Clinical Actionability of non-BRCA1/2 Mutations Uncovered in Multigene Tests for Hereditary Breast Cancer

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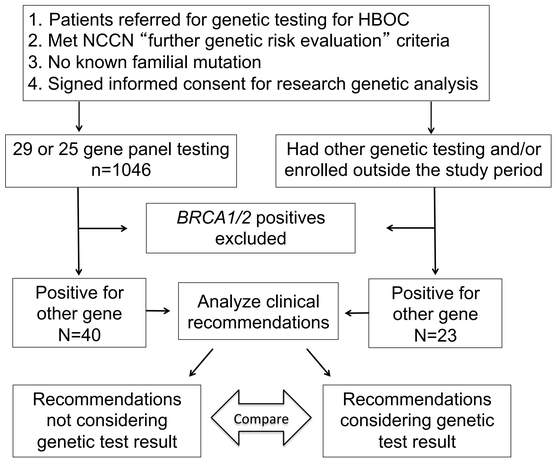
Background and Objectives

The practice of genetic testing is rapidly evolving with the recent introduction of multigene panels. While the prevalence of non-*BRCA1/2* mutations in patients with suspected hereditary breast and ovarian cancer (HBOC) is now well documented, the clinical validity and clinical utility of these tests is not yet fully understood.

We sought to measure how often and in which ways non-*BRCA1/2* findings from multigene tests could change patient management in a representative clinical cohort. We further analyzed the analytic and clinical validity of multigene testing by comparison with traditional genetic tests on the same patients.

Study Design/Methods

We tested up to 29 genes in over 1000 *BRCA1/2*-negative patients, all of whom were enrolled prospectively at three academic medical centers and all of whom met NCCN guidelines for HBOC evaluation. We established a uniform algorithm based on current practice guidelines to recommend management actions for the non-*BRCA1/2* positive individuals, and we evaluated which of these actions would represent a change in management over and above any recommendations based on personal and family history alone.



	MGH+Stanford	BIDMC
Total patients	669	377
Gender		
Male	6 (0.9)	3 (0.8)
Female	663 (99.1)	374 (99.2)
Ethnicity		
African	4 (0.6)	13 (3.4)
Asian	43 (6.4)	6 (1.6)
Asian Indian	15 (2.2)	0 (0)
Caucasian	550 (82.2)	343 (91.0)
Hispanic	27 (4.0)	10 (2.7)
Multiple	17 (2.5)	5 (1.3)
Unknown/other	13 (1.9)	0 (0)
Personal Hx Ca[1]		
Breast Ca	455 (68.0)	377 (100.0)
Ovarian Ca	40 (6.0)	7 (1.8)
Colorectal Ca	9 (1.3)	1 (0.3)
Endometrial Ca	12 (1.8)	4 (1.1)
Pancreatic Ca	2 (0.3)	1 (0.3)
No personal Hx Ca	150 (22.4)	0 (0)

At BIDMC, accrual was restricted to patients with breast cancer and excluded those who had positive clinical testing for *BRCA1/2.*

The presence of non-BRCA1/2 mutations in a subset of these patients was previously reported in our prior work:

- Tung *et al., Cancer* 2014
- Kurian *et al., JCO* 2014

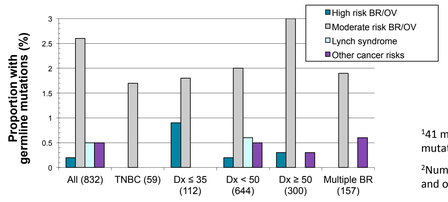
Figures in this table may not add up to 100% due to data not provided or patients with multiple primary tumors.

Mutation Prevalence and Clinical Relevance

63 patients were identified with mutations in non-*BRCA1/2* genes.

- 74% of the cancer-affected patients had a syndromic cancer for the gene they were found to carry. 26% did not.
- However, in 92% of cases, the patient's personal and/or family history was consistent with the syndromic effects of the gene they carry.

Risk category	Any mutation	High risk BR/OV	Moderate risk BR/OV	Lynch syndrome	Other cancer risk genes
Total subjects – 1046	40 (3.9)[1]	3 (0.3)	26 (2.5)	8 (0.8)	4 (0.4)
BR at any age – 832	32 (4.0)[2]	2 (0.2)	23 (2.8)	4 (0.5)	4 (0.5)
OV at any age – 47	5 (10.6)	0 (0)	2 (4.3)	3 (6.4)	0 (0)
Ashkenazi Jewish – 143	1 (0.7)	0 (0)	0 (0)	1 (0.7)	0 (0)
Cancer Unaffected – 150	4 (2.7)	1 (0.7)	1 (0.7)	2 (1.3)	0 (0)



High risk BR/OV: *TP53, PTEN, STK11, CDH1*
Moderate risk BR/OV: *BARD1, CHEK2, PALB2, ATM, BRIP1, RAD51C, RAD51D, NBN*
Lynch syndrome: *MLH1, MSH2, MSH6, PMS2, EPCAM*
Other cancer risks: *APC, BMPR1A, SMAD4, CDK4, CDKN2A, PALLD, MET, MEN1, RET, PTCH1, VHL, MUTYH* (biallelic)

Abbreviations: BR Breast Cancer, OV Ovarian Cancer

[1]41 mutations among 40 patients; one patient had concurrent *ATM* and *BARD1* mutations. The BARD1 mutation was not considered in the management analysis.

[2]Numbers in this column do not total 40, as one patient had breast/ovary cancer, and one Ashkenazi patient had ovarian cancer.

Clinical Management Impact

We found that the majority of these findings would result in consideration of additional screening and/or prevention measures for the patient. Moreover, testing of first-degree family members would also be warranted given the potential management changes in these individuals if found to be mutation positive.

Criteria	Positive genes	Potential change	Patients	Family members
High-risk genes 1.2015 NCCN management guidelines	CDH1, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, APC, BMPR1A, MUTYH (biallelic)	Guidelines-based surveillance/ prevention	20 / 20	19 / 19
≥40% breast cancer risk (and <40% pre-test risk)	PALB2	Surgical prevention candidate	5 / 8	7 / 7
>20% breast cancer risk (<20% pre-test risk) NCCN 1.2015 guidelines	ATM, CHEK2, NBN, RAD51C, BRIP1	Enhanced breast screening candidate	5 / 32	13 / 29
Other cancer risks (pancreas, melanoma)	CDKN2A	Pancreas screening candidate	3 / 3	3 / 3

Management change considered for patient 33/63 (52%)

Family member testing indicated 42/58 (72%)

[2] Family testing recommended if positive result would change management. Only living 1st degree relatives considered.
[3] Risk estimates by IBIS (Tyrer Cuzick).
[4] Risk to age 70. For *PALB2*, risk estimate reflects that all had ≥1 first degree relative with breast cancer.
[5] 3 of 8 patients had undergone prior bilateral mastectomy.
[6]Annual breast MRI per NCCN guidelines.

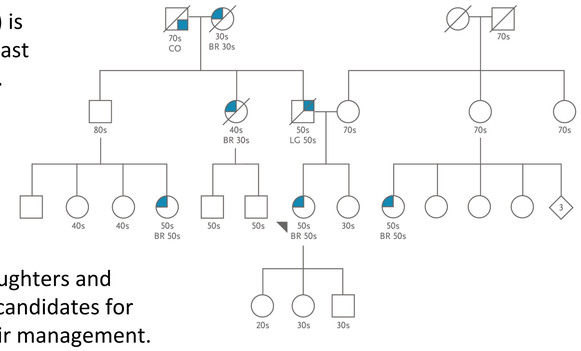
Gene	Category	Management change considered for patient?	Potential management change	Family member testing indicated?[5]
CDH1	High risk breast/ovary	4 of 4	Prophylactic gastrectomy	4 of 4
TP53	High risk breast/ovary	3 of 3	Increased cancer surveillance	3 of 3
PTEN	High risk breast/ovary	1 of 1	Increased cancer surveillance	1 of 1
ATM [2]	Mod/low risk breast/ovary	1 of 11	Increased breast screening	6 of 11
BRIP1	Mod/low risk breast/ovary	0 of 1	N/A	0 of 1
CHEK2	Mod/low risk breast/ovary	2 of 15	Increased breast screening	4 of 13
NBN	Mod/low risk breast/ovary	0 of 2	N/A	0 of 1
PALB2	Mod/low risk breast/ovary	5 of 8	Increased screening or mastectomy	7 of 7
RAD51C	Mod/low risk breast/ovary	2 of 3	Increased breast screening	3 of 3
MLH1	Lynch syndrome	1 of 1	Increased colorectal/endometrial screening	1 of 1
MSH2	Lynch syndrome	2 of 2	Increased colorectal/endometrial screening	1 of 1
MSH6	Lynch syndrome	2 of 2	Increased colorectal/endometrial screening	2 of 2
PMS2	Lynch syndrome	4 of 4	Increased colorectal screening	4 of 4
APC	Other familial cancer	1 of 1	Prophylactic colectomy	1 of 1
BMPR1A	Other familial cancer	1 of 1	Increased gastric cancer screening	1 of 1
CDKN2A	Other familial cancer	3 of 3	Increased pancreatic surveillance	3 of 3
MUTYH	Other familial cancer	1 of 1	Increased colorectal screening	1 of 1
Total		33 of 63		42 of 58

Case Studies

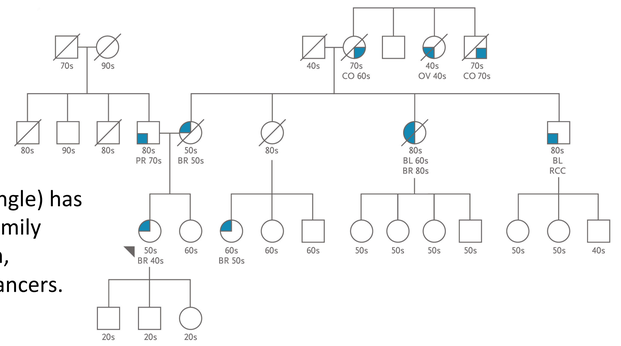
This patient (black triangle) is already a candidate for breast MRI screening based on Fx.

Finding a *PALB2* mutation makes her a possible candidate for prophylactic surgery.

It also makes her sister, daughters and potentially other relatives candidates for testing that could alter their management.



This patient (black triangle) has breast cancer with a family history of breast, colon, uterine, and bladder cancers.



Finding a MSH6 mutation indicates that screening for colorectal, gynecologic, and urologic cancers would be indicated, and prophylactic gynecologic surgery could be considered. Additional family member testing is also recommended.

Validation Study

If multigene panels are to replace traditional tests in appropriate situations, then it is important to understand the analytic and clinical performance of these new tests in comparison with the previous standard of care. Panel test results for the MGH and Stanford patients, adding in the *BRCA1/2* positives, were compared to traditional (Sanger) genetic tests performed on the same patients by another laboratory.

Analytic concordance	
Sensitivity	100.0%
Specificity	100.0%

Analytic concordance: N=750 directly comparable variants in 1105 individuals. 49 of these 750 were copy number del/dups or otherwise technically challenging sequence alterations.

Clinical interpretation: Positive vs. not positive	
Agree	99.8%
Disagree	0.2%

Clinical concordance for alterations in *BRCA1/2* in N=975 directly comparable cases. Positive result means pathogenic or likely pathogenic variant identified. Not positive means only VUS, likely benign, or benign variants identified.

Conclusions

In appropriately referred patients, multigene panel testing yields valid and clinically relevant findings with potential management impact for substantially more patients than does BRCA1/2 testing alone. Additional results and discussion are available in our recent publications from this multicenter study:

- Desmond *et al., JAMA Oncology* 2015
- Swisher, *JAMA Oncology* 2015 (Commentary)
- Lincoln *et al., J Molecular Diagnostics* 2015