Results of Next-Generation Sequencing Panels in a Large Community-Based Hereditary Cancer Risk Program

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Introduction

Next generation sequencing (NGS) allows for broader germline genetic testing for hereditary cancers. Since the Supreme Court decision of Association of Molecular Pathology v. Myriad on June 13, 2013, hereditary cancer multi-gene panels can now include *BRCA1* and *BRCA2*, making these panels an option for first-tier testing. A multi-gene testing approach may reveal that as high as 17% of *BRCA*-negative high-risk breast cancer families and as high as 24% of unselected ovarian cancer cases will have an identifiable mutation through this testing option. [1-3] However, questions have been raised about the clinical utility and implications of panels for medical management, given the inclusion of unknown to moderately penetrant genes. The National Comprehensive Cancer Network (NCCN) released updated guidelines in September of 2014 which address next generation multi-gene testing panels. [4] These guidelines discuss the need to develop an appropriate management plan in patients who have mutations in less well-described genes. Therefore, we investigated how results obtained from the use of multi-gene panels impacted medical recommendations made to our patients.

Methods

We reviewed all patients who underwent genetic counseling and multi-gene panel testing from July 1, 2013 through May 23, 2014. A retrospective chart review was conducted for patients whose indications for testing included personal and/or family history of breast and/or ovarian cancer. The multi-gene panel tests were all ordered through a single genetic testing laboratory (Ambry Genetics, Aliso Viejo, CA) by a single genetics department within a large community-based cancer center.

Patient charts were reviewed and categorized according to what multi-gene panel test was ordered (Table 1), if any previous genetic testing was performed, and if the patient met the current National Comprehensive Cancer Network (NCCN) guidelines for pursuing *BRCA1* and *BRCA2* genetic testing. Genetic test results were categorized into three groups: positive, variant of unknown significance (VUS) and negative. Finally, chart information was reviewed to identify which patients were recommended changes in medical management due to their genetic test results on these panels. This information is represented in Figures 1-3 in the Results section.

BRCAplus™	BreastNext™	OvaNext™	CancerNext™
BRCA1	*BRCA1*	*BRCA1*	*BRCA1*
BRCA2	*BRCA2*	*BRCA2*	*BRCA2*
CDH1	*CDH1*	*CDH1*	*CDH1*
PTEN	*PTEN*	*PTEN*	*PTEN*
TP53	*TP53*	*TP53*	*TP53*
STK11	*STK11*	*STK11*	*STK11*
	PALB2	*PALB2*	*PALB2*
	ATM	*ATM*	*ATM*
	CHEK2	*CHEK2*	*CHEK2*
	MUTYH	*MUTYH*	*MUTYH*
	BARD1	*BARD1*	*BARD1*
	BRIP1	*BRIP1*	*BRIP1*
	MRE11A	*MRE11A*	*MRE11A*
	NBN	*NBN*	*NBN*
	RAD50	*RAD50*	*RAD50*
	RAD51C	*RAD51C*	*RAD51C*
	*RAD51D**	*RAD51D**	*RAD51D**
	*NF1**	*NF1**	*NF1**
		MLH1	*MLH1*
		MSH2	*MSH2*
		MSH6	*MSH6*
		PMS2	*PMS2*
		EPCAM	*EPCAM*
			APC
			SMAD4
			CDK4
			BMPR1A
			CDKN2A

Table 1: *NF1* and *RAD51D* added to panels on October 18th, 2013

Results

Figure 1: Patients with previous testing

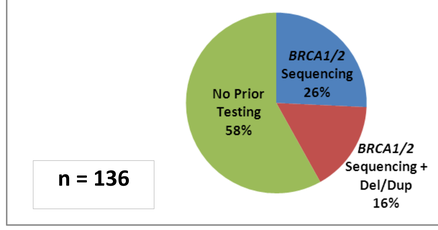


Figure 2: Patients with NO PRIOR testing who had a panel test

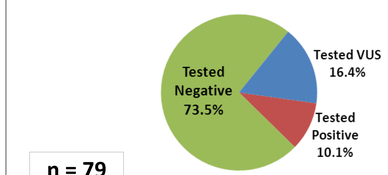


Figure 3: Patients with prior *BRCA1/2* testing who had a panel test

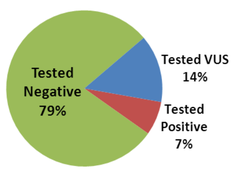


Table 2: 12 patients with medically actionable test results with pre-test treatment and post-test recommendations

Patient ID	Result	Prev. Testing?	Affected/Unaffected?	2014 HBOC NCCN guidelines	Surgery and Treatment Prior to Genetic Testing	Recommended Medical Management Changes after Genetic Testing
1	*PALB2* (c.2257C>T)	Y	Y, br ca dx 58 (right IDC) & 60 (right inflammatory)	Y	Right mastectomy; TAH/BSO at 50	Consider increased breast screening and/or prophylactic mastectomy; possible pancreatic screening
2	*BRCA2* (c.6091dupA)	N	Y, br ca dx 45 (path unknown-TNBC) & 58 (path unknown-TNBC); thyroid ca dx 35	Y	TAH at 35, BSO at 52, bilateral mastectomy	No breast/ovarian medical management changes
3	*ATM* (c.170G>A) & *MUTYH* -VUS (c.821G>A)	N	Y, br ca dx 38 (right IDC) & 70 (left DCIS)	Y	Right lumpectomy; left mastectomy	Increased breast screening and/or R mastectomy. Use radiation sparingly
4	*CDH1* - VLP (c.1118C>T)	N	N	Y, did not meet *CDH1* guidelines	None	Total prophylactic gastrectomy, prophylactic bilateral mastectomy, colonoscopy if colon cancer present in family.
5	*ATM* - VLP (c.7638_7646del TAGAATTTC)	N	Y, br ca dx 44 (left-DCIS) & 49 (right-IDC); pancreatic ca dx 59; desmoid tumor dx 52	Y	Bilateral mastectomy; TAH/BSO at 45; colonoscopy every year	Patient now deceased due to pancreatic cancer. Patient's husband informed of genetic test results. Recommendation of single site testing of at-risk family members.
6	*BRCA2* (c.4936_4939del GAAA) & *ATM* - VLB (c.7294A>T)	N	Y, br ca dx 50 (left-TNBC) & 60 (right-IDC ER positive)	Y	Bilateral mastectomy; TAH at 39	Prophylactic BSO
7	*TP53* (c.155_157dupA AT)	Y	Y, br ca dx 43 (left-IDC)	Y, did not meet *TP53* guidelines	Left mastectomy	Increased breast screening and/or prophylactic R mastectomy. Prophylactic TAH/BSO. Dermatology and neurology exams. Colonoscopy every 2-5 years. Consider whole-body MRI, abdominal U/S and brain MRI. Avoid known carcinogens and radiation.
8	*CHEK2* (c.1100delC)	Y	Y, br ca dx 43y (right-path unknown)	Y	Bilateral mastectomy; TAH/BSO at 44; colonoscopy in 2009	Dermatology exam at least once a year. Colonoscopy every 2-5 years.
9	*BRCA2* (c.9699_9702del TATG)	N	N	Y	None	Increased breast screening and/or prophylactic mastectomy; prophylactic TAH/BSO
10	*TP53* (c.725G>A)	Y	Y, br ca dx 28 (left-IDC)	Y, did meet *TP53* guidelines	Bilateral mastectomy; BSO at 29; radiation treatment	No breast/ovarian medical management changes. Consider TAH, dermatology exam, neurology exam. Colonoscopy every 2-5 years. Avoid radiation and known carcinogens.
11	*BRCA2* (c.3847_3848del GT)	N	N	Y	None	Increased breast screening and/or prophylactic mastectomy; prophylactic TAH/BSO
12	*NBN* (c.657_661delAC AAA)	N	Y, br ca dx 44 (left-IDC with DCIS)	Y	Bilateral mastectomy; TAH/BSO at 48	Consider colonoscopy. Consider dermatology exam. No breast/ovarian management changes.

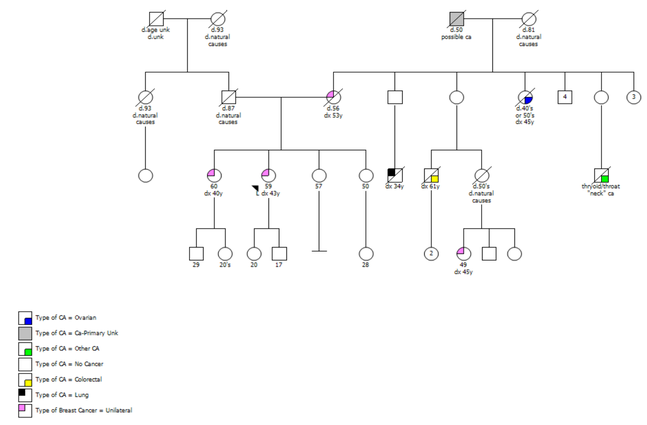
- VLP is defined as "variant likely pathogenic"
- VLB is defined as "variant likely benign"
- Previous testing is defined as *BRCA1* and *BRCA2* sequencing and deletion/duplication analysis
- TNBC is defined as "triple negative breast cancer"

Results Continued

Out of 136 patients who met our criteria, 12 (8.8%) patients were positive for a pathogenic or likely pathogenic mutation in a cancer susceptibility gene; 4 of these had prior negative *BRCA1* and *BRCA2* sequencing and deletion/duplication testing. These positive results included four *BRCA2* mutations, two *TP53* mutations, one *CDH1* mutation, two *ATM* mutations, and one patient each with a *CHEK2, NBN*, or *PALB2* mutation. Of the patients found to have a positive test result, 100% met the National Comprehensive Cancer Network (NCCN) guidelines for Hereditary Breast and Ovarian Cancer (HBOC) genetic testing. The *CDH1* mutation carrier did not meet NCCN guidelines for hereditary diffuse gastric cancer testing and only one of the two *TP53* mutation carriers met NCCN guidelines for Li-Fraumeni syndrome testing. Within our cohort (136), 21 (15.4%) patients had a total of 25 variants of uncertain significance (VUS) and 103 (75.7%) patients had negative test results.

Patient ID 7 Pedigree

Li-Fraumeni-Like



Conclusion

Testing through NGS panels identified 12 patients in our cohort of 136 (8.8%) with an actionable test result (variant likely pathogenic or mutation). Of these 12 patients, 10 out of 12 (83.3%) of those with an actionable test result or 10 out of 136 (7.3%) of the overall cohort were advised with new medical management recommendations based on their multi gene panel test result (see Table 2). This study did not include medical recommendations provided to family members of these patients; future research could investigate the broader impact of these genetic test results on family members. Eight patients out of the 12 (66.6%) had a mutation in a gene other than *BRCA1* or *BRCA2*. Our findings suggest that there is clinical utility of NGS panels for use in this patient population despite the inclusion of unknown to moderate penetrant genes and a higher rate of VUS results than single gene testing.

References

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2. Walsh T, Casadei S, Coats KH et al. Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 families at high risk of breast cancer. JAMA 2006; 295: 1379-1388.
3. Walsh T, Casadei S, Lee MK, et al., Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci 2011;108:18032-18037.
4. Current NCCN guidelines Version 2.2014