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Prospective evaluation of a 27 gene inherited cancer panel across 631 consecutive patients referred for testing in a clinical diagnostic laboratory

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I. Abstract

Extensive heterogeneity in the clinical and genetic spectrum of inherited cancers coupled with the advantages offered by Next Generation Sequencing (NGS) technologies has allowed multi-gene panel testing to become an efficient means for clinical and diagnostic identification of patients with an inherited predisposition to a broad spectrum of syndromic and non-syndromic forms of cancer. We report our experience with a panel of 27 genes selected on the basis of a genetic predisposition towards cancer risk, across a prospective cohort of 631 consecutive patients referred for testing at our laboratory. **Our goal was to evaluate the clinical uptake of this test, the locus heterogeneity of the constituent genes on the panel, and review the clinical sensitivity in our cohort relative to that published in the recent literature**. A family history (hx) of cancer constituted a major reason for referral accounting for 84% (n=529) of our cohort while individuals affected with cancer accounted for 43% (n=273). Further evaluation of the cancer phenotypes revealed that 34% (n=214) had a personal history of Hereditary Breast and Ovarian (HBOC)-associated cancers and 10% (n=64) had a personal history of Lynch Syndrome (LS)-associated cancers, including 34 patients who had a personal history of cancers associated with both syndromes. Similarly, a family history of HBOC-associated tumors and LS-associated tumors respectively accounted for 74% (n=467) and 58% (n=364) of the cohort. Fifty percent (n=314) of the cohort had a family history of cancers associated with both HBOC and LS syndromes, illustrating the utility of panel testing over serial evaluations based on tumor phenotype. Over 27,000 non-unique variants were identified in this cohort of 631 patients, averaging to 43 variants per patient with a variant workload that was linearly correlated to the size of the coding region in the 27 genes tested. Six hundred and fifty variants were classified between pathogenic (P) to likely benign (LB) in this cohort, averaging to at least 1 variant per specimen that would trigger a classification event (range 0-5 variants). Eighty-two variants were classified as likely pathogenic (LP)/pathogenic (P) across the 27 genes. Seven LP/P variants were recurrent (detected in multiple patients), and four patients had more than one LP/P variant. In total, the proportion of patients testing positive for a LP or a P variant was 10.5% (n=66) without including carriers of single pathogenic *MUTYH* variants, which increased to 12.4% when *MUTYH* carriers were included. Forty-six percent (n=36) of the LP/P variants were identified in classic HBOC or LS-related genes, while the remaining 54% (n=42) of LP/P variants were present in other high or moderate to low risk genes on the panel. We observed a 4.0 fold increase in the number of LP/P variants despite a 7.1 fold increase in VUS going from *BRCA1* and *2* genes upwards to the 25 additional genes on the panel. Fifteen affected individuals with personal and family history of both HBOC- and Lynch-associated tumors, tested negative for actionable variants in our panel, illustrating the possible genetic contribution of other loci. Overall, the performance and clinical sensitivity of the 27 gene inherited cancer panel within a referral testing population correlated well with all recently published reports, further corroborating the utility of panel testing in patients with an inherited predisposition to cancer.

II. Methods

- Study subjects included the first consecutive 631 patients referred to our laboratory for the 27 inherited cancer gene panel testing (**Table 1**).
- Clinical information was ascertained from questionnaires provided by the submitting medical professional.
- Patients were stratified based on personal and family history of cancer.
  - HBOC included the following cancers: breast, ovarian and prostate
  - Lynch Syndrome included the following cancers: colorectal, gastric, pancreatic, ovarian, uterine/endometrial
- Variants classified as Likely Benign and Benign were eliminated from further evaluation.
- Findings were grouped and assessed based on variant classification and phenotypes.
- Positive cases were defined as those having at least one LP/P, while VUS cases were defined as cases with at least one VUS, but lacking likely pathogenic or pathogenic variants.
- Negative cases were those which had likely benign and/or benign variants, as well as synonymous variants.
- For variant level data, *MUTYH* variants were included, while for patient level data, *MUTYH* single allele carriers of LP/P mutations were not included as *MUTYH*-associated polyposis is an autosomal recessive condition.

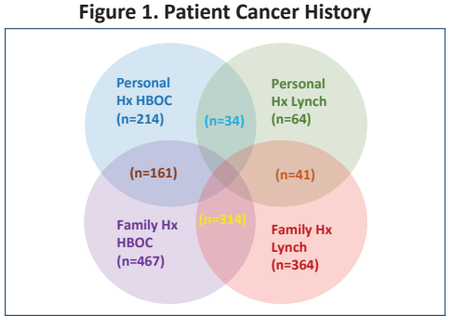
Table 1. Twenty-seven Inherited Cancer Gene Panel

Phenotype	Genes
HBOC	*BRCA1, BRCA2*
Lynch	*MLH1, MSH2, MSH6, PMS2, EPCAM*
High Risk for Breast and Other Hereditary Cancers	*TP53, PTEN, STK11, CDH1, APC, BMPR1A, MUTYH, SMAD4, CDK4, CDKN2A*
Moderate to Low Risk Genes	*ATM, CHEK2, PALB2, BARD1, BRIP1, NBN, RAD51C, RAD51D, FAM175A, PRKAR1A*

III. Results

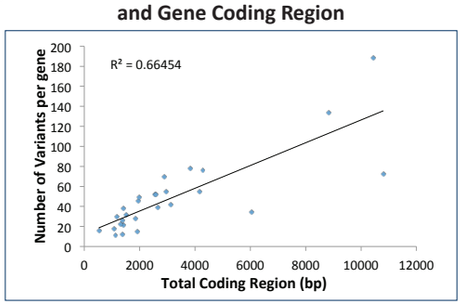
- **Figure 1** depicts the phenotypic representation of our cohort (n=631).
  - A personal history of HBOC-associated cancers was found in 34% of patients (n=214), while only 10% (n=64) had a personal history of LS-associated cancers.
  - Thirty-four patients had a personal history of cancers associated with both syndromes.
  - A family history of cancer constituted a major reason for referral accounting for 84% (n=529) of our cohort while individuals affected with cancer accounted for 43% (n=273).
  - A family history of HBOC-associated tumors and LS-associated tumors respectively accounted for 74% (n=467) and 58% (n=364) of the cohort.
  - Fifty percent (n=314) of the cohort had a family history of cancers associated with both HBOC and LS syndromes.

Figure 1. Patient Cancer History



- Over 27,000 non-unique variants were identified; averaging 43 variants per patient.
  - Six hundred and fifty variants were classified between P to LB; averaging at least 1 variant per specimen that would trigger a classification event (range 0-5 variants).
  - A linear correlation between the variant workload and size of the coding region was observed (**Figure 2**).

Figure 2. Linear Correlation between Variant Workload and Gene Coding Region



- Eighty-two variants were classified as LP/P across 27 genes (**Table 2**).
  - Seven LP/P variants were detected in multiple patients.
  - Four individuals had more than one LP/P variants.
- In total, the proportion of patients testing positive for a LP or P variant was **10.5% (n=66)** (*MUTYH* pathogenic carriers were not captured here) (**Table 3**).
  - Forty-six percent (n=36) of the LP/P variants were identified in classic HBOC -or LS-related genes, while the remaining 54% (n=42) of LP/P variants were present in other high or moderate to low risk genes in the panel.

Table 2: LP/P Variants Broken Down by Patient Phenotype

Gene	Total LP/P patients	Personal Hx HBOC patients	Personal Hx Lynch patients	Fam Hx HBOC patients	Fam Hx Lynch patients
APC	1	0	0	0	1
ATM	8	5	0	5	6
BARD1	1	0	0	0	0
BRCA1	10	5	1	10	5
BRCA2	10	3	0	8	6
BRIP1	5	0	0	4	2
CDH1	1	0	0	1	0
CHEK2	9	3	3	7	3
MSH2	4	1	2	3	4
MSH6	3	0	1	0	2
MUTYH	14	5	1	12	11
NBN	3	0	0	2	2
PALB2	4	0	1	3	2
PMS2	9	2	0	6	6

Table 3: Positive, VUS, and Negative Rate for Datasets

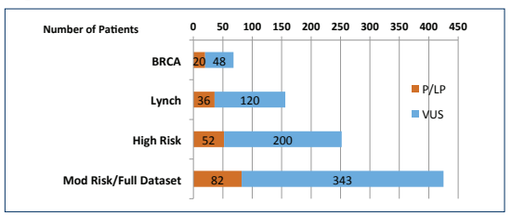
Phenotype	LP/P	VUS	Neg.
Full Dataset (n=631)	10.5%	33.3%	56.3%
Personal Hx HBOC-associated cancer (n=214)	8.9%	38.8%	52.3%
Personal Hx Lynch-associated cancer (n=64)	10.9%	37.5%	51.6%
Personal Hx both HBOC and Lynch (n=34)	8.8%	47.1%	44.1%
Family Hx HBOC-associated cancer (n=467)	10.3%	34.5%	55.2%
Family Hx Lynch-associated cancer (n=364)	10.7%	31.3%	58.0%
Family Hx both HBOC and Lynch (n=314)	9.9%	32.8%	57.3%
Personal and Family Hx HBOC (n=161)	9.3%	42.2%	48.4%
Personal and Family Hx Lynch (n=41)	7.3%	39.0%	53.7%
Personal and Family Hx both HBOC and Lynch (n= 15)	0.0%	60.0%	40.0%

- The positive and VUS rates of this study were comparable to previously published studies (**Table 4**).
- We observed a **4-fold** increase in the number of LP/P variants despite a **7.1**-fold increase in VUS variants from *BRCA1* and *BRCA2* genes upwards to the 25 additional genes on the panel (**Figure 3**).

Table 4: Positive and VUS Rates from the Literature

Laboratory	Gene Panel	Positive Rate	VUS Rate
Current Study	27 genes	10.5%	33.3%
LaDuca, et al. 2014[1]	22 genes	9.6%	23.5%
Lincoln, et al. 2015[2]	29 genes	24.5%	41%
Tung, et al. 2015[3]	25 genes	13.5%	41.7%
Yurgelun, et al. 2015[4]	25 genes	12.4%	38%
Susswein, et al. 2016[5]	29 genes	10.2%	34.7%

Figure 3: LP/P to VUS Ratio in the Different Gene Sets



IV. Conclusions

- Despite an increase in VUS rate, additional LP/P variants were being detected with an increase in panel size, supporting the utility of a larger panel over conventional HBOC or Lynch testing.
- Positive and VUS rate from this study were comparable to previously published studies.
- LP/P variants in the *BRCA1/2* genes were detected in patients with personal/family history of Lynch Syndrome, and classic Lynch gene variants were detected in patients with personal/family history of HBOC, supporting the utility of consolidated panel testing over serial evaluations based on tumor phenotype.
- No positive cases were detected among individuals with both personal and family history of both HBOC- and Lynch-associated cancers, illustrating the possible contribution of other genetic loci to the etiology of disease in these families.

V. References

1. *Genet Med* 2014; 16:830-837.
2. *J Mol Diagn* 2015; 17:533-544.
3. *Cancer 2015*; 121:25-33.
4. *Gastroenterology* 2015; 149:604-613.
5. *Genet Med 2016*; 18:823-832.

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