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Identification of a Variety of Mutations in Cancer Predisposition Genes in Patients with Suspected Lynch Syndrome

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Abstract

Background & Aims—Multigene panels are commercially available tools for hereditary cancer risk assessment that allow for next-generation sequencing of numerous genes in parallel. However, it is not clear if these panels offer advantages over traditional genetic testing. We investigated the number of cancer predisposition gene mutations identified by parallel sequencing in individuals with suspected Lynch syndrome.

Methods—We performed germline analysis with a 25-gene next-generation sequencing panel using DNA from 1260 individuals who underwent clinical genetic testing for Lynch syndrome from 2012 through 2013. All subjects had a history of Lynch syndrome-associated cancer and/or polyps. We classified all identified germline alterations for pathogenicity and calculated the

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frequencies of pathogenic mutations and variants of uncertain significance (VUS). We also analyzed data on patients' personal and family history of cancer, including fulfillment of clinical guidelines for genetic testing.

Results—Of the 1260 subjects, 1112 met National Comprehensive Cancer Network (NCCN) criteria for Lynch syndrome testing (88%; 95% confidence interval [CI], 86%–90%). Multigene panel testing identified 114 probands with Lynch syndrome mutations (9.0%; 95% CI, 7.6% −10.8%) and 71 with mutations in other cancer predisposition genes (5.6%; 95% CI, 4.4%−7.1%). Fifteen individuals had mutations in *BRCA1* or *BRCA2*; 93% of these met the NCCN criteria for Lynch syndrome testing and 33% met NCCN criteria for *BRCA1* and *BRCA2* analysis (*P*=.0017). An additional 9 individuals carried mutations in other genes linked to high lifetime risks of cancer (5 had mutations in *APC*, 3 had bi-allelic mutations in *MUTYH*, and 1 had a mutation in *STK11*); all of these patients met NCCN criteria for Lynch syndrome testing. Four hundred seventy-nine individuals had ≥1 VUS (38%; 95% CI, 35%–41%).

Conclusions—In individuals with suspected Lynch syndrome, multigene panel testing identified high-penetrance mutations in cancer predisposition genes, many of which were unexpected based on patients' histories. Parallel sequencing also detected a high number of potentially uninformative germline findings, including VUS.

Keywords

hereditary nonpolyposis colorectal cancer; HNPCC; colon cancer genetics; inherited cancer

INTRODUCTION

Hereditary cancer syndromes are classically characterized by markedly increased lifetime risks of multiple cancers, typically at young ages. Identifying individuals with specific inherited predispositions to cancer thus greatly impacts risk counseling for affected patients and their families, including the type and timing of cancer surveillance and potential recommendations for prophylactic surgery. Timely implementation of appropriate, enhanced cancer prevention strategies can have a profound impact on decreasing cancer incidence and mortality in such patients.[1–4] Two of the most common inherited cancer syndromes are Lynch syndrome (LS), caused by mutations in one of the DNA mismatch repair (MMR) genes, and hereditary breast/ovarian cancer (HBOC), caused by germline mutations in *BRCA1* or *BRCA2*.[1–3] LS is the most common inherited cause of colorectal cancer (CRC) and is also associated with markedly increased risks of endometrial, ovarian, gastric, pancreatic, small bowel, urinary tract, and other cancers.[1, 2, 5, 6]

The traditional model of hereditary cancer risk assessment involves identifying individuals whose histories fulfill clinical criteria for a specific syndrome, followed by targeted germline testing only on the gene(s) associated with that syndrome.[7] Although clinical guidelines and prediction models can help direct the use of genetic testing for LS, 30–50% of families fulfilling stringent clinical criteria for LS will ultimately have normal germline testing for MMR gene mutations.[1, 8–12] Furthermore, there is increasing recognition that the wide phenotypic spectrum of LS cancers can overlap with other hereditary cancer

syndromes.[13–16] Thus, traditional, criteria-based genetic testing may not be the ideal hereditary cancer risk assessment strategy in individuals with suspected LS.

With recent advances in next-generation sequencing (NGS) technologies, multigene panel testing has emerged as an alternative strategy for hereditary cancer risk assessment, in which numerous cancer susceptibility genes are analyzed in parallel.[7, 17] Whether panel testing offers meaningful advantages over targeted criteria-based genetic testing practices, however, is unknown. This study's aim was to determine the frequency of non-LS gene mutations detected by a multigene hereditary cancer panel among individuals undergoing clinical genetic testing for LS.

METHODS

Study Population

3057 individuals with a history of LS-associated cancer and/or colorectal polyps whose clinicians submitted germline DNA to a CLIA-approved commercial laboratory (Myriad Genetic Laboratories, Inc., Salt Lake City, Utah, USA) for clinical genetic testing for all 5 genes underlying LS (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*) between 2012–2013 were consecutively ascertained. Upon completion of clinical LS testing, samples were anonymized for research-based multigene panel testing. 1615 subjects were excluded since their testing originated from states with legislation mandating destruction of biospecimens after completion of clinical genetic testing. Another 182 subjects were excluded due to technical factors (insufficient remaining DNA after clinical testing; DNA extracted from a non-blood sample) to give an overall cohort of 1260 individuals for this cross-sectional analysis. The study was approved by the Dana-Farber Cancer Institute's institutional review board.

Clinical Data

As part of routine clinical LS genetic testing, subjects' clinicians completed a test request form for each individual describing basic demographics (gender, ancestry), cancer/polyp history, ages at diagnosis, and family history of cancer.

Consistent with prior studies, the following were considered LS-associated cancers: CRC, endometrial cancer (EC), ovarian cancer, gastric cancer, pancreatic cancer, small intestine cancer, urinary tract cancer, hepatobiliary cancer, sebaceous adenomas/carcinomas, and brain tumors.[12] Based on their reported personal/family histories, subjects were assessed as to whether they fulfilled National Comprehensive Cancer Network (NCCN) guidelines for LS testing (Supplementary Methods).[9] A numeric estimate of the likelihood of identifying a germline mutation in *MLH1*, *MSH2*, or *MSH6* was calculated for each subject using the $PREMM_{1,2,6}$ prediction model (http://premm.dfci.harvard.edu/).[12] Each subject was assessed for whether their personal/family histories fulfilled NCCN criteria for HBOC testing for germline *BRCA1/2* mutations (Supplementary Methods).[18]

Germline Sequencing/Interpretation

After completion of clinical LS testing, anonymized genomic DNA samples were PCR-amplified with a custom amplicon library on a Raindance ThunderStorm instrument (RainDance Technologies, Inc., Lexington, MA) for NGS (Supplemental Methods). DNA products were sequenced on an Illumina HiSeq 2500 (Illumina, Inc., San Diego, CA) to detect sequence variations and large rearrangements among twenty-five cancer susceptibility genes with at least 1000x average coverage.

All sequence variations and large rearrangements detected were classified for pathogenicity into the following categories, as previously described: deleterious mutation, suspected deleterious mutation, variant of uncertain clinical significance (VUS), favor polymorphism, and polymorphism (Supplemental Methods).[19, 20] Individuals with deleterious or suspected deleterious genomic alterations were collectively defined as having “pathogenic” mutations. Alterations were classified as VUS if data were insufficient to support either a deleterious or benign interpretation.

Genes analyzed with the multigene panel were categorized as high- or moderate-penetrance based on expected lifetime risks of cancer (≥40% versus <40% or unknown) associated with the respective cancer predisposition syndrome (Table 1).[21–26] The genes underlying LS, adenomatous polyposis (*APC* and *MUTYH*) and hamartomatous polyposis (*BMPR1A*, *PTEN*, *SMAD4*, and *STK11*) syndromes, *BRCA1/2*, familial atypical multiple mole melanoma syndrome (*CDKN2A* and *CDK4*), hereditary diffuse gastric cancer (*CDH1*), and Li-Fraumeni syndrome (*TP53*) were categorized as high-penetrance, whereas the remaining eight genes (*ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NBN*, *PALB2*, *RAD51C*, and *RAD51D*) were considered moderate-penetrance. Biallelic *MUTYH* mutations were considered high-penetrance whereas monoallelic *MUTYH* mutations were not.[26–29]

Statistical Methods

The primary outcome was detection of pathogenic mutations in ≥1 cancer susceptibility genes on the multigene panel. Subjects’ ages and $PREMM_{1,2,6}$ scores were described as continuous variables, and mean $PREMM_{1,2,6}$ scores were compared using the Student’s *t*-test. All other clinical characteristics were described as categorical variables, and proportions were compared with Fisher’s exact test. All *P*-values were two-tailed, and *P*-values <0.05 were considered statistically significant.

RESULTS

Clinical Characteristics

915/1260 (73%) participants were female (Table 2). All subjects had a personal history of ≥1 LS-associated cancer and/or colorectal polyps with a median age at first cancer/polyp diagnosis of 47 years. 790 subjects (63%) had a history of CRC and 172 (14%) had a history of ≥2 primary cancers. 930 subjects (74%) had a family history of any LS-associated cancer, including 726 (58%) with a family history of CRC and 191 (15%) with a family history of EC. Based on reported personal/family histories, the cohort’s mean $PREMM_{1,2,6}$ score was

11.2% (95% CI: 10.4%–12.0%), and 1112/1260 (88%; 95% CI: 86%–90%) fulfilled NCCN guidelines for LS testing.

Germline Findings

182/1260 (14.4%; 95% CI: 12.6–16.5%) subjects were found to carry ≥1 pathogenic mutation with the multigene panel (Supplemental Table 1), including 114 (9.0%; 95% CI: 7.6–10.8%) with a LS mutation and 71 (5.6%; 95% CI: 4.4–7.1%) with a non-LS mutation (3 subjects carried both a LS and non-LS mutation; Figure 1A, Table 3). Of the 182 mutation carriers identified, 137 (75%; 95% CI: 68–81%) had ≥1 high-penetrance gene mutations.

Of the 114 LS mutations identified, there were 31 (27%) *MLH1* mutations, 40 (35%) *MSH2* mutations, 26 (23%) *MSH6* mutations, 14 (12%) *PMS2* mutations, and 3 (3%) *EPCAM* mutations (Figure 1B).

Of the 71 non-LS mutations, 24 (34%; 95% CI: 23–46%) were in high-penetrance genes (Figure 1C), including *BRCA1/2* (N=15), *APC* (N=5), biallelic *MUTYH* mutations (N=3), and *STK11* (N=1). There were 20/71 (28%; 95% CI: 18–40%) non-LS mutations in moderate-penetrance cancer susceptibility genes and another 27 (38%; 95% CI: 27–50%) individuals were monoallelic *MUTYH* mutation carriers. The three individuals with two germline mutations included one subject with pathogenic *MSH2* and *ATM* mutations, one with *MSH6* and *STK11* mutations, and one with *MSH2* and a monoallelic *MUTYH* mutation.

The clinical significance of monoallelic *MUTYH* mutation carriage is a matter of debate.[27–34] If monoallelic *MUTYH* mutation carriers are excluded from the tally of pathogenic mutations in this study, then a total of 156 (12.4% of the overall 1260 patient cohort; 95% CI: 10.6–14.4%) mutation carriers were identified, including 44 (3.5% of the cohort; 95% CI: 2.6–4.7%) with a non-LS mutation, two of whom had both a LS and non-LS mutation.

The 15 *BRCA1/2* probands represented 8% of all mutation carriers identified with the multigene panel, and *BRCA1/2* mutations were found in 1.2% (15/1260; 95% CI: 0.7–2.0%) of the entire cohort. Eight (53%) *BRCA1/2* mutation carriers were female and 7 (47%) were male. Five (33%) of the *BRCA1/2* mutations were Ashkenazi founder mutations (three *BRCA1* 5382insC and two *BRCA2* 6174delT), though only 1 of the 15 *BRCA1/2* probands was identified on the test request form as being of Ashkenazi descent. 9/15 (60%) *BRCA1/2* probands had a history of CRC, including 6/7 (86%) male *BRCA1/2* carriers. 4/15 (27%) *BRCA1/2* probands had a history of EC, 1 (7%) had a history of ovarian cancer, and none had a history of breast or pancreatic cancer. Ten (67%) *BRCA1/2* carriers had a family history of any LS cancer, including 7 (47%) with a family history of CRC. Seven (47%) *BRCA1/2* carriers had a family history of breast cancer. *BRCA1/2* carriers were significantly more likely to fulfill NCCN criteria for LS testing than for HBOC testing (93% vs 33%; P=0.0017).

Nine individuals were found to carry mutations in high-penetrance non-LS cancer susceptibility genes other than *BRCA1/2*. One had both pathogenic *STK11* and *MSH6*

mutations, with a personal history of CRC, EC, and breast cancer. Of the remaining 8 who carried either germline *APC* mutations (N=5) or biallelic *MUTYH* mutations (N=3), all had a family history of CRC and fulfilled NCCN criteria for LS testing, and 6 (75%) had a personal history of CRC. Three (38%) reported prior colorectal polyps, although details on polyp number and histology were not available.

Of the 26 individuals found to carry a monoallelic *MUTYH* mutation (excluding the proband with both a *MSH2* and monoallelic *MUTYH* mutation), 12 (46%; 95% CI: 27–66%) had a personal history of CRC and 11 (42%; 95% CI: 24–63%) had a family history of CRC.

682 VUS were detected in 479 individuals (38% of the cohort; 95% CI: 35–41%) (Supplemental Table 2). The most common genes in which VUS were discovered were *ATM* (N=128), *APC* (N=51), *NBN* (N=51), and *BRIP1* (N=50) (Figure 2).

$PREMM_{1,2,6}$ scores and NCCN criteria

The majority of mutation carriers had a $PREMM_{1,2,6}$ score ≥5% (the cutoff recommended by NCCN guidelines for consideration of LS evaluation), regardless of whether they carried a LS or a non-LS mutation (Table 4).[9] 52% of LS carriers had a $PREMM_{1,2,6}$ score ≥15%, versus 26% of non-LS probands (*P*=0.001). There was no significant difference between the proportion of LS carriers who fulfilled NCCN criteria for LS testing compared to *BRCA1/2* carriers (*P*=1.00) or other high-penetrance mutation carriers (*P*=1.00).

DISCUSSION

Multigene panel testing identified clinically unsuspected mutations in non-LS cancer susceptibility genes in 71/1260 (5.6%) individuals undergoing LS genetic testing, including 3 with both LS and non-LS mutations. In total, 75% of pathogenic mutations identified by the multigene panel were in high-penetrance genes.[25] The most common unexpected findings in our cohort were *BRCA1/2*, *APC*, and biallelic *MUTYH* mutations in individuals with clinical features of LS.

The growing availability of multigene panels provides clinicians with the option of broad-based genetic analysis for hereditary cancer risk assessment, rather than traditional, phenotype-driven genetic testing. The benefits of such comprehensive testing strategies have been debated and are only beginning to be scientifically evaluated.[7, 35] Clinical guidelines, such as NCCN criteria, and prediction models, such as $PREMM_{1,2,6}$, have been developed to select individuals for LS evaluation, based on their personal/family histories.[9, 12] Our study, where the vast majority of both LS and non-LS mutation carriers fulfilled NCCN criteria for LS and had a $PREMM_{1,2,6}$ score ≥5%, demonstrates that such criteria, although very useful for identifying which individuals should be referred for genetic evaluation, ultimately may not be specific for underlying LS.

In the only prior study to specifically examine panel testing in patients with suspected hereditary gastrointestinal cancer, actionable mutations were detected in 42/586 (7.2%) patients, 23 of which were LS mutations.[36] All patients in this study, however, were specifically selected by their clinicians to undergo testing with a panel of 13 CRC

susceptibility genes, rather than targeted, phenotype-directed testing, suggesting that this was a particularly high-risk cohort. Furthermore, the panel used did not include *BRCA1/2* testing, thereby precluding analysis regarding phenotypic overlap between LS and HBOC.

In other recent analyses studying panel testing in women with suspected HBOC, the identification of mutations in high-penetrance genes other than *BRCA1/2* was uncommon.[37–39] As such, a recent editorial cautioned that identifying unexpected, clinically useful, high-penetrance mutations with multigene panel testing is likely to be rare.[35] Panel testing in our cohort, however, found >1 high-penetrance non-LS gene mutation for every 5 LS mutations identified, demonstrating that unexpected actionable findings are not uncommon in patients with LS-like phenotypes.

The identification of pathogenic *BRCA1/2* mutations in 8% of mutation carriers and 1.2% of our overall cohort is unexpected, and raises important clinical questions. The carrier rate of *BRCA1/2* mutations is known to be particularly high (1.1–2.5%) in Ashkenazi Jewish individuals, but is considerably lower (0.22–0.33%) in the general population.[40–43] Since only 2% of our cohort was identified as being of Ashkenazi descent and only 5 *BRCA1/2* mutations identified were Ashkenazi founder mutations, it seems unlikely that the unexpected identification of *BRCA1/2* mutations in our study can be attributed to simply detecting their background population prevalence. Even if the 5 Ashkenazi founder mutations are excluded from the analysis, the 10 non-founder *BRCA1/2* mutations identified in this study is substantially higher (0.8%; 10/1260) than the expected prevalence in the general population.

Prior studies have shown no increased CRC risk in *BRCA1/2* probands, and the traditional thinking has thus been that LS and HBOC are phenotypically distinct syndromes, aside from both conferring increased risks of ovarian cancer.[44] In this study, however, *BRCA1/2* probands had phenotypes that were markedly more “Lynch-like” than “HBOC-like,” suggesting that standard clinical evaluation would not have identified most of these individuals as needing *BRCA1/2* testing. Such atypical phenotypes may be more common in men, since 86% of the male *BRCA1/2* probands in our study had a history of CRC. Our findings thus raise the hypothesis that a subset of *BRCA1/2* probands may have particularly atypical phenotypes that can mimic LS.

The identification of such patients with “unexpected” high-penetrance germline mutations that do not seem concordant with their clinical histories raises the question as to whether hereditary cancer syndromes should be defined based on genotypic data, phenotypic data, or both. Prior to the identification of specific genes linked to familial cancer risks, assessment of an individual’s clinical phenotype was the primary means of diagnosing a particular hereditary cancer syndrome (e.g. fulfillment of Amsterdam criteria for Lynch syndrome).[45] With the discovery of specific cancer susceptibility genes linked to particular syndromes and the availability of clinical genetic testing, it has become clear that such criteria are often too stringent and insensitive.[1] As such, the current gold standard for diagnosing a hereditary cancer syndrome is now the identification of a germline mutation in the associated gene (e.g. Lynch syndrome is defined by the presence of a germline MMR mutation), and cancer surveillance recommendations are usually made based on genotype more so than family

history.[9] If multigene panel testing routinely identifies a subset of patients with pathogenic mutations in the setting of highly atypical clinical histories, however, such patients' management recommendations may need to take into account phenotype as well as genotype. For example, prophylactic total gastrectomy is the current recommendation for *CDH1* mutation carriers from hereditary diffuse gastric cancer families, though this recommendation may be overly aggressive in the context of an "incidental" *CDH1* mutation in an individual with no personal or family history of diffuse gastric cancer.[46] Larger studies with more detailed clinical histories will be needed to address this more definitively.

In order to fully assess the potential benefits and downsides of multigene panel testing compared to traditional hereditary cancer risk assessment strategies, the cost of testing must be taken into consideration. Although rigorous cost-effectiveness analyses were beyond the scope of this study, multigene panel testing offers a lower cost of testing per gene and may also decrease some of the ancillary costs of genetic testing, such as additional physician and counselor visits, by analyzing genes in parallel, rather than sequentially.[47] One recent analysis concluded that multigene panel testing was cost-effective as an initial diagnostic test for patients with suspected hereditary CRC syndromes, particularly for panels that include genes associated with high-penetrance CRC syndromes.[48] Such potential cost savings, however, must be weighed carefully against the costs (both financial and non-financial) that are likely to arise from the increased identification of VUSs and mutations in moderate-penetrance genes.

The discovery of uninformative and potentially anxiety-provoking results remains a primary limitation of multigene panel testing, and the identification of ≥1 VUS in 38% of our cohort validates such concerns.[7, 35] Other results of debatable clinical utility include the detection of mutations in moderate-penetrance cancer susceptibility genes, which may not account for subjects' clinical phenotypes, and the identification of monoallelic *MUTYH* mutations in 2.1% of participants.[35] The population prevalence of monoallelic *MUTYH* mutation carriage is estimated to be 1%,[28] and prior studies have shown a roughly 2-fold increase in CRC risk among monoallelic carriers with an estimated 7.2% and 5.6% risk of CRC by age 70 for male and female carriers, respectively.[27, 31, 32] Recent data have also suggested that monoallelic *MUTYH* mutation carriers with a first-degree relative with early-onset CRC are at particularly increased CRC risk (12.4% and 9.9% risk of CRC by age 70 for male and female carriers, respectively).[27, 30] Other studies, however, have found no significant increase in the risk of CRC or other cancers among monoallelic *MUTYH* mutation carriers, thus leaving the clinical significance of such findings up for debate.[29, 33, 34] While the clinical utility of detecting monoallelic *MUTYH* carrier status for the proband themselves is thus uncertain, such results at the very least may prompt family members with a history of CRC to be evaluated for biallelic carriage.

Our study's main strength is its use of a large, consecutive cohort of individuals with clinical histories suggestive of LS, which makes its findings generalizable to other populations of patients with suspected LS. The use of a CLIA-certified laboratory with extensive experience in clinical genetic testing and interpretation of germline cancer susceptibility gene alterations allowed for rapid and comprehensive genetic analysis of a large panel of cancer susceptibility genes. The availability of linked personal/family cancer history data

allowed for determination of whether mutation carriers fulfilled various clinical guidelines for hereditary cancer risk assessment.

We recognize that our study has limitations. Data regarding subjects' personal/family histories of cancer were obtained via clinician report on a test request form, and we were thus unable to confirm its accuracy or completeness. Although this is a potential limitation, the same approach was used to develop the $PREMM_{1,2,6}$ prediction model for LS risk assessment, and $PREMM_{1,2,6}$ has been subsequently validated in clinic- and population-based cohorts where clinical data were extensively verified.[12] Furthermore, all subjects in this cohort were ascertained from a large commercial laboratory which receives genetic testing referrals from academic medical centers as well as community practices. Given that patients from academic cancer centers may have higher-risk clinical histories than those from smaller practices, we are unable to account for the possibility that the performance of multigene panel testing may vary across different healthcare settings.

The specific frequencies of mutation carriers detected by panel testing are also likely to vary depending on the genes included in a given multigene panel. Although there is a growing array of commercially available multigene panels for hereditary cancer risk assessment, almost all such panels include the same high-penetrance cancer susceptibility genes (i.e. MMR genes, *BRCA1/2*, *APC*, *MUTYH*, *STK11*, *PTEN*, *CDH1*, and *TP53*), and thus the key findings of our study are likely generalizable to testing performed with other multigene panels.[47]

Another limitation of our study is that we did not have data on tumor testing results that may have prompted referral for germline testing. NCCN guidelines[9] recommend that all CRC specimens undergo MMR IHC or MSI testing as an initial screen for LS. Roughly 20% of the MSI-H/MMR-deficient CRCs identified with such testing will be due to LS, and additional tumor testing for *BRAF* V600E mutations or *MLH1* promoter hypermethylation can help identify the 80% of MSI-H/MMR-deficient cases that are likely sporadic and thus do not need LS germline testing. Without such data, we are unable to extrapolate our study's findings on multigene panel testing into contemporary LS diagnostic algorithms which rely heavily on MMR IHC and MSI screening of tumor specimens. Multiple studies, however, have found that the uptake and efficacy of universal tumor testing strategies are highly variable, even within large academic medical centers.[49–51] Furthermore, most studies examining universal tumor testing have only performed germline LS testing on individuals with MSI-H/MMR-deficient CRC, and thus the mutation rate amongst patients with normal or absent tumor testing results is not well-studied.[52–54]

Within our cohort of patients with a history of LS-associated cancer/polyps, MSI and MMR IHC tumor testing likely would have identified individuals where targeted germline LS testing would have been indicated, rather than panel testing, although this would still miss the rare individual with both a LS and non-LS mutation. Future research is needed to determine the yield of multigene panel testing in patients for whom MSI, MMR IHC, and other tumor testing results are available. Universal tumor testing algorithms only screen for LS, however, and our results demonstrate that a substantial fraction of patients with Lynch-like clinical histories will actually have other inherited cancer syndromes. Thus, the practice

of using tumor testing to distinguish between patients with “familial” and “sporadic” cancers will ultimately miss some individuals with actionable mutations in non-Lynch cancer susceptibility genes.

In spite of these limitations, our findings provide novel insight about the evaluation of patients with suspected LS in the era of multigene panel testing. Since clinical criteria for LS analysis appear to identify a substantial number of probands with unexpected actionable mutations in high-penetrance non-LS cancer susceptibility genes, panel testing may ultimately replace targeted genetic testing in patients with suspected LS, except when tumor testing suggests a specific underlying MMR mutation. Increased use of panel testing, however, will undoubtedly lead to more patients being diagnosed with VUS and other germline findings of uncertain clinical utility. Furthermore, with expanded use of panel testing, the question as to how patients with “unexpected,” high-penetrance germline mutations identified by panel testing (e.g. *BRCA1/2* mutations in individuals with a clinical history suggestive of hereditary colorectal cancer) should be managed is likely to become an increasingly common dilemma for practicing clinicians.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Palomaki GE, McClain MR, Melillo S, et al. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med. 2009; 11:42–65. [PubMed: 19125127]
2. Vasen HF, Blanco I, Aktan-Collan K, et al. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut. 2013; 62:812–23. [PubMed: 23408351]
3. Nelson HD, Pappas M, Zakher B, et al. Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer in women: a systematic review to update the U.S. Preventive Services Task Force recommendation. Ann Intern Med. 2014; 160:255–66. [PubMed: 24366442]
4. Jarvinen HJ, Aarnio M, Mustonen H, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology. 2000; 118:829–34. [PubMed: 10784581]
5. Win AK, Young JP, Lindor NM, et al. Colorectal and other cancer risks for carriers and noncarriers from families with a DNA mismatch repair gene mutation: a prospective cohort study. J Clin Oncol. 2012; 30:958–64. [PubMed: 22331944]

6. Kastrinos F, Mukherjee B, Tayob N, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA. 2009; 302:1790–5. [PubMed: 19861671]
7. Domchek SM, Bradbury A, Garber JE, et al. Multiplex genetic testing for cancer susceptibility: out on the high wire without a net? J Clin Oncol. 2013; 31:1267–70. [PubMed: 23460708]
8. Kastrinos F, Steyerberg EW, Balmana J, et al. Comparison of the clinical prediction model PREMM(1,2,6) and molecular testing for the systematic identification of Lynch syndrome in colorectal cancer. Gut. 2013; 62:272–9. [PubMed: 22345660]
9. Levy DE, Byfield SD, Comstock CB, et al. Underutilization of BRCA1/2 testing to guide breast cancer treatment: black and Hispanic women particularly at risk. Genet Med. 2011; 13:349–55. [PubMed: 21358336]
10. Lindor NM, Rabe K, Petersen GM, et al. Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X. JAMA. 2005; 293:1979–85. [PubMed: 15855431]
11. Balmana J, Balaguer F, Castellvi-Bel S, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients with Lynch syndrome in a population-based cohort of colorectal cancer patients. J Med Genet. 2008; 45:557–63. [PubMed: 18603628]
12. Kastrinos F, Steyerberg EW, Mercado R, et al. The PREMM(1,2,6) model predicts risk of MLH1, MSH2, and MSH6 germline mutations based on cancer history. Gastroenterology. 2011; 140:73–81. [PubMed: 20727894]
13. Morak M, Heidenreich B, Keller G, et al. Biallelic MUTYH mutations can mimic Lynch syndrome. Eur J Hum Genet. 2014; 22:1334–7. [PubMed: 24518836]
14. Mester JL, Moore RA, Eng C. PTEN germline mutations in patients initially tested for other hereditary cancer syndromes: would use of risk assessment tools reduce genetic testing? Oncologist. 2013; 18:1083–90. [PubMed: 24037976]
15. Weissman SM, Bellcross C, Bittner CC, et al. Genetic counseling considerations in the evaluation of families for Lynch syndrome--a review. J Genet Couns. 2011; 20:5–19. [PubMed: 20931355]
16. Castillejo A, Vargas G, Castillejo MI, et al. Prevalence of germline MUTYH mutations among Lynch-like syndrome patients. Eur J Cancer. 2014; 50:2241–50. [PubMed: 24953332]
17. Stadler ZK, Schrader KA, Vijai J, et al. Cancer genomics and inherited risk. J Clin Oncol. 2014; 32:687–98. [PubMed: 24449244]
18. Hall MJ, Reid JE, Burbidge LA, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer. 2009; 115:2222–33. [PubMed: 19241424]
19. Eggington JM, Bowles KR, Moyes K, et al. A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. Clin Genet. 2013
20. Richards CS, Bale S, Bellissimo DB, et al. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. Genet Med. 2008; 10:294–300. [PubMed: 18414213]
21. Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet. 2006; 38:873–5. [PubMed: 16832357]
22. Stoffel EM, Kastrinos F. Familial colorectal cancer, beyond Lynch syndrome. Clin Gastroenterol Hepatol. 2014; 12:1059–68. [PubMed: 23962553]
23. Chun N, Ford JM. Genetic testing by cancer site: stomach. Cancer J. 2012; 18:355–63. [PubMed: 22846738]
24. Mukherjee B, Delancey JO, Raskin L, et al. Risk of non-melanoma cancers in first-degree relatives of CDKN2A mutation carriers. J Natl Cancer Inst. 2012; 104:953–6. [PubMed: 22534780]
25. Stadler ZK, Thom P, Robson ME, et al. Genome-wide association studies of cancer. J Clin Oncol. 2010; 28:4255–67. [PubMed: 20585100]
26. Nieuwenhuis MH, Vogt S, Jones N, et al. Evidence for accelerated colorectal adenoma--carcinoma progression in MUTYH-associated polyposis? Gut. 2012; 61:734–8. [PubMed: 21846783]
27. Win AK, Dowty JG, Cleary SP, et al. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. Gastroenterology. 2014; 146:1208–11. e1–5. [PubMed: 24444654]

28. Casper M, Plotz G, Juengling B, et al. MUTYH hotspot mutations in unselected colonoscopy patients. Colorectal Dis. 2012; 14:e238–44. [PubMed: 22469480]
29. Lubbe SJ, Di Bernardo MC, Chandler IP, et al. Clinical implications of the colorectal cancer risk associated with MUTYH mutation. J Clin Oncol. 2009; 27:3975–80. [PubMed: 19620482]
30. Win AK, Cleary SP, Dowty JG, et al. Cancer risks for monoallelic MUTYH mutation carriers with a family history of colorectal cancer. Int J Cancer. 2011; 129:2256–62. [PubMed: 21171015]
31. Croitoru ME, Cleary SP, Di Nicola N, et al. Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. J Natl Cancer Inst. 2004; 96:1631–4. [PubMed: 15523092]
32. Jones N, Vogt S, Nielsen M, et al. Increased colorectal cancer incidence in obligate carriers of heterozygous mutations in MUTYH. Gastroenterology. 2009; 137:489–94. 494 e1. quiz 725–6. [PubMed: 19394335]
33. Balaguer F, Castellvi-Bel S, Castells A, et al. Identification of MYH mutation carriers in colorectal cancer: a multicenter, case-control, population-based study. Clin Gastroenterol Hepatol. 2007; 5:379–87. [PubMed: 17368238]
34. Peterlongo P, Mitra N, Chuai S, et al. Colorectal cancer risk in individuals with biallelic or monoallelic mutations of MYH. Int J Cancer. 2005; 114:505–7. [PubMed: 15578699]
35. Robson M. Multigene panel testing: planning the next generation of research studies in clinical cancer genetics. J Clin Oncol. 2014; 32:1987–9. [PubMed: 24868020]
36. Cragun D, Radford C, Dolinsky JS, et al. Panel-based testing for inherited colorectal cancer: a descriptive study of clinical testing performed by a US laboratory. Clin Genet. 2014
37. Walsh T, Casadei S, Lee MK, et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A. 2011; 108:18032–7. [PubMed: 22006311]
38. Kurian AW, Hare EE, Mills MA, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol. 2014; 32:2001–9. [PubMed: 24733792]
39. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 2015; 121:25–33. [PubMed: 25186627]
40. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med. 1997; 336:1401–8. [PubMed: 9145676]
41. Metcalfe KA, Poll A, Royer R, et al. Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. J Clin Oncol. 2010; 28:387–91. [PubMed: 20008623]
42. Roa BB, Boyd AA, Volcik K, et al. Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nat Genet. 1996; 14:185–7. [PubMed: 8841191]
43. McClain MR, Palomaki GE, Nathanson KL, et al. Adjusting the estimated proportion of breast cancer cases associated with BRCA1 and BRCA2 mutations: public health implications. Genet Med. 2005; 7:28–33. [PubMed: 15654225]
44. Garber JE, Syngal S. One less thing to worry about: the shrinking spectrum of tumors in BRCA founder mutation carriers. J Natl Cancer Inst. 2004; 96:2–3. [PubMed: 14709725]
45. Vasen HF, Watson P, Mecklin JP, et al. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. 1999; 116:1453–6. [PubMed: 10348829]
46. Fitzgerald RC, Hardwick R, Huntsman D, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. 2010; 47:436–44. [PubMed: 20591882]
47. Hall MJ, Forman AD, Pilarski R, et al. Gene panel testing for inherited cancer risk. J Natl Compr Canc Netw. 2014; 12:1339–46. [PubMed: 25190699]
48. Gallego CJ, Shirts BH, Bennette CS, et al. Next-Generation Sequencing Panels for the Diagnosis of Colorectal Cancer and Polyposis Syndromes: A Cost-Effectiveness Analysis. J Clin Oncol. 2015
49. Ward RL, Hicks S, Hawkins NJ. Population-based molecular screening for Lynch syndrome: implications for personalized medicine. J Clin Oncol. 2013; 31:2554–62. [PubMed: 23733757]

50. Cragun D, DeBate RD, Vadaparampil ST, et al. Comparing universal Lynch syndrome tumor-screening programs to evaluate associations between implementation strategies and patient follow-through. Genet Med. 2014; 16:773–82. [PubMed: 24651603]

51. Beamer LC, Grant ML, Espenschied CR, et al. Reflex immunohistochemistry and microsatellite instability testing of colorectal tumors for Lynch syndrome among US cancer programs and follow-up of abnormal results. J Clin Oncol. 2012; 30:1058–63. [PubMed: 22355048]

52. Moreira L, Balaguer F, Lindor N, et al. Identification of Lynch syndrome among patients with colorectal cancer. JAMA. 2012; 308:1555–65. [PubMed: 23073952]

53. Perez-Carbonell L, Ruiz-Ponte C, Guarinos C, et al. Comparison between universal molecular screening for Lynch syndrome and revised Bethesda guidelines in a large population-based cohort of patients with colorectal cancer. Gut. 2012; 61:865–72. [PubMed: 21868491]

54. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol. 2008; 26:5783–8. [PubMed: 18809606]

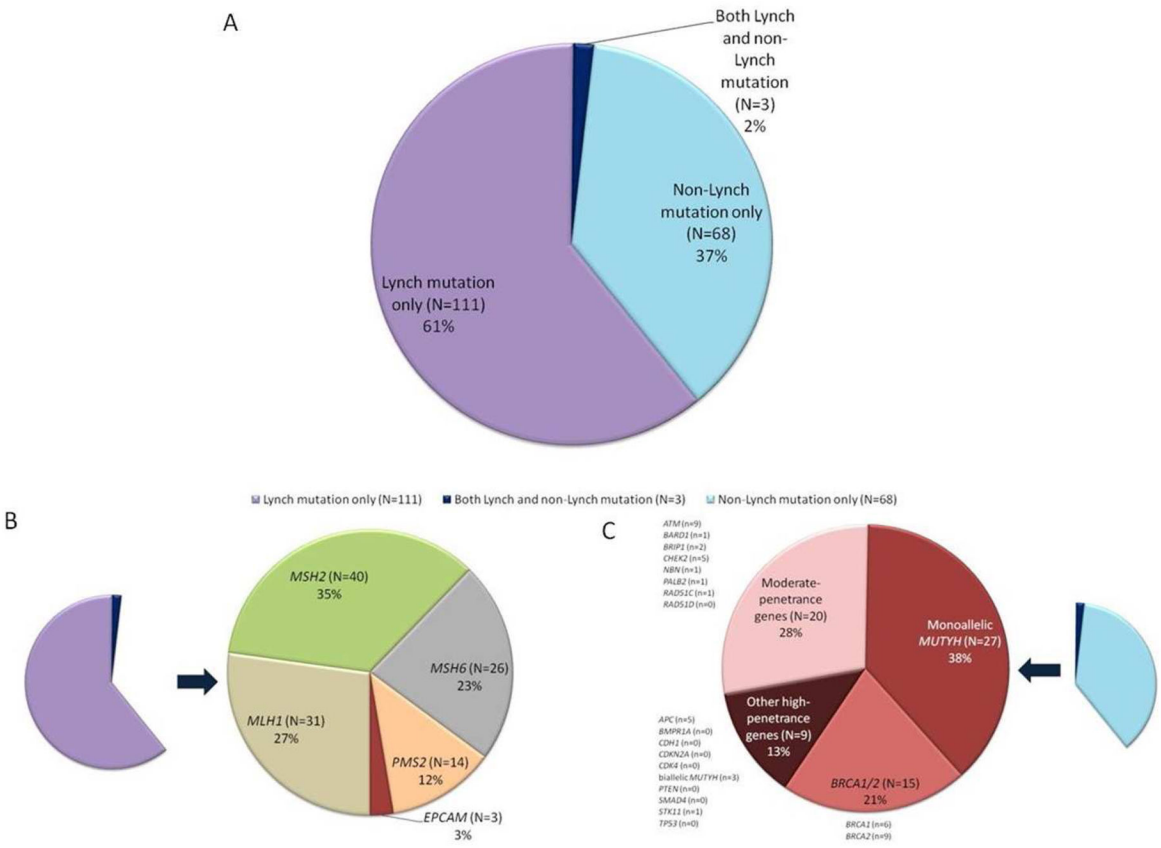

Figure 1.

Pathogenic mutations identified with a multigene panel among 1260 individuals with suspected Lynch syndrome. (A) Proportion of mutation carriers with Lynch syndrome mutations (purple), non-Lynch syndrome mutations (blue), or both Lynch and non-Lynch syndrome mutations (dark purple). (B) Distribution of Lynch syndrome mutation carriers by specific gene. (C) Distribution of non-Lynch syndrome mutation carriers by gene type (*BRCA1/2*, monoallelic *MUTYH*, other high-penetrance genes, or moderate-penetrance genes).

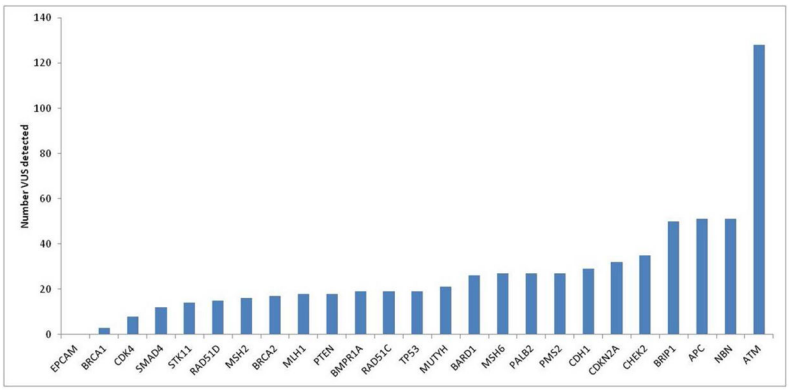

Figure 2.

Number of variants of uncertain significance (VUS), per gene, detected with a multigene panel in 1260 individuals undergoing Lynch syndrome testing.

Table 1

Genes analyzed by a multigene hereditary cancer panel

High-penetrance genes	Moderate-penetrance genes
Lynch syndrome	*ATM*
MLH1	*BARD1*
MSH2	*BRIP1*
MSH6	*CHEK2*
PMS2	*NBN*
EPCAM	*PALB2*
Adenomatous polyposis syndromes	*RAD51C*
APC	*RAD51D*
MUTYH (biallelic)	
Hamartomatous polyposis syndromes	
BMPR1A	
PTEN	
SMAD4	
STK11	
Hereditary breast/ovarian cancer (HBOC)	
BRCA1	
BRCA2	
Familial atypical multiple mole melanoma syndrome	
CDKN2A	
CDK4	
Hereditary diffuse gastric cancer syndrome	
CDH1	
Li-Fraumeni syndrome	
TP53	

Table 2

Characteristics of 1260 individuals undergoing clinical testing for Lynch syndrome

	Total cohort (N=1260) N (%)
Female	915 (73)
Median age (years) at first cancer diagnosis [IQR]	47 [39 – 55.5][†]
Personal history[*]	
Colorectal cancer, any age	790 (63)
Colorectal cancer, age <50	434 (34)
Endometrial cancer	292 (23)
Ovarian cancer	84 (7)
Multiple primary cancers	172 (14)
Colorectal polyps	280 (22)
Family history[*]	
Any Lynch cancer	930 (74)
Colorectal cancer	726 (58)
Endometrial cancer	191 (15)
Ovarian cancer	142 (11)
Breast cancer	294 (23)
No/unknown family history	161 (13)
Met NCCN Lynch criteria	1112 (88)

[†] Age data missing for 56 subjects

[*] Personal and family history classifications are not mutually exclusive

Table 3

Clinical characteristics of mutation carriers identified by multigene panel testing

				Personal history*								Family history*					
	Female N (%)	**Median age† (yrs) at first cancer/ polyp**	**Met NCCN Lynch criteria N (%)**	**CRC, any age N (%)**	**CRC, age <50 N (%)**	**EC N (%)**	**Ovarian cancer N (%)**	**Breast cancer N (%)**	**Multiple primary cancers N (%)**	**Colorectal polyps N (%)**	**Any Lynch cancer N (%)**	**CRC N (%)**	**EC N (%)**	**Ovarian cancer N (%)**	**Breast cancer N (%)**	**None/ unknown N (%)**
High-penetrance genes																
Lynch (N=111)	75 (68)	45	105 (95)	81 (73)	58 (52)	33 (30)	3 (3)	4 (4)	26 (23)	12 (11)	95 (86)	82 (74)	29 (26)	9 (8)	22 (20)	4 (4)
BRCA1 (N=6)	4 (67)	55	6 (100)	3 (50)	0	2 (33)	1 (17)	0	1 (17)	3 (50)	5 (83)	4 (67)	2 (33)	1 (17)	2 (33)	0
BRCA2 (N=9)	4 (44)	42	8 (89)	6 (67)	5 (56)	2 (22)	0	0	0	2 (22)	5 (56)	3 (33)	0	1 (11)	5 (56)	2 (22)
APC (N=5)	3 (60)	44	5 (100)	3 (60)	2 (40)	0	0	0	0	2 (40)	5 (100)	5 (100)	0	1 (20)	0	0
Biallelic *MUTYH* (N=3)	2 (67)	58	3 (100)	3 (100)	1 (33)	0	0	1 (33)	1 (33)	1 (33)	3 (100)	3 (100)	0	0	1 (33)	0
Moderate-penetrance genes																
ATM (N=8)	6 (75)	47.5	7 (88)	6 (75)	3 (38)	3 (38)	1 (13)	0	2 (25)	1 (13)	5 (63)	4 (50)	1 (13)	0	0	2 (25)
CHEK2 (N=5)	4 (80)	52	4 (80)	4 (80)	1 (20)	1 (20)	0	0	0	0	3 (60)	3 (60)	0	0	1 (20)	1 (20)
BRIP1 (N=2)	0	42	2 (100)	2 (100)	2 (100)	0	0	0	0	0	1 (50)	1 (50)	0	0	0	0
BARD1 (N=1)	0	66	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)	1 (100)	0	0	0	0
NBN (N=1)	1 (100)	35	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	0	0	0	0	0
PALB2 (N=1)	1 (100)	41	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	0	0	0	1 (100)	0
RAD51C (N=1)	1 (100)	48	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	0	0	0	0	1 (100)
Dual mutation carriers																
MSH6 and *STK11* (N=1)	1 (100)	50	1 (100)	1 (100)	0	1 (100)	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0
MSH2 and *ATM* (N=1)	0	43	1 (100)	1 (100)	1 (100)	0	0	0	1 (100)	0	1 (100)	1 (100)	0	0	1 (100)	0
MSH2 and monoallelic *MUTYH* (N=1)	1 (100)	47	1 (100)	0	0	1 (100)	0	0	0	0	1 (100)	1 (100)	0	0	0	0
Monoallelic *MUTYH* only (N=26)	17 (65)	48.5	19 (73)	12 (46)	5 (19)	9 (35)	2 (8)	2 (8)	3 (12)	5 (19)	19 (73)	11 (42)	4 (15)	2 (8)	9 (35)	2 (8)

*Personal and family history classifications are not mutually exclusive

†Age data missing for 4 mutation carriers

Abbrevations: CRC = colorectal cancer; EC = endometrial cancer

Table 4

$PREMM_{1,2,6}$ scores and fulfillment of NCCN criteria for Lynch syndrome testing among individuals† with pathogenic mutations identified by a multigene panel

	Mean $PREMM_{1,2,6}$ score, % (95% CI)	*P*-value¥	$PREMM_{1,2,6}$ score ≥5%, N (%)	*P*-value¥	$PREMM_{1,2,6}$ score ≥15%, N (%)	*P*-value¥	Met NCCN Lynch criteria, N (%)	*P*-value¥
Lynch mutation carriers (N=111)†	28.6 (23.7–33.5)	-	96 (86)	-	58 (52)	-	105 (95)	-
All non-Lynch mutation carriers (N=68)†	13.4 (9.9–16.8)	0.00002	51 (75)	0.07	18 (26)	0.001	58 (85)	0.056
BRCA1/2 mutation carriers (N=15)	12.4 (6.9–17.8)	0.02	12 (80)	0.45	3 (20)	0.03	14 (93)	1.00
Other high-penetrance gene mutation carriers (N=8)†	27.8 (10.1–45.5)	0.93	6 (75)	0.32	5 (63)	0.72	8 (100)	1.00
Moderate-penetrance gene mutation carriers (N=19)†	11.7 (5.4–18.0)	0.007	14 (74)	0.17	4 (21)	0.01	17 (89)	0.33
Monoallelic *MUTYH* mutation carriers (N=26)†	10.8 (7.0–14.6)	0.001	19 (73)	0.13	6 (23)	0.009	19 (73)	0.0032

¥ *P*-values for comparison with Lynch carriers

† Excluding 3 subjects with both a Lynch mutation and a non-Lynch mutation: one subject with both *MSH2* and *ATM* mutations, one with both *MSH 6* and *STK11* mutations, and one with both *MSH2* and a monoallelic *MUTYH* mutation