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Multi-Gene Panel Testing of 23,179 Individuals for Hereditary Cancer Risk Identifies Pathogenic Variant Carriers Missed by Current Genetic Testing Guidelines

Cynthia L. Neben, Anjali D. Zimmer, Will Stedden, Jeroen van den Akker, Robert O'Connor, Raymond C. Chan, Elaine Chen, Zheng Tan, Annette Leon, Jack Ji, Scott Topper, and Alicia Y. Zhou

From Color Genomics, Inc., Burlingame, California

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Address correspondence to Alicia Y. Zhou, Ph.D., Color Genomics, Inc., 831 Mitten Rd., Ste. 100, Burlingame, CA 94010. E-mail: alicia@color.com.

Recent advancements in next-generation sequencing have greatly expanded the use of multi-gene panel testing for hereditary cancer risk. Although genetic testing helps guide clinical diagnosis and management, testing recommendations are based on personal and family history of cancer and ethnicity, and many carriers are being missed. Herein, we report the results from 23,179 individuals who were referred for 30-gene next-generation sequencing panel testing for hereditary cancer risk, independent of current testing guidelines—38.7% of individuals would not have met National Comprehensive Cancer Network criteria for genetic testing. We identified a total of 2811 pathogenic variants in 2698 individuals for an overall pathogenic frequency of 11.6% (9.1%, excluding common low-penetrance alleles). Among individuals of Ashkenazi Jewish descent, three-quarters of pathogenic variants were outside of the three common *BRCA1* and *BRCA2* founder alleles. Across all ethnic groups, pathogenic variants in *BRCA1* and *BRCA2* occurred most frequently, but the contribution of pathogenic variants in other genes on the panel varied. Finally, we found that 21.7% of individuals with pathogenic variants in genes with well-established genetic testing recommendations did not meet corresponding National Comprehensive Cancer Network criteria. Taken together, the results indicate that more individuals are at genetic risk for hereditary cancer than are identified by current testing guidelines and/or use of single-gene or single-site testing. *(J Mol Diagn 2019, 21: 646—657; https://doi.org/10.1016/j.jmoldx.2019.03.001)*

Recent advancements in next-generation sequencing have greatly expanded the use of multi-gene testing panels in clinical diagnosis and management. Multi-gene panels are more sensitive and efficient than traditional testing paradigms and are increasingly more affordable. Furthermore, multi-gene panels increase the likelihood of detecting an underlying germline genetic component in diseases with genetic heterogeneity, such as cancer.[1]

Approximately 5% to 10% of all cancers are associated with hereditary cancer syndromes, most of which are inherited in an autosomal-dominant manner with high-to-moderate penetrance.[2] As such, the current approach to germline cancer testing is to recommend testing only for those individuals with a personal or family history that indicates an increased risk of disease or presence of a known familial pathogenic variant. However, because of phenotypic variability, age-related penetrance, and sex-specific cancer risks, this approach misses many carriers.[3–5] For example, recent studies have shown that up to half of women carrying *BRCA1* and *BRCA2* pathogenic variants had no family history of breast cancer.[6,7] In another study of 360 ovarian cancer patients, three women with

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C.L.N. and A.D.Z. contributed equally to this work.

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TP53 pathogenic variants had no family history of Li-Fraumeni syndrome, and two individuals with *MSH6* pathogenic variants had no family history of Lynch syndrome.[8] These prior studies indicate a need in the field to perform a more systemic evaluation of the efficacy of personal- and family history–based screening as a prequalifier for genetic testing.

This study analyzed the results of 23,179 individuals who received a 30-gene next-generation sequencing panel for risk of hereditary breast, ovarian, uterine/endometrial, colorectal, melanoma, pancreatic, prostate, and stomach cancer. Herein, we provide data on the frequency and spectrum of pathogenic or likely pathogenic variants by variant type, personal history of cancer, and ethnicity. More important, as these individuals were referred for genetic testing independent of testing guidelines, the results were also evaluated with respect to the genetic/familial high-risk assessments provided by the National Comprehensive Cancer Network (NCCN).[9–11]

Materials and Methods

Participants

The cohort in this retrospective study included 23,179 individuals who had Color Hereditary Cancer Test (Color Genomics, Inc., Burlingame, CA) results reported between May 2016 and September 2017. All individuals were ordered the test by a health care provider and gave informed consent to have their deidentified information used in anonymized studies. This population was not specifically selected for any particular metric, including sex, age, ethnicity, or history of cancer.

Data Collection

All phenotypic information was reported by the individual through an interactive, collaborative online health history tool; information not provided was noted as such. Individuals who reported more than one ethnicity were counted as multiple ethnicities, with the following exception: any individuals who reported Ashkenazi Jewish in addition to any other ancestry were counted as Ashkenazi Jewish.

Multi-Gene Panel

The Color Hereditary Cancer Test was used to analyze 30 genes in which pathogenic variants have been associated with an elevated risk of hereditary cancer, including breast, ovarian, uterine/endometrial, colorectal, melanoma, pancreatic, prostate, and stomach. This test is adapted from the multi-gene panel validated in Crawford et al.[12] The 30 genes are *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*. These genes were selected on the basis of published evidence of association with hereditary cancer and technical feasibility using the methods described below. Most of these genes were assessed for variants within all coding exons (±20 bp flanking each exon) and noncanonical splice regions. In *PMS2*, exons 12 to 15 could not be reliably assessed with the standard target enrichment protocol and, therefore, were not reported. In several genes, only specific positions known to impact cancer risk were analyzed (genomic coordinates in GRCh37): *CDK4*, only chromosome 12: g.58145429-58145431 (codon 24); *MITF*, only chromosome 3: g.70014091 (including c.952G>A); *POLD1*, only chromosome 19: g.50909713 (including c.1433G>A); *POLE*, only chromosome 12: g.133250250 (including c.1270C>G); *EPCAM*, only large deletions and duplications, including the 3′ end of the gene; and *GREM1*, only duplications in the upstream regulatory region.

Laboratory Procedures

Laboratory procedures were performed at the laboratory of Color Genomics, Inc. (Burlingame, CA) under Clinical Laboratory Improvement Amendments (number 05D2081492) and College of American Pathologists (number 8975161) compliance. DNA was extracted from blood or saliva samples and purified using the Perkin Elmer Chemagic DNA Extraction Kit (Perkin Elmer, Waltham, MA) automated on the Hamilton STAR (Hamilton, Reno, NV) and the Chemagic Liquid Handler (Perkin Elmer) instruments. The quality and quantity of the extracted DNA were assessed by UV spectroscopy (BioTek, Winooski, VT). High molecular weight genomic DNA was enzymatically fragmented and prepared using the Kapa HyperPlus Library Preparation Kit (Kapa Biosciences, Cape Town, South Africa) automated on the Hamilton STAR liquid handler. Target enrichment was performed with an automated (Hamilton STAR) hybrid capture procedure using SureSelect XT probes (Agilent, Santa Clara, CA) before being loaded onto the NextSeq 500/550 instrument (Illumina, San Diego, CA) for 150-bp paired-end sequencing.

Bioinformatics Analysis

Sequence reads were aligned against human genome reference GRCh37.p12 with the Burrows-Wheeler Aligner version 0.7.15,[13] and duplicate and low-quality reads were removed. Single-nucleotide variants and small insertions and deletions (2 to 50 bp) were called by the HaplotypeCaller module of GATK3.4.[14] Variants in homopolymer regions were called by an internally developed algorithm using SAMtools version 1.8.[15] Large structural variants (>50 bp) were detected using dedicated algorithms based on read depth (CNVkit version 0.8.5),[16] paired reads, and split reads (LUMPY version 0.2.13,[17] in-house developed algorithms). On pipeline completion, the sequencing run quality was checked. A no template control and two positive controls

containing a set of known variants were concurrently run within every batch of samples. The coverage requirements for reporting were ≥20 unique reads (20×) for each base. Median coverage typically ranged between 200× and 300×.

Variant Interpretation

Variants were classified according to the American College of Medical Genetics and Genomics 2015 guidelines for sequence variant interpretation.[18] Every variant was reviewed by at least two variant scientists (R.O., R.C.C., E.C., Z.T., A.L., J.J., and A.Y.Z.), and all variant classifications were signed out by a board-certified medical geneticist or pathologist (Z.T., A.L., J.J., and S.T.). All pathogenic and likely pathogenic variants were confirmed on an orthogonal technology at an independent Clinical Laboratory Improvement Amendments–certified laboratory. Specifically, single-nucleotide variants and insertions and deletions were confirmed by Sanger sequencing, and structural variants were confirmed by variant-specific PCR, array comparative genomic hybridization, or multiplex ligation-dependent probe amplification.

Results were reported as positive if one or more pathogenic or likely pathogenic variants (hereafter referred to as pathogenic variants) were detected and negative if no variant and/or only benign variants, likely benign variants, or variants of uncertain significance were detected at the time of data collection. Among the 30 genes tested, there are several alleles that are classified as pathogenic or likely pathogenic by multiple submitters in ClinVar but are known in the field to be commonly occurring and of low penetrance. Specifically, these include a single allele in *APC*, *APC* c.3920T>A (p.I1307K),[19] and all monogenic pathogenic or likely pathogenic variants in *MUTYH*.[20] This group of known high-frequency, low-penetrance alleles will be referred to as common low-penetrance alleles for brevity in the remainder of the article. Therefore, all pathogenic and likely pathogenic variant counts and frequency analyses have been reported as two values: one that includes all reported pathogenic variants and one that excludes the common low-penetrance alleles. To note, individuals with a monoallelic *MUTYH* pathogenic variant or APC p.I1307K were provided genetic testing reports that were distinct from genetic testing reports for biallelic *MUTYH* (ie, homozygous or compound heterozygous) pathogenic variants and other alleles in *APC*, respectively.

NCCN Consideration of Genetic Testing

Health history was assessed to determine whether individuals met or did not meet NCCN consideration for genetic testing, as provided by the Genetic/Familial High-Risk Assessment: Breast and Ovarian Version 2.2017 (*BRCA1*, *BRCA2*, *TP53*, and *PTEN*),[9] the Genetic/Familial High-Risk Assessment: Colorectal Version 2.2016 [*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC* (excluding APC p.I1307K), biallelic *MUTYH*, *SMAD4*, and *BMPR1A*],[10] and the Gastric Cancer Version 1.2017 (*CDH1*).[11] Phenotypic information, used to evaluate if an individual met or did not meet criteria, is outlined in Supplemental Table S1. Individuals who did not provide sufficient health history information were excluded from analyses or noted as such.

Data Statement

The data that support the findings in this study are available on request from the corresponding author (A.Y.Z.). The data are not publicly available as they contain information that could compromise research participant privacy or consent. All reported variants have been submitted to ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/submitters/505849*).

Results

Population Study

Our cohort of 23,179 individuals received a 30-gene next-generation sequencing panel for detection of pathogenic variants associated with elevated risk of hereditary cancer. The demographics of these individuals are described in Table 1. Most were women (83.1%) and older than 40 years (73.3%), and approximately half were Caucasian (52.1%). Two-fifths of individuals in the cohort (42.4%) reported no personal history of cancer. A total of 3845 individuals (16.6%) had a personal history of breast cancer, 341 (1.5%) had ovarian cancer, 438 (1.9%) had colorectal cancer, and 1476 (6.4%) had a personal history of another hereditary cancer associated with genes on the panel.

Variants Detected

In this cohort, 2811 pathogenic variants were identified in 2698 individuals, and an overall pathogenic frequency of 11.6% was reported (9.1%, excluding common low-penetrance alleles) (Table 1). The majority of individuals with a positive result had a pathogenic variant in genes with high-to-moderate penetrance (76.0%) (Figure 1A). *BRCA1* and *BRCA2* pathogenic variants accounted for 31.4% of positive results, which is not surprising given the number of individuals in the cohort with a personal history of breast cancer. Pathogenic variants in *MLH1*, *MSH2*, *MSH6*, and *PMS2*, which are associated with Lynch syndrome, accounted for 7.0% of positive results. A total of 647 positive results (24.0%) were monoallelic *MUTYH* pathogenic variants or APC p.I1307K. These alleles are classified as pathogenic or likely pathogenic by multiple submitters in ClinVar but are known in the field to be commonly occurring and of low penetrance. Because these alleles might confound the analysis, all subsequent calculations of pathogenic allele frequency and count will be explicitly reported with and without these common low-penetrance alleles. Finally, the frequency of individuals with a variant of uncertain significance in the cohort, irrespective of additional pathogenic variants, was 19.0% ($n = 4414$).

Table 1 Demographics of Individuals Tested with the 30-Gene Next-Generation Sequencing Hereditary Cancer Panel

Variable	Individuals, *n*	Population, %	Individuals with PV, *n* (excluding common low-penetrance alleles, *n*)	Pathogenic frequency, % (excluding common low-penetrance alleles, %)
Total	23,179	100.0	2698 (2116)	11.6 (9.1)
Sex				
Female	19,263	83.1	2156 (1694)	11.2 (8.8)
Male	3916	16.9	542 (422)	13.8 (10.8)
Age, years				
18–30	1747	7.5	245 (208)	14.0 (11.9)
31–40	4447	19.2	517 (427)	11.6 (9.6)
41–50	5544	23.9	611 (489)	11.0 (8.8)
51–65	7255	31.3	825 (627)	11.4 (8.6)
>65	4186	18.1	500 (365)	11.9 (8.7)
Ethnicity				
Caucasian	12,083	52.1	1413 (1166)	11.7 (9.6)
Ashkenazi Jewish	2301	9.9	364 (218)	15.8 (9.5)
Hispanic	1458	6.3	201 (180)	13.8 (12.3)
Multiple ethnicities	852	3.7	61 (46)	7.2 (5.4)
Asian	824	3.6	96 (80)	11.7 (9.7)
African	234	1.0	29 (23)	12.4 (9.8)
Native American	64	0.3	9 (8)	14.1 (12.5)
Unknown*	5363	23.1	525 (395)	9.8 (7.4)
Personal cancer history†				
Breast	3845	16.6	602 (471)	15.7 (12.2)
Ovarian‡	341	1.5	68 (59)	19.9 (17.3)
Endometrial/uterine	204	0.9	24 (18)	11.8 (8.8)
Colorectal	438	1.9	101 (75)	23.1 (17.1)
Melanoma	446	1.9	63 (48)	14.1 (10.8)
Pancreatic	107	0.5	18 (14)	16.8 (13.1)
Prostate	672	2.9	92 (73)	13.7 (10.9)
Stomach	47	0.2	11 (9)	23.4 (19.1)
Other cancer§	1324	5.7	172 (126)	13.0 (9.5)
No cancer	9824	42.4	1005 (696)	10.2 (7.1)
Information not provided	6963	30.0	710 (492)	10.2 (7.1)

Number of individuals with a PV and pathogenic frequency when excluding common low-penetrance alleles (monoallelic *MUTYH* pathogenic variant and APC p.I1307K) are in parentheses.

*Unknown includes information not provided.

†Number of individuals with personal history of cancer exceeds 23,179 because of multiple reported cancer types.

‡Ovarian cancer includes fallopian cancer and primary peritoneal cancer.

§Other cancer includes hematological malignancies, kidney cancer, thyroid cancer, and other cancers.

PV, pathogenic variant.

A large majority [1730 (61.5%)] of the 2811 pathogenic variants identified were in *CHEK2*, *BRCA2*, *MUTYH*, and *BRCA1*, with the next most frequent in *APC* and *ATM* [268 (9.5%) and 174 (6.2%), respectively] (Figure 1C). No pathogenic variants were identified in *EPCAM*, *POLD1*, or *POLE* genes, which are primarily associated with colorectal cancer. Single-nucleotide variants accounted for 58.9% ($n = 1657$) of all pathogenic variants, whereas insertions and deletions and structural variants accounted for 35.4% ($n = 995$) and 5.7% ($n = 159$), respectively. Approximately half of all insertions and deletions were found in *BRCA2* and *BRCA1* [514 (51.7%)], and nearly one-third [51 (32.1%)] of structural variants were also in *BRCA1*. The functional consequences of the pathogenic variants identified were primarily missense and frameshift effects (38.7% and 33.7%, respectively) (Figure 1B). Of the 1088 missense variants, 612 (56.3%) were pathogenic and 476 (43.8%) were likely pathogenic. Copy number variants accounted for 5.7% ($n = 159$) of variants, of which 30.2% ($n = 48$) affected only a single exon.

Most individuals with a positive result carried a single pathogenic variant [2576 (95.5%); 2630 (97.5%), excluding common low-penetrance alleles]. However, 119 individuals (4.4%) who carried two concurrent pathogenic variants (Figure 2) and 3 individuals (0.1%) who carried three concurrent pathogenic variants were identified. Not surprisingly, 45.3% (54/119) of the individuals who carried two concurrent pathogenic variants carried at least one common low-penetrance allele (Figure 2). More important, 23.5% of individuals (28/119) carried a pathogenic variant in *BRCA1* or *BRCA2* and another concurrent pathogenic

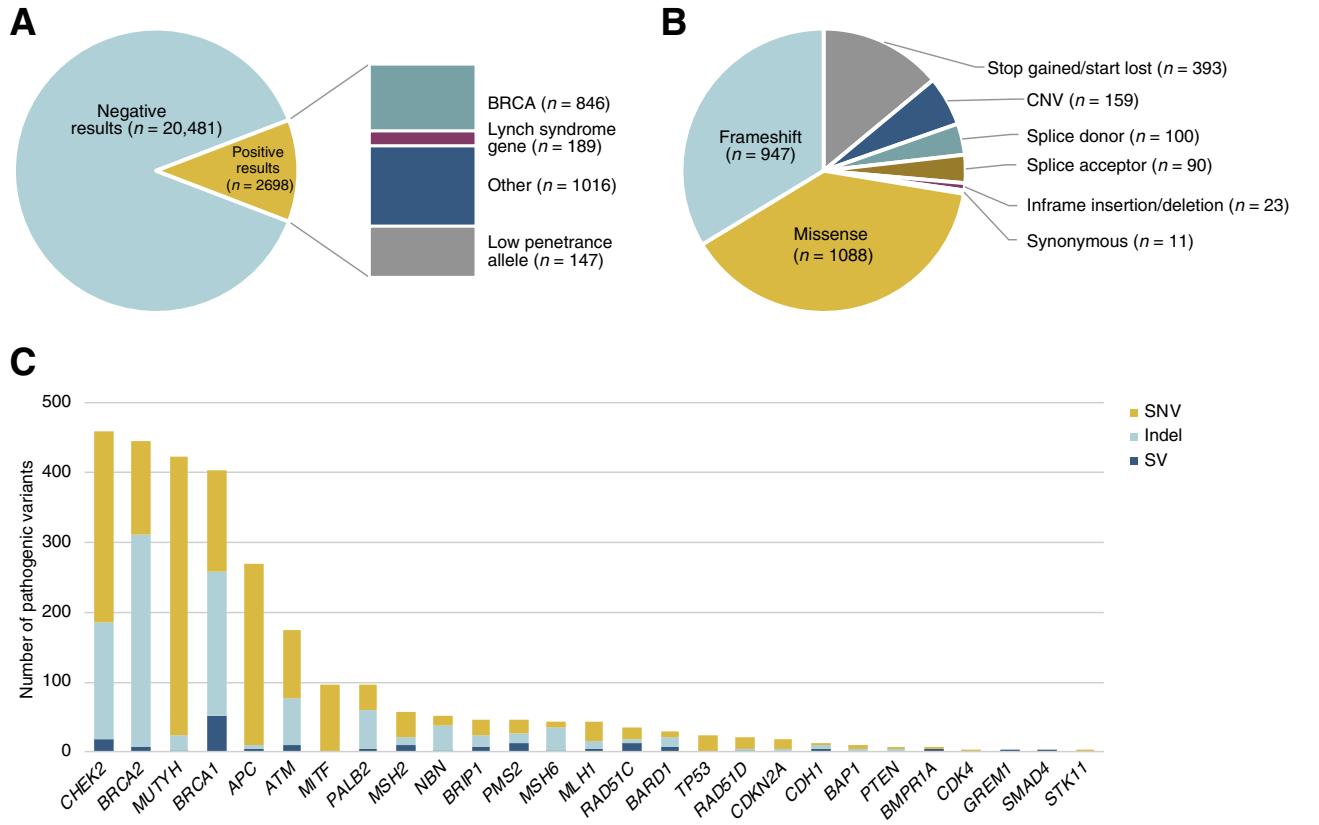

Figure 1 Test outcomes by result type, pathogenic variant type, and effect. **A:** Number of negative and positive results in the cohort. Positive results were stratified into *BRCA* (*BRCA1* and *BRCA2*), Lynch syndrome gene (*MLH1*, *MSH2*, *MSH6*, and *PMS2*), low-penetrance allele (a monoallelic *MUTYH* pathogenic variant or APC p.I1307K), and other (*APC*, *ATM*, *BAP1*, *BARD1*, *BMPR1A*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *GREM1*, *MITF*, biallelic *MUTYH* pathogenic variants, *NBN*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *SMAD4*, *STK11*, and *TP53*). **B:** Effects of pathogenic variants in the cohort. Effect was predicted by SnpEff version 4.0d.[21] A total of 251 missense variants were APC p.I1307K. There were 16 stop gained/start lost, 1 copy number variant (CNV), 4 splice donors, 27 splice acceptors, 8 in-frame deletions/insertions, 340 missense mutations, and 21 frameshift mutations that were monoallelic *MUTYH* variants. **C:** Number of pathogenic variants by gene, stratified by variant type: single-nucleotide variant (SNV; 1 bp), small insertions and deletion (indel; 2 to 50 bp), and large structural variant (SV; >50 bp). A total of 251 SNVs were APC p.I1307K. A total of 395 SNVs, 21 indels, and 2 SVs were monoallelic *MUTYH* variants. SVs include CNVs.

variant outside of the common low-penetrance alleles (Figure 2).

Frequency and Spectrum of Pathogenic Variants in Ethnic Populations

After Caucasian, the second largest ethnic population in our cohort was individuals of Ashkenazi Jewish descent (9.9%), with a pathogenic frequency of 15.8% (9.5%, excluding common low-penetrance alleles) (Table 1 and Figure 3A). Three founder alleles in *BRCA1* and *BRCA2* are known to occur at a high population frequency (approximately 2.5%, collectively)[22–24] in Ashkenazi Jewish individuals: *BRCA1* c.68_69delAG, *BRCA1* c.5266dupC, and *BRCA2* c.5946delT. In our cohort, these founder alleles were identified in 3.6% of Ashkenazi Jewish individuals (Figure 3A), and they accounted for 81.4% ($n = 83$) of the *BRCA1* and *BRCA2* pathogenic variants in Ashkenazi Jewish individuals—including one individual with two founder alleles (Figure 3B). More important, of individuals with pathogenic variants outside of common low-penetrance alleles, approximately 49.8% ($n = 100$) had pathogenic variants in genes other than *BRCA1* and *BRCA2* (Figure 3A).

A total of 14.8% of individuals reported non-Caucasian and non−Ashkenazi Jewish ethnicity (Table 1). The pathogenic frequency for Asians (11.7%; 9.7%, excluding common low-penetrance alleles) was similar to the overall pathogenic frequency (11.6%; 9.1%, excluding common low-penetrance alleles), whereas the pathogenic frequencies for Hispanics (13.8%; 12.3%, excluding common low-penetrance alleles), Africans (12.4%; 9.8%, excluding common low-penetrance alleles), and Native Americans (14.1%; 12.5%, excluding common low-penetrance alleles) were slightly higher (Table 1). As expected, the Ashkenazi Jewish BRCA founder alleles were largely absent in non−Ashkenazi Jewish individuals. They composed only 10.0% ($n = 42$) of *BRCA1* and *BRCA2* pathogenic variants for Caucasians and 5.0% ($n = 5$) for Hispanics, and they were absent in Asians, Africans, and Native Americans (Figure 3B). However, approximately half (54.1%) of the pathogenic variants outside of common low-penetrance alleles in these populations were in *BRCA1* and *BRCA2*

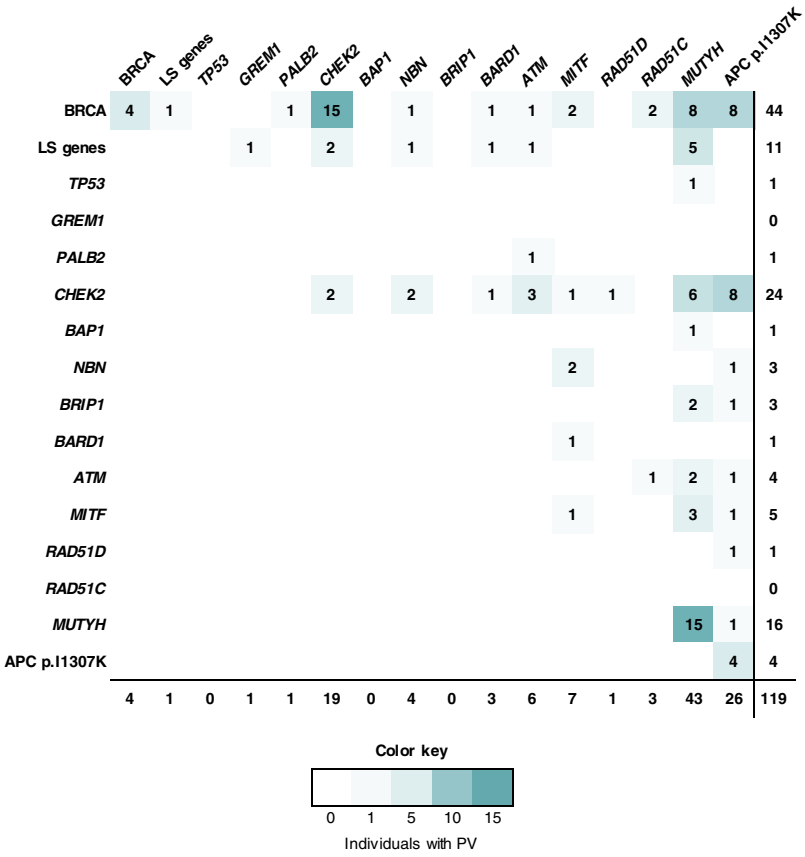

Figure 2 Heatmap of the number of individuals with two pathogenic variants by gene, including homozygotes. Genes with pathogenic variants (PVs) among concurrent carriers are listed on the *x* and *y* axes. The number of individuals with pathogenic variants in a set of two genes is specified by number and visualized by color gradient. BRCA includes BRCA1 and BRCA2. LS genes, Lynch syndrome genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*).

(Figure 3C). Hispanic individuals also had a large number of Lynch syndrome gene pathogenic variants [32/181 (17.7%)] (Figure 3C), likely because of an enrichment of colorectal cancer in this subset of the cohort. Ashkenazi Jewish individuals also had a large number of pathogenic variants in *CHEK2* [57/204 (27.9%)] (Figure 3C), most of which were the Ashkenazi Jewish *CHEK2* founder alleles c.470T>C (p.I157T)[25] and c.1283C>T (p.S248F).[26]

Individuals with a Personal History of Cancer

A total of 5649 individuals (24.4%) in the cohort reported a personal history of cancer associated with genes on the panel (Table 1). A personal history of stomach or colorectal cancer correlated with the highest pathogenic frequencies at 23.4% and 23.1%, respectively (19.1% and 17.1%, excluding common low-penetrance alleles, respectively). The pathogenic variants most frequently identified in these individuals were in well-established stomach or colorectal cancer genes, including *MLH1*, *MSH2*, *APC* (excluding p.I1307K), *CHEK2*, *MSH6*, *PMS2*, and *BMPR1A* [61 (70.1%)] (Supplemental Table S2); five individuals were identified as biallelic *MUTYH* carriers. However, pathogenic variants were also identified in genes not typically associated with these cancers, such as *BRCA1*, *BRCA2*, *MITF*, *CDKN2A*, *PALB2*, *ATM*, *NBN*, and *BARD1* [24 (27.6%)]. *BRCA1* and *BRCA2* pathogenic variants accounted for 13.8% ($n = 12$), indicating the utility of broader gene panel testing regardless of clinical phenotype. Individuals with a personal history of ovarian cancer had a similarly high pathogenic frequency at 19.9% (17.3%, excluding common low-penetrance alleles) (Table 1). A total of 50 (67.6%; 78.1%, excluding common low-penetrance alleles) pathogenic variants in individuals with a history of ovarian cancer were in *BRCA1*, *BRCA2*, *BRIP*, *RAD51C*, *RAD51D*, or Lynch syndrome genes. *BRCA1* and *BRCA2* pathogenic variants accounted for 51.4% (59.4%, excluding common low-penetrance alleles) (Supplemental Table S2).

A total of 9824 individuals in our cohort (42.4%) reported that they did not have a personal history of cancer. This subpopulation had a pathogenic frequency of 10.2% (7.1%, excluding common low-penetrance alleles) (Table 1). A total of 766 pathogenic variants (73.4%) were in genes with high-to-moderate penetrance, including *BRCA1* and *BRCA2* [297 (28.5%)] and Lynch syndrome genes [57 (5.5%)] (Supplemental Table S2). These data suggest that personal history alone is a poor indicator of pathogenic variant carrier status. Monoallelic *MUTYH* pathogenic variants ($n = 177$) and APC p.I1307K ($n = 100$) accounted for 26.6% of pathogenic variants in individuals with no personal history of cancer, consistent with known population frequencies for these alleles.[27,28]

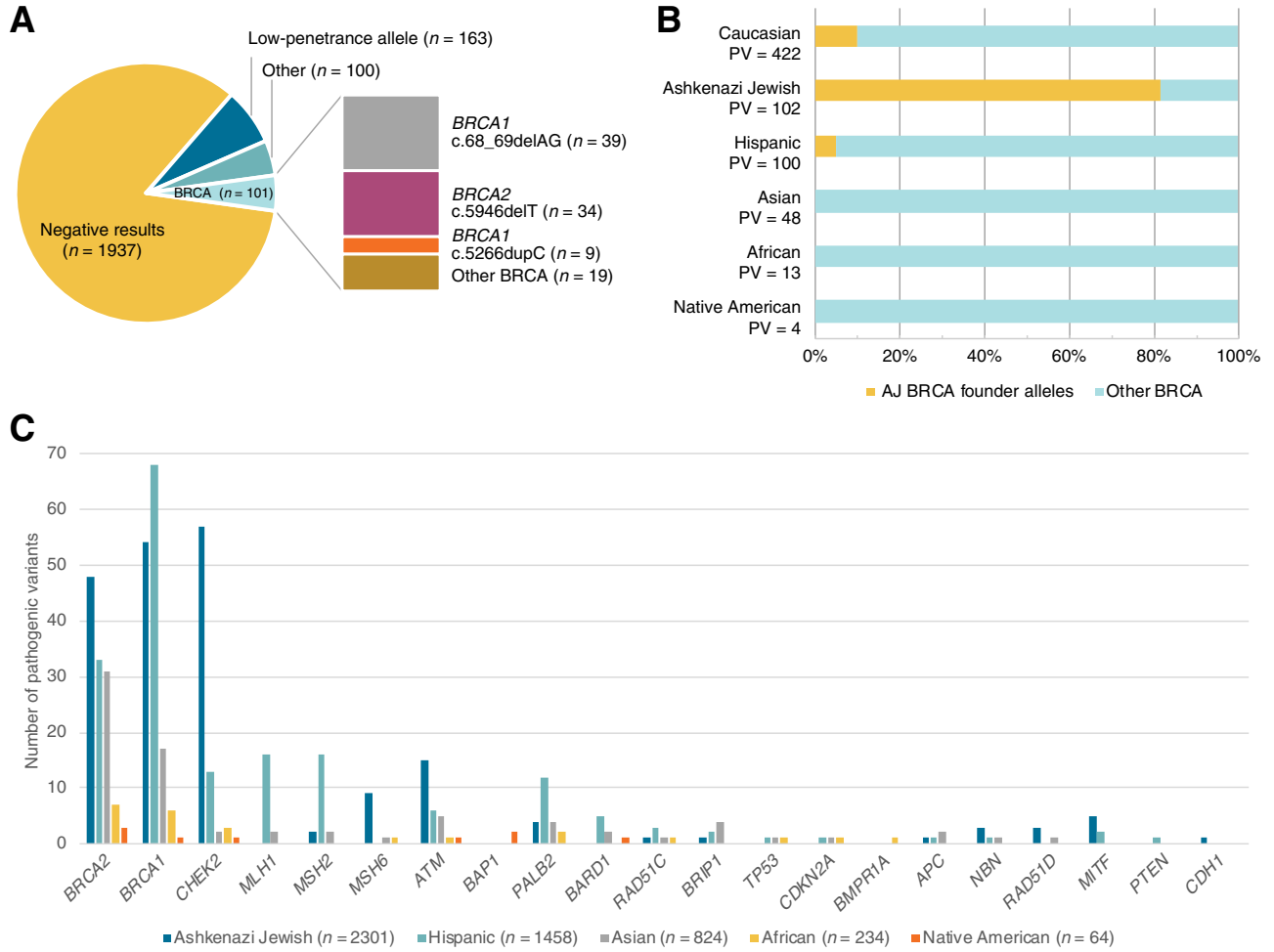

Figure 3 Pathogenic variants and frequency in ethnic subpopulations. **A:** Number of negative and positive results in the Ashkenazi Jewish (AJ) population. Positive results are BRCA (*BRCA1* and *BRCA2*), low-penetrance allele (monoallelic *MUTYH* pathogenic variant or APC p.I1307K), and other (*APC*, *ATM*, *BRIP1*, *CDH1*, *CHEK2*, *MITF*, *MSH2*, *MSH6*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*). BRCA was stratified into Ashkenazi Jewish BRCA founder alleles (*BRCA1* c.68_69delAG, *BRCA1* c.5266dupC, and *BRCA2* c.5946delT) and other BRCA. **B:** Percentage of AJ BRCA founder alleles among *BRCA1* and *BRCA2* pathogenic variants (PVs) by ethnicity. **C:** Number of pathogenic variants by gene in ethnicity minority groups, excluding common low-penetrance alleles (monoallelic *MUTYH* pathogenic variants and APC p.I1307K).

Genetic Testing Recommendations by NCCN

NCCN guidelines provide recommendations for genetic testing and counseling for hereditary cancer syndromes and risk management recommendations for patients who are suspected to be at high risk for a genetic syndrome on the basis of personal and family history. To assess how many individuals in our cohort would or would not have met criteria for genetic testing, the number of individuals who provided insufficient health history information was determined

Table 2 Individuals Who Would Have Met the Clinical Criteria for Genetic Testing for Breast and Ovarian, Colorectal, or Gastric Cancer, Those Who Would Not, and Those Who Did Not Provide Enough Information to Determine

NCCN	Negative result, *n* (excluding common low-penetrance alleles, *n*)	Positive result, *n* (excluding common low-penetrance alleles, *n*)	Total, *n*	Pathogenic frequency, % (excluding common low-penetrance alleles, %)
Met criteria	9502 (9824)	1645 (1323)	11,147	14.8 (11.9)
Did not meet criteria	6453 (6594)	576 (435)	7029	8.2 (6.2)
Not enough information	4526 (4645)	477 (358)	5003	9.5 (7.2)
Total	20,481 (21,063)	2698 (2116)	23,179	11.6 (9.1)

Number of individuals with a pathogenic variant and pathogenic frequency when excluding common low-penetrance alleles (monoallelic *MUTYH* pathogenic variant and APC p.I1307K) are in parentheses. NCCN guidelines are outlined in Supplemental Table S1.

NCCN, National Comprehensive Cancer Network.

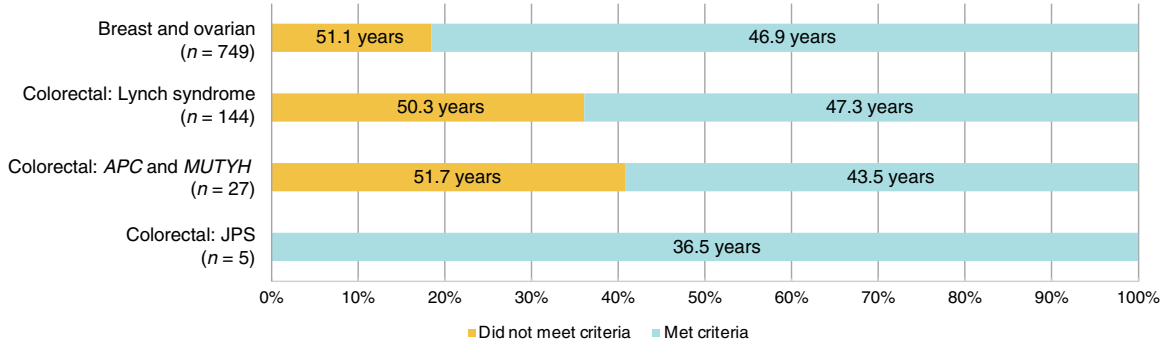

Figure 4 Individuals with a pathogenic variant in genes with National Comprehensive Cancer Network (NCCN) guidelines, by guideline. Percentage of individuals with a pathogenic variant in *BRCA1*, *BRCA2*, *TP53*, or *PTEN* who would not have met the clinical criteria for genetic testing of breast and ovarian cancer (means ± SD age, 51.1 ± 15.3 years) and those who would (means ± SD age, 46.9 ± 14.7 years). Percentage of individuals with a pathogenic variant in *MLH1*, *MSH2*, *MSH6*, or *PMS2* who would not have met the clinical criteria for genetic testing for Lynch syndrome (means ± SD age, 50.3 ± 16.3 years) and those who would (means ± SD age, 47.3 ± 14.6 years). Percentage of individuals with a pathogenic variant in *APC* or biallelic *MUTYH* pathogenic variants who would not have met the clinical criteria for genetic testing for colorectal cancer (means ± SD age, 51.7 ± 16.5 years) and those who would (means ± SD age, 43.5 ± 12.4 years). *APC* does not include APC p.I1307K. Percentage of individuals with a pathogenic variant in *SMAD4* or *BMPR1A* who would not have met the clinical criteria for genetic testing for juvenile polyposis syndrome (JPS; $n = 0$) and those who would (means ± SD age, 36.5 ± 18.2 years). NCCN guidelines are outlined in Supplemental Table S1.

(Supplemental Table S1), which excluded 5003 individuals (Table 2). Among the remaining 18,176 individuals, 11,147 (61.3%) would have met criteria for genetic testing for breast and ovarian,[9] colorectal,[10] or gastric cancer,[11] and 7029 (38.7%) would not (Table 2). The pathogenic frequency of the NCCN-ineligible population was 8.2% (6.2%, excluding common low-penetrance alleles) (Table 2). Strikingly, 21.7% ($n = 201$) of individuals with pathogenic variants in genes with well-established genetic testing recommendations did not meet corresponding NCCN criteria for genetic testing, and this frequency varied greatly by hereditary cancer syndrome (Figure 4 and Supplemental Table S3). For example, of the 749 individuals who had a pathogenic variant in *BRCA1*, *BRCA2*, *TP53*, or *PTEN*, 138 (18.4%) would not have met criteria for genetic testing for breast and ovarian cancer (Figure 4 and Supplemental Table S3). Of the 144 individuals who had a pathogenic variant in *MLH1*, *MSH2*, *PMS2*, or *MSH6*, 52 (36.1%) would not have met criteria for genetic testing for Lynch syndrome (Figure 4 and Supplemental Table S3). An additional 57 individuals in the cohort, who would not have met corresponding NCCN criteria for the pathogenic variant that we identified, would have met criteria for genetic testing for another hereditary cancer indication (Supplemental Table S3). These data indicate that, given the phenotypic and genotypic heterogeneity that exists within various cancers, it is difficult to definitively categorize genes for specific indications without missing potential carriers.

Discussion

The advancements in next-generation sequencing technologies and reductions in the cost of sequencing have greatly expanded the use of multi-gene panel testing for hereditary cancer risk in the clinic. Herein, we analyzed the results of 23,179 individuals who received physician-ordered genetic testing via a 30-gene panel for hereditary cancer risk. Compared with other studies to date on hereditary cancer panel testing, our cohort included a high proportion of men,[5,29–31] unaffected individuals, and individuals of non-Caucasian ethnicity. Taken together, the data presented herein advance our understanding of the frequency and spectrum of pathogenic variants in these genes and highlight the utility of multi-gene panels.

High-Frequency Alleles with Low Penetrance

The rapid uptake of genetic testing has revealed that several less penetrant but more frequently occurring alleles are associated with hereditary cancer; however, the risks and/or screening recommendations for these alleles are different from those reported for high-to-moderate penetrance pathogenic variants.

For example, in contrast to other germline pathogenic variants in *APC*, APC p.I1307K is not associated with familial adenomatous polyposis, in which hundreds to thousands of adenomatous colonic polyps develop during adolescence.[32] This nonsynonymous variant is primarily found in individuals of Ashkenazi Jewish descent, with an estimated prevalence of 5% to 10%.[33] Although APC p.I1307K has been shown to increase risk of colorectal cancer in this subpopulation,[27] the frequency and penetrance in individuals who are not of Ashkenazi Jewish descent is unclear. In our cohort, 175 individuals of Ashkenazi Jewish descent (7.6%) had APC p.I1307K, and of those who were not concurrent carriers ($n = 155$), 16 individuals (10.3%) reported a personal history of colorectal cancer. However, only 1 non–Ashkenazi Jewish individual with APC p.I1307K reported a personal history of colorectal cancer, suggesting that this variant may have variable risk for colorectal cancer outside of the Ashkenazi Jewish subpopulation. Further longitudinal studies are warranted to determine the true associated risk in diverse populations.

MUTYH-associated polyposis is an autosomal-recessive syndrome that is characterized by significantly increased lifetime risk of colorectal cancer, up to 100% in the absence of timely surveillance.[20] The risk of developing colorectal cancer in individuals with a heterozygous germline *MUTYH* pathogenic variant is less clear, but several studies have demonstrated an increased cancer risk in monoallelic family members compared with the general population.[34,35] In our cohort, only 1.8% ($n = 7$) of individuals with a heterozygous *MUTYH* pathogenic variant reported a personal history of colorectal cancer, suggesting that monoallelic *MUTYH* carriers are not at significantly higher risk for colorectal cancer. This is consistent with the NCCN guidelines, which do not propose specific screening recommendations for individuals with a heterozygous *MUTYH* pathogenic variant.[10] Regardless, returning positive results for these variants has important implications for carrier testing, as affected offspring are often missed because of lack of family history.

Concurrent Pathogenic Variant Carriers

One advantage of multi-gene panel testing compared with single-gene testing is the detection of additional pathogenic variants in other genes that may also contribute to cancer risk. Recent reports have estimated that up to 3.1% of individuals who test positive on a multi-gene hereditary cancer test have more than one pathogenic variant.[5,29,30] In our cohort, 122 individuals with a positive result (4.5%) had two or more pathogenic variants ($n = 68$; 2.5%, excluding common low-penetrance alleles). More important, a second concurrent pathogenic variant in a different gene with high-to-moderate penetrance would have been missed in 55.4% ($n = 36$) of individuals if they had been tested for only *BRCA1*, *BRCA2*, or Lynch syndrome genes. The knowledge of a second pathogenic variant could lead to additional preventions and more accurate genetic counseling. Identifying individuals with multiple pathogenic variants also has important implications for family members. Family members who had previously undergone single-gene or single-gene testing may have been erroneously informed they were true negatives. Further detailed studies are warranted to determine the clinical implications of carrying more than one pathogenic variant, especially with regard to variation in expressivity.

Pathogenic Variant Spectrum in the Ashkenazi Jewish Subpopulation

One ethnicity in which single-site, single-gene, and *BRCA1*- and *BRCA2*-only testing has been commonly used is the Ashkenazi Jewish subpopulation. Initial reports suggested that three founder alleles in *BRCA1* and *BRCA2* accounted for 98% to 99% of *BRCA1* and *BRCA2* pathogenic variants in individuals of Ashkenazi Jewish descent, with a pathogenic frequency of 1 in 40.[36–38] Furthermore, these founder alleles were estimated to account for up to 30% of early-onset breast cancer and 60% of ovarian cancer in this subpopulation.[39] As a result, there were suggestions that genetic testing for the BRCA founder alleles within the Ashkenazi Jewish population may be sufficient. However, the data presented herein indicate that this approach would be insufficient, as 18.6% of *BRCA1* and *BRCA2* pathogenic variants in individuals of Ashkenazi Jewish descent were non-*BRCA* founder alleles (Figure 3B). Furthermore, 59.2% of pathogenic variants outside of common low-penetrance alleles were non-*BRCA* founder alleles (Figure 3A). The most prevalent pathogenic variant in this subpopulation other than the three *BRCA1* and *BRCA2* founder alleles (when excluding low-penetrance alleles) was *CHEK2* c.1283C>T (p.S428F), which has previously been reported to increase breast cancer risk in Ashkenazi Jewish women by approximately twofold.[26] Taken together, these data support emerging research that other gene pathogenic variants contribute to the high incidence of breast and ovarian cancer within this subpopulation[12,40,41] and suggest that multi-gene panels are a more efficient testing paradigm.

Limited Genetic Testing and Ethnicity

Much of the literature on multi-gene panel testing is composed of cohorts that are predominantly Caucasian and of non-Hispanic ancestry. Recent efforts have been made to correct this ascertainment bias and have demonstrated that multi-gene panel testing is relevant across racial and ethnic groups.[42] This study adds support to these claims as the pathogenic frequency from our multi-gene panel was elevated among all reported ethnicities in the cohort. The pathogenic frequencies for Caucasians and Asians were similar, whereas the pathogenic frequencies for other ethnicities were slightly higher, perhaps revealing a selection bias for high-risk individuals who have, to date, had limited genetic testing for hereditary cancer risk. The combined frequency of pathogenic variants in *BRCA1* and *BRCA2* was remarkably similar across all ethnicities, despite large discrepancies in the number of individuals who underwent genetic testing. In contrast, the other pathogenic variants were distributed non-uniformly across other genes. This suggests that the association of true high-penetrance genes, such as *BRCA1* and *BRCA2*, with cancer risk is conserved across ethnic groups, whereas low- or moderate-penetrance genes show selected variability. As genetic testing volume expands and more data become available about genetic variants in non-European ethnicities, multi-gene panel testing will likely continue to show improved utility in all ethnicities and reveal if the prevalence and penetrance that had been previously established in individuals of Caucasian and of non-Hispanic ancestry stand true.

Broadening Access of Genetic Testing

Current recommendations and reimbursements for genetic testing for hereditary cancer risk are based on personal and family clinical history or presence of a known family pathogenic variant. However, several groups have recently

proposed broadening access of genetic testing beyond these high-risk populations for highly penetrant conditions that have well-defined genetic causes and well-established clinical interventions. Indeed, several studies focused on the US Centers for Disease Control and Prevention Tier 1 genomic cancer conditions (hereditary breast and ovarian cancer and Lynch syndrome) have demonstrated that population testing leads to early detection and intervention, improved survival rates, and reduced cost.[4,6,7,43] Furthermore, it has been reported that approximately 50% of individuals with pathogenic variants in *BRCA1*, *BRCA2*, and Lynch syndrome genes would not have met clinical criteria for genetic testing.[3,44–46] In our cohort, among those who provided sufficient health history information, 21.3% ($n = 190$) of individuals with a pathogenic variant in one of these genes would not have met NCCN criteria for genetic testing for hereditary breast and ovarian cancer or Lynch syndrome. One common concern with broadening access to population-level genetic testing is the possibility of a negative psychological impact. However, recent studies have shown that screening does not adversely affect short-term psychological or quality-of-life outcomes and that study participation was associated with decreased anxiety and uncertainty linked to genetic testing.[47–49] The lack of detrimental psychological outcomes coupled with clinically actionable findings supports population-based genetic testing for these hereditary cancers.

Study Limitations

This study may be limited by self-reporting of demographics and health history by the individual as opposed to a health care provider. Health care provider reports are considered the gold standard for collection of patient medical history data. The data collected in our study are entirely self-reported, which we recognize may be a limitation of this study. However, several studies have shown that self-reported data on personal and family history of cancer have high concordance with data reported by a health care provider or electronic medical records. Concordance varied by cancer site and was highest (>85.1%) in breast, prostate, and colorectal cancer and lowest in melanoma.[50–52] Reliability and accuracy of self-reported family history were also high (up to 95.4%) and dependent on type of cancer and relationship to the individual.[53–55] Similarly, several recent studies have reported discrepancies in how individuals self-report their race/ethnicity and the subjective assignments made by health care providers or administrators.[56–58] Misclassifications were highest among those individuals who are racial/ethnic minority or multiple ethnicities, suggesting that the way in which information on race and ethnicity is collected may need to be reevaluated. Finally, our analyses also assumed that individuals represent unrelated probands; however, it is possible that two or more family members may have been referred for genetic testing for hereditary cancer risk and were present in the cohort.

Future Directions

Most individuals in this cohort self-reported as female and Caucasian, indicating a need in the broader community for better outreach and education of genetic testing in males and minorities. The paucity of younger test takers (aged <40 years) in our cohort highlights the importance of increasing awareness and uptake of genetic testing within this population when preventative care is more relevant. Finally, as the cost of sequencing continues to decrease and genetic testing becomes more accessible to a broader population, we will gain a better understanding of the genes associated with elevated risk for hereditary cancer. With this additional knowledge, it will be imperative to reevaluate the genes included on multi-gene panels. The genes on this panel were selected on the basis of studies in high-risk cohorts and, thus, the population-based prevalence and penetrance of many of these genes are unknown. The data presented herein advance our understanding of the true frequency and spectrum of pathogenic variants in these genes and highlight the clinical actionability and utility of multi-gene panels for hereditary cancer risk.

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A.Y.Z. designed the study; C.L.N., A.D.Z., W.S., J.v.d.A., and S.T. acquired and analyzed data; R.O., R.C.C., E.C., Z.T., A.L., J.J., and A.Y.Z. performed variant classification; C.L.N., A.D.Z., J.v.d.A., S.T., and A.Y.Z. drafted or critically revised the manuscript for important intellectual content; A.Y.Z. is the guarantor of this work and, as such, has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Supplemental Data

Supplemental material for this article can be found at *https://doi.org/10.1016/j.jmoldx.2019.03.001*.

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