ASSESSMENT OF THE CLINICAL PRESENTATION OF PATIENTS WITH AT LEAST TWO PATHOGENIC MUTATIONS ON MULTIGENE PANEL TESTING

Jeffrey N. Weitzel,[1] Kathleen R. Blazer,[1] Bita Nehoray,[1] John Kidd,[2] Thomas P. Slavin,[1] Ilana Solomon,[1] Mariana Niell-Swiller,[1] Christina Rybak,[1] Jennifer Saam[2]

1) City of Hope, Duarte, CA 2) Myriad Genetic Laboratories, Inc., Salt Lake City, UT

BACKGROUND

- Technical advances have upended the established paradigm of testing high penetrance cancer predisposition genes based on syndromic features.
- National guidelines now include discussion of multigene panels.[1] However, significant gaps in our knowledge of gene-specific phenotypes have been identified, and the effects of various gene combinations is unknown.
- The aim of this analysis was to assess patients who underwent hereditary cancer predisposition testing with a 25-gene panel and were found to have at least two pathogenic mutations.

METHODS

Genetic Testing

- We examined results from 80,829 sequential patients tested with a 25 hereditary cancer gene panel in a CLIA certified laboratory.
- The 25-gene panel included *APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, PTEN, SMAD4, STK11, RAD51C, RAD51D and TP53*.
- Sequencing and large rearrangement was performed for all the genes in the panel (large rearrangement only for *EPCAM*).

Patients with Multiple Mutations

- Patients with more than one pathogenic mutation were identified.
  - Patients with biallelic *MUTYH* mutations were considered to have one high-penetrance gene condition and were included in the analyses if found in combination with another pathogenic mutation.
  - 107 patients with a monoallelic *MUTYH* mutation and a pathogenic mutation in another gene were considered to have just one pathogenic mutation and were not included in the analyses.
- Clinical history was obtained from the test requisition forms submitted by health care provider report on.
- Phenotype manifestation was assessed in multiple mutation carriers by a team of cancer genetics clinicians to determine whether a patient's phenotype was consistent with one or both mutations.[2]
  - Any cancer indicated in NCCN guidelines as associated with mutations in that gene was counted as consistent with the expected phenotype.

Statistical Methods

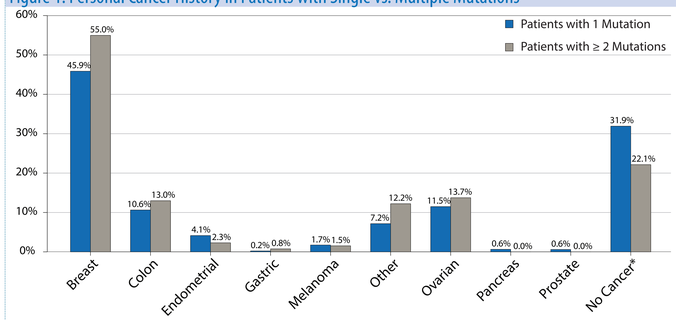
- Pearson's chi-square tests were performed to determine a difference between affected status, multiple cancer status, and age of diagnosis across multiple mutation status. A p-value <0.05 was considered statistically significant.

Table 1. Combinations of Genes in Patients with Multiple Pathogenic Mutations

(*1a*) Two High Penetrance Genes*		(*1b*) Two Moderate Penetrance Genes		(*1c*) Combination of High* and Moderate Penetrance Genes			
Mutations	**Count**	**Mutations**	**Count**	**Mutations**	**Count**	**Mutations**	**Count**
BRCA2 - PMS2	7	*CHEK2 - PALB2*	8	*BRCA2 - ATM*	8	*PMS2 - ATM*	2
BRCA1 - BRCA2	6	*ATM - PALB2*	3	*BRCA1 - ATM*	6	*PMS2 - PALB2*	2
BRCA1 - PMS2	6	*ATM - CHEK2*	2	*BRCA1 - BRIP1*	6	*BRCA1 - RAD51D*	1
BRCA1 - MSH2	3	*BARD1 - BRIP1*	2	*BRCA1 - CHEK2*	6	*BRCA2 - BARD1*	1
BRCA1 - MSH6	2	*BRIP1 - CHEK2*	2	*BRCA1 - PALB2*	5	*BRCA2 - RAD51D*	1
BRCA2 - MLH1	2	*CHEK2 - RAD51C*	2	*BRCA2 - CHEK2*	5	*CDH1 - CHEK2*	1
BRCA2 - MSH2	2	*ATM - RAD51C*	1	*BRCA1 - NBN*	3	*CDKN2A - ATM*	1
APC - BRCA1	1	*BARD1 - NBN*	1	*BRCA2 - PALB2*	3	*MLH1 - RAD51C*	1
APC - BRCA2	1	*BRIP1 - NBN*	1	*BRCA1 - BARD1*	2	*PMS2 - BARD1*	1
APC - MSH2	1	*CHEK2 - RAD51D*	1	*BRCA2 - BRIP1*	2	*PMS2 - BRIP1*	1
APC - CDKN2A	1	*NBN - RAD51D*	1	*BRCA2 - NBN*	2	*PMS2 - CHEK2*	1
BRCA1 - CDH1	1			*CDKN2A - PALB2*	2	*PMS2 - NBN*	1
BRCA1 - MLH1	1	biallelic *MUTYH***	23	*MSH2 - NBN*	2	biallelc *MUTYH*** - *ATM*	1
BRCA1 - CDKN2A	1						
BRCA1 - TP53	1						
BRCA2 - MSH6	1						
BRCA2 - CDKN2A	1						
BRCA2 - TP53	1						
biallelic *MUTYH*** - *BRCA2*	*1*						

*High penetrance genes are: *APC, BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, CDKN2A, PMS2, TP53, biallelic MUTYH*
***MUTYH* is low penetrance in monoallelic state; biallelic *MUTYH* causes *MUTYH* associated polyposis (MAP), a highly penetrant recessive condition

Figure 1. Personal Cancer History in Patients with Single vs. Multiple Mutations

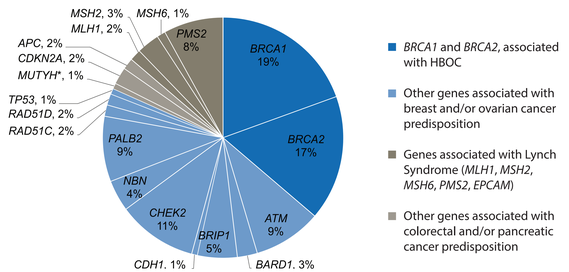


*Patients with multiple pathogenic mutations were statistically more likely to be affected with cancer compared with patients with one mutation (p= 0.017) . No significant differences observed in other comparisons.

RESULTS

- Of 80,829 sequential patients who underwent panel testing, 5,703 (7.1%) had a single pathogenic mutation; 154 (0.19%) had multiple pathogenic mutations.
- Pathogenic mutations were identified in 19 different genes among multiple mutation carriers (Table 1).
  - 40 patients had mutations in two high penetrance genes (*APC*, *BRCA1*, *BRCA2*, *CDH1*, *MLH1*, *MSH2*, *MSH6*, *CDKN2A*, *PMS2*, *TP53*, biallelic *MUTYH*) (Table 1a).
  - 24 patients had mutations in two moderate penetrance genes (Table 1b).
  - 67 patients had mutations in both high and moderate penetrance genes (Table 1c).
  - 23 patients carried only biallelic *MUTYH* mutations.

Figure 2. Distribution of Genes with Pathogenic Variants in Multiple Mutation Carriers



*Represents only biallelic *MUTYH* carriers who also had a pathogenic mutation on an additional gene; Patients with only biallelic *MUTYH* mutations are not included.

Table 2. Number of Primary Cancers in Patients with Single vs. Multiple Mutations

Single Mutation (n = 5,703)		Multiple Mutations (n=131)**	
Number of Primary Cancer Diagnoses*	**n (%)**	**Number of Primary Cancer Diagnoses***	**n (%)**
0	1820 (31.9%)	0	29 (22.1%)
1	2747 (48.2%)	1	68 (51.9%)
2	854 (15.0%)	2	25 (19.1%)
3	208 (3.6%)	3	7 (5.3%)
4	55 (1.0%)	4	1 (0.8%)
5	16 (0.3%)	5	1 (0.8%)
6	2 (0.04%)	6	0
7	1 (0.02%)	7	0
Total Affected	3,883 (68.1%)	**Total Affected**	102 (77.9%)

*Patients with more than one primary cancer diagnosis of the same type (e.g. breast) are counted multiple times. Patients with only biallelic *MUTYH* mutations are not included.
**Patients with multiple mutations appeared more likely to be affected with multiple cancers, though this did not reach significance (p=0.0881).

RESULTS (continued)

- Given the well-defined phenotype of biallelic *MUTYH* carriers (MAP), patients who carried only biallelic *MUTYH* mutations were excluded from all subsequent analyses.
  - Notably, of 23 patients with only biallelic *MUTYH* mutations, 8 had a history of colorectal cancer, 11 had polyps but no colorectal cancer.
  - 4 biallelic *MUTYH* carriers had no reported clinical history of colorectal cancer or polyps.
- Breast or ovarian cancer were the most common cancers among the patients with single (45.9% and 11.5%, respectively) or multiple (55.0% and 13.7%, respectively) pathogenic mutations (Figure 1).
- 89/131 (67.9%) patients had at least one mutation in *BRCA1* or *BRCA2*.
  - 6 had a mutation in both *BRCA1* and *BRCA2* (Table 1a).
- The most common second mutations were in *CHEK2*, *ATM* and *PALB2* (Figure 2).
- Of 40 patients with mutations in two high penetrance genes:
  - 27/40 showed a mixed phenotype, consistent with both.
  - 12/40 only reflected one phenotype.
- Patients with multiple mutations were statistically more likely to be affected with cancer ($p = 0.017$) (Figure 1, Table 2).
- Patients with multiple mutations appeared more likely to be affected with multiple primary cancers, although this did not reach significance ($p = 0.0881$) (Table 2).
- There was no significant difference in age at cancer onset (< or ≥ age 50 years) ($p = 0.7645$).

STUDY LIMITATIONS

- Patient and family phenotype data was based on health information collected and documented on test requisition forms by ordering clinicians.

CONCLUSIONS

- Discovery of more than one deleterious mutation by multigene panel testing confounds accurate prediction of the phenotype or magnitude of risk.
- Dual mutation carriers were statistically more likely to be affected with cancer.
- Some cases with two high penetrance gene mutations only manifested the phenotype of one of the genes.
- There was no obvious effect of moderate penetrance genes (e.g., *ATM*) on high penetrance gene phenotypes.
- A formal, prospective assessment of the impact of moderate penetrance genes, alone and in combination, on carrier phenotype is needed to identify any definitive effect.

REFERENCES

1. Daly M, Pilarski R, Axilbund JE, et al. Genetic/Familial High-Risk Assessment: Breast and Ovarian. *NCCN Clinical Practice Guidelines in Oncology*. 2015. http://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf.
2, Weitzel J, Blazer K, MacDonald D, Culver J, Offit K. Genetics, genomics, and cancer risk assessment: State of the Art and Future Directions in the Era of Personalized Medicine. *CA Cancer J Clin*. 2011;61(5):327-359.

Abstract # 1514: Contact JWeitzel@coh.org with additional questions.

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