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Breast cancer epidemiology Indian perspective

87,090 (12.11%) deaths due to breast cancer

No:1 cancer in terms of incidence, prevalence and mortality

Cancer is a genetic disease although mostly not heritable.
Germline vs Somatic Genetics

- **Germline – the genes you are born with (Inherited)**
  - Can be passed on to relatives.
  - Does not mean that disease will happen.
  - Increased risk of disease.
  - There is no one “breast cancer gene”

- **Somatic – changes in tumors that are acquired over time.**
- **(Combination of Environmental & Genetic Factors)**
  - Can not pass on to relatives
  - Can be tested as part of decision making for therapy for cancer
# GERMLINE SUSCEPTIBILITY

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Susceptibility</th>
<th>Germline alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary</td>
<td>Strong</td>
<td>High/moderate penetrance, one gene</td>
</tr>
<tr>
<td>Familial</td>
<td>Modest</td>
<td>Low penetrance, many genes</td>
</tr>
<tr>
<td>Sporadic</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

![Pie chart showing germline susceptibility](chart.png)
HEREDITARY VS SPORADIC CANCER

• Hereditary cancers, as compared to corresponding sporadic cancers, tend to be characterised by
  • Earlier onset
  • Multiple primary tumours
  • Family history of same cancers in relatives

• Consistent with a first, germline mutation
  • Already present at birth (hence earlier onset)
  • In all cells of the body (hence multiple primaries in susceptible tissues)
  • Including germ line of the patient (hence heritable in relatives)

• Cf Knudson model of retinoblastoma*
Genetics: Cancer Risk Variants

- **Common Variants**
- **Single nucleotide polymorphisms**
- **Rare variants (moderate)**
  - CHEK2, ATM, NBN
- **Rare variants (high)**
  - BRCA1, BRCA2, TP53
Inherited cancer syndromes

- Autosomal dominant
  - Li Fraumeni syndrome
- Familial (BRCA1&BRCA2)
- Autosomal recessive
  - Ataxia Telengectasia
Associated Gene Mutations in Breast Cancer.

**BRCA mutations**
- Early-onset breast cancer
- Triple-negative breast cancer
- Bilateral breast cancer
- Family history of breast cancer

**Non BRCA mutations**
- Li-Fraumeni syndrome
- Cowden disease
- Peutz-Jeghers syndrome

Most documented genes:
- TP53
- PTEN
- SKT-11

BRCA mutations
Types of BRCA mutations in Hereditary Breast and Ovarian Cancer (HBOC)
BRCA1 Gene (17q21)

- Responsible for up to 1/2 of "inherited" breast cancers (5% of cancers)

- Increased risk of ovarian and colon cancers ("Breast-Ovarian" cancer gene)

- Breast cancer develops in >50% of these women by age 50 ("Early onset" breast cancer gene). Lifetime risk of breast cancer is 85%.

- Majority are Triple negative.

- 20% are ER/PR positive while approx 03% are Her-2 positive/amplified.
BRCA2 Gene (13q)

- Responsible for up to 70% of inherited breast cancer **NOT** due to BRCA1 (3.5% of cancers)

- Characterized by increased risk of breast cancer in women and MALE breast cancer (“Male Breast Cancer” gene)

- 30-40% lifetime risk of breast cancer.

- Unlike BRCA1; ER/PR positivity is similar to sporadic cancer, while Her-2 positivity is similar to that of BRCA1(3%)
### BRCA1/2-associated cancers: lifetime risk

#### LIFETIME CANCER RISKS
**KUCHENBÄECKER, 2017**

<table>
<thead>
<tr>
<th>Cancer</th>
<th>General population</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>11%</td>
<td>50-85%</td>
<td>50-85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65-79%</td>
<td>61-77%</td>
</tr>
<tr>
<td>OC</td>
<td>1.4%</td>
<td>20-50%</td>
<td>10-30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36-53%</td>
<td>11-25%</td>
</tr>
<tr>
<td>2nd BC (20y risk)</td>
<td>n/a</td>
<td>35-45%</td>
<td>20-33%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>3%</td>
<td>3%</td>
<td>6%</td>
</tr>
<tr>
<td>Prostate</td>
<td>13%</td>
<td>13%</td>
<td>26%</td>
</tr>
</tbody>
</table>
BRCA mutations increase the risk of developing cancer

In the general population, approximately 12% of women will develop breast cancer in their lifetime. In comparison, 55-65% of women carrying a BRCA1 mutation and ~45% of women carrying a BRCA2 mutation will develop breast cancer by age 70.

**Lifetime risk of developing breast and ovarian cancer (%)**

- **Breast cancer**
  - By age 70
  - By age 50
  - Second cancer
  - Male breast cancer

- **Ovarian cancer**
  - By age 70
  - By age 50
  - Secondary to breast cancer
PENETRANCE OF THE BRCA GENE DEFECT

Estimated Risk of Breast Cancer

Estimated Risk of Ovarian Cancer

Breast cancer in BRCA1 carrier:
✓ Typically triple negative (TNBC)
✓ 2-3 recurrence /100 women/year
✓ Shorter interval if earlier onset

Ovarian cancer in BRCA1/2 carrier:
✓ Serous, papillary epithelioma
✓ Most tumours start in tubes
✓ No efficient surveillance tools

BRCA mutations

**Subtypes:**
BRCA1 and BRCA2.

**Other associated cancers:**
Ovaries, uterine tubes, male breast cancers, pancreatic & melanoma

**Increase risk:**
Ashkenazi Jewish, hispanic individuals.

**Indications for genetic testing in women with and without cancer**

**Guidelines by:**
American Society of Breast Surgeons (ASBrS)
The National Comprehensive Cancer Network (NCCN)
US Preventive Services Task Force (USPSTF)

**Basis of recommendation:**
- Family history of BRCA1 and BRCA2
- Early onset breast cancer
- 2 or more primary cancers
- Ashkenazi Jewish heritage


NON- BRCA mutations
# Non BRCA mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Location</th>
<th>Gene Function</th>
<th>Hereditary Syndrome; Associated Cancers</th>
<th>Associated Lifetime Risk for BC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>TP53</em></td>
<td>17p13.1</td>
<td>Tumor suppressor gene (cell growth regulator)</td>
<td>Li-Fraumeni syndrome; BC, adrenocortical carcinomas, brain cancer, leukemias, sarcomas</td>
<td>90</td>
</tr>
<tr>
<td><em>PTEN</em></td>
<td>10a23.3</td>
<td>Phosphatase tension homologue; specific function unclear; mutation associated with improper cell cycle arrest</td>
<td>Cowden disease; BC, disseminated benign and malignant hamartomas, endometrial and thyroid cancers</td>
<td>~ 50</td>
</tr>
<tr>
<td><em>SKT-11</em></td>
<td>19p13.3</td>
<td>Tumor suppressor gene; associated with apoptosis; also negative regulator of mTOR pathway</td>
<td>Peutz-Jeghers syndrome; BC, ovarian, pancreatic, gastric, small intestine, and colorectal cancers</td>
<td>~ 50</td>
</tr>
<tr>
<td><em>CDH1</em></td>
<td>16q22.1</td>
<td>Epithelial cell-cell adhesion molecule</td>
<td>Hereditary diffuse gastric cancer; BC</td>
<td>39</td>
</tr>
<tr>
<td><em>MLH1, MSH2, MSH6, PMS2</em> (MMR genes)</td>
<td>3p22.2, 2p21-p16, 2p16.3, 7p22.1</td>
<td>DNA mismatch repair</td>
<td>Lynch syndrome; BC, ovarian, endometrial, stomach, and colorectal cancers</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Non BRCA mutations

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<thead>
<tr>
<th>Gene</th>
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<th>Gene Function</th>
<th>Hereditary Syndrome; Associated Cancers</th>
<th>Associated Lifetime Risk for BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEK2 (checkpoint kinase 2)</td>
<td>22q12</td>
<td>Serine threonine kinase associated with DNA double-strand break repair; also phosphorylates BRCA1</td>
<td>BC</td>
<td>37%</td>
</tr>
<tr>
<td>ATM</td>
<td>11q22</td>
<td>Associated with DNA double-strand break repair and cell cycle progression</td>
<td>Ataxia telangiectasia; BC</td>
<td>3- to 5-Fold increased risk</td>
</tr>
<tr>
<td>PALB2, BRIP1</td>
<td>16p12.2,17q23.2</td>
<td>PALB2: binding partner and localizer of BRCA2 associated with DNA homologous recombination repair; BRIP1: encodes protein serving as binding partner of BRCA1</td>
<td>Fanconi anemia; BC, solid tumors, leukemias; PALB2: various additional cancers</td>
<td>2-Fold increased risk</td>
</tr>
<tr>
<td>RAD51C</td>
<td>17q22</td>
<td>Associated with DNA double-strand break repair via homologous recombination and Fanconi anemia, BRCA pathway</td>
<td>BC</td>
<td>NA</td>
</tr>
<tr>
<td>RAD51D</td>
<td>17q12</td>
<td>Associated with DNA double-strand break repair via homologous recombination</td>
<td>BC</td>
<td>No statistically significant increased risk</td>
</tr>
<tr>
<td>BARD1</td>
<td>2q35</td>
<td>Associated with DNA double-strand break repair via homologous recombination; also interacts with BRCA1</td>
<td>BC</td>
<td>Conflicting data</td>
</tr>
<tr>
<td>MRE11, RAD50, NBS1 (MRN complex)</td>
<td>11q21,5q.31.1, 8q21.3</td>
<td>Associated with DNA double-strand break repair</td>
<td>MRE11: ataxia telangiectasia-like disorder; weak association with BC; RAD50: Nijmegen breakage syndrome-like disorder, weak association with BC; NBS1: Nijmegen breakage syndrome, association with BC</td>
<td>2- to 3-Fold increased risk (limited evidence)</td>
</tr>
<tr>
<td>FANCM</td>
<td>14q21</td>
<td>Associated with DNA repair</td>
<td>Fanconi anemia; BC, solid tumors, leukemias</td>
<td>Increased risk (not quantified)</td>
</tr>
</tbody>
</table>

**NCCN:** Individuals who test negative for BRCA1 and BRCA2 and are suspected of having 1 or more inherited syndromes may be considered for multi-gene assessment for efficiency and cost-effectiveness

Cowden Disease

- mutation in PTEN (601728 OMIM), a phosphate tensin homologue located at 10q23.3
- > 1% of breast cancer diagnoses.
- Lifetime risk of breast cancer is approximately 50%
- Other associated cancers: disseminated benign and malignant hamartomas, endometrial and thyroid cancer, and mucous membrane lesions.

Screening criteria

- Family history of PTEN mutations
- Various combinations of
  - major criteria (eg: breast cancer, macrocephaly, or follicular thyroid carcinoma)
  - minor criteria (eg: nonmalignant thyroid lesions, colon cancer, or autism spectrum disorder)
Germline genetic screening as a paradigm for individualized care

- Risk Assessment.
- Disease Prevention.
- Therapeutics.

*BRCA1/2* as the prototype
CANCER PATIENT TRAJECTORY WITH GENETIC COUNSELLING (GC)

Suspected hered c.
- Personal history
- Family history

Pre-test GC
- Family tree
- Inform patient about outcomes of genetic test
- Consent signed.

Genetic Test
- Blood sample
- DNA analysis

Post-test GC
- Give results + interpretation
- Family issues
- Psychological support
Pre-test counseling includes:

- Collection of a comprehensive family history
  - Note that when assessing family history, close blood relatives include first-, second-, and third-degree relatives on each side of the family (See BR/OV-B)
- Evaluation of a patient's cancer risk
- Generating a differential diagnosis and educating the patient on inheritance patterns, penetrance, variable expressivity, and the possibility of genetic heterogeneity
- Preparing the patient for possible outcomes of testing including positive (pathogenic, likely pathogenic), negative, and uncertain findings and obtaining informed consent
RISK ASSESSMENT MODELS

- Computerised algorithms
- Breast Cancer Risk Assessment Tool (BCRAT)
- (Gail model) BRCAPRO
- IBIS
- BOADICEA
- Ontario Family History Assessment Tool

USPSTF recognizes that each risk assessment tool has limitations and found insufficient evidence to recommend one tool over another.

- Help in deciding
- whether to test or not to test for gene mutation
- What surveillance and prevention, e.g. breast MRI, especially if no mutation found

- Case-by-case pedigree-based analysis remains mandatory
RISK FACTORS USED IN THE MODIFIED GAIL MODEL, AGE ≥35 YEARS

- Current age
- Age at menarche
- Age at first live birth or nulliparity
- Number of female first-degree relatives with breast cancer
- Number of previous benign breast biopsies
- Atypical hyperplasia in a previous breast biopsy
- Race

## Pedigree Assessment Tool

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer at age $\geq 50$ y</td>
<td>3</td>
</tr>
<tr>
<td>Breast cancer at age $&lt; 50$ y</td>
<td>4</td>
</tr>
<tr>
<td>Ovarian cancer at any age</td>
<td>5</td>
</tr>
<tr>
<td>Male breast cancer at any age</td>
<td>8</td>
</tr>
<tr>
<td>Ashkenazi Jewish heritage</td>
<td>4</td>
</tr>
</tbody>
</table>
PEDIGREE: SYMBOLS USED

- Normal male, female
- Sex unknown
- Points to proband
- Affected male, female
- Abortion or stillbirth
- Female carrier (heterozygous) for x-linked trait
- Pregnancy
- Adopted
- Two normal males and three normal female sibs
- Sibs in chronological order of birth
- Consanguineous marriage
- Illegitimacy
- Marriage
- No offspring
- Monozygotic twins
- Dizygotic twins
- Zygosity uncertain
PEDIGREE FIRST, SECOND AND THIRD RELATIVES OF PROBAND

PEDIGREE: FIRST-, SECOND-, AND THIRD-DEGREE RELATIVES OF PROBAND

- Paternal grandfather
- Paternal grandmother
- Maternal grandfather
- Maternal grandmother
- Great aunt
- Great uncle
- Aunt
- Father
- Mother
- Uncle
- First cousin (male)
- Sister
- Nephew
- Niece
- Brother
- Son
- Daughter
- Granddaughter
- Grandson

Proband
### Results

#### Classification

1. Positive for a deleterious mutation
2. Genetic variant
3. No deleterious mutation

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for a deleterious mutation</td>
<td>Mutation deemed to be clinically relevant; should prompt discussion regarding risk-reducing strategies and/or therapy</td>
</tr>
<tr>
<td>Genetic variant</td>
<td>Varied clinical significance</td>
</tr>
<tr>
<td>Suspected deleterious</td>
<td>Mutation likely, but not proven to be, deleterious</td>
</tr>
<tr>
<td>Favor polymorphism</td>
<td>Mutation will likely not augment breast cancer risk to great extent</td>
</tr>
<tr>
<td>Uncertain significance</td>
<td>Mutation lacks documented clinical significance</td>
</tr>
<tr>
<td>Specific variant/mutation not identified</td>
<td>Mutation or variant not detected in individual</td>
</tr>
<tr>
<td>No deleterious mutation</td>
<td>Negative test; does not predispose the individual to developing BRCA-associated breast cancer but does not guarantee an absent risk of breast cancer due to other genes</td>
</tr>
</tbody>
</table>

Assuming a 10% probability of a positive test result, the likelihood of an incorrect result is reported as <1%
**GENETIC VARIANTS**

**VUS = Variant of Uncertain clinical Significance**


- Most are expected to be harmless, statistically

VUS classification will require epidemiology of variant and/or functional data (bioinformatics, machine learning approach)

<table>
<thead>
<tr>
<th>VARIANT type</th>
<th>Frequency</th>
<th>Penetrance (functional effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Rare</td>
<td>High</td>
</tr>
<tr>
<td>VUS</td>
<td>Rare</td>
<td>??</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>Frequent</td>
<td>Low or null</td>
</tr>
<tr>
<td>« Rare polymorphism »</td>
<td>Rare</td>
<td>Low or null</td>
</tr>
</tbody>
</table>
HOW TO HANDLE VUS?

- Do not report variant if no clear evidence that it is disease-causing
- Protein-truncating mutation, and/or
- Already reported in other patients
- In-house data
- Publically available databases, e.g. ClinVar-NCBI
- Periodically re-assess VUS for reclassification as benign (most) or disease-causing (few) in large databases
- And recall patients to the clinic for update if disease-causing
Post-test counseling includes discussions of:
- Results along with their significance and impact and recommended medical management options
- Interpretation of results in context of personal and family history of cancer
- Informing and testing at-risk family members
- Available resources such as disease-specific support groups and research studies
Genetic Tests for Breast cancers

INDICATIONS

Genetic testing is a powerful tool that allows for BRCA and non-BRCA germline mutations in high risk individuals.
Indications for patients with a personal h/o breast cancer

ASBrS Guidelines (September 2016)\(^2\)
1. Age 50 years or younger at time of BC onset
2. Triple-negative BC and age 60 years or younger
3. Two or more primary BCs (including asynchronous, synchronous, bilateral, or multicentric)
4. First-degree relative with BC at 50 years or younger
5. Two relatives on same side of family with BC and/or pancreatic cancer
6. Family or personal history of ovarian cancer, fallopian cancer, or primary peritoneal cancer
7. Male BC
8. Ashkenazi Jewish heritage and family history of BC at any age
9. Family member with a known mutation

NCCN Guidelines (December 2016)\(^3\)
1. BC diagnosis at 50 years or younger
2. Two or more primary BCs (including bilateral tumors or ≥2 clearly separate ipsilateral tumors, synchronous or asynchronous), with the first at 50 years or younger
3. BC diagnosis at 50 years or younger with 1 or more close relatives\(^4\) with BC at any age, pancreatic cancer, or prostate cancer (Gleason score ≥7), or with a limited/unknown family history
4. Triple-negative BC at 60 years or younger
5. BC at any age with 1 or more close relatives with BC at 50 years or younger
6. BC at any age with 2 or more close relatives with BC at any age
7. BC at any age with 1 or more close relatives with ovarian carcinoma (including fallopian tube and primary peritoneal cancer) at any age
8. BC at any age with 2 or more close relatives with pancreatic cancer and/or prostate cancer (Gleason score ≥7) at any age
9. Close relative with male BC
10. Personal history of BC and Ashkenazi Jewish heritage (no other family history required)
11. Personal history of ovarian carcinoma
12. Personal history of male BC

USPSTF Guidelines (February 2014)

Excludes

- Patients post diagnosis of breast cancer.
- Men with breast cancer.

Indications for patients without a personal h/o breast cancer

ASBrS Guidelines (September 2016)\textsuperscript{12}
1. First- or second-degree relative with BC, onset at 45 years or younger (early-age onset)
2. Two or more primary BCs (includes asynchronous, synchronous, bilateral, or multicentric) in 1 relative
3. Two or more relatives on the same side of the family with BC and/or pancreatic cancer
4. Family or personal history of ovarian cancer, fallopian cancer, or primary peritoneal cancer
5. Male BC
6. Ashkenazi Jewish heritage and family history of BC at any age
7. Family member with a known mutation

NCCN Guidelines (December 2016)\textsuperscript{3, a}
1. Presence of known deleterious BRCA1 or BRCA2 germline mutation in family
2. Presence of BRCA1 or BRCA2 mutation in tumor profiling without germline mutation
3. Personal history of ovarian carcinoma
4. Ashkenazi Jewish heritage and personal history of pancreatic cancer
5. Personal history of prostate cancer (Gleason score $\geq 7$) at any age with 1 or more close relatives\textsuperscript{5} with ovarian carcinoma at any age, or BC at 50 years or younger, or 2 close relatives with BC, pancreatic cancer, or prostate cancer (Gleason score $\geq 7$) at any age
6. Family history only\textsuperscript{5}:
   a. First- or second-degree relative with any criteria listed above
   b. Third-degree relative who has BC and/or ovarian carcinoma and who has 2 or more family members with BC (at least 1 with BC at 50 years or younger) and/or ovarian carcinoma

USPSTF Guidelines (February 2014)\textsuperscript{4}
1. Family history\textsuperscript{a} of BC and ovarian cancer
2. Family history of bilateral BC
3. Family history of BC diagnosis at younger than 50 years
4. Multiple cases of BC in family
5. At least 1 family member with 2 primary cancers associated with a BRCA mutation
6. At least 1 male family member with BC
7. Ashkenazi Jewish heritage

SOME GENERAL INDICATIONS FOR TESTING

HBOC suspected any of the following:
• TNBC < 50 years
• Ovarian epithelial, serous, high grade cancer Breast and ovarian cancer, any age
• 2 (first-degree) relatives with breast cancer
• before 50 years
• Breast cancer <50 years and (first degree) relative with ovarian cancer

Lynch suspected any of the following
Amsterdam criteria
3 affected, over 2 generations, 1 < 50 yrs. (Giardiello FM, et al. 2001 Gastroenterology)

Bethesda criteria
If present, test tumour for MSI (DNA analysis; IHC)
If MSI+, test patient for germ-line MMR gene mutation

With room for clinical judgement in deciding to test or not to test
Underdiagnosis of Hereditary Breast Cancer: Are Genetic Testing Guidelines a Tool or an Obstacle?
BRCA1/2 TESTING CRITERIAa,b

Meeting one or more of these criteria warrants further personalized risk assessment, genetic counseling, and often genetic testing and management. Testing of an individual without a cancer diagnosis should only be considered when an appropriate affected family member is unavailable for testing.

- Individual from a family with a known BRCA1/2 pathogenic/likely pathogenic variant, including such variants found on research testingb
- Personal history of breast cancera + one or more of the following:
  - Diagnosed ≤45 y
  - Diagnosed 46-50 y with:
    - An additional breast cancer primary at any aged
    - ≥1 close blood relative6 with breast cancer at any age
    - ≥1 close blood relative6 with high-grade (Gleason score ≥7) prostate cancer
    - An unknown or limited family historya
  - Diagnosed ≤50 y with:
    - Triple-negative breast cancer
  - Diagnosed at any age with:
    - ≥1 close blood relative6 with:
      - breast cancer diagnosed ≤50 y; or
      - ovarian carcinoma; or
      - male breast cancer; or
      - metastatic prostate cancer; or
      - pancreatic cancer
    - ≥2 additional diagnoses6 of breast cancer at any age in patient and/or in close blood relatives
    - Ashkenazi Jewish ancestryh
    - Personal history of ovarian carcinomaf

- Personal history of male breast cancer
- Personal history of pancreatic cancer
- Personal history of metastatic prostate cancer
g
- Personal history of high-grade prostate cancer (Gleason score ≥7) at any age with:
  - ≥1 close blood relatives6 with ovarian carcinoma, pancreatic cancer, or metastatic prostate cancer9 at any age or breast cancer <50 y; or
  - ≥2 close blood relatives6 with breast, or prostate cancer (any grade) at any age; or
  - Ashkenazi Jewish ancestryf
- BRCA1/2 pathogenic/likely pathogenic variant detected by tumor profiling on any tumor type in the absence of germline pathogenic/likely pathogenic variant analysis
- Regardless of family history, some individuals with an BRCA1/2-related cancer may benefit from genetic testing to determine eligibility for targeted treatmentf
- An individual who does not meet the other criteria but with ≥1 first- or second-degree blood relative meeting any of the above criteria. The significant limitations of interpreting test results for an unaffected individual should be discussed.
ASBrS Recent Recommendations

ASBrS RECOMMENDATIONS ON GENETIC TESTING

• 1. Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling, can make recommendations to their patients regarding genetic testing, and can arrange testing.

• 2. Genetic testing should be made available to all patients with a personal history of breast cancer.

• 3. Patients who have previously had genetic testing may benefit from updated testing.

• 4. Genetic testing should be made available to patients without a history of breast cancer who meet NCCN Guidelines.

• 5. Variants of uncertain significance are not clinically actionable.

Adapted from American Society of Breast Surgeons.¹ March 25, 2019
Practical consensus recommendation on when to do BRCA testing

Broad question title

Question 1 - Will you do BRCA testing in all breast cancers under age 40 years?

Question 2 - Will you do BRCA testing for sporadic postmenopausal triple negative breast cancer 55 years?

Question 3 - Will you go for extended germline mutation testing in triple negative 35-year-old female?

Question 4 - Will you do BRCA testing for postmenopausal breast cancer 60 years old with one maternal cousin having ovarian cancer
Will you do BRCA testing for postmenopausal breast cancer 60 years old with one maternal cousin having ovarian cancer?

Question 5 - Will you do BRCA testing for postmenopausal breast cancer 60 years with one paternal cousin having prostate cancer or pancreatic cancer?

Update in oncology-X-2017
### RECOMMENDATIONS BY THE PANEL

<table>
<thead>
<tr>
<th></th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The expert panel recommended not to do BRCA testing in all breast cancers under the age of 40 years.</td>
</tr>
<tr>
<td>2</td>
<td>BRCA testing should be done for all breast cancer patients above the age of 60 years.</td>
</tr>
<tr>
<td>3</td>
<td>Extended germline mutation testing (beyond BRCA) should be done for triple negative young patients with breast cancer so as not to miss out on other syndromes.</td>
</tr>
<tr>
<td>4</td>
<td>The expert panel recommended BRCA testing in breast cancer patients with maternal family history of ovarian cancer.</td>
</tr>
<tr>
<td>5</td>
<td>BRCA testing is recommended in selected cases with breast cancer who have paternal family history of prostate or pancreatic cancer (based on published guidelines).</td>
</tr>
</tbody>
</table>
BENEFITS OF A MOLECULAR DIAGNOSIS

• For the patient
  • Identify risk of cancer in other organ (ovary; uterus, CRC) for secondary prevention (BRCA; PTEN, and all syndromic BC genes; MMR genes)
  • Refine risk of recurrence (CHEK2, ATM)
  • Individualised drugs (olaparib in BRCA-linked ovarian cancer)
  • Individualised therapy (avoid radiotherapy in TP53)

• For the patient’s relatives
  • Prevention of cancer if mutation present (surveillance; surgery)
  • Reassurance (population risk; or really?) if mutation absent
  • Primary prevention in future offspring
  • (pregestational diagnosis, prenatal diagnosis)
Screening for BRCA mutated women

- Clinical Breast examination, beginning at age 25.
- Mammogram once per year, beginning at age 30.
- Breast MRI once per year, beginning at age 25.
- "Breast awareness," beginning at age 18, which involves paying attention to changes in breasts and may include regular breast self-exams.
### Table 1. Prevention and screening strategies for specific mutations

<table>
<thead>
<tr>
<th>Screening</th>
<th>Prevention/risk reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Li Fraumeni Syndrome</strong></td>
<td></td>
</tr>
<tr>
<td>- <em>p53</em> mutation</td>
<td></td>
</tr>
<tr>
<td>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</td>
<td>1) Avoid ionising radiation, e.g. CT</td>
</tr>
<tr>
<td>2) Annual breast MRI at age 20–75. If MRI is not available, mammography may be considered [V]</td>
<td>2) Consider offering PGD before pregnancies</td>
</tr>
<tr>
<td>3) Colonoscopy every 5 years from the age of 25 or as clinically indicated</td>
<td>3) Consider risk-reducing mastectomy</td>
</tr>
<tr>
<td>4) Annual dermatological and neurological examination</td>
<td></td>
</tr>
<tr>
<td>5) Consider annual whole-body MRI and 6-monthly complete blood count</td>
<td></td>
</tr>
<tr>
<td><strong>PTEN/Cowden Syndrome</strong></td>
<td></td>
</tr>
<tr>
<td>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</td>
<td>1) Consider risk-reducing mastectomy</td>
</tr>
<tr>
<td>2) Annual breast MRI and/or mammogram at age 30–75 [V]</td>
<td>2) Consider risk-reducing hysterectomy</td>
</tr>
<tr>
<td>3) Annual endometrial ultrasound ± biopsies from age 30–35</td>
<td>3) Consider offering PGD before pregnancies</td>
</tr>
<tr>
<td><strong>ATM mutation</strong></td>
<td></td>
</tr>
<tr>
<td>1) Consider annual breast MRI (no evidence regarding the age of onset)</td>
<td></td>
</tr>
<tr>
<td><strong>Lynch Syndrome</strong></td>
<td></td>
</tr>
<tr>
<td>- <em>MLH1, MSH2, MSH6, EPCAM</em> and <em>PMS2</em> mutations</td>
<td></td>
</tr>
<tr>
<td>1) Annual colonoscopy from age 20–25</td>
<td>1) Consider risk-reducing hysterectomy and RRSO after completion of childbearing</td>
</tr>
<tr>
<td>2) Annual neurological examination for screening of CNS tumours may be considered</td>
<td></td>
</tr>
<tr>
<td>3) Annual endometrial ultrasound ± biopsies from age 30–35 may be considered</td>
<td></td>
</tr>
<tr>
<td><strong>RAD51 mutation</strong></td>
<td></td>
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<tr>
<td><strong>BRIPI mutation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>PALB2 mutation</strong></td>
<td></td>
</tr>
<tr>
<td>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</td>
<td>1) Consider RRSO after the age of 45</td>
</tr>
<tr>
<td>2) Annual breast MRI from age 20–29</td>
<td>1) Consider RRSO after the age of 45</td>
</tr>
<tr>
<td>3) Annual breast MRI and/or mammogram at age 30–75 [V]</td>
<td>1) Consider risk-reducing mastectomy</td>
</tr>
</tbody>
</table>
**Prevention and screening in BRCA mutation carriers and other breast/ovarian hereditary cancer syndromes:**

ESMO Clinical Practice Guidelines for cancer prevention and screening

S. Paluch-Shimon¹, F. Cardoso², C. Sessa³, J. Balmana⁴, M. J. Cardoso², F. Gilbert⁵ & E. Senkus⁶, on behalf of the ESMO Guidelines Committee

<table>
<thead>
<tr>
<th>CHEK2 mutation</th>
<th>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2) Annual breast MRI from age 20–29</td>
</tr>
<tr>
<td></td>
<td>3) Annual breast MRI and/or mammogram at age 30–75 [V]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STK11 mutation (Peutz–Jeghers Syndrome)</th>
<th>1) Consider risk-reducing mastectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</td>
</tr>
<tr>
<td></td>
<td>2) Annual breast MRI from age 20–29</td>
</tr>
<tr>
<td></td>
<td>3) Annual breast MRI and/or mammogram at age 30–75 [V]</td>
</tr>
<tr>
<td></td>
<td>4) Upper endoscopy and colonoscopy every 2–3 years from late teens</td>
</tr>
<tr>
<td></td>
<td>5) Screening for pancreatic cancer with EUS or MRI from the age of 30</td>
</tr>
<tr>
<td></td>
<td>6) Annual testicular examination from childhood</td>
</tr>
<tr>
<td></td>
<td>7) Routine annual gynaecological surveillance</td>
</tr>
<tr>
<td></td>
<td>8) Counselling to reduce lung cancer risk</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CDH1 mutation</th>
<th>1) Consider risk-reducing mastectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1) Clinical breast examination every 6–12 months starting from age 20–25 [V]</td>
</tr>
<tr>
<td></td>
<td>2) Annual breast MRI from age 20–29</td>
</tr>
<tr>
<td></td>
<td>3) Annual breast MRI and/or mammogram at age 30–75 [V]</td>
</tr>
</tbody>
</table>

MRI, magnetic resonance imaging; CT, computed tomography; PGD, pre-implantation genetic diagnosis; CNS, central nervous system; RRSTO, risk-reducing salpingo-oophorectomy; EUS, endoscopic ultrasound.
GENETIC TESTS: History of test development

- Developed by Myriad Genetic Laboratories in 1999
- Patent protected predominant genetic test for BRCA1 and 2 till 2013
- In 2013: US supreme court ruled that genes were naturally occurring and cannot be patented
- University based gene mutation panel
- Other private laboratories:
  - Ambry genetics
  - Gene Dx
Test Limitations

• Sequences that can be read only in the forward or reverse direction.

• Less frequent polymorphisms.

• Inversions or regulatory mutations, and insertions without duplication will not be detected.

• Turn around time 1- several weeks

• A worldwide leader in the field of genetic diagnostics for rare hereditary diseases

• Cento MD (Mutation Database)
  • Bridges the gap between genetic variants and clinical interpretation
  • Follows American College of Medical Genetics and Genomics (ACMG) guidelines for variant classification
  • Access to more than 5.2 million variants, based on clinically diagnosed individuals worldwide.
  • Significant number (58%) of unpublished relevant variants from a worldwide cohort of patients.
CentoCard®

- A unique **CE-labeled filter card** product for sample collection
- Samples collected on CentoCard® are easy to handle
  - Dried samples are stable, can be mailed in regular post
  - Collected samples are not sensitive to temperature over time and not considered as biohazard
- Reusable for future analysis
- Barcode labeled filter cards, ensure accurate tracking and easy monitoring through CentoPortal.

- Genetic counselling services.
Genetic Diagnostics

Analysis scope

- Single/ Few Defined Variations
- Multiple/ Unknown Variations

Low resolution

- Cytogenetics
  - FISH
  - Karyotyping

High resolution

- Molecular Genetics
  - PCR
  - MLPA
  - Sanger Sequencing
  - Microarrays
  - Next Gen Sequencing
Single-marker molecular test

- Identifies only one gene (E.g., GATA2 in MDS)
- Other genes causing the same disease will not be identified
“Hot-spot”

- Multi-gene assay
- Includes the most commonly occurring mutations (E.g., TET2, Tp53, RUNX1, ASXL1 for MDS)
- Will miss the less commonly pathogenic mutation analysis
Comprehensive Gene Profiling

- Whole Genome / Exome
- Most of the pathogenic genetic alterations are identified
Sanger Sequencing

- Gold Standard sequencing method
- The Sanger method has **separate steps** for:
  - Sequencing,
  - Separation (by electrophoresis) and
  - Detection
- **Disadvantages**
  - Difficult to automate the sample preparation
  - Limited in throughput, scalability and resolution
Next Generation Sequencing (NGS)

- Also known as high-throughput sequencing

- Enables a broad range of applications:
  - Sequence DNA and RNA much more **quickly** and **cheaply**
  - Rapidly sequence whole genome / Exome
  - Zoom in to **deeply sequence target regions (increased read depth)**
  - Helps in identifying **rare hereditary and somatic variants**
Next Generation Sequencing (NGS) uses massive parallel sequencing to generate the DNA (or RNA) sequences of many genes simultaneously.
(likely) pathogenic gene variants, sometimes also unclear variants (VUS)
With new knowledge and new classifying software: updated interpretation and possibly new diagnosis.

Interpretation

List of identified gene variants
Exons

- Genes are made up of alternating regions of
  - Introns (noncoding sequences)
  - Exons (coding sequences)

- There are ~21,000 exons in human genome

- Exons constitute ~1-2% of the genome

- Mutations in genes in about 6,800 exons are known to cause various diseases.
Advantages of Clinical Exome Sequencing (CES) versus Whole Genome Sequencing (WGS)

• Whole Genome Sequencing (WGS) analyses introns (non coding regions) & Exons (Coding regions) of the gene
  • Large data after NGS
  • Costlier than Exome sequencing
  • May confuse interpretation.

• Clinical Exome Sequencing (CES) analyses only the coding regions
  • Simplified interpretation versus whole genome sequencing
  • Cheaper and faster than genome sequencing.
Assess patient

Test for most likely gene(s)

Disclose result and reassess

Test for most likely gene(s)

Revolution of genetic testing
New approach?

1. Assess patient
2. Send multigene panel
3. Disclose result and reassess
Why do Multigene testing?

- **More cost effective** (for the testing) to do multigene rather than serial testing
- **Patients (and providers!)** can get testing fatigue
- **The same cancer can be seen with different genes mutations**
  - Ovarian cancer in both BRCA1/2 and Lynch
  - Uterine cancer in Lynch and Cowden
  - Breast in Li-Fraumeni and BRCA1/2
- **Isn’t more better?**
Risk reducing strategies for BRCA mutation but without a diagnosis of breast cancer

• Increased surveillance
• Chemoprevention (e.g., tamoxifen citrate, raloxifene hydrochloride)
• Surgical intervention (i.e., bilateral mastectomy, bilateral salpingo-oophorectomy)
• If hereditary breast cancer syndrome: screening of family members with indicated risk factors.

Women with breast cancer DX and +ve BRCA mutation:
Survival benefits of surgical intervention+ chemotherapy is well documented

Prophylactic surgery: Recommendations

- Risk-reducing bilateral - salpingo-oophorectomy (RRSO) in BRCA mutation carriers.

- BRCA1 mutation: To undergo RRSO by age 35-40 or once the completion of childbearing.

- BRCA2 mutation: Can delay surgery until their 50s.

- Prophylactic Mastectomy
  Prophylactic mastectomy (PM) results in up to a 97% risk reduction of contralateral breast cancer (CBC).
## Risk Reducing Salpingo-Oophorectomy and the risk of breast cancer

<table>
<thead>
<tr>
<th></th>
<th>No Prior Breast Cancer</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>BRCA1</td>
<td>BRCA2</td>
</tr>
<tr>
<td>Total Participants</td>
<td>1,370</td>
<td>869</td>
<td>501</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.54 (0.37-0.79)</td>
<td>0.63 (0.41-0.96)</td>
<td>0.36 (0.16-0.82)</td>
</tr>
</tbody>
</table>

## RRSO and the risk of ovarian cancer

<table>
<thead>
<tr>
<th></th>
<th>Breast cancer prior</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>BRCA1</td>
<td>BRCA2</td>
</tr>
<tr>
<td>Total Participants</td>
<td>1060</td>
<td>684</td>
<td>376</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.14 (0.04-0.59)</td>
<td>0.15 (0.04-0.63)</td>
<td>No cancer events</td>
</tr>
</tbody>
</table>

PROSE Consortium  Domchek et al, *JAMA* 2010
# RRSO and all-cause mortality

<table>
<thead>
<tr>
<th></th>
<th>All eligible women</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>BRCA1</td>
<td>BRCA2</td>
</tr>
<tr>
<td><strong>Total Participants</strong></td>
<td>2,482</td>
<td>1,587</td>
<td>895</td>
</tr>
<tr>
<td><strong>HR (95% CI)</strong></td>
<td>0.40 (0.26-0.61)</td>
<td>0.38 (0.24-0.62)</td>
<td>0.52 (0.22-1.23)</td>
</tr>
</tbody>
</table>

Domchek et al, *JAMA* 2010
“Angelina Jolie effect”

The New York Times

"My doctors estimated that I had an 87 percent risk of breast cancer and a 50 percent risk of ovarian cancer."

Angelina Jolie in "My Medical Choice"
Published on May 14, 2013
Medications

- Tamoxifen.
  - Recommended as adjuvant in BRCA positive, ER+ breast cancers.
  - Reduces risk of contralateral breast cancer (CBC) by 40-70%.

Know the lifetime risk of breast cancer conferred by the mutation

Genetic counselling

Surveillance  Risk reduction strategies
Duties of a genetic counsellor

1. Assesses personal and familial risk for disease.

2. Explains the pros, cons, and limitations of genetic testing.

3. Helps the patient understand the test results and make an informed decision.

4. Identifies potential strategies for risk reduction.

5. Guides the patient through the emotional aspects of genetic testing, which have the potential to alter or even halt the diagnosis and treatment process.
Benefits of genetic counselling

• Decreased risk perception.

• Decreased intention for mutation testing among unlikely carriers.

• Decreased cancer-associated anxiety and depression

Genetic counselling and preventive strategies be carefully recommended to patients with less common mutations in the absence of strong consensus or official guidelines

Genetic testing can be very useful to patients and their family members – Both the prevent and to treat cancer.

Genetic testing is continuously evolving.

BRCA1 and BRCA2 mutations are the most commonly found and we have reasonable data on how to manage.

New genetics tests are often less clear in terms of how to change patients care – and improve patient outcome.

Variants of unknown significance should NOT be managed as mutations.

In the face of rising prophylactic mastectomies, we need to emphasize to patients how mutations in these genes are different from those in BRCA1/2.
Thank you!!!!