Paired DNA and RNA sequencing from 450,000 consecutive individuals: Impact on yield in hereditary breast, ovarian, pancreatic, and prostate cancer (HBOP) genes

Magan Trottier*, Carolyn Horton*, Lily Hoang, Jessica Grzybowski, Heather Zimmermann, Jesus Ramirez Castano, Sami Belhadj, Marcy Richardson, Rachid Karam *Co-first authors

Ambry Genetics

BACKGROUND

- Paired DNA-RNA sequencing has shown promise in improving detection and interpretation of DNA variants across clinical indications[1]
- Medically significant upgrades and variant of uncertain significance (VUS) downgrades are important for clinical care and cascade testing
  - › Medically significant upgrade: Newly detected germline intronic pathogenic/likely pathogenic variant (PV) or VUS reclassified to PV based on RNA evidence
  - › VUS downgrade: Reclassification from VUS to benign/likely benign based on RNA evidence

AIM: Characterize the impact of large-scale RNA analysis on hereditary breast, ovarian, pancreatic, and prostate cancer (HBOP) genes

METHODS

455,378 consecutive cases with hereditary cancer multigene panel testing from April 2019 - December 2023

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430,475 cases with concurrent RNA analysis

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40,292 total PVs and 97,613 total VUS assessed

Excluded autosomal recessive heterozygotes; low risk alleles (e.g., *CHEK2* I157T)' pancreatitis, limited evidence, non-loss of function genes

RNA impact determined for:

Homologous Recombination Deficiency (HRD) 9 genes
ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, PALB2, RAD51C, RAD51D

HBOP Core 13 genes
+ CDH1, NF1, PTEN, STK11, TP53, minus *BRIP1*

HBOP NCCN 18 genes
+ BRIP1, EPCAM, MLH1, MSH2, MSH6, PMS2

RESULTS

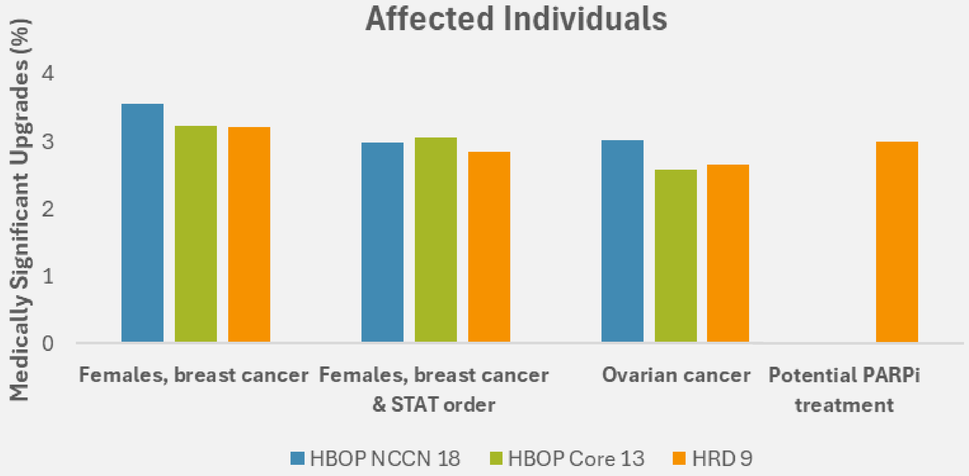

Figure 1. Medically significant upgrades based on RNA evidence (%) for all females with breast cancer or with a STAT (urgent) order and upcoming surgical date, females with ovarian cancer, and individuals with a cancer that may respond to PARP inhibitors (breast, ovarian, prostate, pancreas).

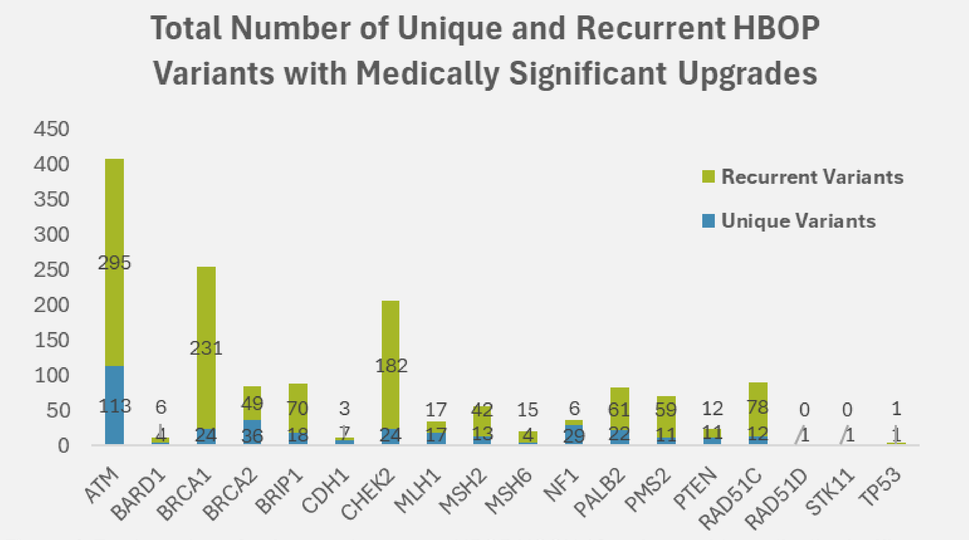

Figure 4. Total number of unique and recurrent HBOP NCCN 18 variants with medically significant upgrades based on RNA evidence, with number of recurrent (top) and unique (bottom) PVs indicated.

Characteristic	N	%
Sex Assigned at Birth		
Female	384,588	84.5
Male	70,742	15.5
Unknown	48	0.0
Age at Testing, Years		
Median (Range)	54 (1-105)	
Number of Genes Tested		
Median (Range)	40 (1-91)	
Race, Ethnicity, & Ancestry		
African American/Black	35,496	7.8
Ashkenazi Jewish	15,427	3.4
Asian	15,107	3.3
Hispanic	28,173	6.2
White	264,868	58.2
Multiple	5,917	1.3
Other	6,345	1.4
Unknown	84,045	18.5
Personal History of Cancer		
Breast	140,454	30.8
Ovarian	13,458	3.0
Prostate	19,766	4.3
Pancreatic	13,286	2.9
Total Cases	445,378	

Figure 2. Summary of cohort demographics.

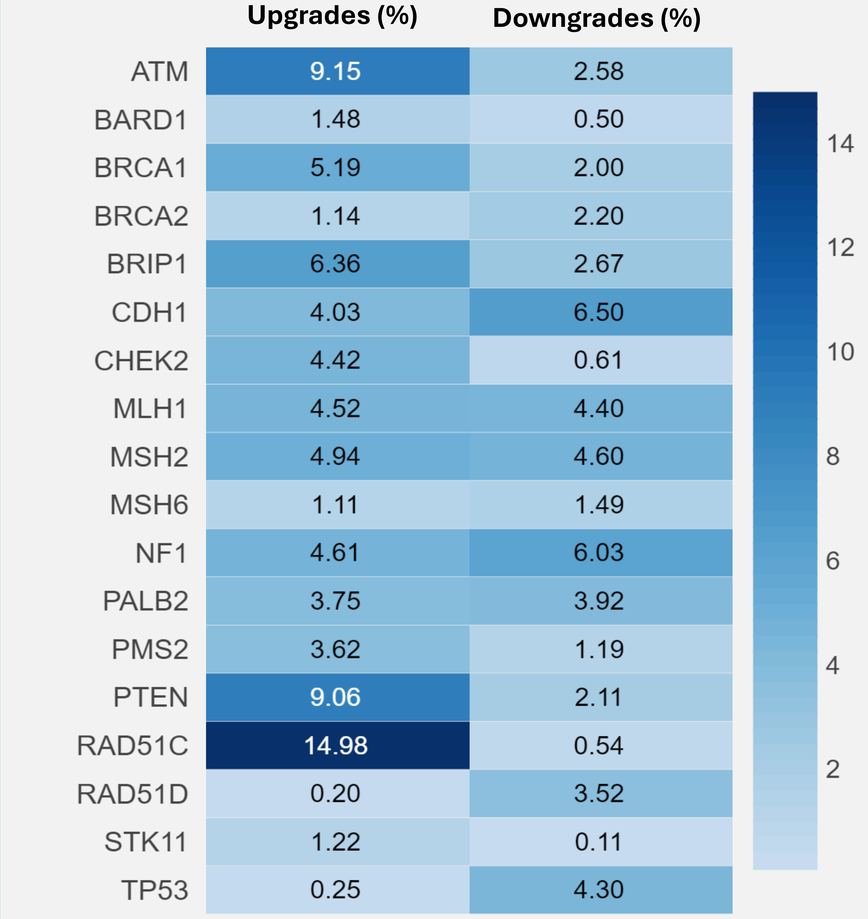

Figure 3. Heat map depicting medically significant upgrades and VUS downgrades based on RNA evidence (%) for HBOP NCCN 18 genes.

TAKE HOME POINTS

- Approximately 1 in 4 (23.6%) HBOP PVs identified using RNA are unique variants.
- RNA-dependent PVs were most common in *RAD51C*, *PTEN*, *BRIP1,* and *BRCA1* & VUS resolution was most common in *CDH1*, *NF1*, *MSH2*, *MLH1,* and *TP53*.
- Approximately 1 in 34 (2.96%) HBOP PVs in females with breast cancer and a DNA-only STAT (urgent) order were dependent on RNA evidence.

REFERENCES

1. Diagnostic Outcomes of Concurrent DNA and RNA Sequencing in Individuals Undergoing Hereditary Cancer Testing. Horton C, Hoang L, Zimmermann H, Young C, Grzybowski J, Durda K, Vuong H, Burks D, Cass A, LaDuca H, Richardson ME, Harrison S, Chao EC, Karam R. JAMA oncology, 2024, Vol 10 (2), 212-21.