Title: GENE VARIANTS ASSOCIATED WITH BREAST CANCER IN INDIVIDUALS AT HIGH BREAST CANCER RISK

Description/Background

Breast Cancer and Genetics
In 2016, researchers estimated breast cancer would be diagnosed in 252710 women and 40610 would die from the disease;¹ a woman's lifetime risk is 12.4%.² Breast cancers can be classified as sporadic, familial, or hereditary.³ Most breast cancers, however, are sporadic (70% to 75%), occurring in women without a family history of the disease. Familial cancers (15% to 25%) aggregate within families but lack clearly discernable patterns of inheritance and are likely polygenic. Hereditary cancers have discernable inheritance patterns, often occur at younger ages, may be bilateral, and comprise between 5% and 10% of breast cancers. Pathogenic BRCA1 and BRCA2 variants appear responsible for 20% to 25% of hereditary breast cancers,⁴ while small proportions are attributed to pathogenic variants in other highly penetrant genes (e.g., TP53, CDH1, PTEN, STK11).

Penetrance of Pathogenic Variants
Penetrance is the risk conferred by a pathogenic variant or the proportion of individuals with the variant expected to develop cancer. Variant penetrance is considered high, moderate, or low according to lifetime risk: high (>50%), moderate (20% to 50%), and low (<20%) (corresponding relative risks of approximately ≥5, 1.5 to 5, and <1.5).⁵ Variants in only a few breast cancer-susceptibility genes (BRCA1 and BRCA2 [hereditary breast/ovarian cancer syndrome], TP53 [Li-Fraumeni syndrome], PTEN [Cowden syndrome], CDH1 [hereditary diffuse gastric cancer], STK11 [Peutz-Jeghers syndrome]) are considered highly penetrant. For example, a woman with a BRCA1 or BRCA2 variant has roughly a 75% lifetime risk of developing breast cancer and a relative risk of 11 to 12 compared with the general population.⁶ Penetrance can be modified by environmental factors and by family history, which is a particularly important modifier for low and moderate penetrance genes. Moreover, specific pathogenic variants within a gene may confer somewhat different risks.
Determining Variant Pathogenicity
Determining the pathogenicity of variants in a more commonly detected cancer susceptibility gene (e.g., founder sequence mutations) is generally straightforward because associations are repeatedly observed. For uncommonly identified variants, such as those found in a few individuals or families, defining pathogenicity can be more difficult. For example, predicting the pathogenicity of previously unidentified variants typically requires in silico (computational) analysis predicting protein structure/function, evolutionary conservation, and splice site prediction. The approach to defining pathogenicity is clearly outlined in standards and reporting guidelines.\textsuperscript{7} Still, distinctions between a variant of uncertain significance and a pathogenic one from different laboratories may not always be identical.\textsuperscript{8}

Genes Associated With a Moderate-to-High Penetrance of Breast Cancer

**PALB2 Gene**
The *PALB2* gene (partner and localizer of *BRCA2*) encodes for a protein first described in 2006.\textsuperscript{9} The gene is located at 16p12.2[a] and has 13 exons. *PALB2* protein assists *BRCA2* in DNA repair and tumor suppression. Heterozygous pathogenic *PALB2* variants increase the risk of developing breast and pancreatic cancers; homozygous variants are found in Fanconi anemia.[b] Most pathogenic *PALB2* variants are truncating frameshift or stop codons, and are found throughout the gene. Pathogenic *PALB2* variants are uncommon in unselected populations and prevalence varies by ethnicity and family history. For example, Antoniou et al (2014) assumed a prevalence of 8 per 10000 in the general population when modeling breast cancer risks.\textsuperscript{10} Variants are more prevalent in ethnic populations where founder mutations have persisted (e.g., Finns, French Canadians, Poles), while infrequently found in others (e.g., in Ashkenazi Jews\textsuperscript{11,12}). In women with a family history of breast cancer, the prevalence of pathogenic *PALB2* variants ranges between 0.9% and 3.9%, or substantially higher than in an unselected general population. Depending on population prevalence, *PALB2* may be responsible for as much as 2.4% of hereditary breast cancers;\textsuperscript{10} and in populations with founder mutations cause 0.5% to 1% of all breast cancers.\textsuperscript{13}

**CHEK2 Gene**
The *CHEK2* (checkpoint kinase 2) gene is activated in response to DNA double-strand breakage and plays a role in cell-cycle control, DNA repair, and apoptosis.

In 2002, a single recurrent truncating variant in the *CHEK2* gene (c.1100delC) was first reported as a cause of breast cancer, and studies have since confirmed this. The incidence of *CHEK2* variants varies widely among populations. It is most prevalent in Eastern and Northern Europe, where the population frequency of the c.1100delC allele ranges from 0.5% to 1.4%; the allele is less frequent in North America and virtually absent in Spain and India.

Although most data for truncating *CHEK2* variants are limited to the c.1100delC allele, 3 other founder mutations of *CHEK2* (IVS2+1G>A, del5395, I157T) have been associated with breast cancer in Eastern Europe. Both IVS2+1G>A and del5395 are protein-truncating variants, and I157T is a missense variant. The truncating variants are associated with breast cancer in the Slavic populations of Poland, Belarus, Russia, and the Czech Republic. The I157T variant has a wider geographic distribution and has been reported to be associated with breast cancer in Poland, Finland, Germany, and Belarus.\textsuperscript{14}
**ATM Gene**

*ATM* (ataxia-telangiectasia mutated), located on chromosome 11q22.3, is associated with the autosomal recessive condition ataxia-telangiectasia syndrome. This condition is characterized by progressive cerebellar ataxia with onset between the ages of one and four years, telangiectasias of the conjunctivae, oculomotor apraxia, immune defects, and cancer predisposition. Female *ATM* heterozygotes carriers have a risk of breast cancer about twice as high as that of the general population; however, they do not appear to have an elevated ovarian cancer risk.

**Identifying Women at Risk of an Inherited Susceptibility to Breast Cancer**

Breast cancer risk can be affected by genetic and nongenetic factors. The risk is increased in women experiencing an earlier age at menarche, nulliparity, late age of first pregnancy, fewer births, late menopause, proliferative breast disease, menopausal hormone therapy, alcohol, obesity, inactivity, and radiation. A family history of breast cancer confers between a 2- and 4-fold increased risk varying by several factors: the number and closeness of affected relatives, age at which cancers developed, whether breast cancers were bilateral and if other cancers occurred (e.g., ovarian). For a woman without breast cancer, the probability of detecting a pathogenic variant can be estimated from a detailed multigenerational pedigree (e.g., Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), screening tools (e.g., BRCAPRO, Ontario Family History Assessment Tool, Manchester Scoring System, Referral Screening Tool, Pedigree Assessment Tool, Family History Screen), or by referring to guidelines that define specific family history criteria. For women with breast cancer, family history also affects the likelihood of carrying a pathogenic variant.

**Regulatory Status**

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. *PALB2, CHEK2,* and *ATM* testing are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories offering to test and voluntarily listing is available through the National Center for Biotechnology Genetic Testing Registry. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

Customized next-generation sequencing panels provide simultaneous analysis of multiple cancer predisposition genes, and typically include both moderate- and high-penetrant genes.

**Medical Policy Statement**

The safety and effectiveness of testing for *PALB2* variants for breast cancer risk assessment in adults have been established. It may be considered a useful diagnostic option when indicated.
Inclusionary and Exclusionary Guidelines (Clinically based guidelines that may support individual consideration and pre-authorization decisions)

Inclusions:
Testing for PALB2 variants for breast cancer risk assessment in adults who
• meet criteria for genetic risk evaluation (BRCA testing);

Exclusions:
• Testing for PALB2 sequence variants in individuals who do not meet the criteria outlined above is considered experimental/investigational.
• Testing for CHEK2 and ATM variants in the assessment of breast cancer risk is considered experimental/investigational.

Criteria for Genetic Risk Evaluation
The National Comprehensive Cancer Network (NCCN) provides criteria for genetic risk evaluation for individuals with no history or breast cancer and for those with a breast cancer. Updated versions of the criteria are available on the NCCN website, https://www.nccn.org/professionals/physician_gls/default.aspx.

CPT/HCPCS Level II Codes (Note: The inclusion of a code in this list is not a guarantee of coverage. Please refer to the medical policy statement to determine the status of a given procedure.)

Established codes:
81307 81308 81406

Other codes (investigational, not medically necessary, etc.):
81408 81479

Note: Individual policy criteria determine the coverage status of the CPT/HCPCS code(s) on this policy. Codes listed in this policy may have different coverage positions (such as established or experimental/investigational) in other medical policies.

Rationale
Evidence reviews assess whether a medical test is clinically useful. A useful test provides information to make a clinical management decision that improves the net health outcome. That is, the balance of benefits and harms is better when the test is used to manage the condition than when another test or no test is used to manage the condition.

The first step in assessing a medical test is to formulate the clinical context and purpose of the test. The test must be technically reliable, clinically valid, and clinically useful for that purpose. Technical reliability is outside the scope of these reviews, and credible information on technical reliability is available from other sources.
PALB2 AND BREAST CANCER RISK ASSESSMENT

Clinical Context and Test Purpose
The purpose of testing for PALB2 variants in women at high-risk of hereditary breast cancer/ovarian cancer (HBOC) is to evaluate whether an abnormal variant is present and, if so, to determine whether the variant conveys a sufficiently high-risk such that changes in surveillance and/or treatment that are likely to decrease the risk of mortality from breast cancer are warranted.

Potential benefit derives from interventions (screening, chemoprevention, risk-reducing surgery) that can prevent first breast cancer, contralateral breast cancer, or cancer in a different organ caused by the same variant. Whether benefit outweighs harms depends on the risk of developing breast cancer (first cancer or a contralateral one), the effectiveness and the harms of interventions.

Assessing the net health outcome requires:
• that a test accurately identifies variants and pathogenicity can be determined;
• that a variant alters (increasing or decreasing) a woman's risk of developing breast cancer (including contralateral disease in women already diagnosed) sufficient to change decision making, and
• of a magnitude that management changes informed by testing can lead to improved health outcomes.

The question addressed in this evidence review is: Does genetic testing for PALB2 variants improve the net health outcome in women at high-risk of HBOC?

The following PICOs were used to select literature to inform this review.

Patients
Genetic testing can be considered for women at increased risk of developing hereditary breast cancer based on their family history or in women with breast cancer whose family history or cancer characteristics (e.g., triple-negative disease, young age) increase the likelihood that the breast cancer is hereditary. Testing may also be considered for women from families with known variants.

The relevant population of interest for this review are patients who are undergoing assessment for HBOC syndrome.

Interventions
The intervention of interest is PALB2 variant testing.

Comparators
The alternative would be to manage women at high-risk of HBOC with no PALB2 genetic testing.

Outcomes
The outcomes of interest are overall survival (OS), disease-specific (breast and ovarian cancer) survival, and test validity.
Study Selection Criteria
For the evaluation of the clinical validity of the tests, studies that meet the following eligibility criteria were considered:

- Included a suitable reference standard
- Patient/sample clinical characteristics were described
- Patient/sample selection criteria were described
- Included a validation cohort separate from development cohort.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Systematic Reviews
Suszyńska et al (2019) reported a systematic review of variants identified in panels of breast and ovarian cancer-related genes. Results were reported for PALB2, CHEK2, and ATM. CHEK2 and ATM results will be discussed in the following sections. The systematic review included studies published through July 2017 reporting on genetic test results of breast and ovarian cancer patients who were referred for evaluation by a multi-gene panel. Given that the Suszyńska et al (2019) report included only studies reporting on test results from a panel, it does not substantially overlap with the studies described in the following section including other PALB2 association studies. The studies of panel results were used to calculate mutation frequencies by the gene. As a control, population mutation frequencies were extracted from the Genome Aggregation Database. Forty-three studies included panels in breast cancer patients. In the breast cancer studies, 95853 patients were included in the analysis of PALB2. PALB2 variants were identified in 0.9% of breast cancer patients. The meta-analytic estimate odds ratio (OR) of the association between PALB2 variants and risk of breast cancer was OR = 4.8 (95 CI, 4.1 to 5.6).

Observational Studies
A number of studies (see Tables 1 and 2) reporting relative risks (RR) or ORs for the association between PALB2 and breast cancer were identified (two reported penetrance estimates). Study designs included family segregation, kin-cohort, family-based case-control, and population-based or multicenter case control. The two multinational studies included individuals from up to five of the single-country studies. The number of pathogenic variants identified varied from 1 (founder mutations examined) to 48 (see Table 1). Studies conducted from single-country samples are described first followed by the two multinational collaborative efforts.

Single-Country Samples
Lu et al (2019) included analysis of 11416 patients with breast cancer and/or ovarian cancer who were referred for genetic testing from 1200 U.S. hospitals and clinics and of 3988 controls referred for genetic testing for noncancer conditions between 2014 and 2015. Whole-exome
sequencing was used for sequencing and suspected pathogenic variants in the breast or ovarian cancer-associated genes were confirmed by Sanger sequencing.

Kurian et al (2017) reported the association between pathogenic variants and breast or ovarian cancer using a commercial laboratory database of 95561 women tested clinically for hereditary cancer risk using a multi-gene panel that included PALB2, CHEK2 and ATM. Although the country is not stated, the patients underwent testing between 2013 and 2015 performed at a Clinical Laboratory Improvement Amendments laboratory and thus will be assumed to include patients from the U.S. Cases were women with a single diagnosis of breast or ovarian cancer. Controls were women from the same database (i.e., being tested for hereditary cancer) with no cancer history at the time of genetic testing. The multivariable models for breast cancer risk are reported here. Among the breast cancer patients, 244 (0.92%) had a PALB2 variant. The association between PALB2 and breast cancer adjusting for age, ancestry, personal and family cancer histories, and Lynch and adenomatous polyposis colon cancer syndromes were OR = 3.39 (2.79 to 4.12).

Thompson et al (2015) evaluated Australian women with breast cancer (n=1996) referred for genetic evaluation from 1997 to 2014. A control group was accrued from participants in the LifePool study (n=1998) who were recruited for a mammography screening program. All PALB2 coding exons were sequenced by next-generation sequencing and novel variants verified by Sanger sequencing. Large deletions or rearrangements were not evaluated. Nineteen distinct pathogenic variants were identified, including 6 not previously described in 26 (1.3%) cases and in 4 (0.2%) controls with an odds for breast cancer of 6.58 (95% confidence interval [CI], 2.3 to 18.9). Moreover, 54 missense variants identified were slightly more common in cases (OR=1.15; 95% CI, 1.02 to 1.32).

Cybulski et al (2015) examined 2 loss-of-function PALB2 variants (c.509_510delGA, c.172_175delTTGT) in women with invasive breast cancer diagnosed between 1996 and 2012 in Poland. From 12529 genotyped women, a PALB2 variant was identified in 116 (0.93%) cases (95% CI, 0.76% to 1.09%) vs. 10 (0.21%, 95% CI, 0.08% to 0.34%) of 4702 controls (OR=4.39; 95% CI, 2.30 to 8.37). A BRCA1 variant was identified in 3.47% of women with breast cancer and in 0.47% of controls (OR=7.65; 95% CI: 4.98 to 11.75). Authors estimated that a PALB2 sequence variant conferred a 24% cumulative risk of breast cancer by age 75 (in the setting of age-adjusted breast cancer rates slightly more than half that in the U.K. or the U.S.). A PALB2 variant was also associated with poorer prognosis-10-year survival of 48.0% vs. 74.7% when the variant was absent (hazard ratio [HR]=2.27; 95% CI, 1.64 to 3.15; adjusted for prognostic factors).

Catucci et al (2014) performed population-based case-control studies in Italy (Milan or Bergamo) among women at risk for hereditary breast cancer and no BRCA1 or BRCA2 variant. In Milan, 9 different pathogenic PALB2 variants were detected in 12 of 575 cases and none in 784 controls (blood donor); in Bergamo, PALB2 c.1027C>T variants were detected in 6 of 113 cases and in 2 of 477 controls (OR=13.4; 95% CI, 2.7 to 67.4). Performed in two distinct populations, the combined sample size was small, and uncertainty as indicated by the large effect estimate.

Casadei et al (2011) studied 959 U.S. women (non-Ashkenazi Jewish descent) with a family history of BRCA1- or BRCA2-negative breast cancer and 83 female relatives using a family-based case-control design. Using conventional sequencing, pathogenic PALB2 variants were
detected in 31 (3.2%) women with breast cancer and none in controls. Compared with their female relatives without PALB2 variants, the risk of breast cancer increased 2.3-fold (95% CI, 1.5 to 4.2) by age 55 and 3.4-fold (95% CI, 2.4 to 5.9) by age 85. Mean age at diagnosis was not associated with the presence of a variant (50.0 years with vs. 50.2 years without). Casadei et al (2011) provided few details of analyses. Additionally, participants reported over 30 ancestries and, given intermarriage in the U.S. population, stratification may have had an impact on results. Generalizability of the RR estimate is therefore unclear.

Heikkinen et al (2009) conducted a population-based case-control study at a Finnish university hospital employing 2 case groups (947 familial and 1274 sporadic breast cancers) and 1079 controls.23 The study sample was obtained from 542 patients with familial breast cancer, a series of 884 oncology patients (79% of consecutive new cases), and 986 surgical patients (87% of consecutive new cases); 1706 were genotyped for the PALB2 c.1592delT variant. All familial cases were BRCA1- and BRCA2-negative-but among controls, there were 183 BRCA carriers. PALB2 variant prevalence varied with family history-2.6% when 3 or more family members were affected and 0.7% in all breast cancer patients. Variant prevalence was 0.2% among controls. In women with the hereditary disease, a PALB2 c.1592delT variant was associated with an increased risk of breast cancer (OR=11.0; 95% CI, 2.65 to 97.78), and was higher in women with the strongest family histories (women with sporadic cancers OR=4.19; 95% CI, 1.52 to 12.09). Although data were limited, survival was lower among PALB2-associated cases (10-year survival, 66.5% [95% CI, 44.0% to 89.0%] vs. 84.2% [95% CI, 83.1% to 87.1%] in women without a variant, p=0.041; HR, 2.94, p=0.047). A PALB2 variant was also associated with triple-negative tumors-54.5% vs. 12.2% with familial disease and 9.4% in sporadic cancers.

Multinational Samples
Yang et al (2019) performed a complex segregation analysis to estimate relative and absolute risks of breast cancer from data on 524 families with PALB2 pathogenic variants from 21 countries, the most frequent being c.3113G>A.28 Female breast cancer RR was found to be 7.18 (95% CI, 5.82 to 8.85; P=6.5x10-75) when assumed to be constant with age. The age-trend model provided the best fit (P=2x10-3) and demonstrated a pattern of decreasing RR with each increased decade in age. The RR was found to be 4.69 (95% CI, 3.28 to 6.70) in those 75 years of age per the age-trend model.

Southey et al (2016) examined the association of 3 PALB2 variants (2 protein-truncating: c.1592delT and c.3113G>A; 1 missense c.2816T>G) with breast, prostate, and ovarian cancers.26 The association with breast cancer was examined among participants in the Breast Cancer Association Consortium (BCAC; 42671 cases and 42164 controls). BCAC (part of the larger Collaborative Oncological Gene-environment Study) included 48 separate studies with participants of multiple ethnicities, but mainly European, Asian, and African American. Most studies were population- or hospital-based case-control with some oversampling cases with family histories or bilateral disease. A custom array was used for genotyping at four centers, with 2% duplicate samples. The ORs were estimated adjusting for study among all participants, and excluding those studies selecting patients based on family history or bilateral disease (37039 cases, 38260 controls). The c.1592delT variant was identified in 35 cases and 6 controls (from 4 studies in the U.K., Australia, U.S., Canada; OR=4.52; 95% CI, 1.90 to 10.8; p<0.001); in those with no family history or bilateral disease (OR=3.44; 95% CI, 1.39 to 8.52; p=.003). The c3113G>A variant was identified in 44 cases and 8 controls (9 studies from Finland and Sweden; OR=5.93; 95% CI, 2.77 to 12.7; p<0.001) and in those with no family
history or bilateral disease (OR=4.21; 95% CI, 1.84 to 9.60; p<.001). There was no association between the c2816T>G missense variant and breast cancer (found in 150 cases and 145 controls).

These results, derived from a large sample, used a different analytic approach than Antoniou et al (2014), described next, and examined only 2 pathogenic variants. The magnitude of the estimated RR approaches that of a high penetrance gene but is accompanied by wide CIs owing to the study design and low carrier prevalence. The lower estimates obtained following exclusion of those selected based on family history or bilateral disease are consistent with the importance of carefully considering the risk of hereditary disease prior to genetic testing.

Antoniou et al (2014) analyzed data from 362 members of 154 families with deleterious PALB2 variants.10 Individuals with benign variants or variants of uncertain significance were excluded. Families were recruited at 14 centers in 8 countries (U.S., U.K., Finland, Greece, Australia, Canada, Belgium, Italy) and had at least 1 member with a BRCA1- or BRCA2-negative PALB2-positive breast cancer. There were 311 women with PALB2 variants-229 had breast cancer; 51 men also had PALB2 variants (7 had breast cancer). Of the 48 pathogenic (loss-of-function) variants identified, two were most common (c.1592delT in 44 families, c.3113G>A in 25 families); 39 of the 48 pathogenic variants were found in just 1 or 2 families.

Carriers of PALB2 variants (men and women) had a 9.47-fold increased risk for breast cancer (95% CI, 7.16 to 12.57) compared with the U.K. population under a single-gene model and age-constant RR; 30% of tumors were triple-negative. For a woman ages 50 to 54, the estimated RR was 6.55 (95% CI, 4.60 to 9.18). The RR of breast cancer for males with PALB2 variants, compared with the male breast cancer incidence in the general population, was 8.3 (95% CI, 0.77 to 88.5; p=0.08). The cumulative risk at age 50 of breast cancer for female PALB2 carriers without considering family history was 14% (95% CI, 9% to 20%); by age 70, it was 35% (95% CI, 26% to 46%). A family history of breast cancer increased the cumulative risk: if a woman with a PALB2 variant has a sister and mother who had breast cancer at age 50, by age 50 she would have a 27% (95% CI, 21% to 33%) estimated risk of developing breast cancer; and by age 70, a 58% (95% CI, 50% to 66%) risk. These results emphasize that family history affects penetrance. Authors noted that the study "includes most of the reported families with PALB2 variant carriers, as well as many not previously reported...."

**Variant Interpretation**

Valid variant classification is required to assess penetrance and is of particular concern for low prevalence variants including PALB2. Although the more common founder mutations were identified in many patients in the clinical validity studies, some specific variants were infrequent in the samples. While there are guidelines for variant classification, the consistency of interpretation among laboratories is of interest. Balmaña et al (2016) examined the agreement in variant classification by different laboratories from tests for inherited cancer susceptibility from individuals undergoing panel testing.31 The Prospective Registry of Multiplex Testing registry is a volunteer sample of patients invited to participate when test results were provided to patients from participating laboratories. From 518 participants, 603 variants were interpreted by multiple laboratories and/or found in ClinVar. Discrepancies were most common with CHEK2 and ATM. Of 49 missense PALB2 results with multiple interpretations, 9 (18%) had at least 1 conflicting interpretation-3 (6%) had pathogenic variants of uncertain significance or likely benign interpretations from different sources. Given the nature of the sample, there was a significant potential for biased selection of women with either reported variants of uncertain
significance or other uncertainty in interpretation. In addition, discrepancies were confined to missense variants. It is therefore difficult to draw conclusions concerning the frequency of discrepant conclusions among all tested women.

Section Summary: Clinically Valid

Identified studies differed by populations, designs, sample sizes, analyses, and variants examined. While estimates of the magnitude of the association between PALB2 and breast cancer risk varied across studies, their magnitudes are of moderate to high penetrance.

Errors in missense variant classification have been reported. False-negatives would result in risk determined by family history alone or may offer incorrect reassurance; the consequences of false-positives may have adverse consequences due to incorrect management decisions.

Finally, of interest is how variant detection affects penetrance estimates compared with family history alone. As with BRCA variants, model-based estimates allow estimating risks for individual patient and family characteristics. To illustrate using the Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm model, a woman age 30 whose mother had breast cancer at age 35 has an estimated 14.4% risk of breast cancer at age 70; if she carries a PALB2 variant, the risk increases to 51.1%. A woman, age 50, with breast cancer whose mother had breast cancer at age 50, has an estimated 11.7% risk of contralateral cancer by age 70, increasing to 28.7% if she carries a PALB2 variant.

Table 1. Included Association Studies of Pathogenic PALB2 Variants

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Design</th>
<th>N</th>
<th>Families</th>
<th>PALB2 Variants</th>
<th>Totals</th>
<th>Pathogenic Variants Identified</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
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<th>Prevalence Cases, %</th>
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<td>?</td>
<td>1</td>
<td>(c1592delT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antoniou10</td>
<td>2014</td>
<td>Multi- national</td>
<td>Kin-cohort</td>
<td>2980</td>
<td>154</td>
<td>229</td>
<td>82</td>
<td>542</td>
<td>2438</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southey30</td>
<td>2016</td>
<td>Multi- national</td>
<td>Multicenter CC</td>
<td>84835</td>
<td>35</td>
<td>6</td>
<td>42671</td>
<td>42164</td>
<td>1</td>
<td>(c1592delT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kurian28</td>
<td>2017</td>
<td>U.S.</td>
<td>CC</td>
<td>95561</td>
<td>257</td>
<td>NR</td>
<td>26384</td>
<td>Unclear</td>
<td>NR</td>
<td>0.97%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CC: case-control; NR: not reported.

* All or selected families included in Antoniou et al (2014).
* Participants included in Southey et al (2016).
The purpose of limitations tables (see Tables 3 and 4) is to display notable limitations identified in each study. This information is synthesized as a summary of the body of evidence following each table and provides the conclusions on the sufficiency of the evidence supporting the position statement.

Table 2. Measures of Association and Penetrance for Breast Cancer and PALB2

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Analysis</th>
<th>RR or OR (95% CI)</th>
<th>Penetrance at Age 70 (95% CI), %</th>
<th>Mean (Median) Age Onset, y</th>
<th>Triple-Negative Tumors, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang</td>
<td>2019</td>
<td>Segregation</td>
<td>7.18 (5.82 to 8.85)</td>
<td>52.8 (43.7 to 62.7) ^d</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Lu</td>
<td>2019</td>
<td>Standard CC</td>
<td>5.5 (2.2-17.7)</td>
<td>47.5 (38.6-57.4) ^d</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Antoniou</td>
<td>2014</td>
<td>Segregation ^b</td>
<td>6.6 (4.6-9.2)^c</td>
<td>54.3 (+FH)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Erkko</td>
<td>2008</td>
<td>Segregation</td>
<td>6.1 (2.2-17.2)^a</td>
<td>54.3 (FH Unavailable)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rahman</td>
<td>2007</td>
<td>Segregation ^b</td>
<td>2.3 (1.4-3.9)^e</td>
<td>46 (IQR, 40-51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casadei</td>
<td>2011</td>
<td>Relative Risk</td>
<td>2.3 (1.5-4.2)^f</td>
<td>50.0 (SD = 11.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson</td>
<td>2015</td>
<td>Standard CC</td>
<td>6.6 (2.3-18.9)</td>
<td>50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cybulski</td>
<td>2015</td>
<td>Standard CC</td>
<td>4.4 (2.3-8.4)</td>
<td>50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catucci</td>
<td>2014</td>
<td>Standard CC</td>
<td>13.4 (2.7-67.4)</td>
<td>53.1 (95%CI, 33.4-79.9)</td>
<td>54.5</td>
<td>9.4, 12.2^g</td>
</tr>
<tr>
<td>Heikkinen</td>
<td>2009</td>
<td>Standard CC</td>
<td>11.0 (2.6-97.8)</td>
<td>53.1 (95%CI, 33.4-79.9)</td>
<td>54.5</td>
<td>9.4, 12.2^g</td>
</tr>
<tr>
<td>Southey</td>
<td>2016</td>
<td>Standard CC</td>
<td>4.5 (1.9-10.8) (c.1592delT)</td>
<td>53.1 (95%CI, 33.4-79.9)</td>
<td>54.5</td>
<td>9.4, 12.2^g</td>
</tr>
<tr>
<td>Kurian</td>
<td>2017</td>
<td>Standard CC</td>
<td>3.39 (2.79-4.12)</td>
<td>53.1 (95%CI, 33.4-79.9)</td>
<td>54.5</td>
<td>9.4, 12.2^g</td>
</tr>
</tbody>
</table>

CC: case-control; CI: confidence interval; FH: family history; IQR: interquartile range; OR: odds ratio; RR: relative risk; SD: standard deviation.

^a Using an "augmented" dataset assuming no cases among families without recorded histories. Analyses limited to those with recorded histories yielded a RR of 14.3 (95% CI, 6.6 to 31.2).

^b Modified.

^c Estimate for women age 50.

^d Estimates varied according to family history. For women with a mother and sister with breast cancer at age 50, cumulative risk was estimated at 58% (95% CI, 50% to 66%); for women with no family history, 33% (95% CI, 26% to 46%).

^e For women <50 years, RR of 3.0 (95% CI, 1.4 to 3.9); for women >50 years, RR of 1.9 (95% CI, 0.8 to 3.7).

^f At age 85 years, RR of 3.4 (95% CI, 2.4 to 5.9).

^g In sporadic and familial cancers without PALB2 variants.
Table 3. Relevance Limitations of Individuals Studies of Pathogenic *PALB2* Variants

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
<th>Duration of FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang⁵⁸</td>
<td>4. No case-control group</td>
<td>1. Not clear which variants were included</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lu⁷⁷</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td>1. Not clear which variants were included</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kurian⁵⁸</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td>1. Not clear which variants were included</td>
<td></td>
<td></td>
<td>1. Control chosen from patients being tested for hereditary cancer; unclear how many developed cancer</td>
</tr>
<tr>
<td>Southey³⁰</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson²⁵</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cybulski¹³</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catucci¹¹</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antoniou¹⁰</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casadei¹²</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erkko²²</td>
<td>4. No case-control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

FU: follow-up.

a Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

b Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

c Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

d Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

e Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 4. Study Design and Conduct Limitations of Individuals Studies of Pathogenic PALB2 Variants

<table>
<thead>
<tr>
<th>Study</th>
<th>Selectiona</th>
<th>Blindingb</th>
<th>Delivery of Testc</th>
<th>Selective Reportingd</th>
<th>Data Completenesse</th>
<th>Statisticalf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang28</td>
<td>1. Incomplete descriptions of how family groups selected</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lu27</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kurian28</td>
<td></td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southey20</td>
<td></td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thompson25</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cybulski13</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catucci11</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antoniou10</td>
<td>2. Kin-cohort controls not randomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casadei12</td>
<td>2. Family groups: controls not randomized</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heikkinen23</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erkko22</td>
<td>2. Family groups: selection not randomized</td>
<td>1. Registration not reported; number of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13
Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence
Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Evidence of clinical utility limited to women with PALB2 variants was not identified.

Chain of Evidence
Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Rosenthal et al (2017) reported an analysis of the impact of testing for genes other than BRCA1/2 and by calculating whether carriers of these gene variants would have been identified as candidates for enhanced screening based family history alone.32 The database included 194107 women who were tested using a hereditary cancer panel between 2013 and 2016. The women were referred by their health care providers for clinical suspicion of hereditary cancer. It is unclear what proportion of the women met professional society criteria for genetic testing for breast cancer risk; baseline information regarding family history was not reported. Of the women in the database, 893 had PALB2 variants and were eligible for Claus assessment to estimate the risk of breast cancer. Approximately 27% of women with PALB2 variants would have had an estimated risk of breast cancer of 20% or higher based on the Claus model. The report did not include health outcomes and it is unclear whether enhanced screening in women who had a moderate penetrance variant but did not have estimated risk of breast cancer of 20% or greater based on the Claus model would have improved health outcomes from enhanced surveillance.

Studies of women at high-risk based on family history alone or in those with BRCA1 and BRCA2 variants are relevant to the clinical utility of PALB2 testing given the penetrance estimates for PALB2 and related molecular mechanism ("BRCA-ness"). Interventions to decrease breast cancer risk in asymptomatic high-risk women include screening,33 (e.g., starting at an early age, the addition of magnetic resonance imaging to mammography, and...
screening annually), chemoprevention, and prophylactic mastectomy. In women with breast cancer, contralateral prophylactic mastectomy is of interest; other treatment decisions are dictated by clinical, pathologic, and other prognostic factors.

In women at high-risk of hereditary breast cancer, including BRCA1 and BRCA2 carriers, evidence supports a reduction in subsequent breast cancer after bilateral or contralateral prophylactic mastectomy. Decision analyses have also concluded the impact on breast cancer incidence extends life in high, but not average risk, women. For example, Schrag et al (1997, 2000) modeled the impact of preventive interventions in women with BRCA1 or BRCA2 variants and examined penetrance magnitudes similar to those estimated for a PALB2 variant. Compared with surveillance, a 30-year-old BRCA carrier with an expected 40% risk of breast cancer and 5% risk of ovarian cancer by age 70 would gain an expected 2.9 years following a prophylactic mastectomy alone and an additional 0.3 years with a prophylactic oophorectomy (see Table 5). A 50-year-old female BRCA carrier with node-negative breast cancer and a 24% risk of contralateral breast cancer at age 70 would anticipate 0.9 years in improved life expectancy (0.6 years for node-negative disease) following a prophylactic contralateral mastectomy.

Table 5. Model Results of the Effects of Bilateral Risk-Reducing Mastectomy vs. Surveillance on Life Expectancy in BRCA Carriers According to Penetrance

<table>
<thead>
<tr>
<th>Risk Level and Strategy</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% risk of breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>2.9</td>
<td>2.0</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Mastectomy delayed 10 y</td>
<td>1.8</td>
<td>0.8</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>60% risk of breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>4.1</td>
<td>2.9</td>
<td>1.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Mastectomy delayed 10 y</td>
<td>2.4</td>
<td>1.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>85% risk of breast cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mastectomy</td>
<td>5.3</td>
<td>3.7</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Mastectomy delayed 10 y</td>
<td>2.6</td>
<td>1.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Adapted from Schrag et al (1997).

Section Summary: Clinically Useful
Evidence concerning preventive interventions in women with PALB2 variants is indirect, relying on studies of high-risk women and BRCA carriers. In women at high-risk of hereditary breast cancer who would consider preventive interventions, identifying a PALB2 variant provides a more accurate estimated risk of developing breast cancer compared with family history alone and can offer a better understanding of benefits and potential harms of interventions.

CHEK2 AND BREAST CANCER RISK ASSESSMENT

Clinical Context and Test Purpose
The purpose of testing for CHEK2 variants in individuals at high-risk of HBOC is to evaluate whether an abnormal variant is present and, if so, to determine whether the variant conveys a sufficiently high-risk that changes in surveillance and/or treatment likely to decrease the risk of mortality from breast and/or ovarian cancer are warranted.
The question addressed in this evidence review is: Does genetic testing for \textit{CHEK2} variants improve the net health outcome in women at high-risk of HBOC?

The following PICO\textsuperscript{s} were used to select literature to inform this review.

\textbf{Patients}  
Genetic testing can be considered for women at increased risk of developing hereditary breast cancer based on their family history or in women with breast cancer whose family history or cancer characteristics (e.g., triple-negative disease, young age) increase the likelihood that the breast cancer is hereditary. Testing may also be considered for women from families with known variants.

The relevant population of interest in this review are patients who are undergoing assessment for hereditary breast and/or ovarian cancer syndrome.

\textbf{Interventions}  
The intervention of interest is \textit{CHEK2} variant testing.

\textbf{Comparators}  
The alternative would be to manage women at high-risk of HBOC with no \textit{CHEK2} genetic testing.

\textbf{Outcomes}  
The outcomes of interest are OS, disease-specific (breast and ovarian cancer) survival, and test validity.

\textbf{Study Selection Criteria}  
For the evaluation of the clinical validity of the tests, studies that met the eligibility criteria outlined for indication 1 were considered.

\textbf{Technically Reliable}  
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

\textbf{Clinically Valid}  
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

\textbf{Risk of Developing Breast Cancer}  
For genetic susceptibility to cancer, clinical validity can be established if the variants that the test is intended to identify are associated with disease risk, and if so, if these risks are well quantified.\textsuperscript{6} Most studies assessing the risk of breast cancer associated with \textit{CHEK2} are population- and family-based case-control studies.

\textbf{Systematic Reviews}
Systematic reviews of CHEK2 and breast cancer risk have been reported. Characteristics are shown in Table 6 and the results are shown in Table 7.

The Suszynska et al (2019) systematic review described previously also included association estimates for CHEK2 variants. In the 43 breast cancer studies included in the review, 94845 patients contributed to the meta-analysis of CHEK2 in breast cancer patients. The OR of breast cancer for CHEK2 variants including variants c.470T>C and c.1283C>T was OR=0.96 (95% CI, 0.90 to 1.03); after excluding variants c.470T>C and c.1283C>T, the association between the remaining CHEK2 variants and breast cancer was OR=1.73 (95% CI, 1.58 to 1.89). Given that the Suszynska et al (2019) report included only studies reporting on test results from a panel, it does not substantially overlap with the studies described in the following section including other CHEK2 association studies.

Liang et al (2018) conducted a meta-analysis to investigate the link between CHEK2 and breast cancer. Two researchers independently searched 7 online databases and selected for analysis 26 published studies representing a pooled sample of 118735 cancer patients and 195807 controls, all case-control studies conducted in Europe or the Americas. The meta-analysis revealed that CHEK2 variants are more common in patients with breast cancer (OR=2.89; 95% CI, 2.63 to 3.16), with variants 5.9% more likely in female patients with breast cancer than in male patients with breast cancer. Limitations of the study included a study population that might not represent the general population, inaccurate control sampling methods in some original studies, selection biases, and unclear criteria for breast cancer diagnoses.

An article by Schmidt et al (2016) evaluated data on CHEK2 variant status and breast cancer risk from BCAC. The analysis included 44777 breast cancer patients and 42997 controls from 33 studies in which individuals were genotyped for CHEK2 variants. The estimated odds for invasive breast cancer in patients with and without the CHEK2 1100delC variant was 2.26 (95% CI, 1.90 to 3.10).

A meta-analysis by Yang et al (2012) examined the risk of breast cancer in whites with the CHEK2 c.1100delC variant. Twenty-five case-control studies conducted in Europe and North and South America published in 16 articles were analyzed, with a total of 29154 breast cancer cases and 37064 controls. Of the cases, 13875 patients had unselected breast cancer, 7945 had familial breast cancer, and 5802 had early-onset breast cancer. In total, 391 (1.3%) of the cases had a CHEK2 c.1100delC variant and 164 (0.4%) of the controls. The association between the CHEK2 c.1100delC variant and breast cancer risk was statistically significant (OR=2.75; 95% CI, 2.25 to 3.36). By subgroup, odds were 2.33 (95% CI, 1.79 to 3.05) for unselected, 3.72 (95% CI, 2.61 to 5.31) for familial, and 2.78 (95% CI, 2.28 to 3.39) for early-onset breast cancer.

Weischer et al (2008) performed a meta-analysis of studies on CHEK2 c.1100delC heterozygosity and the risk of breast cancer among patients with unselected (including the general population), early-onset (<51 years of age), and familial breast cancer. The analysis identified prospective cohort and case-control studies on CHEK2 c.1100delC and the risk of breast cancer published before March 2007. Inclusion criteria were women with unilateral breast cancer who did not have a known multicancer syndrome, Northern or Eastern European descent, availability for CHEK2 genotyping, BRCA1 and BRCA2 sequence variant-negative or unknown status, and breast cancer-free women as controls. The meta-analysis included 16
studies with 26488 patient cases and 27402 controls. Presenting both fixed and random-effect models, for CHEK2 c.1100delC heterozygotes vs. noncarriers, the aggregated ORs for breast cancer were 2.7 (95% CI, 2.1 to 3.4) and 2.4 (95% CI, 1.8 to 3.2) in studies of unselected breast cancer, 2.6 (95% CI, 1.3 to 5.5) and 2.7 (95% CI, 1.3 to 5.6) in studies of early-onset breast cancer, and 4.8 (95% CI, 3.3 to 7.2) and 4.6 (95% CI, 3.1 to 6.8) in studies of familial breast cancer, respectively.

Table 6. Characteristics of Systematic Reviews of CHEK2 and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Dates</th>
<th>Population</th>
<th>Designs Included</th>
<th>No. of Studies</th>
<th>No. of Participants</th>
<th>Pathogenic Variants Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suszynska21</td>
<td>To Jul 2017</td>
<td>Cases: patients with breast and/or ovarian cancer referred for evaluation by a multi-gene panel</td>
<td>Studies reporting prevalence of genetic variants</td>
<td>48 (overall)</td>
<td>94845</td>
<td>37 CHEK2 variants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls: patients from the Genome Aggregation Database</td>
<td></td>
<td>43 (breast cancer)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liang53</td>
<td>To Jan 2018</td>
<td>Mixed (including men and women with breast cancer)</td>
<td>Case-control</td>
<td>26</td>
<td>314542</td>
<td>c.1100delC</td>
</tr>
<tr>
<td>Schmidt39,40</td>
<td>NR</td>
<td>European women in the Breast Cancer Association Consortium</td>
<td>Case-control</td>
<td>33</td>
<td>87754</td>
<td>c.1100delC</td>
</tr>
<tr>
<td>Yang39</td>
<td>To May 2012</td>
<td>Mixed</td>
<td>Case-control</td>
<td>16</td>
<td>66218</td>
<td>c.1100delC</td>
</tr>
<tr>
<td>Weischer41</td>
<td>To Mar 2007</td>
<td>Unilateral breast cancer, Northern or Eastern European descent, BRCA1 or BRCA2 negative or unknown, and breast cancer free controls</td>
<td>Prospective cohort and case-control</td>
<td>16</td>
<td>26488</td>
<td>c.1100delC</td>
</tr>
</tbody>
</table>

NR: not reported.

Table 7. Results of Systematic Reviews of CHEK2 and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Relative Risk/Odds Ratio (95% CI)</th>
<th>Penetrance at Age 70 (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suszynska21</td>
<td>1.73 (95% CI, 1.58-1.89)a</td>
<td>NR</td>
</tr>
<tr>
<td>Schmidt40</td>
<td>Overall</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total N</td>
<td>81700</td>
</tr>
<tr>
<td></td>
<td>Pooled estimate (95% CI)</td>
<td>2.4 (2.1-2.9)</td>
</tr>
<tr>
<td></td>
<td>Non-BRCA 1 or BRCA2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total N</td>
<td>72334</td>
</tr>
<tr>
<td></td>
<td>Pooled estimate (95% CI)</td>
<td>2.3 (2.0-2.8)</td>
</tr>
<tr>
<td>Yang39</td>
<td>Unselected for family history</td>
<td></td>
</tr>
</tbody>
</table>
Individual Studies Not Included in Systematic Reviews

Individual studies not included in the previous meta-analyses have also reported on the association between breast cancer development and CHEK2 variants; they are summarized in Tables 8 and 9. The number of included patients ranged from over 5500 to over 95000. The prevalence of CHEK2 variants was approximately 2% to 3% in breast cancer patients. The OR, HR, or RR ranged from approximately two to three, although it was higher in subgroups of women with a family history of breast cancer and in biallelic carriers of CHEK2 pathogenic variants.

Table 8. Characteristics of Studies of CHEK2 and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Dates</th>
<th>Population</th>
<th>No. of Participants</th>
<th>Pathogenic Variants Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainville et al (2020)</td>
<td>2013-2019</td>
<td>Monoallelic and biallelic female carriers of CHEK2 pathogenic variants identified through clinical panhereditary cancer panel testing</td>
<td>6515</td>
<td>c.1100delC and unclear</td>
</tr>
<tr>
<td>Lu27</td>
<td>2014-2015</td>
<td>Cases with breast and/or ovarian cancer referred for genetic testing and controls referred for genetic testing for noncancer conditions</td>
<td>15404</td>
<td>&quot;known breast or ovarian gene&quot;</td>
</tr>
<tr>
<td>Kurian28</td>
<td>2013-2015</td>
<td>Cases and controls referred for testing for hereditary cancer. Control were those without cancer at the time of testing</td>
<td>95561</td>
<td>Unclear</td>
</tr>
<tr>
<td>Fan54</td>
<td>2003-2015</td>
<td>Breast cancer patients at Chinese university cancer hospital who received gene panel sequencing</td>
<td>8085</td>
<td>c.1100delC</td>
</tr>
<tr>
<td>Hauke42</td>
<td>NR</td>
<td>Met inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer for germ-line testing</td>
<td>5589</td>
<td>Unclear</td>
</tr>
<tr>
<td>Decker43</td>
<td>After 1991</td>
<td>U.K.; diagnosed with invasive breast cancer from SEARCH study and controls from 3 population-based studies</td>
<td>18575</td>
<td>c.1100delC plus 14 rare truncating variants</td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Description</td>
<td>N</td>
<td>Variant Details</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Rainville et al (2020)²²</td>
<td>2020</td>
<td>Monoallelic carriers of CHEK2 variants</td>
<td>6473/6515</td>
<td>2668/6473 (99.4%) monoallelic carriers of CHEK2 variants</td>
</tr>
<tr>
<td>Monoallelic</td>
<td></td>
<td>Ductal invasive: 2.02 (1.90 to 2.15)</td>
<td>2668/6473</td>
<td>2534/6473 (50.0%) in breast cancer patients</td>
</tr>
<tr>
<td>Biallelic</td>
<td></td>
<td>Ductal invasive: 8.69 (3.69 to 20.47)</td>
<td>2531/31</td>
<td>21 (9.7%) in no personal cancer history</td>
</tr>
<tr>
<td>Lu²⁷</td>
<td>2012</td>
<td>0.8% in breast or ovarian cancer cases</td>
<td>0.3% in controls</td>
<td>2.19 (1.40 to 3.56)</td>
</tr>
<tr>
<td>Kurian²⁸</td>
<td>2019</td>
<td>1.2% in breast cancer patients</td>
<td>1% in patients without breast or ovarian cancer</td>
<td>1.99 (1.70 to 2.33)</td>
</tr>
<tr>
<td>Fan⁵⁴</td>
<td>2014</td>
<td>Total N</td>
<td>7657</td>
<td>NR</td>
</tr>
<tr>
<td>Hauke⁴²</td>
<td>2016</td>
<td>Estimate (95% CI)</td>
<td>0.34% in breast cancer patients</td>
<td>NR</td>
</tr>
<tr>
<td>Decker⁴³</td>
<td>2017</td>
<td>Total N</td>
<td>5589</td>
<td>NR</td>
</tr>
<tr>
<td>Couch⁴⁴</td>
<td>2018</td>
<td>Estimate (95% CI)</td>
<td>1.8% in breast cancer patients 0.6% and 0.4% in control datasets</td>
<td>2.9 (2.3-3.8)</td>
</tr>
<tr>
<td>Naslund-Koch⁴⁵</td>
<td>2019</td>
<td>Total N</td>
<td>54305</td>
<td>NR</td>
</tr>
<tr>
<td>Estimate (95% CI)</td>
<td>1.6% in breast cancer patients 0.5% in controls</td>
<td>3.1 (2.2-4.7)</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Results of Individuals Studies of CHEK2 and Risk of Breast Cancer
Study design and conduct limitations are shown in Tables 10 and 11. Only one study included population-based sampling in a prospective cohort. The remaining studies were case-control studies. Several studies did not adequately describe the selection of cases and/or controls. A complete disposition of patients or samples eligible for inclusion and those appearing in the analysis was also not provided in several studies.

### Table 10. Relevance Limitations of Individuals Studies of CHEK2 and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention</th>
<th>Comparator</th>
<th>Outcomes</th>
<th>Duration of FU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainville (2020)42</td>
<td>4. No control population, likely overestimated risk</td>
<td>1. Not clear which variants were included</td>
<td>1. Unclear if follow-up duration is sufficient due to retrospective review</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lu27</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimating risk</td>
<td>1. Not clear which variants were included</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kurian28</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimating risk</td>
<td>1. Not clear which variants were included</td>
<td></td>
<td>1: Control chosen from patients being tested for hereditary cancer; unclear how many developed cancer</td>
<td></td>
</tr>
<tr>
<td>Fan54</td>
<td>4. Case-control population of breast cancer patients (and controls), likely overestimated risk;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
only included Chinese patients

Hauke\textsuperscript{42} 4. Case-control population of breast cancer patients (and controls), likely overestimated risk; only included participants of European ancestry

Decker\textsuperscript{43} 4. Case-control population of breast cancer patients (and controls), likely overestimated risk

Couch\textsuperscript{44} 4. Case-control population of breast cancer patients referred to genetic testing (and controls), likely overestimated risk

Naslund-Koch\textsuperscript{45} 4. Includes only White participants and those of Danish descent

Cybulski\textsuperscript{14} 4. Case-control population of breast cancer patients (and controls), likely overestimated risk

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

FU: follow-up.

\textsuperscript{a} Population key: 1. Intended use population unclear; 2. Clinical context is unclear; 3. Study population is unclear; 4. Study population not representative of intended use.

\textsuperscript{b} Intervention key: 1. Classification thresholds not defined; 2. Version used unclear; 3. Not intervention of interest.

\textsuperscript{c} Comparator key: 1. Classification thresholds not defined; 2. Not compared to credible reference standard; 3. Not compared to other tests in use for same purpose.

\textsuperscript{d} Outcomes key: 1. Study does not directly assess a key health outcome; 2. Evidence chain or decision model not explicated; 3. Key clinical validity outcomes not reported (sensitivity, specificity and predictive values); 4. Reclassification of diagnostic or risk categories not reported; 5. Adverse events of the test not described (excluding minor discomforts and inconvenience of venipuncture or noninvasive tests).

\textsuperscript{e} Follow-Up key: 1. Follow-up duration not sufficient with respect to natural history of disease (true-positives, true-negatives, false-positives, false-negatives cannot be determined).

Table 11. Study Design and Conduct Limitations of Individuals Studies of CHEK2 and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection\textsuperscript{a}</th>
<th>Blinding\textsuperscript{b}</th>
<th>Delivery of Test\textsuperscript{c}</th>
<th>Selective Reporting\textsuperscript{d}</th>
<th>Data Completeness\textsuperscript{e}</th>
<th>Statistical\textsuperscript{f}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainville (2020)\textsuperscript{42}</td>
<td>1. Registration not reported</td>
<td>1. Only exclusion criteria are provided</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lu\textsuperscript{27}</td>
<td>1. Incomplete description of how controls selected</td>
<td>1. Registration not reported</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kurian\textsuperscript{28}</td>
<td>1. Registration not reported</td>
<td>1. Only exclusion criteria are provided</td>
<td>1. Registration not reported</td>
<td>1. No description of disposition of eligible patients/samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Limitations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fan&lt;sup&gt;54&lt;/sup&gt;</td>
<td>1. Incomplete description of how controls selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hauke&lt;sup&gt;42&lt;/sup&gt;</td>
<td>1. Incomplete description of how controls selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decker&lt;sup&gt;43&lt;/sup&gt;</td>
<td>1. No description of how cases or controls selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Couch&lt;sup&gt;44&lt;/sup&gt;</td>
<td>1. Incomplete description of how controls selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naslund-Koch&lt;sup&gt;45&lt;/sup&gt;</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cybulski&lt;sup&gt;14&lt;/sup&gt;</td>
<td>1. Registration not reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The study limitations stated in this table are those notable in the current review; this is not a comprehensive limitations assessment.

*Selection key: 1. Selection not described; 2. Selection not random or consecutive (i.e., convenience).

*Blinding key: 1. Not blinded to results of reference or other comparator tests.

*Test Delivery key: 1. Timing of delivery of index or reference test not described; 2. Timing of index and comparator tests not same; 3. Procedure for interpreting tests not described; 4. Expertise of evaluators not described.


*Data Completeness key: 1. Inadequate description of indeterminate and missing samples; 2. High number of samples excluded; 3. High loss to follow-up or missing data.

*Statistical key: 1. Confidence intervals and/or p values not reported; 2. Comparison with other tests not reported.

---

**Breast Cancer Prognosis in an Individual With a CHEK2 Sequence Variant**

Studies of survival between breast cancer patients with and without CHEK2 variants have shown differing results. Breast cancer patients with CHEK2 variants may have a worse prognosis than noncarriers.

Fan et al (2018) investigated the clinical relevance of CHEK2 variants in breast cancer patients.<sup>54</sup> In this observational study, the genomes of 7657 Chinese BRCA1- and BRCA2-negative breast cancer patients were analyzed. Researchers reported a CHEK2 germline variant rate of 0.34%, and those with the variants were significantly more likely (p=0.022) to have family histories of cancer and to develop lymph node-positive and progesterone receptor-positive cancers. Limitations include sample homogeneity and retrospective design.

A study by Huzarski et al (2014) estimated the 10-year survival rate for patients with early-onset breast cancer, with and without CHEK2 variants.<sup>46</sup> Patients were consecutively identified women with invasive breast cancer diagnosed at or below the age of 50, between 1996 and 2007, in 17 hospitals throughout Poland. Patients were tested for four founder mutations in the CHEK2 gene after diagnosis, and their medical records were used to retrieve tumor characteristics and treatments received. Dates of death were retrieved from a national registry. A total of 3592 women were eligible for the study, of whom 487 (13.6%) carried a CHEK2 variant.
variant (140 with truncating variants, 347 with missense variants). Mean follow-up was 8.9 years. Ten-year survival for CHEK2-variant carriers (78.8%; 95% CI, 74.6% to 83.2%) was similar to noncarriers (80.1%; 95% CI, 78.5% to 81.8%). After adjusting for other prognostic features, the hazard ratio comparing carriers of the missense variant with noncarriers was similar, as was the hazard ratio for carriers of a truncating variant and noncarriers.

A study by Kriege et al (2014) compared breast cancer outcomes in patients with and without CHEK2 variants. Different study cohorts were combined to compare 193 carriers with 4529 noncarriers. Distant disease-free survival and breast cancer-specific survival were similar in the first six years after diagnosis. After 6 years, both distant disease-free survival (multivariate HR=2.65; 95% CI 1.79 to 3.93) and breast cancer-specific survival (multivariate HR=2.05; 95% CI, 1.41 to 2.99) were worse in CHEK2 carriers. No interaction between CHEK2 status and adjuvant chemotherapy was observed.

Weischer et al (2012) reported on breast cancer associated with early death, breast cancer-specific death, and the increased risk of a second breast cancer (defined as a contralateral tumor) in CHEK2-variant carriers and noncarriers in 25571 white women of Northern and Eastern European descent who had invasive breast cancer, using data from 22 studies participating in BCAC conducted in 12 countries. The 22 studies included 30056 controls. Data were reported on early death in 25571 women, breast cancer-specific death in 24345, and a diagnosis of second breast cancer in 25094. Of the 25571 women, 459 (1.8%) were CHEK2 c.1100delC heterozygous and 25112 (98.2%) were noncarriers. Median follow-up was 6.6 years, over which time the following was observed: 124 (27%) early deaths occurred, 100 (22%) breast cancer-specific deaths occurred, and 40 (9%) second breast cancers among CHEK2 c.1100delC variant carriers were observed. Corresponding numbers among noncarriers were 4864 (19%), 2732 (11%), and 607 (2%), respectively. At the time of diagnosis, CHEK2-variant carriers vs. noncarriers were on average four years younger (p<0.001); additionally, CHEK2-variant carriers were more likely to have a family history of cancer (p<0.001). Multifactorially adjusted hazard ratios for CHEK2 vs. noncarriers were 1.43 (95% CI, 1.12 to 1.82; p=0.004) for early death and 1.63 (95% CI, 1.24 to 2.15; p<0.001) for breast cancer-specific death.

Section Summary: Clinically Valid
Studies have shown that a CHEK2 variant is of moderate penetrance and confers a risk of breast cancer two to four times that of the general population; this risk appears to be higher in patients who also have a strong family history of breast cancer. Although the CHEK2 variant appears to account for approximately one-third of variants identified in BRCA1- and BRCA2-negative patients, it is relatively rare with estimates ranging from 1.5 to 4.7% of breast cancer patients in the included studies, and risk estimates, which have been studied in population- and family-based case-controls, are subject to bias and overestimation. One systemic review and 2 studies published since the review estimated the risk of breast cancer by age 70 years in women with CHEK2 variants was close to 20%. However, another review estimated that it may be as high as 37% (95% CI, 26% to 56%) in women with familial breast cancer. Several studies have suggested that CHEK2 carriers with breast cancer may have worse breast cancer-specific survival and distant-recurrence free survival, with about twice the risk of early death.

Clinically Useful
A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

**Direct Evidence**

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence of clinical utility for genetic testing in individuals with *CHEK2* variants was not identified.

**Chain of Evidence**

Indirect evidence on clinical utility rests on clinical validity. If the evidence is insufficient to demonstrate test performance, no inferences can be made about clinical utility.

Weidner et al (2020) conducted a retrospective, consecutive study on 69 *CHEK2* carriers enrolled in the Inherited Cancer Registry (ICARE) at Vanderbilt University and their relatives. Eligibility for annual breast MRI surveillance was based on ≥20% lifetime risk of breast cancer based on family cancer history alone as calculated by the BOADICEA predictive model, or family cancer history and proband *CHEK2* variant status, utilizing an updated version of the BOADICEA model (BWA v4). Among the *CHEK2* carriers and family history alone, 21 first-degree relatives (FDRs) (14.9%) and 14 second-degree relatives (SDRs) (13.9%) had a lifetime cancer risk ≥20%. Inclusion of the proband's variant status significantly increased identification of FDRs to 78 (55.3%; *P*<0.0001) and SDRs to 22 (21.8%; *P*=0.008), respectively. While the study revealed that family history alone may be insufficient to appropriately identify at-risk FDRs and SDRs of *CHEK2* carriers, the study authors note that the expanded BOADICEA predictive model (BWA v4) is not intended for clinical use. Additionally, this version has not been licensed for commercial use. Additional study limitations include the retrospective study design, lack of clarity regarding to what extent study participants met society criteria for genetic testing for breast cancer risk, and no reporting of outcomes associated with enhanced screening for *CHEK2* variant carriers.

As outlined in the section on *PALB2*, for women with high-risk hereditary cancer syndromes, interventions to decrease breast cancer risk in high-risk women include screening (e.g., starting at an early age, the addition of magnetic resonance imaging to mammography, and screening annually), chemoprevention, prophylactic mastectomy, and prophylactic oophorectomy. In contrast to the case of *PALB2*, where the penetrance approaches that of a *BRCA* variant, there is unlikely to be a similar benefit-to-risk calculus for preventive interventions in women with a *CHEK2* variant. Surveys assessing adherence to guideline-based recommendations have explored this relationship but are limited in sample size and generally have not reported variant-stratified long-term outcomes of prophylactic or preventative interventions in controlled studies to support standard actionable thresholds for *CHEK2*. Findings from other studies point to potential overtreatment through risk-reducing bilateral mastectomy among those with *ATM/CHEK2* variants, with over half of all carriers reporting use of prophylactic surgery independent of family history or personal breast cancer history.
Section Summary: CHEK2 and Breast Cancer Risk Assessment
Despite some studies showing potentially poorer outcomes for breast cancer patients who have CHEK2 variants, it is unclear how such knowledge would be used to alter the treatment of such a patient. Furthermore, updated predictive models utilizing information on CHEK2 status have not been approved for widespread clinical use. No evidence is available to support the clinical utility of genetic testing for CHEK2 variants in breast cancer patients to guide patient management. There is no strong chain of evidence supporting CHEK2 testing in breast cancer patients.

ATM AND BREAST CANCER RISK ASSESSMENT

Clinical Context and Test Purpose
The purpose of testing for ATM variants in individuals at high-risk of HBOC is to evaluate whether an abnormal variant is present and, if so, to determine whether the variant conveys a sufficiently high-risk that changes in surveillance and/or treatment likely to decrease the risk of mortality from breast and/or ovarian cancer are warranted.

The question addressed in this evidence review is: Does genetic testing for ATM variants improve the net health outcome in women at high-risk of HBOC?

The following PICOs were used to select literature to inform this review.

Patients
Genetic testing can be considered for women at increased risk of developing hereditary breast cancer based on their family history or in women with breast cancer whose family history or cancer characteristics (e.g., triple-negative disease, young age) increase the likelihood that the breast cancer is hereditary. Testing may also be considered for women from families with known variants.

The relevant population of interest in this review are patients who are undergoing assessment for HBOC syndrome.

Interventions
The intervention of interest is ATM variant testing.

Comparators
The alternative would be to manage women at high-risk of HBOC with no ATM genetic testing.

Outcomes
The outcomes of interest are OS, disease-specific (breast and ovarian cancer) survival, and test validity.

Study Selection Criteria
For the evaluation of the clinical validity of the tests, studies that met the eligibility criteria outlined for indication 1 were considered.

Technically Reliable
Assessment of technical reliability focuses on specific tests and operators and requires a review of unpublished and often proprietary information. Review of specific tests, operators, and unpublished data are outside the scope of this evidence review and alternative sources exist. This evidence review focuses on the clinical validity and clinical utility.

Clinically Valid
A test must detect the presence or absence of a condition, the risk of developing a condition in the future, or treatment response (beneficial or adverse).

Systematic Reviews
The Suszynska et al (2019) systematic review described previously also included association estimates for \textit{ATM} variants. In the 43 breast cancer studies included in the review, 94787 patients contributed to the meta-analysis of \textit{ATM} in breast cancer patients. The OR of breast cancer for \textit{ATM} variants was 2.42 (95% CI, 2.16 to 2.71). Given that the Suszynska et al (2019) report included only studies reporting on test results from a panel, it does not substantially overlap with the studies described in the following section including other \textit{ATM} association studies.

Marabelli et al (2016) reported on a meta-analysis of the penetrance of \textit{ATM} variants in breast cancer, which used a model allowing the integration of different types of cancer risk estimates to generate a single estimate associated with heterozygous \textit{ATM} gene variants. The meta-analysis included 19 studies, which were heterogeneous in terms of population, study designs, and baseline breast cancer risk. The estimated cumulative absolute risk of breast cancer in heterozygous \textit{ATM} variant carriers was 6.02% by age 50 (95% credible interval, 4.58% to 7.42%) and 32.83% by age 80 (95% credible interval, 24.55% to 40.43%).

In another meta-analysis, van Os et al (2016) included 7 studies and found that \textit{ATM} variants were associated with an increased risk of developing breast cancer in women (RR=3.0; 95% CI, 2.1 to 4.5) and a decreased life expectancy (RR=1.7; 95% CI, 1.2 to 2.4).

Association Studies
Individual studies published after the meta-analyses have also reported on the association between breast cancer development and pathogenic \textit{ATM} variants. The study characteristics of Lu et al (2019), Kurian et al (2017), Decker et al (2017), Couch et al (2017), Hauke et al (2018), were included in the previous section on \textit{CHEK2} (see Tables 8, 10, and 11). Study results are shown in Table 12.

<table>
<thead>
<tr>
<th>Study</th>
<th>Prevalence of ATM Variants</th>
<th>RR/OR (95% CI)</th>
<th>Penetrance at Age 70 (95% CI), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu (2019)</td>
<td>0.7% in breast and ovarian cancer cases</td>
<td>2.97 (1.67-5.68)</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.2% in controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3% in breast cancer cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hauke (2018)</td>
<td>0.4% and 0.2% in control samples</td>
<td>3.63 (2.67-4.94)</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>0.6% in breast cancer patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2% in controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decker (2017)</td>
<td></td>
<td>3.26 (1.82-6.46)</td>
<td>NR</td>
</tr>
<tr>
<td>Study</td>
<td>ATM Heterozygotes</td>
<td>Odds Ratio (CI)</td>
<td>RR</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------</td>
<td>----------------</td>
<td>----</td>
</tr>
<tr>
<td>Couch (2017) 46</td>
<td>0.9% in breast cancer patients referred for testing</td>
<td>2.78 (2.22-3.62)</td>
<td>NR</td>
</tr>
<tr>
<td>Kurian (2017) 29</td>
<td>0.92% in breast cancer patients referred for testing</td>
<td>1.74 (1.46-2.07)</td>
<td>NR</td>
</tr>
</tbody>
</table>

CI: confidence interval; NR: not reported; OR: odds ratio; RR: relative risk.

Section Summary: Clinically Valid

*ATM* heterozygotes appear to have an RR of breast cancer from 2 to 3 times that of the general population, with an estimated absolute risk of 6% by age 50 and 33% by age 80, although estimates come from the population- and family-based case-controls, which are subject to bias and overestimation.

Clinically Useful

A test is clinically useful if the use of the results informs management decisions that improve the net health outcome of care. The net health outcome can be improved if patients receive correct therapy, or more effective therapy, or avoid unnecessary therapy, or avoid unnecessary testing.

Direct Evidence

Direct evidence of clinical utility is provided by studies that have compared health outcomes for patients managed with and without the test. Because these are intervention studies, the preferred evidence would be from randomized controlled trials.

Direct evidence of clinical utility for genetic testing in individuals with *ATM* variants was not identified.

Weidner et al (2020) conducted a retrospective, consecutive study on 56 *ATM* carriers enrolled in the Inherited Cancer Registry (ICARE) at Vanderbilt University and their relatives. Eligibility for annual breast MRI surveillance was based on ≥20% lifetime risk of breast cancer based on family cancer history alone as calculated by the BOADICEA predictive model, or family cancer history and proband *CHEK2* variant status, utilizing an updated version of the BOADICEA model (BWA v4). Among the *ATM* carriers and family history alone, 24 first-degree relatives (FDRs) (22.6%) and 15 second-degree relatives (SDRs) (13.6%) had a lifetime cancer risk ≥20%. Inclusion of the proband’s variant status significantly increased identification of FDRs to 60 (56.6%; \( P < 0.0001 \)) and SDRs to 31 (28.1%; \( P < 0.0001 \)), respectively. While the study revealed that family history alone may be insufficient to appropriately identify at-risk FDRs and SDRs of *ATM* carriers, the study authors note that the expanded BOADICEA predictive model (BWA v4) is not intended for clinical use. Additionally, this version has not been licensed for commercial use. Additional study limitations include retrospective study design, lack of clarity regarding to what extent study participants met society criteria for genetic testing for breast cancer risk, and no report of outcomes associated with enhanced screening for *ATM* variant carriers.

As outlined in the section on *PALB2*, for women with high-risk hereditary cancer syndromes, interventions to decrease breast cancer risk in high-risk women include screening (e.g.,
starting at an early age, the addition of magnetic resonance imaging to mammography, and screening annually), chemoprevention, prophylactic mastectomy, and prophylactic oophorectomy. In contrast to the case of PALB2, where the penetrance approaches that of a BRCA variant, there is unlikely to be a similar benefit-to-risk calculus for preventive interventions in women with an ATM variant. Surveys assessing adherence to guideline-based recommendations have explored this relationship but are limited in sample size and generally have not reported variant-stratified long-term outcomes of prophylactic or preventative interventions in controlled studies to support standard actionable thresholds for ATM.\textsuperscript{53,54} Findings from a study by Cragun et al (2020) point to potential overtreatment through risk-reducing bilateral mastectomy among those with ATM/CHEK2 variants, with over half of all carriers reporting use of prophylactic surgery independent of family history or personal breast cancer history.\textsuperscript{55}

**Section Summary: ATM and Breast Cancer Risk Assessment**

Updated predictive models utilizing information on ATM status for enhanced screening have not been approved for widespread clinical use. No evidence is available to support the clinical utility of genetic testing for ATM variants in breast cancer patients to guide patient management, and there is no strong chain of evidence supporting ATM testing in breast cancer patients.

**SUMMARY OF EVIDENCE**

For individuals with a risk of hereditary breast/ovarian cancer who receive genetic testing for a PALB2 variant, the evidence includes studies of clinical validity and studies of breast cancer risk, including a meta-analysis. The relevant outcomes are OS, disease-specific survival, and test validity. Evidence supporting clinical validity was obtained from numerous studies reporting RR or ORs (two studies estimated penetrance). Study designs included family segregation, kin-cohort, family-based case-control, and population-based case-control. The number of pathogenic variants identified in studies varied from 1 (founder mutations) to 48. The RR for breast cancer associated with a PALB2 variant ranged from 2.3 to 13.4, with the 2 family-based studies reporting the lowest values. Evidence of preventive interventions in women with PALB2 variants is indirect, relying on studies of high-risk women and BRCA carriers. These interventions include screening with magnetic resonance imaging, chemoprevention, and risk-reducing mastectomy. Given the penetrance of PALB2 variants, the outcomes following bilateral and contralateral risk-reducing mastectomy examined in women with a family history consistent with hereditary breast cancer (including BRCA1 and BRCA2 carriers) can be applied to women with PALB2 variants—with the benefit-to-risk balance affected by penetrance. In women at high-risk of hereditary breast cancer who would consider risk-reducing interventions, identifying a PALB2 variant provides a more precise estimated risk of developing breast cancer compared with family history alone and can offer women a more accurate understanding of benefits and potential harms of any intervention. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with risk of hereditary breast/ovarian cancer who receive genetic testing for a CHEK2 variant, the evidence includes studies of variant prevalence and studies of breast cancer risk. The relevant outcomes are OS, disease-specific survival, and test validity. The available studies on clinical validity have demonstrated that CHEK2 variants are of moderate penetrance, with lower RR for breast cancer than PALB2, and confer a risk of breast cancer two to four times that of the general population. Direct evidence for the clinical utility of genetic
testing for \textit{CHEK2} variants in individuals with risk of hereditary breast/ovarian cancer was not identified. It is unclear the RR associated with the moderate penetrance variants other than \textit{PALB2} would increase risk enough beyond that already conferred by familial risk to change screening behavior. In contrast to the case of \textit{PALB2}, where the penetrance approaches that of a \textit{BRCA} variant, there is unlikely to be a similar benefit-to-risk calculus for risk-reducing mastectomy in women with a \textit{CHEK2} variant. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals with risk of hereditary breast/ovarian cancer who receive genetic testing for an \textit{ATM} variant, the evidence includes studies of variant prevalence and studies of breast cancer risk. The relevant outcomes are OS, disease-specific survival, and test validity. The available studies on clinical validity have demonstrated that \textit{ATM} variants are of moderate penetrance, with lower RR for breast cancer than \textit{PALB2}; moreover, \textit{ATM} variants confer a risk of breast cancer two to four times that of the general population. Direct evidence for the clinical utility of genetic testing for \textit{ATM} variants in individuals with risk of hereditary breast/ovarian cancer was not identified. It is unclear that the RR associated with the moderate penetrance variants-other than \textit{PALB2}-would increase risk enough beyond that already conferred by familial risk to change screening behavior. In contrast to the case of \textit{PALB2}, where the penetrance approaches that of a \textit{BRCA} variant, there is unlikely to be a similar benefit-to-risk calculus for preventive interventions in women with an \textit{ATM} variant. The evidence is insufficient to determine the effects of the technology on health outcomes.

**SUPPLEMENTAL INFORMATION**

**Clinical Input From Physician Specialty Societies and Academic Medical Centers**

While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted.

In response to requests, input was received from 5 specialty societies and 2 academic medical centers (total of 7 reviewers) while this policy was under review in 2014. The input was limited on whether \textit{PALB2} testing to estimate the risk of developing breast cancer should be medically necessary, and whether testing results alter patient management. Reviewer input on both questions was mixed.

**Practice Guidelines and Position Statements**

**American College of Radiology**

The American College of Radiology (ACR) has established Appropriateness Criteria® for breast cancer screening.\textsuperscript{58} This includes high-risk women with a BRCA gene mutation and their untested first-degree relatives, women with a history of chest irradiation between 10 to 30 years of age, and women with 20% or greater lifetime risk of breast cancer as follows:

Table 13. ACR Appropriateness Criteria for Breast Cancer Screening in High-Risk Women

<table>
<thead>
<tr>
<th>Screening Procedure</th>
<th>Appropriateness Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammography</td>
<td>Usually appropriate</td>
</tr>
<tr>
<td>DBT</td>
<td>Usually appropriate</td>
</tr>
</tbody>
</table>
Specific recommendations for *PALB2*, *CHEK2*, or *ATM* variant carriers are not available.

**American Society of Breast Surgeons**
A consensus guideline on genetic testing for hereditary breast cancer was updated in February 2019. Guidelines state that genetic testing should be made available to all patients with a personal history of breast cancer and that such testing should include *BRCA1/BRCA2* and *PALB2*, with other genes as appropriate for the clinical scenario and patient family history. Furthermore, patients who had previous genetic testing may benefit from updated testing. Finally, genetic testing should be made available to patients without a personal history of breast cancer when they meet National Comprehensive Cancer Network (NCCN) guideline criteria. The guidelines also note that variants of uncertain significance are not clinically actionable. For patients with mutations in *ATM* and *CHEK2*, enhanced screening may be recommended, however, the data are not sufficient to support risk-reducing mastectomy in the absence of other factors such as strong family history.

**American Society of Clinical Oncology**
In a policy statement update on genetic and genomic testing for cancer susceptibility, the American Society of Clinical Oncology (2015) stated that testing for highly penetrant variants in appropriate populations has clinical utility in that variants inform clinical decision making and facilitate the prevention or amelioration of adverse health outcomes. The update noted: "Clinical utility remains the fundamental issue with respect to testing for variants in moderate penetrance genes. It is not yet clear whether the management of an individual patient or his or her family should change based on the presence or absence of a variant. There is insufficient evidence at the present time to conclusively demonstrate the clinical utility of testing for moderate penetrance variants, and no guidelines exist to assist oncology providers."

**National Comprehensive Cancer Network**
The National Comprehensive Cancer Network (v.1.2020) guidelines on genetic/familial high-risk assessment for breast and ovarian cancer review single-gene tests for *PALB2*, *CHEK2*, or *ATM*. The guidelines state that for those that meet hereditary cancer testing criteria, testing for a specific familial pathogenic/likely pathogenic variant may be recommended for appropriate genes. For patients with who meet criteria with no known familial variants, comprehensive testing a multigene panel may be considered. This testing may consider a number of genes, including but not limited to *PALB2*, *CHEK2*, and *ATM*. However, the inclusion of certain genes in the guideline does not imply the endorsement “for or against multigene testing for moderate-penetrance genes” and that there are limited data on the degree of cancer risk associated with some genes in multigene panels. The guidelines state that the panel recommends an annual mammogram for women with mutated *PALB2* gene beginning at age 30 and with mutated *ATM* or *CHEK2* gene beginning at age 40 with consideration of annual breast MRI.
The National Comprehensive Cancer Network guidelines on breast cancer screening and diagnosis (v.1.2019)\textsuperscript{61}, and on genetic/familial high-risk assessment for breast and ovarian cancer (v.1.2020)\textsuperscript{3}, recommend the following:

- Annual mammogram.
- Annual breast magnetic resonance imaging if the patient has >20% risk of breast cancer based on models largely dependent on family history.
- Consideration of a risk-reducing mastectomy based on family history.

The guidelines also state there is insufficient evidence to draw conclusions on risk-reducing mastectomy in individuals with \textit{CHEK2}, or \textit{ATM} and that patients should be managed based on family history. For patients with \textit{PALB2}, the option of a risk-reducing mastectomy should be discussed.

**Ongoing and Unpublished Clinical Trials**

Some currently unpublished trials that might influence this review are listed in Table 14.

**Table 14. Summary of Key Trials**

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td>Implementation of a Model for Personalized Risk-Based Breast Cancer Prevention and Screening</td>
<td>28,389</td>
<td>Dec 2020</td>
</tr>
<tr>
<td>NCT03989258</td>
<td>Enabling a paradigm shift: a preference-tolerating RCT of personalized vs. annual screening for breast cancer (Wisdom Study)</td>
<td>100,00</td>
<td>Dec 2020</td>
</tr>
<tr>
<td>NCT02620852</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NCT: national clinical trial.

**Government Regulations**

**National:**

There is no national coverage determination.

**Local:**

There is no local coverage determination.

\textit{(The above Medicare information is current as of the review date for this policy. However, the coverage issues and policies maintained by the Centers for Medicare & Medicare Services [CMS, formerly HCFA] are updated and/or revised periodically. Therefore, the most current CMS information may not be contained in this document. For the most current information, the reader should contact an official Medicare source.)}

**Related Policies**

- Genetic Testing for BRCA1 or BRCA2 for Hereditary Breast/Ovarian Cancer Syndrome
- Genetic Cancer Susceptibility Panel Using Next-Generation Sequencing
- Genetic Testing SNV to Predict Risk of Nonfamilial Breast Cancer
• Genetic Testing-Expanded Molecular Panel Testing of Cancers to Identify Targeted Therapies
• Genetic Testing and Counseling
• Genetic Testing for CHEK2 Mutations for Breast Cancer (retired)

References


The articles reviewed in this research include those obtained in an Internet based literature search for relevant medical references through January 2020, the date the research was completed.
### Joint BCBSM/BCN Medical Policy History

<table>
<thead>
<tr>
<th>Policy Effective Date</th>
<th>BCBSM Signature Date</th>
<th>BCN Signature Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1/20</td>
<td>10/15/19</td>
<td></td>
<td>Joint policy established</td>
</tr>
<tr>
<td>5/1/20</td>
<td>2/18/20</td>
<td></td>
<td>Added codes 81307 and 81308 as established, effective 1/1/20.</td>
</tr>
<tr>
<td>5/1/21</td>
<td>2/16/21</td>
<td></td>
<td>Routine policy maintenance, added references # 28, 51-52 and 54-55. No change in policy status.</td>
</tr>
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</table>

Next Review Date: 1st Qtr. 2022

### Pre-Consolidation Medical Policy History

<table>
<thead>
<tr>
<th>Original Policy Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCN:</td>
<td>Revised:</td>
</tr>
<tr>
<td>BCBSM:</td>
<td>Revised:</td>
</tr>
</tbody>
</table>
I. Coverage Determination:

<table>
<thead>
<tr>
<th>Plan Description</th>
<th>Coverage Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial HMO (includes Self-Funded groups unless otherwise specified)</td>
<td>Covered per policy</td>
</tr>
<tr>
<td>BCNA (Medicare Advantage)</td>
<td>See government section</td>
</tr>
<tr>
<td>BCN65 (Medicare Complementary)</td>
<td>Coinsurance covered if primary Medicare covers the service.</td>
</tr>
</tbody>
</table>

II. Administrative Guidelines:

- The member’s contract must be active at the time the service is rendered.
- Coverage is based on each member’s certificate and is not guaranteed. Please consult the individual member’s certificate for details. Additional information regarding coverage or benefits may also be obtained through customer or provider inquiry services at BCN.
- The service must be authorized by the member’s PCP except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Services must be performed by a BCN-contracted provider, if available, except for Self-Referral Option (SRO) members seeking Tier 2 coverage.
- Payment is based on BCN payment rules, individual certificate and certificate riders.
- Appropriate copayments will apply. Refer to certificate and applicable riders for detailed information.
- CPT - HCPCS codes are used for descriptive purposes only and are not a guarantee of coverage.