DETECTION OF LARGE REARRANGEMENTS IN A PAN CANCER GENE PANEL USING NEXT GENERATION SEQUENCING

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BACKGROUND

- The advent of Next Generation Sequencing (NGS) has facilitated the detection of sequencing variants across multiple genes simultaneously.[1]
- To date, however, large deletions and duplications are largely detected by alternative technologies such as Multiplex Ligation-dependent Probe Amplification (MLPA)[2] and microarray Comparative Genomic Hybridization (CGH).[3]
- Accurate detection of large rearrangements using NGS requires thorough validation of the technical assay and informatics analysis pipeline.
- Here we describe the detection of large rearrangements (LR) in a clinically validated pan cancer panel test using NGS.

METHODS

- A consecutive series of patients who received clinical testing with the pan-cancer panel between September 2013 and August 2015 were assessed.
- The panel included *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *BMPR1A*, *CDH1*, *CDKN2A*, *MUTYH*, *SMAD4*, *STK11*, *CHEK2*, *PALB2*, *ATM*, *NBN*, *BARD1*, *BRIP1*, *CDK4*, *RAD51C* and *RAD51D*.
- Sequencing and large rearrangement testing was performed for all genes in the panel except *EPCAM*, for which only large rearrangement analysis of the terminal exons was performed.
- Dosage analysis was performed across gene coding exons and select promoters using NGS.
- All LRs were confirmed using targeted microarray CGH, MLPA, or confirmatory PCR and sequencing of the mutant product.
- MLPA was initially used as the primary assay for dosage analysis in *PMS2* and *CHEK2*, which are genes with highly homologous pseudogene regions.
- *PMS2*-specific sequencing analysis and/or long range PCR were used where appropriate to confirm the presence of LRs in *PMS2*.

RESULTS

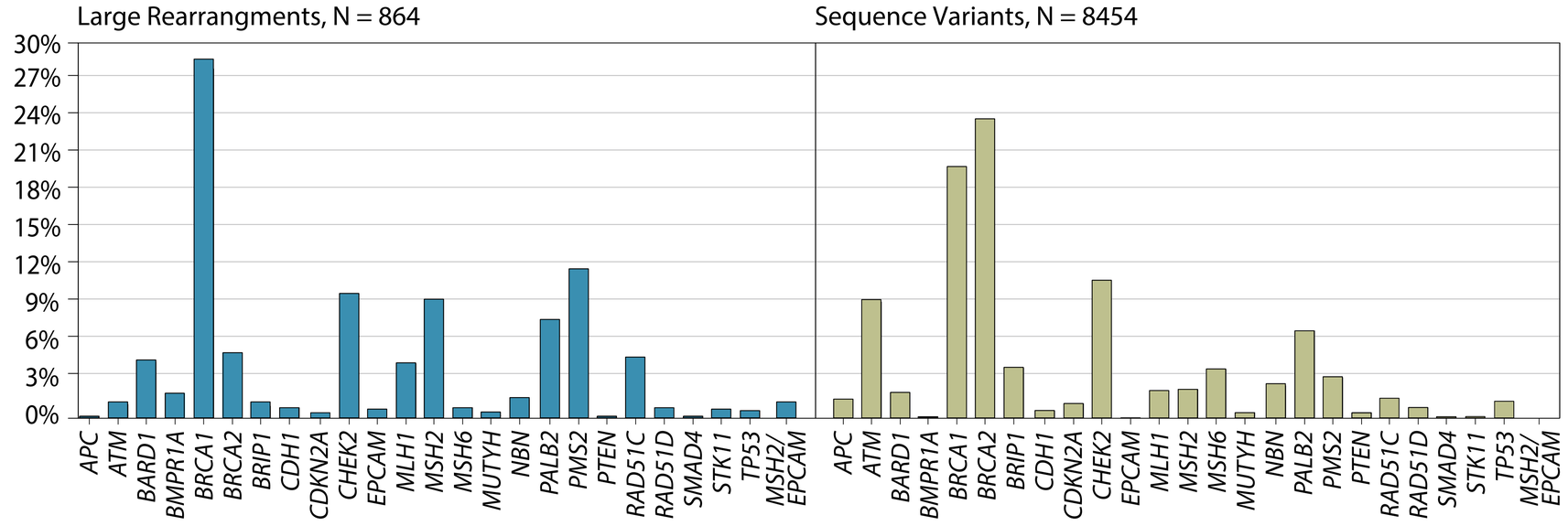
- The proportion of clinically significant defects attributable to large genomic rearrangements across 25 genes (Figure 1) was determined to be 9.3% using the methods described above.
- 85.8% of all LRs detected were deletions and 12.5% were duplications (Table 1).

TABLE 1. NUMBER OF PATHOGENIC LR AND SEQ. VARIANTS DETECTED BY GENE

	TOTAL LR	TOTAL SEQ.
APC	11 (8.0%)	127 (92.0%)
ATM	40 (4.8%)	800 (95.2%)
BARD1	17 (9.0%)	172 (91.0%)
BMPR1A	1 (12.5%)	7 (87.5%)
BRCA1	248 (12.7%)	1700 (87.3%)
BRCA2	45 (2.2%)	2023 (97.8%)
BRIP1	11 (3.1%)	342 (96.9%)
CDH1	7 (12.3%)	50 (87.7%)
CDKN2A	3 (3.0%)	97 (97.0%)
CHEK2	86 (8.5%)	931 (91.5%)
EPCAM	6 (100%)	0 (0%)*
MLH1	38 (17.0%)	185 (83.0%)
MSH2	82 (29.8%)	193 (70.2%)
MSH6	7 (2.1%)	331 (97.9%)
MUTYH	4 (10.3%)	35 (89.7%)
NBN	14 (5.7%)	231 (94.3%)
PALB2	68 (10.4%)	589 (89.6%)
PMS2	103 (27.0%)	278 (73.0%)
PTEN	1 (2.9%)	34 (97.1%)
RAD51C	42 (24.0%)	133 (76.0%)
RAD51D	7 (9.1%)	70 (90.9%)
SMAD4	1 (10.0%)	9 (90.0%)
STK11	6 (42.9%)	8 (57.1%)
TP53	5 (4.4%)	109 (95.6%)
MSH2/EPCAM	11 (100%)	0 (0%)*
TOTAL	**864 (9.3%)**	**8454 (90.7%)**

**EPCAM was not sequenced*

FIGURE 1. DISTRIBUTION OF PATHOGENIC MUTATIONS AMONG ALL GENES TESTED



- Seven distinct insertions were also detected, including the *BRCA2* Portuguese founder mutation (c.156_157insAlu), and five triplications.
- Importantly, there were a large number of clinically significant LRs detected in *PMS2*, of which 39.8% occurred in exons 11-15 alone, a region that shares significant sequence homology to the pseudogene, *PMS2CL*.
- This finding stresses the importance of being able to accurately detect mutations in this region and distinguish between clinically significant LRs that occur in *PMS2*, and benign LRs in *PMS2CL*.
- Comprehensive coverage of exonic regions in this NGS assay also facilitated the detection of 18 unique partial deletions.

DISCUSSION

- NGS technology has been demonstrated as a reliable method to detect sequencing variants and has facilitated simultaneous, multigene analysis.
- We have shown that dosage analysis by NGS can accurately detect the presence of genomic LRs in our pan cancer panel test.
- This study shows that LRs constitute a significant proportion of mutations found in individuals with a personal or family history of hereditary cancer, and should be part of a comprehensive genetic testing strategy.

REFERENCES

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