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BRCA vs Multi-Gene Panel in HBOC

SUNIL TADEPALLI

11TH AUGUST 2019

National Comprehensive Cancer Network®

NCCN Guidelines Version 2.2019
BRCA-Related Breast and/or Ovarian Cancer Syndrome

NCCN Guidelines Index
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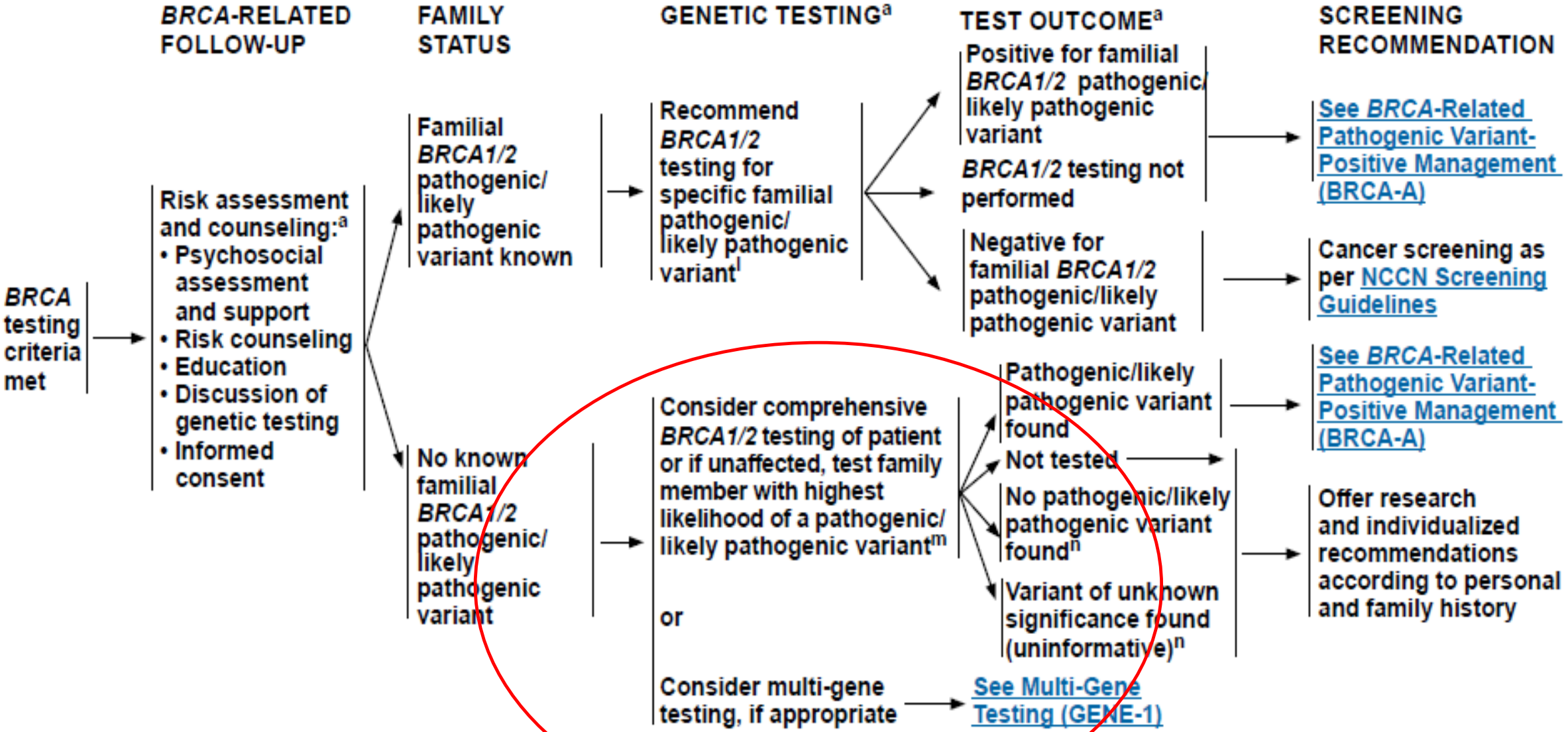

Next Generation Sequencing replacing Sanger Sequencing …

- Lower cost
- Faster
- More genes at the same price

Next-Generation Sequencing of the *BRCA1* and *BRCA2* Genes for the Genetic Diagnostics of Hereditary Breast and/or Ovarian Cancer

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Accepted for publication November 25, 2014.

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Genetic testing for hereditary breast and/or ovarian cancer mostly relies on laborious molecular tools that use Sanger sequencing to scan for mutations in the *BRCA1* and *BRCA2* genes. We explored a more efficient genetic screening strategy based on next-generation sequencing of the *BRCA1* and *BRCA2* genes in 210 hereditary breast and/or ovarian cancer patients. We first validated this approach in a cohort of 115 samples with previously known *BRCA1* and *BRCA2* mutations and polymorphisms. Genomic DNA was amplified using the Ion AmpliSeq *BRCA1* and *BRCA2* panel. The DNA Libraries were pooled, barcoded, and sequenced using an Ion Torrent Personal Genome Machine sequencer. The combination of different robust bioinformatics tools allowed detection of all previously known pathogenic mutations and polymorphisms in the 115 samples, without detecting spurious pathogenic calls. We then used the same assay in a discovery cohort of 95 uncharacterized hereditary breast and/or ovarian cancer patients for *BRCA1* and *BRCA2*. In addition, we describe the allelic frequencies across 210 hereditary breast and/or ovarian cancer patients of 74 unique definitely and likely pathogenic and uncertain *BRCA1* and *BRCA2* variants, some of which have not been previously annotated in the public databases. Targeted next-generation sequencing is ready to substitute classic molecular methods to perform genetic testing on the *BRCA1* and *BRCA2* genes and provides a greater opportunity for more comprehensive testing of at-risk patients. *(J Mol Diagn 2015, 17: 162–170; http://dx.doi.org/10.1016/j.jmoldx.2014.11.004)*

Trujiallano et at, J Mol Diag, 2014

Presentation Outline

HBOC – Criteria for genetic testing

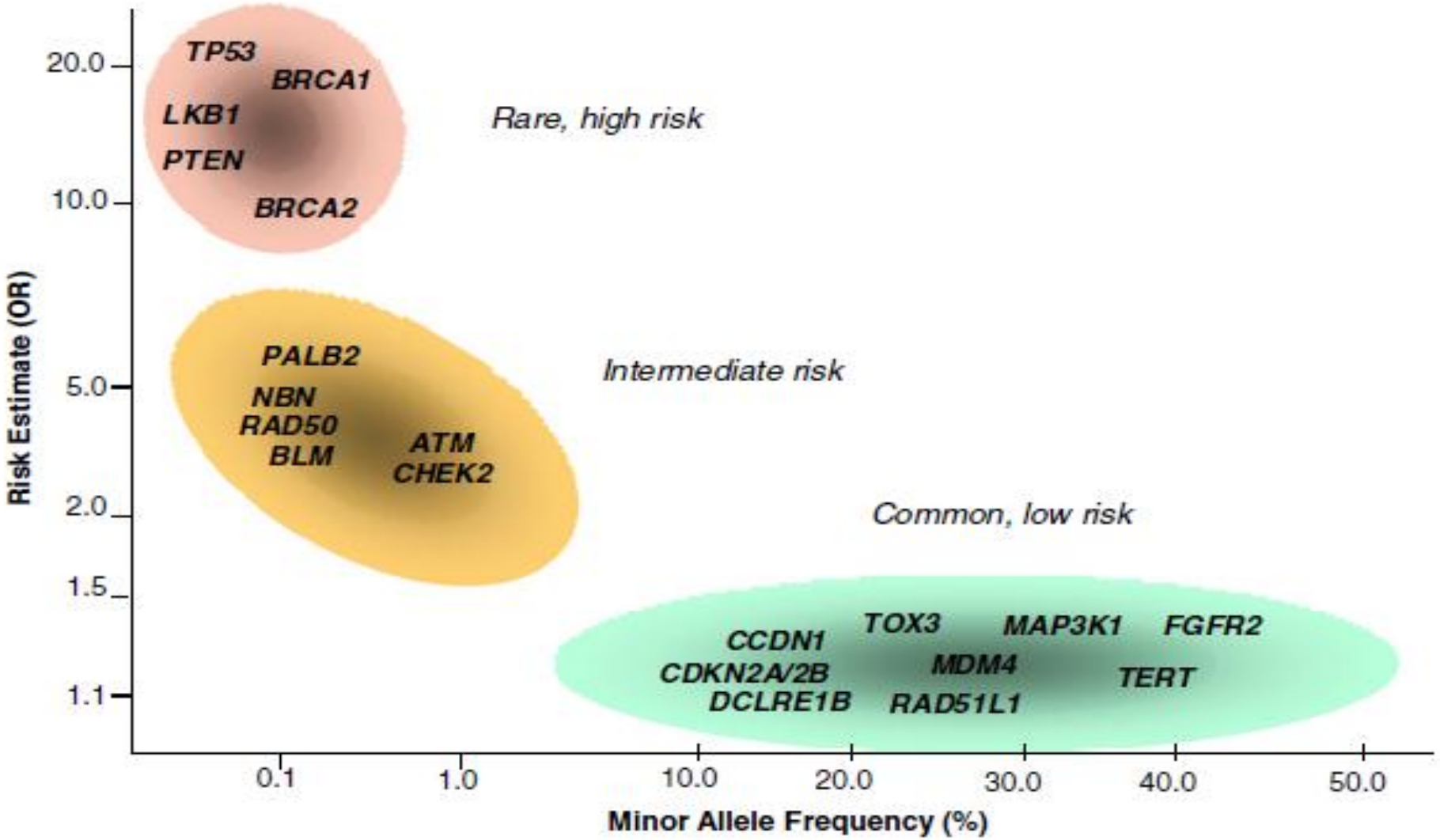
Testing Options & how do you decide?

Understanding the Genetic Test Report & Next Steps

Our results of BRCA testing from India

Genetics of HBOC

- Around 10% of all breast cancer patients have germline mutations in one of several different genes.
- >50% of pathogenic variants are in BRCA1 and BRCA2 genes
- Other genes like TP53, PTEN (high penetrance) and CHEK2, ATM (moderate penetrance) are found to mostly carry pathogenic variants in high risk cohorts
- Management guidelines exist only for a few of the genes implicated



Bogdanova et al. Hereditary Cancer in Clinical Practice 2013

Who should be offered referral for genetic counselling and/or genetic testing?

- Multiple cases of breast and/or ovarian cancer in family
  - closely related relatives
  - more than one generation
  - Breast cancer diagnosed at < age 50
  - Family member with both breast and ovarian cancers
- Breast cancer diagnosed at age < 45
- Personal history of ovarian cancer
- History of male breast cancer in the family
- Triple negative breast cancer
- Pancreatic cancer with breast or ovarian cancer in the same individual or on the same side of the family
- Previously identified pathogenic BRCA1/2 variant in the family

BRCA1 and BRCA2 Associated Cancers & Penetrance

Cancer Type	General Population Risk	Mutation Risk	
		BRCA1	**BRCA2**
Breast	12% (in India 5-8%)	50%-80%	40%-70%
Second primary breast	3.5% within 5 years Up to 11%	27% within 5 years	12% within 5 years 40%-50% at 20 years
Ovarian	1%-2%	24%-40%	11%-18%
Male breast	0.1%	1%-2%	5%-10%
Prostate	15%-18%	<30%	<39%
Pancreatic	0.5%	1%-3%	2%-7%

Testing Options for HBOC

BRCA1/BRCA2

- Sequencing by NGS
- Sanger fill-in to ensure 100% coverage
- Deletion Duplication testing by MLPA included
- Rs 21,420/-

CentoBreast

- ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN, RAD51C, STK11, TP53 by NGS
- Coberage >99.5% at 20x
- Includes Copy Number Variant detection (by NGS)
- Rs 79,600/-

Patient Clinical Information / Familial Mutations Information

Patient Name: _____

Date of birth: _____

CentoCard Number: _____

Personal history of cancer (check all that apply)

☐	No personal history of cancer
☐	History of breast cancer
	Age at Diagnosis _____ years
	Bilateral – Yes / No
	Premenopausal – Yes / No
	Immunohistochemistry Markers: ER – Pos/Neg ; PR – Pos/Neg; Her2 – Pos/Neg
☐	History of ovarian cancer
	Age at Diagnosis _____ years
	Histopathology _____
☐	History of any other cancer
	Age at Diagnosis _____ years

Family history of cancer

☐	No known family history
☐	Family history of cancer present (please provide details for parents, grandparents, siblings & children below)

Relationship	Maternal	Paternal	Cancer Site	Age at Diagnosis (years)
	☐	☐		
	☐	☐		
	☐	☐		
	☐	☐		
	☐	☐		

BRCA Report Decoded

Result	AKA	Probability of Pathogenicity	Implication	Next Steps
Pathogenic or Likely Pathogenic mutation	Class 1 or Class 2; Positive	P (>99%) & LP (95-99%)	• Susceptibility to HBOC can be confirmed	• Genetic counseling of the patient and further family members • Predictive analysis is now available • See HBOC management guidelines
Variant of Uncertain Significance	Class 3; Indeterminate	VUS (5-94.9%)	• Variant has not been reported earlier and we cannot make projections about its pathogenicity	• Genetic counseling of patient and further family members • Testing of other affected family members to establish segregation of the variant in the family • Variant Reclassification
Likely Benign or Benign	Class 4 or Class 5; Negative	LB (0.1-4.9%) or Benign (<0.1%)	• Susceptibility to HBOC cannot be confirmed	• Consider deletion / duplication testing for BRCA • Consider testing for other susceptibility genes – (TP53, CDH1, RAD51C, CHEK2 & STK11) • Genetic counseling

According to NCCN Guideline V.3.2019 Multigene Panels may be considered

- When more than one gene can explain an inherited cancer syndrome.
- In individuals who have tested negative (indeterminate) for a single syndrome, but whose personal or family history remains suggestive of an inherited susceptibility.
- Choosing the right panel is important- specific genes analyzed (as well as classification of variants and many other factors)
- Not all genes included on panel tests are necessarily clinically actionable.
- Increased likelihood of finding variants of unknown significance when testing for multiple genes.
- It is for these and other reasons that multi-gene testing is ideally offered in the context of professional genetic expertise for pre- and post- test counseling.

Benefits and Limitations of Multi-gene testing

BENEFITS

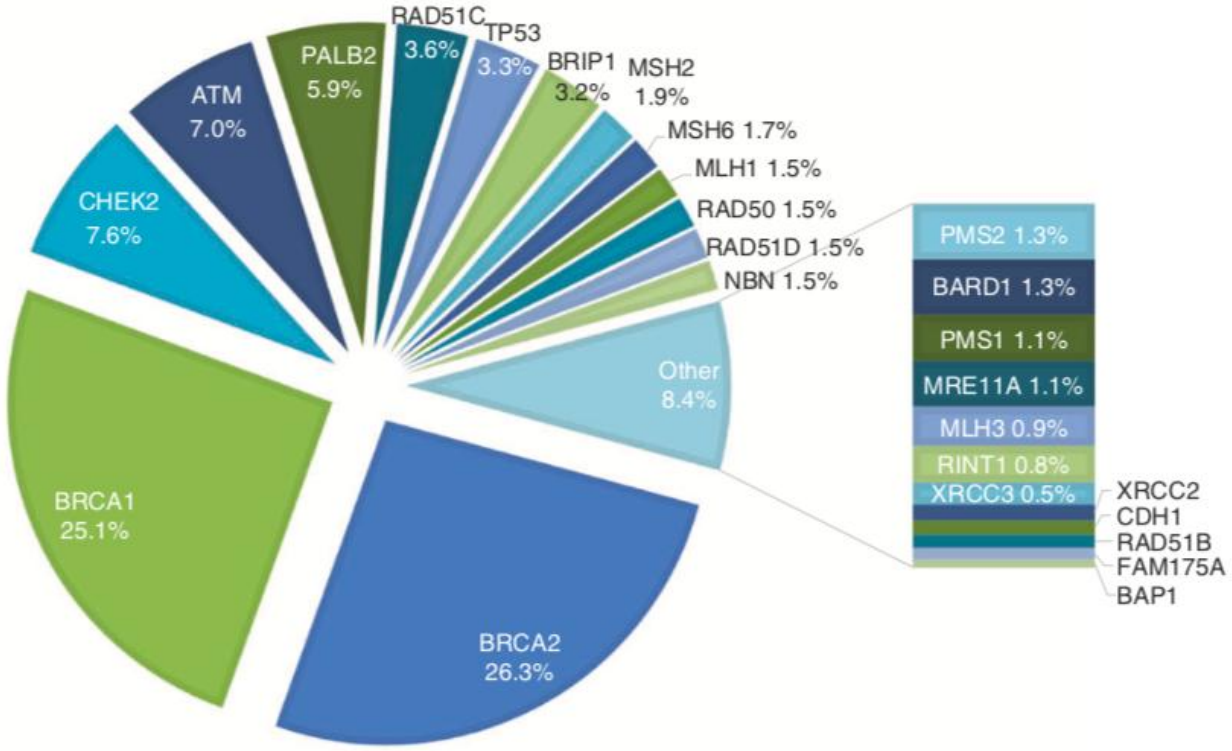
- Cost effective
- Overall short TAT compared to serial gene testing
- Increased rate of detection of pathogenic variants
- Improved surveillance for patients found to be carriers for “actionable genes”

LIMITATIONS

- Patient management recommendations not available for all genes
- In some cases NGS may miss some Pathogenic/ likely pathogenic variants (allele drop-out)
- Higher rates of Variants of uncertain significance
- Variants may be identified in >1 gene- adds complexity

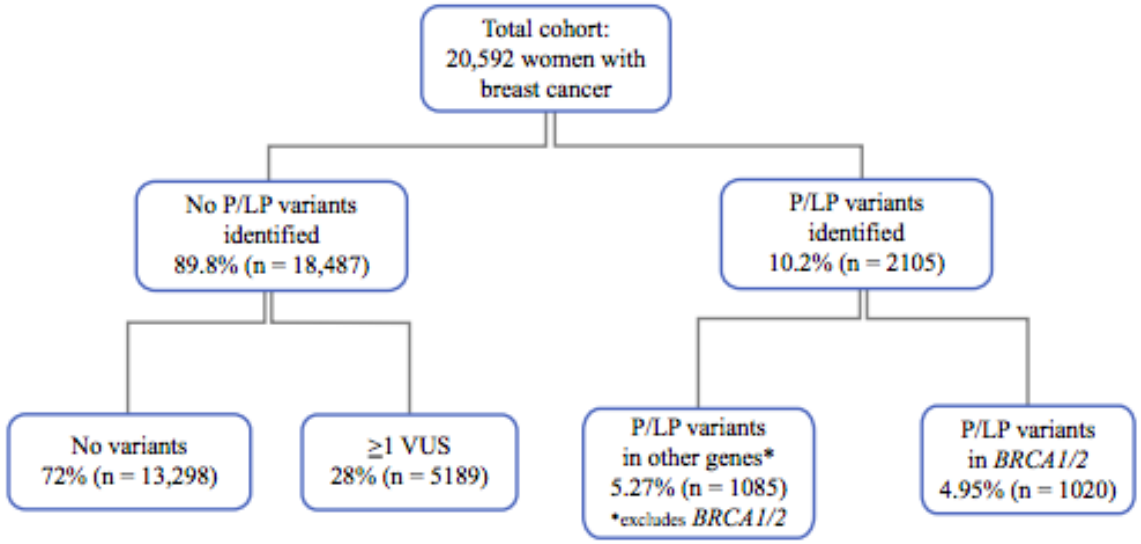
Data in support of Multi-gene testing

Castera L et al. 2018 analyzed 4409 patients using a 34 HBOC gene panel. 647 pathogenic variants identified



Depending on the number of genes in a panel and the patients who are tested, VUS rates from panel testing have been reported to range from 6.7%-41.7%

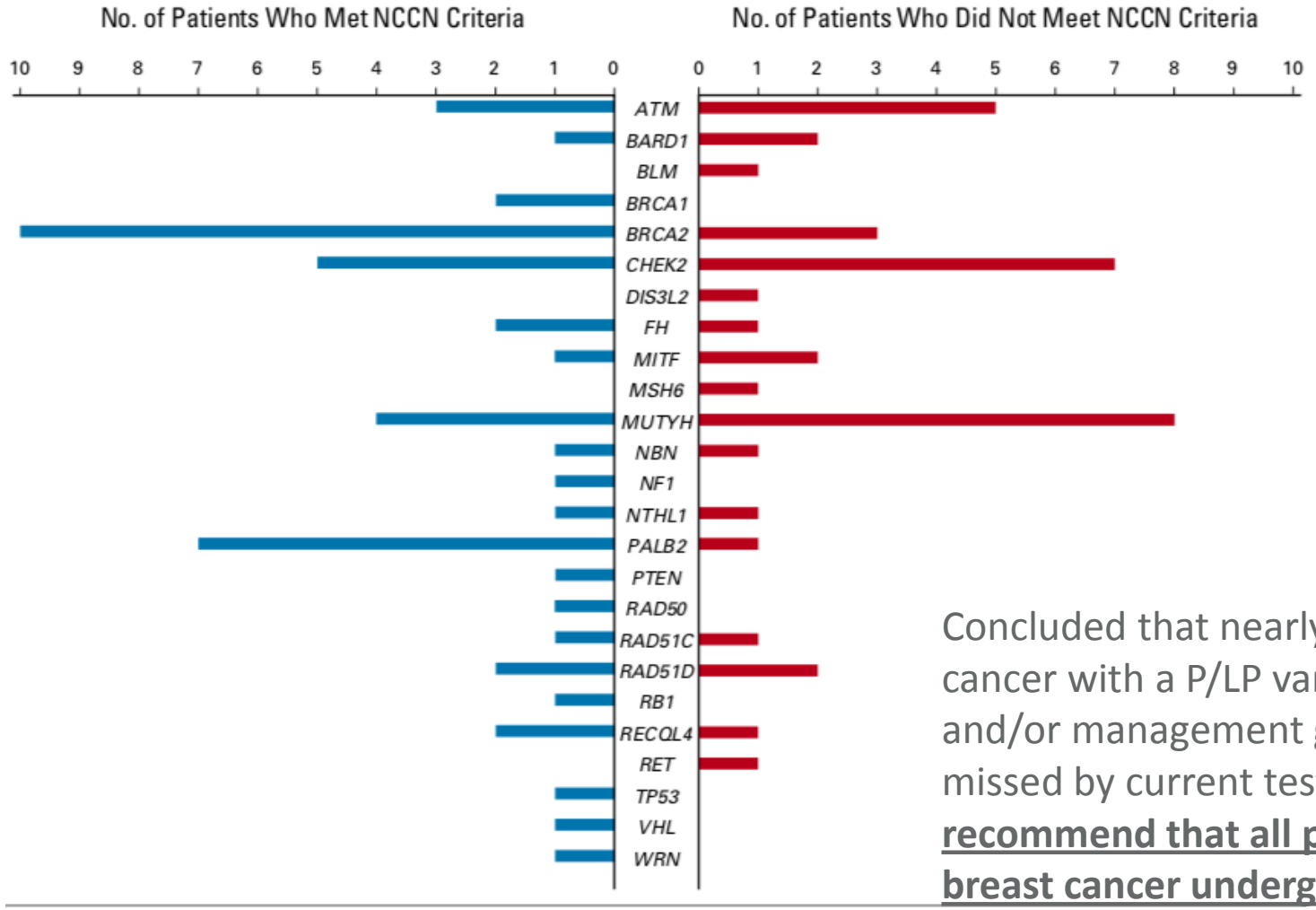
O'Leary et al. 2017 (Commercial Laboratory data published in Ann Surg Oncol)



Study group	No. of patients evaluated	BRCA 1/2 +ve (%)	Non-BRCA +ve (%)
Tung et al. 2015 (14-21 gene panel)	2000	9.3%	4.2%
Kapoor NS et al.2015 (5-53 gene panel)	966	4%	3.6%
Castera L et al. 2014 (27 gene panel)	708	Detection rate- 15.4%	
		59%	41%

Castera et al. Genetics in Medicine, 2018

Beitsch et al. 2018 published a multicenter prospective study on 959 Br Ca patients tested with a 80 gene panel- 49.95% met NCCN criteria and 50.05% did not. Over all 8.65% patients had pathogenic/ likely pathogenic variants.



9.39 %

7.9 %

Concluded that nearly half of patients with breast cancer with a P/LP variant with clinically actionable and/or management guidelines in development are missed by current testing guidelines. They **recommend that all patients with a diagnosis of breast cancer undergo expanded panel testing.**

Beitsch et al. JCO, 2018

Summary of NCCN recommendations for pathogenic variation carriers

Gene	Breast cancer risk	Ovarian cancer risk	Other cancers	Recommendations for breast/ovarian cancer risk reduction
ATM[1]	Increased	Not increased	Unknown/insufficient evidence for prostate and pancreas cancer	• Annual mammogram/consider breast MRI starting at the age of 40 years • Consider RRM (based on family history)
BRCA1	Increased	Increased	Prostate	• Annual mammogram starting at the age of 30 years/annual breast MRI starting at the age of 25 years • Consider RRM • Recommend RRSO at the age of 35-40 years
BRCA2	Increased	Increased	Prostate, pancreas, melanoma	• Annual mammogram starting at the age of 30 years/annual breast MRI starting at the age of 25 years • Consider RRM • Recommend RRSO at the age of 35–40 years (can extend to 40–45 years)
BRIP1	Not increased	Increased	N/A	• Consider RRSO at the age of 45–50 years
CDH1	Increased	Not increased	Diffuse gastric cancer	• Annual mammogram/consider breast MRI starting at the age of 30 years • Consider RRM (based on family history)
CHEK2[2]	Increased	Not increased	Colon cancer	• Annual mammogram/consider breast MRI starting at the age of 40 years • Consider RRM (based on family history)
MSH6	Unknown/insufficient	Not increased	Colorectal cancer, endometrial cancer	• Breast cancer management based on family history
NBN[3]	Increased	Unknown/insufficient	Unknown/insufficient	• Annual mammogram/consider breast MRI starting at the age of 40 years

Fountzilas C et al. Multi-gene Panel Testing in Breast Cancer Management. Cancer Treat Res.2018;173:121-140

Gene	Breast cancer risk	Ovarian cancer risk	Other cancers	Recommendations for breast/ovarian cancer risk reduction
				• Consider RRM (based on family history)
NF1	Increased	Not increased	Gastrointestinal stromal tumors, malignant peripheral nerve sheath tumors	• Annual mammogram starting at the age of 30 years/consider breast MRI starting at the age of 30–50 years • Consider RRM (based on family history)
PALB2	Increased	Unknown/insufficient	Unknown/insufficient	• Annual mammogram/consider breast MRI starting at the age of 30 years • Consider RRM (based on family history)
PTEN	Increased	Not increased	Thyroid cancer, endometrial cancer	• Annual mammogram/breast MRI starting at the age of 30–35 years • Consider RRM
RAD51C	Unknown	Increased	N/A	• Consider RRSO at the age of 45–50 years
RAD51D	Unknown	Increased	N/A	• Consider RRSO at the age of 45–50 years
STK11	Increased	Increased (non-epithelial)	Colorectal cancer	• Annual mammogram/breast MRI starting at the age of 25 years
P53	Increased	Not increased	Adrenocortical carcinoma, leukemia, brain tumors, soft tissue sarcomas	• Annual mammogram starting at the age of 30 years/annual breast MRI starting at the age of 20–29 years • Consider RRM

Fountzilas C et al. Multi-gene Panel Testing in Breast Cancer Management. Cancer Treat Res.2018;173:121-140

Patients Screened and Cases identified for BRCA1 and BRCA2 from India at Centogene

Gene	Patients Screened (India)	Cases* (India)	%age (India)	Patient Screened (total)	Cases* (total)	Cases% (total)
BRCA1	1469	261	18%	4479	701	16%
BRCA2	1463	170	12%	4456	495	11%
Grand Total	**1473**	**431**	**29%**	**4825**	**1196**	**25%**

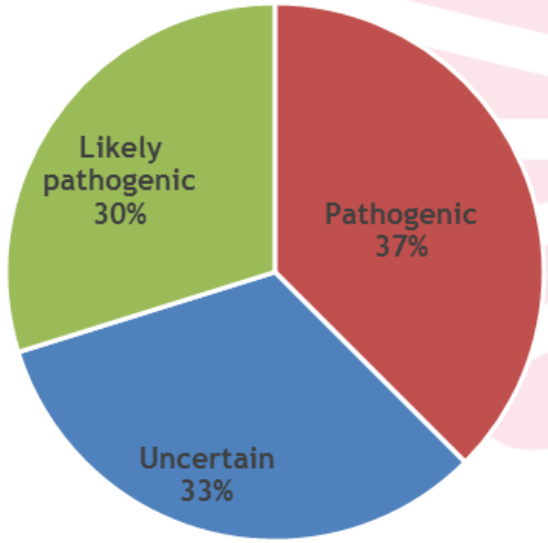
*Case: Indicates an individual where diagnosis was confirmed by genetic testing at Centogene

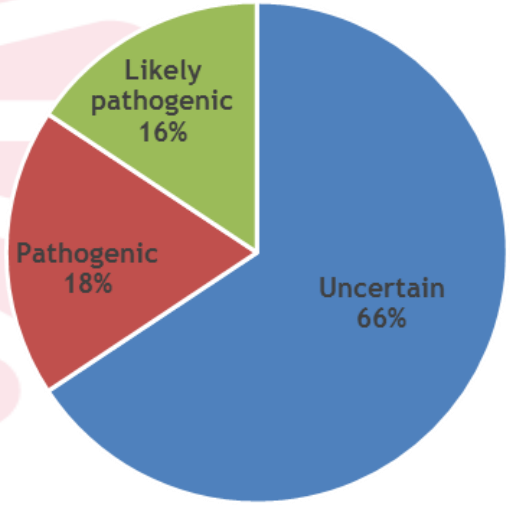
Clinical and family history spectrum of cases from India		
	BRCA 1 (n = 261)	BRCA 2 (n = 170)
Abnormality of the breast	102	61
Abnormality of the ovary	67	24
Abnormality of other organs	12	4
Suspected / Affected	6	4
No information	73	77

Number of Clinical relevant and uncertain BRCA1 and BRCA2 variants at Centogene

BRCA1	No. of variants (India)	No. of variants (total)
Pathogenic	39	124
Uncertain	34	100
Likely pathogenic	31	72
Total	104	296

BRCA2	No. of variants (India)	No. of variants (total)
Uncertain	67	175
Pathogenic	19	82
Likely pathogenic	16	49
Total	102	306





Take-home Message

For the Clinician

- Record detailed family and personal history in every case of breast, ovarian, colon & endometrial cancer and offer genetic testing in appropriate families
- Multi-gene panels may be considered especially if warranted by personal / family history and specific associated findings. We can expect to see a lot of VUS from cases in India
- Genetic testing offers the possibility of risk evaluation & management even in the pre-symptomatic stage
- Many patients are quite aware of the possibility of familial cancers and request testing – but misinformation is widespread
- Germline mutations can be diagnosed easily and have defined inheritance patterns.
- Both Somatic & Germline mutations are useful in guiding management of patients.

For the Gynecologic Oncology Societies

- Issue guidelines for genetic testing in hereditary cancers
- Identify more cases and carriers (present estimate <1%)
- Promote sharing of variant classification to reduce VUS

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