HEREDITARY BREAST AND OVARIAN CANCER: CURRENT CONCEPTS OF PREVENTION AND TREATMENT

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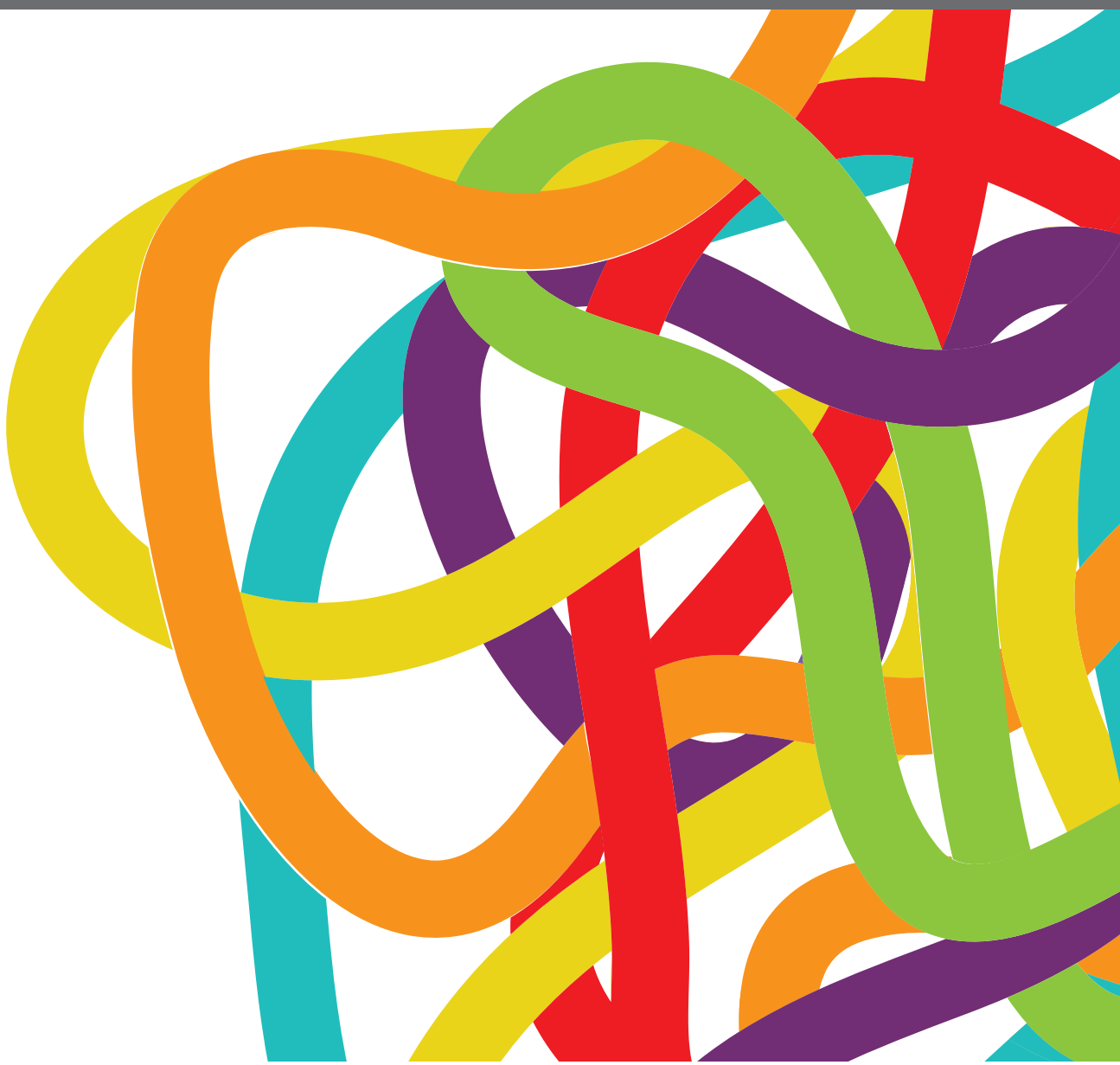

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HEREDITARY BREAST AND OVARIAN CANCER: CURRENT CONCEPTS OF PREVENTION AND TREATMENT

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Table of Contents

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EDITORIAL
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Editorial: Hereditary Breast and Ovarian Cancer: Current Concepts of Prevention and Treatment

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Editorial on the Research Topic

Hereditary Breast and Ovarian Cancer: Current Concepts of Prevention and Treatment

Breast cancer is the leading cause of cancer-related death in women worldwide. Whilst ovarian cancer is less common, it remains challenging due to late detection and high mortality (1). Most cases are considered sporadic; however, both tumor types may occur in patients with inherited mutations in cancer susceptibility genes (2). Hereditary breast and ovarian cancer syndrome (HBOC) accounts for 90% of the hereditary neoplasms and is predominantly associated with germline mutations in *BRCA1* or *BRCA2* genes (3). The mean cumulative risk of breast cancer is 57% in *BRCA1* mutation carriers and 49% in *BRCA2* mutation carriers, while ovarian cancer risk in women with *BRCA1* and *BRCA2* mutations is 40% and 18%, respectively. HBOC can also increase the risk, albeit to a lesser extent, for other neoplasms such as prostate or pancreatic cancer and malignant melanoma (3). Since the discovery of the *BRCA* genes and the development of clinical testing, the health advantages of identifying individuals at risk for HBOC have been well documented leading to the investigation and implementation of genetic counseling and screening, enhanced surveillance as well as surgical and non-surgical risk reduction options. We hope that a collection of review (including systematic review), mini-review, perspective and original research articles in this Research Topic will provide further insight on HBOC, including clinicopathologic features of associated cancers, genetic testing and treatment modalities, and their impact on patient outcomes.

The most common and well-characterized genes implicated in HBOC are *BRCA1* and *BRCA2*. Hatano et al. provide a comprehensive summary of the molecular biology of these genes, with a brief history of their discovery and review of the clinical implications of mutations (4). They also discuss the emerging research into the concept of "mutational signatures," representing the characteristic combination of mutation types in somatic cells. Deciphering mutational signatures in cancer provides insight into the mechanisms of cancer progression and this comprehensive genome analysis enables researchers to not only learn the current status of cancer predisposing genes but potentially to predict their future behavior through the understanding of the molecular underpinnings from which they arose.

The advancement of molecular techniques and gene sequencing platforms has enabled the discovery of additional genes, beyond *BRCA1* and *BRCA2*, that play a role in the development of hereditary breast and ovarian cancers. In a review of *PALB2*, a functional protein partner of *BRCA2*, Wu et al. discuss its function and role in breast cancer (5). Patients with monoallelic *PALB2* mutations are susceptible to breast, pancreatic, and ovarian cancer. *PALB2* mutation carriers are predisposed to breast cancer with a similar cumulative risk as *BRCA2* and having as much as a nine-fold higher than average lifetime risk,

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particularly in males. Emphasizing that *PALB2*-mutated breast cancers are associated with aggressive clinicopathologic features and poor prognosis, the authors recommend the inclusion of *PALB2* in multigene panels. Importantly, they introduce the possibility of effectiveness of poly (ADP-ribose) polymerase (PARP) inhibitors for *PALB2*-deficient breast tumors.

PARP is essential in the repair of single-stranded DNA breaks by homologous recombination (HR). However, in HR-deficient tumors PARP inhibitors prevent DNA repair *via* synthetic lethality. In their meta-analysis, Wang et al. assessed the efficacy of PARP inhibitors in newly diagnosed advanced stage ovarian cancers (6). Analysis of three randomized controlled trials revealed that maintenance therapy by PARP inhibitors improved progression free survival when compared to placebo, with only minimal adverse events. These findings were also confirmed on subgroup analysis, which showed improved survival regardless of age and stage at diagnosis, especially in patients with HR-deficiency and *BRCA* mutations.

The relationship between PARP inhibitor efficacy and *BRCA* mutations was further investigated in original research by Peixoto et al. who sought to determine the frequency of somatic and germline *BRCA* mutations in non-mucinous ovarian cancers, with focus on those with Portuguese ancestry (7). They discovered pathogenic variants in 19.3% of patients (13.3% germline, 5.9% somatic), with higher prevalence in tumors with high-grade serous morphology. In addition, they determined that identification of the most common deleterious variants in their population would be the most efficacious strategy for early detection and management.

In addition to PARP inhibitors, HR-deficient tumor cells can be sensitive to platinum compounds. In their mini-review, Pouptsis et al. describe the most recent systemic treatment advances and clinical outcomes of hereditary breast cancer patients treated with platinum-based regimens and PARP inhibitors (8). In addition, they discuss risk-reducing surgical management options and challenges associated with such interventions in young patients.

HBOC may be suspected in different clinical scenarios, including cancer diagnosis before the age of 50 years or in multiple first and/or second degree relatives on the same side of the family, diagnosis of second ipsilateral or contralateral breast cancer or both breast and ovarian cancers, diagnosis of breast cancer in a male relative, or cancer history in a family of Ashkenazi Jewish ancestry. However, approximately one-third of HBOC patients would not qualify for germline mutation testing based on family history alone. One effective way to triage patients for genetic screening is through microscopic examination of their tumors. A perspective article by Hodgson and Turashvili describe the unique pathologic features of *BRCA*-associated breast and ovarian carcinomas (9). They contrast the *BRCA1* mutated breast cancers which are frequently high-grade and triple-negative with medullary morphology with those of *BRCA2* carriers which are more similar to sporadic ER-positive luminal-type tumors. *BRCA*-associated ovarian tumors are almost exclusively high-grade serous carcinomas, often exhibiting the so-called "SET (Solid, pseudo-Endometrioid, and Transitional cell carcinoma-like) features." They emphasize the importance of accurate pathologic assessment to ensure that patients receive optimal management, including genetic screening.

As screening programs, genetic testing and preventative measures have demonstrated to reduce HBOC-related mortality by half since the discovery of the *BRCA* genes and their role in HBOC, the data presented in this Research Topic will hopefully serve as an important reminder to clinicians, pathologists, geneticists, and other medical professionals as well as trainees that a multidisciplinary approach is critical in order to help *BRCA* mutation carriers make informed decisions regarding the screening, prevention, and treatment of hereditary breast and ovarian cancer.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Molecular Mechanisms of PALB2 Function and Its Role in Breast Cancer Management

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Partner and localizer of BRCA2 (PALB2) is vital for homologous recombination (HR) repair in response to DNA double-strand breaks (DSBs). PALB2 functions as a tumor suppressor and participates in the maintenance of genome integrity. In this review, we summarize the current knowledge of the biological roles of the multifaceted PALB2 protein and of its regulation. Moreover, we describe the link between *PALB2* pathogenic variants (PVs) and breast cancer predisposition, aggressive clinicopathological features, and adverse clinical prognosis. We also refer to both the opportunities and challenges that the identification of *PALB2* PVs provides in breast cancer genetic counseling and precision medicine.

Keywords: PALB2, homologous recombination, breast cancer, precision medicine, pathogenic variants

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INTRODUCTION

Partner and localizer of BRCA2 (PALB2) is encoded on chromosome 16p12.2 and comprises 1186 residues (1). As a major BRCA2 binding partner, PALB2 licenses the function of BRCA2 and participates in homologous recombination (HR), a faithful DNA double-strand break (DSB) repair pathway in mammalian cells (2–4). Numerous studies have demonstrated that biallelic mutations in *PALB2* resulted in a subtype of Fanconi anemia (FA-N), while monoallelic *PALB2* mutations predispose carriers to multiple cancers such as breast, pancreatic, and ovarian cancers (5–8).

Breast cancer is the most frequently diagnosed cancer and the major cause of cancer death among women worldwide (9). Approximately 10–15% of breast cancer cases are due to familial and genetic factors, underscoring the great significance of genetic susceptibility in breast cancer development (10). Previous studies have identified a broad range of breast cancer susceptibility genes, including *BRCA1*, *BRCA2*, and *TP53* (11). However, the high-penetrance *BRCA1* and *BRCA2* are responsible for only ∼20% of the familial aggregation of breast cancer (12, 13), and syndromic breast cancer susceptibility genes such as *TP53*, *PTEN*, and *CDH1* are estimated to explain just 5% of familial breast cancers (14). Large-scale analyses of multigene panel testing recently confirmed *PALB2* as a high-risk breast cancer susceptibility gene (15), and the odds ratio (OR) of *PALB2* mutations for breast cancer was comparable to that of *BRCA2* mutations (16). Hence, a comprehensive understanding of the biological functions of PALB2 is vital for breast cancer management and precision medicine.

STRUCTURES OF PALB2 AND ITS BIOLOGICAL FUNCTIONS IN HR

PALB2, first described by Xia et al. in 2006 (1), has an important role in HR. It mainly serves as a bridging molecule that connects the BRCA complex (BRCA1-PALB2-BRCA2-RAD51) and facilitates the function of RAD51, a protein vital for strand invasion during HR (**Figure 1**). The role of PALB2 in HR has been shown to involve several protein domains, including a coiled-coil domain, a WD40 domain, and a chromatin-association motif (ChAM) (**Figure 2**).

The coiled-coil domain is located in the N terminus of PALB2 (residues 9–42) and is responsible for its interaction with BRCA1 (2–4). The L21A, Y28A, and L35A mutations in the PALB2 coiled-coil domain disrupt the BRCA1-PALB2 interaction, impairing the function of PALB2 in HR repair and inducing hypersensitivity to mitomycin C (MMC) treatment (3). In addition to positively regulating HR, the BRCA1-PALB2 interaction is required for preventing single-strand annealing (SSA), which is a deletion-causing DSB repair pathway. Using U2OS/DR-GFP and U2OS/SA-GFP reporter cells, Anantha et al. demonstrated that depletion of either PALB2 or BRCA2 led to impaired HR activity and a substantial increase in SSA, whereas BRCA1 depletion caused a reduction of both HR and SSA activity (17). These results established that BRCA1 is essential for DSB repair, while PALB2 serves to direct the DSB repair toward the HR pathway following resection.

The WD40 domain is located in the PALB2 C-terminus and in the shape of a WD40-type β-propeller with seven blades (18). This domain is involved in the interaction with BRCA2, DNA polymerase η, RAD51, RAD51C, and the ubiquitin ligase RNF168 (5, 19–21). Even a single nucleotide change within the WD40 region (e.g., L939W) can disturb the PALB2-BRCA2 interaction and causes HR deficiency (20). The WD40 domain of PALB2 is also crucial for the interaction with DNA polymerase η, which is vital for the initiation of HR-mediated DNA synthesis and D-loop extension (19). Recently, a hidden nuclear export sequence (NES) was found in the WD40 domain of PALB2. The breast cancer-associated *PALB2* truncating mutation, W1038X, exposes this NES, resulting in PALB2 translocation to the cytoplasm and defects in HR (22).

The ChAM is an evolutionarily conserved domain located in the middle region of PALB2 (23). ChAM-deleted PALB2 has a compromised role in supporting MMC-induced RAD51 focus formation, suggesting that ChAM promotes the function of PALB2 through chromatin association (23). The ChAM binds to nucleosomes and participates in the formation of the PALB2-BRCA2-RAD51 complex on chromatin, which rapidly transforms into an active BRCA complex following DSBs (23).

In addition to BRCA complex formation, PALB2 also directly interacts with RAD51 and enhances its strand invasion activity (24, 25). *In vitro* D-loop assays revealed increased product formation when PALB2 was included in the RAD51 reaction. Moreover, Buisson et al. also identified two DNA-binding domains in PALB2 (24) (**Figure 2**). More recently, Deveryshetty et al. (26) showed that the main DNA-binding domain (DBD) of PALB2 is located in its N-terminus (N-DBD, residues 1–200). Mutation of just four amino acids in the N-DBD significantly disrupts the HR activity of PALB2. Surprisingly, the authors discovered that the N-DBD of PALB2 enhances RAD51-mediated strand exchange and also promotes a similar reaction in the absence of RAD51. Using strand exchange fluorescent assays, they further demonstrated that PALB2 N-DBD promotes both forward and inverse strand exchange using either DNA or RNA as substrate (26).

These studies uncovered multiple effects of PALB2 during HR. On the one hand, PALB2 serves as the bridging molecule in the BRCA complex; on the other hand, it potently stimulates strand invasion in HR.

PALB2: A VERSATILE PLAYER IN BIOLOGICAL REGULATION

PALB2 and Chromatin Association

Chromatin association is considered indispensable for the biological function of PALB2. In addition to the ChAM, MRG15 is another PALB2-interacting factor involved in PALB2 chromatin association (27) (**Figure 3A**). In 2009, Sy et al. unveiled MRG15 and another MORF-related gene product, MRGX, as PALB2 cooperators through tandem affinity purification and mass spectrometry analysis (28). MRG15 belongs to the highly conserved MRG protein family (29) and has two functional domains: one is an MRG domain that binds to PALB2 as well as multiple transcriptional regulators (28, 30), the other is N-terminal chromodomain that binds lysine 36-trimethylated histone H3 (H3K36me3) (31), which is mediated by lysine methyltransferase SET domain containing 2 (SETD2) (32). The MRG15-binding region was roughly mapped to the middle region of PALB2 (residues 611–764) and exactly matched two highly conserved regions named MBD-I (residues 611–629) and MBD-II (residues 724–737) (33). Sy et al. found that reconstitution of MRG15-binding domain deleted PALB2 could restore RAD51 foci formation and cell survival after MMC treatment in EUFA1341F PALB2-deficient cells (28). Strikingly, a concurrent study reached a contradictory conclusion, whereby siRNA-mediated MRG15 depletion in cells compromised HR repair efficiency and sensitization to MMC (34). Furthermore, MRG15 knockout murine embryonic fibroblasts exhibited moderate sensitivity to γ-irradiation and decreased capacity for RAD51 nuclear foci formation (35). Considering the studies above, we could propose that the

Abbreviations: ATM, ataxia telangiectasia mutated protein; ATR, ATM and Rad3-related kinase; CDK, cyclin-dependent kinase; ChAM, chromatin-association motif; CI, confidence interval; DSBs, DNA double-strand breaks; FA, Fanconi anemia; FPC, familial pancreatic cancer; H3K36me3, lysine 36-trimethylated histone H3; HR, homologous recombination; MBC, male breast cancer; MMC, mitomycin C; MRN, Mre11–RAD50–Nbs1 complex; NES, nuclear export sequence; OR, odds ratio; PALB2, partner and localizer of BRCA2; PARP, poly (ADP-ribose) polymerase; PARPi, PARP inhibitor; PV, pathogenic variant; ROS, reactive oxygen species; RPA, replication protein A; SETD2, SET domain containing 2; SSA, single-strand annealing; ssDNA, single-stranded DNA; VUSs, variants of unknown significance.

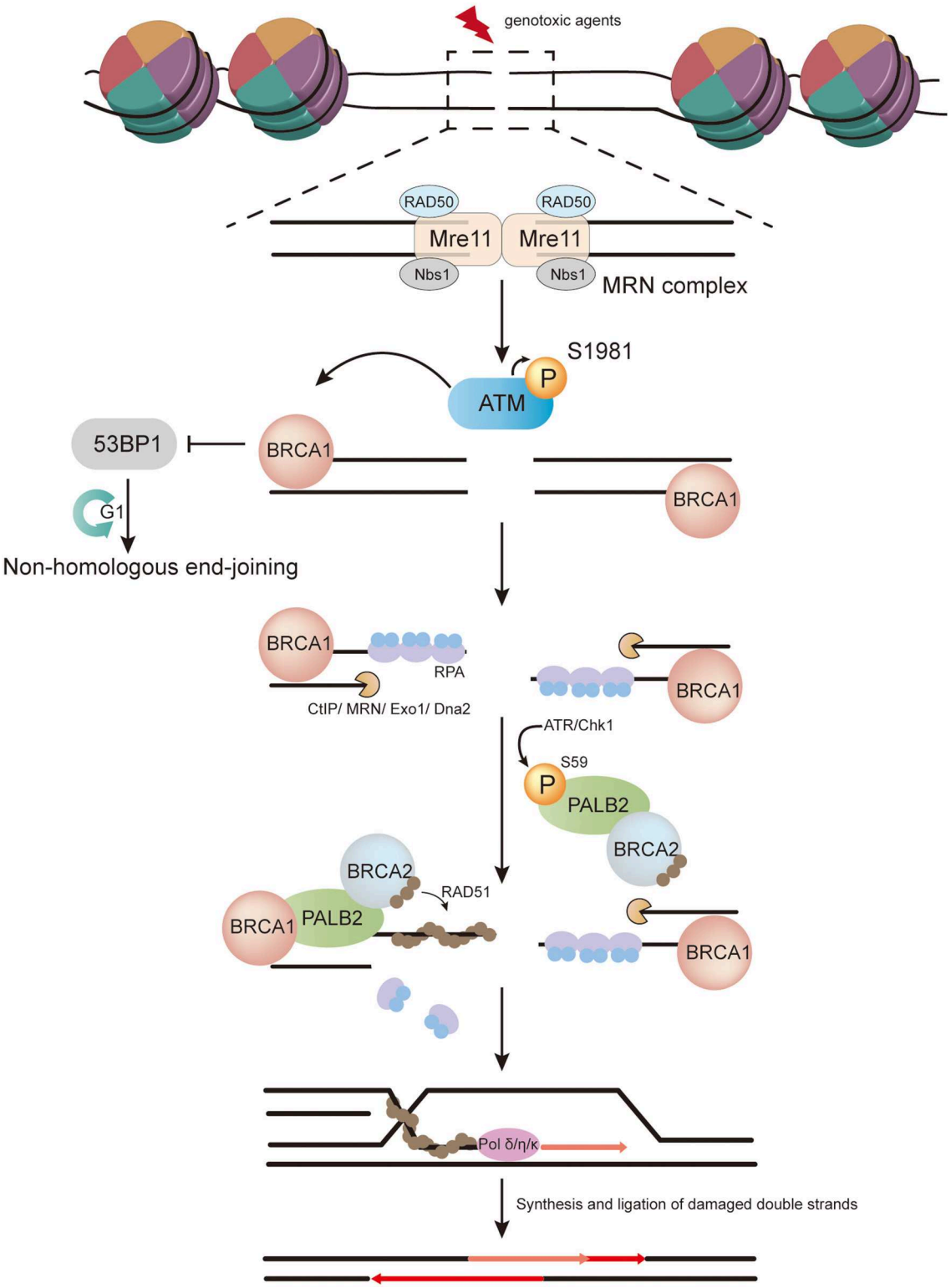

FIGURE 1 | The role of PALB2 in homologous recombination (HR). In response to DNA double-strand breaks (DSBs) induced by genotoxic agents in the S/G2 phase, the Mre11–RAD50–Nbs1 (MRN) complex is recruited to DSBs and promotes ATM recruitment. The inactive ATM dimer then dissociates into active monomers through autophosphorylation at serine 1981. Active ATM monomers phosphorylate H2AX in regions of DSBs and create a platform to recruit BRCA1, which facilitates a shift

(Continued)

FIGURE 1 | from non-homologous end-joining to HR. Meanwhile, CtBP-interacting protein (CtIP), in conjunction with the MRN complex, catalyzes 5′-3′ resection at DSBs to generate single-stranded DNA (ssDNA), and further resection is completed by Exo1 exonuclease and Dna2 nuclease/helicase in cooperation with BLM helicase. The resulting ssDNA is then covered by replication protein A (RPA). PALB2 is phosphorylated on S59 by ATR/Chk1, which accelerates its recruitment to sites of damage. Thereafter, BRCA2 is recruited by PALB2. PALB2 and BRCA2 further promote RPA removal and RAD51 loading. The resulting RAD51-ssDNA filament invades the intact sister chromatid and extends the strand with the help of DNA polymerase δ/η/κ. Finally, further restoration and ligation of double strands are carried out.

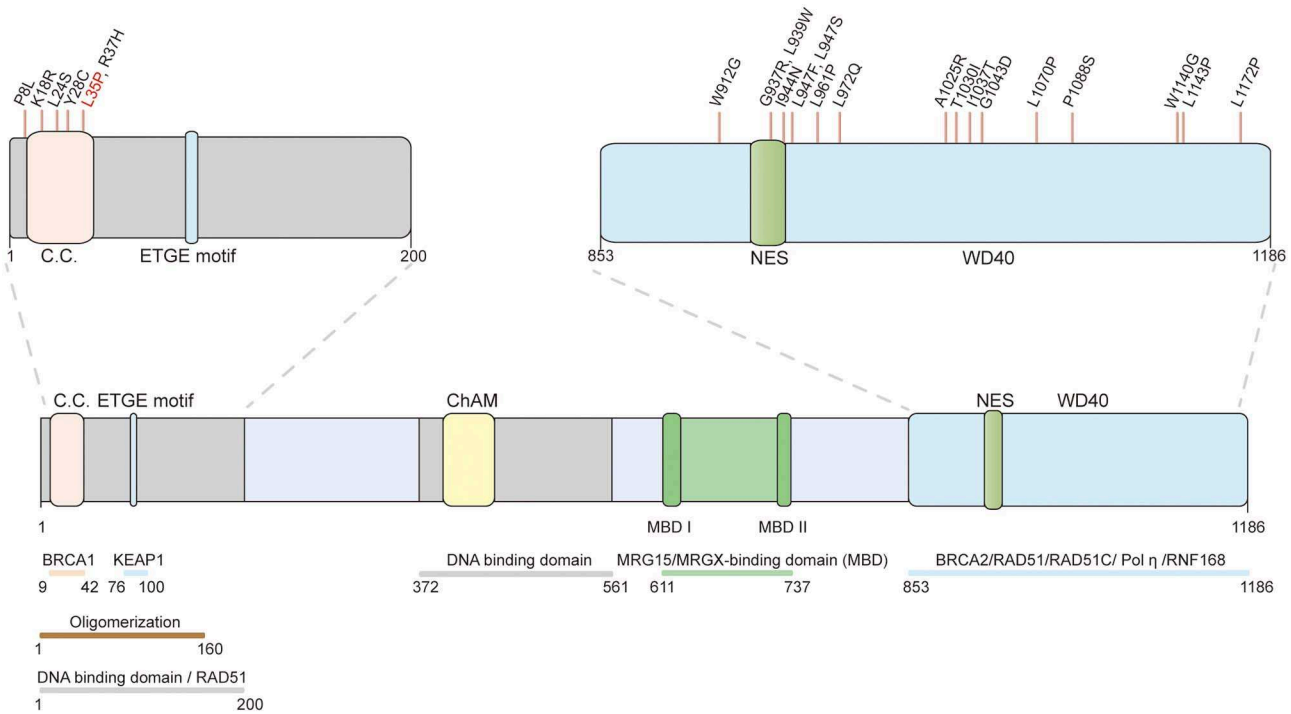

FIGURE 2 | Schematic representation of the PALB2 protein and the position of functionally validated *PALB2* pathogenic missense variants. The structural motifs and functional domains of PALB2. C.C.: coiled-coil motif (9–42); ETGE motif (88–94); ChAM: chromatin-association motif (395–446); WD40: WD40-repeats (853–1186); NES: nuclear export sequence (928–945). The validated pathogenic missense variants are marked on top. The only recognized *PALB2* pathogenic missense variant (p.L35P) validated by systematic *in vitro* functional assays is highlighted in red.

MRG15-PALB2 interaction is involved in HR repair, but may not be critical for this process.

Intriguingly, a genome-wide analysis evaluating PALB2 chromatin residence revealed a tight relationship between PALB2 chromatin residence and transcriptionally active genes (36). This result confirmed that MRG15-PALB2 interaction is associated with unperturbed chromatin. In 2017, Bleuyard et al. hypothesized the innovative concept that the MRG15-PALB2 interaction within undamaged chromatin maintains chromatin stability during DNA replication (33) (**Figure 3A**). This idea was supported by genome-wide PALB2 chromatin immunoprecipitation-sequencing analysis, which indicated gathering of PALB2 at H3K36me3-modified genes through the SETD2/H3K36me3/MRG15 axis. Moreover, expression of MRG15 binding-defective PALB2 leads to compromised proliferation, DNA stress, and genome instability when compared with wild-type PALB2 expression in EUFA1341 cells (33). These findings indicate that the MRG15-PALB2 complex may be a genomic stabilizer within active genes, which renders PALB2 immediately available following DNA damage and guarantees a rapid response to replication stress, thereby maintaining genome stability. In addition to MRG15-PALB2 interaction, PALB2 is also recruited by phosphorylated replication protein A (RPA) during replication stress. Murphy et al. revealed that phosphorylation of RPA during replication stress stimulates the recruitment of PALB2 and increases the stability of PALB2 chromatin binding, making PALB2 available to alleviate replication stress and facilitating the recovery of stalled replication forks (37).

PALB2 and Oxidative Stress

KEAP1, an oxidative stress mediator that negatively regulates the function of the antioxidant transcription factor NRF2, was revealed to bind PALB2 by coimmunoprecipitation (38). Surprisingly, a highly conserved 7-aa motif (LDEETGE) within the KEAP1 binding domain of PALB2 (residues 76–100) was identical to the ETGE motif of NRF2 that binds KEAP1,

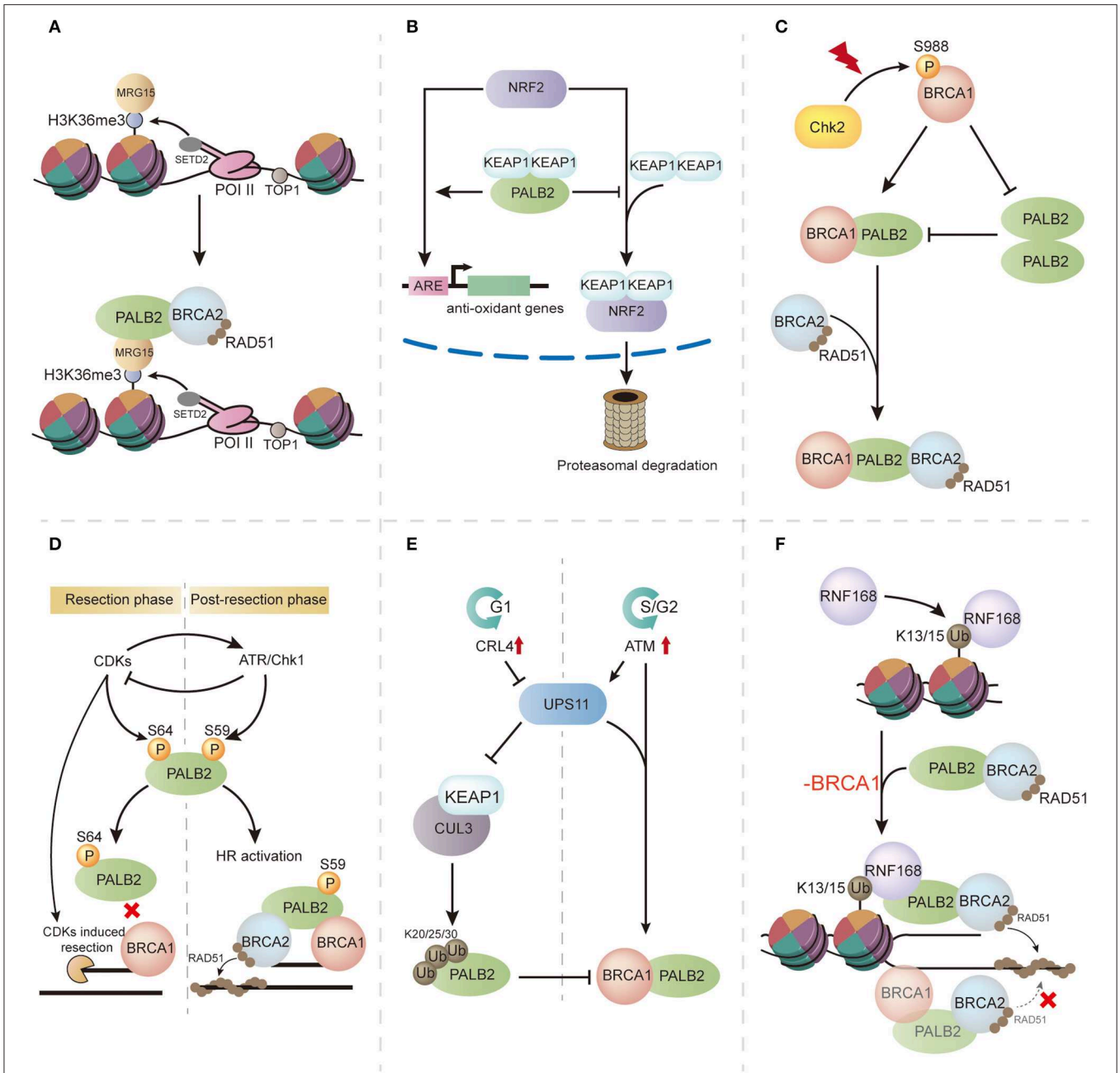

FIGURE 3 | The multifaceted functions of PALB2 and its regulation. **(A)** PALB2 is recruited through the SETD2/H3K36me3/MRG15 axis and protects transcriptionally active genes from replication stress. **(B)** PALB2 promotes NRF2 function during oxidative stress by competitively binding KEAP1. **(C)** Following ionizing radiation (IR), the switch from PALB2 oligomerization to BRCA1-PALB2 interaction is regulated by S988 phosphorylated BRCA1. **(D)** Phosphorylation events in PALB2 regulation. In the resection phase, high CDKs induce PALB2 phosphorylation at S64, inhibiting its interaction with BRCA1, whereas in post-resection phase, ATR-induced PALB2 phosphorylation at S59 promotes BRCA1-PALB2 binding and enhances HR activity. **(E)** In the G1 phase of the cell cycle, PALB2 is ubiquitylated by the CUL3–KEAP1 complex, which disrupts BRCA1-PALB2 interaction, whereas in the G2 phase, PALB2 ubiquitylation is neutralized by USP11. **(F)** RNF168 mediates PALB2 recruitment and RAD51 loading in BRCA1-deficient cells.

implying that PALB2 may promote the role of NRF2 by competitively binding KEAP1 (**Figure 3B**). This was supported by increased levels of reactive oxygen species (ROS) and reduced expression of NRF2-regulated genes after PALB2 depletion. Thus, this study unveiled a unique function of PALB2 during oxidative stress and provided a possible link between the oxidative stress response and PALB2-associated cancer formation.

PALB2 and Cell-Cycle Checkpoint Control

Cell-cycle checkpoints are essential for DNA damage repair following genotoxic exposure because of their role in constraining cell-cycle progression and providing time for accurate DSB repair by HR, thereby guaranteeing genome stability (39). Menzel et al. performed a high-throughput siRNA screen to explore potential G2 checkpoint modulators, and identified PALB2 as a main G2 checkpoint maintainer (40). Depletion of PALB2 led to G2 checkpoint dysregulation and premature checkpoint recovery. In the same study, the role of PALB2 in the maintenance of the G2 checkpoint was seemly independent of the HR pathway, as RAD51 depletion did not compromise G2 checkpoint control. More recently, Simhadri et al. proposed a novel model in which PALB2 serves as a nexus that connects BRCA1 and BRCA2 in G2/M checkpoint control (41). Consistent with this view, disturbing the interactions of BRCA1-PALB2 or BRCA2-PALB2, using the L35P or A1025R mutant of PALB2, respectively, severely impaired the checkpoint response. Notably, BRCA1-PALB2 interaction seems to be critical for checkpoint initiation, whereas BRCA2-PALB2 interaction plays a more significant role in checkpoint maintenance. Although these studies have unveiled the role of PALB2 in checkpoint control, it remains unclear how exactly PALB2 participates in the pathway.

REGULATION OF PALB2

The biological functions of PALB2 are strictly regulated. To date, many mechanisms of its regulation have been elucidated, including PALB2 oligomerization, phosphorylation, ubiquitylation, and interaction with RNF168.

PALB2 Oligomerization

PALB2 oligomerization negatively regulates HR through its coiled-coil domain (42, 43) (**Figure 3C**). Overexpression of the PALB2 coiled-coil domain markedly impairs RAD51 filament formation, suggestive of competition between PALB2 oligomerization and BRCA1-PALB2 interaction. Meanwhile, immunoprecipitation analyses showed that the presence of BRCA1 completely abrogated PALB2 self-interaction, indicating that PALB2 self-interaction can be inhibited by its interaction with BRCA1 (43). As the Chk2-induced BRCA1 phosphorylation of S988 is important for HR activity (44), Buisson et al. proposed that BRCA1 phosphorylation may lead to a molecular switch from PALB2 homodimerization to BRCA1-PALB2 interaction, thereby promoting HR (43) (**Figure 3C**). Song et al. recently reported that PALB2 homodimerization is mediated by an antiparallel coiled-coil leucine zipper (45). Mutation of residue Leu24, a key stabilizer at the dimer interface, greatly reduces PALB2 homodimer stability and results in genomic instability in mutated cells, suggesting an important role of PALB2 oligomerization in HR regulation.

PALB2 Phosphorylation

PALB2 phosphorylation is also critical for its modulation. Three N-terminal S/Q sites of PALB2 (S59, S157, and S376) were found to be phosphorylated following ionizing radiation, and the phosphorylation events were mediated by ataxia telangiectasia mutated protein (ATM) and ATM and Rad3-related kinase (ATR) (46, 47). Phosphorylation-deficient PALB2 failed to promote RAD51 foci formation, leading to impaired HR and genome instability, highlighting the role of phosphorylation in PALB2 regulation (47). Strikingly, Buisson et al. (48) demonstrated a phosphorylation conversion at S59 and S64 on PALB2 during the phosphorylation process (**Figure 3D**). In this model, PALB2 is first phosphorylated at S64, a cyclin-dependent kinase (CDK) site, and high CDK activity actuates DNA end resection and ATR activation. Activated ATR then induces S59 phosphorylation and suppresses CDK activity, followed by hypo-phosphorylation of S64 and a strengthened BRCA1-PALB2 interaction (48). This CDK-ATR switch is crucial for attaining optimal levels of PALB2 at DSBs.

PALB2 Ubiquitylation

Ubiquitylation has also been reported to regulate PALB2 function via cell-cycle control (**Figure 3E**). In the G1 phase, the CUL3–KEAP1 complex ubiquitylates PALB2 on its N terminus, which is the BRCA1-binding region, to suppress BRCA1-PALB2 interaction, ultimately inhibiting HR. As cells enter the S/G2 phase, PALB2 ubiquitylation is neutralized by USP11, a deubiquitylase that is antagonized by CRL4 in the G1 phase. Restoration of BRCA1-PALB2 interaction facilitates BRCA complex formation and induces HR repair (49).

PALB2 and RNF168

The E3 ubiquitin ligase RNF168 was recently found to promote PALB2 accumulation in the S/G2 phase and facilitate DNA repair. It was supported by the restoration of PALB2 foci in endogenous RNF168-depleted S/G2 cells after re-expression of RNF168. The intrinsic mechanism is a physical interaction between the WD40 domain of PALB2 and the newly uncovered PALB2-interacting domain of RNF168 (21). Recently, Zong et al. revealed that RNF168-driven PALB2 recruitment, a BRCA1-independent pathway, serves as a backup to maintain DNA repair by HR (50) (**Figure 3F**). In this model, PALB2 recruitment is mainly orchestrated by BRCA1 in BRCA1-proficient cells, and an RNF168-driven pattern is applied as an auxiliary. Nevertheless, in *BRCA1* mutated cells, RNF168-mediated PALB2 recruitment plays a vital alternative role for RAD51 loading and genome stability. Considering the unambiguous association between RNF168 and PALB2, inhibiting RNF168 signaling in BRCA1-insufficient cancers may be an effective therapeutic strategy (51).

PALB2 AND DISEASES

PALB2 and Fanconi Anemia

Fanconi anemia (FA) is a rare human genetic instability syndrome associated with diverse developmental defects, early-onset bone marrow failure, and cancer predisposition, mainly to acute myeloid leukemia and head and neck squamous cell carcinoma. Cells derived from FA patients are hypersensitive to DNA crosslinking agents such as MMC and cisplatin, and this hallmark is commonly used for the clinical diagnosis of FA (52). To date, 22 FA-related proteins have been identified

in the FA-BRCA pathway for DNA interstrand cross-link repair (53–55), and PALB2 serves as a mediator in the BRCA pathway (56). In 2007, Xia et al. (5) reported a new subtype of Fanconi anemia (FA-N) resulted from biallelic mutations in *PALB2* (also known as *FANCN*). A PALB2-deficient Fanconi anemia cell line showed impaired RAD51 foci formation and hypersensitivity to MMC treatment (5). Notably, FA-N patients are at a high risk of developing embryonal cancer, similar to that seen in patients with biallelic *BRCA2* mutations, but differing from that observed for patients of other FA subtypes (57). These findings emphasize the important role of PALB2 in maintaining genomic stability and tumor suppression.

PALB2 and Breast Cancer

PALB2 is tightly correlated with breast cancer and has been associated with breast cancer predisposition, clinicopathological features, and prognosis.

In 2007, Rahman et al. (6) provided a profile of *PALB2* mutations in breast cancer predisposition. The authors determined the frequency of *PALB2* monoallelic truncating variants in a familial breast cancer cohort negative for *BRCA* mutations (10/923, 1.1%), which was far more common than in controls (0/1,084, 0%; $p = 0.0004$). They also revealed that individuals with monoallelic *PALB2*-mutations had a 2.3-fold increased risk of breast cancer compared with controls (95% confidence interval [CI], 1.4–3.9; $p = 0.0025$) (6), hinting that monoallelic *PALB2* mutations may have a more moderate role in breast cancer predisposition than monoallelic *BRCA2* variants (58, 59). At the same time, research in Finland identified a recurrent mutation, c.1592delT, in 1% of unselected breast cancer patients (60). This frameshift mutation resulted in a 40% cumulative risk of developing breast cancer by age 70 (95% CI, 17–77) (61), similar to that for *BRCA2* mutation carriers (∼45%; 95% CI, 31–56) (62), implying a striking role of *PALB2* in predisposition to breast cancer. Subsequently, multiple population-based screenings of *PALB2*-truncating mutations reported 2–30-fold increases in breast cancer risk for *PALB2*-truncating mutations carriers (6, 63–67). In 2014, Antoniou et al. (64) estimated the age-specific relative risk for *PALB2* mutation carriers, which was highest among women before age 40 years (relative risk, 8–9) then gradually declined with age, with the lowest risk after the age of 60 years (relative risk, ∼5). Meanwhile, female *PALB2* mutation carriers showed an estimated cumulative breast cancer risk of 35% (95% CI, 26–46) by age 70 (64). A recent international study from 21 countries that comprised 524 families with *PALB2* pathogenic variants (PVs) revealed that the estimated relative risk associated with *PALB2* PVs for breast cancer in females was 7.18 (95% CI, 5.82–8.85; $p = 6.5 \times 10^{-76}$). The authors also showed that the estimated relative risk for female breast cancer declined with age, varying from 13.1 at young ages to 4.69 for older ages, and the estimated female breast cancer risk was 53% (95% CI, 44–63) to age 80 years (68).

Male breast cancer (MBC) is a rare disease that accounts for <1% of all breast cancer cases (69). However, ∼20% of MBC patients have a family history of breast cancer (70), highlighting a strong correlation between genetic susceptibility genes and MBC. Two high-penetrance breast cancer genes, *BRCA1* and *BRCA2*, are thought to be responsible for only 13% of MBC (71), and multiple genetic factors remain unknown. To date, many PVs of *PALB2* have been reported in MBC patients (72–76). In 2017, Pritzlaff et al. uncovered that *PALB2* variants significantly increased the risk of MBC (OR, 6.6; $p = 0.01$) (77). This viewpoint was further supported by an Italian population-based multicenter study in 2019 (78). Rizzolo et al. found that *PALB2* was the most frequently mutated gene (1.2%) among non-BRCA1/2 altered MBC patients, and deleterious *PALB2* variants conferred a 9.63- to 17.30-fold increased risk of MBC (78). More recently, Yang et al. further showed an estimated MBC relative risk of 7.34 (95% CI, 1.28–42.18; $p = 0.026$) for *PALB2* PVs carriers by analyzing data from 524 families with *PALB2* PVs from 21 countries (68).

Several studies have also found that *PALB2*-mutated breast cancer is associated with aggressive clinicopathological features. In 2009, Heikkinen et al. reported that breast cancer patients harboring the *PALB2* c.1592delT mutation were more likely to present the triple-negative phenotype (54.5%, $p < 0.0001$), characterized by the absent expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (79), than other familial (12.2%) or sporadic (9.4%) breast cancer patients (80). This finding was further supported by other population-based screening studies (10, 81–84). Heikkinen et al. also showed that *PALB2*-mutated breast cancer patients were more likely to present at an advanced disease stage ($p = 0.0027$ and $p = 0.0017$, respectively) and have a higher Ki67 level ($p = 0.0004$ and $p = 0.0490$, respectively) compared with other familial or sporadic patients (80).

In 2015, Cybulski et al. first evaluated the prognostic effects of two *PALB2* deleterious mutations (509_510delGA and 172_175delTTGT) in Poland (83). In this study, the 10-year survival rate for female breast cancer patients with a *PALB2* mutation was 48.0% (95% CI, 36.5–63.2), significantly lower than that for *PALB2* mutation-negative female breast cancer patients (74.7%; 95% CI, 73.5–75.8). The 10-year adjusted hazard ratio for all-cause mortality was 2.27 (95% CI, 1.64–3.15; $p < 0.0001$), indicating an adverse prognostic effect of *PALB2* in breast cancer (83). More recently, a population-based screening of breast cancer susceptibility genes in China further confirmed the prognostic value of *PALB2* in breast cancer; patients with a *PALB2* mutation presented shorter overall survival compared with noncarriers (adjusted hazard ratio, 8.38; 95% CI, 2.19–32.11; $p = 0.002$) (85).

PALB2 and Other Cancers

In addition to breast cancer, *PALB2* has also been identified as a susceptibility gene for pancreatic cancer. Jones et al. (86) first discovered a germline *PALB2*-truncating mutation (c.172_175delTTGT) in a familial pancreatic cancer (FPC) patient, and three *PALB2*-truncating mutations were further identified in 96 additional FPC patients (3.1%). In contrast, no *PALB2*-truncating mutation was found in 1,084 normal individuals (86). Subsequent studies also revealed the prevalence of *PALB2* deleterious mutations in patients with FPC (∼3–4%) (87, 88), validating the role of *PALB2* mutations in pancreatic

cancer predisposition. To date, several studies have indicated that *PALB2* mutations are associated with ovarian cancer (8, 89, 90). Although the mutation frequency was low, Norquist et al. demonstrated that *PALB2* mutation carriers had a significantly higher risk of ovarian cancer compared with the NHLBI Exome Sequencing Project (OR, 10.2; 95% CI, 2.2–47.0; $p < 0.001$) or the Exome Aggregation Consortium database (OR, 4.4; 95% CI, 2.1–9.1; $p < 0.001$) (8). Pathogenic *PALB2* mutations have also been identified in patients with other cancers, such as gastric and prostate cancer; however, whether these mutations confer an increased cancer risk for these cancer types requires further research (91–94).

PALB2 AND PRECISION MEDICINE

Excluding *BRCA1/2*, PVs in *PALB2* contribute most significantly to the mutation detection rate in multigene testing panels for hereditary breast cancer (95). Thus, germline *PALB2* status is crucial for breast cancer risk assessment in individuals with an apparent family history of breast cancer. To date, several ethnic-specific *PALB2* recurrent mutations have been reported, and related cancer predisposition risks have been established in distinct territories (60, 63, 82, 83, 96, 97) (**Table 1**). In these regions, genotyping for specific *PALB2* PVs can be applied as a cost-effective tactic in high-risk individuals. For most high-risk people who are not in these specific regions, multigene panel testing that includes *PALB2* is an advisable choice for genetic counseling (98–100). According to National Comprehensive Cancer Network (NCCN) guidelines, annual mammogram with consideration of tomosynthesis and breast magnetic resonance imaging with contrast are recommended for people with *PALB2* PVs/likely PVs from the age of 30 to detect cancer at an early stage (101).

Poly (ADP-ribose) polymerases (PARPs) act as DNA damage sensors and regulators and play important roles in the repair of single-stranded DNA breaks through the base-excision repair pathway (102). PARP inhibitor (PARPi) treatment prevents the repair of single-stranded DNA breaks and leads to DSBs in cells. *BRCA1/2*-deficient cells are unable to repair DSBs via the HR pathway, resulting in cell death (103). Consequently, PARP inhibition is considered a promising strategy for the treatment of *BRCA1/2*-deficient tumors through synthetic lethality. More recently, the PARPi olaparib and talazoparib have been approved for germline BRCA-mutated (gBRCAm) HER2-negative metastatic breast cancer in clinic (104). Similar to *BRCA1/2*, *PALB2* is an essential component in HR-based DNA repair, and *PALB2* loss of function was shown to be synthetic lethal in combination with PARPi (105). Recently, PARPi sensitivity of *PALB2* missense variants has been partially elucidated *in vitro*. Foo et al. identified a PARPi-hypersensitive *PALB2* variant (p.L35P) using EUFA1341 cells, an FA-N patient-derived skin fibroblast cell line with biallelic mutations in *PALB2* (106). In 2019, Rodrigue et al. (107) utilized siRNA-mediated RNA interference to generate PALB2-depleted HeLa cells, and exogenous siRNA-resistant PALB2 variants were complemented before PARPi sensitivity assay. Cells expressing the *PALB2*

TABLE 1 | Ethnic-specific *PALB2* recurrent mutations and related breast cancer predisposition.

Exon	Nucleotide change	Protein effect	Ethnicity	Familial breast cancer		Cancer predisposition (95% confidence interval; *p*-value)	Unselected breast cancer		Cancer predisposition (95% confidence interval; *p*-value)	References
				Carriers/total probands studied	Controls		Carriers/total probands studied	Controls		
Ex4	c.1592delT	L531fs	Finnish	3/113 (2.7%)	6/2,501 (0.2%)	Odds ratio: 11.3 (1.8–57.8; $p = 0.005$)	18/1,918 (0.9%)	6/2,501 (0.2%)	Odds ratio: 3.94 (1.5–12.1; $p = 0.003$)	(60)
Ex4	c.509_510delGA	R170fs	Polish	4/648 (0.6%)	1/1,310 (0.08%)	– (–; $p = 0.044$)	–	–	–	(82)
Ex4	c.509_510delGA	R170fs	Polish	–	–	–	76/12,529 (0.61%)	7/4,702 (0.15%)	Odds ratio: 4.09 (1.89–8.88; $p < 0.0001$)	(83)
Ex4	c.1027C>T	Q343X	Italian (Bergamo)	6/113 (5.3%)	2/477 (0.4%)	– (–; $p < 0.01$)	–	–	–	(97)
Ex5	c.2323C>T	Q775X	French-Canadian	–	–	–	2/356 (0.56%)	0/6,440 (0%)	– (3.4–∞; $p = 0.0027$)	(96)
Ex10	c.3113G>A	W1038X	Australian	8 /779 (1%)	0/764 (0%)	–	5/1,403 (0.4%)	0/764 (0%)	Hazard ratio: 30.1 (7.5–120; $p < 0.0001$)	(63)

variants p.T1030I or p.W1140G showed significantly higher olaparib sensitivity than those expressing wild-type *PALB2* (107). A concurrent study conducted by Wiltshire et al. revealed four new PARPi-hypersensitive variants in *PALB2* (p.L24S, p.I944N, p.A1025R, and p.L1070P) using PALB2-deficient B400 mouse mammary tumor cells (108). Boonen et al. (109) developed a cDNA-based system for the functional analysis of *PALB2* variants. By evaluating the ability of *PALB2* variants to rescue PARPi sensitivity in *PALB2* knockout mouse embryonic stem cells, they identified twelve *PALB2* variants (p.Y28C, p.L35P, p.W912G, p.G937R, p.I944N, p.L947S, p.L961P, p.L972Q, p.A1025R, p.T1030I, p.G1043D, and p.L1172P) that showed hypersensitivity to PARPi (109).

In spite of the lack of clinical evidence for PARPi treatment efficacy in *PALB2*-deficient breast cancer patients, the response of some other *PALB2*-deficient solid tumors to PARPi in clinical/preclinical studies have been remarkable. A preclinical study of BMN673 (talazoparib) for the panel of the Pediatric Preclinical Testing Program showed that a maintained complete response was observed *in vivo* in a Wilms tumor xenograft model, characterized by a truncating mutation in *PALB2* (p.Y1108fs) (110). de Bono et al. (111) conducted a phase I trial of the PARPi talazoparib in patients with advanced solid tumors, and reported an objective response rate of 20% in 10 pancreatic cancer patients treated with 1.0 mg/day talazoparib. Of the two patients who showed a partial response, one harbored a mutation in *BRCA2*, and the other harbored a mutation in *PALB2* (111). These results suggest that PARPi exerts a synthetic lethal effect in *PALB2*-deficient tumors. Several clinical trials of PARPi are currently in progress for breast cancer with mutations in DNA repair genes, including *PALB2*. A phase II trial is underway for the evaluation of PARPi olaparib monotherapy in the treatment of metastatic breast cancer patients harboring germline/somatic mutations in non-*BRCA1/BRCA2* DNA repair genes (NCT03344965). A different phase II clinical trial of the PARPi talazoparib is being performed for non-*BRCA1/BRCA2*-mutated patients with advanced triple-negative breast cancer and HR deficiency or advanced HER2-negative solid tumors harboring a germline/somatic mutation in a HR pathway gene, such as *PALB2* (NCT02401347). The outcomes of these trials are expected to expand the potential applications for PARPi therapy.

Overall, these data indicate that *PALB2* status should be assessed and included in genetic counseling and patient treatment regimens for best clinical outcome.

THE CHALLENGES OF PALB2 RESEARCH IN CLINICAL APPLICATION

Population-based screening has identified numerous *PALB2* variants, and the frequency-penetrance profiles of some ethnic-specific *PALB2* PVs have been described. At least 604 distinct variants in *PALB2* have been discovered according to an established database (https://databases.lovd.nl/shared/variants/PALB2/unique); however, only ∼140 of the variants are thought to be pathogenic, whereas more than 400 are missense variants of unknown significance (VUSs). The lack of verification toward these VUSs challenges genetic counseling (27). Here, we summarize the breast cancer-associated missense variants of *PALB2* that have been functionally verified (**Figure 2**). Pathogenic *PALB2* missense variants are mainly located in the N- and C-terminus. In the PALB2 N-terminus, p.L35P (c.104T>C) disrupts BRCA1-PALB2 interaction and abolishes the HR activity of PALB2, resulting in sensitivity to the PARPi (106). Moreover, p.P8L (c.23C>T), p.K18R (c.53A>G), p.L24S (c.71T>C), p.Y28C (c.83A>G), and p.R37H (c.110G>A) compromise HR activity of PALB2 and are suggested to be pathogenic (106–108). In the PALB2 C-terminus, p.W912G, p.G937R, p.L939W, p.I944N (c.2831T>A), p.L947F (c.2841G>T), p.L947S (c.2840T>C), p.L961P, p.L972Q, p.A1025R, p.T1030I (c.3089C>T), p.I1037T, p.G1043D, p.L1070P (c.3209T>C), p.P1088S (c.3262C>T), p.W1140G (c.3418T>C), p.L1143P, and p.L1172P promoted a decrease in the HR activity of PALB2 (20, 107–109, 112). Among these mutations, p.L939W, p.A1025R, p.T1030I, p.P1088S, and p.L1143P disrupt BRCA2-PALB2 interaction; p.W912G, p.G937R, p.I944N, p.L961P, p.L972Q, p.T1030I, p.I1037T, p.G1043D, and p.L1172P are associated with PALB2 protein instability; and p.I944N, p.L947F, p.L947S, p.T1030I, p.L1070P, and p.W1140G result in the mislocalization of PALB2 to the cytoplasm. However, p.L35P remains the only recognized *PALB2* pathogenic missense variant validated by systematic *in vitro* functional assays. Further functional analysis and people-based screening data are needed to properly evaluate the pathogenicity of *PALB2* VUSs.

CONCLUSIONS AND PERSPECTIVES

To date, the specific structures, multifaceted functions, and complex regulatory networks of PALB2 have been elaborated by multiple studies. PALB2 is a crucial regulator in maintaining genome integrity, while its dysfunction leads to breast cancer predisposition. The clinical relevance of PALB2 has been partially described, and *PALB2* is reported to be a high-risk breast cancer susceptibility gene comparable to *BRCA2* (16). With the identification of deleterious *PALB2* recurrent mutations and PARPi, individualized risk assessment and precision medicine for *PALB2* mutation-associated breast cancer become possible. Nevertheless, caution is warranted before promoting specific treatments, such as preventive surgery, as the existing experimental and clinical data are not sufficient. Moreover, plenty of *PALB2* VUSs emerged in large-scale *PALB2* screenings; however, their pathogenicity remains undefined, thereby precluding their clinical application. Altogether, to deliver individualized precision medicine, further long-term, population-based *PALB2* mutation studies combined with systematic functional verification are required.

AUTHOR CONTRIBUTIONS

YC and SW designed the subject of the review. SW, JZ, and KZ wrote the manuscript. SW, HC, ML, YL, and YS compiled the figures. JZ and YC reviewed the manuscript. All the authors read and approved the final manuscript.

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REVIEW
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Molecular Trajectory of *BRCA1* and *BRCA2* Mutations

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Every cancer carries genomic mutations. Although almost all these mutations arise after fertilization, a minimal count of cancer predisposition mutations are already present at the time of genesis of germ cells. Of the cancer predisposition genes identified to date, *BRCA1* and *BRCA2* have been determined to be associated with hereditary breast and ovarian cancer syndrome. Such cancer predisposition genes have recently been attracting attention owing to the emergence of molecular genetics, thus, affecting the strategy of cancer prevention, diagnostics, and therapeutics. In this review, we summarize the molecular significance of these two *BRCA* genes. First, we provide a brief history of *BRCA*1 and *BRCA2*, including their identification as cancer predisposition genes and recognition as members in the Fanconi anemia pathway. Next, we describe the molecular function and interaction of BRCA proteins, and thereafter, describe the patterns of *BRCA* dysfunction. Subsequently, we present emerging evidence on mutational signatures to determine the effects of *BRCA* disorders on the mutational process in cancer cells. Currently, *BRCA* genes serve as principal targets for clinical molecular oncology, be they germline or sporadic mutations. Moreover, comprehensive cancer genome analyses enable us to not only recognize the current status of the known cancer driver gene mutations but also divulge the past mutational processes and predict the future biological behavior of cancer through the molecular trajectory of genomic alterations.

Keywords: breast, ovary, pancreas, prostate, *BRCA1*, *BRCA2*, cancer predisposition gene, mutational signature

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INTRODUCTION

Cancer cells harbor several genetic mutations and epigenetic modifications, which are believed to have arisen from sequential and multistage neoplastic processes (1). However, the mechanism of cellular transformation remains unclear because each oncogenic event is broken down into a molecular reaction, which seems to occur stochastically and independently during oncogenic events in each cancer case. Even if comprehensive genomic data are available, it is still difficult to determine the correct order of genomic alteration.

A possible breakthrough in the understanding of the evolutional process of cancer cells *in vivo* was provided by studies conducted on the hereditary cancer syndrome, which is due to a germline mutation of the cancer predisposition gene (2, 3). Before the establishment of molecular evidence, clinicians had insights into the familial breast cancer (4). Subsequently, genetic and reverse-genetic research revealed the initial and the following steps in the neoplastic process, which has contributed to novel strategies for cancer prevention, diagnostics, and therapeutics.

The main purpose of this review is to summarize the molecular biology associated with the representative cancer predisposition genes, *BRCA1* and *BRCA2*, and to speculate on the missing link between normal and cancer cells.

DISCOVERY OF *BRCA1* AND *BRCA2*

Cancer predisposition genes, *BRCA1* and *BRCA2,* were first discovered in the genetic study on familial breast cancer (5) (**Table 1**). At that time, linkage analyses with DNA polymorphic markers were detecting the causal relationships between certain genetic diseases and specific genomic loci (6). Similarly, a variable number tandem repeat marker known as *D17S74*, revealed that the candidate familial breast cancer gene is located at chromosome 17q21 (7). Thereafter, this locus, also called the "breast cancer, early onset," or *BRCA1*, was indexed for the comprehensive genetic disease database, Mendelian inheritance in Man (MIM), and was given the reference number 113705 (8). After the inter-laboratory competition over 4 years (9), positional cloning of *BRCA1* was first achieved using an emerging technique which required the use of bacterial artificial chromosomes (10). In contrast, the second breast cancer predisposition gene, *BRCA2*, was discovered at chromosome 13q12 by other DNA polymorphic markers, *D13S260,* and *DS13S263* (11), and registered with the MIM number 600185. The discovery of the second breast cancer predisposing gene was followed by the *BRCA1* cloning, and subsequently, the race to clone *BRCA2* was completed the following year by the same research team (12).

TABLE 1 | Summary of *BRCA1* and *BRCA2*.

	BRCA1	***BRCA2***
Location	Chromosome 17q21	Chromosome 13q12
Functional domains (their main binding partners)	RING domain (BARD1) Coiled coil domain (PALB2) BRCT domain (ABRA1, CtIP, and BRIP1)	Eight BRC repeats (RAD51) DNA binding domain (DSS1)
Synonym as FA genes	*FANCS*	*FANCD1*
Cardinal function as a cancer predisposition gene	Homologous recombination	Homologous recombination
Association between promoter methylation and silencing	Established	Not established
Reversion of the mutated gene	Sometimes	Sometimes
Mutational signatures associated	Signature 3 or SBS3/ID6	Signature 3 or SBS3/ID6
Association with breast cancer	Basal-like and/or triple negative breast tumor, high grade histology	Lobular neoplasia, moderate to high grade histology
Association with ovarian cancer	High-grade serous carcinoma, SET-type	High-grade serous carcinoma, SET-type Possibly clear cell carcinoma
Association with pancreatic cancer	Not established	Rarely, high grade histology
Association with prostate cancer	Not established	Rarely, high grade histology

RING, really interesting new gene; BARD1, BRCA1-associated RING domain protein; PALB2, partner and localizer of BRCA2; BRCT, BRCA1 C terminus; ABRA1, abraxas; CtIP, CtBP interactive protein; CtBP, C-terminal binding protein; BRIP1, BRCA1-interacting protein C-terminal helicase 1; DSS1, deleted in split-hand/split foot protein 1; FA, Fanconi anemia; SBS, Single base substitution; ID, Small insertion and deletion; SET, solid, pseudoendometrioid, and transitional cell carcinoma-like histology.

FUNCTIONAL SIMILARITIES AND DIFFERENCES BETWEEN *BRCA1* AND *BRCA2*

Both *BRCA1* and *BRCA2* are large genes, which consist of ∼100 and 70 kb, respectively; the largest exon of both the *BRCA* genes is exon 11. Although these genetic features resemble the proof of breast and ovarian cancer predisposing gene family at the first glance, there is no homology between *BRCA1* and *BRCA2* (13). *BRCA1* contains a nuclear localization sequence (NLS) and three functional domains; RING, coiled coil, and BRCT domains interact with the BRCA1-associated RING domain protein (BARD1), the partner and localizer of BRCA2 (PALB2), and several other proteins that include abraxas (ABRA1), CtBP interactive protein (CtIP), and BRCA1-interacting protein C-terminal helicase 1 (BRIP1), respectively (13). These interactions lead to versatile functions of BRCA1: DNA damage sensing, cell cycling regulation, E3 ubiquitin ligase activity, chromatin remodeling, and homologous recombination (HR). In contrast, *BRCA2* has NLS, eight BRC repeats (14), and a DNA binding domain. Unlike BRCA1, the functional domains of BRCA2 are principally associated with the HR-related proteins, including RAD51 and deleted in split-hand/split foot protein 1 (DSS1) (15, 16). Therefore, the unique molecular traits of each BRCA protein create a difference between *BRCA1*- and *BRCA2*-mutated cancers.

As a common function between *BRCA1* and *BRCA2*, HR is an essential DNA repair system that enables the error-free recovery of double strand breaks (DSBs) (17). DSBs are the most severe DNA damage, the accumulation of which results in genetic translocation and cell death (18). In the condition of homologous recombination deficiency (HRD) by BRCA dysfunction, restoration of DSBs depends on an error-prone repair machinery, known as non-homologous end joining (NHEJ). Such an HRD, also called genomic instability, is advantageous for the progression of *BRCA*-associated cancer to effectively gain sequence and structural variance, especially in the early phase.

OTHER INHERITED BREAST AND OVARIAN CANCER GENES ASIDE FROM *BRCA1* AND *BRCA2*

Because *BRCA1* and *BRCA2* account for ∼25% of the familial breast and ovarian cancers (19), this section describes other breast and ovarian cancer predisposition genes. A linkage analysis study revealed that the third candidate hereditary breast cancer gene, *BRCA3*, was suspected at the *BRCA2* neighboring locus, 13q21-22, in intact *BRCA1*/*BRCA2* Nordic cohorts (20); however, the replication study failed to demonstrate the cancer susceptibility (21). These findings suggest that the current genetics-based research has been unable to identify the next cancer predisposition gene or that all *BRCA* genes have already been found.

Another technique to identify novel breast and ovarian cancer genes is to identify a gene cluster, such as *BRCA* genes, that play a role in the DNA repair system. Remarkably, HR is related to the Fanconi anemia (FA) pathway, which mediates repair of the interstrand crosslink (ICL) (22). FA is an inherited hematopoietic disorder that gives rise to myelodysplastic syndrome and leukemia. To date, over 20 genes have been identified as FA predisposing genes, and the germline mutation of the *FANCA* gene accounts for approximately two-thirds of FA cases (23). Most of the FA genes play an important role in the formation of the FA core complex, which binds at the ICL site and then activates the downstream signaling to repair this severely damaged DNA. Finally, the damaged sequence is removed by HR. Therefore, the defective FA pathway leads to cancer predisposition through genetic instability, such as BRCA1 and BRCA2 dysfunction.

Considering the functional significance of HR in the FA pathway, *BRCA1* and *BRCA2* have been refocused as FA susceptibility genes. Of the eight FA genes detected, *FANCD1* was identified as *BRCA2* (24). In contrast, *BRCA1* has been recently recognized as *FANCS* (25). However, germline mutations of *BRCA* genes lead to bone marrow failure less frequently than mutations in other FA genes, likely because they are absent from the FA core complex.

Individuals with germline mutations of the FA genes are susceptible not only to hematopoietic but also to solid malignancies. Multiple gene panel studies have revealed that inherited breast and ovarian cancers rarely harbor germline mutations of FA genes, including *BRIP1/FANCJ*, *PALB2/FANCN*, and *RAD51C/FANCO* (26). Based on the latest National Comprehensive Cancer Network Guideline (27), *PALB2* is categorized as a gene associated with breast cancer risk, whereas *BRIP1* and *RAD51C* are categorized as genes associated with ovarian cancer risk.

The remaining clinically significant, inherited breast and ovarian cancer genes are the so-called cancer predisposition genes: *ATM*, *CDH1*, *CHEK2*, mismatch repair genes, *NBN*, *NF1*, *PTEN*, *RAD51D*, *STK11*, and *TP53* (27). Therefore, an investigation of these cancer predisposition genes is effective in detecting the pathogenic allele in the case of the non-*BRCA* inherited breast and ovarian cancer.

SIGNIFICANCE OF *BRCA* MUTATIONS

Although numerous germline *BRCA* mutations, also called sequence variants, have been reported to date, not all the variants lead to predisposition to cancer. Therefore, interpretation of the clinical significance of the detected mutation is a challenge in medical practice. To determine whether the detected sequence variant is pathogenic or not, the American College of Medical Genetics and Genomics (ACMG), together with the Association for Molecular Pathology and the College of American Pathologist, issued the revised universal guidelines for the interpretation of sequence variants (28). Based on the evidence of pathogenicity or benignity, this guideline classifies the sequence variants into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. In practice, pathogenic and likely pathogenic variants require further medical management, whereas other variants do not require such intervention. Nevertheless, variants of uncertain significance, which are found in up to 20% of *BRCA1*/*BRCA2* genetic tests (29), need follow-up to monitor the manifestation of the true nature of the variants; e.g., variant reclassification programs. The major databases and platforms that contain information on *BRCA1* and *BRCA2* variants are as follows: BRCA Exchange (30), ClinVar (31), the Human Gene Mutation Database (HGMD) (32), the Leiden Open Variation Database (LOVD) (33), the Consortium of Investigators of Modifiers of BRCA (CIMBA) (34), and the Evidence-based Network for the Interpretation of Germline Mutant Allele (ENIGMA) (35).

Recently, the international collaboration study conducted by CIMBA clarified different cancer risks related to *BRCA* genes (36). Consistent with the previous studies (37–39), both *BRCA1* and *BRCA2* genes contain several cancer risk regions. The ovarian cancer cluster region (OCCR) of both *BRCA1* and *BRCA2* largely overlaps with exon 11, whereas the breast cancer cluster regions (BCCRs) are located on the exterior of exon 11. The mutation in these cancer cluster regions leads to increased cancer risk of the corresponding organ. Additionally, the mutational type of the *BRCA* genes also affects the breast and ovarian cancer risk. Collectively, the diversity of the *BRCA* sequence variants implies not only the general cancer risk but also the specific susceptible organ, and, therefore, the detailed classification of the pathogenic variants would be effective to determine the optimal medical management.

DNA METHYLATION STATUS OF THE *BRCA* GENES

The dysregulation of the *BRCA* genes arises not only from genetic alternations but also from epigenetic modifications. At the transcriptional level, *BRCA1* is regulated by the DNA methylation status at its upstream CpG island (40–42). Consistent with the promoter hypermethylation, *BRCA1* is silenced in sporadic breast and ovarian cancer (43, 44). The aberrant *BRCA1* promoter methylation is found in approximately one-ninth of ovarian cancer tumors (45–47) and in one-fourth of breast basal-like tumors (48), suggesting that *BRCA1* silencing is considered a leading non-genetic case of *BRCA1* inactivation in sporadic wild-type *BRCA* cancer. The comprehensive ovarian cancer genomic studies revealed that hypermethylated-*BRCA1* ovarian cancer with platinum therapy had a similar prognosis as the intact *BRCA* cancer, whereas *BRCA1*/*BRCA2*-mutated ovarian cancer showed better prognosis than the wild-type cancer (46, 47). On the other hand, cancer with homologous *BRCA1* hypermethylation showed a good response to an emerging therapeutic agent (described in a later section), the PARP inhibitor, which was same as the response of cancer with *BRCA* germline mutation (49). These evidences suggest that quantitative methylation analysis of *BRCA1* promoter would be needed to predict the clinical behavior of hypermethylated *BRCA1* cancer.

Conversely, the functional significance of the nearest CpG islands of *BRCA2* still remains unclear. Unlike *BRCA1*, *BRCA2* promoter methylation is not considered a leading cause of *BRCA2* dysfunction (45–48, 50). However, the specific CpG site methylation is a possible marker of germline *BRCA* mutations (51). Because functional significance of the aberrant methylation still remains unclear, further investigations would be needed.

REVERSION OF THE *BRCA* MUTATION

Reversion is defined as the secondary mutation of an inherited mutant gene, which restores normal function in somatic cells (52). For example, the pathogenic *BRCA* allele sometimes reverts to the wild-type sequence via an additional point mutation (back mutation) (53, 54). Conversely, additional insertion/deletion of *BRCA* genes amends the altered reading frame normally (in-frame mutations), thus, converting it to the non-pathogenic allele. These genetic alterations are considered to be a late stage oncogenic event to reactivate the HR pathway, and it consequently renders the cancer cells resistance to lethal DNA damage. Interestingly, approximately a quarter to half of ovarian cancers with germline *BRCA1*/*BRCA2* mutations exhibit the reversion of the inherited mutation and chemoresistance after chemotherapy (47, 55, 56), suggesting that *in vivo* retrieval of *BRCA* function is a potent oncogenic event to resist unwanted DNA damage.

MUTATIONAL SIGNATURE OF *BRCA* DYSFUNCTION

The forthcoming breakthrough in carcinogenesis research is the "mutation signature," which stands for a unique pattern of genetic alterations in somatic cells. Given that every mutation arises from a specific molecular reaction, the characteristic sets of the genetic alterations are good evidence for mutational processes in cancer cells. This concept enables researchers to convert the vast genomic data on cancer cells into evidence on the current status of cancer-related genes and sheds light on the history of cancer progression.

Owing to the emerging technology, such as next-generation sequencing (57), the first series of comprehensive somatic mutation research successfully demonstrated the close relationship between a certain type of cancer and mutational signatures. Briefly, the melanoma cell line frequently carried C>T and/or CC>TT transition, which is consistent with the effect of ultraviolet light exposure on pyrimidine bases (58). Conversely, the small-cell lung cancer cell line harbored predominantly G>T, G>A, and A>G transitions, which are interpreted as the modification of purine bases by tobacco smoke carcinogens (59). Interestingly, both studies also highlighted the presence of other mutational signatures, suggesting that somatic cells experience multiple mutational processes *in vivo*.

In the last decade, the classification of mutational signatures has rapidly progressed (**Figure 1**). The classification of mutational signatures was first initiated in a whole-genome study of human breast cancers (60). Owing to the complementation between pyrimidine and purine nucleobases in the double helices, all single base substitutions (also known as point mutations) can be summarized into the following six patterns: C>A/G>T, C>G/G>C, C>T/G>A, T>A/A>T, T>C/A>G, and T>G/A>C transitions. Additionally, to consider the sequence context of the mutated base, these six mutation classes are further subdivided into 96 trinucleotides patterns by referring to the neighboring bases: the 5′- and 3′-base (each base has four types). By analyzing these 96 trinucleotides patterns in 21 different types of breast cancers with mathematical models, five distinctive molecular signatures were extracted. The mutational spectrum of these signatures possibly reflected either aging (spontaneous deamination of 5-methyl-cytosine: Signature A), overexpression of cytidine deaminase belonging to the APOBEC family (Signatures B and E), or *BRCA1*/*BRCA2* mutations (Signatures C and D). Unlike the other mutational signatures, the *BRCA1*/*BRCA2* mutation-associated signatures were unique in regard to the relatively equal distribution of the 96 trinucleotides patterns. Additionally, *BRCA1*/*BRCA2*-mutated cancer carried microhomology-mediated deletions more frequently compared with the wild-type cancers. These genomic abnormalities are likely due to the dysfunction of HR when double strand breaks occur. Subsequently, the additional breast cancer genome study failed to reproduce the Signature C-like pattern; thus, the *BRCA1*/*BRCA2* mutation associated Signatures C and D was combined into Signature 3 (61).

Thereafter, the international collaborative research group analyzed the large collection of somatic mutations for various cancer types to identify further detailed classes of mutational signatures (62). Although this study identified the 21 distinctive patterns of mutational signatures, the etiology remained unknown for approximately half of the mutational signatures. The mutational signature for *BRCA1*/*BRCA2* mutations, or Signature 3, was reconfirmed in this study, and documented in version 2 of the Catalog of Somatic Mutations in Cancer (COSMIC) mutational signatures (63).

To date, the classification of mutational signatures continues to evolve. Version 3 of COSMIC mutational signatures is composed of three conceptual sets: Single Base Substitution (SBS), Double Base Substitution (DBS), and Small Insertion and Deletion (ID) Signatures (64). This detailed scheme sorts the *BRCA1*/*BRCA2*-associated mutational signature into SBS and ID. In other words, the relatively equal SBS distribution and microhomology-mediated deletions of Signature 3 are interpreted as SBS3 and ID6, respectively.

The analysis of mutational signatures reveals the DNA damage and repair processes of the cancer genome, which arise from the specific molecular reaction. Given the close relationship between Signature 3 and *BRCA* mutations, this genome-wide mutational pattern would be applied to the analysis of cancer genome (50, 65). Increased Signature 3 activity was observed not only in the dysfunction of *BRCA1* and *BRCA2* but also in the inactivation of other HR-related genes, including the *PALB2* germline mutation and *RAD51C* hypermethylation. Remarkably, the increased Signature 3 activity is significantly associated with biallelic mutation, loss of heterozygosity, or epigenetic silencing of the HR-related genes. In contrast, HR-related incomplete

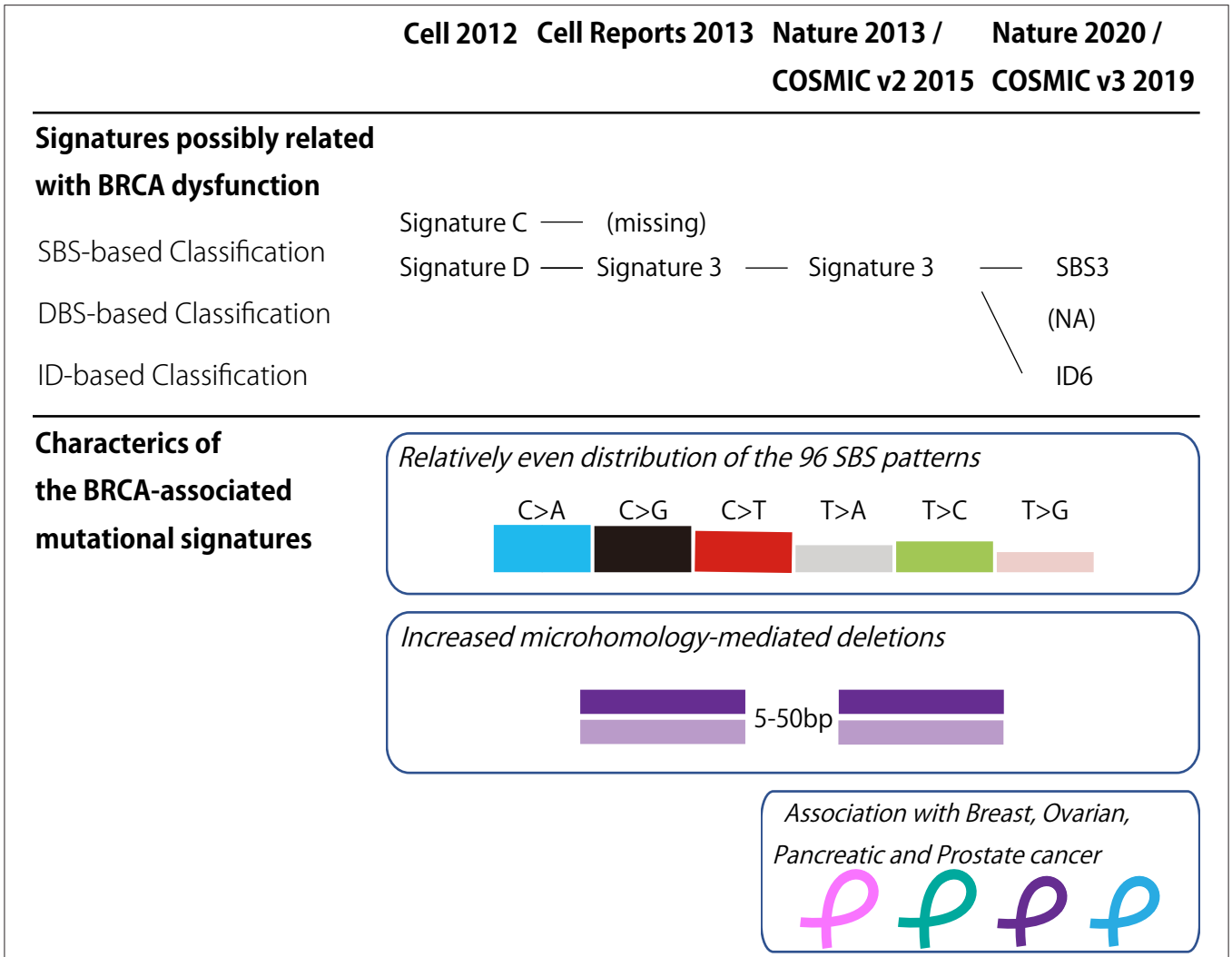

FIGURE 1 | BRCA-associated mutational signature. **(Upper panel)** Classification of the mutational signatures possibly related with BRCA dysfunction. The details of each classification are found in the references 60, 61, 62, and 64. **(Lower panel)** Characteristics of the BRCA-associated mutational signatures. COSMIC, the Catalog of Somatic Mutations in Cancer; v2, version 2; v3, version 3; SBS, Single base substitution; DBS, Double base substitution; ID, Small insertion and deletion; NA, not applicable.

inactivation of the gene, e.g., a monoallelic mutation, did not achieve significant Signature 3 enrichment. Therefore, Signature 3 is the circumstantial evidence of HRD, and a good predictor of pathogenic variants of HR-related genes.

CHARACTERISTICS OF CANCER WITH *BRCA* DYSFUNCTIONS

The link between *BRCA* mutations and specific types of cancer has been emerging. The recent TCGA study addressed the molecular classification of gynecologic and breast cancers, and acknowledged the existence of a subset of cancers with *BRCA*-associated mutational signatures (66). In breast cancer, *BRCA1*-mutated carcinoma is significantly associated with the basal-like subtype that exhibits negative expression of the estrogen receptor (ER), progesterone receptor (PGR), and ERBB2/HER2 (67–69). Additionally, *BRCA1*-mutated and basal-like breast cancer are high grade carcinomas with frequent *TP53* mutations (70, 71), indicating that coexisting *BRCA1* and *TP53* mutations facilitate breast cancer progression. In comparison with *BRCA1*-mutated cancer, *BRCA2*-mutated breast carcinomas frequently express ER and PGR; additionally, HER2 is expressed at the same frequency (69). Furthermore, the histological grade of *BRCA2*-mutated breast carcinoma is generally lower than that of *BRCA1*-mutated breast carcinoma. Regarding the histological type, lobular carcinoma is typically prevalent in the *BRCA2*-carriers, whereas medullary carcinoma is more common in *BRCA1*-carriers.

Lately, in the breast surgical specimens of *BRCA*-carriers, the dedicated histological examination revealed a distinctive pathologic condition known as "hyaline fibrous involution" (72). Lee et al. reported that hyaline fibrous involution was frequently

associated with *BRCA*-mutated perimenopausal women. This unusual histological finding, including diffuse thickening of the fibrous band in the benign breast lobule, likely arises from the abnormal DNA repair state in non-neoplastic breast epithelium. Although this atrophic-like alteration is a promising premalignant lesion that is rarely found in the benign breast disease, we believe that hyaline fibrous involution is an unexpected chance to suspect inherited cancer in cases without genetical test and clinical history.

Conversely, ovarian cancer among *BRCA*-carriers tends to be the most frequent histological type; it is a high-grade serous carcinoma (HGSC) (73). HGSC is a representative type II carcinoma (74), which almost always exhibits high grade nuclear atypia arising from *TP53* mutations (46). The prophylactic surgical specimens revealed that the fallopian tube sometimes contained serous intraepithelial carcinoma (STIC) with *TP53* mutations even in asymptomatic *BRCA*-carriers (75, 76). Interestingly, the putative precursor lesion of STIC, or the p53 signature (77), which already carries the *TP53* mutation, is also sometimes found in the fallopian tube, regardless of the *BRCA* genotype. Additionally, the *TP53* mutation type of the p53 signature is occasionally discordant with that of HGSC (78). These findings suggest that the functional significance of *BRCA* mutations is the promotion of neoplastic cells rather than the initiation of minute precursors. Additionally, they suggest that inherited ovarian cancer is most probably an inherited "tubal" cancer, on the basis of the tubal origin theory of HGSC (79).

Notably, HGSC with *BRCA* dysregulations, including *BRCA1*/*BRCA2* mutations and *BRCA1* promoter hypermethylation, are associated with specific morphological "SET" patterns: Solid, pseudoEndometrioid, and Transitional cell carcinoma-like histology (80, 81). Recognition of the SET variant is in line with the recent diagnostic concept for ovarian carcinoma; there are five major histological types that reflect unique molecular characteristics and precursor lesions, and mixed-type ovarian carcinoma accounts for a rare fraction of ovarian epithelial malignancies (82). Although ovarian transitional cell carcinoma was a distinct entity (83), this malignant tumor was incorporated into HGSC in the World Health Organization 2014 classification because of the similarity of the molecular characteristics between the two carcinomas (84). Importantly, SET-type HGSC shows good therapeutic response compared to the conventional-type HGSC, likely due to the HRD arising from *BRCA* dysregulation. Thus, the SET pattern is a diagnostic and therapeutic predictor for HGSC; therefore, pathological examination still remains important in the era of molecular oncology.

Another possible genetic-pathologic correlation between clear cell carcinoma (CCC) and *BRCA2* mutations (85, 86) seems contradictory to the findings of other research groups (87, 88). Because CCC is a type I carcinoma that originates from endometriosis-related cysts or lesions (74), the pathogenesis of CCC is generally unrelated to the above-mentioned high-grade serous carcinogenesis. Nevertheless, three mixed CCC and HGSC cases were reported based on immunohistochemical and genetic analyses (82). The two of the three mixed CCC and HGSC cases were true combined type I and II carcinomas, whereas the remaining case was pure HGSC. These findings suggest that CCC and HGSC might arise from the common precursor cells or that CCC is sometimes misinterpreted as a HGSC by histological assessment only. Therefore, further data are needed to confirm this ovarian genetic-pathologic correlation.

In addition to breast and ovarian cancers, pancreatic and prostate cancers rarely harbor *BRCA* mutations (89, 90) and *BRCA*-associated signatures (62, 64). The clinical sequence studies reveal that the germline *BRCA2* mutation is detected in ∼5% of metastatic prostate carcinoma cases (91–93). Histologically, *BRCA2*-mutated prostate carcinoma is associated with high grade histology (94, 95), including ductal (96) and endocrine (97, 98) differentiation. On the other hand, pancreatic cancer also harbors *BRCA2* mutations. Of the common types of cancer, including pancreatic ductal adenocarcinoma (PDAC) and neuroendocrine tumors (PanNET), ∼4 and 1% of PDAC and PanNET possess germline *BRCA2* mutations, respectively (99, 100). These findings suggest that mutated *BRCA2*-carriers should exercise caution regarding the development of extra-mammary and uterine adnexal cancers.

THERAPEUTIC APPROACH TO THE *BRCA*-MUTATED CANCER

Because breast and ovarian cancer predisposition genes were identified, the principal strategy of hereditary cancer management involves the early detection of cancer by frequent medical checks, including mammogram, breast MRI, transvaginal ultrasound, and serum CA-125 test, frequently referred to as surveillance. In some cases, this entails the surgical removal of the susceptible organs, if deemed medically necessary. Traditionally, pathogenic *BRCA*-carriers require a prophylactic surgery to prevent breast and/or ovarian cancer even in their reproductive age (101). The resected breasts and uterine adnexa contain premalignant lesions and/or microscopic carcinomas (102, 103), which imply the presence of candidates for future malignancy. Indeed, *BRCA*-carriers sometimes suffer from contralateral breast cancer after the first breast cancer. Therefore, bilateral mastectomy is effective to prevent multiple and hererochronous cancer. In addition, in a recent study, it was revealed that oophorectomy slightly assisted in decreasing contralateral breast cancer (104).

Recent molecular and clinical evidence endorses molecular therapy for *BRCA*-mutated cancer. As described previously, *BRCA*-mutated cancer generally exhibits high-grade histology and aggressive phenotypes but responds favorably to platinum-containing chemotherapy (105–108). Such platinum sensitivity is probably due to *BRCA*-associated HRD that fails to recover platinum-induced ICL (109).

A novel molecular treatment using poly (ADP–ribose) polymerase (PARP)-inhibitor is also based on HRD in the *BRCA*-mutated cancer cells. PARP1 is a cardinal DNA repair molecule in the case of single strand breaks (SSB) (110). Inhibition of PARP1 results in the occurrence of DSBs, which is the failure of the replication fork through SSB repair (111, 112), as well as the disturbance of the NHEJ repair pathway,

by blocking the chromatin remodeler known as CHD2 (113). Together, PARP1 inhibitor and HRD accumulate the critical DSB damage in the *BRCA*-mutated cancer cells. Consistent with the results of these *in vitro* studies, PARP inhibitors have the effect of suppressing the *BRCA*-mutated cancer regardless of the cancer type (114–117). Currently, clinical use of these promising drugs has been approved by the FDA (118). In the future, genetic testing of the *BRCA* mutation would be necessary to determine the optimal therapeutic plan for individuals with advanced cancer.

CONCLUSION

As the molecular functions of *BRCA1* and *BRCA2* have been elucidated, the clinical focus on these cancer predisposition genes shifts toward the development of therapeutic strategies. Additionally, comprehensive cancer genome analysis illustrates not only the present status of cancer related genes but also the past and ongoing mutational processes arising from specific molecular reactions. In the future, the prospective biological behavior of cancer will be predicted via the molecular trajectory of genomic alteration.

AUTHOR CONTRIBUTIONS

YH contributed to the conception of the work and wrote the manuscript. MT, MM, and AH contributed to the revisions of the manuscript. All authors have read and approved the submitted manuscript.

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Tumor Testing for Somatic and Germline *BRCA1/BRCA2* Variants in Ovarian Cancer Patients in the Context of Strong Founder Effects

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Deleterious variants in the *BRCA1/BRCA2* genes and homologous recombination deficiency (HRD) status are considered strong predictors of response to poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi). The introduction of PARPi in clinical practice for the treatment of patients with advanced ovarian cancer imposed changes in the molecular diagnosis of *BRCA1/BRCA2* variants. *BRCA1/BRCA2* tumor testing by next-generation sequencing (NGS) can detect simultaneously both somatic and germline variants, allowing the identification of more patients with higher likelihood of benefiting from PARPi. Our main goal was to determine the frequency of somatic and germline *BRCA1/BRCA2* variants in a series of non-mucinous OC, and to define the best strategy to be implemented in a routine diagnostic setting for the screening of germline/somatic variants in these genes, including the *BRCA2* c.156_157insAlu Portuguese founder variant. We observed a frequency of 19.3% of deleterious variants, 13.3% germline, and 5.9% somatic. A higher prevalence of pathogenic variants was observed in patients diagnosed with high-grade serous ovarian cancer (23.2%). Considering the frequencies of the c.3331_3334del and the c.2037delinsCC *BRCA1* variants observed in this study (73% of all *BRCA1* pathogenic germline variants identified) and the limitations of NGS to detect the *BRCA2* c.156_157insAlu variant, it might be cost-effective to test for these founder variants with a specific test prior to tumor screening of the entire coding regions of *BRCA1* and *BRCA2* by NGS in patients of Portuguese ancestry.

Keywords: *BRCA1/BRCA2,* ovarian cancer, PARPi, NGS, founder variants, tumor testing

INTRODUCTION

Pathogenic germline variants in the breast cancer susceptibility genes *BRCA1* and *BRCA2* increase the risk for the development of ovarian cancer (OC) in carriers. The cumulative OC risk at age 80 years is 44 and 17% for *BRCA1* and *BRCA2* variant carriers, respectively (1). Women unselected for family history present germline *BRCA1*/*BRCA2* variants in 14% of the cases when having any epithelial OC and in ∼17% of the cases with a high-grade serous ovarian cancer (HGSOC) diagnosis (2, 3). Furthermore, somatic mutations were observed in these genes in an additional 3% of HGSOC (2). In total, up to 50% of HGSOC have homologous recombination defects related with loss of function of BRCA1 or BRCA2 or other homologous recombination (HR) pathway proteins (2).

BRCA1 and BRCA2 are critical proteins in the process of HR repair of double-strand DNA breaks (DSBs). BRCA1/BRCA2-deficient cancers are recognized as the main responders to a class of drugs known as poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) (4, 5). PARPi blocks the base excision repair (BER) pathway, which is involved in the repair of DNA single-strand breaks, leading to the formation of DSBs that cannot be accurately repaired in HR-deficient cells and consequently to cell death. (4, 6). Deleterious variants in the *BRCA1*/*BRCA2* genes and homologous recombination deficiency (HRD) status are strong predictors of response to PARPi (7). The PARPi olaparib (Lynparza) was the first-in-class agent to gain approval for treatment in OC by the European Medicines Agency (EMA) for use as maintenance therapy of patients with platinum-sensitive relapsed, BRCA-mutated advanced epithelial ovarian, fallopian tube or primary peritoneal cancer and by the U.S. Food and Drug Administration (FDA) as monotherapy for patients with germline BRCA mutations, who have received three or more prior lines of chemotherapy (8). Consequently, it became mandatory to determine the *BRCA1*/*BRCA2* mutational status to be able to select HGSOC patients for PARPi therapy. At that time, however, the regulatory approvals of FDA and EMA differed, as the latter also considered HGSOC patients with somatic *BRCA1*/*BRCA2* mutations as eligible for PARPi therapy. After that, FDA and EMA approved olaparib for the maintenance treatment in the recurrent setting, regardless of BRCA status, and, more recently, in patients with newly diagnosed BRCA-mutated advanced OC. Therefore, molecular diagnosis algorithms in OC patients had to be updated, not only because of the availability of the new therapy for HGSOC, but also because molecular diagnostic labs would have to consider the detection of somatic *BRCA1*/*BRCA2* mutations. Currently, there is no consensus regarding in which order one should undertake germline and tumor *BRCA1*/*BRCA2* testing in HGSOC patients, but it is generally recommended to perform both (9, 10). Although the tumor testing strategy would need subsequent test in a blood sample of specific variants to evaluate if they are of germline or somatic origin, this would be more cost effective than performing full tumor testing after a negative full germline test to identify the rarer somatic variants. Since *BRCA1*/*BRCA2* tumor testing can detect simultaneously both somatic and germline variants, with the exception of some variants like rearrangements, a higher number of patients who may benefit from PARPi can be identified at a faster turnaround time and at a lower cost (9).

In this study, we aimed to estimate the prevalence of germline and somatic *BRCA1* and *BRCA2* variants in a consecutive series of non-mucinous ovarian cancer patients and to evaluate the advantages and limitations of the tumor testing first strategy.

MATERIALS AND METHODS

Patient Samples

A consecutive series of patients with non-mucinous OC treated at the Portuguese Oncology Institute of Porto from January 2016 to December 2017 (135 patients), from whom formalin–fixed and paraffin–embedded (FFPE) tissue and a peripheral blood sample were available, were analyzed. All patients included in the study were referred for genetic counseling and written informed consent was obtained together with collection of cancer family history and subsequent calculation of the Manchester Score, which estimates the probability of finding a germline *BRCA1*/*BRCA2* variant (11). Tumor samples from 10 patients with known pathogenic germline variants in *BRCA1*/*BRCA2* were collected as validation controls. FFPE samples were obtained, with hematoxylin and eosin-stained slides carefully reviewed by an experienced pathologist in gynecological tumors, who delimited areas with >50% tumor cells. DNA extraction was performed from tumor tissue using the *cobas*® DNA Sample Preparation Kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol and DNA quality was evaluated using the Qubit® 2.0 Fluorometer with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA was extracted from peripheral blood leucocytes using a standard protocol. Blood samples were used to confirm whether the variants found in the tumor samples were germline or somatic, to search for the *BRCA1/BRCA2* germline founder variants and to test for large genomic rearrangements (LGRs), the latter in patients with a Manchester score equal or higher than 15.

One hundred and nine cases (80.7%) had tumors with a pure serous histology, including 95 (70.4%) HGSOC and 14 (10.4%) LGSOC. Twenty-one cases (15.6%) were of non-serous histology, including 10 (7.4%) clear cell, nine (6.7%) endometrioid, and two (1.5%) mixed with clear cell, and endometrioid histology. There were also four (3%) carcinosarcomas, and one (0.7%) mixed carcinoma with clear cell and HGSOC components. Ninety-one FFPE samples (67.4%) were obtained prior to treatment, 27 (20%) post treatment with chemotherapy, and for 17 samples (12.6%) it was not possible to obtain that information.

Next-Generation Sequencing

Next-generation sequencing (NGS) was performed in all FFPE tumor samples using the BRCA Tumor MASTR™ Plus Dx (Multiplicom, Niel, Belgium), an amplicon based NGS kit targeting the full coding sequence and adjacent intronic regions of the *BRCA1*/*BRCA2* genes, following the manufacturer's protocol. Sequencing was carried out using a standard flow cell in the MiSeq platform (Illumina, Inc., San Diego, CA, USA) in 2 × 250 bp paired-end runs. Sequencing and bioinformatic analysis was carried out as previously described (12). All deleterious

variants and variants of uncertain significance (VUS) identified by NGS were confirmed by Sanger sequencing following a standard protocol.

Large Genomic Rearrangements and Founder Variants Screening

The detection of *BRCA1*/*BRCA2* LGRs and Portuguese founder variants was performed in DNA extracted from peripheral blood samples. Multiplex Ligation-dependent Probe Amplification (MLPA; MRC-Holland, Amsterdam, Netherlands) was used to detect *BRCA1*/*BRCA2* LGRs, according to the manufacturer's instructions. Screening of the *BRCA2* c.156_157insAlu variant was performed in all patients according to the protocol previously described by us (13). Screening of the *BRCA1* c.2037delinsCC and c.3331_3334del variants was performed using KASPar SNP genotyping technology (LGC, Teddington, UK) on a Roche LightCycler 480 Real-Time PCR System, according to manufacturer's instructions. KASPar assay primers were designed using the Primer-BLAST design tool (14) and are available upon request. Genotyping results were analyzed using the LightCycler 480 Software 1.5.0. Positive samples were confirmed by Sanger sequencing following a standard protocol.

Loss of Heterozygosity (LOH)

VAF was used to infer biallelic inactivation by deletion of the second allele. LOH presence was evaluated in patients with *BRCA1* or *BRCA2* germline pathogenic variants and VUS that were called in a heterozygous state in the tumor samples. LOH was considered present when the germline *BRCA1*/*BRCA2* VAF was >60%, and/or at least two informative (heterozygous) single nucleotide variants (SNVs) showed a VAF <0.4 or >0.6 (15).

RESULTS

Variant Detection

A total of 10 FFPE tumor DNA samples from OC patients with known pathogenic germline variants were used to validate the NGS assay, including the bioinformatic analysis. This sample set included deletions, duplications, point mutations, and the *BRCA2* c.156_157insAlu Portuguese founder variant (**Table 1**), which is not detectable using standard sequencing methodologies in FFPE samples (12). Regarding the known germline point mutations, the concordance between Sanger sequencing in peripheral blood samples and the NGS-based tumor test on FFPE samples was 100% (8/8). As expected, the germline *BRCA2* c.156_157insAlu variant was not called by the NGS tumor assay pipeline described above.

The NGS-based tumor test was performed in 136 ovarian tumor samples derived from 135 patients. We detected 27 pathogenic variants in 26 patients (19.3%; **Table 2**): 16 patients with a deleterious *BRCA1* variant (61.5%) and 10 patients with a deleterious *BRCA2* variant (38.5%). A total of 18 (13.3%) patients had germline variants (11 in the *BRCA1* gene and seven in the *BRCA2* gene) and eight (5.9%), including one patient with two pathogenic variants in the *BRCA1* gene, presented mutations that were found to be somatic (five in *BRCA1* and three in *BRCA2)*. The most frequent deleterious variant was the *BRCA1* c.3331_3334del, detected in 4.4% (6/135) of the tumors and representing 22.2% of the pathogenic variants found in this series. This variant together with c.2037delinsCC represents 73% (8/11) of all the *BRCA1* pathogenic germline variants identified.

We also detected 12 VUS in 11 patients (8.1%). Within this group, eight (5.9%) patients had a germline VUS and four (3%) patients had a somatic VUS (**Table 3**). One patient had one VUS in each of the genes, one somatic *BRCA1* VUS and one germline *BRCA2* VUS.

In one of the samples, no deleterious variant was identified using the variant filters previously described. However, when reviewing the data, a pathogenic variant (*BRCA1* c.1459_1463delinsTAT) with a 4% VAF was identified that had been discarded by the software due to low VAF (<5%). This tumor sample was obtained post neoadjuvant chemotherapy (paclitaxel and carboplatin) and another available sample, prior to treatment, was subsequently analyzed. The same *BRCA1* pathogenic mutation was detected in the second analysis, but now with a 19% VAF.

LOH

LOH was evaluated in patients with *BRCA1* or *BRCA2* germline pathogenic variants ($n = 11$ for *BRCA1*; $n = 6$ for *BRCA2*) and VUS ($n = 1$ for *BRCA1*; $n = 7$ for *BRCA2*) in the tumor samples. In the sample with *BRCA2* c.156_157insAlu, LOH was not possible to evaluate since this variant was not called by the software and there were no informative SNVs. The subset of germline pathogenic variants had a mean VAF of 80% and 69% for *BRCA1* and *BRCA2* genes, respectively. The subset of germline VUS had a mean VAF of 53%. We considered that LOH occurred in 10 out of 11 patients (91%) and in 4 out of 6 patients (67%) with a *BRCA1* and *BRCA2* germline pathogenic variant, respectively. Loss of the wild type allele was not observed in the tumor from the patient with a germline *BRCA1* VUS. In patients with germline *BRCA2* VUS, loss of the wild type allele was seen in 29% (2/7) of the tumor samples.

Manchester Score

The Manchester score was calculated for 133 patients (three patients belonged to the same family) and a median score of 15 was obtained. The median score was 14 for patients where no germline pathogenic variants or VUS were identified ($n = 107$), 15 for patients with a germline VUS ($n = 8$, **Table 3**), and 21 for patients with a germline pathogenic variant ($n = 18$, **Table 2**).

Frequency of Mutations by Histology

A higher prevalence of pathogenic variants was observed in patients diagnosed with HGSOC, namely 17 of 95 (17.9%) patients with germline variants and 22 of 95 (23.2%) patients with germline/somatic variants. Four additional tumors, out of the 40 with other histologies (10%), had a deleterious germline or somatic BRCA mutation, namely 2 of 9 (22.2%) endometrioid carcinomas, both of which were high grade, and 2 of 4 (50%) carcinosarcomas.

TABLE 1 | Known pathogenic germline variants used to validate the NGS assay.

Gene	HGVS coding	HGVS protein	Tumor	Blood	RD tumor	VAF tumor %
BRCA1	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	2,812	62
BRCA1	c.2490_2497dup	p.(Leu833CysfsTer16)	Positive	Positive	457	86
BRCA1	c.2086dup	p.(Thr696AsnfsTer16)	Positive	Positive	1,247	70
BRCA1	c.5278-1G>T		Positive	Positive	840	71
BRCA1	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	534	80
BRCA1	c.470_471del	p.(Ser157Ter)	Positive	Positive	3,729	80
BRCA1	c.3817C>T	p.(Gln1273Ter)	Positive	Positive	2,275	68
BRCA1	c.2906del	p.(Asn969IlefsTer31)	Positive	Positive	5,917	85
BRCA2	c.156_157insAlu		Negative	Positive		
BRCA2	c.156_157insAlu		Negative	Positive		

RD, read depth; VAF, variant allele frequency.

DISCUSSION

The National Authority of Medicines and Health Products (Infarmed) approved olaparib in Portugal as maintenance therapy only in HGSOC patients with a germline or somatic BRCA mutation. Therefore, it became important to evaluate whether a tumor-testing-first strategy would be the most cost-effective option, allowing for the simultaneous detection of both germline and somatic *BRCA1*/*BRCA2* variants. However, the detection of somatic mutations depends on DNA extraction from FFPE tumor material, which is usually of poor quality and highly fragmented. Additionally, tumor samples are very heterogeneous and contamination with DNA from normal tissue is often an issue. In order to detect somatic mutations in addition to the germline variants, it is necessary to use a methodology with high sensitivity and specificity, such as the use of NGS after tumor macrodissection of the tumor areas marked by a pathologist. However, accurate detection of LGRs in tumor samples with NGS is not straightforward. Moreover, the specific variant c.156_157insAlu represents about 50% of the *BRCA2* pathogenic variants identified in the Portuguese population, but it is not detected by standard sequencing technologies neither by common bioinformatic approaches using NGS data (12). In this study, we used Multiplicom BRCA MASTR Dx assay for the detection of *BRCA1*/*BRCA2* variants using DNA extracted from FFPE tumor samples, for which it has CE-IVD marking. Furthermore, our bioinformatic analysis used Sophia Genetics software which also obtained CE-IVD marking. Our main goal was to determine the frequency of somatic and germline *BRCA1*/*BRCA2* variants in a series of non-mucinous OC, and to define the best strategy to be implemented in a routine diagnostic setting for screening of germline/somatic variants in these genes, including the *BRCA2* c.156_157insAlu founder variant.

The first task of this work consisted in the analysis of FFPE tumor DNA samples from OC patients with known pathogenic germline variants to validate the NGS assay. All germline point mutations were detected by the NGS-based tumor test, allowing us to implement this technique in a routine diagnostic setting. However, as expected, the germline *BRCA2* c.156_157insAlu variant was not called by the NGS tumor assay pipeline, using the software Sophia DDM®, in two tumor samples from the validation series. Taking this into account, blood samples from the 135 patients were analyzed to search for the *BRCA2* c.156_157insAlu germline variant. In this study, we detected the presence of germline pathogenic variants in 13.3% of the 135 patients studied, which is comparable to previous studies. The frequency of *BRCA1* and *BRCA2* germline variants in women with ovarian cancer varies in the literature (6–41%), with the lowest prevalence observed in unselected series of patients with OC (16–18). A higher prevalence of *BRCA1/BRCA2* variants (>15%) has been consistently described in patients with HGSOC (16, 19). Although we observed a predominance (23.2%) of *BRCA1*/*BRCA2* variants in patients with HGSOC, these alterations were not exclusively associated with this group, as they were also frequently found in carcinomas with other histologies (10%). These findings corroborate those obtained by Pennington et al. (20), which found HR gene variants (germline and somatic) to be also common in carcinomas with non-HGSOC histologies. In this work, we identified a *BRCA1/BRCA2* deleterious variant frequency of 50% (2/4) in ovarian carcinosarcomas. Although this frequency can be overestimated due to the limitation of a small sample size, the association of ovarian carcinosarcomas with *BRCA1/BRCA2* pathogenic variants has already been described in the literature (20–22), including two (17%) out of 12 ovarian carcinosarcomas, one with a germline and the other with a somatic mutation. Indeed, there are a few studies indicating that ovarian carcinosarcomas and HGSOC may arise from the same precursor lesion in the Fallopian tube (serous intraepithelial carcinoma) (23).

The identification of specific and recurrent/founder variants in any given population allows a more efficient and cost-saving mutational screening approach. In our previous work, we demonstrated that two variants in *BRCA1* (c.2037delinsCC and c.3331_3334del) and one in *BRCA2* (c.156_157insAlu) together represent about 50% of all deleterious variants found in Portuguese hereditary breast and ovarian cancer families mostly originated from northern Portugal (13). These data allowed us to define our current strategy of starting the

TABLE 2 | Pathogenic variants identified.

Patient	Histological type	Gene	HGVS coding	HGVS protein	Tumor	Blood	RD tumor	VAF tumor %	MS
1	HGSOC	*BRCA1*	c.1192_1193del	p.(Ser398ThrfsTer2)	Positive	Negative	5,885	52	15
2	Endometrioid	*BRCA1*	c.1459_1463delinsTAT	p.(Val487TyrfsTer2)	Positive	Negative	13,929	19	12
3	HGSOC	*BRCA1*	c.1058G>A	p.(Trp353Ter)	Positive	Positive	5,170	55	15
4	HGSOC	*BRCA1*	c.2037delinsCC	p.(Lys679AsnfsTer4)	Positive	Positive	12,302	63	28
5	HGSOC	*BRCA1*	c.2037delinsCC	p.(Lys679AsnfsTer4)	Positive	Positive	6,419	77	18
6	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	522	87	47
7	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	6,015	92	18
8	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	6,118	86	28
9	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	7,995	87	17
10	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	3,544	84	31
11	HGSOC	*BRCA1*	c.3331_3334del	p.(Gln1111AsnfsTer5)	Positive	Positive	4,700	71	19
12	Carcinosarcoma	*BRCA1*	c.211A>G	p.(Arg71Gly)	Positive	Positive	2,048	84	22
13	Carcinosarcoma	*BRCA1*	c.1016dup	p.(Val340GlyfsTer6)	Positive	Negative	3,887	57	15
14	HGSOC	*BRCA1*	c.3817C>T	p.(Gln1273Ter)	Positive	Positive	5,687	91	41
15	HGSOC	*BRCA1*	c.4411_4412del	p.(Gly1471ProfsTer4)	Positive	Negative	8,921	25	15
15	HGSOC	*BRCA1*	c.4485-2A>C		Positive	Negative	3,610	6	
25	HGSOC	*BRCA1*	c.116G>T	p.(Cys39Phe)	Positive	Negative	30,150	24	18
16	HGSOC	*BRCA2*	c.8488-1G>A		Positive	Positive	2,838	49	23
17	HGSOC	*BRCA2*	c.5073dup	p.(Trp1692MetfsTer3)	Positive	Positive	6,900	78	23
18	HGSOC	*BRCA2*	c.4964dup	p.(Tyr1655Ter)	Positive	Positive	3,592	80	12
19	HGSOC	*BRCA2*	c.5073dup	p.(Trp1692MetfsTer3)	Positive	Positive	9,183	78	19
20	HGSOC	*BRCA2*	c.4964dup	p.(Tyr1655Ter)	Positive	Positive	2,708	70	20
21	Endometrioid	*BRCA2*	c.5436del	p.(Glu1812AspfsTer3)	Positive	Negative	5,638	69	19
22	HGSOC	*BRCA2*	c.5950_5961delinsTGCT	p.(Lys1984CysfsTer16)	Positive	Negative	21,046	65	15
23	HGSOC	*BRCA2*	c.9379_9400del	p.(Trp3127AlafsTer29)	Positive	Negative	1,357	6	18
24	HGSOC	*BRCA2*	c.156_157insAlu		Negative	Positive			21
34	HGSOC	*BRCA2*	c.7975A>G	p.(Arg2659Gly)	Positive	Positive	873	56	18

HGSOC, high-grade serous ovarian cancer; RD, read depth; VAF, variant allele frequency; MS, Manchester score.

genetic study of all families by testing these variants before the screening of the entire coding regions of the *BRCA1* and *BRCA2* genes. In this study, we identified six patients with the *BRCA1* c.3331_3334del (4.4%), two with the *BRCA1* c.2037delinsC variant (1.5%), and one patient with the *BRCA2* c.156_157insAlu variant (0.7%). Together, these three variants represent 50% (9/18) of all germline deleterious variants found in this series, indicating that it might be cost-effective to test for these founder variants with a specific test prior to tumor screening of the entire coding regions of *BRCA1* and *BRCA2* by NGS in patients of Portuguese ancestry (**Figure 1**). Furthermore, since the detection of LGRs by NGS in FFPE samples, including the *BRCA2* c.156_157insAlu variant, is not yet optimized due to the low quality of FFPE samples and the possibility of somatic chromosomal deletions and gains that might shield germline LGRs, blood samples from these patients must be collected to test for this specific variant and for germline LGRs [which are relatively rare in our population (13)] at least in patients with a Manchester score higher than 14. Nevertheless, any strategy for the detection of *BRCA1* and *BRCA2* variants must be adapted to specific populations, considering the presence and nature of recurrent and/or founder variants and the ability of current methodologies to detect them in FFPE tissue. In the future, it might be time-saving to optimize the bioinformatics pipeline to detect all variant types in FFPE tissue, making the blood sample only necessary to determine the eventual germline origin of a variant identified in the initial tumor testing by NGS (eventually preceded by founder variant testing in the tumor if considered cost-effective).

In this study, Manchester score was determined for 133 patients. The median of this score was higher for patients with pathogenic germline variants in comparison to patients where no germline variants or a VUS was identified, reflecting that a family history of breast and/or ovarian cancer increases significantly the chance of identifying women with a germline *BRCA1*/*BRCA2* variant. In general, a 10% estimated probability of finding a germline *BRCA1*/*BRCA2* variant is considered to be cost-effective for DNA testing (24). Although a strong family history increases the chance of identifying these variants, it has been reported that family history may be absent in a significant percentage of germline *BRCA1*/*BRCA2* variant carriers (25). Recently, an overall probability of a germline *BRCA1*/*BRCA2* variant above 10% was described for all women with epithelial OC (26). Therefore, it is recommended to refer

TABLE 3 | Variants of unknown significance identified.

Patient	Histological type	Gene	HGVS coding	HGVS protein	Tumor	Blood	VAF tumor %	MS
26	HGSOC	*BRCA1*	c.898G>A	p.(Glu300Lys)	Positive	Negative	19	12
27	HGSOC	*BRCA1*	c.994C>T	p.(Arg332Trp)	Positive	Positive	19	15
29	HGSOC	*BRCA1*	c.5420T>G	p.(Ile1807Ser)	Positive	Negative	38	13
30	Clear cell	*BRCA2*	c.19G>C	p.(Glu7Gln)	Positive	Negative	15	10
31	HGSOC	*BRCA2*	c.1343G>A	p.(Arg448His)	Positive	Positive	49	21
32	HGSOC	*BRCA2*	c.3256A>T	p.(Ile1086Leu)	Positive	Positive	64	15
33	HGSOC	*BRCA2*	c.4933_4935del	p.(Lys1645del)	Positive	Positive	45	12
23	HGSOC	*BRCA2*	c.6351_6377del	p.(Val2118_Cys2126del)	Positive	Negative	69	
26	HGSOC	*BRCA2*	c.7435+6G>A		Positive	Positive	56	
35	HGSOC	*BRCA2*	c.8036A>G	p.(Asp2679Gly)	Positive	Positive	50	13
36	LGSOC	*BRCA2*	c.8902A>G	p.(Thr2968Ala)	Positive	Positive	50	14
37	HGSOC	*BRCA2*	c.9364G>C	p.(Ala3122Pro)	Positive	Positive	85	27

HGSOC, high-grade serous ovarian cancer; LGSOC, low grade serous ovarian cancer; VAF, variant allele frequency; MS, Manchester score.

all women with these tumors for genetic risk evaluation and DNA analysis. In the present series, however, only 1 out of 18 (6%) patients with a *BRCA1*/*BRCA2* pathogenic germline variant had a prior probability lower than 10%. Although BRCA variant testing for ovarian cancer patients must be done in the context of targeted therapy to estimate the potential clinical benefit, in our population the majority of patients with a germline BRCA variant would have been identified based on personal and family history of breast and/or ovarian cancer, revealing that the current model for genetic testing based in risk assessment using familial risk models is still an accurate tool to select patients for germline genetic testing in our population.

It is still not entirely clear if the magnitude of benefit from PARPi for a patient with OC harboring *BRCA1*/*BRCA2* somatic mutations is the same as for those with a germline variant (27). Phase 3 trials that included germline and somatic BRCA mutated patients revealed similar outcomes between these two groups (28, 29). Somatic mutations were ascertained in several studies, with report rates varying from 4 to 7% (20, 30). Our study revealed the presence of somatic *BRCA1*/*BRCA2* pathogenic mutations in 5.9% of the 135 patients studied and in 30.8% of all the patients with pathogenic variants, which is comparable to previous studies. Testing both tumor and blood samples increased the proportion of pathogenic variants identified in OC patients from 13.3 to 19.3% (17.9 to 23.2% in patients with HGSOC), allowing the identification of more patients with higher likelihood of benefiting from PARPi.

Several factors can influence the number of variants detected in a tumor. A factor that must be taken into account is the quality of FFPE samples for DNA analysis (9). For instance, the age of the FFPE block has a significant impact on the quality and the number of variants detected (31). Despite the limited number of studies evaluating the mutation profile in pre- and post-chemotherapy OC specimens, tumor mutational shifts have been described after chemotherapy (32). This phenomenon may be due to pre-existing intra-tumoral heterogeneity and sampling bias, cytotoxic therapy applying selective pressure, or direct drug-induced genetic aberrations (32). One patient from our series was tested in two different samples, one obtained prior to and the other after treatment with chemotherapy. No mutations were detected in the post-treatment sample using the cutoff of a minimum 5% VAF, although a *BRCA1* pathogenic mutation was present with a 4% VAF. In the sample that was obtained prior to treatment, the same *BRCA1* pathogenic variant was present with a VAF of 19%. This finding highlights the importance of selecting the most suitable sample for *BRCA1*/*BRCA2* tumor testing in OC patients. Although the analysis of metastatic tissue at the time of progression may provide a more accurate indication of tumors likely to respond to PARPi treatment, the information available from clinical trials relates to the analysis of primary ovarian tumors (9). While there are no recommendations about the timing of *BRCA1*/*BRCA2* mutation analysis concerning pre- and post-therapeutic tumor samples, an adequate collection of tumor samples with a high tumor content prior to surgery is advised (9).

It is accepted that tumors with *BRCA1*/*BRCA2* germline pathogenic variants usually exhibit LOH, resulting from deletion of the wild type allele, which can be inferred from the high VAF of the mutant allele. BRCA locus-specific LOH in germline *BRCA1*/*BRCA2* carriers has been associated with sensitivity to DNA damaging agents. In a recent work, absence of LOH was observed in 7% of *BRCA1* and 16% of *BRCA2* ovarian tumors and was correlated with decreased overall survival in ovarian cancers treated with platinum chemotherapy (33). In this study, LOH was observed in 91 and 67% of ovarian tumors with a *BRCA1* and *BRCA2* germline pathogenic mutation, respectively, which is in accordance with previous reports. Given that most ovarian tumors with germline BRCA deleterious variants show LOH and loss of the wildtype allele in tumor tissue provides strong evidence for a deleterious germline mutation, we can use LOH status to provide some evidence about the clinical significance of VUS in ovarian cancer tumors. Whereas, LOH was observed in more than 80% of the patients with *BRCA1*/*BRCA2* germline pathogenic variants, loss of the

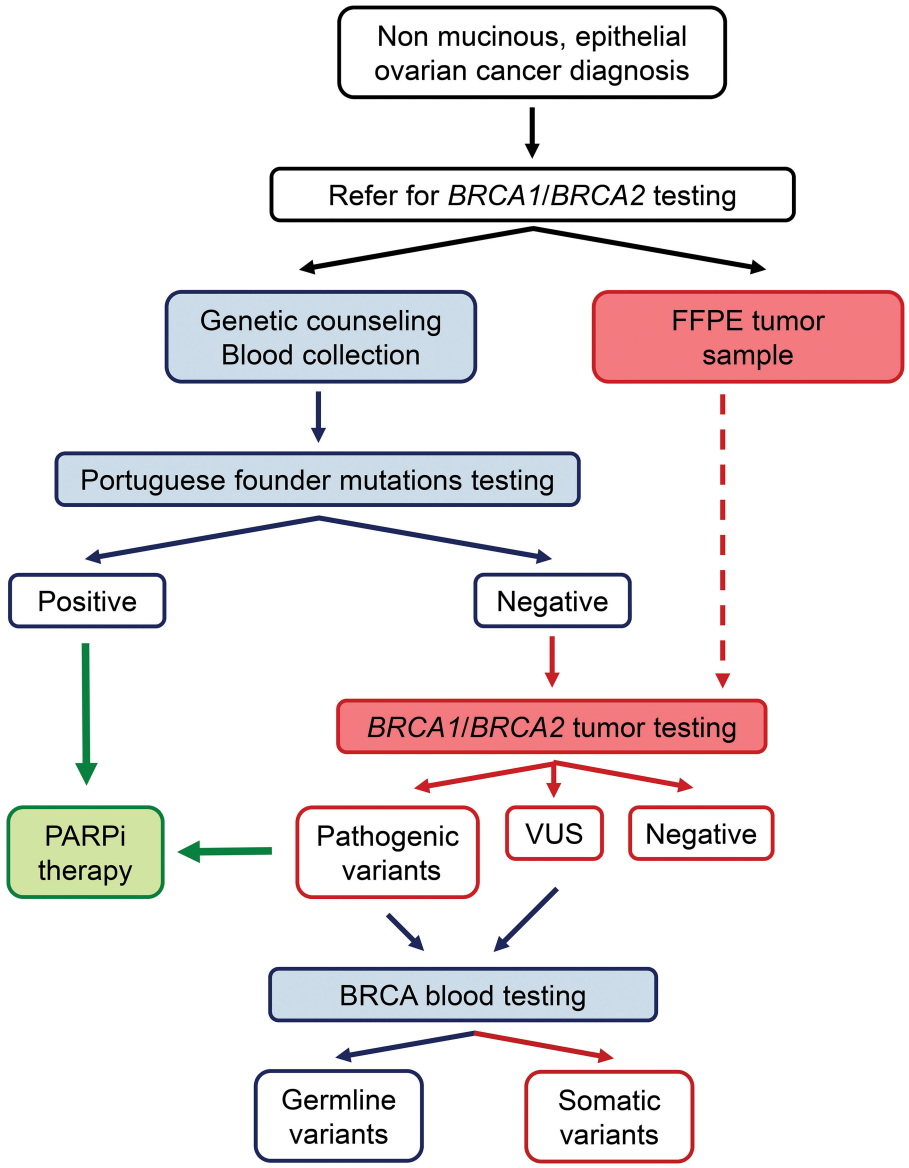

FIGURE 1 | Strategy for detection of germline and somatic *BRCA1/BRCA2* mutations in ovarian cancer patients: A specific blood test for the detection of the three most common mutations in our population is performed before tumor screening of the entire coding regions of *BRCA1* and *BRCA2* by NGS. Blood samples from these patients are used to confirm whether the variants found in the tumor samples are germline or somatic. Patients with pathogenic variants in *BRCA1/BRCA2* genes are eligible for PARPi therapy.

wild type allele was observed in about 25% of the patients with *BRCA1/BRCA2* VUS. These results suggest that most of the germline VUS identified are probably not pathogenic. One of these variants is the c.994C>T in the *BRCA1* gene, which is described in ClinVar (ID 55775) as a VUS and was detected in the tumor sample with a VAF of 19%, which is relatively low for a germline variant. On the other hand, the *BRCA2* c.9364G>C variant was identified with a VAF of 85%, which is suggestive of LOH and pathogenicity. Nevertheless, LOH might be the result of genomic instability, therefore, additional studies will be required to further characterize these variants.

In conclusion, we have characterized the mutation spectrum of *BRCA1/BRCA2* in a consecutive series of ovarian carcinomas, observing a frequency of 19.3% of deleterious variants, 13.3% germline, and 5.9% somatic. Considering the frequencies of the variants observed in our study and the limitations of NGS, we recommend performing a specific blood test for the detection of the three most common variants in our population prior to tumor

screening of the entire coding regions of *BRCA1* and *BRCA2* by NGS. Any deleterious variant identified in the tumor testing, which by itself is predictive of better response to PARPi, should subsequently be evaluated for its germline or somatic origin.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: European Nucleotide Archive (ENA) with accession number PRJEB38270 (https://www.ebi.ac.uk/ena/data/view/PRJEB38270).

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

APei, PP, and JG performed experiments. APei, PP, JG, MP, CS, CP, RS, and CE analyzed data. CB, RC, AB, AG, APet, MA, SS, DP, and JS provided samples and data. APei, PP, and MT wrote the manuscript. MT designed and supervised the study. All authors critically revised and approved the manuscript.

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SYSTEMATIC REVIEW
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PARP Inhibitors in Patients With Newly Diagnosed Advanced Ovarian Cancer: A Meta-Analysis of Randomized Clinical Trials

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Background: The efficacy of poly(adenosine diphosphate–ribose) polymerase inhibitors (PARPi) as a maintenance therapy in patients with newly diagnosed advanced ovarian cancer remains unclear. We conducted a meta-analysis to assess the benefits and safety of PARPi maintenance therapy in patients with newly diagnosed advanced ovarian cancer.

Methods: We searched the PubMed, EMBASE, and Cochrane databases for randomized controlled trials (RCTs), which assessed the efficacy of PARPi as a maintenance therapy for newly diagnosed advanced ovarian cancer. Progression-free survival (PFS) was the primary endpoint, which was assessed using hazard ratios (HRs) with 95% confidence intervals (95% CI). Progression-free survival was extracted independently, and the pooled results were used to compare the prognoses of patients who received PARPi maintenance therapy and those who received a placebo.

Results: Three RCTs, SOLO1, VELIA/GOG-3005, and PRIMA, which included 1,881 patients with newly diagnosed advanced ovarian cancer, were included in the meta-analysis. The overall analysis showed that PARPi maintenance therapy significantly increased PFS (HR, 0.51; 95% CI, 0.33–0.80; $P = 0.004$) compared to placebo. Subgroup analyses confirmed this result. We also observed an improved PFS in patients with homologous recombination deficiency (HR, 0.50; 95% CI, 0.38–0.66; $P < 0.001$) and in patients with BRCA mutations (HR, 0.42; 95% CI, 0.31–0.57; $P < 0.001$). Moreover, there were no significant differences in health-related quality of life between the PARPi and placebo groups.

Conclusions: Patients with newly diagnosed advanced ovarian cancer who received PARPi maintenance therapy had a better prognosis than did those who received a placebo. Moreover, no significant changes in health-related quality of life were seen in PARPi-treated individuals.

Keywords: newly diagnosed advanced ovarian cancer, PARP inhibitors, homologous recombination deficiency, BRCA mutation, meta-analysis

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INTRODUCTION

Ovarian cancer is the most lethal gynecological malignancy (1, 2). There were ∼22,000 new cases and 14,000 deaths due to ovarian cancer during 2019 in the United States (3). More than 70% of ovarian cancer patients are diagnosed in the advanced stage (4). Currently, the primary treatment for newly diagnosed advanced ovarian cancer is a combination of optimal debulking surgery and platinum/taxane-based chemotherapies (5). Unfortunately, the majority of patients with advanced ovarian cancer will have a recurrence within 3 years (6).

Targeted therapies are a new treatment option for patients with ovarian cancer (7). Poly(adenosine diphosphate–ribose) polymerase (PARP) can prevent DNA repair in tumors with homologous recombination deficiency (HRD), including those with BRCA1 or BRCA2 mutations (8). Approximately 13% of ovarian cancers are caused by a mutation in BRCA1 or BRCA2 (9). Poly(adenosine diphosphate–ribose) polymerase inhibitors (PARPi) including niraparib, rucaparib, and olaparib have been approved as maintenance therapies for relapsed platinum-sensitive ovarian cancer patients regardless of BRCA status (10). However, it is unclear if PARPi can improve the prognosis of patients with newly diagnosed advanced ovarian cancer.

Recently, results from two separate phase 3, multicenter, randomized trials of PARPi in patients with newly diagnosed advanced ovarian cancer were published, and they both found that PARPi could improve the progression-free survival (PFS) of patients with newly diagnosed advanced ovarian cancer when PARPi were used as a maintenance therapy (11, 12). Therefore, we performed a meta-analysis to assess the efficacy of PARPi maintenance treatment for patients with newly diagnosed advanced ovarian cancer.

METHODS

Search Strategy and Data Sources

A comprehensive search of clinical trials published before December 1, 2019, in the PubMed, EMBASE, and Cochrane databases was performed in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (**Supplementary Table 1**). The following search terms were used: "poly(ADP-ribose) polymerase inhibitors," "inhibitors of poly(ADP-ribose) polymerases," "poly(ADP-ribosylation) inhibitors," "PARP inhibitors," "inhibitors, PARP," "olaparib," "niraparib," "veliparib," "rucaparib," and "ovarian neoplasm," "ovarian cancer," "cancer of ovary." There were no restrictions with regard to language. The references in the selected studies were also scrutinized to further identify relevant studies.

Inclusion and Exclusion Criteria

PICOS (population, intervention, comparison, outcomes, and study design) guidelines were used to formulate inclusion and exclusion criteria. The inclusion criteria were as follows: (1) population: patients with newly diagnosed high-grade serous or endometrioid ovarian cancer of FIGO (International Federation of Gynecology and Obstetrics) stage III or IV; (2) intervention: PARPi were used as a maintenance treatment; (3) comparison: patients receiving oral PARPi as a maintenance treatment vs. patients receiving a placebo; (4) outcomes: PFS was compared between the PARPi group and the placebo group; and (5) study design: randomized controlled trials (RCTs).

The exclusion criteria were as follows: (1) Population: patients with relapsed ovarian cancer and patients with bevacizumab as maintenance treatment in first line; (2) Intervention: patients did not receive oral PARPi as maintenance treatment; (3) Comparison: there were no control or placebo groups; (4) Outcomes: studies without PFS measurements; (5) Study design: studies that were not RCTs.

Data Extraction and Study Quality Assessment

Two reviewers independently reviewed the included studies and extracted the following data: first author, year of publication, trial acronym, study period, follow-up time, number of total patients enrolled, FIGO stage, and PFS. The risk of bias approach proposed by the Cochrane Collaboration (13) was used to assess the quality of the included RCTs. Any discrepancies were discussed among all authors and identified by consensus.

Statistical Analysis

The primary endpoint of this meta-analysis was PFS, which was assessed using hazard ratios (HRs). Stata software, version 12.0 (2011; Stata Corp., College Station, TX, USA), was used to perform the meta-analysis. Hazard ratios are presented with 95% confidence intervals (CIs). A random-effects model was used in all analyses. Significant two-tailed $P < 0.05$ was considered significant. We used Cochran's Q test and the I^2 statistic to evaluate the heterogeneity among the studies (14, 15). The robustness of the results was assessed using sensitivity analyses (16). Subgroup analyses were conducted based on age, FIGO stage, the timing of chemotherapy in relation to surgery, BRCA mutation status, and homologous recombination status. Funnel plots that are used to assess publication bias were not performed for the limited number of included studies.

RESULTS

Study Selection

A total of 1,226 studies were identified using our search strategy. After screening of the abstracts or titles, the full texts of five studies were further reviewed. Three RCTs that met the study inclusion criteria were selected for analysis (PARPi group = 1,129, placebo group = 752; total = 1,881 patients): VELIA/GOG-3005 (PARPi group = 382, placebo group = 375; total = 757 patients), PRIMA (PARPi group = 487, placebo group = 246; total = 733 patients), and SOLO1 (PARPi group = 260, placebo group = 131; total = 391 patients) (11, 12, 17). A flow diagram of the trial selection is illustrated in **Figure 1**. The quality of the RCTs was evaluated using the "risk of bias" tool according to the *Cochrane Handbook* (**Figure 2**). The main characteristics of the population involved in the studies are represented in **Table 1**.

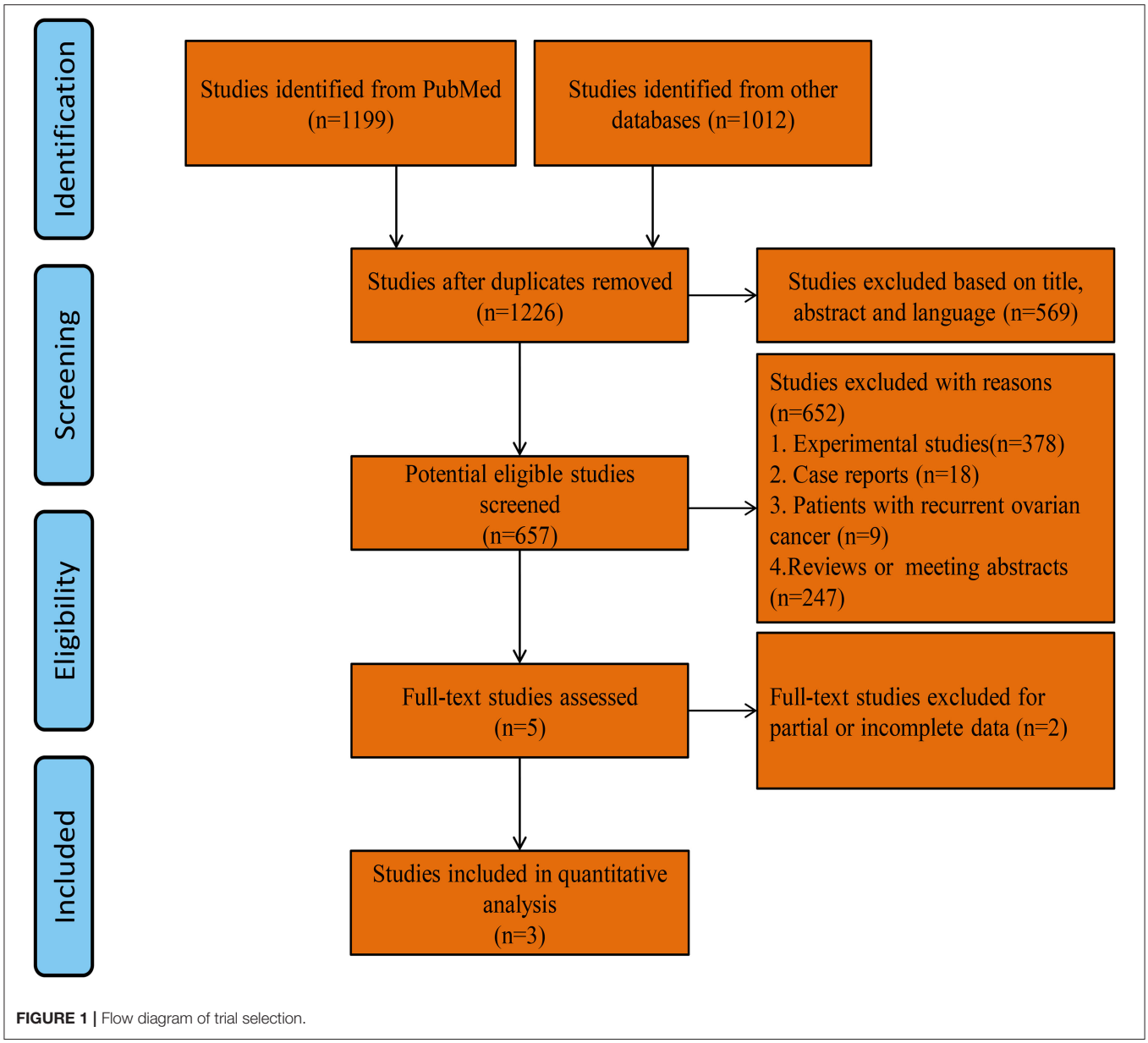

FIGURE 1 | Flow diagram of trial selection.

PARPi vs. Placebos for Ovarian Cancer Patients

All three of the selected trials provided PFS data. The pooled analysis indicated that PARPi maintenance treatment could significantly improve PFS compared to the placebos (HR, 0.51; 95% CI, 0.33–0.80; $P = 0.004$; **Figure 3**). Although substantial heterogeneity existed ($\chi^2 = 24.29$; $P < 0.01$, $I^2 = 91.8\%$), sensitivity analyses were conducted, which demonstrated that the result was robust.

Which Ovarian Cancer Patients Could Benefit From PARPi?

We conducted a subgroup analysis based on age, comparing PFS in patients categorized as <65 and >65 years of age. There were 1,242 patients <65 years old and 639 patients >65 years old. And we found that PARPi improved PFS in both groups (<65 years: HR, 0.51; 95% CI, 0.34–0.76; $P = 0.001$; >65 years: HR, 0.61; 95% CI, 0.45–0.83; $P = 0.002$).

All three trials conducted separate analyses of patients with FIGO stage III and IV ovarian cancer. There were 1,388 patients of stage III and 492 patients of stage IV included. Subgroup analysis based on the FIGO stage showed that PARPi improved PFS in both stage III ovarian cancer patients (HR, 0.49; 95% CI, 0.33–0.74; $P = 0.001$) and stage IV patients (HR, 0.74; 95% CI, 0.58–0.94; $P = 0.016$) compared to patients receiving placebos.

Patients in all three trials received PARPi as a maintenance therapy, whereas VELIA/GOG-3005 added PARPi to first-line chemotherapy. Subgroup analysis of the other two studies that did not combine PARPi with chemotherapy demonstrated a

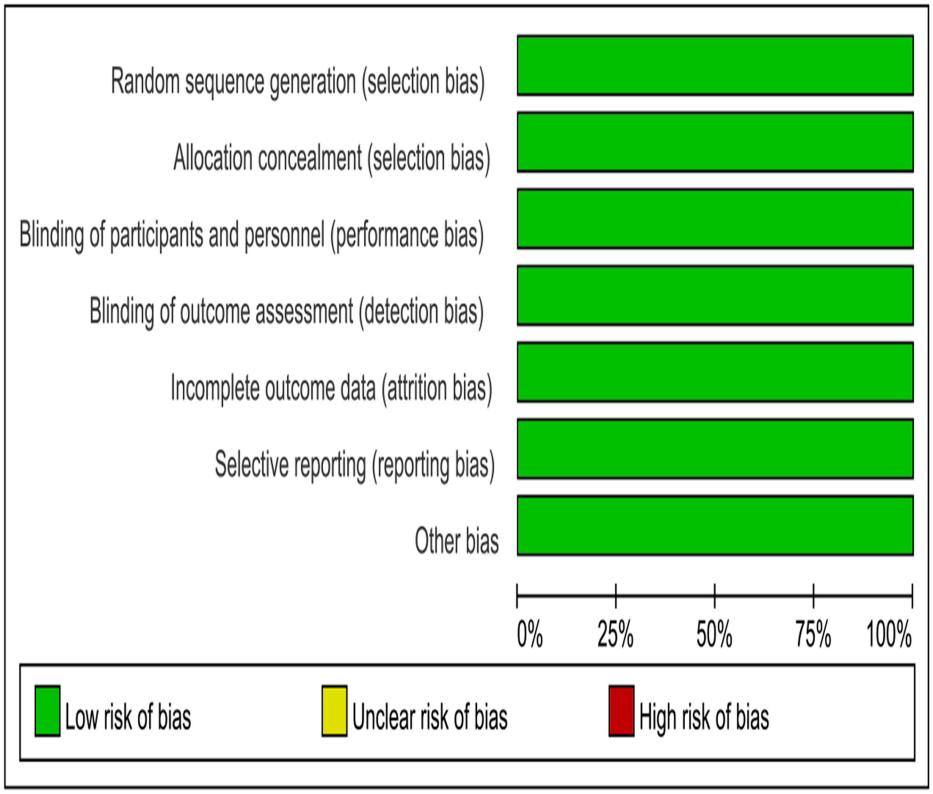

FIGURE 2 | Risk-of-bias graph.

TABLE 1 | Main characteristics of the study populations in the included studies.

Study	Trial acronym	Medication	Study period	Follow-up (median months)	Total patients	Age at baseline		Stage		PARPi	Placebo	BRCA mutation		HRD	
						<65 year	>65 year	III	IV			PARPi	Placebo	PARPi	Placebo
Coleman et al. (12)	VELIA/GOG-3005	Veliparib	2015–2017	28	757	461	296	587	169	382	375	108	92	214	207
González-Martín et al. (11)	PRIMA	Niraparib	2016–2018	13.8	733	444	289	476	257	487	246	152	71	169	80
Moore et al. (17)	SOLO1	Olaparib	2013–2015	40.7	391	337	54	325	66	260	131	260	NA	131	NA

PARPi, poly(adenosine diphosphate–ribose) polymerase inhibitors; HRD, homologous recombination deficiency; NA, not available.

significant improvement in PFS in PARPi group compared to the control group (HR, 0.43; 95% CI, 0.21–0.88; $P = 0.022$).

VELIA/GOG-3005 and PRIMA used PARPi in patients who underwent interval surgery and primary surgery. We performed subgroup analysis based on the timing of chemotherapy in relation to surgery, and we found that PARPi improved PFS in both the interval surgery group (HR, 0.61; 95% CI, 0.50–0.74; $P < 0.001$) and the primary surgery group (HR, 0.70; 95% CI, 0.57–0.86; $P < 0.001$).

Additionally, we performed subgroup analyses based on BRCA mutation and homologous recombination status. VELIA/GOG-3005 and PRIMA analyzed PARPi use in patients with or without BRCA mutations. Subgroup analyses based on BRCA mutation status were conducted, and the results indicated that PARPi significantly improved PFS in patients with BRCA mutations (HR, 0.42; 95% CI, 0.31–0.57; $P < 0.001$), but not in patients without BRCA mutations (HR, 0.67; 95% CI, 0.43–1.04; $P = 0.077$) compared to the placebo groups. In addition, VELIA/GOG-3005 and SOLO1 analyzed BRCA1 and BRCA2 separately. Hence, we conducted subgroup analyses of BRCA1 and BRCA2 patients and found that PARPi improved PFS in patients with BRCA1 mutations (HR, 0.39; 95% CI, 0.30–0.52; $P < 0.001$), but not in patients with BRCA2 mutations (HR, 0.35; 95% CI, 0.11–1.08; $P = 0.067$) compared to the placebo groups. Moreover, VELIA/GOG-3005 and PRIMA analyzed PARPi as a maintenance treatment in patients with HRD and homologous recombination proficiency. We also performed a subgroup analysis and found that PARPi maintenance therapy

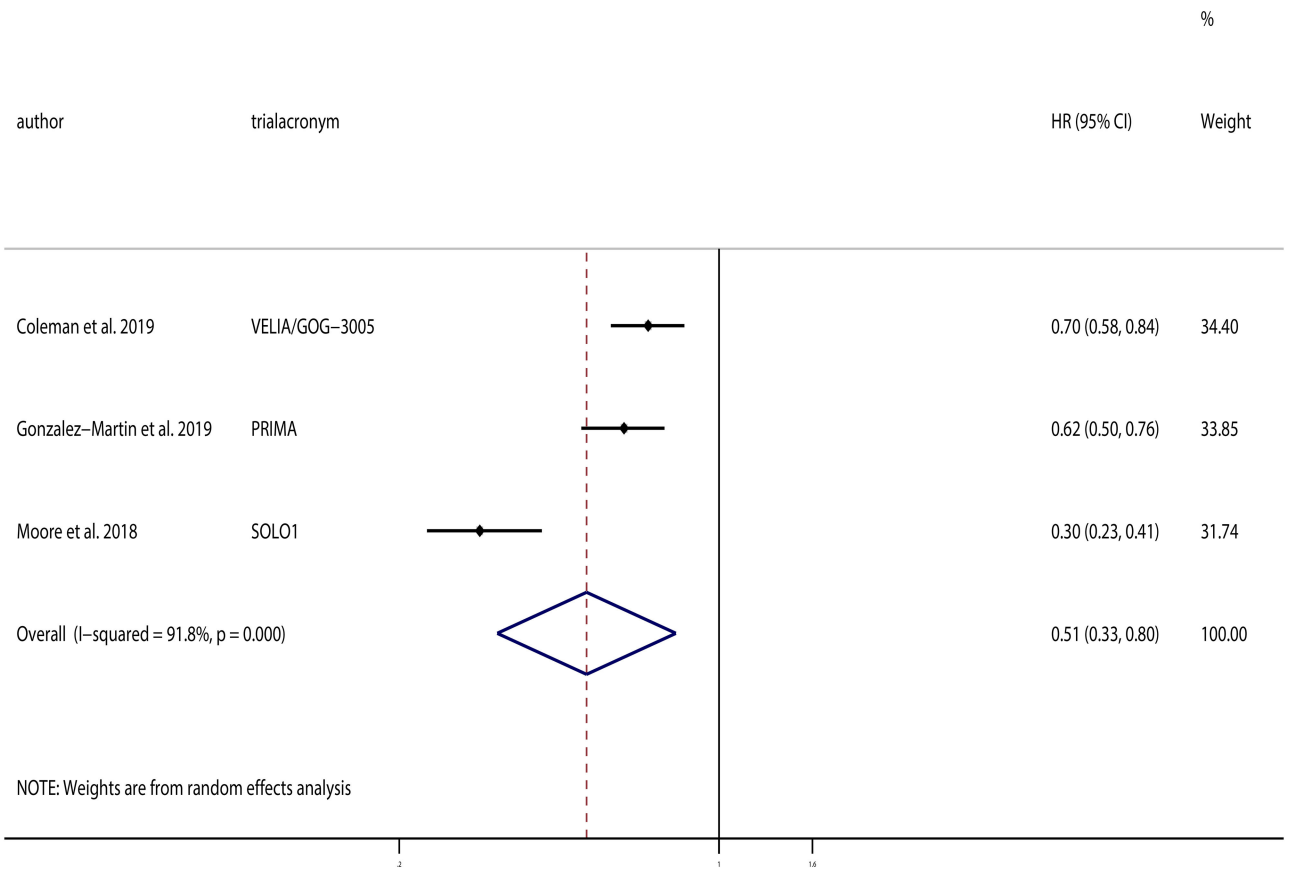

FIGURE 3 | Progression-free survival (PFS) of all patients.

was associated with improved prognosis both in patients with HRD (HR, 0.50; 95% CI, 0.38–0.66; $P < 0.001$) and in patients with homologous recombination proficiency (HR, 0.75; 95% CI, 0.60–0.93; $P = 0.010$). The results from the subgroup analyses are illustrated in **Figure 4**.

Systematic Review of Safety and Health-Related Quality of Life

All the three RCTs investigated adverse events during the trials. In VELIA/GOG-3005 and SOLO1, most adverse events were grade 1 or 2, and the percentages of patients experiencing adverse events were similar in the PARPi group and the control group. Anemia was the most common serious adverse event in the SOLO1 trial, with 22% of patients presenting anemia grade 3 or more in the olaparib arm as compared to 2% in the placebo arm. And the main reasons for discontinuation of olaparib therapy were anemia (2.3%) or nausea (2.3%). In the VELIA/GOG-3005 trial, 28% of patients in the niraparib arm presented thrombocytopenia grade 3 or grade 4 compared to 8% in the control arm, and the main reason for discontinuation of veliparib therapy was nausea (8%). PRIMA reported that 70% of patients in the niraparib group had grade 3 or higher adverse events compared to 18.9% in the placebo group. The most common severe adverse event was hematological toxicity, which was also the most common reason for discontinuation. The proportion of discontinuation was 12.0% in the niraparib arm and 2.5% in the placebo arm. All three trials also assessed health-related quality of life, and they all found that there were no clinically significant differences between the PARPi and the control groups (**Figures 5**, **6**).

DISCUSSION

Over the past decade, many trials of targeted agents have been conducted in order to improve prognosis of ovarian cancer (18), including vascular endothelial growth factor inhibitors (19) and bevacizumab maintenance therapy after first-line chemotherapy for advanced disease (2). Poly(adenosine diphosphate–ribose) polymerase inhibitors have been proven to be effective in patients with platinum-sensitive recurrent ovarian cancer regardless of BRCA and HRD status (10). However, studies examining PARPi maintenance treatment in patients with newly diagnosed ovarian cancer are limited.

Several trials have investigated PARPi use in relapsed ovarian cancer patients, and Tomao et al. (10) conducted a meta-analysis to assess the efficacy of PARPi in platinum-sensitive recurrent ovarian cancer. Since the first report of the olaparib maintenance

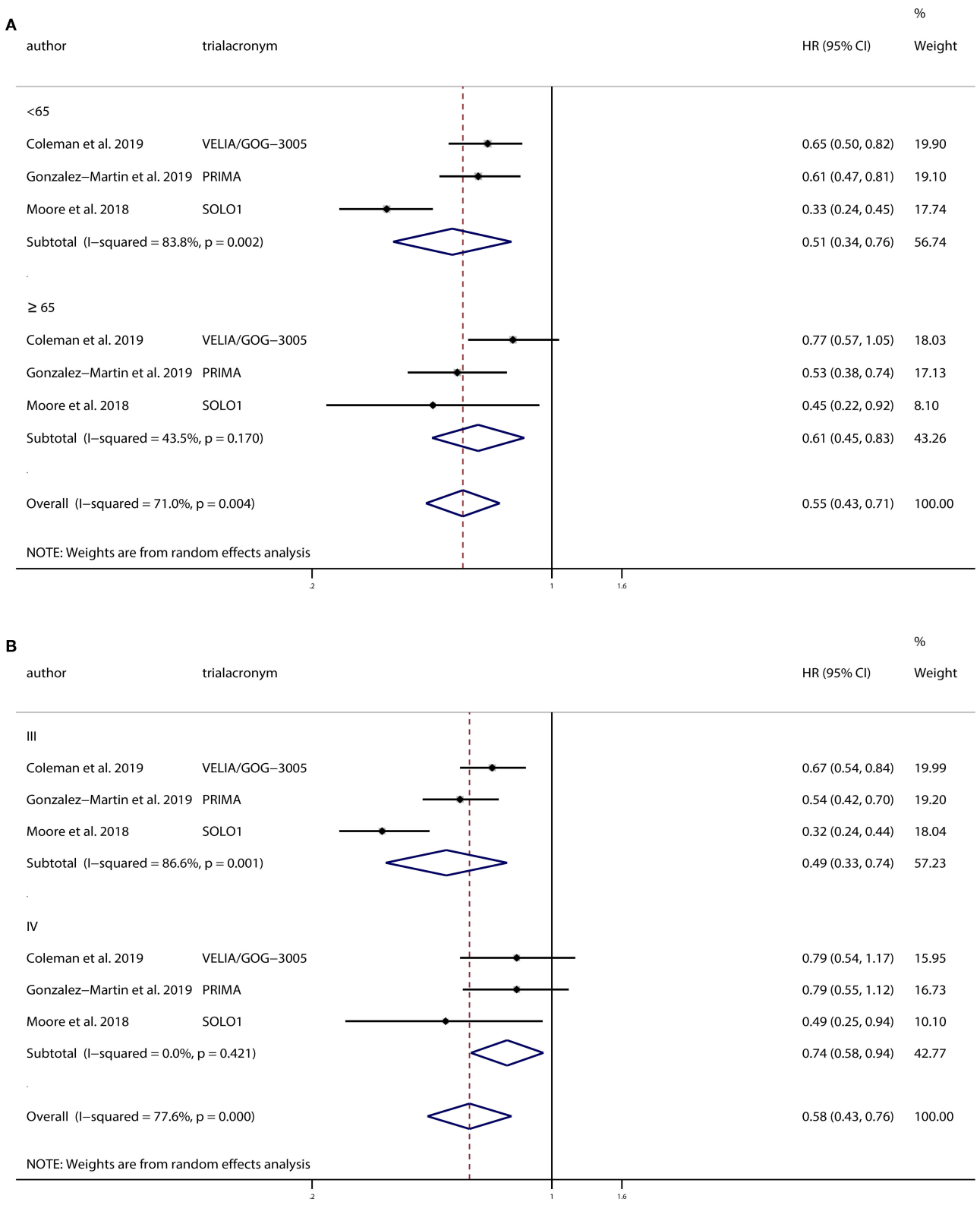

FIGURE 4 | Progression-free survival (PFS) of **(A)** subgroups based on age and **(B)** subgroups based on International Federation of Gynecology and Obstetrics stage.

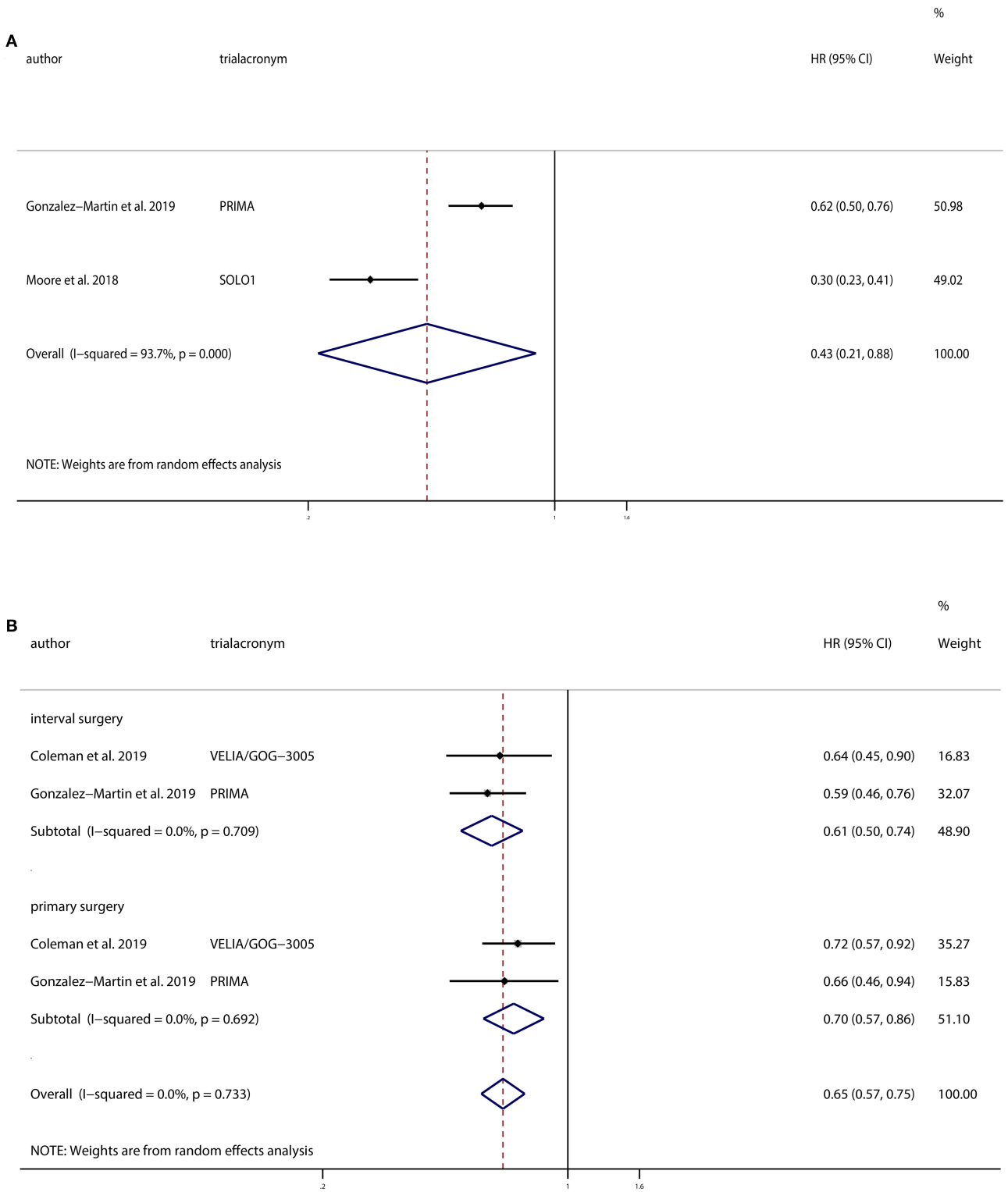

FIGURE 5 | Progression-free survival (PFS) of **(A)** subgroup of patients without PARPi in combination with chemotherapy and **(B)** subgroups based on the timing of chemotherapy in relation to surgery.

therapy trial results in newly diagnosed advanced ovarian cancer patients in 2018 (17), physicians have begun to use veliparib and niraparib in newly diagnosed patients (11, 12). Therefore, it is important and timely to assess the benefits associated with PARPi maintenance therapy in newly diagnosed advanced-stage ovarian cancer patients.

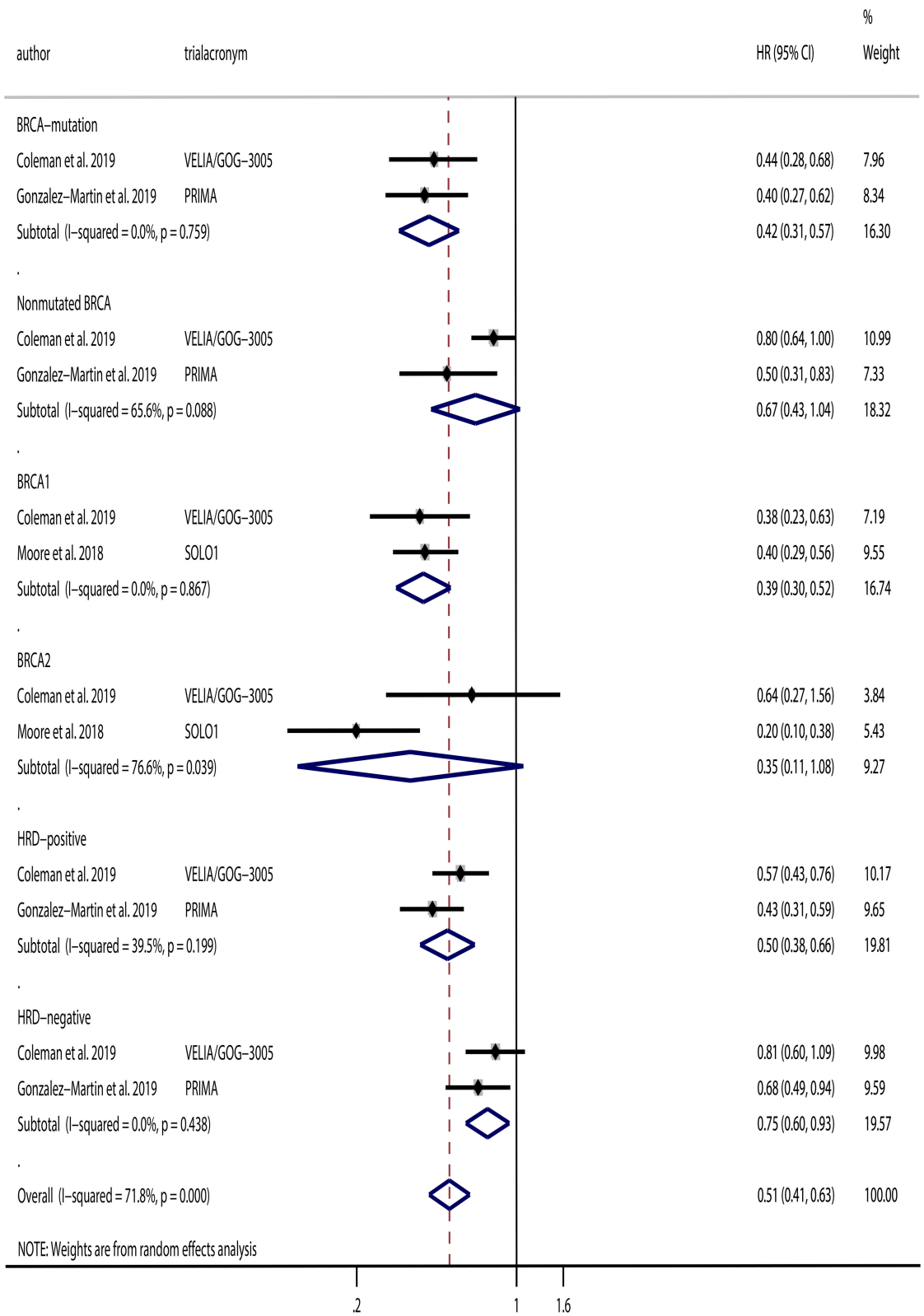

FIGURE 6 | Progression-free survival (PFS) of subgroups based on BRCA mutation and homologous recombination status.

Three studies (SOLO1, VELIA/GOG-3005, and PRIMA) with a combined total of 1,881 advanced ovarian cancer patients were included in this meta-analysis. Our pooled results showed that PARPi maintenance therapy could improve PFS of patients with newly diagnosed advanced ovarian cancer. Subgroup analyses based on age also demonstrated an improvement in PFS in patients both <65 and >65 years of age. We next performed subgroup analyses based on FIGO stage, and all the three trials indicated that patients with stage III ovarian cancer who received PARPi had an improved PFS compared to patients who received placebos. For stage IV patients, SOLO1 (17) found a significant improvement in PFS in the PARPi group, but VELIA/GOG-3005 (12) and PRIMA (11) did not find any differences between the PARPi and placebo groups. When we conducted the subgroup analyses based on FIGO stage, we found that PARPi maintenance therapy was associated with an improvement in PFS in stage III regardless BRCA mutation and stage IV in BRCA mutation alteration.

Poly(adenosine diphosphate–ribose) polymerase inhibitors have been shown to improve PFS when added to chemotherapy and followed as a maintenance treatment in recurrent ovarian cancer (20). Patients in VELIA/GOG-3005 received veliparib combined with chemotherapy, and then veliparib was used as maintenance treatment (12). Thus, we conducted a subgroup analysis of the other two studies that did not combine PARPi with chemotherapy, and we found that maintenance therapy with PARPi significantly improved PFS.

The use of neoadjuvant chemotherapy in advanced ovarian cancer continues to be debated. Although some studies have reported an inferior prognosis in patients with neoadjuvant chemotherapy compared to primary surgery (21, 22), a recent meta-analysis found no difference in overall survival (OS) or PFS between patients who underwent neoadjuvant chemotherapy or primary surgery (23). Poly(adenosine diphosphate–ribose) polymerase inhibitors have also been researched in patients undergoing both neoadjuvant chemotherapy and primary surgery. Our subgroup analysis based on the timing of chemotherapy in relation to surgery demonstrated that PARPi maintenance therapy was associated with an improved prognosis both in patients who underwent interval surgery and in those who underwent primary surgery.

Konstantinopoulos et al. (24) had reported that approximately half of epithelial ovarian cancers have defective repair pathways of homologous recombination including BRCA1/2 mutations. Seo et al. (25) found an improved PFS in BRCA2 mutation patients compared to BRCA2 wild-type patients, but this was not seen in patients with BRCA1 mutations. Poly(adenosine diphosphate–ribose) polymerase inhibitors exhibit greater therapeutic effects in patients with germline or somatic BRCA mutations than those with wild-type BRCA (26). Poly(adenosine diphosphate–ribose) polymerase inhibitors may cause tumor cell death through regulation of DNA repair in BRCA1/2 mutant–selected tumors (27). Previous RCTs have shown that BRCA-mutated patients with recurrent ovarian cancer could benefit from PARPi (28–31). The trials included in our analysis all tested the BRCA mutation status of ovarian cancer patients. A majority of patients (388 of the 391 patients) included in SOLO1 had germline BRCA mutations. SOLO1 observed an improved PFS both in patients with BRCA1 mutations and in patients with BRCA2 mutations. In addition, VELIA/GOG-3005 and SOLO1 provided comparisons of PFS between the PARPi and placebo groups in patients with BRCA1 and BRCA2 mutations separately. VELIA/GOG-3005 observed an improvement in PFS in patients with BRCA mutations and patients with BRCA1 mutations, but not in patients with BRCA2 mutations or without BRCA mutations. PRIMA observed an improved PFS in patients with niraparib maintenance therapy compared to placebo regardless of HRD status. However, VELIA/GOG-3005 observed an improvement in PFS only in patients with HRD. When we conducted subgroup analyses based on homologous recombination and BRCA mutation status, we found that PARPi significantly improved PFS in patients with BRCA mutations or HRD, particularly those with BRCA1 mutations. However, there were no differences between PARPi and placebos in patients with BRCA2 mutations or patients without BRCA mutations. In patients without HRD, we observed an improved PFS in the PARPi group, which seemed to be inconsistent. As the two trials (PRIMA and VELIA/GOG-3005) had different criteria for HRD and had different results, and the upper limit of the 95% CIs of our pooled results was ∼1.00, we cannot confirm the clinical significance of these findings.

To the best of our knowledge, this was the first meta-analysis to explore PARPi maintenance therapy in newly diagnosed advanced ovarian cancer. This meta-analysis was conducted according to PRISMA, and we used PICOS to determine the inclusion criteria. The studies we included were all well-designed, high-quality RCTs.

However, some limitations in our meta-analysis should be stated. First, the heterogeneity of population among the included trials was significant. The tumor characteristics of patients enrolled in the three trials were not consistent. For example, SOLO1 observed the PFS of presence or not of residual tumor after debulking surgery, whereas VELIA/GOG-3005 observed them in primary surgery group and interval surgery group, respectively, and PRIMA did not specify presence or absence of residual tumor after surgery. Thus, we could not combine the results according to the presence or absence of residual tumor after surgery. Second, the heterogeneity of inclusion criteria and exclusion criteria was not consistent in different studies. Although PRIMA and VELIA/GOG-3005 tested the homologous recombination status, they used a different criterion to define HRD. These issues contribute to the heterogeneity of the meta-analysis. Third, the trials researched on the maintenance therapy with different drug (olaparib, niraparib, and veliparib). Fourth, we could not determine OS because of the lack of OS data in the RCTs, which may have provided a more convincing result. Fifth, the number of the studies included was limited, and more, larger and high-quality RCTs are needed to confirm our conclusion.

CONCLUSION

Poly(adenosine diphosphate–ribose) polymerase inhibitor maintenance therapy may improve PFS in patients with

newly diagnosed advanced ovarian cancer, especially patients with BRCA mutations or HRD regardless age, stage at diagnosis, and time to surgery performed. There were no clinically significant differences in health-related quality of life between the PARPi and placebo groups.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

YW and LO designed the study idea and the study methodology. YW, ZS, and FR performed the research and analyzed the data. XW and CZ screened full texts and performed quantitative data analyzing. YW wrote the manuscript. All authors read and approved the version of the manuscript, contributed to the article, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.01204/full#supplementary-material

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Pathology of Hereditary Breast and Ovarian Cancer

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Hereditary breast and ovarian cancer (HBOC) syndrome is most commonly characterized by deleterious germline mutations in *BRCA1* and *BRCA2*. HBOC patients are prone to the development of malignant neoplasms in multiple organs including the breast, ovary, and fallopian tube. From a pathological perspective, a number of morphological features have been described in *BRCA*-associated breast and tubo-ovarian cancers. For example, breast cancers diagnosed in *BRCA1*-mutation carriers are frequently of a high Nottingham grade and display medullary morphology and a triple-negative and/or a basal-like immunophenotype. In contrast, breast cancers in *BRCA2*-mutation carriers are similar to sporadic luminal-type tumors that are positive for hormone receptors and lack expression of human epidermal growth factor receptor 2. Cancers arising in the fallopian tube and ovary are almost exclusively of a high-grade serous histotype with frequent Solid, pseudo-Endometrioid, and Transitional cell carcinoma-like morphology ("SET features"), marked nuclear atypia, high mitotic index, abundant tumor infiltrating lymphocytes, and necrosis. In addition, pushing or infiltrative micropapillary patterns of invasion have been described in *BRCA*-associated metastases of tubo-ovarian high-grade serous carcinomas. Besides *BRCA1* and *BRCA2* mutations, alterations in a number of other homologous recombination genes with moderate penetrance, including *PALB2, RAD51C, RAD51D, BRIP1*, and others, have also been described in HBOC patients with varying frequency; however, distinct morphological characteristics of these tumors have not been well characterized to date. In this review, the above pathological features are discussed in detail and a focus is placed on how accurate pathologic interpretation plays an important role in allowing HBOC patients to receive the best possible management.

Keywords: BRCA, hereditary breast cancer, hereditary tubo-ovarian cancer, high-grade serous carcinoma, triple-negative breast cancer

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INTRODUCTION

Hereditary breast and ovarian cancer (HBOC) is a genetic tumor syndrome most commonly caused by germline deleterious mutations in *BRCA1* and *BRCA2*. The *BRCA1* and *BRCA2* tumor suppressor genes (chromosome 17q21 and 13q12.3, respectively) (1–6) encode for proteins involved in DNA double strand break repair by homologous recombination, one of the critical maintenance mechanisms of DNA integrity (7). In order to complete this function, the BRCA proteins interact with a host of other molecules which together form a protein complex; without

a functional BRCA complex, the cell relies on alternative mechanisms for DNA repair, some of which are error prone and may further contribute to the development of genetic aberrations (8). Because of this phenomenon, HBOC patients with germline *BRCA1* and *BRCA2* mutations have an increased risk for the development of a number of neoplasms, particularly those arising in the breast as well as ovary and fallopian tube (9) (hereby referred to as "tubo-ovarian cancer").

In the general population, the risk for the development of breast and tubo-ovarian cancer is approximately 10–15% and 1–2%, respectively. In *BRCA1* and *BRCA2* mutation carriers, the risk increases to approximately 45–65% and 20–50%, respectively (10–14). Germline mutations in other homologous recombination genes including *BARD1, BRIP1, PALB2, RAD51C, RAD51D,* and others (all encoding proteins involved in BRCA protein stability and/or function), have also been identified to varying degrees in breast and tubo-ovarian cancer patients. Studies evaluating the lifetime risk of disease development in these patients have estimated a range of at least 15–35% for breast cancer (15, 16) and 5–10% for tubo-ovarian cancer (17–20). Mutations in some of these genes impart an increased risk for either breast or tubo-ovarian cancer with minimal to no increased risk for the development of the other tumor type (i.e., increased risk of breast cancer without risk of tubo-ovarian cancer, and vice versa) (21–24). For example, *BRIP1, RAD51C*, and *RAD51D* mutation carriers have an increased risk for tubo-ovarian cancer, while there is insufficient evidence for an increased risk for breast cancer development. In contrast, *BARD1* and *PALB2* mutation carriers have an increased risk for breast cancer development without an associated increased risk for tubo-ovarian cancer (25).

A number of morphological features perceived at the time of microscopic examination have been described in *BRCA*-related breast and tubo-ovarian cancers, and the discussion of these characteristic features and their clinical relevance will be the main topic of this review. In addition, tumor and germline genetic testing will also be discussed.

TABLE 1 | Morphological and molecular features of *BRCA1* and *BRCA2*-associated breast cancer.

Morphological features	*BRCA1*	*BRCA2*
Tubule formation	Minimal to none, "medullary" solid growth	Abundant
Nuclear grade	High	Variable, usually low to intermediate
Mitotic rate	High	Variable
Overall Nottingham grade	High	Variable, usually grade 1 or 2
Intrinsic molecular subgroup	Basal-like	Luminal-like (luminal A)
Biomarker profile	ER-, PR-, HER2-	ER+, PR+, HER2-

ER, estrogen receptor; PR, progesterone receptor; and HER2, human epidermal growth factor receptor 2.

HBOC-ASSOCIATED BREAST CANCER

Breast carcinoma is the most common malignancy arising in female patients with HBOC as a result of germline *BRCA1/2* mutations. For risk reduction, bilateral mastectomy is recommended for all *BRCA1/2* mutation carriers (26). From a pathological perspective, *BRCA1* and *BRCA2*-associated breast tumors have been shown to differ on both morphological and molecular levels (**Table 1**). Furthermore, *BRCA1*-associated tumors tend to be more difficult to visualize on mammographic studies compared to *BRCA2*-associated tumors which more commonly present with microcalcifications and/or isolated ductal carcinoma in situ (27).

BRCA1-Associated Breast Cancer

Morphologically, *BRCA1*-associated breast carcinomas are most commonly a high-grade invasive ductal carcinoma of no special type and display minimal if any tubule or glandular formation, markedly pleomorphic nuclei (significant variation in size and shape), vesicular chromatin, prominent nucleoli, and high mitotic activity. A "medullary" appearance with a sheet-like proliferation of tumor cells, pushing borders, necrosis, and prominent peri- and intra-tumoral lymphocytes has also been described (**Figure 1**). Of note, classical criteria for medullary carcinoma of the breast include syncytial architecture composing >75% of the tumor mass, histological circumscription with pushing margins, lack of tubular differentiation and in situ carcinoma, a prominent and diffuse lymphocytic infiltrate, and round tumor cells with abundant cytoplasm and pleomorphic high-grade vesicular nuclei containing one or several nucleoli (28, 29). Given that these diagnostic criteria are difficult to apply and lead to high interobserver variability, the World Health Organization (WHO) proposes the term "invasive carcinoma of no special type with medullary pattern" to describe a tumor exhibiting some or all of the above characteristics (30). From a molecular perspective, the majority of these *BRCA1*-associated breast tumors fall into the "basal-like" subtype of breast cancer, one of the four common intrinsic molecular subtypes (31). "Basal-like" tumors are characterized by overexpression of genes associated with basal epithelium and proliferation and minimal to no expression of genes associated with estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2). This gene expression profile is reflected in the immunohistochemical expression of basal markers including cytokeratin 5/6 and epidermal growth factor receptor (EGFR), in addition to lack of expression of ER and progesterone receptor (PR) as well as HER2 (27, 32–35). Metaplastic carcinomas have also been reported in *BRCA1* mutations carriers (36, 37).

BRCA2-Associated Breast Cancer

In contrast to *BRCA1*-associated breast cancers, *BRCA2*-associated tumors are very similar to sporadically-occurring "luminal-type" tumors (31). This group comprises the most common of the intrinsic molecular subtypes of breast cancer (luminal A) and is characterized by variable expression of genes typically expressed in luminal breast epithelium and those associated with ER (31). Morphologically, these tumors are most commonly invasive ductal carcinoma of no special type of variable grade and do not appear to have a specific morphology, although lobular carcinomas have been

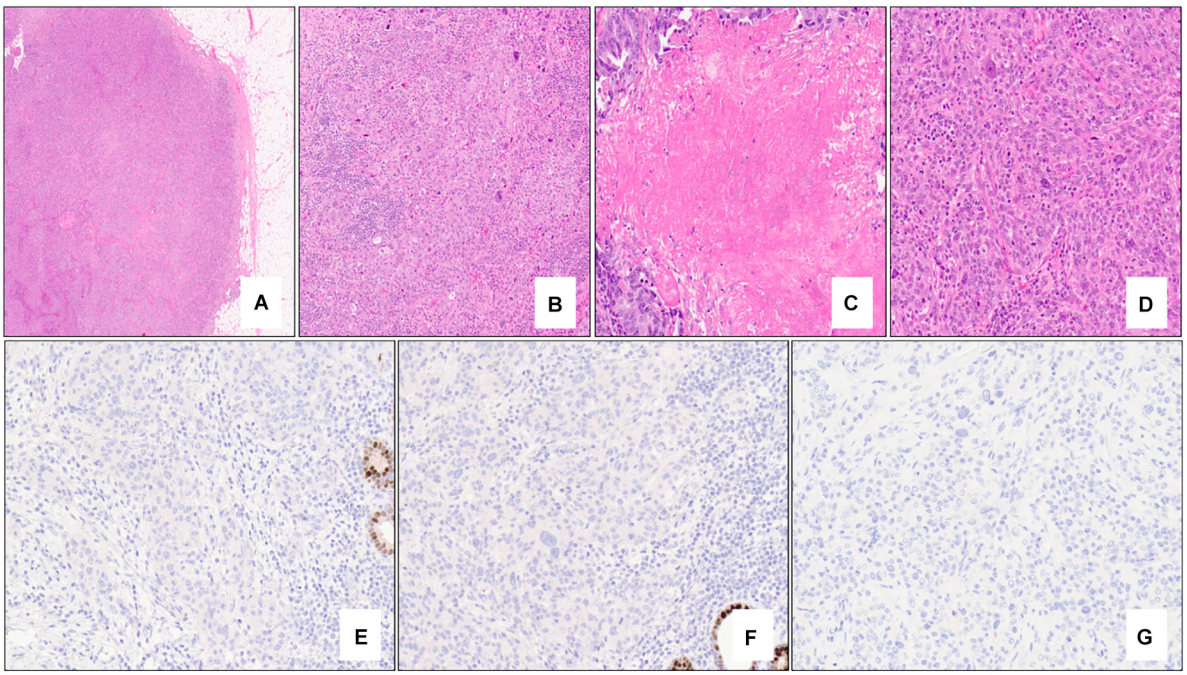

FIGURE 1 | Nottingham grade 3 invasive ductal carcinoma (no special type) of the breast associated with *BRCA1* germline mutation and "triple-negative" biomarker profile. The tumor exhibits solid architecture and pushing border (**A** – 2x mag), prominent intra-tumoral lymphocytes (**B** – 10x mag), large areas of necrosis (**C** – 15x mag), and high-grade nuclear atypia with prominent nucleoli (**D** – 20x mag). The tumor is triple-negative lacking expression of estrogen receptor (**E** – 20x mag), progesterone receptor (**F** – 20x mag), and HER2 (**G** – 20x mag). Note the positive internal control cells (benign terminal duct lobular units) in **(E,F)**. **(A–D)** hematoxylin-eosin stain; **(E–G)** immunohistochemistry.

reported to be more likely related to *BRCA2* mutations (32). Immunohistochemically, *BRCA2*-associated tumors are typically positive for low molecular weight keratins, ER and PR and lack HER2 protein overexpression (38) (**Figure 2**).

Non-BRCA-Associated Breast Cancer

To date, no specific morphological features have been described in tumors associated with mutations in non-*BRCA* genes which impart increased risk for breast cancer development.

HBOC-ASSOCIATED TUBO-OVARIAN CANCER

General Tumor Morphology

Germline *BRCA1/2* mutations are found in approximately 15% of women with ovarian epithelial neoplasms, the most common tubo-ovarian tumor subtype (39). The hallmark histopathologic diagnosis of HBOC-related tubo-ovarian cancer due to *BRCA* mutations is that of high-grade serous carcinoma (40–42), and the frequency of *BRCA1* and *BRCA2* germline mutations increases to approximately 25% in patients diagnosed with these neoplasms (43–45). In addition to high-grade serous carcinoma, other ovarian tumor histotypes including those with endometrioid, mucinous and clear cell differentiation (and others) have also been described to varying degrees in *BRCA*-associated cohorts (32, 33, 39, 46, 47), although some of these studies did not have central review of all pathological specimens (48).

Morphologically, classical high-grade serous carcinoma shows expansile and infiltrative growth of glands and papillae with slit-like spaces. Tumor nuclei are generally enlarged and irregular with prominent nucleoli and brisk mitoses, including atypical forms (**Figure 3A**). Immunohistochemically, high-grade serous carcinomas express p53 in an aberrant pattern (most commonly either nuclear overexpression or complete absence of expression, and less commonly cytoplasmic pattern expression) (**Figures 3B–D**), in addition to CK7, PAX8, and WT-1. ER (and much less commonly PR) usually shows diffuse and strong expression, although staining may be variable in some cases. P16 expression is typically diffuse, strong and block-like.

Specific Tumor Characteristics

A variety of specific morphological characteristics have been described in the context of *BRCA*-associated high-grade serous carcinoma (**Table 2**). Fujiwara et al. showed that tubo-ovarian carcinomas in a cohort of *BRCA1* germline mutation carriers tended to exhibit high-grade and serous/undifferentiated histology, prominent tumor infiltrating lymphocytes (TILs), marked nuclear atypia with giant/bizarre forms, and abundant mitotic figures; these features had a negative predictive value of >94% and a positive predictive value of 21% for *BRCA1* germline mutation status (49). Soslow et al. studied tumors

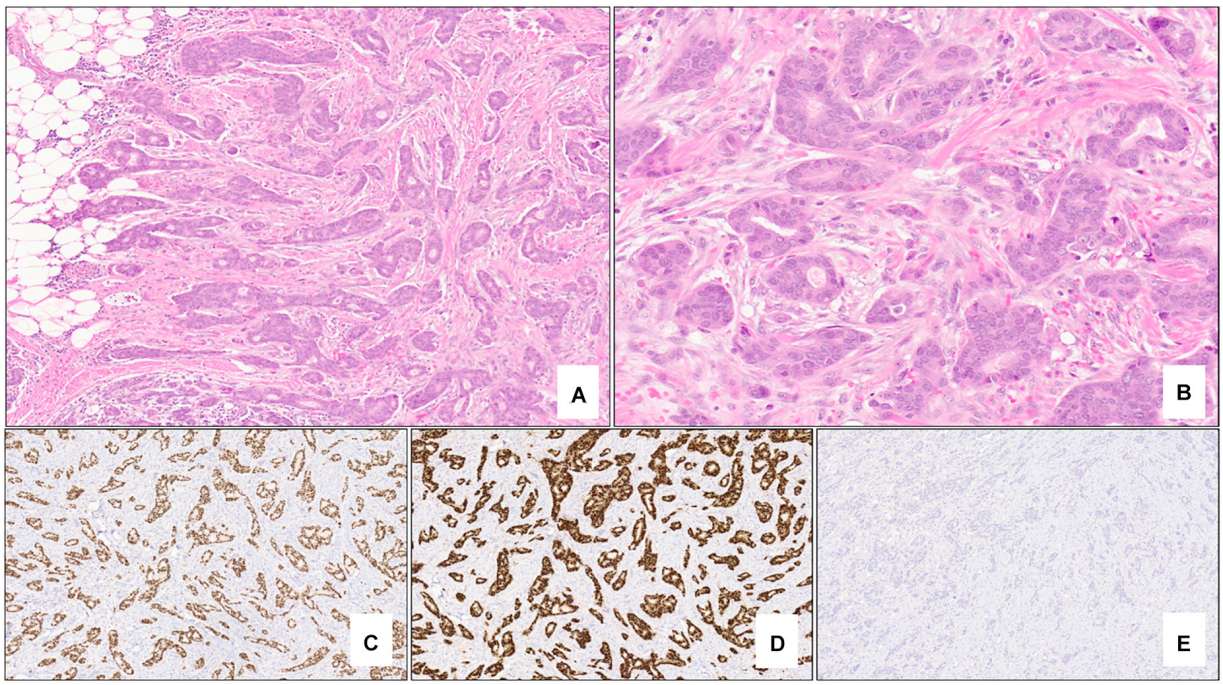

FIGURE 2 | Nottingham grade 2 invasive ductal carcinoma (no special type) of the breast associated with *BRCA2* germline mutation. The tumor shows >75% tubule formation (**A** – 5x mag) with moderate nuclear pleomorphism and inconspicuous mitotic activity (**B** – 20x mag). Almost 100% of the neoplastic cells are strongly positive for estrogen receptor (**C** – 5x mag) and progesterone receptor (**D** – 5x mag), and negative (score 0) for HER2 protein overexpression (**E** – 5x mag). **(A,B)** hematoxylin-eosin stain; **(C–E)** immunohistochemistry.

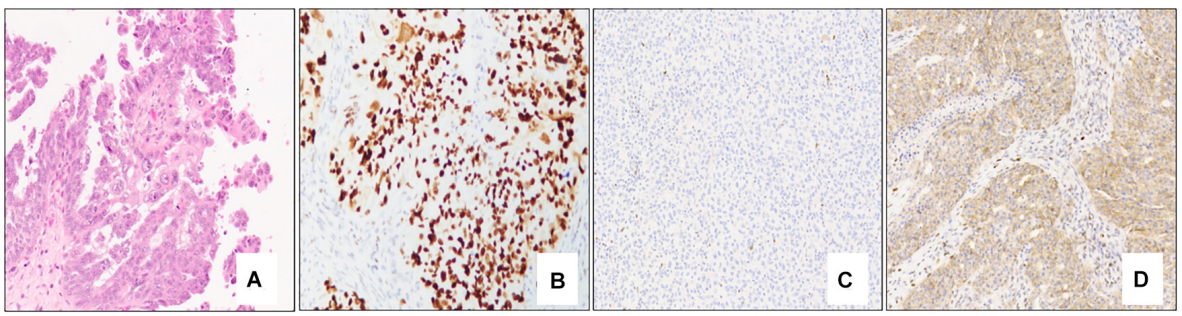

FIGURE 3 | Classical high-grade serous carcinoma composed mostly of papillae lined by atypical epithelial cells with irregular and pleomorphic nuclei and prominent nucleoli (**A** – 10x mag). Immunohistochemical staining patterns of p53 in high-grade serous carcinoma: Strong, diffuse nuclear staining (**B** – 10x mag), complete absence of staining/null pattern (**C** – 10x mag), and cytoplasmic staining (**D** – 10x mag); note the positive internal control (lymphocytes) in **(C)**. **(A)** hematoxylin-eosin stain; **(B–D)** immunohistochemistry.

from patients with germline *BRCA1/2* mutations in addition to tumors with somatic *BRCA1/2* mutation or promoter hypermethylation and found that *BRCA1*-associated high-grade serous carcinomas exhibit high mitotic rates, increased TILs, geographic/comedo-type necrosis, and non-traditional architectural patterns including Solid, pseudo-Endometrioid, and Transitional-like (SET) features. *BRCA2*-mutated tumors also had SET features but tended to have a relative deficiency of TILs and necrosis (42). Examples of SET features are shown in **Figures 4A–F**; note the sheet-like growth of the solid pattern, the glandular spaces in the pseudo-endometrioid pattern, and broad and multi-layered papillary-like structures of the transitional-like pattern.

Prior to the recognition of SET features in high-grade serous carcinoma, tumors exhibiting these morphological findings were often misdiagnosed as high-grade endometrioid, transitional cell, or undifferentiated carcinomas. In a more recent study, Ritterhouse et al. confirmed that tumors with homologous recombination deficiency, including those diagnosed in *BRCA1* and *BRCA2* mutation carriers, are six times more likely to exhibit non-classical (SET or ambiguous) features of high-grade serous carcinoma (50).

TABLE 2 | Morphological features of *BRCA1* and *BRCA2* associated high-grade serous carcinoma.

Morphological features	*BRCA1*	*BRCA2*
Architecture	Frequent SET morphology	
Nuclear atypia	Marked	
Necrosis	Abundant	Relatively deficient
TILs	Abundant	Relatively deficient
Morphology of metastases	Pushing invasion or infiltrative invasion composed exclusively of micropapillae	
Immunophenotype	CK7 +, PAX8 +, WT-1 +, ER +, PR +/-, aberrant expression pattern of p53, and diffuse p16	

SET, Solid, pseudo-Endometrioid, and Transitional-like; TILs, tumor infiltrating lymphocytes; CK7, cytokeratin 7; ER, estrogen receptor; and PR, progesterone receptor.

Although the aforementioned features are associated with *BRCA*-mutated tumors, the data to date have not been able to demonstrate differences to accurately distinguish tumors associated with germline mutations versus somatic mutations and *BRCA* promoter methylation based on morphology alone. As such, confirmatory genetic testing is necessary. However, identification of morphological features associated with *BRCA1/2* is useful for clinical guidance and potential genetic screening.

In addition to morphological features identified at the primary tumor site, specific architectural patterns (metastatic deposits with rounded and pushing contours/"medullary-like" invasion or infiltrative invasion composed exclusively of micropapillae) identified at metastatic sites have also been found to be highly concordant with *BRCA1/2* mutation status and display a high level of agreement among observers (kappa >0.9) (51) (**Figures 4G–I**). Cases which displayed those features at metastatic sites most commonly also exhibited SET features in both the metastatic and primary tumors. Distinction between these two patterns appears to be prognostically relevant as an infiltrative micropapillary pattern has been more commonly identified in metastatic tumor foci from patients who suffered recurrence or death from disease, compared to those with

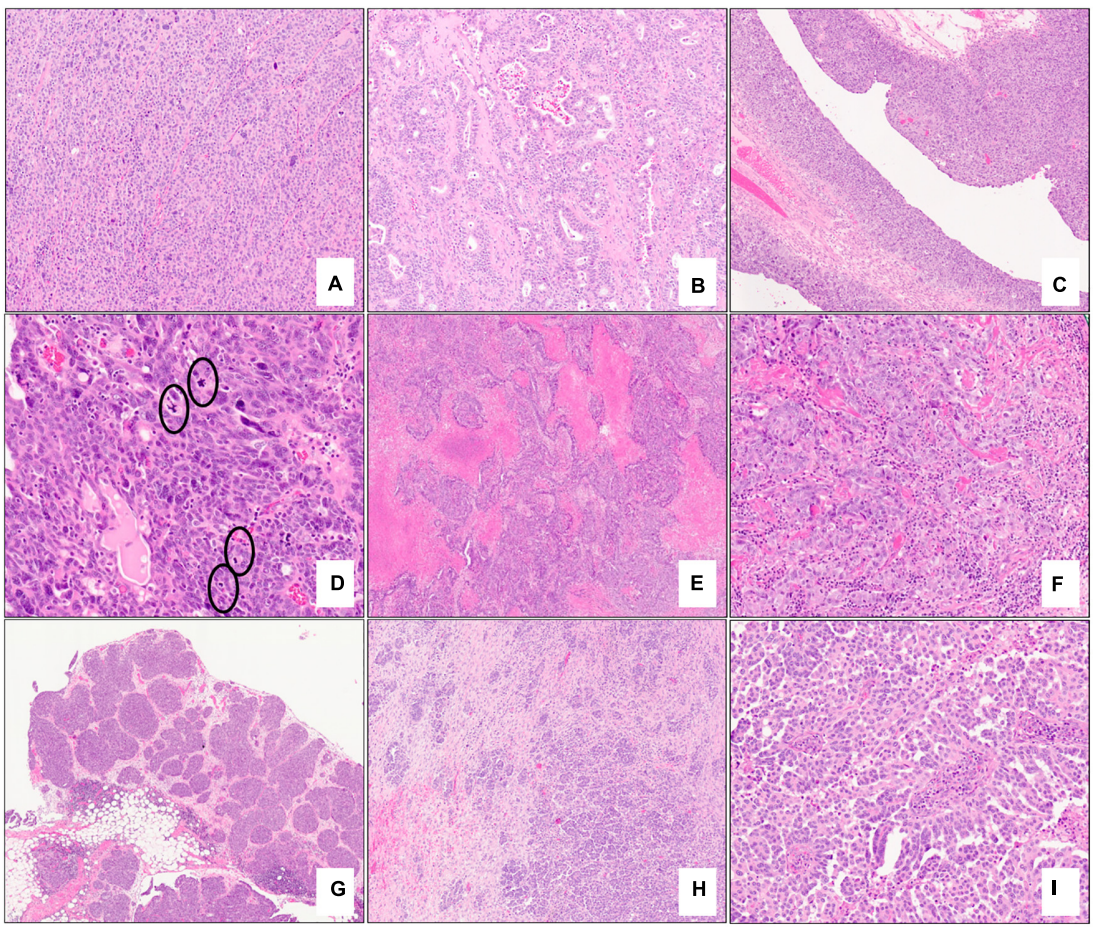

FIGURE 4 | Examples of morphologic features of primary and metastatic high-grade serous carcinoma with *BRCA* mutations. Solid (**A** – 10x mag), pseudo-endometrioid (**B** – 10x mag), and transitional cell carcinoma-like (**C** – 5x mag) architectural patterns; note features reminiscent of papillary urothelial carcinoma in **(C)**. Brisk mitotic activity (**D** – 20x mag), geographic necrosis (**E** – 2x mag), and increased tumor infiltrating lymphocytes (**F** – 10x mag) are present. Omental involvement by *BRCA*-associated high-grade serous carcinoma. A well-circumscribed tumor nodule with a rounded edge and pushing border (**G** – 2x) and an infiltrative focus composed exclusively of micropapillae (**H** – 5x mag, **I** – 15x mag). **(A–I)** hematoxylin-eosin stain.

pushing pattern metastases (52). Interestingly, it has been hypothesized that metastatic tumor architecture may influence the ease of resection of these deposits and thus may contribute to surgeons' ability to achieve optimal tumor debulking in these patients (51, 53).

Interestingly, loss of BRCA1 protein expression by immunohistochemistry has been shown to correlate with *BRCA1* mutation status or *BRCA1* promoter hypermethylation with negative predictive values ranging from 95% to 100% (54, 55). Despite these findings, this technique is not used in routine clinical practice, likely because of a number of limitations including internal control issues, the requirement for nuanced interpretation, and because at least some *BRCA1* clones are not helpful in detecting mutations in certain parts of the gene (54, 56). Immunohistochemistry for the assessment of BRCA2 expression also exists; however, studies to date which have evaluated its use appear to be heterogeneous and have shown mixed results (57).

Role of the Fallopian Tube in the Pathogenesis of High-Grade Serous Carcinoma

It is now widely accepted that the majority of high-grade serous carcinomas arise from fallopian tube epithelium (58–60). Serous tubal intraepithelial carcinoma (STIC) has been recognized as an early form/precursor of high-grade serous carcinoma (61, 62). Approximately 40–60% of all women with high-grade serous carcinoma will harbor a STIC lesion (63, 64). STICs are most commonly identified in the fimbriated end of the fallopian tube near the tubal-peritoneal junction. Although precursor lesions in the fallopian tube had been described prior to the implementation of risk reducing bilateral salpingo-oophorectomy (rrBSO), the possible relationship with ovarian high-grade serous carcinoma was only noted after implementation in the management of patients with germline *BRCA1/2* mutations. Approximately 5–10% of patients with *BRCA1/2* mutations who undergo rrBSO will harbor some form of early serous neoplasia (discussed below), most commonly STIC (60, 65, 66). It should be noted that rrBSO is recommended by multiple guidelines for *BRCA1/2* mutation carriers between the ages of 35 to 40 (or once childbearing is complete or 10 years younger than the age of the youngest first degree relative diagnosed with tubo-ovarian cancer). The age of prophylactic surgery may be delayed until 40 to 45 years of age in some *BRCA2* carriers in addition to *RAD51C, RAD51D*, and *BRIP1* mutation carriers (26, 67). Salpingectomy only followed by interval oophorectomy is another therapeutic alternative being actively investigated (68).

Microscopically, STICs exhibit multilayered epithelium with minimal to mild tufting and stratification, loss of polarity, hyperchromatic and often pleomorphic nuclei, and prominent nucleoli; cilia are absent, and mitotic figures and apoptotic bodies are usually seen (69). A morphological and immunohistochemical algorithm was proposed in 2011 and validated in 2012 for standardization of the classification of STIC, according to which STICs should exhibit an elevated Ki-67 proliferation index (>10%) and aberrant expression of p53 protein (overexpressed in >75% of cells or completely absent/null–pattern) (**Figure 5**) (69, 70). Precursor lesions that do not meet the morphological and/or immunohistochemical criteria for STIC are categorized as serous tubal intraepithelial lesion (STIL) or p53 signature (70). STIL may be diagnosed in a number of different scenarios: (a) tubal epithelium with unequivocal features of STIC and aberrant p53 expression and a low (<10%) Ki-67 proliferation index, or wild-type p53 expression and high (>10%) Ki-67 index, or wild-type p53 expression and low (<10%) Ki-67 index; (b) atypical tubal epithelium suspicious for STIC and either aberrant p53 expression and low (<10%) proliferation index, or wild-type p53 expression and high (>10%) Ki-67 index; and (c) morphologically normal epithelium with aberrant p53 expression and a high (>10%) Ki-67 index. P53 signature is defined by morphologically normal (or near normal) epithelium with aberrant p53 expression and a low (<10%) Ki-67 proliferation index. These lesions have been shown to share *TP53* mutations with adjacent invasive carcinomas (64, 71, 72).

An alternate classification scheme exists which does not rely on Ki-67 proliferation index and which rather focuses on epithelial atypia combined with aberrant p53 immunohistochemical staining. Some forms of benign epithelial atypia (i.e., secretory or stem cell outgrowths, i.e., SCOUTs) are discrete proliferations which may lose cilia but lack aberrant p53 immunohistochemical expression. When aberrant p53 staining is detected in a discretely altered epithelium lacking cilia, the lesion may be classified one of three ways: (a) as a STIC when a loss of polarity is detected, (b) as a serous tubal epithelial proliferation/lesion of uncertain significance when polarity is retained but atypia is present, or (c) benign serous tubal intraepithelial proliferation/p53 signature when polarity is retained and atypia is absent (73).

It should be noted that there is still considerable work being done with regard to the origin of tubo-ovarian high-grade serous carcinoma as in some patients (especially those diagnosed with high stage tumors), no evident STIC is ever identified. In particular, tumors exhibiting SET morphology have a lower level of correlation with the presence of STIC compared to tumors with classical morphology (74, 75). This finding suggests that tumors with SET morphology may derive from a number of different mechanisms including rapid overgrowth of STIC or an alternate tubal precursor lesion (75). This has led to some investigators to question whether the carcinogenic sequence leading to high-grade serous carcinoma is more complex (76). Currently, assignment of the fallopian tube as a primary site is based on the finding of STIC, invasive mucosal carcinoma with or without STIC, or if the fallopian tube is partially or entirely incorporated into tubo-ovarian mass. Tumors lacking STIC or invasive mucosal carcinoma in either fallopian tube in presence of macroscopic or microscopic ovarian involvement can be classified as primary ovarian regardless of presence and size of peritoneal disease (77). High-grade serous carcinoma can be classified as primary peritoneal if both fallopian tubes and ovaries have been examined entirely, and are macroscopically and microscopically normal.

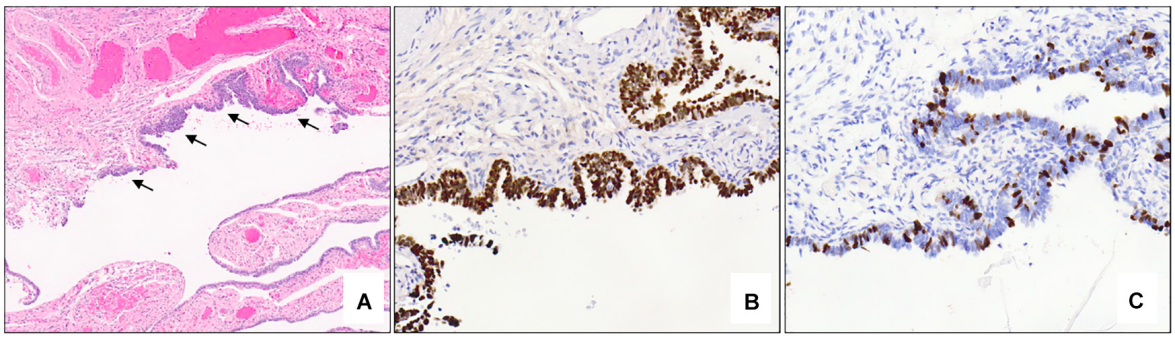

FIGURE 5 | Serous tubal intraepithelial carcinoma. Low power examination of the fimbriated end of the fallopian tube shows an atypical focus (black arrows) which appears darker and crowded compared to the adjacent benign epithelium (**A** – 5x mag). Immunohistochemical stains show aberrant expression of p53 with strong, diffuse nuclear staining (**B** – 10x mag) and an elevated (>10%) Ki-67 proliferation index (**C** – 10x mag) in the atypical area. **(A)** hematoxylin-eosin stain, **(B,C)** immunohistochemistry.

Non-BRCA-Associated Tubo-Ovarian Cancer

Similar to breast cancers associated with mutations of non-*BRCA* genes involved in homologous recombination, limited data currently exists which definitively describes any specific morphological features associated with these tumors.

ADDITIONAL TUMOR CONSIDERATIONS

Although the prototypical female cancers associated with HBOC are those arising in the breast and ovary/fallopian tube/peritoneum, recent work suggests that some endometrial carcinomas may be associated with underlying BRCA alterations. de Jonge et al. have shown homologous recombination deficiency in 24% of endometrial cancers, all with non-endometrioid morphology (78). In addition, endometrial carcinomas in germline *BRCA1* or *BRCA2* mutation carriers have also been reported to be of non-endometrioid subtype in 58% of cases and grade 3 histology in 79% of cases, and most commonly fall into the *TP53*-mutated molecular subgroup defined by The Cancer Genome Atlas Research Network (TCGA) in 92% of cases (79). Overall, these interesting findings warrant additional studies to establish whether endometrial cancer patients may benefit from treatment targeting homologous recombination deficiency. The findings also raise important potential consequences from counseling/surveillance perspectives.

Besides malignancies arising in the breast and gynecological organs, an increased risk of other neoplasms, including pancreatic carcinoma, gastric carcinoma and cutaneous malignant melanoma (80–82), has been reported in HBOC patients, particularly in individuals with germline *BRCA2* mutations. However, in contrast to the better characterized phenotype-genotype correlations in female breast and gynecological tumors discussed above, no particular *BRCA*-associated morphological features have been described in these tumors, to the best of our knowledge. Nevertheless, awareness of the potential association that these tumors may have with an underlying *BRCA* mutation is very important, especially when they are identified in a patient without a known family history.

CLINICAL IMPLICATIONS

Pathological Processing

The concept that the many high-grade serous carcinomas arise from the fallopian tube has played a major role in driving the evolution of how prophylactic surgical specimens from HBOC patients are evaluated. Currently, it is standard practice to examine these specimens according to the SEE-FIM (Sectioning and Extensively Examining the Fimbriated end of the fallopian tube) protocol (60) which dictates that the distal 2 cm of each fimbriated end should be sectioned at 2 mm intervals along the long axis and entirely submitted for microscopic examination. The remainder of the fallopian tube is also to be sectioned at 2 mm intervals and entirely submitted, in addition to both ovaries (in the absence of any grossly evident lesion). The purpose of the SEE-FIM protocol is to maximally expose tubal fimbrial epithelium for microscopic evaluation, as STIC lesions are typically focal and not grossly evident.

Treatment

Importantly, the identification of an germline-associated *BRCA1/2*-mutated tumor indicates not only an underlying germline defect (in the patient and perhaps also in her family members), but also implies certain important prognostic and treatment connotations. For example, mutations involving genes whose protein products are involved in homologous recombination have been shown to be associated with chemotherapeutic platinum sensitivity and improved survival in both breast and tubo-ovarian cancer patients (46, 50, 83). Similarly, triple-negative breast cancer patients harboring defects in homologous recombination proteins have been shown to exhibit increased sensitivity to both platinum-based and standard chemotherapy regimens (84, 85), although the effect on prognosis is more complex (86). The underlying molecular

abnormalities due to homologous recombination deficiency indicate that these malignancies can be treated with novel poly ADP-ribose polymerase (PARP) inhibitors which act to limit repair of single strand breaks (87) and thus lead to tumor cell death due to the overwhelming genetic instability (88). Recent studies have also shown that *BRCA*-deficient tumors have elevated expression levels of programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) in tumor-associated immune cells, indicating that checkpoint inhibitors may be useful in the treatment of *BRCA*-associated cancers (89–91).

Genetic Testing

Tumor and germline genetic testing is variably performed in patients affected by breast (92) and ovarian carcinomas (93). It has been shown that triaging women for genetic testing based on family history alone will miss up to 30% of affected individuals (94). Nevertheless, genetic testing for breast cancer patients is still largely based on family history risk (**Table 3**) (26). However, it has been suggested that all patients with breast cancer should undergo genetic testing in order to optimize treatment and improve survival, but also to mitigate risk for family members that are healthy mutation carriers (95).

For tubo-ovarian cancer, an approach driven by histological tumor features has been adopted in a number of institutions given the strong association between high-grade serous morphology and *BRCA1/2* mutations. It is generally recommended that every tubo-ovarian/primary peritoneal high-grade serous carcinoma be tested for at least somatic *BRCA1/2* gene mutations, in addition to mutations in other high-risk genes (96). At some institutions, this testing is done reflexively once a diagnosis of high-grade serous carcinoma has been made. Diagnostic accuracy is therefore critical. Importantly, mutation testing should be done regardless of the presence or absence of the morphological features discussed above. Some organizations have recommended germline testing in all patients with invasive non-mucinous epithelial ovarian, fallopian tube or peritoneal cancers (i.e., not only high-grade serous carcinomas, but also endometrioid, clear cell, and seromucinous subtypes) (97–99). If tumor testing is undertaken and a mutation is identified, referral to genetic counseling for consideration of additional germline testing is necessary (if not already done) as a proportion of pathogenic mutations identified in tumor tissue will be of germline origin. A number of genetics referral models exist with each model having its advantages and disadvantages (100).

In the past, single gene testing was used for the purpose of assessing underlying mutations. However, the use of comprehensive multigene panel testing has become increasingly prevalent and has helped to identify additional patients with *BRCA1/2* mutations as well as patients with mutations in other genes associated with an increased risk. Although a multigene testing approach provides advantages in terms of comprehensive assessment, cost and turnaround time, the goals of practical clinical utility and ease of interpretation should always be kept in mind (101, 102), in addition to the care that should be taken to ensure extensive and in-depth clinical and analytical validation (103).

TABLE 3 | Criteria for breast and/or ovarian cancer genetic assessment.

Any individual (at any age):	• With a known pathogenic or likely pathogenic variant in a cancer susceptibility gene within the family • With a known pathogenic or likely pathogenic variant in a cancer susceptibility gene discovered on tumor testing
Any individual (at any age) who develops the following:	• Ovarian cancer • Pancreatic cancer • Metastatic prostate cancer • Is of Ashkenazi Jewish ancestry and develops breast cancer or high-grade prostate cancer
Any individual with breast cancer and the following:	• Diagnosis is at ≤50 years of age • Development of a triple-negative cancer at ≤60 years of age • Two separate breast cancers (either in the same or contralateral breast, synchronous or metachronous) • ≥2 close blood relatives diagnosed with breast cancer at any age
Any individual with breast cancer at any age and ≥1 close blood relative with the following:	• A diagnosis of breast cancer at ≤50 years of age • Ovarian cancer • Male breast cancer • Pancreatic cancer • High-grade or metastatic prostate cancer
Any individual who does not meet the above criteria but who has a first or second degree relative with any of the following:	• Diagnosis of breast cancer at ≤45 years of age • Ovarian cancer • Male breast cancer • Pancreatic cancer • Metastatic prostate cancer • ≥2 separate breast cancers in a single individual • ≥2 individuals with breast cancer on the same side of the family with at least one diagnosed ≤50 years of age
Any individual with a personal and/or family history on the same side of the family of ≥3 of a variety of malignant neoplasms	

A close blood relative includes a first, second, or third degree relative.

CONCLUSION

In this review, we have discussed the HBOC syndrome from a pathological perspective and have described specific characteristics of *BRCA1* and *BRCA2*-associated breast and tubo-ovarian neoplasms. Pathologists play a critical role in the identification and triage of affected patients, particularly those without a known family history, as a number of morphological features associated with these *BRCA*-mutated tumors have been reproducibly described and are easily recognized. Accurate and timely pathological assessment and interpretation is critical given the implications for prognosis, therapy and genetic testing. Ongoing research will continue to refine our understanding of HBOC syndrome pathology, including how non-*BRCA* gene mutations affect tumor morphology, behavior and prognosis. In addition, our understanding will continue to develop regarding precursor lesions of high-grade serous carcinoma and other neoplasms arising in the context of the syndrome, including endometrial carcinoma and other non-gynecologic tract tumors.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

AH and GT contributed equally to the conception, initial drafting, and final editing of this review article. Both authors approved the submitted version.

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MINI REVIEW
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Surgical and Systemic Treatment of Hereditary Breast Cancer: A Mini-Review With a Focus on BRCA1 and BRCA2 Mutations

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Hereditary breast cancer accounts for 5%–10% of breast cancer cases. The majority of familial cases have been linked to germline mutations in BRCA1 and BRCA2 genes, though other high penetrance susceptibility genes have also been identified through genomic testing advances. Optimal surgical treatment for these patients, who are of a younger age, has several challenges as it usually involves aggressive therapeutic and risk reducing interventions. At the same time, the therapeutic armamentarium for BRCA1/2 mutation carriers apart from platinum salts, has been enriched with the addition of poly-ADP ribose polymerase (PARP) inhibitors with promising outcomes. In this review we provide a succinct and comprehensive overview of the surgical and systemic treatment options for patients with BRCA1/2 mutation related breast cancer and an update on the most recent systemic treatment advances.

Keywords: genes, hereditary, breast cancer, BRCA1, BRCA2, surgical management, systemic treatment

INTRODUCTION

Breast cancer (BC) is the most common female malignancy, with more than 2 million cases being diagnosed world-wide annually (1). Hereditary syndromes account for approximately 5-10% of the cases and are associated with the presence of germ-line mutations. The majority of hereditary breast cancer cases result from mutations in BRCA1 and BRCA2 genes, whereas the rest have been linked to less frequent germline mutations in other high penetrance genes such as TP53, STK11, PTEN, CDH1, and PALB2, as well as moderate penetrance genes like ATM and CHEK2 (2).

Abbreviations: N, number; BCT, breast conserving treatment; UBC, unilateral breast cancer; M, mastectomy; BC, breast cancer; CRRM, Contralateral risk reducing mastectomy; OS, Overall survival;CBC, contralateral breast cancer; PYO, person years of observation; LR, local recurrence; EBC, early breast cancer; BPO, bilateral salpingo-oophorectomy.

Both BRCA1 and BRCA2 are tumour suppressor genes encoding proteins involved in homologous recombination repair (3). Pathogenic variants in both genes affect 1 in 400 persons in the general population and 1 in 40 in the Ashkenazi Jewish population. They get inherited by an autosomal dominant pattern and carry a lifetime cumulative breast cancer risk of 72% for BRCA1 and 69% for BRCA2 (4).

This review will focus on the surgical and systemic treatment of hereditary breast cancer with a particular focus on BRCA1 and BRCA2 mutations.

SURGICAL TREATMENT

Surgery on Locoregional Disease

The optimal surgical treatment for operable BC in BRCA1/2 mutation carriers depends on several factors and remains a topic of debate. Although breast conserving surgery (BCS) is the preferred surgical treatment for early stage disease in sporadic breast cancer, its oncological safety in BRCA1/2 mutation carriers has not been extensively studied. A meta-analysis of 10 studies, demonstrated a significantly higher risk for ipsilateral breast recurrence (IBR) in

TABLE 1 | Summary of main studies investigating the role of breast conserving surgery and risk reducing mastectomy in breast cancer patients with BRCA1/2 mutations.

Author, year	Study design	Endpoints	Outcome
Nilsson et al. (6)	Retrospective cohort study Women with stage I-III BC N=162, BCT=45/M=117 Comparison between BCT vs mastectomy in BRCA1/2 carriers	LR as first recurrence Death resulting from BC Overall survival Distant recurrence	Increased risk for LR in BCT (new primary in most cases) No difference in BC related death, overall survival or distant recurrence
Pierce et al. (7)	Retrospective cohort study N= 655, BCT=302/M=353 Comparison between BCT vs mastectomy in BRCA1/2 carriers	LR as first recurrence Development of CBC BC specific survival	LR more likely in BCT but in 70% of cases new primary No difference in CBC or BC specific survival
van den Broek et al. (8)	Retrospective cohort study *BRCA1* (N = 191) and *BRCA2* (N = 70) Non carriers= 5820 Comparison between BCT vs mastectomy in BRCA1/2 carriers and non carriers	Ipsilateral recurrence Overall survival BC specific survival	No difference between BRCA1 carriers (10-year risk = 7.3%) and noncarriers (10-year risk = 7.9%) No difference in OS or BC specific survival Data for BRCA2 carriers insufficient for conclusions
Evans et al. (9)	Observational study N=718 patients with UBC BRCA1 (N=357)/BRCA2 (N=361) Comparison between N= 105 who underwent CRRM to controls with no CRRM: 593 carriers and 105 specifically matched	Overall survival	CRRM group 10-year survival: CRRM only: 83% CRRM + RRBSO: 92% Non CRRM group 10-year survival: No RRBSO:65% + RRBSO: 81% CRRM appears to confer a survival advantage. But warrants prospective validation in larger cohort.
Heemskerk- Gerritsen et al. (10)	Prospective cohort study N=583 patients with BRCA-associated BC. CRRM: N=242 patients (42%) underwent Surveillance: N 341 patients (58%) Examined efficacy of CRRM on OS	Overall survival (measured in PYO) CBC Incidence	CRRM: 4 patients developed CBC (2%). Surveillance: 64 patients developed CBC (19%) OS: 8% of patients died in CRRM group; 16% in the surveillance group Mortality was lower in CRRM group, 21.6 vs. 9.6 per 1000 PYO (Adjusted HR=0.49, 95% CI 0.29-0.82)
Metcalfe et al. (11)	Retrospective cohort study N= 390 BRCA1/2 carriers with EBC Unilateral M: N= 309 Bilareral M: N= 181	Breast cancer related death	Survival rate at 20 years: Contralateral M: 88% (95% CI, 83% to 93%) Unilateral M: 66% (95% CI, 59% to 73%)
van Sprundel et al. (12)	Retrospective cohort study N= 148 women with a BRCA1 or BRCA2 previously treated for invasive stage I-III BC N=79 opted for CRRM N=69 women remained under close surveillance. Mean follow-up was 5 years post diagnosis	Risk of CBC CBC specific and overall survival	CRRM: One case (1.3%) of invasive CBC Surveillance: 6 cases (14%) (P < 0.001) Risk of CBC in CRRM group vs. risk of CBC in surveillance group HR=0.09 (95% CI 0.01-0.78) P=0.03 Breast cancer-specific survival not significantly better in CRRM group (P=0.11) At 5 years follow-up, OS was 94% in CRRM group vs 77% in the surveillance group (P=0.027). With adjustment for BPO, CRRM group did not have significantly better survival vs. surveillance group HR 0.35, P=0.14

BCRA1/2 mutation carriers compared to non-carriers following BCS at a median follow up greater than 7 years, but no difference for shorter follow up periods (5). The risk for contralateral breast cancer was also found to be increased in BRCA1/2 mutation carriers (5). Although BCS is associated with higher IBR risk compared to mastectomy in BRCA1/2 mutation carriers, no difference was found between the two treatment options for overall survival, breast cancer death, or distant recurrence **(Table 1)** (5–8). Data from a meta-analysis indicate that the risk of IBR in BRCA1/2 mutation carriers who have undergone BCS was found to be reduced with adjuvant chemotherapy (RR 0.51, 95%CI 0.31–0.84) and oophorectomy (RR 0.42, 95%CI 0.22–0.81) (5).

BCS could be considered a safe and reasonable option for BRCA1/2 mutation carriers but this should be discussed on an individual basis and further factors need to be taken into account. These include patient's understanding of the increased risk for an ipsilateral new primary breast cancer with all potential emotional implications, as well as their ability to undergo appropriate breast surveillance.

International guidelines recommend that early breast cancer patients carrying mutations in moderate penetrance breast cancer susceptibility genes, should be offered BCS if appropriate. However, patients carrying TP53 germline mutations should avoid BCS followed by radiation as they are at high risk of developing radiation induced malignancies such us angiosarcoma (13).

Risk Reducing Mastectomy

The term "risk reducing" has been deemed more appropriate than "prophylactic" in recent times as no mastectomy can remove all breast tissue. Several studies demonstrated a reduction in the risk of breast cancer by ~95% in BRCA1/2 mutation carriers who underwent bilateral risk reducing mastectomy (BRRM) in combination with oophorectomy and by ~90% in those with intact ovaries (14–17). A recent systematic review confirms the benefit of BRRM in reducing both incidence and mortality from breast cancer in high risk patients, such as BRCA1/2 carriers, but calls for rigorous prospective studies due to methodological flaws of the existing literature (18). Data for contralateral risk reducing mastectomy (CRRM) for patients who have had breast cancer in one breast are less conclusive as existing studies show a reduction in the incidence of contralateral breast cancer but no definitive survival benefit **(Table 1)** (9–12, 18).

For high risk patients such as BRCA1/2 mutation carriers, international guidelines recommend RRM with appropriate counselling on risks and benefits. When assessing the risk for developing contralateral breast cancer (CBC) the following factors need to be taken into account: age at diagnosis of primary breast cancer, family history, ability to undergo indicated surveillance imaging, prognosis from this or other malignancies, comorbidities and life expectancy (13, 19). RRM cannot completely eliminate the risk of breast cancer and can have a negative impact on body image and quality of life due to potential complications such as multiple surgeries, chronic pain, sexual dysfunction and poor cosmetic outcomes (20). Women considering this procedure should be well informed and weigh the risks and benefits compared to other alternatives such as risk reducing bilateral salpingo-oophorectomy, chemoprevention and intensive screening. For women who wish to avoid or delay RRM, MRI-based breast screening is a reasonable option (19, 21). For patients who undergo RRM, skin sparing mastectomy with or without preservation of the nipple-areolar complex has been found to be a safe option for BRCA carriers while achieving better cosmesis (22, 23).

There is a lack of data in the existing literature on the risk for CBC in breast cancer patients carrying mutations in cancer susceptibility genes other than BRCA1/2. Limited data exist for the CHEK2 1100elC frameshift mutation which is associated with a 3-fold increase in the risk of CBC (24). Decisions on CRRM for patients with moderate risk mutations should not be extrapolated from existing data on BRCA1/2, but should be balanced on several factors (age at diagnosis of primary breast cancer, family history, ability to undergo surveillance imaging) and involve appropriate patient counselling (13).

Risk Reducing Bilateral Salpingo-Oophorectomy

Risk reducing bilateral salpingo-oophorectomy (rrSBO) is recommended for female BRCA1/2 carriers who have completed childbearing and should be completed by age 35 to 40 for BRCA1, 40 to 45 for BRCA2 carriers or earlier as per patient's relevant family history (25). It has been demonstrated that rrBSO reduces the risk for ovarian cancer by 80% and all-cause mortality by 68% in female BRCA1/2 carriers (26, 27). The beneficial effect of rrBSO on breast cancer risk reduction has also been assessed but current data are less conclusive. Some prospective studies confirmed that rrBSO reduces BC risk for both BRCA1/2 carriers (25, 28). However, a large case-control study showed a benefit for rrBSO only for BRCA1 carriers when performed before the age of 40, while a more recent study identified a benefit only for BRCA2 carriers when performed prior to 50 years old (29). Oophorectomy has been associated with a significant decrease in the risk of IBR and CBC (5).

SYSTEMIC TREATMENT

Germline mutations of BRCA1 and BRCA2 genes lead to the decreased capacity of the cell to repair double strand breaks (DSBs), as they are key elements of the homologous recombination (HR), one of the two main mechanisms of DSB repair (30, 31). This formed the basis for the development of new therapeutic strategies and the development of novel treatments for this specific breast cancer patient subgroup (**Table 2**).

Platinum Salts

Since the introduction of cisplatin in the 1970s, platinum compounds have been the cornerstone in the treatment of

TABLE 2 | BRCA1/2 associated breast cancer and systemic treatment.

Authors, year	Phase	Treatment	Setting	Endpoint	Results
Tutt et al. (TnT) (32)	III	Carboplatin vs Docetaxel	Metastatic	ORR	BRCAm group ORR 68% vs 33% PFS 6.4 vs 4.4 months
Byrski et al. (33)	Retrospective	Cisplatin	Neoadjuvant	pCR	pCR = 83%
Hahnen et al. (34) (GeparSixto)	II	Carboplatin vs SoC ChT	Neoadjuvant	pCR	pCR 65.4% vs 66.7%
Tung et al. (35) (INFORM)	II	Cisplatin vs Doxorubicin & Cyclophosphamide	Neoadjuvant	pCR	18% vs 26%
Robson et al. (36) (OlympiAD)	III	Olaparib vs SoC ChT	Metastatic	PFS	7 vs 4.2 months
Litton et al. (37) (EMBRACA)	III	Talazoparib	Metastatic	PFS	8.6 vs 5.6 months
Drew et al. (38)	II	Rucaparib	Metastatic	RR	15%
Dieras et al. (39) (BROCADE3)	III	Veliparib + paclitaxel carboplatin vs Placebo + paclitaxel Carboplatin	Metastatic	PFS	14.5 vs 12.6 months
Vinayak et al. (40)	II	Niraparib + pembrolizumab	Metastatic	RR	BRCAm group PR 47%

ORR, overall response rate; pCR, pathological complete response; PFS, progression free survival; RR, response rate; BRCAm, BRCA mutated; ChT, chemotherapy; SoC, standard of care.

various tumour types. Platinum agents form intra-strand adducts by binding with the purines leading to DSBs. This triggers various repair mechanisms including that of homologous recombination (HR) (41). Consequently, cells with HR deficiency can be particularly sensitive to platinum compounds (42, 43).

In a small phase II open label study, 20 BRCA1 mutation carriers with metastatic breast cancer (mBC) received cisplatin 75 mg/m2 on a 3-weekly basis with 35% achieving partial response and 45% complete response with acceptable toxicity profile (44). In the Phase II TBCR009 trial, 86 previously treated triple negative mBC patients received either cisplatin or carboplatin. Response rates in the BRCA1/2 mutation carrier patient subgroup were significantly higher compared to the total study population (54% versus 26%) (45).

The triple negative breast cancer trial (TNT) was the largest trial examining the role of platinum compounds in the treatment of triple negative and BRCA1/2 mutated mBC patients. In this Phase III study, 376 mBC patients were randomised to receive first line chemotherapy with carboplatin or docetaxel. In the BRCA1/2 mutation subgroup the overall response rates were higher for the carboplatin group (68% vs 33%). Similarly, PFS was also improved in the BRCA1/2 mutation carriers who received carboplatin (6.4 vs 4.4 months) (32).

The use of platinum compounds has also been assessed in the neoadjuvant setting. In 2010, Byrski et al. reported a pathological complete response (pCR) rate of 83% for women with BRCA1 positive BC treated with neoadjuvant cisplatin (33). This was further echoed in the findings of a single arm study including 107 BC patients carrying BRCA1 mutation who were treated with 4 cycles of neoadjuvant chemotherapy with 61% achieving pCR (46).

In GeparSixto, a phase II randomised trial, triple negative stage II-III breast cancer patients were given anthracycline and taxane based neoadjuvant chemotherapy with or without carboplatin (47). In a secondary analysis, BRCA1/2 mutation carriers did not gain any additional benefits in terms of pCR with the addition of carboplatin (65.4% vs 66.7%) with similar impact on DFS. On the contrary, carboplatin conferred significant improvement in response rates to non-carriers (34). In the phase II CALBG 40603 trial, although the addition of carboplatin to NACT achieved superior pCR rates in patients with II-III triple negative BC, an improvement in long term survival outcomes was not demonstrated (48). Results from the recent randomized Phase II INFORM trial, demonstrated that in BRCA1/2 carriers with HER2 negative stage I-III BC, neoadjuvant single agent cisplatin did not achieve better pCR compared to doxorubicin and cyclophosphamide (AC) (35). All things considered, the use of platinum compounds as part of neoadjuvant chemotherapy does not clearly improve the rates of pCR in breast cancer patients carrying BRCA1/2 mutations.

PAPR Inhibitors

The concept that some genes can be "synthetically lethal" has been well known since early preclinical studies. In order for two genes to be synthetically lethal, both have to carry mutations leading to cell death. As a result, the targeting of one gene, combined with a known genetic mutation could be a tempting field for the development of new anticancer drugs (49).

Under this scope, the inhibition of single strand (SS) DNA repair with the use of the enzyme poly (ADP) ribose polymerase (PARP) inhibitors, in combination with known homologous recombination (HR) deficiency, can result in cell death (50).

Over the past 6 years multiple PARP inhibitors have been approved for the treatment of ovarian cancer (51). Olaparib is the PARP inhibitor which has been studied more extensively in breast cancer patients with BRCA1/2 mutations. In the early phase clinical trial olaparib showed efficacy in advanced solid tumors with 22 patients having breast cancer and 9 of them being BRCA1/2 mutant (52). In a proof of concept trial 54 pretreated metastatic breast cancer patients with BRCA1/2 mutation were treated with olaparib 400 mg twice daily (BD) or 100 mg BD. On the 400 mg BD arm, overall response rate was 41% and 22% in the cohort of 100 mg BD with acceptable toxicity profile (53). In another phase II basket trial 62 women with advanced breast cancer received olaparib. ORR was achieved in 13% of patients and stable disease for more than 8 weeks was observed in 47% (54). The ORR was lower in patients with previous exposure to

platinum compounds suggesting that there is cross-resistance with PAPR inhibitors.

In the randomized open label phase III OlympiAD trial, olaparib 300 mg BD monotherapy was compared with standard chemotherapy (eribulin, capecitabine, gemcitabine) in 302 patients with metastatic, HER2 negative, BRCA1/2 related breast cancer. All patients had received anthracycline and taxane based chemotherapy. Median progression free survival was significantly improved for the olaparib arm (7 months vs 4.2 months). The response rates were 59.9% for the olaparib group and 28.8% for the chemotherapy group (36). Of note, olaparib was not compared to cisplatin or carboplatin.

Talazoparib is a potent PARP inhibitor which has been studied for the treatment of BRCA1/2 mutation related breast cancer. In the early clinical trial, talazoparib showed promising activity in BRCA1/2 mutation related solid tumors including patients with breast cancer (55). EMBRACA was a phase III open label clinical trial, which randomised 431 metastatic breast cancer patients with germline BRCA1/2 mutations to talazoparib or physician's choice chemotherapy. Median PFS was significantly improved in the talazoparib arm (8.6 months vs. 5.6 months) (37). ABRAZO was a phase II, trial assessing the efficacy of talazoparib in germline BRCA1/2 mutant breast cancer patients with previous response to platinum-based chemotherapy or in patients with 3 or more previous lines of cytotoxic treatment and demonstrated promising anti-tumour activity (56).

Talazoparib has also been tested in the early breast cancer setting. After the promising results of a feasibility study in which 2 months of neoadjuvant treatment with talazoparib before the initiation of standard neoadjuvant chemotherapy, showed median decrease in tumor size of 88% (57), a separate pilot study was organized. Twenty patients with germline BRCA1/2 mutant HER2 negative breast cancer received 6 months of neoadjuvant treatment with talazoparib before proceeding with surgery. Pathological complete response was achieved in 53% of the patients with acceptable toxicity (58).

Another PARP inhibitor, rucaparib has been evaluated for the treatment of patients with metastatic breast cancer. In a phase II, open-label, multicentre trial of rucaparib in BRCA1/2 mutation carriers with advanced breast or ovarian cancer, the range of dosing schedules, safety and tolerability were assessed. The treatment schedule included intravenous and subsequently oral rucaparib. In the intravenous only schedule response rated was only 2%, with 15% on the continuous oral schedule. The authors concluded that in order to achieve optimal response continuous dosing schedule is required (38).

Veliparib has also been tested in germline BRCA1/2 mutation carrier breast cancer patients. In a phase II trial, veliparib was given as a monotherapy at 400 mg BD and at the time of progression carboplatin at a dose of AUC5 was added. Partial response rate was 17% for BRCA1 and 23% for BRCA2 mutation carries who had at least 4 cycles of follow-up (59).

Recently the results of phase III BROCADE3 trial were presented. In this trial 509 germline BRCA1/2 mutation carriers with metastatic breast cancer were randomised 2:1 to receive paclitaxel/carboplatin plus intermittent veliparib or paclitaxel/carboplatin plus placebo. Median PFS was improved by 1.9 months (14.5 vs 12.6 months) (39).

The results of a phase II open label trial of niraparib in combination with pembrolizumab were recently announced (40). In this study, 55 women with triple negative metastatic breast cancer were treated with niraparib at a dose of 200 mg once daily combined with pembrolizumab 200 mg every 3 weeks. Fifteen patients had somatic or germline BRCA1/2 mutation with 7 achieving partial response (47%).

There are no data to support the use of systemic treatments in patients with moderate-risk breast cancer susceptibility mutations. This is currently investigated in a Phase II clinical trial which explores the effectiveness of olaparib in mBC patients with somatic or germline mutations in DNA repair genes. Preliminary data shown efficacy in patient with somatic BRCA1/2 and germline PALB2 mutations but not in those with ATM or CHEK2 mutations (60).

CONCLUSION

Treating hereditary breast cancer entails more challenges than sporadic cases. High risk patients such as BRCA1/2 germline mutation carriers, present at a young age and their optimal surgical management yet remains an individualized and debatable area. BRCA1/2 mutation carriers face more aggressive surgical interventions for therapeutic and risk reducing purposes due to their high risk of developing primary or contralateral breast cancer. Breast conserving surgery as well as skin sparing mastectomies with or without preservation of the nipple-areolar complex have been proven to be safe and achieve better cosmesis. Selecting the best surgical approach for this patient population requires taking into account several factors including patient's genetic risk, family history, previous BC biology, as well as patient's own preferences.

Due to defects in homologous recombination, BRCA1/2 related BC is highly susceptible to treatment with platinum compounds. Several clinical trials demonstrated higher response rates with platinum in BRCA1/2 mutation carriers with metastatic BC. However, this finding was not replicated in the neoadjuvant setting, where an additive benefit of platinum compounds in achieving pCR has not been demonstrated for BRCA1/2 mutation carriers.

The therapeutic landscape of BRCA1/2 related breast cancer has been enriched with the addition of PARP inhibitors which led to improvements in survival outcomes. Olaparib and talazoparib have already gained regulatory approval while other such as niraparib and rucaparib and veliparib are undergoing clinical trial assessment. Combinatorial strategies involving PARP inhibitors with chemotherapy or immunotherapy are also being under investigation and hold promise for the future management of BRCA1/2 related breast cancer.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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