History, Advancements, and Future Strategies

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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

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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,

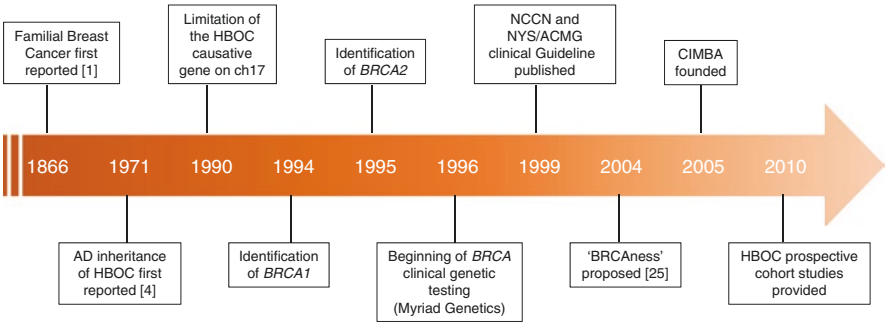

Fig. 1.1 Timeline of HBOC discovery and clinical setting. *AD* autosomal dominant, *NCCN* National Comprehensive Cancer Network, *NYS* New York State, *ACMG* American College of Medical Genetics, *CIMBA* Consortium of Investigators of Modifiers of BRCA1/2

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

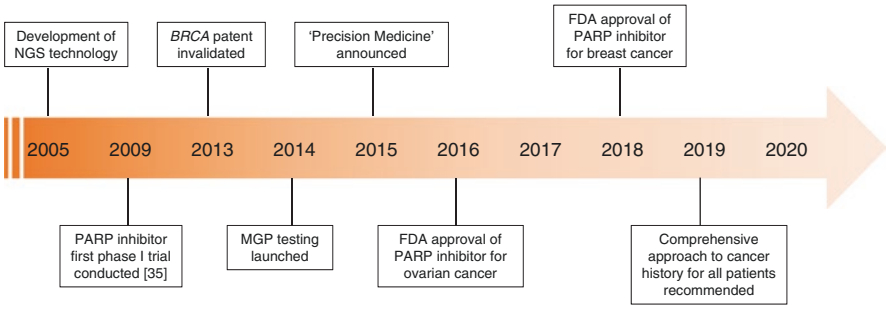

Fig. 1.2 Timeline of HBOC development. *NGS* next-generation sequence, *PARP* poly ADP ribose polymerase, *MGP* multi-gene panel, *FDA* US Food and Drug Administration

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

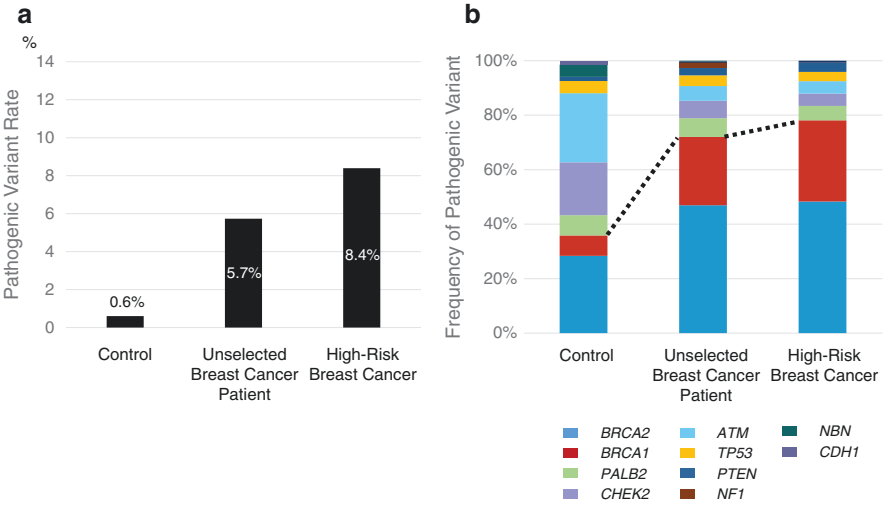

Fig. 1.3 The results of MGP testing in a Japanese biobank cohort. The data of the result of MGP testing including 11 breast cancer-susceptibility genes (*BRCA1*, *BRCA2*, *PALB2*, *TP53*, *PTEN*, *CHEK2*, *NF1*, *ATM*, *CDH1*, *NBN*, and *STK11*) using the Japanese biobank cohort was illustrated in bar 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

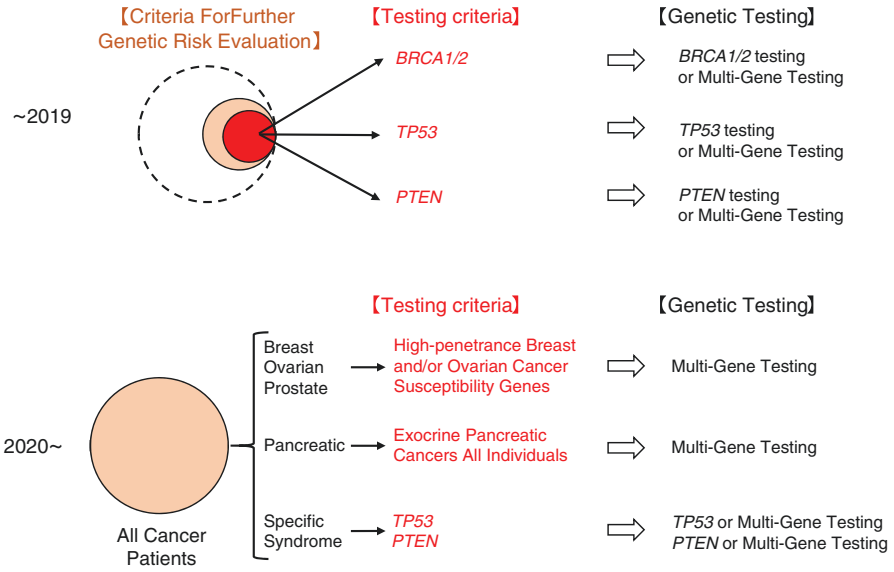

Fig. 1.4 Changes in genetic risk assessment and testing criteria in NCCN guideline. NCCN guideline 2020 edition has radically shifted away from *BRCA* genes toward a broad screening of other genes, consistent with current practice. Hereditary pancreatic cancer has also been added to this guideline, and 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

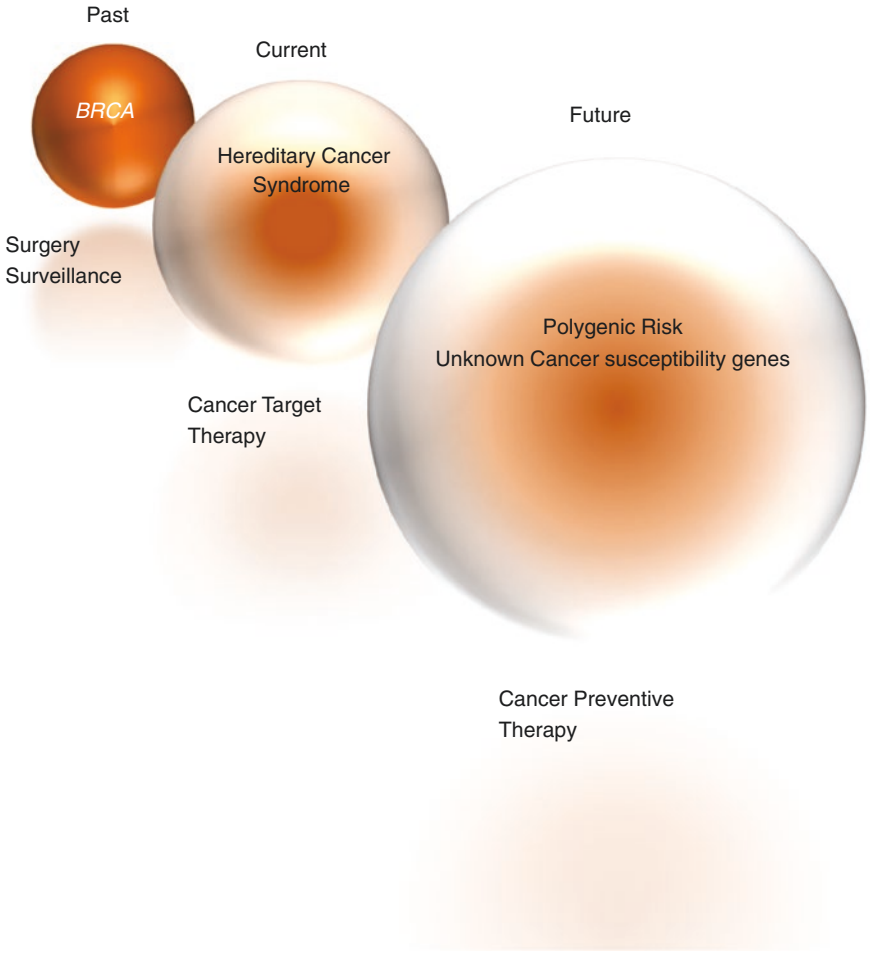

Fig. 1.5 HBOC strategy past, current, future. Targets for considering the risk of cancer susceptibility and effective medical practices are expanding

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.

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