Testing for three founder mutations in *BRCA1* and *BRCA2* in Ashkenazi Jewish individuals misses half of breast and ovarian cancer risk variants

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Introduction

People of Ashkenazi Jewish (AJ) descent have a high occurrence of three founder mutations (*BRCA1* c.5266dupC, *BRCA1* c.68_69delAG, and *BRCA2* c.5946delT, collectively referred to here as "AJ3") that are associated with hereditary breast and ovarian cancer (HBOC). The National Comprehensive Cancer Network (NCCN) genetic testing guidelines for HBOC recommend first testing high-risk AJ individuals for the AJ3 mutations, followed by panel testing if negative[1,2]. However, little is known about the distribution and frequency of HBOC genetic variants in this population outside of *BRCA1* and *BRCA2*. Follow-up panel testing has historically not been feasible given the high cost of genetic testing and variable insurance reimbursement rules. Here, we report the spectrum of HBOC risk variants identified in a cohort of AJ individuals using a comprehensive next-generation sequencing (NGS) panel with 19 genes associated with increased risk of hereditary breast and/or ovarian cancer. We also assess the personal and family histories of cancer in this cohort, and qualification status according to NCCN guidelines.

Study Design and Methods

Color's NGS panel includes 19 genes in which pathogenic mutations are associated with an elevated risk for breast and/or ovarian cancer (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53*). The reportable range contains the entire length of every coding exon of all major transcripts, including 20bp flanking each exon and non-canonical splice regions, with the following exceptions: in *PMS2* exons 12-15 were not analyzed, and in *EPCAM* only large deletions and duplications including the 3' end of the gene were analyzed.

Laboratory procedures were performed at Color's CLIA certified and CAP accredited laboratory (Burlingame, CA). Saliva specimens were collected and transported using the Oragene DX (OGD-510) saliva collection device. DNA extraction was performed using standard methods. Library preparation was performed using Kapa Biosystems HyperPlus reagents and target enrichment was performed using the Agilent SureSelect XT chemistry. Sequencing was performed on a Illumina NextSeq 500 instrument using the paired-end 150bp, High Output kit.

Sequence reads were aligned against GRCh37.p12 with the Burrows-Wheeler Aligner [BWA-MEM][3]. SNVs and indels were called by the HaplotypeCaller module of GATK3[4]. CNVs were detected using dedicated algorithms[5]. The coverage requirements for reporting were ≥20X for each base of the reportable range and ≥50X for 99% of the reportable range. Median coverage typically ranges between 200-300X. Variants were classified according to the guidelines for sequence variant interpretation of the American College of Medical Genetics and Genomics[6]. All variants are evaluated by a board certified medical geneticist or pathologist. Reported variants classified as pathogenic or likely pathogenic were confirmed by a secondary technology (Sanger sequencing, aCGH or MLPA).

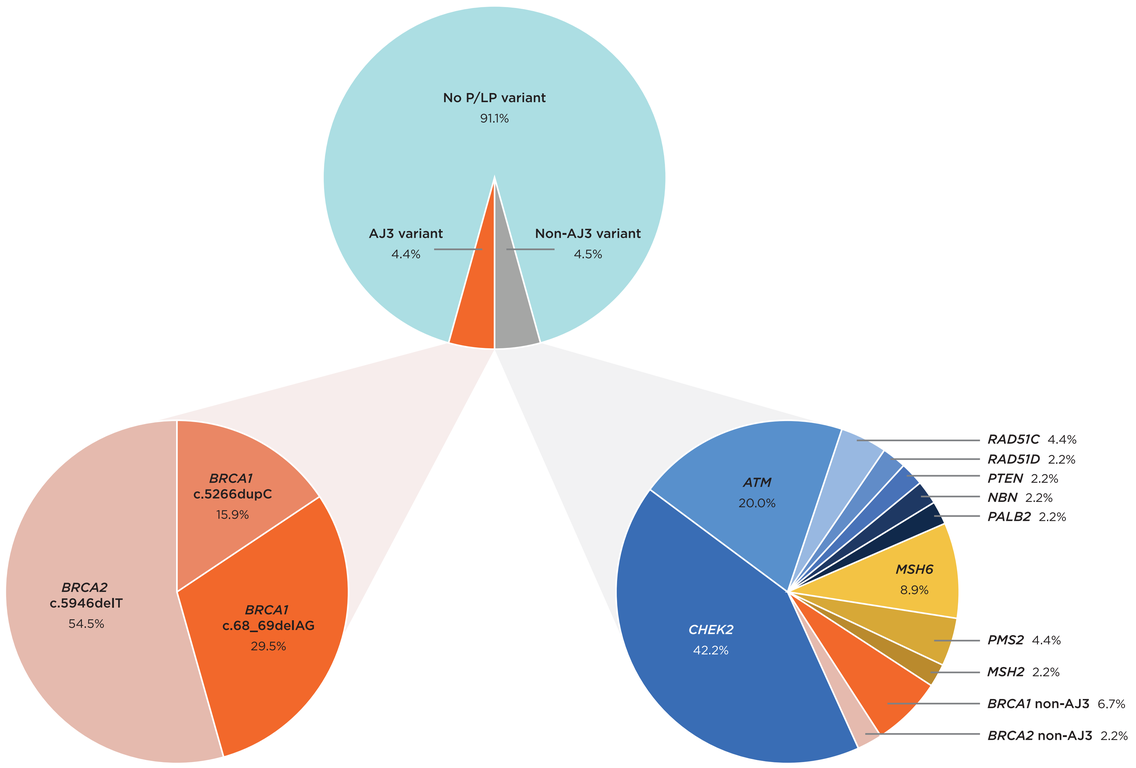
Ethnicity assignments and personal or family history of cancer were based on self-reported information. All individuals who indicated Ashkenazi Jewish ancestry were assigned to the Ashkenazi Jewish category.

Discussion and Conclusions

- The results from this study show that testing for only the AJ3 *BRCA1/2* founder mutations in AJ individuals would miss a majority of HBOC associated mutations. Half of reported variants were outside of these founder mutations, in 10 genes beyond *BRCA1* and *BRCA2*, most predominantly in *CHEK2*.
- This study shows a higher population frequency of the AJ3 mutations, 4.5% of AJ individuals (44/980) compared to previously published mutation rates of 2-2.5%[8,9]. This suggests a slight ascertainment bias towards high-risk patients.
- These data show the importance of panel testing in people with AJ ancestry even in the absence of a personal or family history of breast and/or ovarian cancer.
- Further, these data suggest that panel testing is a more efficient testing paradigm for this population than sequentially testing for founder mutations followed by reflex panel testing, as currently recommended.

Results

Figure 1. Pathogenic and likely pathogenic variants in Ashkenazi Jewish cohort



Top: Among the 980 AJ individuals, 8.9% (87/980) were found to carry at least one pathogenic (P) or likely pathogenic (LP) variant in one of the 19 genes. Two individuals had 2 P/LP variants, for a total of 89 variants, described below.

Left: AJ3 variants in *BRCA1* and *BRCA2*, which together comprise 49.4% (44/89) of total variants.
Right: Non-AJ3 variants, which comprise 50.6% (45/89) of the total variants, broken out by gene. Variants found in *BRCA1* and *BRCA2* outside of AJ3 comprise 4.5% (4/89, orange), Lynch Syndrome associated genes comprise 7.9% (7/89, yellow), and all other HBOC associated genes comprise 38.2% (34/89, blue) of total variants.

Table 1. Cohort demographic details

		Number	Percent	# of Mutation Carriers	Mutation Carrier Rate
Gender	Female	768	78.4%	56	7.3%
	Male	212	21.6%	31	14.6%
Personal Health History	Breast Cancer	136	13.9%	8	5.9%
	Ovarian Cancer	8	0.8%	0	0.0%
	Breast and Ovarian Cancer	2	0.2%	0	0.0%
	No Breast or Ovarian Cancer	834	85.1%	79	9.5%
Family Health History	NCCN Qualified	594	60.6%	65	10.9%
	NCCN Not Qualified	357	36.4%	19	5.3%
	Incomplete Information	29	3.0%	3	10.3%

Demographics of the 980 AJ individuals in this study. Family health history is based on NCCN guidelines[1]. 7 of the 8 mutation carriers with a personal history of breast cancer had a single P or LP variant as follows: *ATM* c.6228delT (two individuals), *MSH6* c.3984_3987dupGTCA, *PTEN* c.697C>T, *BRCA2* c.5946delT (two individuals), *CHEK2* c.1283C>T. One individual carried two variants: *APC* c.3920T>A and *CHEK2* c.1283C>T.

Table 2. Non-AJ3 variants found in cohort

Gene	cHGVS	pHGVS	Total
ATM	c.1027_1030delGAAA	p.Glu343Ilefs*2	2
	c.1065+1G>T		3
	c.2638+2T>C		1
	c.6228delT	p.Leu2077Phefs*5	3
BRCA1	c.134+2T>C		1
	c.5503C>T	p.Arg1835*	2
BRCA2	c.9588delA	p.Asp3197Thrfs*20	1
CHEK2	c.1100delC	p.Thr367Metfs*15	3
	c.1283C>T	p.Ser428Phe	13
	c.433C>T	p.Arg145Trp	1
	c.470T>C	p.Ile157Thr	2
MSH2	c.1906G>C	p.Ala636Pro	1
MSH6	c.3959_3962delCAAG	p.Ala1320Glufs*6	1
	c.3984_3987dupGTCA	p.Leu1330Valfs*12	3
NBN	c.1903A>T	p.Lys635*	1
PALB2	c.196C>T	p.Gln66*	1
PMS2	c.137G>T	p.Ser46Ile	1
	deletion of exon 10		1
PTEN	c.697C>T	p.Arg233*	1
RAD51C	c.706-2A>G		1
	c.955C>T	p.Arg319*	1
RAD51D	c.556C>T	p.Arg186*	1
Total			**45**

Non-AJ3 P/LP variants found in the cohort. 28.9% (13/45) were *CHEK2* c.1283C>T, an allele reported to increase breast cancer risk two-fold in AJ women[7].

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