# Clinical Utility of a 30-gene Hereditary Cancer Risk Panel in a Cohort of 23,179 Individuals

Alicia Y. Zhou, Anjali Zimmer, Cynthia L. Neben, Jeroen Van den Akker, Will McFadden, Robert O'Connor, J. Dianne Keen-Kim, Annette Leon, Jack Ji, Jill Hagenkord

Color Genomics, Burlingame, CA

## Introduction

Recent advancements in next generation sequencing (NGS) have greatly expanded the use of multi-gene testing panels in clinical diagnosis, including hereditary cancer risk. Approximately 5-10% of all cancers are associated with hereditary cancer syndromes, the majority of which are inherited in an autosomal-dominant manner with high-to-moderate penetrance[1]. As such, current recommendations for genetic testing of cancer susceptibility genes are primarily based on family and personal history of cancer. However, because of phenotypic variability, age-related penetrance, and gender-specific cancer risks, some carriers may be missed. Clinical utility studies to date have largely focused on these high-risk and affected populations, and thus, the clinical utility of genetic testing in a broader population has yet to be fully defined. To address this, we developed and validated a 30-gene NGS panel for hereditary breast, ovarian, uterine/endometrial, colorectal, melanoma, pancreatic, prostate, and stomach cancer that was offered through a low cost, easy access online delivery model. Here, we report the results of 23,179 high- and average-risk individuals who received this genetic test, providing data on the frequency and spectrum of pathogenic variants, including associations of high frequency alleles and cancer phenotypes. We also evaluate the results with respect to the current recommendations for genetic testing provided by the National Comprehensive Cancer Network (NCCN).

## Methods

All individuals were referred by physician order for the Color Hereditary Cancer Test which analyzes 30 genes in which pathogenic variants have been associated with increased risk for hereditary breast, ovarian, uterine/endometrial, colorectal, melanoma, pancreatic, prostate, and stomach cancer (*APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A* (p14ARF and p16INK4a), *CHEK2, EPCAM, GREM1, MITF, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11,* and *TP53*). The majority of these genes were assessed for variants within all coding exons and non-canonical splice regions. Laboratory procedures were performed at the Color laboratory under CLIA and CAP compliance. Variants were classified according to the American College of Medical Genetics and Genomics 2015 guidelines for sequence variant interpretation[2], and all variant classifications were approved by an American Board of Medical Genetics and Genomics board certified medical geneticist. Pathogenic and likely pathogenic (hereafter referred to as pathogenic) variants were confirmed by an orthogonal technology (Sanger sequencing, aCGH, or MLPA). Ethnicity assignments and personal history of cancer were based on self-reported information. An F test was used to assess equality of variances. Chi-squared test with Yates' correction or Student's t-test was used to determine statistical significance in each population.

## Conclusions

- Pathogenic variants were found in 27 of the 30 genes analyzed, and *BRCA1* and *BRCA2* accounted for 30.3% of pathogenic variants identified.
- *APC* c.3920T>A moderately increases the risk for hereditary colorectal cancer but not age of onset.
- The risk for hereditary breast cancer and age of onset was similar across all pathogenic *CHEK2* alleles, including c.1100delC and c.470T>C.
- The cohort analyzed here included a broader population than has historically qualified for genetic testing. 13.1% of individuals with pathogenic variants in genes with well-established genetic testing recommendations did not meet corresponding NCCN criteria.
- Taken together, these data reinforce the continued need to increase awareness and broaden access to genetic testing within the general population.

## Results

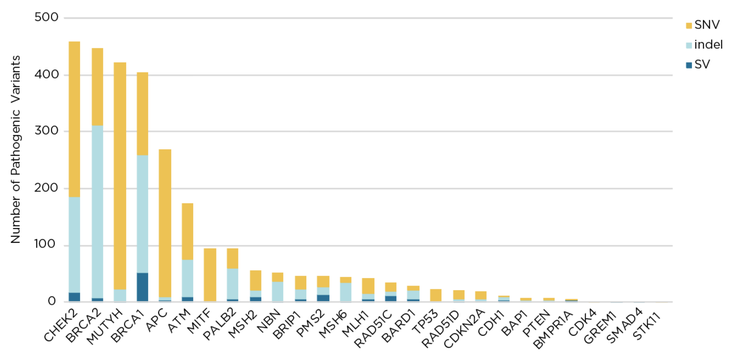
### Table 1. Cohort demographic details

The majority of individuals who received the Color Hereditary Cancer Test were women, over age 40 years, and of Caucasian ethnic background. PV, pathogenic variant.

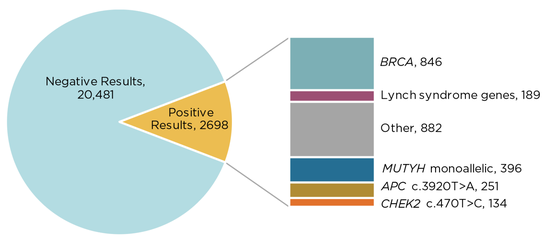
| | | Individuals (n) | Population | Individuals w/ PV (n) | Pathogenic Frequency |
|---|---|---|---|---|---|
| **Total** | | 23,179 | 100.0% | 2698 | 11.6% |
| **Gender** | Female | 19,263 | 83.1% | 2156 | 11.2% |
| | Male | 3916 | 16.9% | 542 | 13.8% |
| **Age (Years)** | 18-30 | 1747 | 7.5% | 245 | 14.0% |
| | 31-40 | 4447 | 19.2% | 517 | 11.6% |
| | 41-50 | 5544 | 23.9% | 611 | 11.0% |
| | 51-65 | 7255 | 31.3% | 825 | 11.4% |
| | 65+ | 4186 | 18.1% | 500 | 11.9% |
| **Ethnicity** | Caucasian | 12,083 | 52.1% | 1413 | 11.7% |
| | Ashkenazi Jewish | 2301 | 9.9% | 364 | 15.8% |
| | Hispanic | 1458 | 6.3% | 201 | 13.8% |
| | Multiple Ethnicities | 852 | 3.7% | 61 | 7.2% |
| | Asian | 824 | 3.6% | 96 | 11.7% |
| | African | 234 | 1.0% | 29 | 12.4% |
| | Native American | 64 | 0.3% | 9 | 14.1% |
| | Unknown | 5363 | 23.1% | 525 | 9.8% |
| **Personal Cancer History** | Breast Cancer | 3845 | 16.6% | 602 | 15.7% |
| | Ovarian Cancer | 341 | 1.5% | 68 | 19.9% |
| | Uterine/Endometrial Cancer | 204 | 0.9% | 24 | 11.8% |
| | Colorectal Cancer | 438 | 1.9% | 101 | 23.1% |
| | Melanoma | 446 | 1.9% | 63 | 14.1% |
| | Pancreatic Cancer | 107 | 0.5% | 18 | 16.8% |
| | Prostate Cancer | 672 | 2.9% | 92 | 13.7% |
| | Stomach Cancer | 47 | 0.2% | 11 | 23.4% |
| | Other Cancer | 1324 | 5.7% | 172 | 13.0% |
| | No Cancer | 9824 | 42.4% | 1005 | 10.2% |
| | Not Enough Information | 6963 | 30.0% | 710 | 10.2% |

### Figure 1. Genes with pathogenic variants, stratified by variant type and results type

(A) Pathogenic variants were most frequently found in *CHEK2, BRCA2, MUTYH,* and *BRCA1.* Pathogenic variant types include single nucleotide variant (SNV, 1 bp), small insertion/deletion (indel, 2-50 bp), and large structural variant (SV, >50 bp).

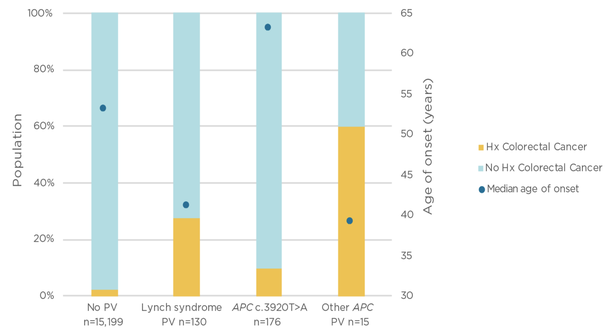



(B) The large majority of positive results were a pathogenic variant in an autosomal-dominant gene with high-to-moderate penetrance (1917, 71.1%). A total of 781 (28.9%) positive results were alleles of high population frequency (*APC* c.3920T>A (p.I1307K) and *CHEK2* c.470T>C (p.I157T)) or were monoallelic *MUTYH* pathogenic variants.

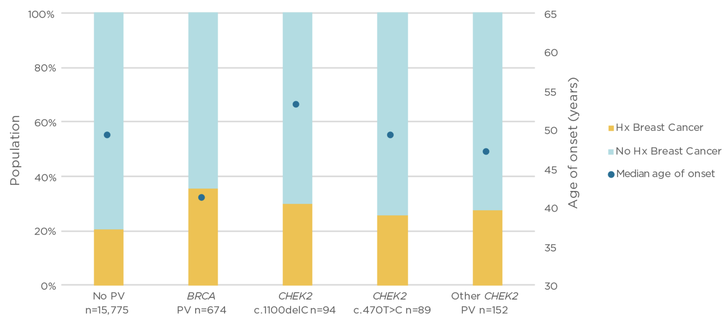



### Figure 2. High frequency alleles and cancer phenotype associations

(A) 9.7% (17/176) of individuals with *APC* c.3920T>A reported a personal history (Hx) of colorectal cancer compared to 2.2% (336/15,199) of individuals with a negative test result ($p < 0.001$). Individuals with *APC* c.3920T>A reported a median age of onset at 63 years compared to individuals with a negative test result at 53 years ($p = 0.429$).




(B) 27.8% (93/335) of individuals with pathogenic *CHEK2* alleles reported a personal history of breast cancer compared to 35.3% (238/674) and 20.5% (3240/15,775) of individuals with *BRCA1* or *BRCA2* pathogenic variants and no pathogenic variant, respectively ($p = 0.0016$, $p = 0.0187$). Individuals with pathogenic *CHEK2* alleles reported a median age of onset at 50 years compared to individuals with a *BRCA1* or *BRCA2* pathogenic variant and no pathogenic variant at 41 years and 49 years, respectively ($p < 0.001$, $p = 0.685$).




### Figure 3. Pathogenic frequency in the cohort by NCCN guidelines

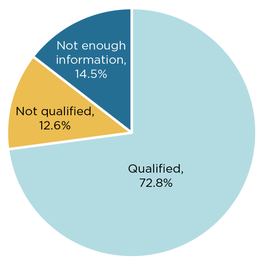
(A) In our cohort, 11,240 (48.5%) individuals would have met NCCN testing criteria, 6978 (30.1%) would not, and 4961 (21.4%) did not provide enough information to determine.

| NCCN Qualification | Negative Result (n) | Positive Result (n) | Total (n) | Pathogenic Frequency |
|---|---|---|---|---|
| Qualified | 9585 | 1655 | 11,240 | 14.7% |
| Not qualified | 6408 | 570 | 6978 | 8.2% |
| Not enough information | 4488 | 473 | 4961 | 9.5% |
| **Total** | 20,481 | 2698 | 23,179 | 11.6% |

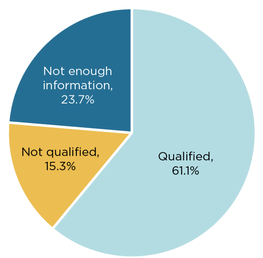
(B) Of the 846 individuals with a pathogenic variant in *BRCA1* or *BRCA2*, 616 (72.8%) would have met NCCN testing criteria, 107 (12.6%) would not, and 123 (14.5%) did not provide enough information to determine.

(C) Similarly, of the 190 individuals with a pathogenic variant in the Lynch syndrome genes, 116 (61.1%) would have met NCCN testing criteria, 29 (15.3%) would not, and 45 (23.7%) did not provide enough information to determine.

BRCA PV: NCCN Guidelines




Lynch Syndrome PV: NCCN Guidelines




## References

[1] Nagy R, Sweet K, Eng C. *Oncogene*. 2004.
[2] Richards S, Aziz N, Bale S, et al. *Genet Med*. 2015.