health insurance in 2020. Presymptomatic mutation carriers may be the best candidates to provide preemptive intervention; however such management is not covered by insurance, which is a major issue to be amended.

The partial coverage of HBOC treatment will provide an opportunity to reexamine Japan's public health insurance system and illustrate future medical system.

Keywords

Genetic test · Multigene testing · Companion diagnosis · Cancer genome profiling · Risk reduction surgery · Presymptomatic mutation carrier · Preemptive medicine

A. Sakurai (✉)
Department of Medical Genetics and Genomics, Sapporo Medical University School of Medicine, Sapporo, Japan
e-mail: sakurai.akihiro@sapmed.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_15

15.1 Breast Cancer in Japanese Women

The incidence of breast cancer in Japanese women has been increasing every year. In Japan, there had been no nationwide cancer registration system until 2016. Thus, we had to estimate cancer incidence from data of regional registry. According to those data, the estimated number of newly diagnosed breast cancer patients in 2005 was about 48,000, and that number was almost doubled to about 92,000 in 2017. The lifetime risk of breast cancer in Japanese women is 10.6%, which has been increased every year, and as is the case with other developed countries, breast cancer is the most frequent cancer in Japanese women. The peak incidence is between the ages of fifth to seventh decade. The incidence is increasing in all age groups, and a loose bimodal pattern is observed (Fig. 15.1) [1].

Hereditary tumors associated with mutations in a responsible gene have been estimated to account for 7–10% of breast cancers. In the United States, mutations of either *BRCA1* or *BRCA2* (*BRCA1/2*) are found in about 5% of breast cancer patients aged 35–64 years [2]. Similar data have recently been reported in Japanese breast cancer patients, suggesting that there is no significant difference in the frequency of pathogenic variant of *BRCA1/2* genes [3].

In this chapter, the genetic background of breast cancer in Japanese women and current status of clinical issues in Japan will be overviewed.

15.2 Patients Suspected of Having Hereditary Breast Cancer

Table 15.1 shows the clinical importance of accurately diagnosing hereditary breast cancer among a large number of breast cancer patients. The diagnosis enables better medical care for the patient, i.e., individualized surveillance, treatment selection, and risk-reducing treatment. Because genetic information remains unchanged

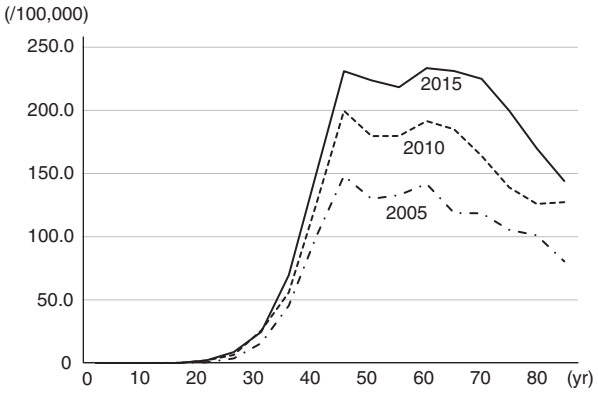


Fig. 15.1 Incidence of breast cancer in Japanese women. Graphs are breast cancer incidence rates per 100,000 population by age group in 2005, 2010, and 2015. The graph is created based on the National Cancer Center's database (Ref. [1])

throughout life and is shared by relatives at a certain rate, it also allows early intervention for at-risk relatives or avoids unnecessary medical intervention for relatives who are identified as not at risk.

In general, conditions that raise suspicion of HBOC include young age of onset; pathological feature, i.e., triple-negative breast cancer; multiple or recurrence of tumors; and a family history of breast or ovarian cancer (family history of prostate and pancreas cancer may also be considered). To pick up suspicious patients with HBOC, relevant societies such as NCCN (National Comprehensive Cancer Network) [4], NICE (National Institute for Health and Care Excellence) [5], and US Preventive Services Task Force [6], as well as guidebook published in Japan [7], have developed and proposed criteria to whom genetic testing of *BRCA1/2* should be provided.

Applications for estimating the probability that the patient carries pathogenic variants of *BRCA1/2* are widely used in clinical settings. Those applications include BRCAPRO (https://projects.iq.harvard.edu/bayesmendel/brcapro), IBIS (https://ibis-risk-calculator.magview.com/), BOADICEA (https://ccge.medschl.cam.ac.uk/boadicea/), Myriad Mutation Prevalence Table (https://s3.amazonaws.com/myriad-library/brac/brca-prevalence-tables.pdf), KOHCal (http://www.kohbra.kr/KOHCal/BRCA_en.html), etc. Those are based on epidemiological data to predict the probability that a patient or a blood relative carries a pathogenic variant. Some studies validated the performance of those models [8–10]. In the United States and Europe, genetic risk assessment has been recommended for patients who are assumed to have more than 5–10% chance of having a *BRCA1/2* germline pathogenic variant [11].

Recently, however, a broader view of who should be offered genetic testing has become the norm. In fact, in a recent revision of the NCCN guidelines, the recommendation was changed from a two-step pick-up approach to suggest genetic testing basically for all patients. The American Society of Breast Surgeons has also announced that genetic testing should be made available to all breast cancer patients [12].

In Japan, genetic testing for *BRCA1/2* has not been covered by the public health insurance system for a long time, and patients who wanted to have the test had to

Table 15.1 Importance of diagnosing hereditary breast cancer

For the proband
Enables personalized medical care
• More accurate prediction of course and prognosis
• Appropriate periodic surveillance
• Choose better treatment option
• Prophylactic intervention to reduce future health risk
Genetic counseling and psychosocial support
For relatives
Genetic counseling, pre-onset genetic testing
• At risk → early response including surveillance and prophylactic treatment
• No risk → avoid unnecessary surveillance
Genetic counseling and psychosocial support

pay approximately 200,000–300,000 yen out-of-pocket. This high cost was a significant burden for many patients, and that was part of the reason *BRCA1/2* genetic testing has not been widely performed in Japan. Since 2020, *BRCA1/2* genetic testing has been covered by public health insurance for patients who have breast or ovarian cancer and meet the conditions listed in Table 15.2. In fact, it is estimated that nearly half of all breast cancer patients meet one of these conditions. Cost of surveillance by MRI, mammography, ultrasonography, and tumor markers for patients with HBOC who have already been diagnosed of breast or ovarian cancer (including fallopian tube and peritoneal cancer) is also covered. In addition, risk-reducing surgeries for the contralateral breast, fallopian tubes, and ovaries of HBOC patients are also covered. Breast reconstruction is covered as well, but simultaneous hysterectomy during tubo-ovarian surgery is not covered. Surveillance and risk reduction surgery for presymptomatic relatives diagnosed as *BRCA1/2* mutation carriers are not covered.

15.3 Japanese *BRCA1/2* Database

The HBOC Consortium, a research group on HBOC in Japan, established a system to register data of patients who have genetic testing of *BRCA1/2*, and the registration started in 2015. Currently, this registration project has been taken over by the Japan Organization for Hereditary Breast and Ovarian Cancer (JOHBOC), and data accumulation and analysis are being conducted continuously [13]. The JOHBOC also conducts open research using the collected database.

The first analysis of this database was reported in 2018 for 830 cases of originators [14]. Of the cases tested, 19.7% had pathogenic variants in *BRCA1/2*. The result of variant of unknown significance (VUS) was reported in 6.5% which was higher compared to overseas data. This may be because that the number of East Asians tested is still low compared to North America and Europe. Regarding the pathogenic variants identified, the most frequent *BRCA1* variant was p.L63*,

Table 15.2 Conditions for public health coverage of *BRCA1/2* genetic testing in Japan

• Has breast or ovarian cancer and has already been found to have a pathogenic variant of *BRCA1* and/or *BRCA2* in the family
• Has developed breast cancer and any of the following apply
• Developed breast cancer at age 45 years or younger
• Triple-negative breast cancer at age 60 years or younger
• Two or more primary breast cancers
• One or more third-degree relatives with breast cancer or ovarian cancer
• Has developed ovarian cancer, fallopian tube cancer, or peritoneal cancer
• Has developed male breast cancer
• Has breast or ovarian cancer and meet the eligibility criteria for companion diagnosis for PARP inhibitors
• Has breast or ovarian cancer, and presumed germline pathogenic variants are found in the *BRCA1* and/or *BRCA2* on tumor tissue profiling

which is considered to be a founder mutation in the Japanese population. This variant accounts for 26% of the *BRCA1* pathogenic variants identified in the Japanese population, and among patients with this variant, 89% had triple-negative breast cancers.

Recently, a study about frequency of hereditary breast cancer in Japanese women with breast cancer was reported [3]. They examined hereditary breast cancer-related genes (*BRCA1, BRCA2, PALB2, TP53, PTEN, CHEK2, NF1, ATM, CDH1, NBN, STK11*) using blood DNA samples from BioBank Japan that include 7093 Japanese women with breast cancer and 11,260 women over 60 years of age without a family history of cancer and 53 men with breast cancer and 12,520 men over 60 years of age without a family history of cancer. The frequency of pathogenic variant in *BRCA1* or *BRCA2* was 1.45% and 2.71%, respectively. Table 15.3 shows a comparison of the clinical findings between the pathogenic variant-positive and variant-negative groups. Factors associated with an odds ratio (OR) > 2 for retention of the pathogenic variants were previous ovarian cancer, bilateral breast cancer, triple-negative breast cancer, and family history of breast cancer, ovarian cancer, bone tumor, and bladder cancer.

About 15% of patients under 39 years of age had pathogenic variants in one of those genes, while about 3% of patients over 80 years of age had pathogenic variants, suggesting that the possibility of hereditary breast cancer should be considered regardless of age of onset. In addition, 0.6% of the control group with no history of breast or ovarian cancer or family history of breast or ovarian cancer had a pathogenic variant in one of these genes.

Table 15.3 Comparison of clinical findings between the group with and without pathogenic variants in the breast cancer high-risk 11 genes including *BRCA1/2* [3]

Variable	Yes	No	*P* value	OR (95% CI)
No. of subjects	404	6647		
Age at entry	51.4 ± 12.8	56.1 ± 11.9	1.00×10^{-10}	
History of ovarian cancer	1.7%	0.6%	0.017	2.9 (1.1–6.6)
Bilateral breast cancer	7.1%	2.4%	6.11×10^{-5}	3.1 (1.8–5.1)
ER positive	66.9%	73.3%	0.028	0.7 (0.6–1.0)
PR positive	47.7%	61.8%	8.45×10^{-6}	0.6 (0.4–0.7)
Triple negative	22.0%	10.1%	2.16×10^{-5}	2.5 (1.6–3.7)
Family history				
Breast cancer	23.3%	11.1%	3.14×10^{-11}	2.4(1.9–3.1)
Ovarian cancer	4.7%	1.0%	1.42×10^{-7}	5.1 (2.8–8.7)
Pancreas cancer	5.9%	3.3%	0.011	1.8 (1.1–2.9)
Gastric cancer	25.0%	20.4%	0.027	1.3 (1.0–1.7)
Liver cancer	9.4%	6.3%	0.017	1.5 (1.1–2.2)
Bone tumor	1.0%	0.2%	0.014	5.1 (1.2–16.6)
Bladder cancer	3.7%	1.5%	3.18×10^{-3}	2.5 (1.4–4.5)

This table is referred and modified with permission of the author from [3]

15.4 Multigene Testing (MGT)

The NCCN guidelines are widely referred to in Japan. In this guideline, the section "Genetic/Familial High-Risk Assessment: Breast and Ovarian" has been referred for management of HBOC for a long time, and it is frequently revised to introduce updated knowledge. This guideline had a major revision in 2019. In the revised version, the title was changed to "Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic." The primary approach to patients was changed to be as comprehensive as possible and to be performed for all cancer patients. In the previous version, the first step was to evaluate and assess those who needed detailed genetic risk assessment and then to assess whether they meet the testing criteria for *BRCA1/2*, *TP53* (Li-Fraumeni syndrome), and *PTEN* (Cowden disease). In the revised version, it is recommended that all cancer patients should be assessed to determine whether they meet the testing criteria and that a multigene panel test should be performed instead of a single gene analysis of *BRCA1/2*. In fact, in the United States, more MGTs had become performed than *BRCA1/2* genetic tests. In Japan, meanwhile, MGTs are not yet widely used mainly because of high cost patients have to pay.

The advantage of MGT is, without mentioning it again, that it increases the likelihood of diagnosing hereditary tumor syndromes that would not have been identified by genetic testing of a single gene. There are reports from Japan, the United States, and China, respectively, on MGP of low to high risk of breast cancer, including *BRCA1/2*, for a large cohort of more than 7000 breast cancer patients without selection bias [3, 15, 16]. The detection rate of *BRCA1/2* pathogenic variants was 4.16%, 4.64%, and 5.34%, respectively, while the detection rate of pathogenic variants of all genes including genes other than BRCA was 5.7%, 9.3%, and 9.2%, respectively. Compared to genetic testing of *BRCA1/2* alone, the detection rate was 1.4 to 2 times higher.

A meta-analysis of 48 MGP reports for breast and ovarian cancers, including cohorts from various backgrounds, showed that *BRCA1/2* accounted for 36% of the detected pathogenic variants in breast cancer and 62% in ovarian cancer. Identified pathogenic variants in genes other than *BRCA1/2* were *CHEK2* (14%), *ATM* (8%), and *PALB2* (8%) in breast cancer and *FANCM* (6%), *BRIP1* (5%), and *ATM/CHEK2/RAD51C/RAD51D* (3% each) in ovarian cancer [17]. Beitsch et al. also reported that only about half of the patients who had pathogenic variants in breast cancer-related genes after MGT met the criteria of the NCCN guidelines. These trials also provide evidence for the superiority of MGT [18]. Although the genes being searched for vary from report to report, it seems certain that MGP will improve the detection rate of pathogenic variants. However, it should be noted that the detection rate of pathogenic variants in Asian patients with breast cancer and ovarian cancer is higher for *BRCA2* and lower for *CHEK2* compared to Western population and that the detection rate of pathogenic variants for all genes does not increase significantly even if the number of low-risk genes searched is increased [19].

On the other hand, the weakness of MGT is the increase in frequency of detecting VUS [20]. In general, as the number of genes to be analyzed increases, the

positive rate of pathogenic variants increases, but the increase gradually slows down after reaching a certain level. In contrast, the detection rate of VUS increases as the number of genes increases (as it should), and this rate remains proportional. In fact, when the detection rates of pathogenic variants and VUS in MGT are examined on a gene-by-gene basis, genes other than *BRCA1/2* are more likely to detect VUS than to detect pathogenic variants, which is not easy to disclose the test results concisely and correctly to patients and their families [21].

Another weakness of MGT is that many of the causative genes detected in MGT have low penetrance and clinical data are scarce, making it difficult to provide evidence-based post-diagnostic management. However, the identification of mutations other than *BRCA1/2* has been reported to lead to changes in risk management and treatment for the majority of cases [22, 23]. In an analysis comparing 2000 cases in the HBOC high-risk group and 1997 cases in the healthy control group, mutations other than *BRCA1/2* were found in 1.6% of cases in the control group and 4.0% in the high-risk group [24]. However, only *PALB2* (26 cases vs. 4 cases, $P < 0.001$) and *TP53* (5 cases vs. 0 case, $P < 0.03$) were found to have significantly higher rates of mutations in the high-risk group, and there were many genes with no difference in detection rates between the two groups, suggesting that mutations in those genes may not largely affect the risk of breast cancer.

15.5 Breast Cancer-Related Genes Other Than *BRCA1/2*

The MGT developed for hereditary breast cancer can analyze the causative genes of the hereditary tumor syndromes shown below. Most of these syndromes are rare and occur in an autosomal recessive manner, but breast and ovarian cancers are more frequently observed because they are often caused by a single-allele mutation alone, which may lead to the identification of affected families. Therefore, it is worthwhile to consider the use of MGT in patients with symptoms suggestive of these hereditary tumor syndromes, after careful consideration of the benefits and disadvantages for each patient:

1. Fanconi's anemia: This disease is transmitted in an autosomal recessive manner. Aplastic anemia, acute myelogenous leukemia, and myelodysplastic syndromes occur in childhood, and a wide variety of cancers develop in adulthood. Among genetic subtypes, D1 is the same gene as *BRCA2*, J is *BRIP1 (BACH1)*, N is *PALB2*, O is *RAD51C*, R is *RAD51*, and S is *BRCA1*. The function of the translated protein is mainly DNA repair.
2. Telangiectatic ataxia: Inherited in an autosomal recessive manner. It is characterized by cerebellar ataxia and telangiectasia with onset in childhood, followed by lymphoma and acute lymphoblastic leukemia in later childhood. The responsible gene is *ATM* which encodes a kinase protein, involved in cell cycle control and DNA repair by phosphorylation signals.
3. Li-Fraumeni syndrome: An autosomal dominant genetic disorder caused by mutations in *TP53*. It is associated with a high incidence of a wide variety of

malignant tumors, including sarcoma, breast cancer, brain tumor, adrenocortical carcinoma, and leukemia, beginning at a young age.

4. Bloom syndrome: Autosomal recessive inheritance. In addition to short stature, sun-sensitive erythema, and immunodeficiency, a variety of carcinomas occur at a high rate. *BLM* has been identified as the responsible gene, and the translated protein is a DNA helicase that is essential for DNA repair. Excess sister chromatid exchange in patient cells is used for diagnosis.
5. Nijmegen syndrome: Autosomal recessive inheritance. In addition to microcephaly, short stature, avian-like facial expression, and immunodeficiency, it is associated with a high incidence of lymphoid malignancies and a variety of solid tumors. The responsible gene is *NBS1* (Nibrin), which is essential for DNA repair.
6. Hereditary diffuse gastric carcinoma syndrome: An autosomal dominant inherited disease caused by mutations in *CDH1*, resulting in a high incidence of young-onset diffuse gastric cancer and breast cancer (lobular carcinoma). The translation protein is E-cadherin, a molecule that regulates cell adhesion.

Other genes known to cause familial malignancies of the digestive organs include *MLH1*, *MSH2*, *MSH6*, *PMS1*, and *PMS2*, the mismatch repair-related genes responsible for Lynch syndrome; *PTEN*, the gene responsible for Cowden syndrome; *LKB1/STK11* responsible for Peutz-Jeghers syndrome; *NF1*, the causative gene of neurofibromatosis; *APC*, the causative gene of familial adenomatous polyposis; and *MUTYH*, the causative gene of MUTYH-related polyposis. These genes can be simultaneously analyzed by the MGT.

MGT may improve the detection rate of genetic mutations. However, when mutations are identified, few have been proven to correlate with risk, and VUS are also identified at a high rate [22, 25, 26]. Individuals without expertise may suffer from unnecessary anxiety and disadvantage due to excessive testing and treatment. Therefore, the use of MGT to identify genetic variants other than *BRCA1/2* should only be recommended for experienced clinical geneticists at this time. However, it may be considered on a case-by-case basis in high-risk groups with a family history and symptoms of hereditary tumor syndromes other than HBOC, after seeking expert opinion.

15.6 Companion Diagnosis

As already mentioned, the conventional diagnosis of hereditary tumors has mainly consisted of extracting suspected patients and proposing genetic testing. Recently, an increasing number of patients have been diagnosed with HBOC through other means. One of them is a “companion diagnosis.”

The enzyme PARP (poly(ADP-ribose) polymerase) is involved in DNA repair, cell death, and differentiation regulation. When DNA is damaged by single-strand breaks, PARP repairs this damage by base excision repair. On the other hand, when the damage is double-strand break, homologous recombination repair is carried out, and BRCA and some other proteins are involved. When single-strand break repair

is inhibited by PARP inhibitors, cells normally convert the break into a double-strand break and then repair it. In HBOC breast cancers with loss of BRCA protein function, double-strand break repair is impaired, so it is expected to lead to more cell death by PARP inhibitors, and its usefulness has been confirmed in clinical trials [27–30].

Olaparib, a PARP inhibitor, was approved in Japan in 2017 for the treatment of breast cancer in patients with *BRCA1/2* germline mutation, HER2-negative, recurrent, or inoperable cancer. In addition, the *BRCA1/2* genetic test to determine drug coverage was also covered by insurance. At the time of 2018, the *BRCA1/2* genetic test for companion diagnostic purposes was covered, but the same test for HBOC diagnostic purposes was not, an unnatural situation that has continued until 2020.

Patients suspected of having HBOC will be offered pre-diagnosis genetic counseling, and once the diagnosis is confirmed by testing, standard HBOC care will be provided. In addition, genetic counseling and presymptomatic test will be provided to at-risk relatives, and if they are carriers of the mutation, surveillance and risk-reducing treatment will be provided as in the case of the originator (at present, such treatment for carriers of the mutation who have not yet developed the disease is not covered by public medical insurance). On the other hand, if a person is found not to carry a mutation, her risk can be considered to be the same as that of the general population, even if there is a strong family history, and unnecessary surveillance can be avoided.

In contrast, in companion diagnostics, the attending physician is basically in charge of explaining the test and obtaining consent. Issues include how to provide advance information to patients for whom treatment is the top priority, the timing of genetic counseling for positive patients, and how to approach blood relatives in the absence of sufficient advance information.

15.7 Cancer Genome Profiling

Several types of cancer genome profiling (cancer gene panel tests) had already been introduced in Japan, and in 2019, two of these tests, OncoGuide™ NCC Oncopanel (NCC-OP) and FoundationOne® CDx (F-One), became covered by insurance. The purpose of these tests is to characterize the molecular genetics of cancer and select drugs based on these characteristics, but they may secondarily lead to the diagnosis of hereditary tumors. In particular, NCC-OP also analyzes blood samples as paired specimens, so if pathogenic variants are detected in the genes that cause hereditary tumors in blood, the patient will be confirmed as a carrier of hereditary tumor variants at that point. On the other hand, as F-One analyzes only tumor tissue, detected pathogenic variants can be either germline or somatic. It is not possible to determine unless variants are reexamined by blood sample.

The probability of having a pathogenic variant in *BRCA1/2* is about 0.2% in the general population in Japan, and the probability is higher if it is limited to cancer patients. In addition, if a pathogenic variant in *BRCA1/2* is identified by profiling using only tumor tissue, it is likely to be of germline origin, regardless of whether it

is a syndrome-related tumor (i.e., breast, ovarian, prostate, and pancreas cancer) or not (such as lung cancer) [31]. Therefore, regardless of allele frequency, it is recommended that the results be disclosed and confirmatory tests using blood be performed when a pathogenic variant in *BRCA1/2* is identified.

With regard to these issues as well, we can raise the following questions: to what extent should the possibility of a diagnosis of hereditary tumor be communicated in the pretest information, how should the information related to the diagnosis of hereditary tumor detected be interpreted and handled, and what should the genetic counseling be in the overall cancer genome profiling?

15.8 Problems in Japan's Genetic Medicine System

In the 2020 revision of Japan's public health insurance system, some of HBOC-related testing, surveillance, and risk-reducing procedures have become covered by the insurance for the first time. However, at present, there are still many inconsistencies and extra burdens to be placed on the patients due to institutional restrictions. Among these, the following three points are discussed.

15.8.1 Repeat Genetic Testing

It has already been mentioned that the *BRCA1/2* genetic test is now being used as a companion diagnostic test for the PARP inhibitor olaparib, but only the *BRCA1/2* genetic test provided by SRL, Inc. (Tokyo, Japan). This is the "BRACAnalysis Diagnostic System" from Myriad, Inc., of the United States, which has been approved for use in Japan. The sample is sent to the United States for analysis.

By the way, even before Myriad's "BRACAnalysis Diagnostic System" was approved in Japan, many Japanese patients have already been diagnosed with HBOC after undergoing *BRCA1/2* genetic testing at their own expense. The majority of these patients have been tested using the contract testing service provided by FALCO Holdings, Co., Ltd. Their test was originally transferred from Myriad's analysis system, and its performance is naturally considered to be equivalent to that of Myriad.

For this reason, the three genetics-related societies (the Japanese Society of Human Genetics, the Japanese Society for Genetic Counseling, and the Japanese Society for Gene Diagnosis and Therapy), the National Liaison Conference for Genetic Medicine, the Japanese Society of Gynecologic Oncology, and the Japanese Society of Hereditary Tumors jointly submitted a letter to the Minister of Health, Labor and Welfare, requesting to avoid unnecessary duplication of genetic testing. However, this issue has not yet been resolved.

15.8.2 Additional Charge for Genetic Counseling

The implementation of *BRCA1/2* genetic testing, which was insured as a companion diagnosis in 2018, is required to be performed at a facility that has submitted a

notification pertaining to the facility criteria for additional genetic counseling charge (notified facility) or has a system of collaboration with a notified facility. If the test is performed at the latter (collaborating facility) and genetic counseling is then requested at the notifying facility, the cost of genetic counseling could not be charged at either facility.

In the 2020 revision of Japan's public health insurance system, some point has changed. If the test is performed at a collaborating facility and patient is referred to notified facility for genetic counseling, the collaborating facility that performed the genetic test can charge an additional fee for genetic counseling. In addition, it was left to a consensus among the institutions to decide how to allocate the reimbursement between the collaborating institution and the notifying institution that actually performed the genetic counseling.

This is an inconvenient, unnatural, and unreasonable rule, but it is because that the fee for genetic counseling is considered as a judgment fee for genetic testing, not as a technical charge for genetic counseling itself. Genetic counseling is not necessarily performed only in cases involving genetic testing, and we have been lobbying for genetic counseling fees to be recognized as a technical fee, mainly through related academic societies, but this has not yet been achieved.

15.8.3 Providing Medical Care to Presymptomatic Mutation Carriers

In the case of Mendelian genetic diseases, such as hereditary tumors, which mostly develop after adulthood and for which effective interventions are available, medical care for presymptomatic mutation carriers can be expected to be the most cost-effective as a preemptive intervention. On the other hand, there is an institutional constraint that those who have not yet developed the disease are not likely to be covered by public health insurance.

For example, let us assume a family as shown in Fig. 15.2, where the medical history of I-2 and I-3 raises the suspicion that HBOC is present in the background of the family. If prostate cancer is found in II-1, the doctor will assume that this could be caused by HBOC, but *BRCA1/2* genetic test for patients with prostate cancer is not covered (male breast cancer is covered because of higher positive predictive value, although the absolute risk of developing breast cancer in male carrier of *BRCA1/2* pathogenic variant is lower than that of prostate cancer). And even if II-1 is mutation-positive, he will still have to pay out-of-pocket for breast cancer surveillance as recommended by the NCCN (National Comprehensive Cancer Network) guidelines.

When II-2 is found to have breast cancer, public health insurance system will cover genetic testing, surveillance of the contralateral breast and ovary when result of genetic testing is positive, as well as risk-reduced contralateral mastectomy and risk-reduced oophorectomy. If breast cancer is found in III-1, a daughter of II-2, her genetic testing, surveillance, and risk-reducing treatment would all be covered if genetic test result is positive, as would II-2. However, she would not have to have full *BRCA1/2* sequencing; rather a less expensive and domestically available

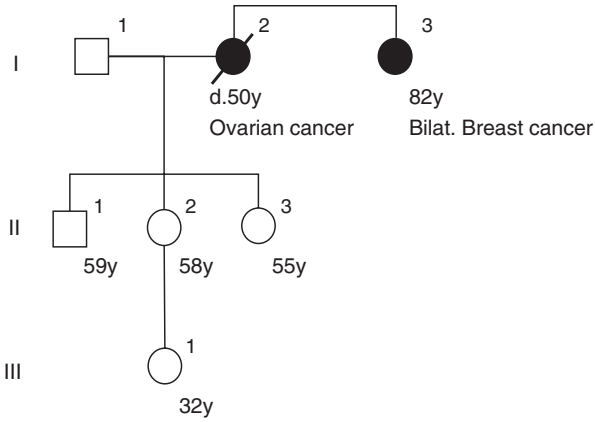


Fig. 15.2 Pedigree of a virtual family with the history of breast and ovarian cancer

single-site test based on her mother's test results should be sufficient, which is not covered by public health insurance.

Furthermore, if II-3 wants to know her own genetic status due to a strong family history, she would have to pay for genetic testing (single-site testing is enough). And if she also turns out to be mutation-positive, surveillance and risk-reducing surgery, if desired, will all be at her own expense since she has not yet developed breast or ovarian cancer. It is not unreasonable for her to think, "If this is the case, why can't they just find me with early breast cancer?" A system that makes healthy citizens expect to be diagnosed with cancer must be corrected immediately.

The reason why II-3 is not covered by insurance is because Article 63 of the Health Insurance Law stipulates that the object of medical treatment benefits is "illness or injury." Of course, carrying the mutation is not a disease, and II-3 is a healthy population, but on the other hand, presymptomatic mutation carriers are also a population that can be expected to benefit from early intervention.

15.9 The Future of Hereditary Tumor Treatment Under Japan's System

Although it is still not enough, partial inclusion of HBOC in the public health insurance system is a major development in Japan, but it has also revealed a number of issues as can be seen in this case study. These facts suggest that this revision will be a turning point not only for management of HBOC but also for the future development of genetic medicine in Japan.

Until now, genetic medicine has focused on chromosomal abnormalities and Mendelian inherited diseases, but multifactorial diseases will account for a larger proportion of genetic medicine in the future. In the field of cancer, the scope of genetic medicine will expand from germline-only information to somatic information and also information of expression/epigenetics. In the future, comprehensive genome analysis will be commonly introduced to general practice in different fields such as cancer, intractable diseases, and multifactorial diseases.

When we envision such a future, it is doubtful that it makes sense to separate those who have developed the disease from those who have not, at least from the perspective of genetic medicine, in a field with high actionability such as hereditary tumors. Of course, the purpose of the Health Insurance Law is valuable and needs to be respected.

For example, a person with high blood pressure or high LDL cholesterol level does not have any complaint of its own (except for severe hypertension). The purpose to control blood pressure and blood LDL cholesterol levels is the "prevention" of cardiovascular disease. Primary prevention is not originally covered by Japan's public health insurance, but by naming the disease "hypertension" or "dyslipidemia," insurance covers the cost for preventive intervention of more serious diseases in the future.

One more thing to consider is infertility. Infertility is a situation in which a healthy man or woman who wants to conceive does not conceive for a certain period of time (usually 1 year), despite the fact that he or she is not using contraception. Infertility in itself is not a disease, but when a couple seeks medical attention for it, the infertility becomes the disease name. Although infertility treatment is not covered by public health insurance (a study has recently been started for future insurance coverage), the cost is currently covered by the public through a system called the Specific Treatment Support Project. This system could be one of answers to provide public support for presymptomatic mutation carriers to receive personalized preemptive medical care. In any case, it is expected that the genetic predisposition of individuals will be clarified through various opportunities (genetic testing of hereditary tumors, MGT, companion diagnoses, and cancer genome profiling), and as a result, there will be more opportunities to identify presymptomatic mutation carriers in blood relatives. It is urgently necessary to establish a system in which those who have not yet developed the disease can receive appropriate health care at the same expense burden as those who have already developed the disease. The partial coverage of HBOC treatment will provide an opportunity to think about the significance of Japan's public health insurance and the public burden of medical costs in the coming era of genomic medicine, and we need to consider the future medical system from such a perspective.

References

1. Katanoda K, Sobue T, Tanaka H, Miyashiro I, editors. JACR monograph supplement no. 2. Tokyo: Japanese Association of Cancer Registries; 2016.
2. Malone KE, Daling JR, Doody DR, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. Cancer Res. 2006;66:8297–308.
3. Momozawa Y, Iwasaki Y, Parsons MT, et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun. 2018;9:4083.
4. NCCN Guidelines: genetic/familial high-risk assessment: breast, ovarian, and pancreatic https://www.nccn.org/professionals/physician_gls/default.aspx#genetics_screening
5. NICE Guidance: familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. https://www.nice.org.uk/guidance/cg164

6. U.S. Preventive Services Task Force: BRCA-Related Cancer: risk assessment, genetic counseling, and genetic testing. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing
7. Guidebook for diagnosis and treatment of hereditary breast and ovarian cancer syndrome 2017. Kanehara Publishing, Tokyo, 2017.
8. Terry MB, Liao Y, Whittemore AS, et al. 10-year performance of four models of breast cancer risk: a validation study. Lancet Oncol. 2019;20:504–17.
9. McInnis RJ, Knight JA, Chung WK, et al. Comparing five-year and lifetime risks of breast cancer in the prospective family study cohort. J Natl Cancer Inst. 2020 Dec 10:djaa178. https://doi.org/10.1093/jnci/djaa178.
10. Lakeman IMM, Rodríguez-Girondo M, Lee A, et al. Validation of the BOADICEA model and a 313-variant polygenic risk score for breast cancer risk prediction in a Dutch prospective cohort. Genet Med. 2020;22:1803–11.
11. George A. UK BRCA mutation testing in patients with ovarian cancer. Br J Cancer. 2015;113:S17–21.
12. The American Society of Breast Surgeons. Consensus guideline on genetic testing for hereditary breast cancer. https://www.breastsurgeons.org/docs/statements/Consensus-Guideline-on-Genetic-Testing-for-Hereditary-Breast-Cancer.pdf
13. Fukushima Y. Genetic counseling of HBOC and Japanese organization of HBOC (in Japanese). Gan To Kagaku Ryoho. 2017;44:107–10.
14. Arai M, Yokoyama S, Watanabe C, et al. Genetic and clinical characteristics in Japanese hereditary breast and ovarian cancer: first report after establishment of HBOC registration system in Japan. J Hum Genet. 2018;63:447–57.
15. Buys SS, Sandbach JF, Gammon A, et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. Cancer. 2017;123:1721–30.
16. Sun J, Meng H, Yao L, et al. Germline mutations in cancer susceptibility genes in a large series of unselected breast cancer patients. Clin Cancer Res. 2017;23:6113–9.
17. 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. Gynecol Oncol. 2019;153:452–62.
18. Beitsch PD, Whitworth PW, Hughes K, et al. Underdiagnosis of hereditary breast cancer: are genetic testing guidelines a tool or an obstacle? J Clin Oncol. 2019;37:453–60.
19. Yoshida R. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer. https://doi.org/10.1007/s12282-020-01148-2.
20. Catana A, Apostu AP, Antemie R-G. Multi gene panel testing for hereditary breast cancer−is it ready to be used? Med Pharm Rep. 2019;92:220–5.
21. van Marcke C, Collard A, Vikkula M, Duhoux FP. Prevalence of pathogenic variants and variants of unknown significance in patients at high risk of breast cancer: a systematic review and meta-analysis of gene-panel data. Crit Rev Oncol Hematol. 2018 Dec;132:138–44. https://doi.org/10.1016/j.critrevonc.2018.09.009.
22. Kurian AW, Hare EE, Mills MA, et al. Clinical evaluation of a multiple–gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol. 2014;32:2001–9.
23. Desmond A, Kurian AW, Gabree M, et al. Clinical Actionability of multigene panel testing for hereditary breast and ovarian cancer risk assessment. JAMA Oncol. 2015;1:943–51.
24. Thompson ER, Rowley SM, Li N, et al. Panel testing for familial breast cancer: calibrating the tension between research and clinical care. J Clin Oncol. 2016;43:1455–9.
25. Lincoln SE, Kobayashi Y, Anderson MJ, et al. A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients. J Mol Diagn. 2015;17:533–44.
26. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015;121:25–33.

27. Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med. 2017;377:523–44.
28. Robson ME, Tung N, Conte P, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol. 2019;30:558–66.
29. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. N Engl J Med. 2018;379:753–63.
30. Dieras V, Han HS, Kaufman B, et al. Veliparib with carboplatin and paclitaxel in BRCA-mutated advanced breast cancer (BROCADE3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2020;21:1269–82.
31. Mandelker D, Donoghue M, Talukdar S, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO precision medicine working group. Ann Oncol. 2019;30:1221–31.

Hereditary Breast and Ovarian Cancer (HBOC) Database in Japan

16

Masami Arai and The Registration Committee of the Japanese Organization of Hereditary Breast and Ovarian Cancer (JOHBOC)

Abstract

In Japan, individuals who underwent *BRCA* genetic testing were first registered in 2015 under a registry project by the Japanese Hereditary Breast and Ovarian Cancer Consortium. In 2019, the Japanese Organization of Hereditary Breast and Ovarian Cancer (JOHBOC) became the parent organization of this nationwide effort, and the data center was transferred to the National Clinical Database. *BRCA*-related data, namely, cancer family history including second-degree relatives; clinicopathological characteristics of breast, ovarian, and other cancers; *BRCA* sequencing results; and risk-reducing surgeries, are registered and are summarized and released annually in August. Data collected from cancer screenings performed in non-cancer diagnosed *BRCA* mutation carriers are also included in the database. By August 2020, 7780 registered individuals underwent the *BRCA* test across 93 medical institutions nationwide (index cases), among whom 726, 645, and 7 were identified as *BRCA1*, *BRCA2*, and *BRCA1* + *BRCA2* mutation-

Registration Committee of the JOHBOC
[Committee (other members)]: Seigo Nakamura (Showa University), Takayuki Enomoto (Niigata University Graduate School of Medical and Dental Sciences), Tadashi Nomizu (Hoshi General Hospital), Akihiro Sakurai (Sapporo Medical University), Masayuki Sekine (Niigata University Graduate School of Medical and Dental Sciences), Hiroyuki Nomura (Fujita Health University), Megumi Okawa (St. Luke's International Hospital), Junko Yotsumoto (Showa University)
[Data center] Chie Watanabe (Showa University)
[NCD] Hiraku Kumamaru (University of Tokyo), Miyata Hiroaki (University of Tokyo)
[Office] Miyuki Shimoda (Showa University), Shiro Yokoyama (Showa University)

M. Arai (✉)
Department of Clinical Genetics, Juntendo University, Graduate School of Medicine, Tokyo, Japan

Registration Committee of the Japanese Organization of Hereditary Breast and Ovarian Cancer (JOHBOC), Tokyo, Japan
e-mail: ms-arai@juntendo.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_16

positive, respectively, which represented a twofold higher registered *BRCA* mutation carriers since 2019. In addition to companion diagnostics and confirmatory secondary cancer assessment, *BRCA* testing is covered under the national health insurance since 2020, which has contributed for the recent marked increase in *BRCA* testing frequency with diverse purposes. In the future, cooperation between the JOHBOC registry and foreign international databases will be explored.

Keywords

JOHBOC registry · *BRCA1* · *BRCA2* · Clinical database · Nationwide database

16.1 Introduction: Importance of a Registry in Hereditary Tumors

The prevalence of hereditary breast and ovarian cancer (HBOC) in the general population is low, for which a definitive diagnosis can be made by *BRCA* genetic testing. Thus, the cooperation of medical institutions nationwide is necessary to study HBOC prevalence and characteristics through their shared clinical data. Moreover, new specific founder mutations and characteristic findings that are different from those in Western countries may be possible in isolated countries such as Japan. Therefore, it is clinically essential to build a nationwide registry system to elucidate the clinical and genetic characteristics of Japanese *BRCA* mutation carriers.

The first effort to register hereditary tumors in Japan dates back to 1975, when the Polyposis Center for the registry of familial adenomatous polyposis (FAP) was established in the Tokyo Medical and Dental University. In 1993, 1104 FAP and 183 Peutz-Jeghers syndrome patients from 722 and 173 families, respectively, were registered [1]. The nationwide registry system revealed new findings on the natural course of FAP, such as low mortality, the possibility of thyroid cancer spontaneous regression as a FAP complication (only one recorded death), and the high recurrence rate of surgically removed desmoids, which have contributed to improving the vital prognosis of this disease.

HBOC is one of the most common hereditary tumors, and HBOC syndrome is defined as individuals who are predisposed to breast and ovarian cancers due to germline mutations in *BRCA1* or *BRCA2* [2]. Hence, the Japanese HBOC database was designed with the aim of elucidating the clinical and genetic characteristics of HBOC in the Japanese population, as well as to improve the health management of *BRCA* mutation carriers [3].

16.2 History of the HBOC Registry in Japan

For 2 years, starting in July 2010, Nakamura and collaborators conducted the project "Management for patients with HBOC and unaffected BRCA mutation carriers in our country" as a study group of the Japanese Breast Cancer Society with the aim

of investigating the details of HBOC in Japan. They found that among breast cancer patients with family history of breast or ovarian cancer who underwent *BRCA* testing in eight Japanese facilities, 30.7% had a *BRCA1* or *BRCA2* mutation. Moreover, of the breast cancers that developed in *BRCA1* mutation carriers, up to 62.2% were triple-negative breast cancers (TNBCs), which was consistent with foreign reports [4].

The Japanese HBOC Consortium (JHC) was established in October 2012 as a co-project of breast oncologists, gynecologists, and clinical geneticists for the improvement of HBOC clinical practice. The main activities of the JHC are (1) building a Japanese HBOC database, (2) research to support and define HBOC management and treatment guidelines, and (3) increasing awareness of HBOC [5]. Initiatives to draft the protocol for the nationwide HBOC registry project started in the registry committee meeting in October 2013, which was finalized and approved by the ethics committee of the JHC in December 2014. Afterward, before conducting an official nationwide registry, a trial registration was conducted in four medical institutes to which the registry committee members were affiliated to test the online entry system and procedures related to the collection of data [3]. The data center was established in the Division of Breast Surgical Oncology of the Showa University Hospital. Minor modifications have been made to the system since then, and data have been compiled once a year since 2016.

Parallel to the activities of the JHC, the Japanese Organization of Hereditary Breast and Ovarian Cancer (JOHBOC) was established in August 2016 [6], and the *BRCA* registry was taken over as a project of the JOHBOC Registration Committee in 2019. The JOHBOC data center was moved to the National Clinical Database (NCD), with cases registered by the JHC that provided consent for registration in future registries also being transferred to the JOHBOC registry. Since 2015, registered cases were increased, but a temporary decrease was observed in 2019 due to a delay in the ethical review procedures regarding the approval of moving registered data in some institutes (Table 16.1).

Nationwide data were compiled for the fifth time in August 2020. The number of *BRCA* tests conducted is increasing dramatically as the HBOC gene testing is covered by the national health insurance since April 2020 and because many are taking it to use as companion diagnostics for PARP inhibitors and germline mutations of *BRCA* are incidentally found in cancer panel testing (Table 16.1).

16.3 Outline of the Current Japanese Registry System

The targets of this registry are individuals who underwent *BRCA* genetic testing [3], who are defined as "subjects." The registry does not ask subjects to specify whether the testing was performed by Sanger sequencing or panel, out-of-pocket or insurance-covered costs, the company that performed the test, or whether it was performed for companion diagnostics or cancer panel testing. However, when a *BRCA* mutation was suspected in a cancer panel testing, it had to be confirmed by *BRCA* genetic testing as a germline mutation in the registry. Data on the

Table 16.1 Transition of registered cases from 2015 to 2020

	Trial registration	Nationwide registration				
	Feb 2015	Aug 2016(first)	Aug 2017(second)	Aug 2018(third)	Aug 2019(fourth)	Aug 2020(fifth)
1 Participating medical institutes	4	7	35	69	62	93
2 Registrees	3935	7118	11,711	16,530(+4819)	15,612(−918)	28,846(+13,234)
3 Subjects	965	1718	2747	3994(+1247)	3929(−65)	7780(+3851)
4 Pedigree	846	1557	2433	3586(+1153)	3629(+43)	7225(+3596)
5 *BRCA1*	135	218	265	429(+164)	398(−31)	726(+328)
6 *BRCA2*	119	197	214	319 (+105)	299 (−20)	645 (+346)
7 *BRCA1* + *BRCA2*	1	1	3	6	6	7

Note: Values within parenthesis represent the difference from the previous year

clinicopathological findings related to breast or ovarian cancer and results of genetic testing were stored (Fig. 16.1a). The entry items are shown in Table 16.2.

Clinical family history, including information within the second-degree relatives and cousins, who had cancer, was also entered. Most of the data about cancer in relatives are based on information reported by the subjects. For *BRCA1/2* mutation carriers without cancer, results of cancer screening tests such as magnetic resonance imaging or mammography are also entered. Therefore, data on individuals who underwent *BRCA* testing (subjects) and their family members were entered into the unit of families (Fig. 16.1b).

Deadline for annual registry is the last day of August. From September, the JOHBOC Registration Committee Office contacts medical institutions on entered data items that are suspected to be erroneous or incomplete to ensure data accuracy. Subsequently, the basic data are analyzed and presented at the JHC Conference held in January the following year. Starting in 2021, the basic data compiled in the previous year is scheduled to be presented at the JOHBOC Conference.

The basic data from the 2018 registry year were the following:

1. Overall, 3477 index cases (the first individuals who underwent *BRCA* testing in a family) were recorded, among whom 423, 312, and 6 individuals had mutations in *BRCA1*, *BRCA2*, and both genes, respectively, representing an overall *BRCA* mutation-positive rate of 21.3%. Variants of uncertain significance (VUS) were observed in *BRCA1*, *BRCA2*, and both genes in 94, 144, and 5 tests, respectively, indicating a 7.0% risk of detecting VUS in *BRCA* testing (Fig. 16.2). *BRCA* mutation-positive rate was summarized according to the format of Myriad Table (Table 16.3), which revealed that the *BRCA* mutation-positive rate was high for all cells compared to the US Myriad Table [7].
2. A total of 164 pathogenic or likely pathogenic *BRCA1* mutations were registered, with c.188T > A (p.L63X) being the most common variant (109 cases). This variant has already been confirmed to be a founder mutation in haplotype analyses [8]. The second most frequent variant was c.2800C > T (p.Q934X), which accounted for 50 cases, while 106 variants were reported only once. A total of 129 pathogenic or likely pathogenic variants of *BRCA2* were registered. The most frequent variant was c.6952C > T (p.R2318*) in 32 cases, followed by c.5576_5579del (p.I1859Kfs*3) in 30 cases. Seven *BRCA1* rearrangements and two *BRCA2* rearrangements could only be diagnosed by multiplex ligation-dependent probe amplification, both of which were exon deletions.
3. Overall, 71 VUS in *BRCA1* were registered, including c.152C > T (p.L52F) in 14 cases and c.5558A > G (p.Y1853C) in 13 cases. Currently, the former is classified as likely benign and the latter as likely pathogenic [9]. For *BRCA2*, 102 VUS were recorded, including c.53G > A (p.R18H) in 19 cases and c.4854T > A (p.D1618E) in 8 cases. Of these, the former is classified as benign. Among the VUS, three *BRCA1* and one *BRCA2* VUS were determined to be inconclusive. These variants cannot be diagnosed by the standard method of analysis by Myriad. The frequency of VUS in *BRCA* testing has not decreased since 2010 and is fairly stable at 5–8%.

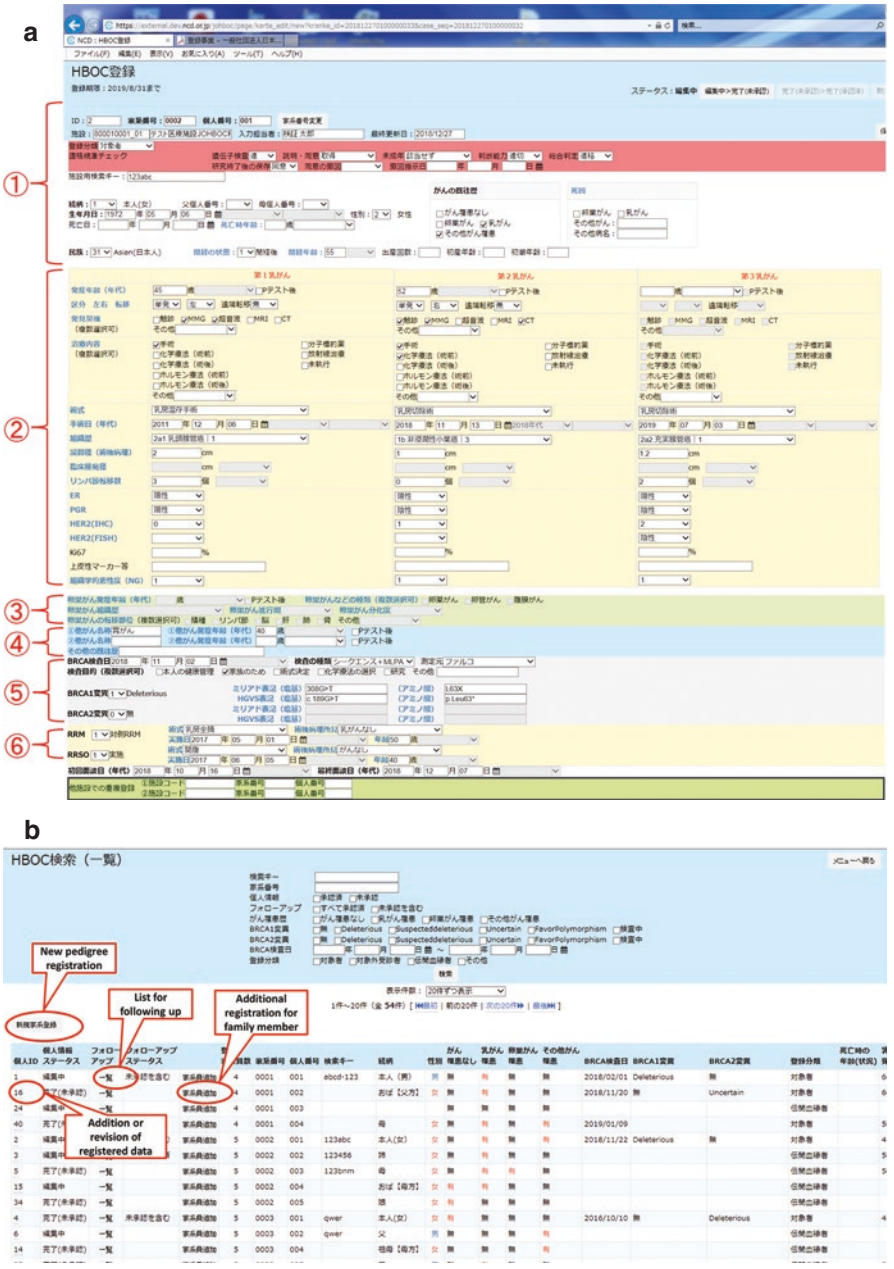


Fig. 16.1 Data registration input screen (**a**) for individuals and (**b**) of the front cover. (**a**) 1, Basic information; 2, Information on breast cancer; 3, Information on ovarian cancer; 4, Other cancers; 5, Information on genetic test; 6, Risk-reducing surgery. (**b**) Registered data group based on the pedigree in each medical institute

Table 16.2 Registration items in the hereditary breast and ovarian cancer (HBOC) registration system

Basic information for the subject or other family members **1. Family relationship, 2. Gender, 3. Race** 4. Father's individual number, 5. Mother's individual number (number issued by this system) **6. Date of birth**[a] 7. Date of death, **8. Age at death**, **9. Cause of death**, **10. History of cancers** **11. Menopausal state**, **12. Menopausal age**, 13. Age at menarche, 14. Times of childbirth, 15. Age at first childbirth
Information on breast cancer[b] **16. Onset age of breast cancer** **17. Single or multiple, unilateral or bilateral, 18. Site (right or left), distant metastasis** **19. Modality of diagnosis (trigger of finding a lesion)** **20. Medical treatment (surgery, preoperative chemotherapy, postoperative chemotherapy, preoperative hormone therapy, postoperative hormone therapy, molecular targeted drug, radiotherapy, no treatment)** **21. Operation procedure, 22. Operation date** **23. Histology, 24. Pathological tumor size (clinical tumor size), 25. Number of lymph nodes positive for cancer, 26. ER, 27. PGR, 28. HER2 (IHC), 29. HER2 (FISH)** 30. Ki67 score, 31. Epithelial marker, **32. Nuclear grade (NG)**
Information on ovarian cancer **33. Onset age of ovarian cancer** **34. Ovarian cancer, tubal cancer, peritoneal cancer** **35. Histology, 36. Stage, 37. Degree of differentiation for cancer, 38. Metastatic sites**
Other cancers than breast and ovary (all cancers should be described if patients have a history of multiple cancers) **39. Type of other cancers, 40. Onset age** **41. Other history associated with HBOC**
Information on genetic tests **42. Date of genetic test for BRCA, 43. Type of examination, source of genetic test (company, medical institute, etc.), 44. Purpose of genetic test** **45. Mutation of *BRCA1*, mutation site**[d] **(DNA and protein), 46. Mutation of *BRCA2*, mutation site**[d] **(DNA and protein)**
Information on prophylactic surgery of the breast and the ovary **47. Risk-reducing mastectomy (RRM), 48. Operation procedure for RRM, 49. Pathological findings of the resected specimen, 50. Date of RRM, 51. Execution age of RRM** **52. Risk-reducing salpingo-oophorectomy (RRSO), 53. Operation procedure for RRSO, 54. Pathological findings of the resected specimen, 55. Date of RRSO, 56. Execution age of RRSO**
Information on visiting a hospital **57. First consultation at the hospital, 58. Final consultation at the hospital**
Information on surveillance examination **59. Examination for breast cancer (date of examination, inspection and palpation, MRI, US, MMG, cytology, biopsy)** **60. Examination for ovarian cancer (date of examination, TVUS, CA-125, MRI, use of oral contraceptives)**

Note 1: Characteristics in bold represent essential registration items for all subjects
Note 2: Items no. 1, 16, 33, 39, and 40 are essential registration items for registrees within the second relatives of the subject or uncles, who suffered from any cancers and registered by interview-based information

Table 16.2 (continued)

Note 3: Items no. 59 and 60 are essential registration items for unaffected BRCA mutation carriers

[a]The date is deleted at submission (only month and year are registered) for protection of individual information

[b]We can register up to the third breast cancer, if patients had suffered from it

[c]We can register up to the third regimen, if patients had received one

[d]Based on the Myriad notation or HGVS notation

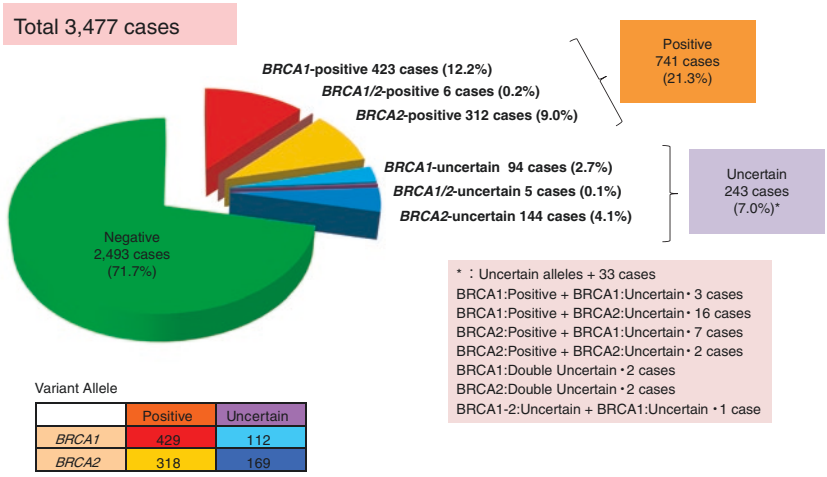


Fig. 16.2 Data Collection Study in Japan (2018). Adapted from [3, 4]

4. About 76.8% of breast cancers in *BRCA1* mutation-positive individuals were triple-negative. The *BRCA1* mutation-positive rate was 26.8% in TNBCs, but the pathogenic mutation rate increased dramatically to 39.8%, 38.1%, and 65.5% in people with additional conditions such as breast cancer onset at <40 years, one or more other family members with breast cancer, and family history of ovarian cancer, respectively. In turn, TNBC accounted for 21.2% of breast cancers in *BRCA2* mutation-positive individuals. The *BRCA2* mutation-positive rate was only 5.5% in TNBC, and the mutation-positive rate remained under 10% even in patients with additive factors such as onset at a young age and family history of breast or ovarian cancer.
5. Additionally, 12.9% of breast cancers in *BRCA* mutation-positive individuals were ductal carcinoma in situ. However, stages III and IV accounted for 80.4% (160 cases) of the total 199 cases with registered disease stage of ovarian cancer in *BRCA* mutation carriers, whereas the percentage was 65.0% in *BRCA* mutation-negative individuals. Regarding histological subtypes of ovarian cancer, serous carcinoma was observed in 137 of 164 (83.5%), 30 of 34 (88.2%), and 144 of 252 (57%) of *BRCA1* mutation-positive, *BRCA2* mutation-positive,

Table 16.3 Prevalence of deleterious mutations in *BRCA1* or *BRCA2* according to 2018 records

Family history	Among 1st- or 2nd-degree relatives				No family history of breast or ovarian cancer	Family history in only 3rd-degree relatives
Breast cancer < 50 years	–	+	–	+		
Ovarian cancer (at any age)	–	–	+	+		
Proband history Breast cancer ≥ 50 years	16/243 6.6%	35/220 15.9%	18/87 20.7%	5/20 25.0%	10/212 4.7%	3/37 8.1%
Breast cancer < 50 years	95/511 18.6%	175/527 33.2%	79/193 40.9%	46/84 54.8%	71/636 11.2%	6/75 8.0%
Ovarian cancer at any age, no breast cancer	8/26 30.8%	9/21 42.9%	58/78 74.4%	6/8 75.0%	21/165 12.7%	2/11 18.2%
Breast cancer and ovarian cancer at any age	13/28 46.4%	6/12 50.0%	15/16 93.8%	6/6 100%	10/46 21.7%	1/8 12.5%
Male breast cancer at any age	1/3 33.3%	2/5 40.0%	0/2 0.0%		1/10 10.0%	0/2 0.0%
No breast cancer or ovarian cancer at any age	2/39 5.1%	10/67 14.9%	5/41 12.2%	3/19 15.8%	2/19 10.5%	0/1 0.0%

and *BRCA* mutation-negative individuals, respectively, among those with distinct histologic types.

6. A total of 113 risk-reducing mastectomies (RRMs) were registered: 103 cases consisted of contralateral and 10 of bilateral RRMs (including 2 cases after partial mastectomy). The mean age at RRM was 42.6 years (26–63 years), and the mean time from the surgical treatment of the first breast cancer to RRMs was 3.5 years (0–23 years). The mean follow-up period after RRM was 23.7 months, and post-RRM onset of breast cancer was observed in two cases. The risk of post-RRM breast cancer onset was 0.9% per year. A total of 216 risk-reducing salpingo-oophorectomies (RRSOs) were registered. The mean age at RRSO was 49.7 years (34–78 years), and the mean post-RRSO follow-up period was 28 months, during which onset of peritoneal cancer was observed in one case (0.3% per year). Occult cancer was detected in the resected specimens of risk-reducing surgery in six cases of RRM (5.3%) and nine cases of RRSO (4.2%).
7. A total of 111 cases of unaffected *BRCA* mutation carriers were registered. The mean follow-up period after *BRCA* testing was 2.7 years (0–16.4 years), and the mean age at genetic testing was 38.8 years (20–67 years). Of these, breast cancer, ovarian cancer, and other cancer onset were observed in nine cases (3.0% per year), one case (0.3% per year), and three cases (1.0% per year), respectively.

16.4 Research Using Registry Data

The JOHBOC Registration Committee uses registered data from each year to select a medical institution that contributed to the registry and requested it to summarize and present the data in an article along with a contemporary topic. Some published manuscripts are introduced below.

Yoshimura et al. investigated the incidence of contralateral breast cancer following initial breast cancer after mastectomy and ipsilateral breast cancer (including primary cancer and recurrence) following breast-conserving surgery in *BRCA* mutation carriers [10]. They found that, over a mean follow-up period of 3 years, the incidence of contralateral breast cancer was 4.0%, 2.9%, and 1.9% per year in *BRCA1* mutation carriers, *BRCA2* mutation carriers, and *BRCA* mutation-negative individuals, respectively, while the risk of ipsilateral recurrence after conservative surgery was 2.7%, 1.4%, and 1.1% per year, respectively. This investigation showed that the risk of contralateral breast cancer or ipsilateral recurrence in *BRCA1* mutation carriers was significantly higher than in *BRCA* mutation-negative individuals; however, no significant difference was observed compared with *BRCA2* mutation carriers. Since the follow-up period was short, a longer-term observation is still required.

Okano et al. focused on the age of onset of breast cancer with *BRCA1/2* mutations to investigate the positive rate of *BRCA* testing [11] and found that the mean age at breast cancer onset in *BRCA1* mutation-positive, *BRCA2* mutation-positive, and *BRCA* mutation-negative individuals were 43.6, 45.2, and 48.8 years, respectively. Although no significant difference between *BRCA1*- and *BRCA2*-positive individuals was observed, they were significantly younger than breast cancer patients without BRCA mutations. Furthermore, they evaluated the *BRCA* mutation-positive rate in cancer patients without a family history of breast or ovarian cancer and found higher *BRCA1* mutation-positive rates in TNBC. In particular, the *BRCA1* mutation-positive rate was 21.1% in patients in their 30s and 12.5% in *BRCA2* mutation carriers younger than 30 years, suggesting the medical significance of *BRCA* testing in TNBC with early onset under 40 years, even without a family history of HBOC-associated cancers.

Inuzuka et al. investigated how the results of preoperative *BRCA* testing conditioned the surgical approach [12]. Among 318 candidates for breast-conserving surgery who underwent *BRCA* testing preoperatively, 45 of 59 *BRCA* mutation-positive patients (76.3%) elected total mastectomy. In turn, only 99 of 250 patients without *BRCA* mutations choose total mastectomy (38.2%). No patients aged 50 years or older at the time of breast cancer onset elected contralateral RRM. According to the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, *BRCA* pathogenic mutations are considered relative contraindications for conservative therapy with radiotherapy. However, the recent American Society of Clinical Oncology guidelines state that *BRCA* germline mutations are not necessarily contraindications for breast-conserving therapy [13]. The availability of data on the long-term prognosis of conservative therapy in Japan should also allow patients to make decisions based on more accurate information.

Yamauchi et al. reported a high rate of occult cancer in resected specimens in 6 of 53 patients who underwent RRM (11.3%). Pathological cutting methods for resected specimens and standardization of preoperative imaging tests are needed [14].

Yoshida et al. conducted a clinicopathological investigation of breast cancer in individuals with L63X, which is a founder mutation of *BRCA1* in Japanese [15].

Mitamura et al. investigated the characteristics of lymph node metastasis of ovarian cancer in *BRCA* mutation-positive individuals. Although the metastasis-positive rate was 20–26% in *BRCA1* mutation-positive and *BRCA1* mutation-negative individuals, ovarian cancer in *BRCA1* mutation-positive individuals without a family history of cancer had a significantly higher incidence of lymph node metastasis [16].

Nomura et al. analyzed the current status of RRSO in Japan. Of 488 individuals diagnosed with HBOC through *BRCA* testing that resulted in the identification of a pathogenic mutation, 153 (31.4%) underwent RRSO. The mean age of patients who underwent RRSO was 49.5 years, and the mean age of individuals who underwent genetic testing was 48.5 years. Significant factors for electing RRSO included birth history, history of breast cancer, and history of RRM. As RRSOs are covered under the national health insurance program since April 2020, the number of *BRCA* mutation carriers who elect RRSO is expected to grow further in the upcoming years [17].

Taken together, these studies demonstrate that the JOHBOC registry database may pave the way for additional findings related to HBOC original to Japan.

16.5 Future Perspectives

Since April 2020, *BRCA* genetic testing has been covered under the national medical insurance for patients with breast and ovarian cancers. The diagnostic criteria for testing indication covered by the medical insurance and the *BRCA* detection rates are presented in Table 16.4. For breast cancer patients who satisfy only one item (and do not meet any other criteria), the detection rate frequently does not reach 10%, but for breast cancer patients who meet either criterion, the detection rates all exceed 20%; thus, the criteria can be considered valid. However, the Myriad Table data were also higher in Japan compared to the United States, most likely due to negative cases that tend to be skipped at the time of registration. In other words, it may be possible that mutation-positive cases are being entered with priority. Henceforth, it is necessary to maintain compliance to continue registration so that the registry project accurately reflects the prevalence of *BRCA* mutations in Japan. In order to respond to the expected rapid increase in *BRCA* testing in the 2022 registry project, registrations are planned to be restricted to individuals with variants that have been classified as *BRCA* mutation-positive or VUS.

Aside from VUS, “inconclusive” is seen occasionally in Myriad reports of registered cases, even though there is no such result in the 2015 trial registry. Four kinds of inconclusive variants are registered, i.e., exon14–19, exon15–18, exon21–24 in *BRCA1*, and exon14–18 in *BRCA2* in 2018 registration. Myriad company explained that an atypical pattern, suggestive of a large

rearrangement, was observed, which could be due to genetic or technical reasons. As such, the interpretation of these regions remains limitations and challenges. There may be common mechanism under these findings and further analysis has to be required.

The data from the JOHBOC registry should also be registered in other registries such as the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) to contribute to international databases [18].

To date, Japanese criteria for national health insurance coverage and clinical management of HBOC have been based on foreign data. Gathering more data to the JOHBOC database is essential to guide the future of clinical management and

Table 16.4 Mutation frequency of each clinical condition in which *BRCA* genetic testing is recommended and public health insurance covers, based on 2019 JOHBOC Registration data and according to the "Guidebook for Diagnosis and Treatment of HBOC Syndrome 2017" [2]

Mutation frequency in subjects who satisfy at least one of the following items							
Clients who satisfy at least one of the following conditions		BRCA1/2+	BRCA1+	BRCA2+	BRCA1/2−	Total	Mutation rate (%)
Patients with breast cancer of the following conditions	Onset <45 years	5	214	169	1218	1606	24.2
	Onset of triple-negative breast cancer <60 years	1	188	38	531	758	29.9
	Double or more primary lesions	1	60	67	425	553	23.1
	Family history of breast or ovarian cancer within third-degree relatives	6	226	207	1429	1868	23.5
Patients with ovarian, tubal, or peritoneal carcinoma		0	128	30	231	389	40.6
Patients with male breast cancer		0	0	4	16	20	20.0
Mutation frequency in subjects who satisfy only one of the following conditions							
Clients who satisfy at least one of the following conditions		BRCA1/2+	BRCA1+	BRCA2+	BRCA1/2−	Total	Mutation rate (%)

Table 16.4 (continued)

Mutation frequency in subjects who satisfy at least one of the following items							
Patients with breast cancer of the following conditions	Onset <45 years	0	6	17	307	330	7.0
	Onset of triple-negative breast cancer <60 years	0	9	4	126	139	9.4
	Double or more primary lesions	0	2	2	73	77	5.2
	Family history of breast or ovarian cancer within third-degree relatives	1	21	47	550	619	11.1
Patients with ovarian, tubal, or peritoneal carcinoma		0	0	89	23	198	36.1
Patients with male breast cancer		0	0	0	0	10	0.0

policies in light of the clinicopathological characteristics of HBOC in the Japanese population.

Acknowledgments We express our sincerest appreciation to the dedicated staff within the 69 participating medical institutions for their cooperation in data entry to the nationwide registry project (2018):

Showa Medical University Hospital, St. Luke's International Hospital, Cancer Institute Hospital, Hoshi General Hospital, Shikoku Cancer Center, Hokkaido Cancer Center, Sapporo Medical Center, Niigata University Faculty of Medicine, Keio University, Kitano Hospital, Kochi University Faculty of Medicine, Tawara IVF Clinic, Kochi Medical Center, Nagoya University Hospital, St. Marianna University School of Medicine, Asahikawa Medical University, Aichi Cancer Center Hospital, Saku Medical Center, Tsukuba Medical University Hospital, Yokohama City University Hospital, Fujita Medical University Hospital, Ehime University Hospital, Gunma Prefectural Cancer Center, Nagasaki University Hospital, Shinshu University Hospital, Juntendo University Hospital, Kitasato University Hospital, Aichi Cancer Center Aichi Hospital, Okayama University Hospital, Saitama Medical University International Medical Center, Tokyo Medical Center, Tokyo Metropolitan Komagome Hospital, Japanese Red Cross Ishinomaki Hospital, Nagoya City University Hospital, Kawasaki Medical School Hospital, Yamanashi Prefectural Central Hospital, Nara Medical University Hospital, Kanagawa Cancer Center, Shiga Medical University, Shizuoka Cancer Center, Gifu University Hospital, Hiroshima Prefectural Hospital, Niigata Cancer Center Hospital, Japanese Red Cross Nagoya Daiichi Hospital, Tottori University Hospital, Kansai Rosai Hospital, Kokura Medical Center, Nippon Medical School Musashi Kosugi

Hospital, Kansai Medical University Hospital, Sagara Hospital Breast Center, Chiba University Hospital, Ibaraki Prefectural Hospital, Fukui University Hospital, Hokkaido University Hospital, Saitama Medical Center, Osaka University, Japanese Red Cross Yamaguchi Hospital, Kyoto University Hospital, Konan Kosei Hospital, Japanese Red Cross Medical Center, Shonan Memorial Hospital, Shizuoka General Hospital, Hiroshima University Hospital, National Hospital Organization Kyoto Medical Center, Shiroyama Hospital, Saitama Cancer Center, National Hospital Organization Osaka National Hospital, Kitakyushu Municipal Medical Center, Iwate Medical University, Tokuyama Central Hospital (69 institutes)

References

1. Iwama T, Mishima Y. The polyposis committee of the Japanese research Society for Cancer of the colon and rectum. Gann Monogr Cancer Res. 1995;43:69–80.
2. The Research Group in Health and Labor, Sciences Research Grant. Research on elucidation of clinical and genetic characteristics of HBOC in Japan, and on improvement of their outcome using genetic information. In: Guidebook for Diagnosis and Treatment of hereditary Breast and Ovarian Cancer Syndrome 2017. Tokyo: Kanehara Co. Ltd.; 2017. p. 7–20.
3. Arai M, Yokoyama S, Watanabe C, Yoshida R, Kita M, Okawa M, et al. Genetic and clinical characteristics in Japanese hereditary breast and ovarian cancer: first report after establishment of HBOC registration system in Japan. J Hum Genet. 2018;63:447–57. https://doi.org/10.1038/s10038-017-0355-1.
4. Nakamura S, Takahashi M, Tozaki M, Nakayama T, Nomizu T, Miki Y, et al. Prevalence and differentiation of hereditary breast and ovarian cancers in Japan. Breast Cancer. 2015;22:462–8. https://doi.org/10.1007/s12282-013-0503-1.
5. The Japanese HBOC Consortium.. http://hboc.jp/summary/index.html (2011). Accessed 31 January 2021.
6. Japan Hereditary Breast Cancer Ovarian Cancer Comprehensive Medical Care System Organization. http://johboc.jp. Accessed 31 January 2021.
7. Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, Lingenfelter B, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol. 2002;20:1480–90. https://doi.org/10.1200/JCO.2002.20.6.1480.
8. Nagata H, Sekine M, Tsuji S, Hirai Y, Fujimoto S, Hatae M, et al. Haplotypes of BRCA1 mutation alleles in Japanese ovarian and breast-ovarian cancer families: a novel method for detecting BRCA1 associated ovarian cancer. Acta Med Biol. 2002;50:33–42.
9. National Center for Biotechnology Information, U.S. national library of medicine: clinvar aggregates information about genomic variation and its relationship to human health. https://www.ncbi.nlm.nih.gov/clinvar. Accessed 29 January 2021.
10. Yoshimura A, Yokoyama S, Iwata H, Takaiso N, Nomizu T, Arai M, et al. Incidence of contralateral and ipsilateral breast cancers and prognosis in BRCA1/2 pathogenic variant carriers based on the Japanese HBOC consortium registration. J Hum Genet. 2020; https://doi.org/10.1038/s10038-020-00850-5.
11. Okano M, Nomizu T, Tachibana K, Nagatsuka M, Matsuzaki M, Katagata N, et al. The relationship between BRCA-associated breast cancer and age factors: an analysis of the Japanese HBOC consortium database. J Hum Genet. 2021;66:307–14. https://doi.org/10.1038/s10038-020-00849-y.
12. Inuzuka M, Watanabe C, Yokoyama S, Kuwayama T, Akashi-Tanaka S, Arai M, et al. A retrospective analysis of the relationship between the result of BRCA1/2 genetic testing and surgical method selection in Japan. Clin Breast Cancer. 2021;21:e48–52. https://doi.org/10.1016/j.clbc.2020.08.004.
13. Tung NM, Boughey JC, Pierce LJ, Robson ME, Bedrosian I, Dietz JR, et al. Management of hereditary breast cancer: American Society of Clinical Oncology, American Society for Radiation Oncology, and Society of Surgical Oncology guideline. J Clin Oncol. 2020;38:2080–106. https://doi.org/10.1200/JCO.20.00299.

14. Yamauchi H, Okawa M, Yokoyama S, Nakagawa C, Yoshida R, Suzuki K, et al. High rate of occult cancer found in prophylactic mastectomy specimens despite thorough presurgical assessment with MRI and ultrasound: findings from the hereditary breast and ovarian cancer registration 2016 in Japan. Breast Cancer Res Treat. 2018;172:679–87. https://doi.org/10.1007/s10549-018-4953-1.
15. Yoshida R, Watanabe C, Yokoyama S, Inuzuka M, Yotsumoto J, Arai M, et al. Analysis of clinical characteristics of breast cancer patients with the Japanese founder mutation *BRCA1* L63X. Oncotarget. 2019;10:3276–84. https://doi.org/10.18632/oncotarget.26852.
16. Mitamura T, Sekine M, Arai M, Shibata Y, Kato M, Yokoyama S, et al. Risk factors for lymph node metastasis of ovarian, fallopian tube and primary peritoneal cancer in hereditary breast and ovarian cancer syndrome. Jpn J Clin Oncol. 2020;50:1380–5. https://doi.org/10.1093/jjco/hyaa124.
17. Nomura H, Sekine M, Yokoyama S, Arai M, Enomoto T, Takeshima N, et al. Clinical background and outcomes of risk-reducing salpingo-oophorectomy for hereditary breast and ovarian cancers in Japan. Int J Clin Oncol. 2019;24:1105–10. https://doi.org/10.1007/s10147-019-01456-4.
18. CIMBA–Consortium of Investigators of Modifiers of BRCA1/2: Centre for Cancer Genetic Epidemiology.. http://cimba.ccge.medschl.cam.ac.uk (2021). Accessed 31 January 2021.

Ethical Issues: Overview in Genomic Analysis and Clinical Context

17

Izen Ri and Kaori Muto

Abstract

This chapter discusses ethical, legal, and social issues (ELSI) centered around hereditary breast and ovarian cancer syndrome (HBOC). In the first half, we discuss ethical considerations in the context of decision-making on genetic testing, debates on incidental/secondary findings (IFs/SFs), and global trends in clinical and/or genetic data sharing, including with patients and their family members. In the second half, from the perspective of clinical ethics of cancer diagnosis and treatment, we introduce the importance of decision-making and care based on the shared decision-making (SDM) approach and practical points in prophylactic surgery. We also discuss dilemmas that arise regarding confidentiality between medical professionals and their patients. This includes disclosure of genetic information with genetic relatives, and challenges in family communication, in which carefully assessed and encouraging support may be needed for patients and family members.

Keywords

Ethical, legal, and social issues (ELSI) · Bioethics · Medical ethics · The right to know/not to know · Shared decision-making (SDM) · Prophylactic surgery Family communication · Incidental findings/secondary findings (IFs/SFs) · Data sharing · Confidentiality

I. Ri · K. Muto (✉)
Department of Public Policy, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
e-mail: izri@ims.u-tokyo.ac.jp; krmt@ims.u-tokyo.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_17

17.1 Introduction: Genomic Medicine/Research and Ethical Issues

From an ethical perspective, genetic and genomic analyses may evoke certain dilemmas, since genetic data are characterized such that they are partially shared among genetic relatives, for not only the diagnosis of current health conditions but also the assessment of future disease risk among individuals. Although "respect for autonomy" is one of the fundamental principles in medical ethics,[1] potential conflicts of interest exist between patients and their family members. Furthermore, diagnosis, treatment, and prevention options may highly influence personal life plans, and various values may depend on individuals, cultural/social context, and historical backgrounds. Multidisciplinary collaborations are required to unravel such questions with no correct answers. Indeed, genetic/genomic studies have always promoted the ethical and psychosocial viewpoint.

The Human Genome Project (HGP) was a remarkable achievement by international collaboration groups from 1990 to 2003, which aimed to determine the complete sequence of the human genome at nucleotide-level resolution. The US Department of Energy (DOE) and National Institutes of Health (NIH) devoted 3–5% of their annual HGP budgets toward studies on ethical, legal, and social issues (ELSI) [1], which consider the policies and examine the implications of genomic analysis technology with respect to individuals, families, and communities. Such studies include various issues, i.e., fairness in the use of genetic data among insurers, employers, and courts; privacy and confidentiality issues; psychological impacts; and stigmatization owing to an individual's genetic differences and reproductive issues. Furthermore, the NIH National Human Genome Research Institute (NHGRI) continued to fund the ELSI program [2].

Today, over 30 years have elapsed since the initiation of the HGP, and genetic testing and genome sequencing have become increasingly popular in basic research and the clinical setting. Although the basic ELSI remains unchanged, numerous technological advantages and changes in the social environment have been brought about. In this chapter, highlighting our research results conducted in Japan, we overview several ethical topics surrounding HBOC (hereditary breast and ovarian cancer) from research and clinical perspectives.

[1] Four ethical principles of medical ethics consisting of "respect for autonomy," "beneficence," "non-maleficence," and "justice," which were advocated by T. L. Beauchamp and J. F. Childress in their book *Principles of Biomedical Ethics* in 1979 (Beauchamp TL and Childress JF. Principles of biomedical ethics. New York: Oxford University Press; 1979.) After decades, although these traditional principles were updated by numerous researchers, their framework still seems applicable to present medicine because of their simplicity and practicality, including genetic and genomic medicine.

17.1.1 The Right to Know/the Right Not to Know: A Basic Ethical Principle in Genetic/Genomic Analysis

Individuals undergoing genetic testing not only have "the right to know" but also "the right not to know" his/her genetic information. This concept is derived from an argument in the 1990s by Dr. Nancy Wexler, who had been at risk of Huntington's disease (HD; a progressive brain disorder without definitive treatment) and had contributed to the assessment of the predisposition to HD on the basis of the *HTT* gene. The great success of Wexler and her colleagues in detecting *HTT* gene facilitated the diagnosis of patients and the prediction of future risk of HD among asymptomatic individuals [3]. There was a debate as to whether at-risk individuals had the "duty to know" their carrier states [4, 5]. Wexler insisted that patients and their family members had the right not to undergo genetic testing, and presymptomatic genetic testing should be accompanied by a careful genetic counseling process with trained counselors [6, 7].

At that time, this discussion also influenced the rules and ethical issues regarding disorders with early onset and with potential preventive and treatment strategies. For instance, the American Society of Human Genetics (ASHG) recommended that predictive genetic testing for hereditary breast and ovarian cancer syndrome (HBOC) and career testing for cystic fibrosis (CF) should be voluntary, with appropriate education and counseling [8, 9].

As one of the global consensuses, *The Universal Declaration on the Human Genome and Human Rights* (1997) of the UNESCO stated the following: "(c) The right of each individual to decide whether or not to be informed of the results of genetic examination and the resulting consequences should be respected" (B. Rights of the persons concerned, Article 5), with the emphasis on respecting human dignity regardless of genetic characteristics [10]. The same principles were included in the *Convention on Human Rights and Biomedicine* by the Council of Europe [11], and the *International Declaration on Human Genetic Data* (2003) by UNESCO confirmed "the right to decide whether or not to be informed" [12].

After the several decades, the germline *BRCA* variant is considered a medically "actionable" finding, i.e., "there is a recognized therapeutic or preventive intervention or other available actions that have the potential to change the clinical course of a disease or condition" [13], or "druggable" for molecular-targeted drugs including PARP (poly(ADP)-ribose polymerase) inhibitors.

Certainly, *BRCA* is a medically actionable variant; hence, it is crucial to carefully assess and balance both risks and benefits of genetic testing of individuals.[2] While

[2] For instance, the US Preventive Services Task Force (USPSTF) revised its recommendation statement on *BRCA*-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing in August 2018: They concluded that with moderate certainty, the net benefits of risk assessment, genetic counseling, and genetic testing outweigh the harms among women whose family or personal history is associated with an increased risk for *BRCA1/2*variants, while the harms outweigh

undergoing genetic testing may relieve a woman's anxiety and uncertainty about whether she has a hereditary cancer risk, especially those with a strong fear for developing cancer, studies have reported that *BRCA*-positive women without a cancer diagnosis may experience long-term uncertainty [14, 15]. A longitudinal study from the life course perspective (LCP) showed how lives were changed among women after knowing that they carried *BRCA* variant, and different emphases on concepts have emerged across different age groups (i.e., 20s, 30s, 40–50s) [16]. A large, prospective analysis performed in 2008–2012 in the USA revealed that nearly one-third of patients did not pursue *BRCA* genetic testing after genetic counseling, with insurance coverage and out-of-pocket cost concerns being the top nonmedical reasons for declining the test [17].

Furthermore, since HBOC is often recognized as a "women's disease," the diagnosis of breast or prostate cancer among male at-risk persons or patients may be confounding or be met with low interest toward such information.

17.1.2 Shared Decision-Making Model: Collaboration of Medical Professionals and Patients for Better Decision-Making

What would be the most effective approach to support the decision-making of patients on matters that significantly affect their way of life?

Shared decision-making (SDM) is a model of decision-making in clinical practice for procedures such as genetic testing [18]. SDM is characterized by having at least both the physician and patient involved in the decision-making process and having both parties share information, take steps to build a consensus, and reach agreement [19]. Of course, one physician and one patient is the most simplified model of SDM. In practice multiple physicians may be included or consulted, and the patient may include or consult with his/her family, friends, counselors, and nurses [20].

In general, informed consent (IC) is aimed at allowing patients or clients to decide whether to consent or dissent (reject) after receiving a complete explanation and understanding of the best-possible treatment plan, as deemed by the clinician. However, SDM emphasizes the process of consensus building wherein the clinician and patient or client collaborate and share information. In this bidirectional process, the clinician provides all potential options from the medical perspective, while the patient shares thoughts on the effect of the illness and treatment on his or her life and cherished values. Although IC and SDM have numerous similarities, IC is a clinician-centered process wherein the patient is relatively passive, whereas SDM requires collaboration between the clinician and patient in decision-making. That is,

---

the benefits among women whose family or personal history is not associated with an increased risk (U.S. Preventive Services Task Force (USPSTF). *BRCA*-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing. In: Recommendation Topics. 2019. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing. Accessed 15 Feb 2021)

the concept of SDM is consistent with the "interpretive" or "deliberative" models, which are situated in-between the "paternalistic" and "informative," in the four models of the physician-patient relationships advocated by Emanuel and Emanuel [21, 22].

SDM is particularly important in a situation with high uncertainty (i.e., lack of clear evidence regarding the best-possible outcomes) and variability in patient values and preferences [23]. It was suggested that SDM would help women make decisions about *BRCA* genetic testing, cancer prevention, and treatment decisions, as there is no single correct plan [24]. As in "anticipatory guidance" in genetic counseling, SDM requires the clinician and patient to collaborate in making the best-possible life plan by considering the potential positive and negative effects of genetic testing, work (employment), marriage plans, plans on conceiving children, and relationships with relatives. Some tools called decision aids (DAs) are used to support an individual in making a shared and informed decision about *BRCA* testing and to clarify values and preferences [25].

17.1.3 Incidental/Secondary Findings (IFs/SFs)

Genomic analysis involving next-generation sequencing (NGS) approaches has been widely introduced in the clinical setting. This technology helps determine the sequence of DNA more rapidly and at a lower cost than conventional Sanger sequencing, and it is used for analyzing panels of multiple genes, exomes, and whole genomes. One of the controversial issues is the management of "incidental findings (IFs)" or "secondary findings (SFs)."

This issue was originally derived from a discussion on whether researchers have a duty to disclose an unexpected finding to research participants of a study, using structural magnetic resonance imaging (MRI) of the brain or computed tomography (CT) colonography, and the discussion extended to the field of genomic research [26, 27]. Wolf et al. defined an IF as a "finding concerning an individual research participant that has potential health or reproductive importance and is discovered in the course of conducting research but is beyond the aims of the study" [28] and led the controversial debates. Since NGS has been widely used in the clinical setting, the discussion also applies to a medical professional's duty to patient and family.

The American College of Medical Genetics and Genomics (ACMG) issued the first clinical recommendations for the return of IFs from whole-genome/whole-exome sequencing and provided a list of a minimum of 56 genes associated with 24 health conditions, which would be extensively screened clinically and reported to the attending physicians, irrespective of the patient's preference [29]. This recommendation emerged controversial, especially regarding mandatory analysis and infringement of the patient's autonomy [30]; consequently, ACMG updated the recommendation that patients should be able to opt out of the analysis of genes unrelated to the indication for testing during the obtainment of informed consent [31].

After the ACMG recommendation and related controversies, the US Presidential Commission for the Study of Bioethical Issues (PCSBI) issued a report called

ANTICIPATE and COMMUNICATE: Ethical Management of Incidental and Secondary Findings in the Clinical, Research, and Direct-to-Consumer Contexts in 2013 [32]. The report verified the taxonomy of IFs/SFs and provided context-specified recommendations for their management in the clinical setting, basic research, and direct-to-consumer (DTC) genetic testing. While the "primary findings" are the results obtained as the primary target of a test or procedure, IFs and SFs are the results that are obtained outside of the original purpose. IFs are unintended discoveries, which can be categorized as "anticipatable" or "unanticipatable" IFs, considering the current state of scientific knowledge. In contrast, SFs refer to a finding which is actively and intendedly sought by a practitioner but is not the primary finding. Furthermore, PCSBI reflects a clinician's ethical and professional responsibilities as to the following points: informed consent, to convey clearly to patients the possibility of discovering IFs/SFs and communicate with their patients regarding follow-up alternatives; shared decision-making, to encourage patients to ask questions, state reservations, and express preferences about the return and management of IFs/SFs; clear communication, to consider incorporating graphs and other visual displays to enhance patient comprehension of risk in medical decision-making; and clinical judgment, to minimize the likelihood of IFs through communication with patients to better understand symptoms and help narrow the list of potential diagnoses [33]. Table 17.1 summarizes the classification of IFs/SFs by the PCSBI, along with suitable examples, which we modified.

Table 17.1 Classification of incidental findings/secondary findings

Type of result discovered	Primary finding	Incidental finding: anticipatable	Incidental finding: unanticipatable	Secondary finding
Description	Practitioner aims to discover A, and result is relevant to A	Practitioner aims to discover A, but learns B, a result known to be associated with the test or procedure at the time it takes place	Practitioner aims to discover A, but learns C, a result not known to be associated with the test or procedure at the time it takes place	Practitioner aims to discover A and also actively seeks D per expert recommendation
Examples	Obtaining positive findings for *BRCA* variants after conducting diagnostic or presymptomatic genetic testing for *BRCA*	Discovering brain tumor when conducting magnetic resonance imaging (MRI)	DTC genetic testing company identifying genetic variants that are not currently associated with the disease	Detecting possibility of germline variants which ACMG recommends that any laboratories conducting genome sequence in clinical purpose should actively screen

See p. 27 in [32]; "Examples" were modified by authors

ACMG revised the terminology to "secondary findings" since the updating of the policy statement in 2016 (ACMG SF v2.0) because the enlisted genes are intentionally being analyzed, as opposed to genetic variants found incidentally or accidentally [34]. ACMG released an updated policy statement and minimum list for reporting of secondary findings (SF v3.0), which include 73 genes in May 2021, and the working group noted its plan to update the list annually [35–37].

In clinical oncology, such genomic analysis may lead to identification of inherited susceptibility to cancer or other diseases through either somatic mutation profiling or germline multigene (multiplex) panel testing, which is also referred to as "germline findings" instead of "incidental" or "secondary findings."

The updated *Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility* (2015) of the American Society of Clinical Oncology (ASCO) [38] requires oncology practitioners to communicate the potential for incidental and secondary germline information to patients before conducting somatic mutation profiling (genomic tumor profiling test) and review the potential benefits, limitations, and risks before testing.[3] Furthermore, a patient's preferences regarding the receipt of germline information, including the choice of declining it, should be carefully ascertained.

In practice, a report of IFs/SFs may include complex considerations, including results returned to whom, how much information to disclose, results returned by whom, and what actions (i.e., follow-up testing and/or care) will take place after disclosure of results [39]. For instance, a disclosure framework as a flowchart in the context of clinical treatment was suggested, which would enable physicians and patients to discuss preferences for receiving IFs/SFs and follow-up options (see p. 290 in [39]).

17.1.4 Secondary Germline Findings in Genomic Tumor Profiling and Public Attitudes

As explained in the previous section, cancer patients undergoing genomic tumor profiling have to make decisions on whether or not to learn about germline SFs and when, to whom, and how to convey the information to their family members, along with decision-making on their own treatment options. This leads to the question of what the attitude is of the cancer patient toward germline findings in such testing. Some qualitative studies have reported that advanced cancer patients were highly interested in learning about secondary germline findings, and they perceived both various benefits and concerns regarding the limitations in clinical utility and the emotional burden or distress derived from such information [40, 41].

[3] The ASCO *Policy Statement* also suggests that oncologists should discuss the possibility of detection of high-penetrance variants among their patients, which has not been suggested by personal and/or family history; less well-understood or lesser-penetrance variants; and variants of uncertain significance (VUS) in multigene (multiplex) panel testing.

We would like to share data obtained from a survey in Japan, where genomic tumor profiling tests to identify tumor-specific genomic changes and find molecular-targeted drugs among patients with advanced cancer have been covered by the national health insurance scheme since June 2019. Some of the tests can identify germline variants including *BRCA* or *TP53*, which are putative candidates owing to their actionable natures, per the recommendations of the ACMG [29, 31]. A cross-sectional survey including Japanese cancer patients, family members of cancer patients, and general adults in 2018 revealed that family members and cancer patients highly evaluated the potential benefits of tumor profiling tests. This was expected to facilitate diagnoses and treatment of patients and their family members, and the detection of any heritable oncogene would facilitate the development of future plans [42]. On the contrary, approximately 20% of respondents in each group did not wish to know whether they had a hereditary disease, and >30% of them worried about the possibility of being discriminated against owing to their genetic condition. However, irrespective of the results, the family members were more willing to share information regarding germline findings than the patients. Owing to concerns regarding anxiety and stress among family members, 3.8% of cancer patients preferred not to share this information. Only 1.8% of family members agreed with this notion, with the most common reason being "It is better for me not to know."

Informed consent forms for the tests provide alternatives for patients regarding whether or not they want to know the test results, including the possibility of hereditary cancers as SFs, and whether or not they would be willing to share this information with their family members. Furthermore, a column is available to provide the names and contact information of the family members, in case the patient is unable to share information with the family members for any reason, including changes in physical conditions.

Since patients tended to overestimate the benefits of tumor profiling for personalized treatments and potentially ended up disappointed, information and decision aids (DAs) are needed to support medical professionals in communicating the realistic benefits and risks associated with the results [43].

17.1.5 Data Sharing and Privacy Issues in Genomic Research and Public Attitudes

The previous sections primarily focused on genomic analysis in the clinical setting; however, here, we discuss this in the context of basic research. Sharing of clinical and genomic data among researchers has been a standard practice in genomic research. Some platforms for global sharing of clinical and genomic data have been developed, such as the Global Alliance for Genomics and Health (GA4GH), where more than 600 organizations and companies from more than 90 countries participate [44]. BRCA Exchange aims to advance understanding of the genetic basis of breast, ovarian, pancreatic, and other cancers by pooling data on *BRCA1/2* variants and corresponding clinical data worldwide [45].

Data operators may adopt an approach such as collecting data in a temporarily closed location within the database for the same disease and releasing it after findings from several studies have accumulated. Institutional review boards (IRBs) also play important roles, and they should require researchers to show their data-sharing plan and check whether the data have been submitted to a database as planned. Furthermore, since social and public understanding is indispensable, ideas are needed to get research participants and the public interested in how genomic and clinical data are used and shared in genetic research. In the USA, the National Institutes of Health (NIH) issued Genomic Data Sharing Policy (GDS Policy) in 2014, which defined responsibilities of investigators, data submission expectations, as well as conditions for research use of controlled-access data (available for users meeting specific requirements, including an approval from a data-access committee) and unrestricted-access data. The data submitter needs to take measures to lower the risk of reidentification by not adding identifiable information to a database initially. The idea of the GDS Policy has been adopted by data repositories in other countries.

In genomic studies on cancer and rare diseases, not only patients but also their family members may provide valuable information. Therefore, protocols to protect both patients and their family members are needed in such studies and on data-sharing platforms. This leads to the question of the concerns regarding sharing data including those of family members. For instance, since cumulative data from both patients and their family members are valuable for genomic analysis, some participants may feel implicit or explicit pressure from researchers or other family members. Although the participant's right to withdraw consent is crucial, withdrawal from the study by certain family members may be difficult when data and samples are already shared internationally. There is risk of identifiability not only for identifying the individual who provided the data but also for his/her family members, especially when family trees are published in the article. In such situations, existing data-sharing policies may not be enough to protect family members.

As data sharing has become increasingly important, confidentiality and privacy issues involved therein have also gained increasing importance. A questionnaire survey in Japan indicated that public (especially patients, compared to healthy adults) concerns were higher with respect to the sharing of their own data with those of their family members, and they expected stronger protection mechanisms, compared with only their own data being shared [46]. A systematic review revealed that research participants and the public attitudes toward genomic data sharing were influenced by various factors, such as their perceptions of sensitivity and controllability of genomic data, perception of potential risk and benefit of genomic data sharing, sensitivity and controllability of genomic data, and governance-level considerations [47]. Global empirical studies showed that general public were most likely to donate their genomic and health data for clinical and research use, but unwilling to donate them to for-profit researchers or company researchers, compared to medical doctors and nonprofit researchers [48, 49]. In a study in the UK,

the public raised concerns about managing flows of information to protect patient confidentiality and guard against unauthorized access to data by third parties, such as employers, marketing companies, and insurers [50].

Cloud computing, a model whereby users rent computers and storage from large data centers, has been expected to promote large-scale collaboration in cancer genomic medicine. We need to argue the challenges of managing genomic data in the cloud and be ready to inform patient and family about data safety and privacy [51, 52].

17.2 Clinical Ethics in the Diagnosis and Treatment of Hereditary and Other Cancers

We further discuss ethical perspectives in the clinical setting with respect to the diagnosis and treatment of HBOC and other hereditary cancers.

17.2.1 Clinical Ethics in Cancer Treatment: Perspective Based on Quality of Life

In making decisions during cancer treatment, careful assessment and improvement of the quality of life (QOL) of the patients are highly significant. Clinical ethics in cancer and oncology nursing have further emphasized the impact of cancer and cancer care on sexuality, sexual behavior, and fertility and on changes in body image resulting from the dissection of organs including the breasts and ovaries [53]. Therefore, the SDM approach is effective here again for patients and medical professionals to predict long-term outcomes of surgery or pharmacotherapy.

In SDM, clinicians and patients are encouraged to use various decision aids (DAs), such as leaflets, video clips, and websites. DAs are not intended to encourage a patient to select or consent to a particular course of action, but rather to support patients and clinicians in identifying and implementing the healthcare options most aligned with the patient's individual preferences and values [54]. The Ottawa Personal Decision Guide (OPDG) was developed as a tool to be used in healthcare [55]. A decision-maker fills out the OPDG form to organize his/her opinions regarding specific treatment options or testing and their merits and demerits, along with his/her knowledge and values, the availability of support, and certainty. The form may be used to promote discussion between the decision-maker and the clinician supporting the decision-making process. Furthermore, DAs have been developed for specific diseases (e.g., a DA is available for decision-making regarding surgery for breast cancer patients).

It is important that the discussion process itself is the means, not the end, for the patient to make a confident decision. The SDM approach may predict patient expectations and concerns, along with long-term effects, not only regarding medical outcomes but also life, work, and QOL.

17.2.2 Prophylactic Surgery: Decision-Making and Follow-Up

Based on the SDM approach and the perspective of QOL, the specific care required for patients with HBOC with a genetic predisposition to breast and ovarian cancer can be evaluated. This information would potentially support decision-making regarding prophylactic surgery and postoperative follow-up among patients. Risk-reducing mastectomy (RRM) and risk-reducing salpingo-oophorectomy (RRSO) are cost-effective preventive strategies in *BRCA1/2* mutation carriers [56]. While prophylactic surgery is an effective lifesaving measure and helps alleviate the fear of developing cancer among women, it may also have a great impact on their QOL and self-image. A systematic review reported that women's decision-making regarding RRSO was affected by demographic, clinical, and psychological factors, as well as family history of cancer, rather than an objective cancer risk [57]. Another systematic review reported that most studies assessing psychosocial aspects reported high levels of satisfaction among women deciding to undergo RRM; however, greater variation was observed in satisfaction levels from a cosmetic perspective, and satisfaction with body image was diminished along with sexual feelings, especially after bilateral risk-reducing mastectomy (BRRM) [58]. An interview-based study in Canada reported that nearly one-half of the women who underwent RRSO did not believe that they were well-informed about postoperative outcomes including anesthetic effects, physical symptoms, menopause symptoms, or return to daily activities, despite fully receiving pre-surgery counseling [59]. Deliberated assessment and support in decision-making before surgery and during postoperative follow-up are required.

According to the HBOC registration system in Japan, only a few *BRCA1/2* carriers have undergone RRM and RRSO in Japan, compared to their European and American counterparts [60]. The guidelines of the Japanese Breast Cancer Society (2018) and Japanese Society of Gynecologic Oncology (2020) recommend that prophylactic surgery for women carrying *BRCA* variants who have not developed cancer, which is not covered by public health insurance (as of 2020), is desirable for the approval of the clinical ethics committee at each institution, although no such requirements are specified in the National Comprehensive Cancer Network (NCCN) guidelines. In clinical conferences, medical professionals have to assess the "beneficence" and "non-maleficence" for prophylactic surgery in each case. In addition, in terms of respect for autonomy, they have to provide a patient with complete explanations of both the benefits and risks of prophylactic surgery and respect the patient's autonomous decision.

17.2.3 What Method May Be Useful in the Clinical Setting? Four-Quadrant Approach of Clinical Ethics

In clinical conference or case studies, the four-quadrant approach for clinical ethics, which was originally introduced by Jonsen et al. (1992) [61], may be a useful approach to better understand the complexities and ethical dilemmas of a case. The method comprises four aspects, medial indication, patient preference, QOL, and

Table 17.2 The four-quadrant approach of clinical ethics: checkpoints for prophylactic surgery

Medical indication	Patient preference
What evidence and data are available worldwide?	Does the patient have a capacity for decision-making or expressing will?
What is the best timing for the patient?	Does the patient fully understand the positive outcomes of *BRCA* variants and RRM or RRSO? Do they have a strong desire and motivation to undergo surgery?
What are the benefits and risks/disadvantages of the surgery?	What is the patient's sexual orientation and gender identity (SOGI)?
Is there a provision for physical/psychosocial care after surgery?	Does the patient intend to become pregnant?
Are follow-up options (surveillance, cost, and medical institution) available for both patients who undergo the surgery and those who do not opt for surgery?	Has the patient been informed of the effects of surgery on sexual activity and gender identity, and how much do they value them?
QOL	**Contextual features**
Subjective QOL: What is the status of the breast and ovarian cancer among the patients?	What intentions do the patient's family or stakeholders have? How do they evaluate the surgery?
What do patients wish to deal with regarding the illness, and how do they want to live their lives?	What are the institution's and medical team's policies? What is the system for research and education?
Objective QOL: What scale and measures should be used to evaluate?	What are the financial aspects of surgery and postsurgical care? Are costs incurred by the patients themselves or public medical services?
By whom should QOL be evaluated and what criteria should it be based on?	What are the religious beliefs and cultural customs, and is there a potential influence on other patients and society?
How would the QOL of the patients change with time and as a consequence of medical intervention?	What are the other factors or concerns (e.g., timing, social background, and communication strategy with the patient's genetic relatives)?

contextual features; several inquiry-based checkpoints are provided for each topic. Muto and Takashima (2017) [62] suggested adapting this approach in considering ethical issues associated with prophylactic surgery. Table 17.2 summarizes illustrative checkpoints for prophylactic surgery.

Regarding "medical indication," medical conditions including diagnosis, prognosis, the aims of intervention and care, and the balance of risk and benefit should be considered. To respect "patient preference," it is important to understand what explanations have been provided to the patients and their understanding of them. It would be helpful for physicians to check casual remarks and questions from the patient. QOL encompasses various components including physical, psychological, social, and spiritual. "Objective QOL" may be measured using certain scales, whereas "subjective QOL" may be better understood through mutual communication with patients. Furthermore, "contextual features," such as family members or other stakeholders, financial aspects,

institutional policies, and any other points potentially influencing decisions, should be considered. From the point of diversity, patients' views of cancer or preferences toward prevention strategies may depend on cultural background, ethnicity, socioeconomic status, religious belief, and generation [63]. This method would help medical professionals collect information and understand the types of conflicts occurring in different cases.

17.2.4 Patient Confidentiality and Disclosure of Genetic Information to At-Risk Relatives

Another challenging controversial debate concerns patient confidentiality and the disclosure of genetic information to their at-risk relatives, since genetic information is partially shared among the genetic relatives of patients. Medical professionals are required to maintain the confidentiality of their patients or clients, and they may face dilemmas of whether they have a duty or are permitted to disclose genetic information with the patient's relatives, especially when patients do not provide consent. Laws and principles vary among different countries, and several lawsuits have emerged regarding this issue.

In the USA, two lawsuits in the mid-1990s yielded different judgments. In the *Pate* v. *Threlkel* case in Florida (1995), the court concluded that a physician's "duty to warn" a patient's (medullary thyroid carcinoma) relatives could be satisfied by simply notifying the patient. However, in the *Safer v. Pack* case in New Jersey (1996), the court held that a physician had a duty to warn those known to be at risk of avoidable harm from a genetically transmissible condition (multiple polyposis) [64]. However, since the Health Insurance Portability and Accountability Act (HIPAA) of 1996 Privacy Rule came into effect in 2003, healthcare providers are neither required nor permitted to warn relatives without the consent of their patients [65]. ASCO updated policy statements (2003; 2015), which indicated that oncologists should explain the importance of sharing test results with at-risk relatives, such that they may benefit from this information during the obtainment of informed consent and pretest education [38]. Similarly, the American Medical Association (AMA) *Code of Medical Ethics* states that physicians should discuss with the patient the medical and psychological implications for the individual's biological relatives, and they will be available to assist in communication with the patient's relatives (Opinion 4.1.1) [66].

A recent case, *ABC v. St George's Healthcare NHS Trust & Ors* (2020), was the first lawsuit that argued patient confidentiality and the duty of medical professionals to disclose genetic information to genetic relatives in the UK [67–69]. Although the High Court concluded that the claimant *ABC* (a daughter of a male patient diagnosed with Huntington's disease) lost the case, it also added that it was reasonable to impose a duty on the medical teams to balance the daughter's interest in being informed of her genetic risk against her father's interest in preserving confidentiality in relation to his diagnosis and the public interest in generally maintaining

medical confidentiality [69]. This duty does not require physicians to directly disclose genetic information to the daughter, nor are the medical professionals generally responsible for the genetic relatives, but rather this duty encourages them to carefully balance the interests of family members.

In Europe, laws and principles are different, i.e., in France, a bioethics law revised in 2011 requires the patient provide information regarding the diagnosis of a pathogenic variant associated with a serious disease that is preventable or treatable among at-risk relatives. This is to be done either directly or by providing consent to healthcare professionals to contact relatives (although in practice, patient disclosure is preferred and it is rare that physicians directly disclose information to the relatives) [70, 71].

In Australia, although state laws differ, healthcare professionals have no legal duty to inform genetic relatives. However, disclosure is allowed under the *Federal Privacy Act 1988* as an exception if there is "reasonable belief that disclosure is necessary to lesson or prevent a serious threat to life, health or safety of a genetic relative" (i.e., disclosing the sister of a woman receiving a positive test result on *BRCA* variant analysis) [72]. This exception was further corroborated by a National Health and Medical Research Council (NHMRC) guideline (2014), which provides a framework and specific steps for healthcare professionals to use or to disclose genetic information (i.e., advise patients to contact relatives, appropriate expertise to assess whether the threat to genetic relatives is serious and disclose as necessary) [73]. Such a practical framework in exercising discretion may also help healthcare professionals to better balance patient confidentiality and benefits of at-risk relatives.

In summary, there is no conclusive evidence regarding the worldwide unconditional invalidity of patient confidentiality. Disclosure to at-risk relatives without patient approval is limited to particular situations, e.g., when a genetic variant is associated with serious and actionable health conditions. In the practical context, patients or index persons may usually take on the primary role of communication among their family members.

17.2.5 Communication of Genetic Risk Within the Family

From the perspective of genetic relatives, being informed about an increased risk of hereditary cancer may be useful for early cancer detection, the choice of whether "to know or not to know" their genetic information, risk management, and future life planning. However, patients or index persons in the family usually face difficulties in communicating with their genetic relatives, which may also lead to conflict among them.

Numerous empirical qualitative and quantitative studies have revealed dilemmas and practices for familial communication about HBOC. Several studies indicated that *BRCA* carriers (both patients and asymptomatic carriers) or at-risk persons often feel responsible for communicating with their genetic relatives [74, 75];

Table 17.3 Factors associated with the teller's view and experience

Categories	Examples
Value and norm	It is beneficial to know everything, ignorance is bliss, openness in the family, the importance of a relationship based on trust, responsibility or the sense of a mission to share information
Knowledge and experience	Medical and genetic literacy, experience with health and illness, educational background, occupation
Health and psychological conditions	Current physical and mental health, disease course, perception of the family history and one's own genetic risk
Benefit/concern	Feeling relieved in keeping the information concealed, sharing one's feelings and worries, providing or gaining support from family members Psychological burden of communication, difficulties in the timing of communication, possibility of receiving a negative response

however, their decision-making of whether to tell, whom to tell, and what and how to tell depends on the case and is dependent on various factors. Situations are different in communicating with offspring (especially young children and adolescents) and with other genetic relatives including siblings, cousins, and parents. Patients may decline to have such communication with their family members in an effort to protect their relatives (e.g., from painful knowledge or potential discrimination), due to difficulties in overcoming preexisting conflicts or rifts within the family or due to feeling that certain relatives did not "need" to be provided such information (e.g., believing that boys and other male relatives do not need to be provided such information) [76]. Studies have reported that sex is an influential factor, since numerous patients speculated that the risk of cancer associated with *BRCA1/2* variants was higher among women than among men [77]. Furthermore, physical/emotional distance (i.e., having no contact) with the relatives matters. Quantitative analysis revealed that the information dissemination rate depended on the type of relative; information dissemination rates were the highest among siblings, followed by parents and children (first-degree relatives), aunts/uncles, nieces/nephews (second-degree relatives), and lowest among cousins (third-degree relatives) [78, 79].

Regarding parent-children relationships, the age of the children, maturity, cognition, personality, and emotional readiness influenced parental decision-making, and sometimes parents decided that it was not the right time to tell their children at least at that point [80].

Based on previous studies of hereditary diseases including those associated with *BRCA* variants and our studies in Japan, we found various factors associated with decision-making related to communication within the family. Views and experiences of the teller (individuals who attempt to share information with their family member, either the patient having undergone genetic testing, at-risk individuals, or sometimes their partners) matter (Table 17.3), as well as the teller's presumption with the listener (e.g., children, siblings, parents, cousins, and aunt/uncle) (Table 17.4).

Table 17.4 Factors associated with the listener presumed by the teller

Categories	Examples
Knowledge and experience	Medical and genetic literacy, experience with health and illness, educational background, occupation
Attribute	Sex/gender, age
Receptivity	Comprehension, readiness, life condition
Relationships	Physical distance (living together or separately), psychological distance (intimacy)
Benefit/concern	Early disease detection, changing/improving lifestyle habits, choice of preventive or therapeutic measures, the opportunity to participate in clinical trials, the choice of presymptomatic or diagnostic genetic testing Psychological burdens, negative impact on life decisions including marriage and childbirth

Therefore, difficulties in and the optimal timing for communication with family members may differ in a case-specific manner. Medical professionals may not only directly contact relatives or encourage clients to share information with their relatives, but they may also assist clients in communicating their relatives and provide psychoeducational guidance or written information aids. Genetic counselors could introduce the topic and discuss the pros and cons of communicating them with children [80]. Furthermore, local programs and books and videos serve as supportive resources for children to learn about cancer [77, 78].

Acknowledgments This work was supported by Japan Agency for Medical Research and Development (AMED) Grant Number 20cm0106001h0005 and Health Labour Sciences Research Grant Number 20AD1001, 19EA1008.

References

1. U.S. Department of Energy. ARCHIVE: ethical, legal, and social issues. In: Human genome project information Archive 1990–2003; 2019. https://web.ornl.gov/sci/techresources/Human_Genome/elsi/index.shtml. Accessed 15 Feb 2021.
2. NIH National Human Genome Research Institute. Ethical, legal and social implications research program. 2020. http://www.genome.gov/10001618. Accessed 15 Feb 2021.
3. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell. 1993;72(6):971–83. https://doi.org/10.1016/0092-8674(93)90585-e.
4. Shaw MW. Testing for the Huntington gene: a right to know, a right not to know, or a duty to know. Am J Med Genet. 1987;26(2):243–6. https://doi.org/10.1002/ajmg.1320260202.
5. Hayden MR, Bloch M, Fox S, Crauford D. Ethical issues in preclinical testing in Huntington disease: response to Margery Shaw's invited editorial comment. Am J Med Genet. 1987;28(3):761–3. https://doi.org/10.1002/ajmg.1320280329.
6. Wexler NS. The Tiresias complex: Huntington's disease as a paradigm of testing for late-onset disorders. FASEB J. 1992;6(10):2820–5. https://doi.org/10.1096/fasebj.6.10.1386047.
7. Wexler NS. Clairvoyance and caution: repercussions from the human genome project. In: Kevles DJ, Hood L, editors. The code of codes: scientific and social issues in the human genome project. Cambridge: Harvard University Press; 1992. p. 211–43.
8. American Society of Human Genetics (ASHG). Statement of the American Society of Human Genetics on cystic fibrosis carrier screening. Am J Hum Genet. 1992;51(6):1443–4.

9. American Society of Human Genetics (ASHG). Statement of the American Society of Human Genetics on genetic testing for breast and ovarian cancer predisposition. Am J Hum Genet. 1994;55(5):i–iv.
10. United Nations Educational, Scientific and Cultural Organization (UNESCO). Universal declaration on the human genome and human rights. 1997. http://portal.unesco.org/en/ev.php-URL_ID=13177&URL_DO=DO_TOPIC&URL_SECTION=201.html. Accessed 15 Feb 2021.
11. Council of Europe. Convention for the protection of Human Rights and dignity of the human being with regard to the application of biology and medicine: convention on human rights and biomedicine. European Treaty Series. 1997. No. 164. http://rm.coe.int/CoERMPublicCommonSearchServices/DisplayDCTMContent?documentId=090000168007cf98. Accessed 15 Feb 2021.
12. United Nations Educational, Scientific and Cultural Organization (UNESCO). International declaration on human genetic data. 2003. http://portal.unesco.org/en/ev.php-URL_ID=17720&URL_DO=DO_TOPIC&URL_SECTION=201.html. Accessed 15 Feb 2021.
13. Knoppers BM, Deschênes M, Zawati MH, Tassé AM. Population studies: return of re-search results and incidental findings policy statement. Eur J Hum Genet. 2013;21(3):245–7. https://doi.org/10.1038/ejhg.2012.152.
14. DiMillo J, Samson A, Thériault A, Lowry S, Corsini L, Verma S, Tomiak E. Living with the BRCA genetic mutation: an uncertain conclusion to an unending process. Psychol Health Med. 2013;18(2):125–34. https://doi.org/10.1080/13548506.2012.687827.
15. Dean M. "It's not if I get cancer, it's when I get cancer": BRCA-positive patients' (un)certain health experiences regarding hereditary breast and ovarian cancer risk. Soc Sci Med. 2016;163:21–7. https://doi.org/10.1016/j.socscimed.2016.06.039.
16. Hamilton RJ, Innella NA, Bounds DT. Living with genetic vulnerability: a life course perspective. J Genet Couns. 2016;25(1):49–61. https://doi.org/10.1007/s10897-015-9877-x.
17. Hayden S, Mange S, Duquette D, Petrucelli N, Raymond VM. BRCA clinical network partners. Large, prospective analysis of the reasons patients do not pursue BRCA genetic testing following genetic counseling. J Genet Couns. 2017;26(4):859–65. https://doi.org/10.1007/s10897-016-0064-5.
18. Geller G, Botkin JR, Green MJ, Press N, Biesecker BB, Wilfond B, Grana G, Daly MB, Schneider K, Kahn MJ. Genetic testing for susceptibility to adult-onset cancer. The process and content of informed consent. JAMA. 1997;277(18):1467–74.
19. Charles C, Gafni A, Whelan T. Shared decision-making in the medical encounter: what does it mean? (or it takes at least two to tango). Soc Sci Med. 1997;44(5):681–92. https://doi.org/10.1016/s0277-9536(96)00221-3.
20. Charles C, Gafni A, Whelan T. Decision-making in the physician-patient encounter: revisiting the shared treatment decision-making model. Soc Sci Med. 1999;49(5):651–61. https://doi.org/10.1016/s0277-9536(99)00145-8.
21. Emanuel EJ, Emanuel LL. Four models of the physician-patient relationship. JAMA. 1992;267(16):2221–6.
22. Moore JA, Denton K, Epner D. Experience matters: a partnership between patient and physician. In: Gallagher C, Ewer M, editors. Ethical challenges in oncology: patient care, research, education, and economics. London: Academic Press; 2017. p. 19–39.
23. Whitney SN, McGuire AL, McCullough LB. A typology of shared decision making, informed consent, and simple consent. Ann Intern Med. 2004;140(1):54–9. https://doi.org/10.7326/0003-4819-140-1-200401060-00012.
24. Schrager S, Torell E, Ledford K, Elezaby M, Barroleit L, Sadowski E. Managing a woman with BRCA mutations? Shared decision-making is key. J Fam Pract. 2020;69(5):237–43.
25. Silverman TB, Kuperman GJ, Vanegas A, Sin M, Dimond J, Crew KD, Kukafka R. An applied framework in support of shared decision making about BRCA genetic testing. AMIA Annu Symp Proc. 2018:961–9.
26. Kohane IS, Masys DR, Altman RB. The incidentalome: a threat to genomic medicine. JAMA. 2006;296(2):212–5. https://doi.org/10.1001/jama.296.2.212.

27. Van Ness B. Genomic research and incidental findings. J Law Med Ethics. 2008;36(2):292–7. 212. https://doi.org/10.1111/j.1748-720X.2008.00272.x.
28. Wolf SM, Lawrenz FP, Nelson CA, Kahn JP, Cho MK, Clayton EW, Fletcher JG, Georgieff MK, Hammerschmidt D, Hudson K, Illes J, Kapur V, Keane MA, Koenig BA, Leroy BS, McFarland EG, Paradise J, Parker LS, Terry SF, Van Ness B, Wilfond BS. Managing incidental findings in human subjects research: analysis and recommendations. J Law Med Ethics. 2008;36(2):219–48. 211. https://doi.org/10.1111/j.1748-720X.2008.00266.x.
29. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, McGuire AL, Nussbaum RL, O'Daniel JM, Ormond KE, Rehm HL, Watson MS, Williams MS, Biesecker LG. American College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15(7):565–74. https://doi.org/10.1038/gim.2013.73.
30. Richardson A. Incidental findings and future testing methodologies: potential application of the ACMG 2013 recommendations. J Law Biosci. 2014;1(3):378–7. https://doi.org/10.1093/jlb/lsu028.
31. ACMG Board of Directors. ACMG policy statement: updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. Genet Med. 2015;17(1):68–9. https://doi.org/10.1038/gim.2014.151.
32. Presidential Commission for the Study of Bioethical Issues (PCSBI). Anticipate and communicate: ethical management of incidental and secondary findings in the clinical, research, and direct-to-consumer contexts. 2013. https://bioethicsarchive.georgetown.edu/pcsbi/sites/default/files/FINALAnticipateCommunicate_PCSBI_0.pdf. Accessed 15 Feb 2021.
33. Presidential Commission for the Study of Bioethical Issues (PCSBI). For clinicians: incidental and secondary findings. In: primers for professionals and the public. 2016. https://bioethicsarchive.georgetown.edu/pcsbi/sites/default/files/Clinician%20Primer%20Incidental%20Findings%2010.30.16.pdf. Accessed 15 Feb 2021.
34. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, Herman GE, Hufnagel SB, Klein TE, Korf BR, McKelvey KD, Ormond KE, Richards CS, Vlangos CN, Watson M, Martin CL, Miller DT. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19(2):249–55. https://doi.org/10.1038/gim.2016.190.
35. Miller DT, Lee K, Gordon AS, Amendola LM, Adelman K, Bale S, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(8):1391–8. https://doi.org/10.1038/s41436-021-01171-4.
36. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(8):1381–90. https://doi.org/10.1038/s41436-021-01172-3.
37. Miller DT, Lee K, Chung WK, Gordon AS, Herman GE, Klein TE, et al. Correction to: ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2021;23(8):1582–4. https://doi.org/10.1038/s41436-021-01278-8.
38. Robson ME, Bradbury AR, Arun B, Domchek SM, Ford JM, Hampel HL, Lipkin SM, Syngal S, Wollins DS, Lindor NM. American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility. J Clin Oncol. 2015;33(31):3660–7. https://doi.org/10.1200/JCO.2015.63.0996.
39. Farroni JS, Moore JA, Gallagher CM. An ethical framework for disclosing individual genetic findings to patients, research participants, and relatives. In: Gallagher C, Ewer M, editors. Ethical challenges in oncology: patient care, research, education, and economics. London: Academic Press; 2017. p. 275–96.
40. Hamilton JG, Shuk E, Genoff MC, Rodríguez VM, Hay JL, Offit K, Robson ME. Interest and attitudes of patients with advanced cancer with regard to secondary germline findings

from tumor genomic profiling. J Oncol Pract. 2017;13(7):e590–601. https://doi.org/10.1200/JOP.2016.020057.
41. Liang R, Meiser B, Smith S, Kasparian NA, Lewis CR, Chin M, Long GV, Ward R, Menzies AM, Harris-Wai JN, Kaur R. Advanced cancer patients' attitudes towards, and experiences with, screening for somatic mutations in tumours: a qualitative study. Eur J Cancer Care (Engl). 2017;26(6) https://doi.org/10.1111/ecc.12600.
42. Nagai A, Ri I, Muto K. Attitudes toward genomic tumor profiling tests in Japan: patients, family members, and the public. J Hum Genet. 2019;64(5):481–5. https://doi.org/10.1038/s10038-018-0555-3.
43. Davies G, Butow P, Napier CE, Bartley N, Juraskova I, Meiser B, Ballinger ML, Thomas DM, Schlub TE, Best MC, members of the PiGeOn Project. Advanced cancer patient knowledge of and attitudes towards tumor molecular profiling. Transl Oncol. 2020;13(9):100799. https://doi.org/10.1016/j.tranon.2020.100799.
44. Global Alliance for Genomics and Health. About us. 2021. https://www.ga4gh.org/about-us/. Accessed 1 Aug 2021.
45. Cline MS, Liao RG, Parsons MT, Paten B, Alquaddoomi F, Antoniou A, Baxter S, Brody L, Cook-Deegan R, Coffin A, Couch FJ, Craft B, Currie R, Dlott CC, Dolman L, den Dunnen JT, SOM D, Domchek SM, Easton D, Fischmann Z, Foulkes WD, Garber J, Goldgar D, Goldman MJ, Goodhand P, Harrison S, Haussler D, Kato K, Knoppers B, Markello C, Nussbaum R, Offit K, Plon SE, Rashbass J, Rehm HL, Robson M, Rubinstein WS, Stoppa-Lyonnet D, Tavtigian S, Thorogood A, Zhang C, Zimmermann M, BRCA Challenge Authors, Burn J, Chanock S, Rätsch G, Spurdle AB. BRCA challenge: BRCA exchange as a global resource for variants in BRCA1 and BRCA2. PLoS Genet. 2018 Dec 26;14(12):e1007752. https://doi.org/10.1371/journal.pgen.1007752.
46. Takashima K, Maru Y, Mori S, Mano H, Noda T, Muto K. Ethical concerns on sharing genomic data including patients' family members. BMC Med Ethics. 2018;19(1):61. https://doi.org/10.1186/s12910-018-0310-5.
47. Shabani M, Bezuidenhout L, Borry P. Attitudes of research participants and the general public towards genomic data sharing: a systematic literature review. Expert Rev Mol Diagn. 2014 Nov;14(8):1053–65. https://doi.org/10.1586/14737159.2014.961917.
48. Milne R, Morley KI, Howard H, Niemiec E, Nicol D, Critchley C, Prainsack B, Vears D, Smith J, Steed C, Bevan P, Atutornu J, Farley L, Goodhand P, Thorogood A, Kleiderman E. Middleton a; participant values work stream of the global Alliance for genomics and health. Trust in genomic data sharing among members of the general public in the UK, USA, Canada and Australia. Hum Genet. 2019 Dec;138(11–12):1237–46. https://doi.org/10.1007/s00439-019-02062-0.
49. Middleton A, Milne R, Almarri MA, Anwer S, Atutornu J, Baranova EE, Bevan P, Cerezo M, Cong Y, Critchley C, Fernow J, Goodhand P, Hasan Q, Hibino A, Houeland G, Howard HC, Hussain SZ, Malmgren CI, Izhevskaya VL, Jędrzejak A, Jinhong C, Kimura M, Kleiderman E, Leach B, Liu K, Mascalzoni D, Mendes Á, Minari J, Wang N, Nicol D, Niemiec E, Patch C, Pollard J, Prainsack B, Rivière M, Robarts L, Roberts J, Romano V, Sheerah HA, Smith J, Soulier A, Steed C, Stefànsdóttir V, Tandre C, Thorogood A, Voigt TH, West AV, Yoshizawa G, Morley KI. Global Public Perceptions of genomic data sharing: what shapes the willingness to donate DNA and health data? Am J Hum Genet. 2020 Oct 1;107(4):743–752. doi: https://doi.org/10.1016/j.ajhg.2020.08.023.
50. Hassan L, Dalton A, Hammond C, Tully MP. A deliberative study of public attitudes towards sharing genomic data within NHS genomic medicine services in England. Public Underst Sci. 2020 Oct;29(7):702–17. https://doi.org/10.1177/0963662520942132.
51. Langmead B, Nellore A. Cloud computing for genomic data analysis and collaboration. Nat Rev Genet. 2018 Apr;19(4):208–19. https://doi.org/10.1038/nrg.2017.113.
52. Carter AB. Considerations for genomic data privacy and security when working in the cloud. J Mol Diagn. 2019 Jul;21(4):542–52. https://doi.org/10.1016/j.jmoldx.2018.07.009.
53. Tadman M, Roberts D, Foulkes M, editors. Oxford handbook of cancer nursing second edition. New York: Oxford University Press; 2019. p. 607–12.

54. Thompson R, Trevena L. Demystifying decision aids: a practical guide for clinicians. In: Elwyn G, Edwards A, Thompson R, editors. Shared decision making in health care: achieving evidence-based patient choice. 3rd ed. New York: Oxford University Press; 2016. p. 51–6.
55. O'Connor AM, Stacey D, Jacobsen MJ. Ottawa personal decision guides. In: Patient decision aids. Ottawa Hospital Research Institute & University of Ottawa; 2015. https://decisionaid.ohri.ca/decguide.html. Accessed 15 Feb 2021.
56. Yamauchi H, Nakagawa C, Kobayashi M, Kobayashi Y, Mano T, Nakamura S, Arai M. Cost-effectiveness of surveillance and prevention strategies in BRCA1/2 mutation carriers. Breast Cancer. 2018;25(2):141–50. https://doi.org/10.1007/s12282-017-0803-y.
57. Park SY, Kim Y, Kim S. Factors associated with the decision to undergo risk-reducing salpingo-oophorectomy among women at high risk for hereditary breast and ovarian cancer: a systematic review. Korean J Women Health Nurs. 2020; https://doi.org/10.4069/kjwhn.2020.11.19.
58. Carbine NE, Lostumbo L, Wallace J, Ko H. Risk-reducing mastectomy for the prevention of primary breast cancer. Cochrane Database Syst Rev. 2018;4(4):CD002748. https://doi.org/10.1002/14651858.CD002748.pub4.
59. Trister R, Jacobson M, Nguyen P, Sobel M, Allen L, Narod SA, Kotsopoulos J. Patient reported experiences following laparoscopic prophylactic bilateral salpingo-oophorectomy or salpingectomy in an ambulatory care hospital. Familial Cancer. 2020; https://doi.org/10.1007/s10689-020-00208-y.
60. Arai M, Yokoyama S, Watanabe C, Yoshida R, Kita M, Okawa M, Sakurai A, Sekine M, Yotsumoto J, Nomura H, Akama Y, Inuzuka M, Nomizu T, Enomoto T, Nakamura S. Genetic and clinical characteristics in Japanese hereditary breast and ovarian cancer: first report after establishment of HBOC registration system in Japan. J Hum Genet. 2018;63(4):447–57. https://doi.org/10.1038/s10038-017-0355-1.
61. Jonsen AR, Siegler M, Winslade WJ. Clinical ethics: a practical approach to ethical decisions in clinical medicine. 3rd ed. New York: McGraw-Hill; 1992.
62. Muto K, Takashima K. Clinical ethics in risk-reducing surgery in hereditary breast and ovarian cancer. J Familial Tumors. 2017;17(1):2–7. (in Japanese)
63. Gibbs HR, Gallagher CM. Diversity and health care. In: Gallagher C, Ewer M, editors. Ethical challenges in oncology: patient care, research, education, and economics. London: Academic Press; 2017. p. 261–74.
64. Schleiter KE. A Physician's duty to warn third parties of hereditary risk. Virtual Mentor. 2009;11(9):697–700. https://doi.org/10.1001/virtualmentor.2009.11.9.hlaw1-0909.
65. Rothstein MA. Reconsidering the duty to warn genetically at-risk relatives. Genet Med. 2018;20(3):285–90. https://doi.org/10.1038/gim.2017.257.
66. American Medical Association. AMA code of ethics, chapter 4: ethics of Genetics & Reproductive Medicine. In: code of medical ethics overview. 2016. https://www.ama-assn.org/delivering-care/ethics/code-medical-ethics-genetics-reproductive-medicine. Accessed 15 Feb 2021.
67. ABC v St George's Healthcare NHS Trust & Ors [2015] EWHC 1394 (QB).
68. ABC v St George's Healthcare NHS Trust & Ors [2017] EWCA Civ 336 (CA).
69. ABC v St George's Healthcare NHS Trust & Ors [2020] EWHC 455 (QB).
70. d'Audiffret Van Haecke D, de Montgolfier S. Genetic diseases and information to relatives: practical and ethical issues for professionals after introduction of a legal framework in France. Eur J Hum Genet. 2018;26(6):786–95. https://doi.org/10.1038/s41431-018-0103-9.
71. Derbez B, de Pauw A, Stoppa-Lyonnet D, Galactéros F, de Montgolfier S. Familial disclosure by genetic healthcare professionals: a useful but sparingly used legal provision in France. J Med Ethics. 2019;45(12):811–6. https://doi.org/10.1136/medethics-2018-105212.
72. Meggiolaro N, Barlow-Stewart K, Dunlop K, Newson AJ, Fleming J. Disclosure to genetic relatives without consent - Australian genetic professionals' awareness of the health privacy law. BMC Med Ethics. 2020;21(1):13. https://doi.org/10.1186/s12910-020-0451-1.
73. Tiller J, Bilkey G, Macintosh R, O'Sullivan S, Groube S, Palover M, Pachter N, Rothstein M, Lacaze P, Otlowski M. Disclosing genetic information to family members without consent: five Australian case studies. Eur J Med Genet. 2020;63(11):104035. https://doi.org/10.1016/j.ejmg.2020.104035.

74. Hallowell N, Foster C, Eeles R, Ardern-Jones A, Murday V, Watson M. Balancing autonomy and responsibility: the ethics of generating and disclosing genetic information. J Med Ethics. 2003;29(2):74–9. Discussion 80–3. https://doi.org/10.1136/jme.29.2.74.
75. Forrest Keenan K, Simpson SA, Wilson BJ, Van Teijlingen ER, Mckee L, Neva Haites N, Matthews E. 'It's their blood not mine': Who's responsible for (not) telling relatives about genetic risk? Health Risk Soc. 2005;7(3):209–26. https://doi.org/10.1080/13698570500229606.
76. Forrest K, Simpson SA, Wilson BJ, van Teijlingen ER, McKee L, Haites N, Matthews E. To tell or not to tell: barriers and facilitators in family communication about genetic risk. Clin Genet. 2003;64(4):317–26. https://doi.org/10.1034/j.1399-0004.2003.00142.x.
77. Seenandan-Sookdeo KA, Hack TF, Lobchuk M, Murphy L, Marles S. Parental decision making regarding the disclosure or nondisclosure of a mutation-positive BRCA1/2 test result to minors. Oncol Nurs Forum. 2016;43(3):330–41. https://doi.org/10.1188/16.ONF.330-341.
78. Julian-Reynier C, Eisinger F, Chabal F, Lasset C, Noguès C, Stoppa-Lyonnet D, Vennin P, Sobol H. Disclosure to the family of breast/ovarian cancer genetic test results: patient's willingness and associated factors. Am J Med Genet. 2000;94(1):13–8. https://doi.org/10.1002/1096-8628(20000904)94:1<13::aid-ajmg4>3.0.co;2-t.
79. Healey E, Taylor N, Greening S, Wakefield CE, Warwick L, Williams R, Tucker K. Quantifying family dissemination and identifying barriers to communication of risk information in Australian BRCA families. Genet Med. 2017;19(12):1323–31. https://doi.org/10.1038/gim.2017.52.
80. Patenaude AF, DeMarco TA, Peshkin BN, Valdimarsdottir H, Garber JE, Schneider KA, Hewitt L, Hamilton J, Tercyak KP. Talking to children about maternal BRCA1/2 genetic test results: a qualitative study of parental perceptions and advice. J Genet Couns. 2013;22(3):303–14. https://doi.org/10.1007/s10897-012-9549-z.

PARP Inhibitors: Mechanism of Action

18

Shigeaki Sunada and Yoshio Miki

Abstract

Synthetic lethal therapy with poly(ADP-ribose) polymerase (PARP) inhibitors continues to attract attention as an effective treatment for hereditary breast and ovarian cancer (HBOC) with reduced DNA repair function because the treatment specifically causes high sensitivity in tumors. This effect is caused by the combination of PARP inhibitor-induced DNA double-strand break (DSB) formation and functional deficiency of homologous recombination (HR) repair, which is the main repair pathway for DNA damage. On the other hand, the clonal evolution in tumors as a tumor heterogeneity causes functional changes at each stage of the synthetic lethal pathway, resulting in the emergence of cancer cells with acquired resistance to PARP inhibitors. Various molecular mechanisms of the acquired resistance have been clarified. This chapter describes the synthetic lethal mechanism of PARP inhibitors, the mechanism of acquired resistance to PARP inhibitors, and the development of various PARP inhibitors.

Keywords

BRCA · Homologous recombination · PARP inhibitor · Synthetic lethality Chemoresistance

S. Sunada (✉) · Y. Miki
Department of Molecular Genetics, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan
e-mail: sunada.mgen@mri.tmd.ac.jp; miki.mgen@mri.tmd.ac.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_18

18.1 Introduction

Induction of DNA damage is one of the main mechanisms of action of anticancer drugs in cancer treatment. There are various types of damage, such as base damage, DNA cross-linking, and DNA strand breaks, and the accumulation of these damages in the cell causes cell death. However, most cells, including cancer cells, have mechanisms for repair of DNA damage and maintenance of genomic homeostasis. Understanding DNA repair mechanisms is important for effective cancer treatment.

Hereditary breast and ovarian cancer syndrome (HBOC) may be caused by DNA repair dysfunction due to mutations in genes such as breast cancer susceptibility gene 1 or 2 (*BRCA1 or BRCA2*) [1, 2]. These genes play a critical role in homologous recombination (HR) repair, which is a major repair pathway for DNA double-strand breaks (DSBs) [3, 4]. In other words, it is indicated that disruption of genome homeostasis by abnormal DNA repair-associated genomic instability increases the risk of carcinogenesis. In contrast, cells with dysfunctional DNA repair are highly sensitive to DNA damage. Accordingly, tumor cells with abnormalities in DNA repair can be selective targets for treatment because the surrounding normal tissues with normal DNA repair capacity are relatively less sensitive to DNA damage. Synthetic lethal therapy with poly(ADP-ribose) polymerase (PARP) inhibitors is a strategy which may reduce side effects by causing lethal damage specific to cancer cells [5, 6]. Clinical studies using PARP inhibitors for HBOC have progressed steadily, and novel PARP inhibitors have been developed.

Although synthetic lethal therapy has been developed as an effective treatment method, reduced therapeutic effect has been reported in several cases. One of the causes of the reduced therapeutic effect is tumor heterogeneity, as a small number of cancer cells acquire resistance to anticancer drugs due to various factors, eventually resulting in cancer recurrence and metastasis [7]. Multiple therapeutic strategies are being proposed to overcome chemoresistance in these tumors.

This chapter explores the entire landscape of events related to treatment with PARP inhibitors, the mechanism of synthetic lethality, the acquisition of chemoresistance, and the development of PARP inhibitors in the market.

18.2 Synthetic Lethality with PARP Inhibitors

Reduction of side effects of anticancer drugs is an important issue in molecular targeted therapy. HBOC with reduced HR repair activity demonstrate higher sensitivity to DNA-damaging anticancer agents such as PARP inhibitors. This specific sensitivity is called synthetic lethality, and several PARP inhibitors have gained attention and have been approved as therapeutic agents for HBOC. The detailed mechanism leading to synthetic lethality with PARP inhibitors is described here.

18.2.1 Function of PARPs

The *PARP* family of genes consists of 17 genes and is involved in various cell functions including DNA damage repair [8]. PARPs play an important role in the repair of DNA single-strand breaks (SSBs) [9–11]. Oxidative stress that spontaneously occurs due to the metabolic process in cells frequently causes endogenous DNA damage, and the most typical damage is base damage. The damaged base is removed by base excision repair (BER), and an SSB is formed in the process (Fig. 18.1). PARPs participate in the repair process of these breaks and contribute to maintenance of genome homeostasis. Specifically, when a base damage occurs, DNA glycosylase

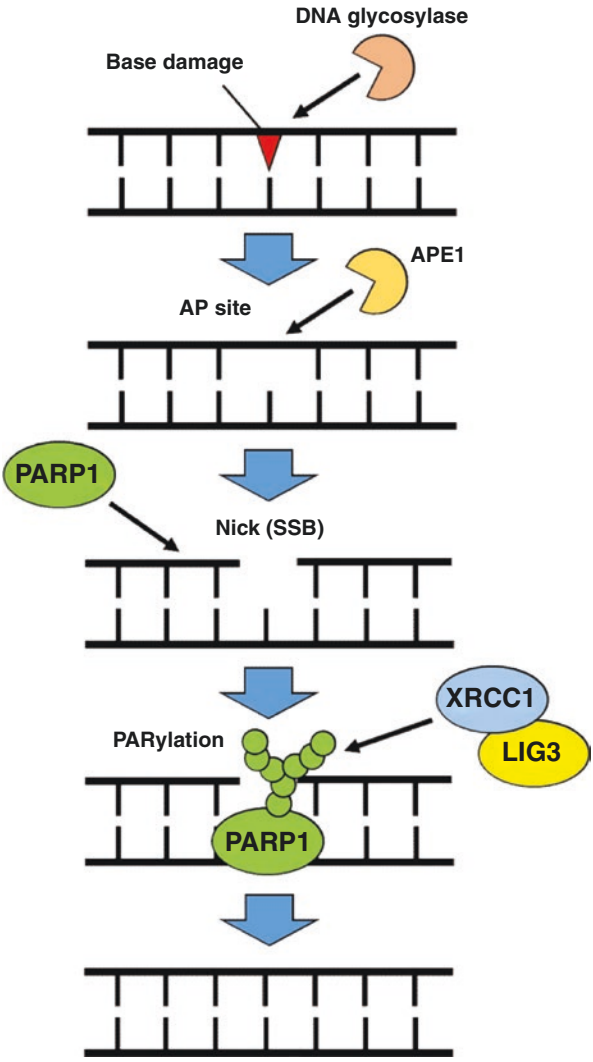


Fig. 18.1 Base excision repair mechanism. Poly(ADP-ribose) polymerase 1 (PARP1) participates in the single-strand break (SSB) repair process after a nick in the DNA is formed

removes the base from the nucleotide, resulting in an apurinic/apyrimidinic (AP) site. The AP site is excised by AP endonuclease 1 (APE1) to form a nick (SSB). PARP1, which is the first cloned and most analyzed PARP family member, is then focused here [12]. In the SSB repair pathway, PARP1 binds to a nicked DNA substrate and poly(ADP-ribose) (PAR) generated using nicotinamide adenine dinucleotide (NAD+) as substrate links to PARP1 in a chained manner to form a PAR chain (PARylation). This branch structure serves as a marker for recruitment of SSB repair factors like X-ray repair cross-complementing protein 1 (XRCC1), DNA ligase III, and DNA polymerase β, thereby facilitating SSB repair [13].

18.2.2 PARP Inhibitor-Induced DNA Double-Strand Break

Inhibition of PARylation and PARP trapping are the major modes of inhibition of PARP function by PARP inhibitors. Benzamide suppresses PARylation as an NAD+ analog, but does not significantly induce DNA damage, while other PARP inhibitors like olaparib with an NAD+ structure induce DNA damage [14, 15]. These PARP inhibitors bind to PARP1 and change its conformation, resulting in a DNA-PARP1 complex called PARP trapping [16, 17]. PARP1 binding to nicked DNA substrate collides with the replication fork and blocks its progression. Furthermore, the stalled replication fork causes activation of endonucleases to form DNA DSB because of the replication fork collapse [18]. The mechanism causing PARP trapping-induced DSB is clinically utilized for synthetic lethal therapy with PARP inhibitors.

18.2.3 DSB Repair Inhibition After Replication Fork Collapse

DSBs in DNA are mainly repaired by either HR or nonhomologous end joining (NHEJ) pathways. HR functions specifically in the S to G2 cell cycle phases where sister chromatids are present. This repair is considered an error-free repair mechanism as it repairs DNA accurately using the homologous DNA sequence in sister chromatids as a template. In contrast, NHEJ is a repair pathway that binds the cleaved DNA ends directly and hence is considered an error-prone repair mechanism. The cleaved ends formed in the paired DNA due to DNA-damaging factors such as radiation, are rejoined with small DNA scrapes to blunt them, resulting in some base deletions. However, NHEJ is an essential repair mechanism for maintenance of genome homeostasis as a rapid repair pathway [19] and as most parts of the DNA contain noncoding genetic information.

DSBs caused by PARP inhibitors form due to replication stress and continuous replication fork collapse. A DSB formed in the process is characterized as a single-ended DSB (seDSB) as there are no paired DNA cleaved ends. Accordingly, seDSB returns to the original DNA sequence when repaired by HR; however, seDSB rejoining with an unpaired DNA end by NHEJ causes chromosomal aberrations like chromosome rearrangement, leading to cell death [20]. Thus, HR repair is essential for accurate repair of DSB caused by replication stress for cell survival.

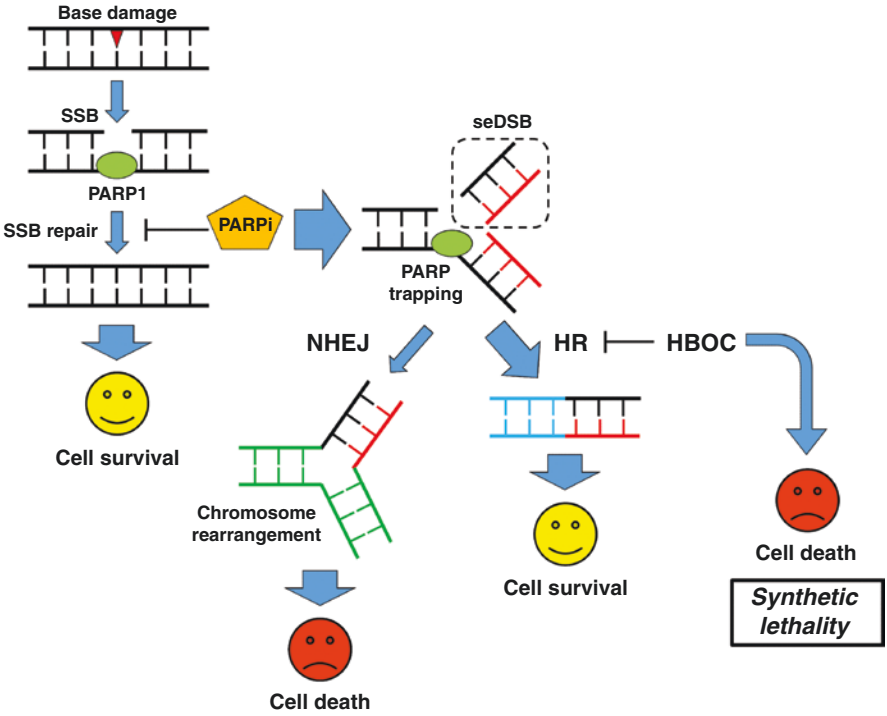


Fig. 18.2 Mechanisms of synthetic lethality with PARP inhibitors. Cell death event selective to tumor cells is caused by a combined phenomenon of PARP inhibitor-induced double-strand break (DSB) formation and dysfunctional HR repair-induced DNA repair inhibition

In summary, PARP inhibitors induce PARP trapping which inhibits BER and SSB repair and form seDSB with high HR repair requirements for accurate DNA repair. HBOC with dysfunctional HR repair cannot repair the DNA damage, resulting in synthetic lethality (Fig. 18.2). Normal tissues around the tumor with normal HR repair functions are less sensitive to PARP inhibitors. Synthetic lethal therapy utilizes this difference in DNA damage sensitivity to reduce side effects.

18.3 Mechanisms of Resistance to PARP Inhibitors

Recent genome analyses have revealed that a large number of subclones form tumors. This phenomenon is called tumor heterogeneity and is known to contribute to cancer chemotherapy resistance. It has been hypothesized that the plasticity of the genome causes cancer recurrence and metastasis by proliferation of a small number of clones that acquire treatment resistance factors [7]. Clinical cases have elucidated mechanisms of treatment resistance in synthetic lethal therapy with PARP inhibitors for HBOC. In this context, various mechanisms by which cancer cells with higher sensitivity to PARP inhibitors acquire chemoresistance have been reported. The mechanisms of acquired resistance in synthetic lethal therapy and

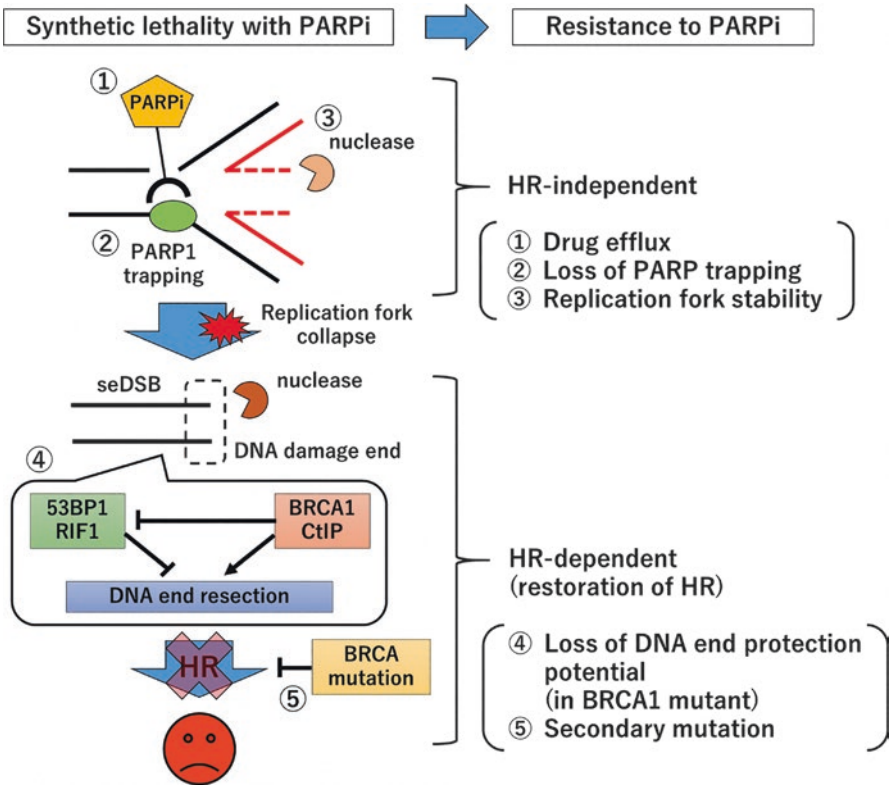


Fig. 18.3 Acquisition of PARP inhibitor resistance in synthetic lethality. Resistance to PARP inhibitors is acquired via homologous recombination (HR)-independent or HR-dependent mechanisms

their classification based on HR repair dependence or independence are described here (Fig. 18.3).

18.3.1 Restoration of HR Repair

Synthetic lethal therapy with PARP inhibitors mainly targets dysfunctional HR repair in cancer cells. Therefore, the mechanism by which loss of HR repair function is restored reduces sensitivity to PARP inhibitors and leads to acquisition of resistance.

18.3.1.1 Secondary Mutation

A mutation, additional to the deleterious mutant of the HR repair function, causes resistance to PARP inhibitors or other DNA-damaging agents. A frequently reported example is the mechanism by which a secondary mutation in a frameshift functional deletion mutation causes return to the functional gene. Several studies found that

secondary mutations in dysfunctional *BRCA2* caused resistance to DNA-damaging agents such as cisplatin and PARP inhibitors [21, 22]. Most cases of resistance were due to the functional restoration of the DNA-binding domain, nuclear localization signal, and RAD51-binding domain, which are important protein domains in the HR repair function of BRCA2. Subsequently secondary mutation-induced restoration of DNA repair function was observed in clinical studies for genes that play critical roles in the HR repair pathway, such as *BRCA1* and *RAD51* [23, 24]. These results indicate that the same phenomenon may also be observed for factors including partner and localizer of BRCA2 (PALB2) [25] and BRCA1-associated RING domain protein 1 (BARD1) [26], which interact with BRCA2 and BRCA1 in the HR repair process.

18.3.1.2 Loss of DNA End Protection Potential

Defective p53-binding protein 1 (53BP1) in BRCA1 mutant cells was reported as a potential acquired resistance mechanism for synthetic lethal therapy [27, 28]. The choice of DSB repair pathway is involved in the background of this phenomenon. DSB is repaired mainly via the HR or NHEJ repair pathways through a controlled mechanism. DNA end resection, which is the gateway to the HR repair pathway, is considered an important turning point in the selection of a repair pathway [29, 30]. The major regulators are 53BP1 and Rap1-interacting factor 1 (RIF1), which protect cleaved ends of the DNA from resection [31], while BRCA1 and C-terminal-binding protein-interacting protein (CtIP) promote resection [32]. When a DSB is formed, 53BP1 immediately attaches in the vicinity of the cleaved end, and the 53BP1-binding factor, RIF1, forms a complex with 53BP1 to prevent resection. In contrast, the complex of CtIP and BRCA1 promotes resection by causing the removal of RIF1 and repositioning of 53BP1. Resection activity is suppressed in cells lacking BRCA1 function, but further deletion of 53BP1 function restores the activity. Hence, the HR repair capacity is restored, resulting in resistance to PARP inhibitors. Interestingly, dysfunctional 53BP1 does not cause resistance in cells lacking BRCA2 function, which is another gene responsible for HBOC [33]. This indicates that BRCA1 plays an important role in HR repair but is mainly involved in the upstream repair pathway of HR repair, while BRCA2 is an indispensable factor in HR repair.

18.3.2 HR Repair-Independent Acquired Resistance

DSB formation due to inhibition of PARP function is a major factor in the synthetic lethality of PARP inhibitors. Recently, various HR repair-independent mechanisms of acquired resistance that avoid DSB formation have been reported.

18.3.2.1 Replication Fork Stability

PARP inhibitors trap PARP on the DNA strand and cause replication stress that arrests the replication fork, resulting in the formation of DSB. Stabilization of the replication fork contributes to the acquisition of resistance in synthetic lethality as a

mechanism for avoiding replication stress [34]. When replication is stalled due to PARP trapping, replication fork reversal forms a four-way junction, as DNA damage tolerance occurs to bypass the damage. On the other hand, BRCA1/2 function to prevent the degradation of nascent DNA during replication stress-induced fork reversal [35]. Meiotic recombination 11 (MRE11) nuclease was activated in BRCA1/2-deficient cells, and the replication fork was degraded. In other words, reduced function of the factor involved in the degradation stabilizes the replication fork and causes resistance to PARP inhibitors. Stabilization of replication forks by inhibition of MRE11 nuclease activity due to dysfunction of PAX transactivation-domain-interacting protein (PTIP) and subsequent methyl methanesulfonate and ultraviolet-sensitive gene clone 81 (MUS81) nuclease activity due to dysfunction of enhancer of zeste homolog 2 (EZH2) in BRCA-deficient cells have been reported [36, 37].

18.3.2.2 Loss of PARP Trapping

Trapping of PARPs on DNA strands is a critical step in induction of DSBs. Several mechanisms of blocking PARP trapping are reported to cause resistance to synthetic lethality. One such mechanism includes a decrease in the binding ability of PARP1 to DNA due to mutations in PARP1 itself. Point mutations in the zinc finger, DNA-binding domain, WGR, and HD domains of PARP1 reduce PARP1 binding to the DNA strand and inhibit PARP trapping [38]. On the other hand, the loss of ubiquitin ligase function targeting PAR reduces PARP1 turnover and promotes PARP trapping, thereby causing a sensitizing effect to PARP inhibitor [39]. This phenomenon indicates that activation of proteasome-mediated PARP1 degradation suppresses PARP trapping and causes resistance to PARP inhibitors.

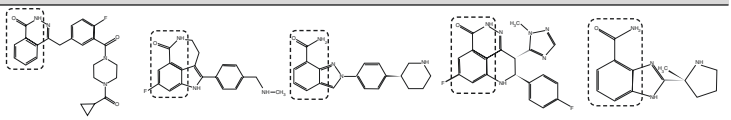
18.3.2.3 Drug Efflux

Although drug efflux is not unique to PARP inhibitors, it is a known drug resistance mechanism. The drug transporter gene ATP-binding cassette subfamily B member 1 (ABCB1) is upregulated in anticancer drug-resistant cells [14]. A study has demonstrated a significant relation between drug efflux by ABCB1 and the resistance acquired against PARP inhibitors in breast and ovarian cancers [40].

18.4 Development of PARP Inhibitors

Various PARP inhibitors have been developed, with approval of some inhibitors achieved after clinical research as therapeutic agents for various types of cancers including HBOC (Table 18.1). Currently, the FDA has approved four PARP inhibitors. The first PARP inhibitor approved was olaparib in December 2014 for the treatment of patients with germline BRCA-mutated advanced ovarian cancer. Since then, the scope of its clinical use has expanded, and it has been approved for metastatic breast cancer with BRCA mutation (January 2018) and metastatic

Table. 18.1 Clinical PARP inhibitors

PARP inhibitor	Olaparib	Rucaparib	Niraparib	Talazoparib	Veriparib
Structure (NAD+ structure in dotted line)					
Clinical practice (approval by FDA)	• Ovarian • Breast • Prostate • Pancreatic	• Ovarian • Prostate	• Ovarian	• Breast	N/A
PARP1 enzyme IC50 (nM) [Ref 44]	1.4	3.2	16.7	1.1	3.3
PARP trapping (relative level)	moderate	moderate	moderate	high	low

castrate-resistant prostate cancer with HR repair gene mutation (May 2020). High efficacy has been demonstrated by synthetic lethal therapy in treatments targeting HR repair function. Other PARP inhibitors approved include rucaparib (December 2016) for advanced ovarian cancer treatment; niraparib (March 2017) for recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer maintenance treatment; and talazoparib (October 2018) for BRCA mutation human epidermal growth factor receptor 2 (HER2)-negative locally advanced/metastatic breast cancer. In addition to these four PARP inhibitors, veliparib has been reported to be a PARP inhibitor and has been well investigated. It is currently under clinical trials as a treatment for non-small cell lung carcinoma (NSCLC), breast, and ovarian cancers. Remarkably, some PARP inhibitors are expected to have therapeutic effects on cancer cells with no BRCA mutations.

The effectiveness of PARP inhibitors as therapeutic agents may be strongly dependent on the PARP trapping potency. For the five representative PARP inhibitors mentioned above, the relationship between PARP trapping potency and inhibitor-induced cytotoxicity has been well studied. These trapping potencies can be roughly divided into three categories, with talazoparib showing the highest trapping potency, veliparib exhibiting the lowest trapping potency, and niraparib, olaparib, and rucaparib demonstrating relatively moderate trapping potencies; and cytotoxicities corresponding to these orders have been confirmed in vitro [41–43]. Interestingly, the IC50 for PARP1 enzyme did not differ in the dose range of action for each PARP inhibitor [44]. In other words, the results indicate that there may be a small correlation between the PARP trapping potency and the inhibitory activity of PARP. Therefore, it may be difficult to predict the PARP trapping potency only by examining the inhibitory activity of PARP, when exploring PARP inhibitors for synthetic lethal therapy for drug discovery. There points remain unclear; however, it is expected that the details will be clarified and more various inhibitors will be developed in the future.

18.5 Perspective

As described above, synthetic lethal therapy with PARP inhibitors for HBOC has attracted attention as an effective treatment, and many PARP inhibitors have been developed. However, the acquisition of resistance through the evolution of cancer cells and tissue is a serious issue which ought to be overcome in the future. Recently, a clinical study against the resistance mechanism causing re-restoration of HR repair capacity, such as secondary mutation, has been reported. The study is based on the strategy of regaining sensitivity of PARP inhibitors combined with a phosphoinositide 3-kinase (PI3K) inhibitor targeting the restored HR repair pathway [45]. To summarize, it is necessary to clarify various resistance mechanisms and establish strategies to overcome chemoresistance.

References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266(5182):66–71.
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature. 1995;378(6559):789–92.
3. Snouwaert JN, Gowen LC, Latour AM, Mohn AR, Xiao A, DiBiase L, et al. BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a brca1 transgene. Oncogene. 1999;18(55):7900–7.
4. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. Mol Cell. 2001;7(2):263–72.
5. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434(7035):917–21.
6. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434(7035):913–7.
7. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. Nat Rev Clin Oncol. 2018;15(2):81–94.
8. Ame JC, Spenlehauer C, de Murcia G. The PARP superfamily. Bioessays. 2004;26(8):882–93.
9. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J. 1999;342(Pt 2):249–68.
10. Fisher AE, Hochegger H, Takeda S, Caldecott KW. Poly(ADP-ribose) polymerase 1 accelerates single-strand break repair in concert with poly(ADP-ribose) glycohydrolase. Mol Cell Biol. 2007;27(15):5597–605.
11. Hegde ML, Hazra TK, Mitra S. Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res. 2008;18(1):27–47.
12. Alkhatib HM, Chen DF, Cherney B, Bhatia K, Notario V, Giri C, et al. Cloning and expression of cDNA for human poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A. 1987;84(5):1224–8.
13. Leppard JB, Dong Z, Mackey ZB, Tomkinson AE. Physical and functional interaction between DNA ligase IIIalpha and poly(ADP-ribose) polymerase 1 in DNA single-strand break repair. Mol Cell Biol. 2003;23(16):5919–27.
14. Rottenberg S, Jaspers JE, Kersbergen A, van der Burg E, Nygren AO, Zander SA, et al. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A. 2008;105(44):17079–84.

15. Evers B, Drost R, Schut E, de Bruin M, van der Burg E, Derksen PW, et al. Selective inhibition of BRCA2-deficient mammary tumor cell growth by AZD2281 and cisplatin. Clin Cancer Res. 2008;14(12):3916–25.
16. Langelier MF, Planck JL, Roy S, Pascal JM. Structural basis for DNA damage-dependent poly(ADP-ribosyl)ation by human PARP-1. Science. 2012;336(6082):728–32.
17. Langelier MF, Zandarashvili L, Aguiar PM, Black BE, Pascal JM. NAD(+) analog reveals PARP-1 substrate-blocking mechanism and allosteric communication from catalytic center to DNA-binding domains. Nat Commun. 2018;9(1):844.
18. Heacock ML, Stefanick DF, Horton JK, Wilson SH. Alkylation DNA damage in combination with PARP inhibition results in formation of S-phase-dependent double-strand breaks. DNA Repair. 2010;9(8):929–36.
19. Shibata A, Conrad S, Birraux J, Geuting V, Barton O, Ismail A, et al. Factors determining DNA double-strand break repair pathway choice in G2 phase. EMBO J. 2011;30(6):1079–92.
20. Patel AG, Sarkaria JN, Kaufmann SH. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci U S A. 2011;108(8):3406–11.
21. Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, et al. Resistance to therapy caused by intragenic deletion in BRCA2. Nature. 2008;451(7182):1111–5.
22. Sakai W, Swisher EM, Jacquemont C, Chandramohan KV, Couch FJ, Langdon SP, et al. Functional restoration of BRCA2 protein by secondary BRCA2 mutations in BRCA2-mutated ovarian carcinoma. Cancer Res. 2009;69(16):6381–6.
23. Swisher EM, Sakai W, Karlan BY, Wurz K, Urban N, Taniguchi T. Secondary BRCA1 mutations in BRCA1-mutated ovarian carcinomas with platinum resistance. Cancer Res. 2008;68(8):2581–6.
24. Kondrashova O, Nguyen M, Shield-Artin K, Tinker AV, Teng NNH, Harrell MI, et al. Secondary somatic mutations restoring RAD51C and RAD51D associated with acquired resistance to the PARP inhibitor Rucaparib in high-grade ovarian carcinoma. Cancer Discov. 2017;7(9):984–98.
25. Hartford SA, Chittela R, Ding X, Vyas A, Martin B, Burkett S, et al. Interaction with PALB2 is essential for maintenance of genomic integrity by BRCA2. PLoS Genet. 2016;12(8):e1006236.
26. Westermark UK, Reyngold M, Olshen AB, Baer R, Jasin M, Moynahan ME. BARD1 participates with BRCA1 in homology-directed repair of chromosome breaks. Mol Cell Biol. 2003;23(21):7926–36.
27. Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol. 2010;17(6):688–95.
28. Jaspers JE, Kersbergen A, Boon U, Sol W, van Deemter L, Zander SA, et al. Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. Cancer Discov. 2013;3(1):68–81.
29. Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, et al. Human CtIP promotes DNA end resection. Nature. 2007;450(7169):509–14.
30. Huertas P, Jackson SP. Human CtIP mediates cell cycle control of DNA end resection and double strand break repair. J Biol Chem. 2009;284(14):9558–65.
31. Chapman JR, Barral P, Vannier JB, Borel V, Steger M, Tomas-Loba A, et al. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. Mol Cell. 2013;49(5):858–71.
32. Isono M, Niimi A, Oike T, Hagiwara Y, Sato H, Sekine R, et al. BRCA1 directs the repair pathway to homologous recombination by promoting 53BP1 Dephosphorylation. Cell Rep. 2017;18(2):520–32.
33. Cruz-Garcia A, Lopez-Saavedra A, Huertas P. BRCA1 accelerates CtIP-mediated DNA-end resection. Cell Rep. 2014;9(2):451–9.
34. Ray Chaudhuri A, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature. 2016;535(7612):382–7.

35. Mijic S, Zellweger R, Chappidi N, Berti M, Jacobs K, Mutreja K, et al. Replication fork reversal triggers fork degradation in BRCA2-defective cells. Nat Commun. 2017;8(1):859.
36. Rondinelli B, Gogola E, Yucel H, Duarte AA, van de Ven M, van der Sluijs R, et al. EZH2 promotes degradation of stalled replication forks by recruiting MUS81 through histone H3 trimethylation. Nat Cell Biol. 2017;19(11):1371–8.
37. Lemacon D, Jackson J, Quinet A, Brickner JR, Li S, Yazinski S, et al. MRE11 and EXO1 nucleases degrade reversed forks and elicit MUS81-dependent fork rescue in BRCA2-deficient cells. Nat Commun. 2017;8(1):860.
38. Pettitt SJ, Krastev DB, Brandsma I, Drean A, Song F, Aleksandrov R, et al. Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. Nat Commun. 2018;9(1):1849.
39. Gatti M, Imhof R, Huang Q, Baudis M, Altmeyer M. The ubiquitin ligase TRIP12 limits PARP1 trapping and constrains PARP inhibitor efficiency. Cell Rep. 2020;32(5):107985.
40. Christie EL, Pattnaik S, Beach J, Copeland A, Rashoo N, Fereday S, et al. Multiple ABCB1 transcriptional fusions in drug resistant high-grade serous ovarian and breast cancer. Nat Commun. 2019;10(1):1295.
41. Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, et al. Trapping of PARP1 and PARP2 by clinical PARP inhibitors. Cancer Res. 2012;72(21):5588–99.
42. Murai J, Huang SY, Renaud A, Zhang Y, Ji J, Takeda S, et al. Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Mol Cancer Ther. 2014;13(2):433–43.
43. Hopkins TA, Ainsworth WB, Ellis PA, Donawho CK, DiGiammarino EL, Panchal SC, et al. PARP1 trapping by PARP inhibitors drives cytotoxicity in both cancer cells and healthy bone marrow. Molecular Cancer Res: MCR. 2019;17(2):409–19.
44. Thorsell AG, Ekblad T, Karlberg T, Low M, Pinto AF, Tresaugues L, et al. Structural basis for potency and promiscuity in poly(ADP-ribose) polymerase (PARP) and Tankyrase inhibitors. J Med Chem. 2017;60(4):1262–71.
45. Konstantinopoulos PA, Barry WT, Birrer M, Westin SN, Cadoo KA, Shapiro GI, et al. Olaparib and alpha-specific PI3K inhibitor alpelisib for patients with epithelial ovarian cancer: a dose-escalation and dose-expansion phase 1b trial. Lancet Oncol. 2019;20(4):570–80.

PARPi: Efficacy in Hereditary Breast Cancer

19

Akiyo Yoshimura

Abstract

Breast cancer (BC) with germline pathogenic variants of *BRCA1* or *BRCA*2 is found in approximately 5% of Japanese BC patients. *BRCA1/2*-associated BC with homologous recombination (HR) deficiency is potentially sensitive to DNA damage agents, including platinum agents and PARP (poly(ADP-ribose) polymerase) inhibitors. In this chapter, we will summarize the clinical evidence supporting the efficacy of chemotherapy and PARP inhibitors (PARPis), as single agents or in combination, in the (neo)adjuvant setting or in the advanced setting of *BRCA1/2*-associated BC. Moreover, we will discuss resistance to PARPi and the development of further approaches to improve the therapeutic efficacy of PARPi.

Keywords

BRCA · Breast cancer · Efficacy · Platinum · PARP inhibitors · Drug resistance

19.1 Introduction

Pathogenic germline variants of *BRCA1* or *BRCA*2 have been found in 1.4% and 2.7%, respectively, of Japanese breast cancer (BC) patients [1].

The prognosis of *BRCA1/2*-associated BC patients who received traditional standard treatment was similar to that of sporadic breast cancer patients after adjustment for age, tumor stage, nodal status, and hormone receptors, based on the literature [2, 3]. The result of a meta-analysis also showed that the status of

A. Yoshimura (✉)
Department of Breast Oncology, Aichi Cancer Center, Nagoya, Japan
e-mail: akiyo@aichi-cc.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_19

germline *BRCA1/2* (g*BRCA1/2*) pathogenic variants does not influence the prognosis [4].

The *BRCA1* and *BRCA*2 proteins play a role in the repair of DNA double-strand breaks (DSBs) by intervening in homologous recombination (HR).

In functional HR repair-deficient cells, nonconservative forms of DNA repair such as nonhomologous end joining (NHEJ) became dominant [5]. Therefore, *BRCA1/2*-deficient BC is potentially sensitive to DNA damage agents such as platinum agents and PARP (poly(ADP-ribose) polymerase) inhibitors (PARPis) [6, 7].

19.2 Traditional Anthracycline- and Taxane-Based Regimens

The anthracyclines used in the treatment of BC are either epirubicin or doxorubicin. The commonly used anthracycline-containing regimens include cyclophosphamide. Anthracyclines can induce DSBs by inhibiting the enzyme topoisomerase II. Anthracyclines stabilize the topoisomerase II complex after the enzyme has induced a break in the DNA chain for replication, thus preventing the DNA double helix from being resealed; this inhibits the process of replication. In vitro data suggest that cells without functional *BRCA1* or *BRCA*2 proteins are particularly sensitive to agents causing DSBs including doxorubicin, with a subsequent increased level of apoptosis [8, 9].

On the other hand, taxanes are anti-microtubule agents which work by inhibiting the depolymerization of the mitotic spindle and by inhibiting the polymerization of tubulin during cell division. Several preclinical studies showed that the inhibition of *BRCA1* leads to increased chemoresistance to microtubule-interfering agents [10, 11]. The *BRCA1* protein is involved in facilitating apoptosis in cells with disrupted mitotic spindle formation. Deficiency of the *BRCA1* protein may lead to paclitaxel resistance through premature inactivation of the spindle checkpoint in BC cells [12].

19.2.1 Neoadjuvant Setting

Studies conducted at the MD Anderson Cancer Center (MDACC) have reported on the pathological complete response (pCR) rate after anthracycline- and taxane-based regimens in *BRCA1/2* pathogenic variant carriers and noncarriers. Twenty-six (46%) of 57 *BRCA1* carriers achieved a pCR, compared with 3 (13%) of 23 *BRCA*2 carriers and 53 (22%) of 237 *BRCA* noncarriers ($P < 0.001$). *BRCA1* status and ER negativity were independently associated with a higher pCR rate in patients with BC [13].

In a retrospective study involving triple-negative breast cancer (TNBC) patients receiving neoadjuvant AC (doxorubicin and cyclophosphamide) followed by paclitaxel, 34 *BRCA1* carriers had pCR rate of 68%, compared with that of 37% among 43 noncarriers ($P = 0.01$). However, this did not translate into superior survival [14].

More recently, another prospective cohort study from MDACC reported the pCR rate after AC or AC-T (AC followed by taxane) in TNBC with and without g*BRCA*

pathogenic variants. The pCR rates in *BRCA*-associated tumors and non-*BRCA*-associated tumors were 58.3% (28/48) and 51.1% (43/84), respectively [15].

Furthermore, the GeparQuinto phase III trial evaluated the efficacy of the addition of bevacizumab on neoadjuvant EC-docetaxel for 493 TNBC patients.

Germline *BRCA1/2* pathogenic variants were detected in 18.3% of patients with TNBC. Overall, the pCR rate was higher in *BRCA1/2* pathogenic variant carriers than in noncarriers (50% vs. 31.5%, $P = 0.001$), and the pCR rate among patients treated with bevacizumab was 61.5% for *BRCA1/2* pathogenic variant carriers and 35.6% for those without pathogenic variants ($P = 0.004$). Disease-free survival (DFS) was also better in those without the *BRCA1/2* pathogenic variants (HR, 0.644; $P = 0.047$) [16].

19.2.2 Advanced or Metastatic Setting

Kriege et al. investigated the sensitivity to standard first-line chemotherapy of 121 metastatic *BRCA1/2*-associated BC patients (93 with *BRCA1* and 28 with *BRCA*2 pathogenic variants), compared to 121 matched sporadic BC patients in a retrospective study from the Family Cancer Clinic database. The chemotherapy regimens most frequently used were anthracycline-based ($n = 147$) and also included cyclophosphamide, methotrexate, and fluorouracil (CMF) ($n = 68$). As compared to sporadic patients, *BRCA*2-associated BC patients had a significantly higher OR (89% vs. 50%; $P < 0.001$) and a longer PFS (HR, 0.64; $P = 0.04$) and OS (HR, 0.53; $P = 0.005$) after start of first-line chemotherapy for metastatic breast cancer (MBC). Statistically significant increase in sensitivity was not observed for *BRCA1*-associated BC [17].

Kriege et al. also assessed the efficacy of either paclitaxel or docetaxel for 48 MBCs with g*BRCA1/2* pathogenic variants (35 with *BRCA1* and 13 with *BRCA*2 pathogenic variants), compared to 95 sporadic MBCs. *BRCA1*-associated, hormone receptor-negative MBC patients were less sensitive to taxane chemotherapy than sporadic HR-negative patients (OR 23% vs. 38%, PD 60% vs. 19%, $P < 0.001$; PFS 2.2 vs. 4.9 months, $P = 0.04$). The sensitivity of *BRCA1*- and *BRCA*2-associated, HR-positive MBC patients to taxane chemotherapy was similar to that of sporadic MBC patients [18].

Clinical data suggest that breast cancer with g*BRCA1/2* pathogenic variants may be more sensitive to anthracyclines and may be less sensitive to taxane monotherapy, which supports preclinical studies. However, these data are not definitive.

19.3 Alkylating Agents

Cyclophosphamide affects the alkylation of DNA and inhibits DNA replication by cross-linking guanine nucleobases in DNA double-helix strands.

Byrski et al. reported that pCR was observed in only 1 patient (7%) among 14 g*BRCA1* pathogenic variant carriers receiving neoadjuvant CMF [19].

From a retrospective study, the status of g*BRCA1/2* pathogenic variants did not influence the sensitivity to first-line CMF for MBC [17]. The specific impact of cyclophosphamide also remains unclear in *BRCA1/2*-associated BC.

19.4 Platinum Agents

Recent evidence suggests that *BRCA*-related BC is particularly sensitive to treatment with inter-strand cross-linking agents such as platinum-based chemotherapy [20, 21].

The cytotoxic actions of platinum drugs involve the binding of platinum to DNA, which interferes with DNA replication and transcription. It seems likely that cross-links cause replication fork stalling when encountered by the DNA replication machinery; this may result in DSBs. *BRCA1/2* are critical genes in the HR repair of DSBs. Hence, *BRCA1/2*-deficient BC may be more sensitive to platinum drugs [22, 23].

Representative clinical trials of platinum agents in *BRCA1/2*-associated BC are summarized in Table 19.1.

19.4.1 Neoadjuvant Setting

Byrski et al. in a retrospective study conducted in 2010 were the first to report a greater sensitivity of g*BRCA1* pathogenic variant carriers to neoadjuvant platinum agents [19]. Among 102 patients with g*BRCA1* pathogenic variants including 12 patients who received cisplatin from the Poland registry, a higher rate of pCR (83%) was seen after treatment with cisplatin (75 mg/m^2 every 3 weeks for 4 cycles) compared to the pCR (22%) for AC (doxorubicin and cyclophosphamide) or FAC (fluorouracil, doxorubicin, and cyclophosphamide). In a larger study of 107 patients with *BRCA1*-related BC treated with neoadjuvant cisplatin, pCR was observed in 65 patients (61%) [24].

On the other hand, the GeparSixto trial assessed the efficacy of adding neoadjuvant carboplatin to a regimen consisting of anthracycline, taxane, and bevacizumab for 291 patients with TNBC including 50 g*BRCA1/2* pathogenic variant carriers. Under the nonstandard GeparSixto polychemotherapy regimen, the high pCR rate observed in *BRCA1/2* pathogenic variant carriers in the non-carboplatin arm (66.7%) was not increased further by adding carboplatin (65.4%) [20, 25].

A secondary analysis of the GeparOcto trial reported an association of germline variant status with therapy response. For TNBC, a positive g*BRCA1/2* variant status was associated with therapy response in both the PMCb arm (74.3% vs. 47.0%; OR, 3.26; 95% CI, 1.44–7.39; $P = 0.005$) and the iddEPC arm (64.7% vs. 45.0%; OR, 2.24; 95% CI, 1.04–4.84; $P = 0.04$). Differences between treatment arms were not significant (74.3% vs. 64.7%; OR, 1.58; 95% CI, 0.56–4.43; $P = 0.39$). Interaction between the g*BRCA1/2* variant and the study arm was not significant ($P = 0.51$). In g*BRCA1/2*-associated TNBC, iddEPC also appears to be effective, though with a

Table 19.1 Clinical trials of platinum agents

Clinical trial	Type of study	Patients	Platinum treatment regimen	Result
Neoadjuvant setting				
Byrski 2010 [19]	Retrospective	12 pts with *gBRCA1* pathogenic variant	CDDP (75 mg/m2 every 3 weeks for 4 cycles)	pCR = 83% (10/12)
Byrski 2014 [24]	Retrospective	107 pts with *gBRCA1* pathogenic variant	CDDP (75 mg/m2 every 3 weeks for 4 cycles)	pCR = 61% (65/107)
Hahnen 2017 GeparSixto [20, 25]	Ph. II RCT Ancillary analysis	TNBC (n = 146) including 50 pts with *g BRCA1/2* pathogenic variant	Adding CBDCA (AUC1.5–2.0 weekly for 18 weeks) with paclitaxel/doxorubicin/ bevacizumab	Additional CBDCA pCR =66.7% (16/24) Non-CBDCA pCR = 65.4% (17/26)
Pohl-Rescigno 2020 GeparOcto [26]	Ph. II RCT Secondary analysis	TNBC (n = 393) including 69 pts with gBRCA1/2 pathogenic variant	CBDCA (AUC1.5 weekly for 18 weeks) with paclitaxel/doxorubicin (PMCb) Intensive-dose-dense epirubicin/paclitaxel/ cyclophosphamide (iddEPC)	PMCb pCR = 74.3% (26/35) iddEPC pCR = 64.7% (22/34)
Tung 2020 TBCRC031 [27]	Ph. II RCT	117 pts (HER2-neagtive) with gBRCA1/2 pathogenic variant	CDDP (75 mg/m2 every 3 weeks for 4 cycles) Doxorubicin-cyclophosphamide (AC)	CDDP pCR = 18%, RCB 0/1 = 33% AC pCR = 26%, RCB 0/1 = 46%
Advanced or metastatic setting				
Byrski 2012 [28]	Ph. II Single arm	20 pts with gBRCA1 pathogenic variant	CDDP (75 mg/m2 every 3 weeks for 6 cycles)	ORR = 80% mPFS = 12 months

(continued)

Table 19.1 (continued)

Clinical trial	Type of study	Patients	Platinum treatment regimen	Result
Isakoff 2015 TBCRC009 [29]	Ph. II Single arm First or second line	mTNBC (n = 86) including 11 pts with gBRCA1/2 pathogenic variant	CDDP (75 mg/m2) or CBDCA (AUC6) once every 3 weeks	BRCA-associated BC ORR = 54.5% mPFS = 3.3mo Non-BRCA-associated BC ORR = 19.7% mPFS = 2.8 mo
Tutt 2018 TNT trial [30]	Ph. III RCT for TNBC First line Subgroup analysis	mTNBC(n = 376) including 43pts with gBRCA1/2 pathogenic variant	CBDCA (AUC 6 every 3 weeks) Docetaxel (100 mg/m2 every 3 weeks)	CBDCA ORR = 68% (17/25) mPFS = 6.8mo Docetaxel ORR = 33% (6/18) mPFS = 4.4mo
Zhang 2020 CBCSG006 [31]	Phase III RCT for TNBC First line Biomarker assessment	mTNBC(n = 236) including 12pts with gBRCA1/2 pathogenic variant	Cisplatin+gemcitabine (GP) Paclitaxel +gemcitabine (GT)	GP ORR = 83.3% (5/6) mPFS = 8.9mo GT ORR = 37.5% (3/8) mPFS = 3.2mo

gBRCA germline *BRCA*, *CDDP* cisplatin, *CBDCA* carboplatin, *pCR* pathological complete response, *Ph* phase, *RCT* randomized clinical trial, *TNBC* triple-negative breast cancer, *mTNBC* metastatic TNBC, *pts* patients, *ORR* overall response rate, *mPFS* median progression-free survival

pCR rate approximately 10 percentage points lower than that observed in the PMCb arm. Whether this difference is associated with survival outcome is yet unclear [26].

A randomized phase II study of neoadjuvant cisplatin (CDDP) versus doxorubicin-cyclophosphamide (AC) in g*BRCA* pathogenic variant carriers with HER2-negative BC (TBCRC 031) demonstrated that the pCR or residual cancer burden (RCB) 0/1 was not significantly higher with CDDP than with AC in *BRCA* carriers for both TNBC and ER+/HER2-negative disease [27].

A meta-analysis showed that the addition of platinum to chemotherapy regimens in the neoadjuvant setting increases the pCR rate in *BRCA*-associated (58.4%, 93/159) as compared to wild-type TNBC patients (50.7%, 410/808). However, this trend did not achieve statistical significance [21].

19.4.2 Advanced or Metastatic Setting

In a phase II single-arm study, 20 patients with *BRCA1*-asscoated MBC, 55% of whom had prior chemotherapy for MBC, were treated with cisplatin at 75 mg/m^2 every 3 weeks for 6 cycles [28]. The overall response rate (ORR) was 80%, including complete clinical response (45%) and partial response (35%). A complete response was achieved in 8 of 15 ER-negative patients (53%), compared to only 1 of 5 ER-positive patients (20%). The median time to progression was 12 months.

The TBCRC009 trial was also a single-arm phase II clinical trial of single-agent platinum for 86 metastatic TNBC patients, including 11 patients with g*BRCA1/2* pathogenic variants. Patients received either cisplatin (75 mg/m^2) or carboplatin (AUC6) as first- or second-line therapy by physician's choice once every 3 weeks. Individuals with *BRCA1/2* mutations were more likely to achieve a response than were those without mutations (54.5% vs. 19.7%, $P = 0.022$). However, PFS was not significantly different between carriers and noncarriers (median 3.3 vs. 2.8 months; $P = 0.92$) [29].

Although there are no randomized controlled trials investigating the efficacy of platinum alone in patients with *BRCA1/2*-associated advanced breast cancer, the randomized phase III CBCSG006 and TNT trials conducted in TNBC patients included patients with g*BRCA1/2* pathogenic variants.

The TNT trial compared first-line carboplatin (AUC6 every 3 weeks) with docetaxel (100 mg/m^2 every 3 weeks) in *BRCA1/2*-associated BC or TNBC patients [30]. In 376 patients, carboplatin was not more efficacious than docetaxel (ORR, 31.4% vs. 34.0%; $P = 0.66$). In subgroup analysis by patients with g*BRCA1/2* pathogenic variants ($n = 43$), carboplatin showed double the ORR compared to docetaxel (68% vs. 33%, $P = 0.03$). PFS also favored carboplatin (6.8 months vs. 4.4 months, interaction $P = 0.002$), but no difference was found in overall survival, which may be due to the crossover design. This trial provided evidence that the platinum agent was better than the current standard chemotherapies for a selected population in whom g*BRCA1/2* pathogenic variants were detected early.

The CBCSG006 trial reported the superior efficacy of cisplatin plus gemcitabine (GP) regimen compared to the paclitaxel plus gemcitabine (GT) regimen (HR. 0.692;

95% CI, 0.523–0.915) as first-line treatment of metastatic triple-negative breast cancer (mTNBC) [31]. In additional biomarker assessment, patients with g*BRCA1/2* mutations (n = 12) had numerically higher ORR and prolonged PFS in the GP arm than in the GT arm (83.3% vs. 37.5%, P = 0.086; 8.90 vs. 3.20 months, P = 0.459).

In summary, the efficacy of platinum in patients with *BRCA1/2*-associated MBC is promising, but there are no randomized controlled trials of platinum limited to patients with *BRCA1/2* germline pathogenic variants; this needs to be studied further.

19.5 PARP Inhibitors

As described in Chap. 18, several PARP inhibitors have been developed based on the concept of "synthetic lethality" and with the expectation of an antitumor effect based on PARP trapping. PARP inhibitors including olaparib, talazoparib, veliparib, niraparib, and rucaparib have undergone clinical investigation for the treatment of BC.

PARPi, either as monotherapy or in combination with cytotoxic chemotherapy, improved efficacy compared to conventional chemotherapy. However, PARPi combination therapy showed increased hematological toxicity as well as fatigue and gastrointestinal toxicities. Adverse events have been a challenge for further development.

Here, we briefly review the clinical data of PARPi in *BRCA1/2*-associated BC (Table 19.2).

19.5.1 Olaparib

Olaparib, a PARP-1, PARP-2, and PARP-3 inhibitor, is the first FDA-approved PARPi for the treatment of *BRCA*-associated ovarian cancer.

In Japan, olaparib was approved in 2018 for maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer and was subsequently approved for MBC patients with a g*BRCA* pathogenic variant based on the results of the OlympiAD study [36].

19.5.1.1 Neoadjuvant Setting

The GeparOLA study was a randomized phase II trial conducted to assess the efficacy of paclitaxel and olaparib (PwO) in comparison to paclitaxel and carboplatin (PwCb) followed by EC as neoadjuvant chemotherapy in patients with HER2-negative early BC with homologous recombination deficiency (HRD). Here, HRD was defined as score high tumors +/− germline (g) or tumor (t) *BRCA* pathogenic variants. The pCR rate with PwO was 55.1% (90% CI, 44.5%–65.3%) vs. that of PwCb which was 48.6% (90% CI, 34.3%–63.2%). An analysis of the stratified subgroups showed higher pCR rates with PwO in the cohorts of patients aged <40 years and hormone receptor-positive tumors [32].

Table 19.2 PARP inhibitors

Clinical trial	Type of study	Patients	PARP inhibiter treatment regimen	Result
1. Olaparib				
Neoadjuvant setting				
Fasching 2019 GeparOLA [32]	Ph. II	102 HER2-negative BC pts with HRD high tumors +/− germline (g) or tumor (t) BRCA1/2 pathogenic variant	Paclitaxel and olaparib (PwO) followed by EC(n = 65) Paclitaxel and CBDCA(PwCb) followed by EC(n = 37) ※Olaparib 100 mg tablets twice daily for 12 weeks	PwO pCR = 55.1% PwCb pCR = 48.6%
Adjuvant setting				
OlympiA (NCT02032823)	Ph. III RCT Post-(neo)adjuvant chemotherapy	1500pts (high risk HER2-negative) with gBRCA1/2 pathogenic variant	Olaparib 300 mg tablets twice daily or placebo for 12 months	Active, not recruiting
Advanced or metastatic setting				
Fong 2009 [33]	Ph. I dose-escalation study	60 pts with advanced solid tumors including 22 pts with gBRCA1/2 pathogenic variant	Olaparib 10–600 mg twice daily	Maximum tolerated dose as 400 mg twice daily
Tutt 2010 [34]	Ph. II Nonrandomized	54 MBC pts with gBRCA1/2 pathogenic variant	Olaparib 400 mg twice daily Olaparib 100 mg twice daily	ORR = 41% ORR = 22%
Kaufman 2015 [35]	Single arm Prior three chemotherapy regimens for MBC	298 solid tumor pts (62 BC) with gBRCA1/2 pathogenic variant	Olaparib 400 mg twice daily	RR = 12.9%(8/62) in MBC
Robson 2017 OlympiAD [36, 37]	Ph. III RCT No more than two prior chemotherapy regimens for MBC Anthracycline and taxane pretreated	302 MBC pts with gBRCA1/2 pathogenic variant	Olaparib 300 mg twice daily Chemotherapy of the physician's choice (capecitabine, eribulin, vinorelbine)	Olaparib ORR = 59.9% mPFS = 7.0mo Chemotherapy ORR = 28.8% mPFS = 4.2mo
2. Niraparib				

(continued)

Table 19.2 (continued)

Clinical trial	Type of study	Patients	PARP inhibiter treatment regimen	Result
Advanced or metastatic setting				
Sandhu 2013 [38]	Ph. I dose-escalation study	100 solid tumors including 22 MBCs	Niraparib 30–400 mg daily	Maximum tolerated dose as 300 mg daily
3. Rucaparib				
Advanced or metastatic setting				
Drew 2016 [39]	Ph. II dose escalation IV and subsequently oral study	78 solid tumor pts including 23 MBCs with gBRCA1/2 pathogenic variant	Rucaparib IV 4–18 mg → oral 92–600 mg twice daily	Well-tolerated doses as oral 480 mg daily No responders by ORR in BC
Wilson 2017 [40]	Ph. I dose-escalation study in combination with chemotherapy	85 pts with advanced solid tumors including 7 pts with gBRCA pathogenic variant	Rucaparib IV 12–24 mg → oral 80–360 mg + chemotherapy	Maximum tolerated dose for the combination was oral 240 mg daily rucaparib and CBDCA
Miller 2015 [41]	Ph. II RCT	128 pts with TNBC or BRCA-associated BC (n = 22) with residual tumor post-neoadjuvant chemotherapy	Cisplatin 75 mg/m2 ± Rucaparib 25–30 mg IV days 1 to 3 (4 cycles) → oral rucaparib 100 mg weekly	Cisplatin alone 2 yr DFS = 58.3% Cisplatin+rucaparib 2 yr DFS = 63.1%
4. Talazoparib				
Neoadjuvant setting				
Litton 2020 [42]	Ph. II	20 HER2-negative BC pts with gBRCA1/2 pathogenic variant	Talazoparib 1 mg once daily for 6 months	RCB0 (pCR) = 53% RCB 0–1 = 63%
Advanced or metastatic setting				
Litton 2018 EMBRACA [43]	Ph. III RCT No more than three cytotoxic regimens for MBC Anthracycline or taxane pretreated	431 advanced/metastatic BC pts with gBRCA1/2 pathogenic variant	Talazoparib 1 mg once daily Chemotherapy of the physician's choice (capecitabine, eribulin, gemcitabine, or vinorelbine)	Talazoparib arm ORR = 62.6% mPFS = 8.6 mo Chemotherapy arm ORR = 27.2% mPFS = 5.6mo
5. Veliparib				

Neoadjuvant setting				
Rugo 2016 I SPY2 [44]	Ph. II adaptive randomized trial	Veliparib group 72 pts with Stage 2–3 BC including 12 BRCA-associated BCs Control group 74 pts with Stage 2–3 BC including 3 BRCA-associated BCs	Weekly paclitaxel+ veliparib 50 mg twice daily + CBDCA followed by AC Weekly paclitaxel followed by AC	Veliparib group pCR = 51% Control group pCR = 26%
Lobi 2016 Brightness [45]	Ph. III RCT	Stage 2–3 TNBC (n = 634) including 92 pts with gBRCA1/2 pathogenic variant	Weekly paclitaxel 12 doses Weekly paclitaxel + CBDCA (AUC6, q3weeks, 4 cycles) Weekly paclitaxel + CBDCA +veliparib (50 mg BID) All patients received followed by AC	Paclitaxel pCR = 31% Paclitaxel + CBDCA pCR = 58% Paclitaxel +CBDCA +veliparib pCR = 53%
Advanced or metastatic setting				
Han 2018 BROCADE [46]	Ph. II RCT	290 advanced/metastatic BC pts with gBRCA1/2 pathogenic variant	Carboplatin/paclitaxel (CP) Carboplatin/paclitaxel veliparib (VCP) Veliparib 120 mg daily plus temozolomide (VT)	CP ORR = 61.3% mPFS = 12.3mo VCP ORR = 78% mPFS = 14.1mo VT ORR = 28.6% mPFS = 7.4mo

g*BRCA* germline *BRCA*, *CBDCA* carboplatin, *pCR* pathological complete response, *Ph* phase, *RCT* randomized clinical trial, *TNBC* triple-negative breast cancer, *MBC* metastatic BC, *pts* patients, *ORR* overall response rate, *mPFS* median progression-free survival

19.5.1.2 Adjuvant Setting

The presence of residual invasive disease after neoadjuvant chemotherapy is a strong predictive factor for survival in TNBC.

A study evaluating the benefit of experimental postoperative PARPi therapy in patients with a high risk of recurrence is being planned.

The OlympiA (NCT02032823) study is a randomized, placebo-controlled phase III trial enrolling *BRCA1/2*-associated, high-risk HER2-negative BC, after completion of local treatment and (neo)adjuvant chemotherapy. Patients were randomized between olaparib (300 mg) and placebo for 12 months. The primary endpoint is invasive DFS. Approximately 1500 patients were randomized, and recruitment was closed in 2019. The result of this study is awaited.

19.5.1.3 Advanced or Metastatic Setting

The first phase 1 trial of the clinical evaluation of olaparib in humans was reported in 2009 [33] and was conducted in 60 patients with advanced solid tumors including 22 g*BRCA1/2* pathogenic variant carriers. The olaparib dose and schedule were increased from 10 mg daily for two of every 3 weeks to 600 mg twice daily continuously. The manifestations of dose-limiting toxicity led to the establishment of a maximum tolerated dose of 400 mg of olaparib twice daily. Clinical response according to three MBC patients with g*BRCA1/2* pathogenic variants was as follows: one patient had CR, and another showed PR.

Tutt et al. assessed the efficacy of olaparib monotherapy in 54 MBC patients with g*BRCA1/2* pathogenic variants in a phase II trial. The first cohort (27 patients) was treated with 400 mg twice daily, and the second cohort (27 patients) was treated with 100 mg twice daily [34]. Most patients had already received anthracycline and taxane regimens. The overall response rate was 41% in the first cohort and 22% in the second cohort.

Kaufman et al. reported that the ORR was 12.9% (8/62) in heavily pretreated *BRCA1/2*-associated MBC. The most common adverse events (AEs) were fatigue, nausea, and vomiting. Severe anemia (grade > 3) was seen in 17% of the patients [35].

In 2017, Robson et al. reported the first randomized, open-label, phase III OlympiAD trial which compared olaparib monotherapy with standard single-agent chemotherapy (eribulin, capecitabine, or vinorelbine) of the physician's choice in patients with HER2-negative MBC carrying g*BRCA1/2* pathogenic variants [36, 37]. Patients had received no more than two previous chemotherapy regimens for MBC and had received anthracycline and a taxane for (neo)adjuvant or metastatic disease. A total of 302 patients were randomized, 205 being assigned to receive olaparib and 97 to receive standard therapy. Olaparib was clinically superior to the standard therapy with mPFS (7.0 months vs. 4.2 months; HR, 0.58; $P \leq 0.001$) and RR (59.9% vs. 28.8%).

While there was no statistically significant improvement in OS with olaparib compared to TPC, a trend of meaningful OS benefit among patients who had not received chemotherapy for metastatic disease was observed. The rate of grade 3 or higher AEs was 36.6% in the olaparib group and 50.5% in the standard-therapy group; the quality of life data were significantly better in the olaparib group. Olaparib was generally well-tolerated.

19.5.2 Niraparib

Niraparib, a high-selective PARP-1 and PARP-2 inhibitor, was approved by the FDA for unselected platinum-sensitive recurrent ovarian cancer patients. It has recently been approved in Japan for ovarian cancer.

In a phase 1 dose-escalation trial evaluating niraparib in 100 solid tumors including 22 MBC patients, 2 MBC patients had PR among 4 MBC patients with g*BRCA* pathogenic variants. The maximum tolerated dose was established to be 300 mg/day [38].

19.5.3 Rucaparib

Rucaparib, a PARP-1, PARP-2, and PARP-3 inhibitor, is a second FDA-approved PARPi for the treatment of patients with *BRCA* (germline and/or somatic)-associated advanced ovarian cancer.

19.5.3.1 Advanced or Metastatic Setting

A phase II trial of rucaparib was conducted in proven *BRCA1/2* mutation carriers with advanced breast and/or ovarian cancer [47]. Rucaparib was well-tolerated in patients up to doses of 480 mg per day. There were no responders to rucaparib as per ORR among the BC patients.

A phase I dose-escalation trial of rucaparib in combination with standard chemotherapy (carboplatin, carboplatin and paclitaxel, cisplatin, and pemetrexed, or epirubicin and cyclophosphamide) has been conducted for the treatment of 85 solid tumors including 22 MBC cases. Maximum tolerated dose for the combination was 240 mg per day of oral rucaparib and carboplatin. Clinical activity (one CR and one PR) was observed among seven cases of heavily pretreated MBC with g*BRCA* pathogenic variants. Neutropenia and thrombocytopenia were the most common grade ≥ 3 toxicities [39].

A randomized phase II trial assessed the efficacy of cisplatin with or without low-dose rucaparib after preoperative chemotherapy (anthracycline and/or taxane) in 128 patients with TNBC or *BRCA*-associated BC ($n = 22$) with residual disease. The addition of rucaparib did not improve the 2-year DFS (58.3% with cisplatin vs. 63.1% with cisplatin and rucaparib, $P = 0.43$). The variant status had no impact, which was thought due to the low-dose schedule of rucaparib [40].

19.5.4 Talazoparib

Talazoparib is an inhibitor of PARP-1 and PARP-2 and shows powerful PARP trapping.

An in vitro comparison of the effects of talazoparib, olaparib, and rucaparib on PARP-1 and PARP-2 showed that talazoparib has the highest efficacy in trapping the PARP-DNA complex [41]. Clinical data supports that the strength of DNA-PARP trapping effect may be associated with enhanced toxicity.

19.5.4.1 Neoadjuvant Setting

In the neoadjuvant setting, the use of the PARPi as a single-agent was reported to minimize toxicity. Litton et al. evaluated the pathologic response and tolerance of talazoparib alone for 6 months in patients with g*BRCA* pathogenic variants [48]. A total of 20 patients were enrolled, including 16 patients with g*BRCA1* and 4 patients with g*BRCA*2 pathogenic variants. Fifteen patients had TNBC. The rate of pCR was 53%, and the RCB 0/1 was 63%. Eight patients (40%) had grade 3 anemia and required a transfusion, three patients had grade 3 neutropenia, and one patient had grade 4 thrombocytopenia. Common grade 1 or 2 toxicities were nausea, fatigue, neutropenia, alopecia, dizziness, and dyspnea. Toxicities were managed by dose reduction and transfusions. Nine patients required dose reduction. Neoadjuvant single-agent oral talazoparib at 1 mg once per day for 6 months without chemotherapy produced a substantial RCB-0 rate with manageable toxicity. Talazoparib monotherapy may be a novel strategy for developing and de-escalating therapy in the neoadjuvant setting.

19.5.4.2 Advanced or Metastatic Setting

The EMBRACA was a randomized, open-label, phase III trial which compared talazoparib (1 mg once daily) or standard single-agent therapy of the physician's choice (capecitabine, eribulin, gemcitabine, or vinorelbine in continuous 21-day cycles) in MBC patients with g*BRCA1/2* pathogenic variants. The median PFS was significantly longer in the talazoparib arm than in the chemotherapy arm (8.6 months vs. 5.6 months; HR, 0.54; $P < 0.001$). The ORR was also better in the talazoparib arm compared to the chemotherapy arm (62.6% vs. 27.2%; $P < 0.001$). Hematologic grade 3–4 AEs occurred in 55% of participants in the talazoparib arm and in 38% of participants in the chemotherapy arm. Patient-reported outcomes favored the talazoparib arm [42].

The results of two RCTs (the OlympiAD and EMBRACA studies) were assessed in a meta-analysis. A total of 733 patients were included, of whom 492 received single-agent PARPi therapy (olaparib in the OlympiAD trial and talazoparib in the EMBRACA trial) and 241 received mono-chemotherapy as per the physician's choice [43]. As compared with mono-chemotherapy, single-agent PARPi therapy significantly improved PFS (HR, 0.56; 95% CI, 0.45–0.70) and ORR (OR, 4.15; 95% CI, 2.82–6.10), with no difference in OS (HR, 0.82; 95% CI, 0.64–1.05). Patients treated with PARPi therapy experienced a significant delayed time to QoL deterioration (HR, 0.40; 95% CI 0.29–0.54). Single-agent PARPi therapy was observed to be an effective, well-tolerated, and useful treatment in maintaining the QoL of patients with *BRCA*-mutated HER2-negative MBC.

19.5.5 Veliparib

Veliparib is an inhibitor of PARP-1 and PARP-2, with the weakest PARP trapping among the clinically tested PARPis, and has been considered as the weakest PARPi. Therefore, this drug has been essentially developed for use in combination

with platinum-based chemotherapy, which is more feasible and is more advantageous.

19.5.5.1 Neoadjuvant Setting

The I-SPY2 trial was the first trial to assess carboplatin-veliparib therapy in a neoadjuvant setting. I-SPY2 is an open-label, adaptive randomized phase II trial for the evaluation of new agents combined with standard neoadjuvant therapy for the treatment of BCs that have a high risk of recurrence. Patients were randomized to combined veliparib-carboplatin and standard chemotherapy (paclitaxel, followed by AC) or standard chemotherapy alone. A total of 72 patients were randomly assigned to receive veliparib-carboplatin including 17% with a deleterious variant in *BRCA1* or *BRCA*2. The rate of pCR in the TNBC population was 51% in the veliparib-carboplatin group, versus 26% in the control group. The toxicity of veliparib-carboplatin was greater than that of the control. This trial showed that veliparib-carboplatin added to standard therapy resulted in higher rates of pCR than standard therapy alone, specifically in TNBC [49].

Based on these results, in the same population, the phase III BrighTNess trial evaluated the addition of carboplatin with and without veliparib to the standard neoadjuvant combination of paclitaxel followed by AC in 634 TNBC patients including 92 patients with a deleterious g*BRCA* mutation [44]. The pCR rates for patients treated with paclitaxel alone, those treated with paclitaxel plus carboplatin, and those treated with paclitaxel plus carboplatin plus veliparib were 31%, 58%, and 53%, respectively. Addition of carboplatin to standard chemotherapy increased the pCR, while veliparib had no further benefit to pCR. The subgroup analyses of patients with a deleterious g*BRCA* mutation showed the pCR rates for paclitaxel alone, paclitaxel plus carboplatin, and paclitaxel plus carboplatin plus veliparib were 41%, 50%, and 57%, respectively.

19.5.5.2 Advanced or Metastatic Breast Cancer

A randomized phase II study (BROCADE) examined the safety and efficacy of carboplatin/paclitaxel (CP) with or without veliparib (VCP) or a third arm with veliparib plus temozolomide (VT) in 290 g*BRCA*-associated advanced/metastatic breast cancer patients. The median PFS and OS were similar for VCP and CP (PFS, 14.1 months vs. 12.3 months, respectively, $P = 0.227$; OS, 28.3 vs. 25.9 months, respectively, $P = 0.156$). The ORR was higher for the VCP regimen compared to that for the CP regimen (77.8% vs. 61.3%; $P = 0.027$). The VT arm was inferior to the CP arm in PFS, OS, and ORR [45].

19.5.6 Potential Mechanisms of Resistance to PARP

Germline *BRCA1/2* pathogenic variants are predictive biomarkers for PARPi response in BC patients; however, the majority of patients had primary and acquired resistance to PARPi. It is essential to identify the mechanism of resistance, to help overcome such resistance.

Several studies have suggested the potential mechanisms of resistance to PARPi in preclinical models and clinical reports. One of the resistance mechanisms in HRR-deficient tumors is associated with a reversion mutation which can cancel the HRR deficiency and restore HRR function. Moreover, increased gene activity such as that of RAD51 that restores the HRR mechanism and genes involved in resistance to PARPi without restoration of the HRR has also been reported. However, we will not describe the mechanisms in detail here, though further information is available in other publications [46]. Combination therapies would be the next options to overcome such resistance.

19.5.7 Combination with Immune Checkpoint Inhibitors

PARPi upregulated PD-L1 expression in *BRCA1/2*-associated BC cell lines and xenograft models. The combination of PARPi and anti-PD-L1 therapy compared with each agent alone significantly increased the therapeutic efficacy in vivo [50].

Meanwhile, *BRCA1*-associated tumors frequently exhibit a triple-negative phenotype with extensive lymphocyte infiltration, with the increased expression of immunomodulatory genes including PD-1 and CTLA4, when compared to TNBCs from *BRCA1* wild-type patients [51].

In these contexts, trials of combination PARPi and immune checkpoint inhibitors (ICIs) have been conducted (Table 19.3).

19.5.7.1 Advanced or Metastatic Setting

The results of two preliminary phase II studies for MBC are already available, and there are several ongoing studies. The phase II, single-arm MEDIOLA basket trial evaluated the efficacy and safety of olaparib in combination with durvalumab (anti-PD-L1 inhibitor) in patients with solid tumors, including ovarian cancer, breast cancer, and gastric cancer. In *BRCA*-associated HER2-negative MBC (n = 30), the 12-week DCR (disease control rate) was 24/30 (80%), and the 28-week DCR was 15/30 (50%). The ORR was 63%. The most common AEs of ≧grade 3 were anemia, neutropenia, and pancreatitis [52].

Another phase II, single-arm TOPACIO trial assessed the clinical activity and safety of niraparib combined with pembrolizumab (anti-PD-1 inhibitor) for TNBC (n = 55), irrespective of *BRCA* status or PD-L1 expression. In patients with *BRCA* pathogenic variants (n = 15), the ORR was 47% (7/15), DCR was 80% (12/15), and the median PFS was 8.3 months. In 27 patients with *BRCA* wild-type tumors, the ORR was 11% (3/27), DCR was 33% (9/27), and the median PFS was 2.1 months. Numerically higher response rates in *BRCA*-associated tumors were observed in a BC cohort. The most common treatment-related AEs of grade 3 or higher were anemia (18%), thrombocytopenia (15%), and fatigue (7%). Immune-related adverse events were reported in 15% (grade 3 in 4%) of patients [53].

Table 19.3 Clinical trials of combinations of PARPi and ICIs

Clinical trial	Type of study	Patients	PARPi and ICIs combination regimen	Result
Advanced or metastatic setting				
Domchek 2019 MEDIOLA [52]	Ph. II Single arm	30 HER2-negative BC pts with gBRCA pathogenic variant	Olaparib 300 mg twice daily Durvalumab (1500 mg) once every 4 weeks	12-week DCR = 80% 28-week DCR = 50% ORR was 63% mPFS = 8.2mo mOS = 20.5mo
Vinayak 2019 TOPACIO [53]	Ph. II Single arm	mTNBC (n = 55) including 15 pts with tBRCA pathogenic variant 27 pts with tBRCA wild type 5 pts with tBRCA unknown	Niraparib 200 mg once daily Pembrolizumab (200 mg) once every 3 weeks	BRCA-associated BC ORR = 47% DCR = 80% mPFS = 8.3mo BRCA wild-type BC ORR = 11% DCR = 33% mPFS = 2.1 mo

PARPi PARP inhibiter, *ICIs* immune checkpoint inhibitors, *gBRCA* germline *BRCA*, t*BRCA* tumor *BRCA*, *Ph* phase, *mTNBC* metastatic triple-negative breast cancer, *pts* patients, *ORR* overall response rate, *DCR* disease control rate, *mPFS* median progression-free survival, *mOS* median overall survival

19.6 Future Direction

In HER2-negative *BRCA*-associated BC, the benefit of PARPi has been validated, and further combination trials are ongoing. In contrast, in HER2-positive *BRCA*-associated BC, the efficacy of PARPi is still unclear. Although data on HER2 expression in *BRCA*-associated tumors vary from series to series, Honrado et al. reported that HER2 positivity was 7% in tumors with *BRCA1* variants and 6% in those with *BRCA*2 variants. Using data from the Japanese hereditary breast and ovarian cancer syndrome registry, we confirmed that HER2 positivity was 4.6% in tumor with *BRCA1* pathogenic variants and 11.3% in those with *BRCA*2 pathogenic variants.

Han et al. reported the efficacy of the combination of olaparib and neratinib in HER2-positive, *BRCA* wild-type ovarian cell lines and xenografts in the 2019 SGO Annual Meeting. Olaparib is approved for the treatment of HER2-negative *BRCA*-associated BC, and neratinib is approved for HER2-positive BC. The effectiveness of PARPi for the treatment of HER2-positive, *BRCA*-associated BC needs to be assessed [54].

Combinations of PARPi with other targeted therapies have the potential to further increase their benefit. PARPis are associated with several oncogenic pathways such as EGFR, IGF, VEGF, or PI3K, and trials evaluating the combination of PARPi with inhibitors of these pathways have been initiated [55].

PARPis are also known to act as radiosensitizing agents, and combination therapy with radiation has been validated in various preclinical models [56].

Moreover, PARPi may potentially have the ability to penetrate the blood-brain barrier, which increases their possible clinical utility in patients with brain metastases [57].

Lastly, pathogenic variants of g*BRCA1/2*, as well as ER, PR, and HER2, have become major, indispensable biomarkers for treatment decisions in BC. In the coming years, further developments in this field will greatly improve the prognosis of hereditary BC and may also lead to improvements in the prognosis of sporadic breast cancer.

References

1. Momozawa Y, et al. Germline pathogenic variants of 11 breast cancer genes in 7,051 Japanese patients and 11,241 controls. Nat Commun. 2018;9(1):4083.
2. Bordeleau L, Panchal S, Goodwin P. Prognosis of *BRCA*-associated breast cancer: a summary of evidence. Breast Cancer Res Treat. 2010;119:13–24.
3. Goodwin PJ, et al. Breast cancer prognosis in *BRCA1* and *BRCA*2 mutation carriers: an international prospective breast cancer family registry population-based cohort study. J Clin Oncol. 2012;30:19–26.
4. Templeton AJ, et al. Interaction between hormonal receptor status, age and survival in patients with *BRCA1/2* germline mutations: a systematic review and meta-regression. PLoS One. 2016;11:e0154789.
5. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001;411:366–74.
6. Rubinstein WS. Hereditary breast cancer: pathobiology, clinical translation, and potential for targeted cancer therapeutics. Familial Cancer. 2008;7:83–9.
7. Keung MYT, Wu Y, Vadgama JV. PARP inhibitors as a therapeutic agent for homologous recombination deficiency in breast cancers. J Clin Med. 2019;8
8. Fedier A, et al. The effect of loss of *BRCA1* on the sensitivity to anticancer agents in p53-deficient cells. Int J Oncol. 2003;22:1169–73.
9. Sylvain V, Lafarge S, Bignon YJ. Dominant-negative activity of a *BRCA1* truncation mutant: effects on proliferation, tumorigenicity in vivo, and chemosensitivity in a mouse ovarian cancer cell line. Int J Oncol. 2002;20:845–53.
10. Lafarge S, et al. Inhibition of *BRCA1* leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. Oncogene. 2001;20:6597–606.
11. Tassone P, et al. *BRCA1* expression modulates chemosensitivity of *BRCA1*-defective HCC1937 human breast cancer cells. Br J Cancer. 2003;88:1285–91.
12. Chabalier C, et al. *BRCA1* downregulation leads to premature inactivation of spindle checkpoint and confers paclitaxel resistance. Cell Cycle. 2006;5:1001–7.
13. Arun B, et al. Response to neoadjuvant systemic therapy for breast cancer in *BRCA* mutation carriers and noncarriers: a single-institution experience. J Clin Oncol. 2011;29:3739–46.
14. Paluch-Shimon S, et al. Neo-adjuvant doxorubicin and cyclophosphamide followed by paclitaxel in triple-negative breast cancer among *BRCA1* mutation carriers and non-carriers. Breast Cancer Res Treat. 2016;157:157–65.
15. Clifton K, et al. Adjuvant versus neoadjuvant chemotherapy in triple-negative breast cancer patients with *BRCA* mutations. Breast Cancer Res Treat. 2018;170:101–9.
16. Fasching PA, et al. *BRCA1/2* mutations and bevacizumab in the neoadjuvant treatment of breast cancer: response and prognosis results in patients with triple-negative breast cancer from the GeparQuinto study. J Clin Oncol. 2018;36:2281–7.

17. Kriege M, et al. Sensitivity to first-line chemotherapy for metastatic breast cancer in *BRCA1* and *BRCA*2 mutation carriers. J Clin Oncol. 2009;27:3764–71.
18. Kriege M, et al. The efficacy of taxane chemotherapy for metastatic breast cancer in *BRCA1* and *BRCA*2 mutation carriers. Cancer. 2012;118:899–907.
19. Byrski T, et al. Pathologic complete response rates in young women with *BRCA1*-positive breast cancers after neoadjuvant chemotherapy. J Clin Oncol. 2010;28:375–9.
20. Hahnen E, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: secondary analysis of the GeparSixto randomized clinical trial. JAMA Oncol. 2017;3:1378–85.
21. Caramelo O, et al. The effect of neoadjuvant platinum-based chemotherapy in *BRCA* mutated triple negative breast cancers -systematic review and meta-analysis. Hered Cancer Clin Pract. 2019;17:11.
22. Nafisi S, Norouzi Z. A comparative study on the interaction of cis- and trans-platin with DNA and RNA. DNA Cell Biol. 2009;28:469–77.
23. Lord CJ, Garrett MD, Ashworth A. Targeting the double-strand DNA break repair pathway as a therapeutic strategy. Clin Cancer Res. 2006;12:4463–8.
24. Byrski T, et al. Pathologic complete response to neoadjuvant cisplatin in *BRCA1*-positive breast cancer patients. Breast Cancer Res Treat. 2014;147:401–5.
25. von Minckwitz G, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol. 2014;15:747–56.
26. Pohl-Rescigno E, et al. Association of germline variant status with therapy response in high-risk early-stage breast cancer: a secondary analysis of the GeparOcto randomized clinical trial. JAMA Oncol. 2020;6:744–8.
27. Tung N, et al. TBCRC 031: randomized phase ii study of neoadjuvant cisplatin versus doxorubicin-cyclophosphamide in germline *BRCA* carriers with HER2-negative breast cancer (the INFORM trial). J Clin Oncol. 2020;38:1539–48.
28. Byrski T, Dent R, Blecharz P, Foszczynska-Kloda M, Gronwald J, Huzarski T, et al. Results of a phase II open—label, non—randomized trial of cisplatin chemotherapy in patients with *BRCA1*—positive metastatic breast cancer. Breast Cancer Res Treat. 2012;14:R110.
29. Isakoff SJ, et al. TBCRC009: a multicenter phase ii clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol. 2015;33:1902–9.
30. Tutt A, et al. Carboplatin in *BRCA1/2*-mutated and triple-negative breast cancer *BRCA*ness subgroups: the TNT trial. Nat Med. 2018;24:628–37.
31. Zhang J, et al. Biomarker assessment of the CBCSG006 trial: a randomized phase III trial of cisplatin plus gemcitabine compared with paclitaxel plus gemcitabine as first-line therapy for patients with metastatic triple-negative breast cancer. Ann Oncol. 2018;29:1741–7.
32. Fasching PA, et al. GeparOLA: a randomized phase II trial to assess the efficacy of paclitaxel and olaparib in comparison to paclitaxel/carboplatin followed by epirubicin/cyclophosphamide as neoadjuvant chemotherapy in patients (pts) with HER2-negative early breast cancer (BC) and homologous recombination deficiency (HRD). J Clin Oncol. 2019;37(Suppl 15):506.
33. Fong PC, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. N Engl J Med. 2009;361:123–34.
34. Tutt A, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA*2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010;376:235–44.
35. Kaufman B, et al. Olaparib monotherapy in patients with advanced cancer and a germline *BRCA1/2* mutation. J Clin Oncol. 2015;33:244–50.
36. Robson M, et al. Olaparib for metastatic breast cancer in patients with a germline *BRCA* mutation. N Engl J Med. 2017;377:523–33.
37. Robson ME, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline *BRCA* mutation and HER2-negative metastatic breast cancer. Ann Oncol. 2019;30:558–66.

38. Sandhu SK, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in *BRCA* mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol. 2013;14:882–92.
39. Wilson RH, et al. A phase I study of intravenous and oral rucaparib in combination with chemotherapy in patients with advanced solid tumours. Br J Cancer. 2017;116:884–92.
40. Miller K, et al. Cisplatin with or without rucaparib after preoperative chemotherapy in patients with triple negative breast cancer: final efficacy results of Hoosier oncology group BRE09-146. J Clin Oncol. 2015;33(Suppl 15):1082.
41. Murai J, et al. Stereospecific PARP trapping by BMN 673 and comparison with olaparib and rucaparib. Mol Cancer Ther. 2014;13:433–43.
42. Litton JK, et al. Talazoparib in patients with advanced breast cancer and a germline *BRCA* mutation. N Engl J Med. 2018;379:753–63.
43. Poggio F, et al. Single-agent PARP inhibitors for the treatment of patients with *BRCA*-mutated HER2-negative metastatic breast cancer: a systematic review and meta-analysis. ESMO Open. 2018;3:e000361.
44. Loibl S, et al. Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. Lancet Oncol. 2018;19:497–509.
45. Han HS, et al. Veliparib with temozolomide or carboplatin/paclitaxel versus placebo with carboplatin/paclitaxel in patients with *BRCA1/2* locally recurrent/metastatic breast cancer: randomized phase II study. Ann Oncol. 2018;29:154–61.
46. Patel M, et al. The role of poly(ADP-ribose) polymerase inhibitors in the treatment of cancer and methods to overcome resistance: a review. Cell Biosci. 2020;10:35.
47. Drew Y, et al. Phase 2 multicentre trial investigating intermittent and continuous dosing schedules of the poly(ADP-ribose) polymerase inhibitor rucaparib in germline *BRCA* mutation carriers with advanced ovarian and breast cancer. Br J Cancer. 2016;114:723–30.
48. Litton JK, et al. Neoadjuvant talazoparib for patients with operable breast cancer with a germline *BRCA* pathogenic variant. J Clin Oncol. 2020;38:388–94.
49. Rugo HS, et al. Adaptive randomization of veliparib-carboplatin treatment in breast cancer. N Engl J Med. 2016;375:23–34.
50. Jiao S, et al. PARP inhibitor upregulates pd-l1 expression and enhances cancer-associated immunosuppression. Clin Cancer Res. 2017;23(14):3711–20.
51. Nolan E, et al. Combined immune checkpoint blockade as a therapeutic strategy for *BRCA1*-mutated breast cancer. Sci Transl Med. 2017;9:eaal4922.
52. Domchek S, et al. 1191O - phase II study of olaparib (O) and durvalumab (D) (MEDIOLA): updated results in patients (pts) with germline *BRCA*-mutated (g*BRCA*m) metastatic breast cancer (MBC). Ann Oncol. 2019;30:v477.
53. Vinayak S, et al. Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. JAMA Oncol. 2019;5:1132–40.
54. Honrado E, Benítez J, Palacios J. The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. Mod Pathol. 2005;18:1305–20.
55. Matulonis UA, et al. Phase I dose escalation study of the PI3kinase pathway inhibitor BKM120 and the oral poly (ADP ribose) polymerase (PARP) inhibitor olaparib for the treatment of high-grade serous ovarian and breast cancer. Ann Oncol. 2017;28:512–8.
56. Jannetti SA, et al. Poly(ADP-ribose)polymerase (PARP) inhibitors and radiation therapy. Front Phamacol. 2020;11
57. Mehta MP, et al. Veliparib in combination with whole brain radiation therapy in patients with brain metastases: results of a phase 1 study. J Neuro-Oncol. 2015;122:409–17.

Efficacy of Poly(ADP-Ribose) Polymerase Inhibitors for Hereditary Ovarian Cancer

20

Koji Matsumoto

Abstract

This chapter describes the efficacy of poly(ADP-ribose) polymerase (PARP) inhibitors, especially in patients with hereditary ovarian cancer. Biomarkers of ovarian cancer (e.g., *BRCA* mutation [both germline and somatic] or homologous recombination deficiency assays) are also reviewed here. The mechanisms of action are reviewed in Chap. 18. Key events, including the publication of important clinical trials and Food and Drug Administration (FDA) approval of each PARP inhibitor, are summarized in one figure. The clinical development of PARP inhibitors for patients with ovarian cancer began in the late-line setting. Then, the development continued to platinum-sensitive recurrence and, finally, to the first-line setting. For better understanding, data on first-line, platinum-sensitive, and platinum-resistant recurrences are reviewed in this order. In each setting, the efficacy results of clinical trials that evaluated PARP inhibitors, as either monotherapy or in combination, with approval by the FDA and Pharmaceuticals and Medical Devices Agency are reviewed. The efficacy endpoints focusing on hereditary ovarian cancer are tabulated in each setting. Ongoing clinical trials evaluating PARP inhibitors with other targeting agents such as antiangiogenic agents and/or immune checkpoint inhibitors are also reviewed at the end of this chapter.

Keywords

Hereditary ovarian cancer · PARP inhibitor · Olaparib · Niraparib · Rucaparib Veliparib · *BRCA* · HRD · FDA approval · PMDA approval

K. Matsumoto (✉)
Division of Medical Oncology, Hyogo Cancer Center, Akashi, Hyogo, Japan
e-mail: kojmatsu2@hyogo-cc.jp

S. Nakamura et al. (eds.), *Hereditary Breast and Ovarian Cancer*,
https://doi.org/10.1007/978-981-16-4521-1_20

20.1 About this Chapter

This chapter describes the efficacy of poly(ADP-ribose) polymerase (PARP) inhibitors, especially in patients with hereditary ovarian cancer. Biomarkers of ovarian cancer (e.g., *BRCA* mutation [both germline and somatic] or homologous recombination deficiency [HRD] assays) are also reviewed here. The mechanisms of action are reviewed elsewhere.

Key events, including the publication of important clinical trials and Food and Drug Administration (FDA) approval of each PARP inhibitor, are summarized in Fig. 20.1. In short, the clinical development of PARP inhibitors for patients with ovarian cancer began in the late-line setting. Then, the development continued to platinum-sensitive recurrence and, finally, to the first-line setting. For better understanding, data on the first-line, platinum-sensitive, and platinum-resistant recurrences are reviewed in the order they have been stated.

In each setting, the efficacy results of clinical trials that evaluated PARP inhibitors, as either monotherapy or in combination, with approval by the FDA and Pharmaceuticals and Medical Devices Agency (PMDA) are reviewed. The efficacy endpoints which focused on hereditary ovarian cancer are also tabulated in each setting. Ongoing clinical trials evaluating PARP inhibitors with other targeting agents such as antiangiogenic agents and/or immune checkpoint inhibitors are also reviewed at the end of this chapter.

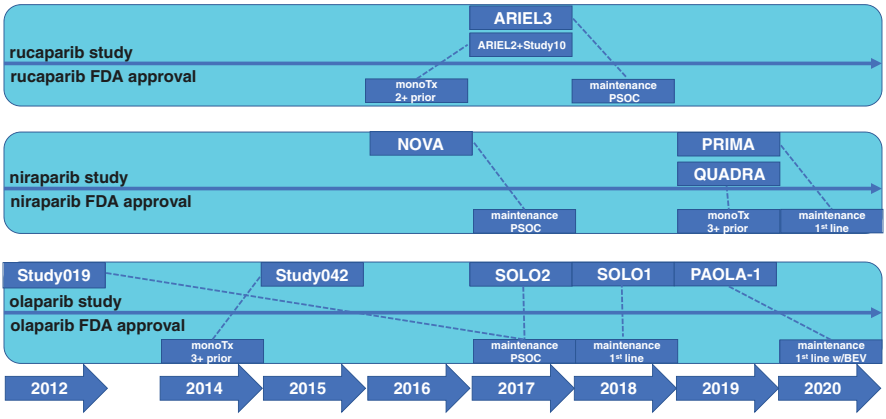


Fig. 20.1 Timeline of the publication of important clinical trials and FDA approval of each PARP inhibitor for ovarian cancer. Abbreviations: *PARPi* PARP inhibitor; *mono Tx* monotherapy; *2+ prior* two or more previous lines of chemotherapy; *PSOC* platinum-sensitive ovarian cancer; *3+ prior* three or more previous lines of chemotherapy; *w/BEV* with bevacizumab

20.2 First Line

20.2.1 Monotherapy

In the SOLO1 trial [1], 2-year olaparib was compared with a placebo as a switch maintenance therapy after response to platinum-combination chemotherapy for patients with advanced ovarian cancer harboring a germline or somatic *BRCA* mutation. Among 391 randomized patients, 388 (99.2%) had a germline *BRCA* mutation. Olaparib significantly improved the 3-year progression-free survival (PFS) from 27% to 60% (hazard ratio [HR] for PFS, 0.30; 95% confidence interval [CI], 0.23–0.41). On December 19, 2018, the FDA gave accelerated approval of olaparib for use in women with advanced ovarian cancer associated with defective *BRCA1/2* genes. The PMDA also approved olaparib for the same indication on June 19, 2019. At the European Society for Medical Oncology (ESMO) 2020 Annual Conference, results of a 5-year follow-up were reported. The 5-year PFS was 48.3% in the patients who received olaparib and 20.5% in those who received the placebo (HR for PFS, 0.33; 95% CI, 0.25–0.43).

In the PRIMA trial [2], 3-year niraparib was compared with a placebo as a switch maintenance therapy after response to platinum-combination chemotherapy for patients with advanced ovarian cancer. In this trial, all eligible patients provided tumor samples to identify HRD using the myChoice test. This test assessed tumor *BRCA* mutation and determined the genomic instability score (GIS), which consists of loss of heterozygosity (LOH), telomeric allelic imbalance, and large-scale state transitions. The GIS ranges from 1 to 100, with a score of ≥42 defined as HRD. The presence of somatic *BRCA* mutation was also considered as HRD. A hierarchical testing method was performed for the primary endpoint, which was PFS in the population with HRD, followed by a test in the overall population. Among 733 patients who underwent randomization, 373 (50.9%) and 223 (30.4%) had HRD and *BRCA* mutation, respectively. Niraparib significantly improved the median PFS (mPFS) from 10.4 months to 21.9 months (HR for PFS, 0.43; 95% CI, 0.31–0.59) as compared with a placebo in the primary endpoint population whose tumor had HRD. The efficacy of niraparib therapy was better in the patients with *BRCA* mutation (HR for PFS, 0.40; 95% CI, 0.27–0.62) but preserved in the patients with homologous recombination-proficient tumors (HR for PFS, 0.68; 95% CI, 0.49–0.94). April 29, 2020, the FDA approved niraparib as a first-line monotherapy maintenance treatment for women with platinum-responsive advanced ovarian cancer regardless of biomarker status. The PMDA also approved niraparib for the same indication on September 25, 2020.

20.2.2 Combination

In the VELIA trial [3], veliparib combined with carboplatin plus paclitaxel (TC) with or without veliparib maintenance therapy administered for 2 years was

compared with a placebo for patients with advanced ovarian cancer. All the eligible patients submitted blood and tumor samples for the assessment of germline *BRCA*, somatic *BRCA*, and HRD status. The primary endpoint was PFS in the veliparib-throughout group as compared with the placebo group and analyzed sequentially in the *BRCA*-mutant (whether germline or somatic), HRD, and intention-to-treat (ITT) groups. Among 1140 patients who underwent randomization, 627 (55%) and 298 (26%) had HRD and *BRCA* mutation, respectively. In the *BRCA* mutation group, 214 patients (71.8%) had a germline *BRCA* mutation. Veliparib as a continuation maintenance therapy compared with the placebo significantly improved the mPFS from 22.0 months to 34.7 months (HR for PFS, 0.44; 95% CI, 0.28–0.68) in the patients with *BRCA* mutation and from 20.5 months to 24.7 months (HR for PFS, 0.57; 95% CI, 0.28–0.68) in the patients with HRD. Although the trial met the primary endpoint, veliparib was yet to be approved.

Combining PARP inhibitors with antiangiogenic agents is promising owing to the conceptual synthetic lethality, especially for tumors with HRD without *BRCA* mutation [4]. In the PAOLA-1 trial [5], olaparib plus bevacizumab (BEV) during maintenance therapy after TC plus BEV was compared with a placebo plus BEV for patients with advanced ovarian cancer. All eligible patients submitted tumor samples for the assessment of HRD status. The primary endpoint was PFS in the ITT population. Among 806 patients who underwent randomization, 387 (48%) and 237 (29%) had HRD and *BRCA* mutation, respectively. In the French cohort of the trial [6], concordance of somatic *BRCA* and germline *BRCA* was investigated. Among 451 patients who submitted both tumor and germline *BRCA* samples, 6.6% had an inconclusive tumor testing. Except for those patients, 391 patients had concordant results (306 had both positive, and 85 had both negative results). Only 30 patients had discordant results. Twenty-nine patients with negative germline results had positive somatic results, and one patient with positive germline result (large genomic rearrangement in exons 1 and 2 in the *BRCA1* gene) had a negative tumor result. For the primary endpoint, 2-year olaparib significantly improved the mPFS from 16.6 months to 22.1 months (HR for PFS, 0.59; 95 CI, 0.49–0.72) in the ITT population. In the pre-planned subgroup analysis, the superiority of the combination with BEV monotherapy was demonstrated regardless of tissue *BRCA* mutation but was limited in patients with HRD. In the *BRCA* mutation and wild-type/unknown subgroup, the HRs for PFS were 0.31 (95% CI, 0.20–0.47) and 0.71 (95% CI, 0.58–0.88), respectively. In the HRD-positive and HRD-negative subgroup, the HRs for PFS were 0.33 (95% CI, 0.25–0.45) and 1.00 (95% CI, 0.75–1.35), respectively. The FDA approved this combination as first-line maintenance treatment for advanced ovarian cancer with HRD on May 8, 2020. The PMDA also approved this combination for the same indication on December 28, 2020.

The efficacy data for hereditary ovarian cancer are summarized in Table 20.1.

Table 20.1 Efficacy of PARP inhibitors for hereditary ovarian cancer in the first-line setting

Trial	PARPi	CTR (m)	INT (m)	HR (PFS)	*BRCA*
SOLO1	Olaparib	13.8	NR	0.3	g only
PRIMA	Niraparib	22.1	10.9	0.4	t or g
VELIA	Veliparib	22	34.7	0.44	t or g
PAOLA-1	Olaparib	21.7	37.2	0.31	t only

Abbreviations: *PARPi* PARP inhibitor; *CTR* median PFS in the control arm; *INT* median PFS in the intervention arm; *NR* not reached; *g only* germline *BRCA* mutation only; *t or g* germline or somatic *BRCA* mutation included; *t only* somatic *BRCA* mutation only

20.3 Platinum-Sensitive Recurrence

20.3.1 Monotherapy as Switch Maintenance Therapy

In the Study 019 [7], olaparib as a switch maintenance therapy was compared with a placebo for patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer after response to platinum-combination chemotherapy. Olaparib improved the mPFS from 4.8 months to 8.4 months (HR for PFS, 0.35; 95% CI, 0.25–0.49). At the time of first publication, only 36.6% (97/265) of the patients reported their germline *BRCA* status. In the follow-up study [8], additional testing for germline and/or somatic *BRCA* revealed the status in 96% (254/265) of the patients. Among 165 patients with both germline and somatic samples, 87% (144/165) had concordant results (71 had both positive and 73 had both negative results). Eighteen patients had negative germline results with positive somatic results. Three patients had positive germline results with negative somatic results. In the subgroup analysis for patients with *BRCA* mutation defined in this follow-up study, olaparib markedly improved the mPFS from 4.3 months to 11.2 months (HR for PFS, 0.19). In the subgroup analysis of the final overall survival (OS) follow-up [9], the efficacy of olaparib was observed in the patients with both germline *BRCA* mutation (HR for OS, 0.62; 95% CI, 0.42–0.93) and wild-type *BRCA* (HR for OS, 0.84; 95% CI, 0.57–1.25). The numerically better efficacy in the *BRCA*-mutant subgroup led to patient enrichment in the next pivotal phase 3 trial, SOLO2. Biomarker analysis of the Study 019 was performed [10]. Among 95 patients with wild-type *BRCA1/2*, 21 had at least 1 loss-of-function gene alteration in one of the homologous recombination-related (HRR) genes such as *BRIP1* (n = 5), *CDK12* (n = 3), *RAD54L* (n = 3), *RAD51B* (n = 2), *FANCL* (n = 2), *ATM* (n = 1), *FANCA* (n = 1), *FANCD2* (n = 1), *RAD51C* (n = 1), *RAD52* (n = 1), and *XRCC3* (n = 1). The subgroup analysis results suggested that olaparib administration was associated with greater PFS benefit (HR for PFS, 0.21; 95% CI, 0.04–0.86) in these patients than in those with no detectable *BRCA* or HRR mutation (HR for PFS, 0.71; 95% CI, 0.37–1.35) who received olaparib.

In the SOLO2 trial [11], olaparib as a switch maintenance therapy was compared with a placebo for patients with platinum-sensitive recurrent ovarian cancer harboring a *BRCA* mutation after response to platinum-combination chemotherapy. Olaparib significantly improved the mPFS from 5.5 months to 19.1 months (HR for

PFS, 0.30; 95% CI, 0.22–0.41). Based on the data from the Study 019 and SOLO2 trials, the FDA approved olaparib as a maintenance therapy for patients with recurrent ovarian cancer who have attained complete or partial response to platinum-based chemotherapy, regardless of *BRCA* status, on August 17, 2017. PMDA also approved olaparib for the same indication on January 19, 2018.

In the NOVA trial [12], niraparib as a switch maintenance therapy was compared with a placebo for patients with platinum-sensitive recurrent ovarian cancer after response to platinum-combination chemotherapy was attained. This study evaluated two independent cohorts, the germline *BRCA*-mutant cohort (g*BRCA* cohort) and the nonmutant cohort (non-g*BRCA* cohort). A hierarchical testing was pre-planned for the non-g*BRCA* cohort in which statistical analysis was first performed in the patients with HRD-positive tumors, and if the results were significant, a test for the overall non-g*BRCA* cohort was performed. Among 553 patients who underwent randomization, 203 (36.7%) had germline *BRCA* mutation and 350 did not. Of the 350 patients in the non-g*BRCA* cohort, 162 (46%) had HRD. In the g*BRCA* cohort, niraparib therapy significantly improved the mPFS from 5.5 months to 21.0 months (HR for PFS, 0.27; 95% CI, 0.17–0.41). In the HRD-positive subgroup of the non-g*BRCA* cohort, which was pre-specified as the primary analysis population, niraparib also significantly improved the mPFS from 3.8 months to 12.9 months (HR for PFS, 0.38; 95% CI, 0.24–0.59). Niraparib proved to have a consistent efficacy in the overall non-g*BRCA* cohort (HR for PFS, 0.45; 95% CI, 0.34–0.61). The FDA approved niraparib as a maintenance therapy for patients with recurrent ovarian cancer who have attained complete or partial response to platinum-based chemotherapy, regardless of *BRCA* status, on March 27, 2017. The PMDA also approved niraparib for the same indication on September 25, 2020.

In the ARIEL3 trial [13], rucaparib as a switch maintenance therapy was compared with a placebo for patients with platinum-sensitive recurrent ovarian cancer after response to platinum-combination chemotherapy. All the randomized patients submitted blood and tumor tissue samples for assessments of germline *BRCA* mutation, somatic *BRCA* mutation, and HRD. In this study, HRD was determined on the basis of genomic LOH by using the Foundation Medicine T5 NGS assay. Based on the results of the preceding phase 2 study, ARIEL2 part 1, a cutoff of ≥16% was determined as high LOH. Among 564 patients who underwent randomization, 130 (23%) and 66 (11%) from the *BRCA*-mutation cohort had germline and somatic *BRCA* mutations. Among 368 patients with wild-type *BRCA*, 158 (42.9%) had HRD. Patients with HRD plus *BRCA* mutation were included in the HRD cohort. Rucaparib significantly improved the mPFS from 5.4 months to 16.6 months (HR for PFS, 0.23; 95% CI, 0.16–0.34) in the *BRCA*-mutation cohort. Rucaparib also proved to have consistent efficacy in the HRD cohort (HR for PFS, 0.32; 95% CI, 0.24–0.42) and ITT population (HR for PFS, 0.36; 95% CI, 0.30–0.45). In the subgroup analysis, the PFS benefit of rucaparib was almost identical in the germline (HR, 0.25) and somatic *BRCA*-mutation cohorts (HR, 0.23). The FDA approved rucaparib as a maintenance therapy for patients with recurrent ovarian cancer who

Table 20.2 Efficacy of the PARP inhibitors as maintenance therapy for platinum-sensitive recurrence of hereditary ovarian cancer

Trial	PARPi	CTR (m)	INT (m)	HR (PFS)	*BRCA*
Study 019	Olaparib	4.3	11.2	0.18	t or g
SOLO2	Olaparib	5.5	19.1	0.3	g only
NOVA	Niraparib	5.5	21.0	0.27	g only
ARIEL3	Rucaparib	5.4	16.6	0.23	t or g

Abbreviations: *PARPi* PARP inhibitor; *CTR* median PFS in the control arm; *INT* median PFS in the intervention arm; *t or g* germline or somatic *BRCA* mutation included; *g only* germline *BRCA* mutation only

have attained complete or partial response to platinum-based chemotherapy, regardless of *BRCA* status, on April 6, 2018 (Table 20.2).

20.3.2 Monotherapy as Salvage Treatment for Patients with Multiple Sensitive Relapses

In the pooled analysis of two phase 1 and four phase 2 studies of olaparib monotherapy for patients with ovarian cancer who had germline *BRCA* mutation, the objective response rate (ORR) and duration of response (DoR) were 48% and 7.8 months, respectively, for the subgroup of 75 patients with platinum-sensitive relapse [14].

In the SOLO3 trial [15], olaparib was compared with non-platinum chemotherapy such as pegylated liposomal doxorubicin for patients with platinum-sensitive relapsed ovarian cancer harboring a germline *BRCA* mutation. Olaparib significantly improved the ORR from 51.4% to 72.2% (odds ratio [OR], 2.53; 95% CI, 1.4–4.58) and PFS from 9.2 months to 14.3 months (HR for PFS, 0.62; 95% CI, 0.43–0.91).

Regarding rucaparib, in the integrated analysis of two phase 2 studies (Study 10 and ARIEL2) for patients with ovarian cancer with *BRCA* mutation [16], the ORR and mPFS were 65.8% and 11.1 months, respectively, in the platinum-sensitive subgroup. Based on these data, the FDA granted accelerated approval of rucaparib on December 19, 2016, for patients with *BRCA*-mutant ovarian cancer treated with two or more lines of chemotherapy.

In the QUADRA trial [17], a single-arm phase 2 study of niraparib monotherapy, patients with ovarian cancer had received three or more lines of chemotherapy. All the patients underwent a blood test for germline *BRCA* mutation and a tumor test for HRD. Among the 463 patients enrolled in this study, 222 (47.9%) and 87 (19%) had HRD and *BRCA* mutation, respectively. Niraparib monotherapy had ORRs of 39%, 26%, and 4% for patients with germline *BRCA* mutation, HRD, and no/unknown HRD, respectively. The FDA approved niraparib on October 23, 2019, for patients with HRD-positive ovarian cancer treated with three or more prior lines of chemotherapies (Table 20.3).

Table 20.3 Efficacy of PARP inhibitor monotherapy for hereditary ovarian cancer as a salvage treatment after multiple platinum-sensitive relapses

Trial	PARPi	ORR (%)	DoR (m)	PFS (m)	*BRCA*
6 trials	Olaparib	48	7.8	–	g only
SOLO3	Olaparib	72.2	9.4	14.3	g only
2 trials	Rucaparib	65.8	–	11.1	g or t[a]
QUADRA	Niraparib	39	9.2	–	g only

Abbreviations: *PARPi* PARP inhibitor; *ORR* objective response rate; *DoR* duration of response; *g only* germline *BRCA* mutation only; *g or t* germline or somatic *BRCA* mutation included
[a]Of the patients, 83% (88/106) from the integrated efficacy population had a germline *BRCA* mutation, and the rest had a somatic *BRCA* mutation

20.3.3 Combination

Olaparib plus cediranib, a multi-VEGF receptor inhibitor, showed an intriguing efficacy in a randomized phase 2 study [18] for patients with recurrent platinum-sensitive ovarian cancer. Among the 90 patients who underwent randomization, 47 (52.2%) had a germline *BRCA* mutation. Compared with olaparib monotherapy, olaparib plus cediranib prolonged the mPFS from 9.0 months to 17.7 months (HR for PFS, 0.42; 95% CI, 0.23–0.76). In a post hoc subgroup analysis, the PFS benefit in the subgroup with germline *BRCA* mutation (HR for PFS, 0.55) seemed smaller than that in the subgroup of wild-type or unknown *BRCA* status (HR for PFS, 0.32).

In the following phase 3 study, the NRG GY-004 trial [19], the combination therapy failed to improve the PFS (HR, 0.856) for the patients with platinum-sensitive recurrent ovarian cancer, as compared with the standard chemotherapy. However, in the subgroup analysis for patients with germline *BRCA* mutation, this combination showed a promising efficacy, with an HR for PFS of 0.55 (95% CI, 0.73–1.30), as compared with the standard chemotherapy.

In the e-Volve trial [20], the same combination was tested in a single-arm, multicohort phase 2 trial, for patients with platinum-sensitive ovarian cancer after progression with PARP inhibitors. Among the 11 patients in cohort 1 (platinum-sensitive recurrence), no response was observed.

Another combination of a PARP inhibitor and antiangiogenic agent, niraparib plus BEV, was tested in the AVANOVA-2 study [21] for patients with platinum-sensitive relapsed ovarian cancer. Among 97 patients who underwent randomization, 33 (34%) had a *BRCA* mutation. Fifteen patients (15.4%) had a germline *BRCA* mutation. Niraparib plus BEV improved the mPFS from 5 months to 11.9 months (HR for PFS, 0.35; 95% CI, 0.21–0.57), as compared with niraparib alone. In a pre-planned subgroup analysis of patients with *BRCA* mutation, the combination therapy showed the same trend (mPFS 14.4 months vs. 9.0 months; HR for PFS, 0.49; 95% CI, 0.21–1.15).

As PARP inhibitors have immunoregulatory effects [22], a combination therapy of PARP and immune checkpoint inhibitors (ICI) is also being

developed. In the MEDIOLA study, the effectiveness of olaparib plus durvalumab was evaluated in patients with platinum-sensitive relapsed ovarian cancer harboring a germline *BRCA* mutation. At the presentation of the ESMO2019, the ORR, mDoR, and mPFS were 71.9%, 10.2 months, and 11.1 months, respectively.

Adding both antiangiogenics and ICI to PARP inhibitors is also promising. In another triplet cohort of the MEDIOLA trial, patients with platinum-sensitive relapsed ovarian cancer with wild-type g*BRCA* received olaparib, durvalumab, and bevacizumab as triplet therapy. The preliminary results of 31 patients were presented at the ESMO2020. The ORR and disease control rate at 24 weeks were 87% and 77%, respectively.

20.4 Platinum-Resistant Recurrence

20.4.1 Monotherapy

In the platinum-resistant subgroup included in the pooled analysis of the aforementioned six trials [14], the ORR and DoR of olaparib monotherapy were 28% and 7.4 months, respectively. Study 042 was one of four phase 2 trials for patients with advanced solid tumor with a germline *BRCA* mutation. The ovarian cancer cohort included 193 patients, most of whom were platinum resistant, and the remaining were intolerant to platinum agents, typically owing to hypersensitivity reactions. The ORR of olaparib monotherapy was 26.2% for the whole cohort [23] and was 34% for patients with measurable disease who had received three or more lines of chemotherapy [24]. Based on this data, the FDA granted accelerated approval of olaparib on December 19, 2014, for patients with advanced ovarian cancer associated with the *BRCA* gene mutation who had received three or more lines of chemotherapy.

In the platinum-resistant subgroup included in the integrated analysis of the aforementioned two trials [16], the ORR and mPFS of rucaparib monotherapy were 25% and 7.4 months, respectively. In the QUADRA trial, niraparib monotherapy showed ORRs 27%, 10%, and 3% for patients with *BRCA* mutation, HRD, or no/unknown HRD, respectively [17].

20.4.2 Combination

In the CONCERTO study, olaparib plus cediranib showed an ORR of 15.3% for patients without g*BRCA* who had received three or more previous lines of chemotherapy [25]. In the TOPACIO study, niraparib plus pembrolizumab, a PD-1 inhibitor, was tested. The ORR and mDoR were 18% and not reached, respectively. The

Table 20.4 Efficacy of PARP inhibitors as salvage treatment for hereditary ovarian cancer in the platinum-resistant setting

Trial	PARPi	ORR (%)	DoR (m)	PFS (m)	*BRCA*
6 trials	Olaparib	28	7.4	–	g only
2 trials	Rucaparib	25	–	7.4[a]	g or t
QUADRA	Niraparib	27	–	–	g only

Abbreviations: *PARPi* PARP inhibitor; *ORR* objective response rate; *DoR* duration of response; *g only* germline *BRCA* mutation only; *g or t* germline or somatic *BRCA* mutation included
[a]Excluding platinum-refractory patients ($n = 7$) whose median PFS was 5.3 months

Table 20.5 Ongoing studies evaluating the efficacy of adding ICI and/or antiangiogenics to PARP inhibitors

Trial	PARPi	ICI	aAngio	Setting	NCT
KEYLINK-001	Olaparib	Pembrolizumab		First line	03740165
ATHENA	Rucaparib	Nivolumab		First line	03522246
FIRST	Niraparib	Dostarlimab		First line	03602859
JAVELIN-100	Talazoparib	Avelumab		First line	03642132
MITO-25	Rucaparib		BEV	First line	03462212
ANITA	Niraparib	Atezolizumab		PSOC	03598270
NItCHE-MITO33	Niraparib	Dostarlimab		PSOC	04679064
NRG-GY021	Olaparib	Tremelimumab		PSOC	04034927
ICON9	Olaparib		Cediranib	PSOC	03278717
COCOS	Olaparib		Cediranib	PROC	02502266
DUO-O	Olaparib	Durvalumab	BEV	First line	03737643
AVATAR	Niraparib	Dostarlimab	BEV	PSOC	03806049
NRG-GY023	Olaparib	Durvalumab	Cediranib	PROC	04739800

Abbreviations: *PARPi* PARP inhibitor; *ICI* immune checkpoint inhibitor; *aAngio* antiangiogenic agent; *PSOC* platinum-sensitive ovarian cancer; *PROC* platinum-resistant ovarian cancer

ORRs of the patients with and without somatic *BRCA* mutation were similar (18% and 19%, respectively) [26] (Table 20.4).

20.5 Ongoing Studies

Many studies are ongoing to evaluate the efficacy of the combination of PARP inhibitors with immune checkpoint inhibitors, antiangiogenic agents, or both (Table 20.5). Other important trials include OReO (NCT03106987) and ARIEL4 (NCT02855944).

OReO (NCT03106987) is a phase 3 study that evaluated whether olaparib as maintenance therapy after progression with PARP inhibitors further improves the PFS in patients with platinum-sensitive recurrent ovarian cancer.

ARIEL4 (NCT02855944) is a phase 3 trial which compares rucaparib with a standard chemotherapy for patients with platinum-resistant/sensitive recurrent

ovarian cancer whose tumor is a germline or somatic *BRCA* mutant. These studies will clarify the optimal strategy for using PARP inhibitors in patients with hereditary ovarian cancer.

References

1. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379(26):2495–505.
2. Gonzalez-Martin A, Pothuri B, Vergote I, DePont Christensen R, Graybill W, Mirza MR, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 2019.
3. Coleman RL, Fleming GF, Brady MF, Swisher EM, Steffensen KD, Friedlander M, et al. Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med. 2019;381:2403–15.
4. Chan N, Pires IM, Bencokova Z, Coackley C, Luoto KR, Bhogal N, et al. Contextual synthetic lethality of cancer cell kill based on the tumor microenvironment. Cancer Res. 2010;70(20):8045–54.
5. Ray-Coquard I, Pautier P, Pignata S, Perol D, Gonzalez-Martin A, Berger R, et al. Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med. 2019;381(25):2416–28.
6. Callens C, Vaur D, Soubeyran I, Rouleau E, Just PA, Guillerm E, et al. Concordance between tumor and germline BRCA status in high-grade ovarian carcinoma patients in the phase III PAOLA-1/ENGOT-ov25 trial. J Natl Cancer Inst 2020.
7. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med. 2012;366(15):1382–92.
8. Ledermann J, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol. 2014;15(8):852–61.
9. Friedlander M, Matulonis U, Gourley C, du Bois A, Vergote I, Rustin G, et al. Long-term efficacy, tolerability and overall survival in patients with platinum-sensitive, recurrent high-grade serous ovarian cancer treated with maintenance olaparib capsules following response to chemotherapy. Br J Cancer. 2018;119(9):1075–85.
10. Hodgson DR, Dougherty BA, Lai Z, Fielding A, Grinsted L, Spencer S, et al. Candidate biomarkers of PARP inhibitor sensitivity in ovarian cancer beyond the BRCA genes. Br J Cancer. 2018;119(11):1401–9.
11. Pujade-Lauraine E, Ledermann JA, Selle F, Gebski V, Penson RT, Oza AM, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18(9):1274–84.
12. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med. 2016;375(22):2154–64.
13. Coleman RL, Oza AM, Lorusso D, Aghajanian C, Oaknin A, Dean A, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2017;390(10106):1949–61.
14. Matulonis UA, Penson RT, Domchek SM, Kaufman B, Shapira-Frommer R, Audeh MW, et al. Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multistudy analysis of response rates and safety. Ann Oncol. 2016;27(6):1013–9.

15. Penson RT, Valencia RV, Cibula D, Colombo N, Leath CA 3rd, Bidzinski M, et al. Olaparib versus nonplatinum chemotherapy in patients with platinum-sensitive relapsed ovarian cancer and a germline BRCA1/2 mutation (SOLO3): a randomized phase III trial. J Clin Oncol. 2020;38(11):1164–74.
16. Oza AM, Tinker AV, Oaknin A, Shapira-Frommer R, McNeish IA, Swisher EM, et al. Antitumor activity and safety of the PARP inhibitor rucaparib in patients with high-grade ovarian carcinoma and a germline or somatic BRCA1 or BRCA2 mutation: integrated analysis of data from study 10 and ARIEL2. Gynecol Oncol. 2017;147(2):267–75.
17. Moore KN, Secord AA, Geller MA, Miller DS, Cloven N, Fleming GF, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol. 2019;20(5):636–48.
18. Liu JF, Barry WT, Birrer M, Lee JM, Buckanovich RJ, Fleming GF, et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol. 2014;15(11):1207–14.
19. Liu JF, Brady MF, Matulonis UA, Miller A, Kohn EC, Swisher EM, et al. A phase III study comparing single-agent olaparib or the combination of cediranib and olaparib to standard platinum-based chemotherapy in recurrent platinum-sensitive ovarian cancer. J Clin Oncol. 2020;38(15_suppl):6003.
20. Lheureux S, Oaknin A, Garg S, Bruce JP, Madariaga A, Dhani NC, et al. EVOLVE: a multicenter open-label single-arm clinical and translational phase II trial of Cediranib plus Olaparib for ovarian cancer after PARP inhibition progression. Clin Cancer Res. 2020;26(16):4206–15.
21. Mirza MR, Avall Lundqvist E, Birrer MJ. dePont Christensen R, Nyvang GB, Malander S, et al. Niraparib plus bevacizumab versus niraparib alone for platinum-sensitive recurrent ovarian cancer (NSGO-AVANOVA2/ENGOT-ov24): a randomised, phase 2, superiority trial. Lancet Oncol. 2019;20(10):1409–19.
22. Huang J, Wang L, Cong Z, Amoozgar Z, Kiner E, Xing D, et al. The PARP1 inhibitor BMN 673 exhibits immunoregulatory effects in a Brca1(−/−) murine model of ovarian cancer. Biochem Biophys Res Commun. 2015;463(4):551–6.
23. Kaufman B, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, Balmana J, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol. 2015;33(3):244–50.
24. Domchek SM, Aghajanian C, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, et al. Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. Gynecol Oncol. 2016;140(2):199–203.
25. Lee J-m, Moore RG, Ghamande SA, Park MS, Diaz JP, Chapman JA, et al. Cediranib in combination with olaparib in patients without a germline BRCA1/2 mutation with recurrent platinum-resistant ovarian cancer: phase IIb CONCERTO trial. J Clin Oncol. 2020;38(15_suppl):6056.
26. Konstantinopoulos PA, Waggoner S, Vidal GA, Mita M, Moroney JW, Holloway R, et al. Single-arm phases 1 and 2 trial of Niraparib in combination with Pembrolizumab in patients with recurrent platinum-resistant ovarian carcinoma. JAMA Oncol 2019.