Moderate penetrance genes complicate genetic testing for breast cancer diagnosis: ATM, CHEK2, PARD1 and RAD51D

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ABSTRACT

Breast cancer risk associated with germline likely pathogenic/pathogenic variants (PV) varies by gene, often by penetrance (high >50% or moderate 20–50%), and specific locus.

Germline PVs in BRCA1 and BRCA2 play important roles in the development of breast and ovarian cancer in particular, as well as in other cancers such as pancreatic and prostate cancers and melanoma. Recent studies suggest that other cancer susceptibility genes, including ATM, CHEK2, PALB2, RAD51C and RAD51D confer differential risks of breast and other specific cancers.

In the era of multigene panel testing, advances in next-generation sequencing technologies have notably reduced costs in the United States (US) and enabled sequencing of BRCA1/2 concomitantly with additional genes. The use of multigene-panel testing is beginning to expand in Europe as well.

Further research into the clinical implications of variants in moderate penetrance genes, particularly in unaffected carriers, is needed for appropriate counselling and risk management with data-driven plans for surveillance and/or risk reduction. For individuals at high risk without any pathogenic or likely pathogenic variant in cancer susceptibility genes or some carriers of pathogenic variants in moderate-risk genes such as ATM and CHEK2, polygenic risk scores offer promise to help stratify breast cancer risk and guide appropriate risk management options.

Cancer patients whose tumours are driven by the loss of function of both copies of a predisposition gene may benefit from therapies targeting the biological alterations induced by the dysfunctional gene e.g. poly ADP ribose polymerase (PARP) inhibitors and other novel pathway agents in cancers with DNA repair deficiencies. A better understanding of mechanisms by which germline variants drive various malignancies may lead to improvements in both therapeutic and preventive management options.

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1. Introduction

Genetic counselling and testing should be included in breast cancer (BC) management in both early and metastatic disease, because of the increasing relevance of the information to patients’ clinical care. Therefore, physicians should begin to consider integrating genetic information into treatment decision-making in the management of BC.

Currently, guidelines for the management of mutation carriers with newly diagnosed BC are lacking, particularly in Europe. Consequently, there are uncertainties about the best choices for surgical, radiation, and systemic treatments.

Furthermore, among countries, there are differences in clinical practice and guidelines for the management of individuals with pathogenic variant (PV) in BRCA1/BRCA2 genes and beyond.

In Europe, there is even less availability of genetic consultation services, and many fewer genetic counsellors. Manchanda et al. reported that only 20–30% of qualified patients underwent genetic testing in the United Kingdom (UK), leaving a large number of possible carriers undetected and missing many personalized prevention medicine opportunities [1].

Another difference between the United States (US) and Europe is the use and cost of multigene testing. In Europe, they are less used due to the expenses that have not decreased as rapidly as in the US.

Another factor that may contribute to this difference is the lack of European updated guidelines to help in the management of women with BC and PV in BRCA1/BRCA2 genes and beyond.

While waiting for the updated ESMO guidelines, this article intends to help European providers to interpret and manage genes beyond BRCA and proposes some screening measures according to the reviewed literature.

The most recent American Society guidelines [2,3], as well as the recently original published paper by Robson [4] and the European guidelines, all include indications on surveillance, prophylactic surgery and systemic management of hereditary BC [5,6].

These guidelines address genetic testing for patients with a new diagnosis of BC in the early stage of disease, prior to surgical decision-making. Bilateral mastectomy may be an option for treatment and risk reduction in PV carriers (e.g. BRCA1/2, TP53, PTEN), perhaps to avoid radiation because of increased carcinogenesis as in TP53 and possibly some ATM variants [2,7].

The NCCN and ESO-ESMO guidelines address genetic testing to support the decision-making process for systemic treatment in the metastatic BC patients setting [8].

Germline BRCA variants in metastatic HER2-negative BC patients are predictors of responsiveness to PARP inhibitors (PARPi). For this reason, olaparib or talazoparib should be made available as an alternative to chemotherapy in first to third-line treatment for metastatic disease [9, 10].

Moreover, genetic testing can have important implications when planning systemic therapy in the metastatic setting. Available evidence shows that single-agent carboplatin yields high response rates in BRCA1/2 carriers with advanced BCs. At the same time, an unclear benefit was observed with the addition of a platinum agent to standard anthracycline- and taxane-based chemotherapy in the neoadjuvant setting [11–16].

Recently, a phase II study demonstrated the effectiveness of olaparib in metastatic BC patients with germline PVs in PALB2 and with somatic mutations in BRCA1/2. Tung et al. concluded that this finding significantly increases the population of patients with BC who can benefit from PARPi in addition to those with germinal BRCA PVs. Therefore, this study emphasizes the importance of performing germline and tumour genomic profiling in patients with metastatic BC for therapeutic decisions [17].

In the last few months, the OlympiA study, a randomised phase III adjuvant trial, showed a significantly improved invasive disease-free survival and distant disease-free survival of olaparib versus placebo in high-risk HER2-negative BC with BRCA PVs [18]. Although primary data indicated that a significant overall survival was not met, a significant overall benefit (HR 0.68: p = 0.009) was reported at the virtual Plenary Session of ESMO in March 2022.

Studies are needed to confirm the high pCR rate observed in a small neoadjuvant study with single-agent talazoparib in BRCA1 PV carriers with triple-negative BC (TNBC) [19].

It is further relevant to consider that ongoing trials will elucidate the effectiveness of PARPi in treating advanced BC patients with somatic or germline PV in genes other than BRCA1 or BRCA2 (NCT02401347).

For these reasons, germline genetic testing for women with BC will be increasingly part of clinical practice, which raises the question of whether all women with BC should be tested at diagnosis.

Therefore, increasing the use of gene panels, breast or breast and ovarian panels will lead to the identification of women with pathogenic/likely pathogenic variants in genes other than BRCA1 or BRCA2 (e.g. ATM, CHEK2, BARD1, RAD51D etc).

In this review, we describe four moderate penetrance genes ATM, CHEK2, BARD1 and RAD51D summarizing the current knowledge and the practical utility by discussing management options during clinical practice.

The choice of these genes is based on recent literature published in the New England Journal and data from the Mayo Clinic that will be mentioned below.

Two recent large studies have shown that PV in 8 genes have a significant association with BC risk: ATM, BRCA1 BRAC2, CHEK2, PALB2, RAD51C and RAD51D. PVS in BRCA1 BRAC2 and PALB2 genes are associated with a high risk of BC (odds ratios ranging from 5.0 to 10.6) and PVS in ATM and CHEK2 with a moderate risk of BC (odds ratios ranging from 2.1 to 2.5) [20,21]; however, ATM and CHEK2 mutations are more common in the general population [22].

Other considerations supporting the reason why these four genes, about which there are fewer data and guidelines, should be examined in addition to the high penetrance genes are: expanding the use of gene-panel testing in Europe, clinical utility (few European management options available about screening, risk reduction surgery, chemoprevention) and the possibility of participation in clinical trials.

2. ATM

The Ataxia-telangiectasia-mutated (ATM) gene encodes a kinase involved in DNA double-strand break repair pathways (Fig. 1).

ATM is associated with a rare autosomal recessive neurodegenerative disease, Ataxia-telangiectasia (A-T), which is caused by the inheritance of bi-allelic deleterious variants in the ATM gene and occurs in approximately 1 per 880 000 live births. While the recessive and severe form of the disease is rare, the carrier frequency is not. Heterozygosity for a pathogenic ATM variant is described in 1–2% of the US Caucasian adult population [23].

Heterozygosity for loss of function variant in ATM has been associated with moderately increased BC risk and excess risk of prostate (PCA), pancreatic (PanC) and ovarian cancers (OC) [24–29]. ATM carriers have also a potential increased risk of gastric and colorectal cancers (CRC) and melanoma, though these are not yet well established [30–32].

A meta-analysis, as well as other studies, reported that truncating and missense variants confer an estimated BC relative risk (RR) of 2.8% (90% CI, 2.2 to 3.7), and an absolute BC risk by 80 years of age of 27% [33,34].

Recently, results of a US-based study and an international study provided data that ATM truncating and missense variants are associated with a higher risk of ER-positive than ER-negative disease (OR = 2.33, 95%CI 1.87 to 2.91 vs 1.01, 95% CI 0.64 to 1.59, P = <0.001) [20,21].

Furthermore, ATM PVS, though not significantly, are more common in women with BC and FH of BC (P = 0.022) and yet less common in women with bilateral BC [35]. Association between PVS carriers in ATM and contralateral BC (CBC) has also been investigated [36]. Available

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2.1. Treatment implications of BC patients with pathogenic ATM gene variants

The relationship between radiation exposure (RT) and the risk of BC is complex in patients with BC with germline ATM PVs. Individuals with ataxia-telangiectasia (ie, biallelic ATM mutation) have an increased sensitivity to ionizing radiation. Otherwise, the data available do not show contraindications to radiation therapy for patients with heterozygous ATM PV. In this context, the Women’s Environmental Cancer and Radiation Epidemiology (WECARE) study analysed the interaction between radiation exposure and genetic predisposition in BC, in particular radiation-induced CBC. Women who carry a common variant in ATM may have a protective effect in reducing the risk of developing CBC. On the contrary, women who carry rare ATM missense variants classified as likely deleterious, are at increased risk for CBC in a dose-dependent manner compared with ATM PV who did not receive RT \[5,37\]. Case reports of radiation toxicity in heterozygous ATM PV carriers are described, otherwise, the correlation is not clear \[39\].

Representative cancers correlated with truncating and missense ATM genes are shown in Table 2.

Management proposals for individuals with ATM PVs are summarized in Table 4.

2.1. Treatment implications of BC patients with pathogenic ATM gene variants

Risk reduction surgery

Available evidence and recommendations

There is insufficient evidence to recommend prophylactic mastectomy in ATM PV carriers. The option of long-term breast surveillance versus a risk reduction surgery might be subject of further debate in the future. Bilateral prophylactic mastectomy might be considered in ATM c.7271T > G PV carriers and if positive FH of BC (first-degree or second-degree relatives).

Considerations for ionizing radiation therapy

Available evidence and recommendations

WECARE data and ASCO ASTRO SSO guidelines support that ionizing radiation therapy for BC treatment in heterozygous ATM PV carriers should not be avoided.

Pharmaceutical agents

Available evidence and recommendations

The usefulness of PARPi for BC patients with ATM PV is under investigation in the metastatic setting. No activity among ATM carriers in the TBCRC048 phase II study was observed, though there were few ATM carriers in the trial cohort \[17\]. Olaparib was approved for patients with metastatic prostate cancer who are carriers of mutations in DNA repair genes including ATM-based largely on the de Bono et al. report though activity specifically in ATM carriers was not observed in that trial \[40\].

2.2. Management of individuals with pathogenic ATM gene variants

Screening

Available evidence and recommendations

Evidence for other cancers risk

Table 1

Adapted from The National Comprehensive Cancer Network guidelines (NCCN) v.2.2022.

| Gene     | Risk                                                                 | Management                        |
|----------|----------------------------------------------------------------------|-----------------------------------|
| ATM      | Increased risk of female BC (with predisposition to ER+)              | RRM: evidence insufficient, manage based on family history |
|          | Potential increased risk of OC                                       | Screening: annual mammogram (tomosynthesis) and breast MRI with contrast starting at age 40+ |
|          | Risk for pancreatic cancer                                           | RRSO: evidence insufficient, manage based on family history |
|          | Insufficient evidence for PCA risk                                    | Pancreatic screening\(^a\) if family history |
| CHEK2    | Increased risk of female BC (with predisposition to ER+)              | RRM: evidence insufficient, manage based on family history |
|          | No increased risk of OC                                              | Screening: annual mammogram (tomosynthesis) and breast MRI with contrast starting at age 40+ |
|          | Risk CRC                                                             | Colonoangiopancreatography and/or endoscopic ultrasound |
| BARD1    | Limited emerging evidence to suggest increased risk of female BC (with predisposition to triple negative) | RRM: evidence insufficient, manage based on family history |
|          | Unknown or Insufficient evidence for OC or other cancers risk         | Screening: annual mammogram (tomosynthesis) and breast MRI with contrast starting at age 40+ |
| RAD51D   | Potential increased risk of female BC (with predisposition to triple negative) | Insufficient evidence for risk management |
|          | Increased risk of OC                                                 | RRSO: consider at 45-50y |
|          | Unknown or Insufficient evidence for other cancers risk               | |

\(^a\) May be modified based on family history (beginning screening 5–10 years earlier than the youngest diagnosis in the family).

\(^b\) Annually contrast-enhanced MRI/magnetic resonance chol-angiopancreatography and/or endoscopic ultrasound.

DNA Damage

DNA Synthesis and Repair

Fig. 1. Intracellular pathways of cell cycle arrest, apoptosis, DNA repair and mitosis in breast cancer.
### Table 2
Representative cancers associated with ATM and CHEK2 variants.

| Cancer Type       | Life-time risk in general population | LTR in ATM carriers | ATM truncating variants (and missense variants) | Case series/case control studies | LTR in CHEK2 carriers | LTR CHEK2 carriers | Case series/case control studies |
|-------------------|--------------------------------------|---------------------|-----------------------------------------------|----------------------------------|------------------------|---------------------|----------------------------------|
| BC                | 12.9%                                | 17.52%              | x (C.7271T > G specific consideration)        | 13087 BCs vs 5952 controls [35]  | 23.48%                 | 31.8%               | 18.3%               | 13087 BCs vs 5952 controls [35]  |
| Second primary    |                                      |                     |                                               |                                  |                        |                     |                                  |                                  |
| Pancreatic cancer |                                      |                     |                                               |                                  |                        |                     |                                  |                                  |
| Prostate Cancer   |                                      |                     |                                               |                                  |                        |                     |                                  |                                  |
| CRC               |                                      |                     |                                               |                                  |                        |                     |                                  |                                  |
| Renal Cell Carcinoma (RCC) | 1.4%                           | Data not correlated |                                               |                                  |                        |                     |                                  |                                  |
| Thyroid cancer (papillary) (TC) | 1%                              | Data not correlated |                                               |                                  |                        |                     |                                  |                                  |
| Male breast cancer (MBC) | 0.1%                             | Data not correlated |                                               |                                  |                        |                     |                                  |                                  |
| Testicular germ cell tumours (TGCT) | 0.4%                       | Data not correlated |                                               |                                  |                        |                     |                                  |                                  |
| Gastric cancer (GC) | 0.8%                             | Not well established |                                               |                                  |                        |                     |                                  |                                  |
| Melanoma          |                                      |                     |                                               |                                  |                        |                     |                                  |                                  |

*Preliminary data.

### Table 3
Representative cancers associated with truncating BARD1 and RAD51D variants.

| Cancer Type       | Life-time risk in general population | Life-time risk in BARD1 Carriers | BARD1 truncating variants | Case series/case control studies with BARD1 | Life-time risk in RAD51D Carriers | Case series/case control studies RAD51D |
|-------------------|--------------------------------------|----------------------------------|---------------------------|---------------------------------------------|----------------------------------|----------------------------------------|
| BC                | 12.4%                                | >20%                             | X                         | 10901 TNBC [69] 15-40%                      | x 10-15%                         | 911 cases (BC/OC families) vs 1060 controls [72] |
| OC                | 1.2%                                 | Not established                  | Not established           | Not established                             | 911 cases (BC/OC families) vs 1060 controls [72] |
| Neuroblastoma     | Not well defined in childhood population | Not established                  | X*                        | –397 high risk neuroblastoma vs 2043 controls [70] | –                                | –                                      |

*Preliminary data.

**Breast Cancer:** The new version of the NCCN and ESMO 2016 guidelines recommend breast screening with clinical breast examinations and 6-monthly radiology surveillance alternating MRI and mammography starting at the age of 40 or 5–10 years prior in the youngest BC diagnosis in the family (Table 1) [2,6].

Supporting evidence of the use of BC screening MRI for women with ATM PVs can be found in a recently conducted comparative modelling analysis. The authors reported that combined annual MRI and mammography starting at age 40 reduce BC mortality above 50% in these women [41].

**Ovarian Cancer:** Ovarian screening with transvaginal ultrasound combined with serum CA 125 may be considered on the clinician’s discretion, despite uncertain benefits.

**Pancreatic Cancer:** the NCCN guidelines, supported by the Canto study, suggest pancreatic screening, beginning at age 50 or 10 years before onset in the family, by alternating annually contrast-enhanced magnetic resonance cholangiopancreatography (MRCP) and endoscopic ultrasound (EUS) if positive FH of PanC (first-degree or second-degree relatives) [42]. In the ongoing US-study CAPSS, in addition to annual imaging surveillance as mentioned above, investigators try to identify early pancreatic cancer or precancerous lesions in high-risk individuals by evaluating pancreatic fluid mutations and circulating pancreatic epithelial cells (NCT02000089).

**Colon Cancer:** Colonoscopy screening to be repeated every 5 years beginning at the age of 40 may be planned if positive FH, as per NCCN colorectal cancer screening guidelines [43].

**Prostate Cancer:** The 2019 Philadelphia Prostate Cancer Consensus Conference, recognizing a potential association between ATM and PCA risk, may consider prostate screening options for ATM PVs carriers if positive FH or participation in screening trials (e.g. NCT03805919) [44].

**Risk reduction surgery for other cancers**

**Available evidence and recommendations**

**Ovarian cancer:** There is insufficient evidence to recommend...
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prophylactic ovarian surgery.

At present, since OC screening is of uncertain benefit in all settings, one might consider bilateral salpingo-oophorectomy at the age of 45–50 if positive FH (first-degree or second-degree relatives).

Risk reduction salpingectomy is not standard of care but may delay oophorectomy; one might discuss participation in future clinical trials.

Risk reduction agents
Available evidence and recommendations

The utility of tamoxifen as a BC risk reduction agent in ATM PV carriers is unknown. A prospective clinical trial for ATM PV carriers may be considered in the future.

Use of the oral contraceptive pill may be planned among those who want contraception during their reproductive years considering that a significant risk-reducing effect on the development of OC by 40%–60% has been demonstrated [45,46]. However, there are conflicting data about increasing BC risk in BRCA1/2 PV carriers associated with the use of the oral contraceptive pill [45].

Reproductive implications
Available evidence and recommendations

Individuals of reproductive age should be advised about options related to prenatal diagnosis and preimplantation genetic diagnosis with respect to the risk of autosomal recessive condition in the offspring.

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Table 4
Management proposals for some moderate penetrance BC genes.

|                 | ATM                  | CHEK2                | BARD1                | RAD51D               |
|-----------------|----------------------|----------------------|----------------------|----------------------|
| Breast          | No data on bilateral mastectomy. RRM might be considered in c.7271T>G ATM PV carriers and if FH | No data on bilateral mastectomy. RRM may be considered in biallelic CHEK2*1100delC or if FH | No data on bilateral mastectomy. It may be considered based on FH | No data on bilateral mastectomy. It may be considered based on FH |
| Clinical breast examination, breast MRI, mammogram at age 40* alternate 6-monthly | Clinical breast examination, breast MRI, mammogram at age 40* alternate 6-monthly | Clinical breast examination, breast MRI, mammogram at age 40* alternate 6-monthly | Clinical breast examination, breast MRI, mammogram at age 40* alternate 6-monthly | Insufficient data managed based on FH |
| Pancreas        | Consider actionable screening for pancreatic cancer risk if FH [42] | No data | No data | No data |
| Colon           | Colonoscopy at the age of 40 if FH | Colonoscopy at the age of 40 every 5 years | No data | No data |
| Ovary           | Ovarian screening on clinician’s discretion, despite uncertain benefit | RRSO if FH | No data | No data |
| Thyroid         | No data | May consider thyroid ultrasound every 2-5 years; 10 years earlier than the youngest first degree relative at diagnosis if FH | No data | No data |
| Kidney          | No data | May consider kidney ultrasound every 2-5 years; 10 years earlier than the youngest first degree relative at diagnosis if FH | No data | No data |
| Prostate        | Consider prostate screening [44]* | Consider prostate screening [44]* | No data | No data |
| Brain           | No data | No data | No data | No data |
| Risk reduction agents | Utility of tamoxifen is unknown. A prospective clinical trial may be considered. Use of the oral contraceptive pill may be planned as contraception to reduce OC risk [45] | Utility of tamoxifen is unknown. A prospective clinical trial may be considered. | - | Use of the oral contraceptive pill may be planned as contraception to reduce OC risk [45] |
| Reproductive implication | To be discussed | - | - | - |
| Life style modifications | Recommended | Recommended | Recommended | Recommended |
| Cascade | Recommended | Recommended | Recommended | Recommended |

In green, our proposal

FH: family history. RRM: risk reduction mastectomy. RRSO: risk reduction salpingo-oophorectomy

Guidelines based on the National Comprehensive Cancer Network (NCCN); European recommendations (e.g. ESMO, NICE) needs to be updated.

* = 5-10 years before diagnosis of earliest BC or CRC

Preliminary data

Strong data
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3. CHEK2

CHEK2 (Checkpoint Kinase 2) is a moderate penetrance BC risk gene. CHEK2 is a tumour suppressor gene encoder for a protein involved in DNA repair, cell cycle arrest or apoptosis in response to DNA damage (Fig. 1).

Heterozygosity for CHEK2 PVs is reported in ~1% of European Caucasian descendants and various aberrations in the CHEK2 gene have been reported: 1100delC (the most studied), I157T, R117G, I160 M, and G167R. In the Dutch and Finnish populations, CHEK2 1100delC is the most common variant whereas p.S428F in Ashkenazi Jews [35]; is less frequent among Asian women [21].

The most common CHEK2 truncating variants (i.e. 1100delC and del5395) confer a greater than twofold increased BC risk [47].

The missense variant I157T is found mainly in Finland and Poland and is associated with a 1.4-fold risk of BC [48].

A Dutch study identified homozygosity for the CHEK2 1100delC variant in 8 women among a cohort of 2554 Dutch non-

Chinese patients. The biallelic variant in 8 women among a cohort of 2554 Dutch non-

European case-control analysis of male patients with and without testicular germ cell tumours (TGCT) provided evidence for CHEK2 as a novel moderate penetrance gene correlated with increased susceptibility to TGCT. Inherited CHEK2 PVs were found in patients with TGCT at a higher rate than expected [63]. Representative cancers correlated with truncating and missense CHEK2 genes are shown in Table 2.

Estimated BC and CRC risk as well as management proposals for individuals with CHEK2 PVs are described in Table 4.

3.1. Treatment implications of BC patients with pathogenic CHEK2 gene variants

Risk reduction surgery
Available evidence and recommendations

There is insufficient evidence to ponder specific recommendations for prophylactic mastectomy in CHEK2 PV carriers. Considering the long-term breast surveillance starting at a young age, bilateral prophylactic mastectomy may be taken into account in biallelic CHEK2 1100delC PV carriers and if positive BC FH (first-degree or second-degree relatives), particularly in CHEK2 truncating PVs.

Considerations for ionizing radiation therapy
Available evidence and recommendations

No data about the association with ionizing radiation therapy for BC treatment and increased risk of second tumours including CBC.

Pharmacological agents
Available evidence and recommendations

The usefulness of PARPi for BC patients with CHEK2 PVs is under investigation in the metastatic setting: no activity was shown in the phase II study [17], differently in the metastatic PCA trial [40].

3.2. Management of individuals with pathogenic CHEK2 gene variants

Screening
Available evidence and recommendations

Breast Cancer: The American and European guidelines recommend breast clinical examinations and annual breast MRI and mammogram starting at the age of 40 or 5–10 years in the youngest BC diagnosis in the family (Table 1).

Moreover, Lowry et al. performed a comparative modelling analysis supporting the use of MRI as a screening for BC in women carrying the mutation in CHEK2. 50% BC mortality reduction was detected in these women with MRI and mammography combined annually from 40 years of age [41].

Colon Cancer: the NCCN recommends colonoscopy screening regularly every 5 years, beginning at the age of 40 or 10 years earlier.
than the youngest first degree relative at CRC diagnosis.

**Prostate Cancer:** Giri et al. identified a potential correlation between CHEK2 and PCA risk. CHEK2 PVs carriers should be encouraged to participate in clinical trials evaluating the efficacy of PCA screening [44].

**Thyroid and kidneys cancers**

There is thus far insufficient evidence of specific recommendations for thyroid and kidney cancer screening.

The following approach may be reasonable given available data and extrapolating from the management of other cancer predisposition genes:

- thyroid and kidney ultrasound may be considered every 2–5 years or 10 years earlier than the youngest first degree relative at diagnosis with thyroid and kidney cancer if positive FH (first-degree or second-degree relatives).

**Risk reduction surgery for other cancers**

Available evidence and recommendations

No data.

**Risk reduction agents**

Available evidence and recommendations

Efficacy of tamoxifen as a chemoprevention agent in CHEK2 PV carriers has not been investigated so far. A prospective clinical trial for CHEK2 PV carriers might be contemplated in the future.

**Reproductive implications**

Available evidence and recommendations

No data are available regarding reproductive implications. Consideration needs to be made regarding women with biallelic CHEK2 PVs that have high BC risk, are more likely diagnosed at or before age 50 and have multiple primary BC compared to monoallelic findings. Furthermore, CHEK2 PV is distinguished by not having a defined recessive phenotype [64].

**Risk to family members**

Recommending genetic testing for family members of an individual who carries a CHEK2 PV to implement personalized screening and early intervention if necessary.

4. **BARD1**

*BARD1* (BRCA1 associated RING domain 1) shares structural and functional similarities with the *BRCA1* protein [65]. The RING finger mediated *BARD1* and *BRCA1* heterodimer appear to be essential for various tumour suppressor functions of *BRCA1*, and both proteins are involved in DNA repair and apoptosis functions. *BARD1* is a low-moderate penetrance gene (Fig. 1).

The occurrence of *BARD1* germline PVs in BC families was investigated by different groups [66].

In a large cohort study, Couch and colleagues reported 9 patients with TNBC and germline *BARD1* truncating variants, unselected for FH [67].

Subsequently, a large study of 65 057 BC patients receiving multigene panel testing showed that PVs in *BARD1* are associated with moderate risk for BC. The authors argued that variants in this gene are particularly rare (<1 in 500 BC patients); therefore, previous studies were unable to adequately assess the association between *BARD1* and BC [68].

Shimelis and colleagues tested 21 and 17 genes in two cohorts of 8573 and 2148 TNBC patients, respectively, and showed that germline PVs in *BARD1* were significantly associated with a high risk of TNBC (OR = 5.92, 95%CI = 3.36 to 10.27, P = 2.2 × 10^-9) and a greater than 20% lifetime risk for BC overall [69].

Results of two population-based analyses demonstrated that *BARD1* was associated with an increased risk of ER-negative BC (P < 0.05), in particular of TNBC (P = 0.044) [20,21].

Two germline *BARD1* truncating variants were identified among 222 patients with aggressive neuroblastoma [70]. This finding raises the question of the role of *BARD1* variants in high-risk neuroblastoma. The possible role of *BARD1* in OC has been studied; however, currently, there is insufficient evidence for increased OC risk. Data from the Mayo Clinic indicate that *BARD1* PVs may confer an increased risk for BC compared to the general population; therefore, particular attention regarding personalized breast surveillance is needed.

*BARD1*-associated cancers are shown in Table 3.

Estimated BC risk and management proposals for individuals with *BARD1* PVs are described in Table 4.

4.1. **Treatment implication of BC patients with pathogenic BARD1 gene variants**

**Risk reduction surgery**

Available evidence and recommendations

There is insufficient evidence to consider prophylactic mastectomy in *BARD1* truncated PV carriers. Risk reduction surgery procedures may be taken into account if positive BC FH (first-degree or second-degree relatives).

**Considerations for ionizing radiation therapy**

Available evidence and recommendations

No data about the association with ionizing radiation therapy for BC treatment and increased risk of second tumours including CBC.

**Pharmacological agents**

Available evidence and recommendations

No data available regarding PARPi in *BARD1* PV carriers with metastatic BC.

4.2. **Management of individuals with pathogenic BARD1 gene variants**

**Screening**

Available evidence and recommendations

Breast cancer: The US guidelines recommend breast screening with clinical breast examination and annual MRI and mammography starting at the age of 40 or 5–10 years before the earliest known BC diagnosis in the family (Table 1). *Benign brain tumour:* There is insufficient evidence to consider screening.

**Risk reduction surgery for other cancers**

Available evidence and recommendations

No data.

**Risk reduction agents**

Available evidence and recommendations

No data available regarding chemoprevention in *BARD1* PV carriers.

**Reproductive implications**

Available evidence and recommendations

No data are available regarding reproductive implications. *BARD1* is not a classic FA gene.

**Risk to family members**

Recommending genetic testing for family members of an individual who carries a *BARD1* PV and proposing early preventive measures if indicated.

5. **RAD51D**

Another example of a DNA repair gene in the homologous recombination pathway is *RAD51D* (Fig. 1). It plays an important role in the maintenance of genomic stability and may be associated with tumorigenesis [71].

Several studies have demonstrated a correlation between *RAD51D* PVs and an increased OC incidence [72].

A Finnish study identified one recurrent PV in *RAD51D* (c.576+1G > A) in BC and OC patients [73].

In some studies, pathogenic *RAD51D* variants were detected in BC patients by gene panel testing [68]. Shimelis et al. introduced a new correlation between TNBC and *RAD51D*. The authors identified five TNBC predisposition genes, including *RAD51D*, with a greater than 20% estimated lifetime risk for BC overall [69].
In a Chinese study, RAD51D deleterious germline variants were found in 29 of 7657 unselected BRCA1/BRCA2 negative BC patients, 18 carried the c.270_271dupTA variant. The authors reported that RAD51D PV carriers in the TNBC cohort were described with positive axillary lymph nodes and high-grade tumours. Likewise, they found that RAD51D PV carriers had an aggressive phenotype and an early onset of BC with a mean age similar to that of BRCA PV carrier patients [74]. Most likely due to the rarity of RAD51D PVs studied among BC and OC families, the relationship between pathogenic RAD51D germline variants and BC risk has been recently validated. Two large studies described that RAD51D had evidence of higher association with ER-negative BC and TNBC than with ER-positive BC (P < 0.05) [20,21].

**Risk to family members**

After identification of a RAD51D PV in an individual, it is strongly recommended that all family members should consider a genetic investigation.

6. Moderate gene mutations in metastatic breast cancer: the challenge

BRCA status indicates responsiveness to platinum-based chemotherapy and to PARPi in the metastatic BC and OC disease setting. Recently, the FDA approved PARPi as a maintenance treatment for patients with advanced PanCa and in mPCA.

Limited data are available on the potential interaction between targeted therapies and chemotherapy effectiveness and mutational status of risk genes other than BRCA1/2. High response rates have been reported with PARPi in castration-resistant mPCA individuals, harbouring alterations in DNA-damage response genes including not only BRCA1 and BRCA2, but also ATM, CHK2, FANCA and PALB2 [75].

Recently, Tung et al. reported that PARPi produced high response rates in BC patients who carry germline PALB2 PVs and somatic BRCA1 and BRCA2 PVs [17].

There are several ongoing phase II studies with PARPi in metastatic BC individuals with mutations in other genes within the BRCA1/2 pathway.

7. Conclusions

Identification and management of individuals and families with moderate risk gene variants have rapidly evolved over the past decade and offer the opportunity to prevent cancer-related morbidity and mortality.

Further studies are required to better understand and quantify cancer risk associated with environmental and clinical risk factors and prognosis. An efficient approach to pre-test genetic counselling and patient education is needed. Studies such as The Prospective Registry of MultiPlex Testing (PROMPT) may help researchers to better define moderate penetrance cancer-susceptibility genes.

The interpretation of genetic testing results requires careful attention and PVs should not all be treated in the same way. Particular attention should be paid to the type and location of different variants and whether they are monoallelic or biallelic. Biallelic variants in some of these genes and PVs should not all be treated in the same way. Particular attention should be paid to the type and location of different variants and whether they are monoallelic or biallelic. Biallelic variants in some of these genes and PVs should not all be treated in the same way. Particular attention should be paid to the type and location of different variants and whether they are monoallelic or biallelic. Biallelic variants in some of these genes and PVs should not all be treated in the same way.

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