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

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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 patients had a history of Lynch syndrome–associated cancer and/or polyps. We classified all identified germline alterations for pathogenicity and calculated the frequencies of pathogenic mutations and variants of uncertain clinical 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 patients, 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. A total of 479 individuals had 1 or more 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.

Hereditary cancer syndromes classically are 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 also is 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 ultimately will 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

Abbreviations used in this paper: **CI, confidence interval; CRC, colorectal cancer; EC, endometrial cancer; HBOC, hereditary breast/ovarian cancer; IHC, immunohistochemistry; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, high microsatellite instability; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PCR, polymerase chain reaction; $PREM_{1,2,6}$, prediction of mismatch repair gene mutations in *MLH1*, *MSH2*, and *MSH6*; VUS, variant of uncertain clinical significance.**

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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.

Materials and Methods

Study Population

A total of 3057 individuals with a history of LS-associated cancer and/or colorectal polyps whose clinicians submitted germline DNA to a clinical laboratory improvement amendments–approved commercial laboratory (Myriad Genetic Laboratories, Inc, Salt Lake City, UT) for clinical genetic testing for all 5 genes underlying LS (*MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*) between 2012 and 2013 were ascertained consecutively. Upon completion of clinical LS testing, samples were anonymized for research-based multigene panel testing. A total of 1615 patients were excluded because their testing originated from states with legislation mandating destruction of biospecimens after completion of clinical genetic testing. Another 182 patients were excluded as a result of technical factors (insufficient remaining DNA after clinical testing, DNA extracted from a nonblood sample), providing 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, patients' clinicians completed a test request form for each individual describing basic demographics (sex, 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, patients were assessed as to whether they fulfilled National Comprehensive Cancer Network (NCCN) guidelines for LS testing (Supplementary Materials and Methods).[9] A numeric estimate of the likelihood of identifying a germline mutation in *MLH1*, *MSH2*, or *MSH6* was calculated for each patient using the prediction of mismatch repair gene mutations in *MLH1*, *MSH2*, and *MSH6* ($PREMM_{1,2,6}$) prediction model (http://premm.dfci.harvard.edu/).[12] Each patient was assessed for whether their personal/family histories fulfilled NCCN criteria for HBOC testing for germline *BRCA1/2* mutations (Supplementary Materials and Methods).[18]

Germline Sequencing/Interpretation

After completion of clinical LS testing, anonymized genomic DNA samples were polymerase chain reaction (PCR)-amplified with a custom amplicon library on a Raindance ThunderStorm instrument (RainDance Technologies, Inc, Lexington, MA) for NGS (Supplementary Materials and Methods). DNA products were sequenced on an Illumina HiSeq 2500 (Illumina, Inc, San Diego, CA) to detect sequence variations and large rearrangements among 25 cancer susceptibility genes with at least 1000 times 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 Materials and Methods).[19,20] Individuals with deleterious or suspected deleterious genomic alterations were defined collectively 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 ($\geq$40% vs <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 8 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 or more cancer susceptibility genes on the multigene

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	
HBOC	
BRCA1	
BRCA2	
Familial atypical multiple mole melanoma syndrome	
CDKN2A	
CDK4	
Hereditary diffuse gastric cancer syndrome	
CDH1	
Li–Fraumeni syndrome	
TP53	

panel. Patient ages and $PREMM_{1,2,6}$ scores were described as continuous variables, and mean $PREMM_{1,2,6}$ scores were compared using the Student *t* test. All other clinical characteristics were described as categoric variables, and proportions were compared with the Fisher exact test. All *P* values were 2-tailed, and *P* values less than .05 were considered statistically significant.

Results

Clinical Characteristics

A total of 915 of 1260 (73%) participants were female (Table 2). All patients had a personal history of 1 or more LS-associated cancer and/or colorectal polyps with a median age at first cancer/polyp diagnosis of 47 years. A total of 790 patients (63%) had a history of CRC and 172 (14%) had a history of 2 or more primary cancers. A total of 930 patients (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% confidence interval [CI], 10.4%–12.0%), and 1112 of 1260 patients (88%; 95% CI, 86%–90%) fulfilled NCCN guidelines for LS testing.

Germline Findings

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

Table 2. Characteristics of 1260 Individuals Undergoing Clinical Testing for Lynch Syndrome

	Total cohort (N = 1260), N (%)
Female	915 (73)
Median age at first cancer diagnosis, *y* [IQR]	47 [39–55.5][a]
Personal history[b]	
Colorectal cancer, any age	790 (63)
Colorectal cancer, age <50 y	434 (34)
Endometrial cancer	292 (23)
Ovarian cancer	84 (7)
Multiple primary cancers	172 (14)
Colorectal polyps	280 (22)
Family history[b]	
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)

IQR, interquartile range.
[a]Age data were missing for 56 patients.
[b]Personal and family history classifications are not mutually exclusive.

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 1*B*).

Of the 71 non-LS mutations, 24 (34%; 95% CI, 23%–46%) were in high-penetrance genes (Figure 1*C*), including *BRCA1/2* (N = 15), *APC* (N = 5), biallelic *MUTYH* mutations (N = 3), and *STK11* (N = 1). There were 20 of 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 3 individuals with 2 germline mutations included 1 subject with pathogenic *MSH2* and *ATM* mutations, 1 subject with *MSH6* and *STK11* mutations, and 1 subject 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, 2 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 of 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 (3 *BRCA1* 5382insC and 2 *BRCA2* 6174delT), although only 1 of the 15 *BRCA1/2* probands was identified on the test request form as being of Ashkenazi descent. Nine of 15 (60%) *BRCA1/2* probands had a history of CRC, including 6 of 7 (86%) male *BRCA1/2* carriers. Four of 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%; $P = .0017$).

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

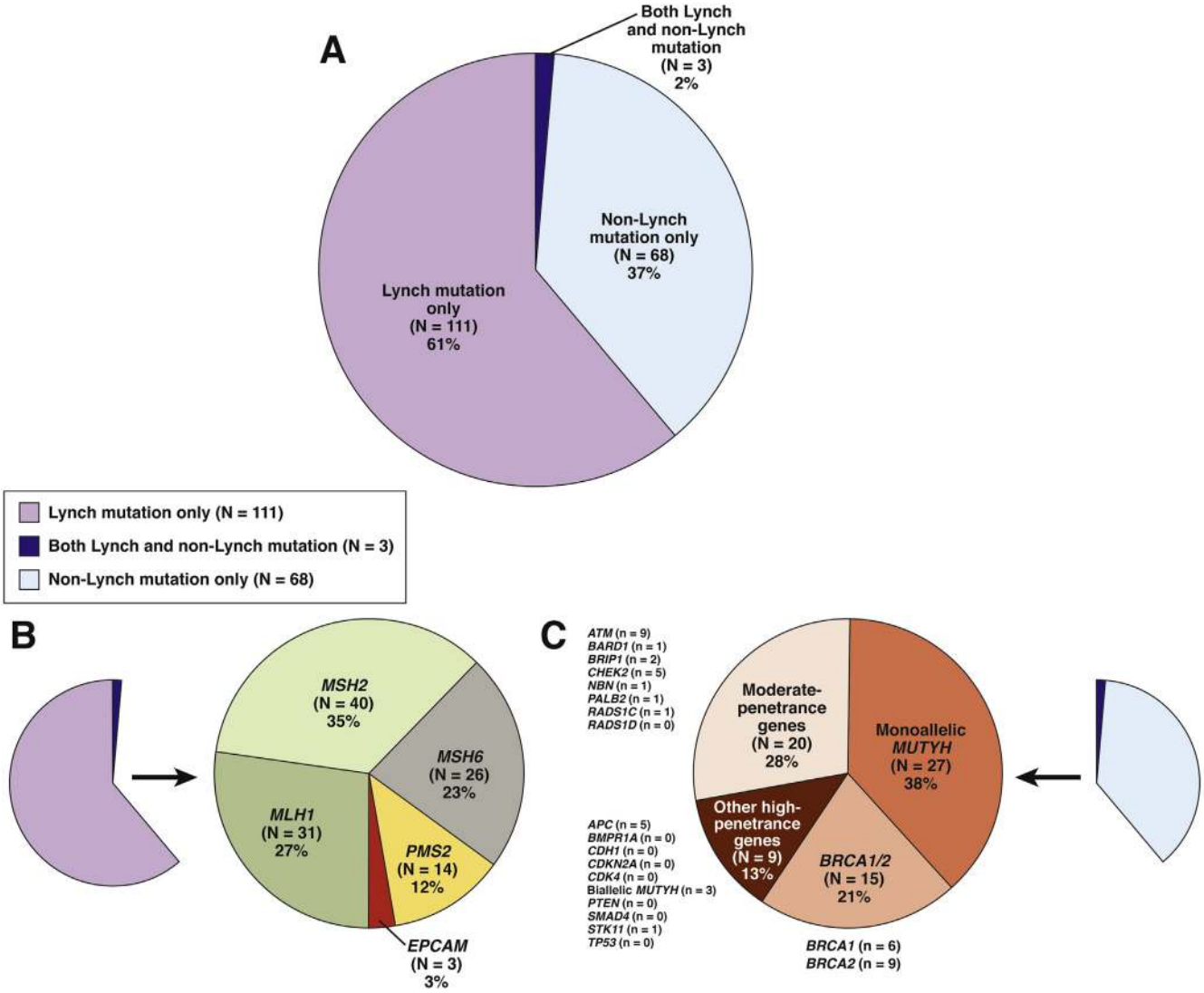

Figure 1. Pathogenic mutations identified with a multigene panel among 1260 individuals with suspected Lynch syndrome. (*A*) Proportion of mutation carriers with Lynch syndrome mutations (purple), non–Lynch syndrome mutations (blue), or both Lynch and non–Lynch syndrome mutations (dark purple). (*B*) 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).

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.

A total of 682 VUS were detected in 479 individuals (38% of the cohort; 95% CI, 35%–41%) (Supplementary 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 of 5% or higher (the cut-off value recommended by NCCN guidelines for consideration of LS evaluation), regardless of whether they carried a LS or a non-LS mutation (Table 4).[9] A total of 52% of LS carriers had a PREMM$_{1,2,6}$ score of 15% or higher, vs 26% of non-LS probands (P = .001). There was no significant difference between the proportion of LS carriers who fulfilled NCCN criteria for LS testing compared with *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 of 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

Table 3. Clinical Characteristics of Mutation Carriers Identified by Multigene Panel Testing

		Median age[b] at first cancer/ polyp, *y*	Met NCCN Lynch criteria	Personal history[a]							Family history[a]					
	Female N (%)	N (%)	N (%)	CRC, any age N (%)	CRC, age <50 y 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)

[a]Personal and family history classifications are not mutually exclusive.
[b]Age data were missing for 4 mutation carriers.

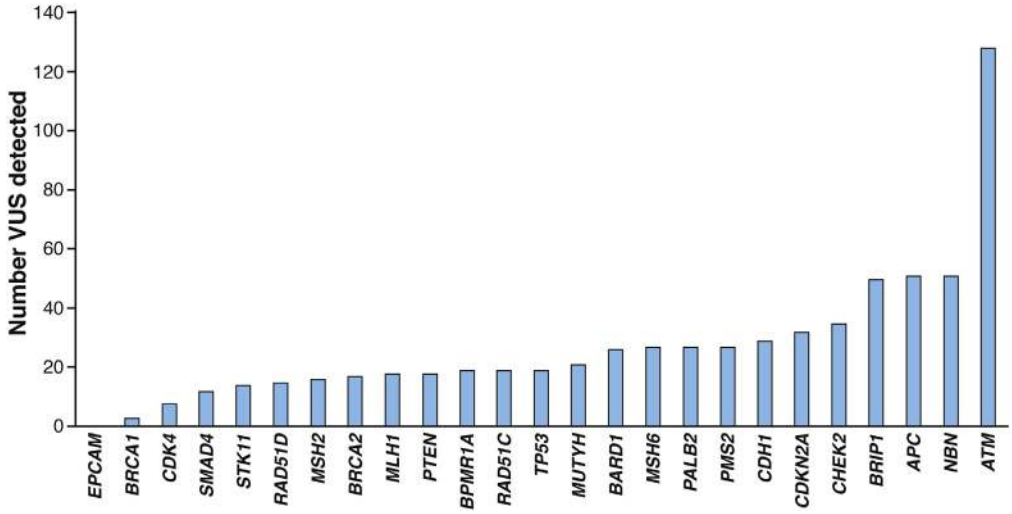

Figure 2. Number of VUS, per gene, detected with a multigene panel in 1260 individuals undergoing Lynch syndrome testing.

traditional, phenotype-driven genetic testing. The benefits of such comprehensive testing strategies have been debated and are only beginning to be evaluated scientifically.[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, in which the vast majority of both LS and non-LS mutation carriers fulfilled NCCN criteria for LS and had a $PREMM_{1,2,6}$ score of 5% or higher, shows 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 a prior study that specifically examined panel testing in patients with suspected hereditary gastrointestinal cancer, actionable mutations were detected in 42 of 586 (7.2%) patients, 23 of which were LS mutations.[36] All patients in this study, however, were selected specifically 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 more than 1 high-penetrance non-LS gene mutation for every 5 LS mutations identified, showing 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 was 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] Because 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 were excluded from the analysis, the 10 nonfounder *BRCA1/2* mutations identified in this study were substantially higher (0.8%; 10 of 1260) than the expected prevalence in the general population.

Prior studies have shown no increased CRC risk in *BRCA1/2* probands, and traditional thinking thus has 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 as suggested by our finding that 86% of the male *BRCA1/2* probands in this 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. Before 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 (eg, 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 often are 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 (eg, Lynch syndrome is defined by the presence of a germline

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[a]	$PREMM_{1,2,6}$ score ≥5%, N (%)	*P* value[a]	$PREMM_{1,2,6}$ score ≥15%, N (%)	*P* value[a]	Met NCCN Lynch criteria, N (%)	*P* value[a]
Lynch mutation carriers (N = 111)[b]	28.6 (23.7–33.5)	-	96 (86)	-	58 (52)	-	105 (95)	-
All non–Lynch mutation carriers (N = 68)[b]	13.4 (9.9–16.8)	.00002	51 (75)	.07	18 (26)	.001	58 (85)	.056
BRCA1/2 mutation carriers (N = 15)	12.4 (6.9–17.8)	.02	12 (80)	.45	3 (20)	.03	14 (93)	1.00
Other high-penetrance gene mutation carriers (N = 8)[b]	27.8 (10.1–45.5)	.93	6 (75)	.32	5 (63)	.72	8 (100)	1.00
Moderate-penetrance gene mutation carriers (N = 19)[b]	11.7 (5.4–18.0)	.007	14 (74)	.17	4 (21)	.01	17 (89)	.33
Monoallelic *MUTYH* mutation carriers (N = 26)[b]	10.8 (7.0–14.6)	.001	19 (73)	.13	6 (23)	.009	19 (73)	.0032

[a]*P* values for comparison with Lynch carriers.
[b]Excluding 3 patients with both a Lynch mutation and a non-Lynch mutation: 1 patient with both *MSH2* and *ATM* mutations, 1 patient with both *MSH6* and *STK11* mutations, and 1 patient with both *MSH2* and a monoallelic *MUTYH* mutation.

MMR mutation), and cancer surveillance recommendations usually are 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, although 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.

To fully assess the potential benefits and downsides of multigene panel testing compared with 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 also may 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 nonfinancial) that are likely to arise from the increased identification of VUS 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 or more 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 patients' 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 also have 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] Although the clinical utility of detecting monoallelic *MUTYH* carrier status for the proband themselves thus is 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 was 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 clinical laboratory improvement amendments–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 had limitations. Data regarding patients' personal/family histories of cancer were obtained via clinician report on a test request form, and therefore we were unable to confirm its accuracy or completeness. Although this was a potential limitation, the same approach was used to develop the $PREMM_{1,2,6}$ prediction model for LS risk assessment, and $PREMM_{1,2,6}$ subsequently has been validated in clinic- and population-based cohorts in which clinical data were verified extensively.[12] Furthermore, all patients in this cohort were ascertained from a large commercial laboratory that 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 patients from smaller practices, we were unable to account for the possibility that the performance of multigene panel testing may vary across different health care settings.

The specific frequencies of mutation carriers detected by panel testing also are 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 (ie, MMR genes, *BRCA1/2*, *APC*, *MUTYH*, *STK11*, *PTEN*, *CDH1*, and *TP53*), and thus the key findings of our study likely are generalizable to testing performed with other multigene panels.[47]

Another limitation of our study was 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 immunohistochemical (IHC) or microsatellite instability (MSI) testing as an initial screen for LS. Roughly 20% of the MSI-high (MSI-H)/MMR-deficient CRCs identified with such testing will be caused by 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 likely are sporadic and thus do not need LS germline testing. Without such data, we were 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 performed germline LS testing only on individuals with MSI-H/MMR-deficient CRC, and thus the mutation rate among patients with normal or absent tumor testing results has not been 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 in whom targeted germline LS testing would have been indicated, rather than panel testing, although this still would 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 show that a substantial fraction of patients with Lynch-like clinical histories actually will have other inherited cancer syndromes. Thus, the practice of using tumor testing to distinguish between patients with familial and sporadic cancers ultimately will miss some individuals with actionable mutations in non-Lynch cancer susceptibility genes.

Despite these limitations, our findings provide novel insight about the evaluation of patients with suspected LS in the era of multigene panel testing. Because 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 ultimately may 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, undoubtedly will 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 (eg, *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

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.05.006.

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Conflicts of interest
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Supplementary Materials and Methods

NCCN Guideline Classification

Patients were considered to fulfill NCCN guidelines for LS testing[1] if they had a personal history of any of the following: CRC or EC at an age younger than 50 years; 2 or more LS-associated cancers, regardless of age; CRC at any age plus a first-degree relative with a LS-associated cancer at an age younger than 50 years; or CRC at any age plus 2 or more first-/second-degree relatives with a LS-associated cancer at any age.

Regardless of their personal history, patients also were considered to fulfill NCCN guidelines for LS testing[1] if they had a family history of any of the following: a first-/second-degree relative with CRC at an age younger than 50 years; a first-/second-degree relative with EC at an age younger than 50 years; a first-/second-degree relative with 2 or more LS-associated cancers at any age; a first-/second-degree relative with CRC at any age plus another first-/second-/third-degree relative with any LS-associated cancer at an age younger than 50 years; a first-degree relative with CRC at any age plus 2 or more first-/second-/third-degree relative with any LS-associated cancer at any age. Unless otherwise specified on the test request form, all family history data were assumed to be from the same side of the patient's family.

Patients were considered to fulfill NCCN guidelines for hereditary breast/ovarian cancer testing for *BRCA1* and *BRCA2* mutations[2] if they had a personal history of any of the following: breast cancer at age 45 years or younger; male breast cancer at any age; ovarian cancer (OC) at any age; breast cancer at any age plus 1 or more first-/second-/third-degree relatives with breast cancer at age 50 years or younger; breast cancer at any age plus 1 or more first-/second-/third-degree relatives with OC or male breast cancer; breast cancer at any age plus 2 or more first-/second-/third-degree relatives with breast cancer at any age; breast cancer at any age plus 2 or more first-/second-/third-degree relatives with pancreatic cancer at any age; pancreatic cancer at any age plus 2 or more first-/second-/third-degree relatives with breast cancer, OC, or pancreatic cancer.

Regardless of their personal history, patients also were considered to fulfill NCCN guidelines for HBOC testing for *BRCA1* and *BRCA2* mutations if they had a family history of any of the following: 1 or more first-/second-degree relatives with OC at any age; 1 or more first-/second-degree relatives with breast cancer at age 45 years or younger; 1 or more first-/second-degree relatives with male breast cancer at any age; 1 or more first-/second-degree relatives with breast cancer plus 1 or more first-/second-/third-degree relatives with breast cancer, at least one of which was at age 50 years or younger; 1 or more first-/second-degree relatives with BC at any age plus 2 or more first-/second-/third-degree relatives with breast cancer at any age; 1 or more first-/second-degree relatives with breast cancer plus 1 or more first-/second-/third-degree relatives with male breast cancer; 1 or more first-/second-degree relatives with breast cancer plus 1 or more first-/second-/third-degree relatives with OC; 1 or more first-/second-degree relatives with pancreatic cancer plus 2 or more first-/second-/third-degree relatives with breast cancer, OC, or pancreatic cancer; 1 or more first-/second-degree relatives with pancreatic cancer plus 1 or more first-/second-/third-degree relatives with a history of 2 or more of the following cancers: breast cancer, OC, or pancreatic cancer. Unless otherwise specified on the test request form, all family history data were assumed to be from the same side of the patient's family.

Design of Custom Primer Library for NGS Target Enrichment

A custom primer library was designed that included regions of interest across 25 genes (Table 1) using the RainDance microdroplet PCR system (RainDance Technologies, Inc). The library design process began with identifying regions of interest, usually coding exons, for a panel of gene targets. This was performed by evaluating results from the Consensus CDS database (http://www.ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi) and comparing them with published literature. In the event of multiple reported transcripts, a review of the available literature was performed to determine the transcript(s) most relevant for patient testing. For coding exons, the sequencing regions were flanked by 20 bases of upstream and 10 bases of downstream intronic sequences to allow for evaluation of variants that occur in conserved, proximal splicing elements. Automated primer design of the specified regions was performed by the supplier (RainDance Technologies, Inc). This design process included the comparison of putative priming sites to public variant databases and the current genome build (http://www.ncbi.nlm.nih.gov/projects/genome/assembly/grc/human/) to avoid nonspecific priming and common SNPs at primer binding sites. Primer sequences also were checked for predicted primer–primer interactions. Primer designs that passed these filters were arranged into multiplexes of 5 amplicons to minimize DNA input requirements and the resulting reagents were dropletized into a custom library (RainDance Technologies, Inc). The RainDance library design involved an iterative process with multiple rounds of testing; the version of the library used in this study contained a combination of multiplexed and single-plex PCR reactions.

Sample Preparation and Next-Generation Sequencing

Genomic DNA was extracted from blood (QIAsymphony; Qiagen, Venlo, The Netherlands) and was fragmented (SonicMan; Brooks Life Science Systems, Spokane, WA) to approximately 3 kb to facilitate NGS sample preparation. Fragmented DNA was combined with PCR master mix containing the necessary buffers, polymerase, and nucleotides. The reaction mix containing DNA was dropletized and merged with droplets containing a custom amplicon primer library using a high-throughput microfluidic emulsion PCR system (RainDance Thunderstorm; RainDance Technologies, Inc). This process generated roughly 40,000 droplets

corresponding to PCR microreactions per patient sample. The resulting emulsion was amplified for 55 cycles on a Mastercycler Pro thermalcycler (Eppendorf, Hamburg, Germany). The emulsion was broken up according to RainDance protocols and the aqueous PCR products were purified using AMPure XP (Beckman Coulter, Brea, CA). A secondary PCR was performed to attach a 6 nucleotide identifier, specific for each sample within a batch, and recognition sites for NGS. The products were purified, pooled, and then sequenced on an Illumina HiSeq 2500 (Illumina, Inc) to generate paired-end, 2 × 150 bp reads according to the manufacturer's instructions.

To sequence portions of the *PMS2* and *CHEK2* genes with highly homologous pseudogenes, target enrichment was modified to include long-range PCR. The primary PCR was performed using LA Taq Hot Start (Takara Bio, Inc, Otsu, Japan) on 50 ng of genomic DNA to generate gene-specific long-range amplicons. The gene-specific long-range amplicon products were diluted 1:10,000 and a second round of PCR was performed to attach an index sequence specific for each sample within a batch and recognition sites for NGS. Equal volumes of each secondary amplicon product for all long-range amplicons were combined per sample. Equal amounts of 96 samples were combined and diluted to 2 nmol/L for sequencing on the Illumina MiSeq (Illumina, Inc) for 2 × 150 bp paired-end sequencing reads.

NGS Data Analysis

DNA sequence reads were assessed using Illumina Sequence Control Software with real-time analysis (Illumina, Inc). Sequence reads were trimmed at the point where quality scores decreased to less than Q30 using an optimized Burrows–Wheeler Aligner trimming approach[3] and then compared with a list of expected amplicon sequences. For *CHEK2* and *PMS2*, JAligner (open source: http://jaligner.sourceforge.net/) was used to determine which DNA target the sequencing read correctly matched; sequencing reads that matched the pseudogene better than the gene target were discarded. Sequence variants were identified by aligning reads using JAligner and comparing with the reference (wild-type) sequence. The average depth of coverage for samples in this study was more than 1000 times.

Large rearrangement detection was performed by relative copy number analysis of the NGS data in this study. The number of reads that mapped back to each exon was normalized using the total number of mapped back reads across all genes for that sample. For each run, or group of similar runs, a median normalized and the read count value was determined for each exon. Samples then were evaluated to see if their normalized read count average, across a given exon or partial exon, was $\geq$1.25 times or $\leq$0.75 times the median value. If a relative copy number value was $\geq$1.25 times the median value, the sample was determined to have a heterozygous duplication. If a value was $\leq$0.75 times the median value, the sample was determined to have a heterozygous deletion.

The median noise of centered normalized read counts for all exons with a normal copy number of 2 was determined. The CV (noise in a sample) was calculated as follows: median (absolute value (1-S) × 2) = noise in a sample (CV); where S (centered normalized read counts for a given exon and sample) = N/(median N across all samples); N (normalized read count for a given exon and sample) = c/C; c = read count for an exon; C = sum of all c for a sample.

PMS2 exons 1–5, 11–15, and *CHEK2* exons 10–14, were excluded from this calculation because they do not hold to the same copy number assumption. If the calculated CV value was less than 0.08 then the sample was included for NGS data analysis of large rearrangements. If the CV value was 0.08 or greater then the sample was rejected for NGS large rearrangement analysis. By these criteria, 90% of the samples in this study were eligible for large rearrangement analysis. Positive and negative calls made by the algorithm were reviewed by human analysts.

Pathogenicity Classification

All sequence variations and large rearrangements detected by the 25-gene panel were classified for pathogenicity into the following categories, as previously described: deleterious mutation, suspected deleterious mutation, variants of uncertain clinical significance (VUS), favor polymorphism, and polymorphism.[4,5] Based on recommendations from the American College of Medical Genetics,[5] deleterious mutations included nonsense and frameshift mutations predicted to result in protein truncation, as well as specific missense and intronic alterations that have been recognized previously as deleterious based on supporting linkage, functional, biochemical, and/or statistical evidence. Suspected deleterious mutations included alterations for which available evidence indicated a high likelihood, but not confirmation, of pathogenicity. Individuals with deleterious or suspected deleterious genomic alterations were defined collectively as having pathogenic mutations (Supplementary Table 1).[5–43] Germline alterations were deemed polymorphisms or suspected polymorphisms if available evidence indicated a low likelihood that such alterations altered normal gene expression and/or function. Alterations were classified as VUS if data were insufficient to support either a deleterious or benign interpretation (Supplementary Table 2 and Figure 2).[5]

Validation of the Multigene Panel

The performance characteristics of the 25-gene panel used in this study were evaluated in a separate validation study.[44] In this validation study, the sequencing component of the 25-gene panel was validated by comparing the results of NGS with Sanger sequencing on 100 anonymized DNA samples. Samples were sequenced for the coding regions and proximal splice sites of all genes except for *EPCAM*, which is evaluated only for large rearrangements involving the terminal exons. A total of 3923 variants were identified including 3884 single-nucleotide substitutions and 39 small insertions or deletions. These results showed 100% concordance between NGS and Sanger sequencing. The

validation study provides an estimated analytic sensitivity of greater than 99.92% (lower limit of the 95% CI) and an estimated analytic specificity of greater than 99.99% (lower limit of the 95% CI) for the clinical assay (Minitab version 15 [Minitab, Inc, State College, Pennsylvania]; 1 proportion test, exact method). Reproducibility, both within and between batches, was confirmed by running 4 samples in triplicate across 3 separate batches and verifying identical results.

Large rearrangement analysis of the 25-gene panel also was validated in a subsequent study.[44] All genes except for *PMS2* and *CHEK2* were validated for large rearrangement review using both microarray comparative genomic hybridization and NGS for large rearrangement dosage analysis. Microarray comparative genomic hybridization was validated by correctly identifying all 51 large rearrangement–positive samples among 212 anonymized samples. NGS dosage analysis requires higher DNA input volumes and therefore was validated on a subset of 49 large rearrangement–positive samples among 110 anonymized samples. One large rearrangement–positive sample failed during laboratory processing but NGS dosage analysis correctly identified all 48 large rearrangement–positive samples that were reviewed as part of the validation and by both large rearrangement detection assays. Large rearrangement review for *PMS2* and *CHEK2* used multiplex ligation-dependent probe amplification and correctly identified all 5 large rearrangement–positive samples among 110 anonymized samples in the validation set.

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Author names in bold designate shared co-first authors.

Supplementary Table 1. Germline Details of 182 Individuals Found to Carry Pathogenic Mutations With a 25-Gene Hereditary Cancer Panel

Patient ID	Gene mutated (Lynch)	HGVS mutation name (Lynch)	Gene mutated (non-Lynch)	HGVS mutation name (non-Lynch)
1095216833	*MLH1*	c.1279C>T (p.Gln427*)		
1095079933	*MLH1*	c.1435_1453del (p.Val479Ilefs*6)		
1086153088	*MLH1*	c.1517T>C (p.Val506Ala)		
0159657500	*MLH1*	c.1746del (p.Phe583Leufs*8)		
1093189976	*MLH1*	c.1852_1854del (p.Lys618del)		
1097149016	*MLH1*	c.1890dupT (p.Asp631*)		
0159893397	*MLH1*	c.1975C>T (p.Arg659*)		
1093190211	*MLH1*	c.1989+3dupC		
1092248139	*MLH1*	c.199G>A (p.Gly67Arg)		
1092247994	*MLH1*	c.208-3C>G		
1093189969	*MLH1*	c.298C>T (p.Arg100*)		
1093189929	*MLH1*	c.350C>T (p.Thr117Met)		
1097162685	*MLH1*	c.380G>T (p.Arg127Ile)		
1092248112	*MLH1*	c.544A>G (p.Arg182Gly)		
1095079935	*MLH1*	c.546-2A>G		
1086153104	*MLH1*	c.589-2A>G		
1095216822	*MLH1*	c.589-2A>G		
1095079895	*MLH1*	c.676C>T (p.Arg226*)		
1086153352	*MLH1*	c.677G>A (p.Arg226Gln)		
1092248097	*MLH1*	c.677G>A (p.Arg226Gln)		
1092248246	*MLH1*	c.677G>A (p.Arg226Gln)		
1086153414	*MLH1*	c.755C>G (p.Ser252*)		
1093189911	*MLH1*	c.790+2T>C		
1097149010	*MLH1*	c.883A>G (p.Ser295Gly)		
1095188508	*MLH1*	c.884+4A>G		
1092248249	*MLH1*	c.971dupA (p.Arg325Alafs*37)		
0159657452	*MLH1*	del exon 13		
1095112480	*MLH1*	del exon 5		
1095112487	*MLH1*	del exons 1-13		
1095188520	*MLH1*	del exons 1-13		
1092248006	*MLH1*	dup exons 6-12		
1086153113	*MSH2*	c.1076+1G>A		
1097162663	*MSH2*	c.1076+1G>A		
1086153044	*MSH2*	c.124_127dupTTCT (p.Tyr43Phefs*40)		
1095188535	*MSH2*	c.1444A>T (p.Arg482*)		
1092248225	*MSH2*	c.1511-1G>A		
1097149017	*MSH2*	c.1552_1553del (p.Gln518Valfs*10)		
0159657472	*MSH2*	c.1700_1704del (p.Lys567Argfs*3)		
0159657450	*MSH2*	c.1760-3C>G		
1095112429	*MSH2*	c.1906G>C (p.Ala636Pro)		
0159891914	*MSH2*	c.1968C>A (p.Tyr656*)		
1097162697	*MSH2*	c.2038C>T (p.Arg680*)		
0159893404	*MSH2*	c.2047G>A (p.Gly683Arg)		
1097149031	*MSH2*	c.2047G>A (p.Gly683Arg)		
1095216850	*MSH2*	c.2210+1G>A		
0159657471	*MSH2*	c.2222_2223del (p.Lys741Argfs*8)		
1086153380	*MSH2*	c.2291G>A (p.Trp764*)		
1093190203	*MSH2*	c.2634+1G>A		
1092248052	*MSH2*	c.367-1G>A		
1092248028	*MSH2*	c.587del (p.Pro196Glnfs*18)		
1086153076	*MSH2*	c.842C>G (p.Ser281*)		
1097162637	*MSH2*	c.914_923del (p.Ala305Glufs*23)		
1097162639	*MSH2*	c.942+2T>A		
1092248051	*MSH2*	c.942+3A>T		
1093189908	*MSH2*	c.942+3A>T		
1093189960	*MSH2*	c.942+3A>T		
1095079883	*MSH2*	c.942+3A>T		
1093190258	*MSH2*	Del exon 3		
1086153348	*MSH2*	Del exon 8		

Supplementary Table 1. Continued

Patient ID	Gene mutated (Lynch)	HGVS mutation name (Lynch)	Gene mutated (non-Lynch)	HGVS mutation name (non-Lynch)
0159657410	*MSH2*	Del exons 1–6		
0159893399	*MSH2*	Del exons 1–6		
1092248055	*MSH2*	Del exons 1–6		
1092248221	*MSH2*	Del exons 1–6		
1092248241	*MSH2*	Del exons 1–6		
1095079914	*MSH2*	Del exons 1–6		
1095216857	*MSH2*	Del exons 1–6		
1097149087	*MSH2*	Del exons 1–6		
1086153066	*MSH2*	Del exons 8–15		
1095188549	*MSH2*	Dup exon 6		
1092248133	*MSH6*	c.10C>T (p.Gln4*)		
1095188551	*MSH6*	c.10C>T (p.Gln4*)		
1086153366	*MSH6*	c.1444C>T (p.Arg482*)		
0159893462	*MSH6*	c.1571dupA (p.Tyr524*)		
1097149067	*MSH6*	c.1634_1635del (p.Lys545Argfs*17)		
0159893401	*MSH6*	c.2057G>A (p.Gly686Asp)		
1097162715	*MSH6*	c.220G>T (p.Gly74*)		
1095079885	*MSH6*	c.2906_2907del (p.Tyr969Leufs*5)		
1093189924	*MSH6*	c.3261del (p.Phe1088Serfs*2)		
0159893435	*MSH6*	c.3439-2A>G		
1095112495	*MSH6*	c.3439-2A>G		
1092247995	*MSH6*	c.3516_3517del (p.Arg1172Serfs*4)		
1095112415	*MSH6*	c.3647-1G>A		
1093190249	*MSH6*	c.3699_3702del (p.Lys1233Asnfs*6)		
1097162643	*MSH6*	c.3802-14_3809del		
1095112405	*MSH6*	c.3934_3937dupGTTA (p.Ile1313Serfs*7)		
1086153080	*MSH6*	c.3939_3957dup (p.Ala1320Serfs*5)		
1092248186	*MSH6*	c.3939_3957dup (p.Ala1320Serfs*5)		
1095112465	*MSH6*	c.3939_3957dup (p.Ala1320Serfs*5)		
1095216873	*MSH6*	c.3939_3957dup (p.Ala1320Serfs*5)		
1086153089	*MSH6*	c.3959_3962del (p.Ala1320Glufs*6)		
1092248103	*MSH6*	c.3959_3962del (p.Ala1320Glufs*6)		
1092248204	*MSH6*	c.4001+1G>A		
0159657467	*MSH6*	c.694C>T (p.Gln232*)		
1095112417	*MSH6*	c.694C>T (p.Gln232*)		
1095079855	*PMS2*	c.137G>T (p.Ser46Ile)		
1092248016	*PMS2*	c.1831dupA (p.Ile611Asnfs*2)		
1092248177	*PMS2*	c.1840A>T (p.Lys614*)		
1092248205	*PMS2*	c.1840A>T (p.Lys614*)		
1092248226	*PMS2*	c.1882C>T (p.Arg628*)		
1092248166	*PMS2*	c.1A>T (p.Met1?)		
1095112410	*PMS2*	c.214G>T (p.Gly72*)		
1092248021	*PMS2*	c.251-2A>T		
1097149076	*PMS2*	c.736_741delins11 (p.Pro246Cysfs*3)		
1095112490	*PMS2*	c.746_753del (p.Asp249Valfs*2)		
0159657458	*PMS2*	c.765C>A (p.Tyr255*)		
1093189907	*PMS2*	c.861_864del (p.Arg287Serfs*19)		
1092248136	*PMS2*	Del exons 8–11		
1093189949	*PMS2*	Del exons 9 and 10		
1095112488	*EPCAM*	Del exons 1–9		
1093189941	*EPCAM*	Del exons 2–9		
0159893426	*EPCAM*	Del exons 6–9		
0159893400			*APC*	c.3927_3931del (p.Glu1309Aspfs*4)
1092248247			*APC*	c.268A>T (p.Lys90*)
1093190205			*APC*	c.531+5G>A
1095188522			*APC*	c.70C>T (p.Arg24*)
1097162691			*APC*	c.667C>T (p.Gln223*)
0159657480			*MUTYH* (biallelic)	c.325C>T (p.Arg109Trp) AND c.1187G>A (p.Gly396Asp)

Supplementary Table 1. Continued

Patient ID	Gene mutated (Lynch)	HGVS mutation name (Lynch)	Gene mutated (non-Lynch)	HGVS mutation name (non-Lynch)
1095112444			*MUTYH* (biallelic)	c.1187G>A (p.Gly396Asp) homozygous
1095216806			*MUTYH* (biallelic)	c.1187G>A (p.Gly396Asp) homozygous
0159657488			*BRCA1*	del exon 21
0159893405			*BRCA1*	c.5266dupC (p.Gln1756Profs*74)
1092248015			*BRCA1*	c.5266dupC (p.Gln1756Profs*74)
1092248047			*BRCA1*	c.5066T>G (p.Met1689Arg)
1093189935			*BRCA1*	c.5266dupC (p.Gln1756Profs*74)
1093189938			*BRCA1*	c.5096G>A (p.Arg1699Gln)
0159893382			*BRCA2*	c.4647_4650del (p.Lys1549Asnfs*18)
1092248111			*BRCA2*	c.9382C>T (p.Arg3128*)
1092248179			*BRCA2*	c.5946del (p.Ser1982Argfs*22)
1093189918			*BRCA2*	c.4631dupA (p.Asn1544Lysfs*4)
1093190286			*BRCA2*	c.7008-2A>G
1095079936			*BRCA2*	c.846_847del (p.Ile283Trpfs*11)
1095112479			*BRCA2*	c.1296_1297del (p.Asn433Glnfs*18)
1095216848			*BRCA2*	c.2971_2983del (p.Asn991Aspfs*3)
1097162706			*BRCA2*	c.5946del (p.Ser1982Argfs*22)
1086153128			*ATM*	c.8371_8374del (p.Tyr2791Glyfs*14)
1092248032			*ATM*	c.1066-3_1072del
1092248117			*ATM*	c.5414G>A (p.Trp1805*)
1093189919			*ATM*	c.1564_1565del (p.Glu522Ilefs*43)
1095188583			*ATM*	c.8395_8404del (p.Phe2799Lysfs*4)
1095214818			*ATM*	c.8824C>T (p.Gln2942*)
1095216856			*ATM*	c.7271T>G (p.Val2424Gly)
1097149023			*ATM*	c.6015dupC (p.Glu2007Argfs*11)
1086153125			*BARD1*	c.1690C>T (p.Gln564*)
1092248067			*BRIP1*	c.2392C>T (p.Arg798*)
1093190284			*BRIP1*	c.484C>T (p.Arg162*)
1092247990			*CHEK2*	c.444+1G>A
1092248022			*CHEK2*	c.1100del (p.Thr367Metfs*15)
1092248202			*CHEK2*	c.444+1G>A
1093189977			*CHEK2*	c.909-2A>G
1095112448			*CHEK2*	c.1100del (p.Thr367Metfs*15)
1092248056			*NBN*	c.657_661del (p.Lys219Asnfs*16)
1095079919			*PALB2*	c.758dupT (p.Ser254Ilefs*3)
1093190269			*RAD51C*	c.890_899del (p.Leu297Hisfs*2)
1093190213	*MSH2*	c.1576del (p.Thr526Profs*17)	*ATM*	del exons 61-62
0159657434	*MSH6*	c.3261dupC (p.Phe1088Leufs*5)	*STK11*	c.375-1C>T
1092248195	*MSH2*	del exons 1-6	*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
0159657427			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
0159657441			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
0159657473			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
0159893402			*MUTYH* (monoallelic)	c.536A>G (p.Tyr179Cys), c.494A>G (p.Tyr165Cys)
0159893438			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1092248023			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1092248192			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1092248228			*MUTYH* (monoallelic)	c.1147del (p.Ala385Profs*23)
1092248266			*MUTYH* (monoallelic)	c.536A>G (p.Tyr179Cys)
1093189965			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1093189978			*MUTYH* (monoallelic)	c.536A>G (p.Tyr179Cys)
1093190192			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1093190226			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095079872			*MUTYH* (monoallelic)	c.1227_1228dupGG (p.Glu410Glyfs*43)
1095079891			*MUTYH* (monoallelic)	c.933+3A>C
1095079913			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095112413			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)

Supplementary Table 1. Continued

Patient ID	Gene mutated (Lynch)	HGVS mutation name (Lynch)	Gene mutated (non-Lynch)	HGVS mutation name (non-Lynch)
1095112432			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095112475			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095112499			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095188584			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095216832			*MUTYH* (monoallelic)	c.1187G>A (p.Gly396Asp)
1095216898			*MUTYH* (monoallelic)	c.536A>G (p.Tyr179Cys)
1097149007			*MUTYH* (monoallelic)	c.536A>G (p.Tyr179Cys)
1097149020			*MUTYH* (monoallelic)	c.734G>A (p.Arg245His)
1097149054			*MUTYH* (monoallelic)	c.1227_1228dupGG (p.Glu410Glyfs*43)

Del, deletion; Dup, duplication; HGVS, Human Genome Variation Society.

Supplementary Table 2. Germline Variants of Uncertain Significance Detected by a Multigene Hereditary Cancer Panel in 1260 Individuals Undergoing Lynch Syndrome Testing

Patient ID	Gene	HGVS alteration name
0159657411	*BMPR1A*	c.1327C>T (p.Arg443Cys)
0159657412	*BRIP1*	c.3293C>A (p.Ala1098Asp)
0159657416	*PALB2*	c.2135C>T (p.Ala712Val)
0159657420	*BARD1*	c.1738G>A (p.Glu580Lys)
0159657423	*BARD1*	c.33G>T (p.Gln11His)
0159657425	*ATM*	c.7313C>T (p.Thr2438Ile)
0159657425	*ATM*	c.334G>A (p.Ala112Thr)
0159657425	*ATM*	c.320G>A (p.Cys107Tyr)
0159657425	*BARD1*	c.1738G>A (p.Glu580Lys)
0159657425	*CDH1*	c.833-16C>G
0159657425	*CDH1*	c.595A>T (p.Thr199Ser)
0159657425	*NBN*	c.37+5G>A
0159657425	*PTEN*	c.802-51_802-14del
0159657425	*RAD51D*	c.904-11T>A
0159657429	*ATM*	c.5675-10T>G
0159657429	*CHEK2*	c.1343T>G (p.Ile448Ser)
0159657429	*NBN*	c.1489A>G (p.Thr497Ala)
0159657430	*NBN*	c.1882G>A (p.Glu628Lys)
0159657432	*TP53*	c.139C>T (p.Pro47Ser)
0159657434	*NBN*	c.1690G>A (p.Glu564Lys)
0159657438	*ATM*	c.2289T>A (p.Phe763Leu)
0159657438	*MSH6*	c.972A>C (p.Lys324Asn)
0159657442	*MLH1*	c.479C>T (p.Ala160Val)
0159657448	*BARD1*	c.1242G>A (p.Met414Ile)
0159657450	*BMPR1A*	c.1327C>T (p.Arg443Cys)
0159657451	*MSH6*	c.3257C>T (p.Pro1086Leu)
0159657454	*RAD51C*	c.376G>A (p.Ala126Thr)
0159657455	*MLH1*	c.-42C>T
0159657456	*ATM*	c.4388T>G (p.Phe1463Cys)
0159657456	*ATM*	c.1810C>T (p.Pro604Ser)
0159657457	*MSH6*	c.743G>C (p.Arg248Pro)
0159657459	*SMAD4*	c.586A>G (p.Ser196Gly)
0159657460	*PMS2*	c.595C>T (p.Arg199Cys)
0159657460	*RAD51D*	c.973G>A (p.Gly325Ser)
0159657462	*ATM*	c.2096A>G (p.Glu699Gly)
0159657462	*NBN*	c.797C>T (p.Pro266Leu)
0159657462	*PTEN*	c.802-51_802-14del
0159657462	*RAD51D*	c.695G>A (p.Arg232Gln)
0159657467	*MSH6*	c.1180T>G (p.Ser394Ala)
0159657469	*NBN*	c.1489A>G (p.Thr497Ala)
0159657470	*PMS2*	c.1715C>T (p.Ala572Val)
0159657470	*RAD51C*	c.706-13C>G
0159657473	*ATM*	c.1229T>C (p.Val410Ala)
0159657474	*APC*	c.6639G>A (p.Met2213Ile)
0159657475	*MUTYH*	c.998-9C>T
0159657476	*ATM*	c.6067G>A (p.Gly2023Arg)
0159657483	*ATM*	c.8915A>G (p.Gln2972Arg)
0159657485	*RAD51C*	c.146-8A>G
0159657487	*CDH1*	c.2413G>A (p.Asp805Asn)
0159657487	*PMS2*	c.2350G>A (p.Asp784Asn)
0159657488	*MSH2*	c.1480T>C (p.Ser494Pro)
0159657491	*APC*	c.1825G>A (p.Val609Ile)
0159657494	*MLH1*	c.1942C>T (p.Pro648Ser)
0159657496	*BRIP1*	c.1198G>T (p.Asp400Tyr)
0159657497	*PALB2*	c.2606C>T (p.Ser869Phe)
0159657501	*CDKN2A (P16)*	c.430C>T (p.Arg144Cys)
0159657503	*PMS2*	c.1864A>G (p.Met622Val)
0159893377	*ATM*	c.5071A>C (p.Ser1691Arg)
0159893378	*ATM*	c.1229T>C (p.Val410Ala)
0159893378	*MSH6*	c.3854T>C (p.Phe1285Ser)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
0159893381	*ATM*	c.6995T>C (p.Leu2332Pro)
0159893381	*CDH1*	c.833-16C>G
0159893382	*ATM*	c.6095G>A (p.Arg2032Lys)
0159893384	*ATM*	c.3175G>A (p.Ala1059Thr)
0159893386	*APC*	c.6724A>G (p.Ser2242Gly)
0159893395	*BMPR1A*	c.1327C>T (p.Arg443Cys)
0159893397	*CDKN2A (P14ARF)*	Dup exons 1 and 2
0159893397	*CDKN2A (P16)*	Dup exons 1–3
0159893399	*MSH6*	c.3961A>G (p.Arg1321Gly)
0159893401	*TP53*	c.704A>G (p.Asn235Ser)
0159893402	*APC*	c.5528C>T (p.Pro1843Leu)
0159893403	*ATM*	c.1229T>C (p.Val410Ala)
0159893403	*ATM*	c.5612C>T (p.Thr1871Ile)
0159893403	*CHEK2*	c.470T>C (p.Ile157Thr)
0159893404	*PTEN*	c.802-51_802-14del
0159893404	*PTEN*	c.1061C>A (p.Pro354Gln)
0159893408	*MSH2*	c.174C>G (p.Phe58Leu)
0159893416	*CHEK2*	c.751A>T (p.Ile251Phe)
0159893416	*PALB2*	c.1696C>T (p.Arg566Cys)
0159893417	*MSH6*	c.1474_1476del (p.Met492del)
0159893421	*CDKN2A (P16)*	c.-33G>C
0159893424	*CDH1*	c.1603A>T (p.Ile535Phe)
0159893427	*BRIP1*	c.2220G>T (p.Gln740His)
0159893428	*APC*	c.4919G>A (p.Arg1640Gln)
0159893429	*BARD1*	c.1738G>A (p.Glu580Lys)
0159893429	*CDH1*	c.833-16C>G
0159893430	*BARD1*	c.2282G>A (p.Ser761Asn)
0159893430	*MSH2*	c.1847C>G (p.Pro616Arg)
0159893432	*NBN*	c.683T>G (p.Ile228Arg)
0159893433	*TP53*	c.665C>T (p.Pro222Leu)
0159893434	*BRCA1*	c.3535A>C (p.Lys1179Gln)
0159893436	*APC*	c.2993G>T (p.Gly998Val)
0159893438	*ATM*	c.7122A>C (p.Glu2374Asp)
0159893439	*ATM*	c.544G>C (p.Val182Leu)
0159893439	*NBN*	c.1882G>A (p.Glu628Lys)
0159893441	*APC*	c.4711_4713del (p.Asp1571del)
0159893444	*CDKN2A (P16)*	c.361C>G (p.Leu121Val)
0159893444	*PTEN*	c.802-51_802-14del
0159893445	*APC*	c.6363_6365dupTGC (p.Ala2122dup)
0159893445	*CDK4*	c.431A>G (p.Glu144Gly)
0159893445	*CDKN2A (P16)*	c.150+6T>C
0159893446	*CDK4*	c.460G>C (p.Val154Leu)
0159893450	*CDH1*	c.2494G>A (p.Val832Met)
0159893450	*TP53*	c.31G>C (p.Glu11Gln)
0159893451	*MSH6*	c.1402C>T (p.Arg468Cys)
0159893453	*TP53*	c.97-6C>T
0159893457	*BMPR1A*	c.1513G>A (p.Ala505Thr)
0159893458	*CDH1*	c.1888C>G (p.Leu630Val)
0159893458	*NBN*	c.1690G>A (p.Glu564Lys)
0159893464	*SMAD4*	c.1573A>G (p.Ile525Val)
0159893467	*ATM*	c.2608A>G (p.Asn870Asp)
1086153037	*MUTYH*	c.724G>T (p.Val242Leu)
1086153040	*BRCA2*	Dup exons 23 and 24
1086153055	*BARD1*	c.1738G>A (p.Glu580Lys)
1086153055	*PMS2*	c.847A>C (p.Ser283Arg)
1086153058	*RAD51C*	c.506T>C (p.Val169Ala)
1086153058	*SMAD4*	c.542C>G (p.Thr181Ser)
1086153059	*ATM*	c.6067G>A (p.Gly2023Arg)
1086153060	*BARD1*	c.1795G>A (p.Glu599Lys)
1086153062	*BMPR1A*	c.1066C>T (p.Pro356Ser)
1086153066	*ATM*	c.1229T>C (p.Val410Ala)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1086153066	*ATM*	c.4066A>G (p.Asn1356Asp)
1086153075	*APC*	c.6637A>G (p.Met2213Val)
1086153076	*MUTYH*	c.998-18G>A
1086153076	*PMS2*	c.1717A>T (p.Thr573Ser)
1086153078	*PMS2*	c.1723A>G (p.Asn575Asp)
1086153078	*PMS2*	c.2386G>A (p.Val796Ile)
1086153086	*BRCA2*	c.898G>A (p.Val300Ile)
1086153092	*CDKN2A (P16)*	c.434T>C (p.Ile145Thr)
1086153100	*CDKN2A (P14ARF)*	c.217A>C (p.Ser73Arg)
1086153101	*ATM*	c.5821G>C (p.Val1941Leu)
1086153106	*APC*	c.1481G>A (p.Ser494Asn)
1086153106	*MSH6*	c.3284G>A (p.Arg1095His)
1086153107	*BARD1*	c.26_40del (p.Asn9_Arg13del)
1086153108	*ATM*	c.8228C>T (p.Thr2743Met)
1086153108	*CHEK2*	c.470T>C (p.Ile157Thr)
1086153112	*MSH6*	c.1858G>A (p.Gly620Ser)
1086153112	*MUTYH*	c.1508G>A (p.Gly503Glu)
1086153113	*BARD1*	c.2008A>G (p.Lys670Glu)
1086153113	*BRCA1*	c.2522G>C (p.Arg841Pro)
1086153114	*CHEK2*	c.428A>G (p.His143Arg)
1086153116	*CHEK2*	c.538C>T (p.Arg180Cys)
1086153122	*ATM*	c.544G>C (p.Val182Leu)
1086153122	*PMS2*	c.123_131del (p.Leu42_Glu44del)
1086153130	*MUTYH*	c.1518+5G>A
1086153131	*SMAD4*	c.788-1G>C
1086153325	*CDKN2A (P16)*	c.-33G>C
1086153332	*MLH1*	c.52C>T (p.Arg18Cys)
1086153345	*STK11*	c.1193C>T (p.Ala398Val)
1086153348	*ATM*	c.8965C>G (p.Gln2989Glu)
1086153353	*APC*	c.3511C>T (p.Arg1171Cys)
1086153356	*RAD51C*	c.376G>A (p.Ala126Thr)
1086153363	*NBN*	c.797C>T (p.Pro266Leu)
1086153363	*TP53*	c.139C>T (p.Pro47Ser)
1086153365	*ATM*	c.290T>C (p.Ile97Thr)
1086153365	*BRIP1*	c.2236A>G (p.Ile746Val)
1086153365	*NBN*	c.797C>T (p.Pro266Leu)
1086153365	*RAD51C*	c.922G>T (p.Ala308Ser)
1086153365	*STK11*	c.894C>A (p.Phe298Leu)
1086153375	*CDKN2A (P14ARF)*	c.361G>A (p.Ala121Thr)
1086153375	*PALB2*	c.94C>G (p.Leu32Val)
1086153376	*MLH1*	c.144A>C (p.Gln48His)
1086153376	*TP53*	c.139C>T (p.Pro47Ser)
1086153377	*ATM*	c.6315G>C (p.Arg2105Ser)
1086153399	*ATM*	c.6995T>C (p.Leu2332Pro)
1086153399	*BARD1*	c.620A>G (p.Lys207Arg)
1086153399	*NBN*	c.505C>T (p.Arg169Cys)
1086153399	*SMAD4*	c.880A>G (p.Met294Val)
1086153399	*TP53*	c.97-6C>T
1086153401	*RAD51C*	c.376G>A (p.Ala126Thr)
1086153404	*BRCA2*	c.292T>G (p.Leu98Val)
1086153404	*RAD51C*	c.211A>T (p.Asn71Tyr)
1086153414	*ATM*	c.1229T>C (p.Val410Ala)
1086153418	*ATM*	c.5071A>C (p.Ser1691Arg)
1086153418	*MSH6*	c.3226C>G (p.Arg1076Gly)
1092247687	*CHEK2*	c.1343T>G (p.Ile448Ser)
1092247989	*MSH2*	c.2006G>A (p.Gly669Asp)
1092247992	*BARD1*	c.1569-13C>G
1092247997	*BARD1*	c.1347A>G (p.Gln449Gln)
1092247998	*ATM*	c.3925G>A (p.Ala1309Thr)
1092248000	*BMPR1A*	c.1327C>T (p.Arg443Cys)
1092248002	*ATM*	c.6919C>T (p.Leu2307Phe)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1092248002	*ATM*	c.1744T>C (p.Phe582Leu)
1092248002	*BMPR1A*	c.1243G>A (p.Glu415Lys)
1092248002	*BRCA2*	c.7444A>G (p.Thr2482Ala)
1092248004	*CDH1*	c.670C>T (p.Arg224Cys)
1092248004	*CDH1*	c.2440-6C>G
1092248004	*MSH2*	c.499G>C (p.Asp167His)
1092248005	*APC*	c.5635G>T (p.Ala1879Ser)
1092248006	*PTEN*	c.892C>G (p.Gln298Glu)
1092248007	*APC*	c.1606G>A (p.Glu536Lys)
1092248007	*NBN*	c.797C>T (p.Pro266Leu)
1092248007	*RAD51D*	c.983C>T (p.Thr328Ile)
1092248008	*BRIP1*	c.2372A>G (p.Asp791Gly)
1092248008	*CDK4*	c.779T>A (p.Val260Glu)
1092248010	*ATM*	c.544G>C (p.Val182Leu)
1092248012	*APC*	c.6679G>T (p.Gly2227Cys)
1092248012	*BRIP1*	c.890A>G (p.Lys297Arg)
1092248022	*CDH1*	c.2440-6C>G
1092248024	*RAD51D*	c.904-3C>T
1092248025	*RAD51C*	c.376G>A (p.Ala126Thr)
1092248031	*CDKN2A (P16)*	c.170C>T (p.Ala57Val)
1092248031	*MSH6*	c.3930G>C (p.Glu1310Asp)
1092248034	*NBN*	c.278C>T (p.Ser93Leu)
1092248043	*PALB2*	c.1846G>C (p.Asp616His)
1092248044	*PMS2*	c.1717A>T (p.Thr573Ser)
1092248044	*PMS2*	c.1714G>A (p.Ala572Thr)
1092248045	*CDH1*	c.1888C>G (p.Leu630Val)
1092248045	*PALB2*	c.3035C>T (p.Thr1012Ile)
1092248045	*PALB2*	c.1273G>A (p.Val425Met)
1092248045	*RAD51C*	c.635G>A (p.Arg212His)
1092248047	*MSH2*	c.1697_1709delinsTTCT (p.Asn566_Tyr570delinsIleLeu)
1092248048	*ATM*	c.7187C>G (p.Thr2396Ser)
1092248054	*BRCA2*	c.8222A>G (p.Lys2741Arg)
1092248060	*CHEK2*	c.470T>C (p.Ile157Thr)
1092248061	*ATM*	c.4709T>C (p.Val1570Ala)
1092248065	*RAD51C*	c.790G>A (p.Gly264Ser)
1092248068	*APC*	c.6679G>T (p.Gly2227Cys)
1092248068	*NBN*	c.321-17C>G
1092248069	*STK11*	c.1211C>T (p.Ser404Phe)
1092248070	*MSH6*	c.3299C>T (p.Thr1100Met)
1092248072	*PALB2*	c.2135C>T (p.Ala712Val)
1092248074	*ATM*	c.1810C>T (p.Pro604Ser)
1092248076	*CDKN2A (P16)*	c.-2G>A
1092248077	*BRIP1*	c.584T>C (p.Leu195Pro)
1092248080	*BRCA2*	c.6158C>A (p.Ser2053Tyr)
1092248082	*ATM*	c.8228C>T (p.Thr2743Met)
1092248082	*MUTYH*	c.700G>A (p.Val234Met)
1092248084	*ATM*	c.4375G>A (p.Gly1459Arg)
1092248085	*CDK4*	c.684-4A>T
1092248086	*APC*	c.4332A>T (p.Gln1444His)
1092248086	*CDH1*	c.88C>A (p.Pro30Thr)
1092248087	*RAD51C*	c.790G>A (p.Gly264Ser)
1092248088	*SMAD4*	c.554C>A (p.Pro185Gln)
1092248091	*CDK4*	c.155G>A (p.Ser52Asn)
1092248097	*STK11*	c.1211C>T (p.Ser404Phe)
1092248099	*ATM*	c.5071A>C (p.Ser1691Arg)
1092248101	*APC*	c.1391A>G (p.His464Arg)
1092248102	*BARD1*	c.2306C>T (p.Ser769Phe)
1092248102	*PMS2*	c.58C>T (p.Arg20Trp)
1092248103	*CDH1*	c.2440-6C>G
1092248115	*ATM*	c.5071A>C (p.Ser1691Arg)
1092248115	*NBN*	c.511A>G (p.Ile171Val)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1092248117	*APC*	c.7797A>C (p.Lys2599Asn)
1092248119	*ATM*	c.8147T>C (p.Val2716Ala)
1092248125	*ATM*	c.280A>G (p.Met94Val)
1092248127	*STK11*	c.970C>G (p.Pro324Ala)
1092248130	*CDH1*	c.2635G>A (p.Gly879Ser)
1092248130	*PMS2*	c.614A>C (p.Gln205Pro)
1092248131	*CHEK2*	c.1567C>T (p.Arg523Cys)
1092248134	*APC*	c.3299C>T (p.Ser1100Phe)
1092248135	*CHEK2*	c.410G>A (p.Arg137Gln)
1092248138	*NBN*	c.1882G>A (p.Glu628Lys)
1092248140	*MUTYH*	c.985G>A (p.Val329Met)
1092248146	*SMAD4*	c.-3C>G
1092248148	*NBN*	c.-2C>A
1092248149	*CHEK2*	c.14C>T (p.Ser5Leu)
1092248151	*ATM*	c.6860G>C (p.Gly2287Ala)
1092248159	*NBN*	c.1262T>C (p.Leu421Ser)
1092248162	*BRIP1*	c.2220G>T (p.Gln740His)
1092248164	*BRIP1*	c.584T>C (p.Leu195Pro)
1092248167	*CDKN2A (P16)*	c.-25C>T
1092248181	*ATM*	c.1810C>T (p.Pro604Ser)
1092248181	*BMPR1A*	c.1235T>C (p.Val412Ala)
1092248181	*CDKN2A (P16)*	c.-25C>T
1092248183	*NBN*	c.1882G>A (p.Glu628Lys)
1092248184	*BRIP1*	c.584T>C (p.Leu195Pro)
1092248184	*CHEK2*	c.593-20_593-18del
1092248185	*NBN*	c.1489A>G (p.Thr497Ala)
1092248185	*PALB2*	c.100C>T (p.Arg34Cys)
1092248185	*PTEN*	c.802-51_802-14del
1092248192	*MSH2*	c.167A>T (p.Glu56Val)
1092248193	*ATM*	c.7475T>G (p.Leu2492Arg)
1092248197	*ATM*	c.1229T>C (p.Val410Ala)
1092248197	*BRIP1*	c.584T>C (p.Leu195Pro)
1092248198	*MLH1*	c.1709A>G (p.Asn570Ser)
1092248205	*NBN*	c.1035C>T (p.Gly345Gly)
1092248206	*BRCA2*	c.8990A>G (p.Tyr2997Cys)
1092248206	*NBN*	c.797C>T (p.Pro266Leu)
1092248206	*PMS2*	c.497T>C (p.Leu166Pro)
1092248217	*STK11*	c.721G>A (p.Ala241Thr)
1092248223	*ATM*	c.5071A>C (p.Ser1691Arg)
1092248225	*APC*	c.5690A>C (p.His1897Pro)
1092248225	*BRIP1*	c.1433A>G (p.His478Arg)
1092248227	*BRIP1*	c.2236A>G (p.Ile746Val)
1092248234	*BMPR1A*	c.749T>C (p.Met250Thr)
1092248238	*NBN*	c.613A>G (p.Ile205Val)
1092248239	*PALB2*	c.2816T>G (p.Leu939Trp)
1092248246	*NBN*	c.1354A>C (p.Thr452Pro)
1092248246	*PTEN*	c.802-51_802-14del
1092248251	*SMAD4*	c.455-6A>G
1092248252	*ATM*	c.496+4T>C
1092248255	*CHEK2*	c.853A>T (p.Ile285Phe)
1092248263	*ATM*	c.3925G>A (p.Ala1309Thr)
1092248265	*BRIP1*	c.1444A>G (p.Ile482Val)
1092248267	*ATM*	c.1810C>T (p.Pro604Ser)
1092248267	*ATM*	c.2494C>T (p.Arg832Cys)
1092248268	*MLH1*	c.1565G>A (p.Arg522Gln)
1092248269	*BRCA2*	c.5537T>C (p.Ile1846Thr)
1092248270	*ATM*	c.544G>C (p.Val182Leu)
1092248270	*NBN*	c.797C>T (p.Pro266Leu)
1092248272	*RAD51D*	c.29C>T (p.Pro10Leu)
1093187329	*PALB2*	c.94C>G (p.Leu32Val)
1093187353	*CDKN2A (P16)*	c.430C>T (p.Arg144Cys)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1093187353	*PMS2*	c.1211C>G (p.Pro404Arg)
1093189904	*RAD51D*	c.695G>A (p.Arg232Gln)
1093189905	*CHEK2*	c.538C>T (p.Arg180Cys)
1093189909	*ATM*	c.6067G>A (p.Gly2023Arg)
1093189909	*CDH1*	c.2590G>A (p.Glu864Lys)
1093189910	*CDH1*	c.1223C>T (p.Ala408Val)
1093189911	*MSH6*	Dup exon 8
1093189912	*MSH6*	Dup exon 8
1093189917	*CHEK2*	c.1091T>C (p.Ile364Thr)
1093189917	*MLH1*	c.482C>T (p.Thr161Met)
1093189917	*MSH6*	c.3802-19_3802-16dupAATA
1093189922	*ATM*	c.544G>C (p.Val182Leu)
1093189922	*MUTYH*	c.1301C>T (p.Thr434Met)
1093189923	*BRIP1*	c.338C>T (p.Thr113Ile)
1093189923	*CDKN2A (P16)*	c.-33G>C
1093189924	*CDKN2A (P16)*	c.430C>T (p.Arg144Cys)
1093189924	*NBN*	c.1489A>G (p.Thr497Ala)
1093189926	*BRIP1*	c.3411_3412delinsCT (p.Asp1138delinsTyr)
1093189926	*CDKN2A (P16)*	c.430C>T (p.Arg144Cys)
1093189933	*ATM*	c.3154-4G>A
1093189934	*CDH1*	c.2440-6C>G
1093189934	*CDKN2A (P16)*	c.-25C>T
1093189939	*BRCA2*	c.6109G>A (p.Glu2037Lys)
1093189939	*BRIP1*	c.1684A>G (p.Ile562Val)
1093189939	*SMAD4*	c.424+5G>A
1093189940	*APC*	c.4336G>A (p.Ala1446Thr)
1093189945	*ATM*	c.6919C>T (p.Leu2307Phe)
1093189946	*ATM*	c.544G>C (p.Val182Leu)
1093189950	*APC*	c.4364A>G (p.Asn1455Ser)
1093189950	*MLH1*	c.83C>T (p.Pro28Leu)
1093189951	*ATM*	c.6572+4T>C
1093189951	*STK11*	c.-1C>T
1093189956	*ATM*	c.6995T>C (p.Leu2332Pro)
1093189962	*CDKN2A (P16)*	c.170C>G (p.Ala57Gly)
1093189962	*CHEK2*	c.922-1G>A
1093189966	*CDK4*	c.122A>G (p.Asn41Ser)
1093189967	*ATM*	c.4388T>G (p.Phe1463Cys)
1093189967	*ATM*	c.1810C>T (p.Pro604Ser)
1093189970	*APC*	c.854A>G (p.Asp285Gly)
1093189971	*BRIP1*	c.728T>C (p.Ile243Thr)
1093189971	*MLH1*	c.53G>C (p.Arg18Pro)
1093189974	*APC*	c.5424_5426del (p.Asn1808del)
1093189974	*CDKN2A (P16)*	c.-33G>C
1093189976	*PMS2*	c.1268C>G (p.Ala423Gly)
1093189977	*BRIP1*	c.584T>C (p.Leu195Pro)
1093189981	*ATM*	c.2401G>A (p.Gly801Ser)
1093189986	*ATM*	c.4424A>G (p.Tyr1475Cys)
1093189992	*RAD51C*	c.376G>A (p.Ala126Thr)
1093189994	*ATM*	c.6114C>G (p.His2038Gln)
1093189997	*BRIP1*	c.2564G>A (p.Arg855His)
1093189999	*ATM*	c.1073A>G (p.Asn358Ser)
1093189999	*ATM*	c.186-7C>T
1093189999	*BRIP1*	c.413T>C (p.Leu138Ser)
1093189999	*NBN*	c.797C>T (p.Pro266Leu)
1093189999	*NBN*	c.37+5G>A
1093189999	*PTEN*	c.-7C>T
1093189999	*RAD51D*	c.695G>A (p.Arg232Gln)
1093190193	*BMPR1A*	c.1420G>C (p.Val474Leu)
1093190196	*BRCA2*	Dup exons 23 and 24
1093190197	*CDH1*	c.833-16C>G
1093190197	*NBN*	c.797C>T (p.Pro266Leu)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1093190201	*APC*	c.2258A>T (p.His753Leu)
1093190201	*CHEK2*	c.246_260del (p.Asp82_Glu86del)
1093190203	*ATM*	c.544G>C (p.Val182Leu)
1093190203	*CDKN2A (P16)*	c.430C>T (p.Arg144Cys)
1093190203	*NBN*	c.2146A>G (p.Asn716Asp)
1093190205	*CHEK2*	c.470T>C (p.Ile157Thr)
1093190206	*ATM*	c.4375G>A (p.Gly1459Arg)
1093190211	*APC*	c.7468G>A (p.Asp2490Asn)
1093190211	*BRIP1*	c.3444C>A (p.Asp1148Glu)
1093190213	*ATM*	c.2396C>T (p.Ala799Val)
1093190220	*BARD1*	c.841C>T (p.Pro281Ser)
1093190223	*STK11*	Dup exons 1–9
1093190228	*ATM*	c.370A>G (p.Ile124Val)
1093190228	*NBN*	c.1317A>G (p.Ile439Met)
1093190230	*ATM*	c.6067G>A (p.Gly2023Arg)
1093190232	*ATM*	c.6974C>T (p.Ala2325Val)
1093190232	*CHEK2*	c.1420C>T (p.Arg474Cys)
1093190232	*CHEK2*	c.1260-8A>T
1093190232	*CHEK2*	c.1333T>C (p.Tyr445His)
1093190232	*CHEK2*	c.1525C>T (p.Pro509Ser)
1093190232	*CHEK2*	c.1348G>A (p.Glu450Lys)
1093190232	*MLH1*	c.52C>T (p.Arg18Cys)
1093190232	*RAD51D*	c.577-13C>A
1093190234	*ATM*	c.6067G>A (p.Gly2023Arg)
1093190239	*BARD1*	c.1738G>A (p.Glu580Lys)
1093190245	*BRCA2*	c.6973G>A (p.Val2325Ile)
1093190245	*PALB2*	c.49-6_49-4del
1093190245	*RAD51D*	c.835G>A (p.Asp279Asn)
1093190246	*BRIP1*	c.890A>G (p.Lys297Arg)
1093190246	*STK11*	c.1211C>T (p.Ser404Phe)
1093190251	*CHEK2*	c.1461G>C (p.Gln487His)
1093190252	*BRIP1*	c.3079G>A (p.Glu1027Lys)
1093190257	*NBN*	c.511A>G (p.Ile171Val)
1093190262	*APC*	c.5894A>G (p.His1965Arg)
1093190263	*BRIP1*	c.3746A>G (p.Lys1249Arg)
1093190265	*CDKN2A (P16)*	c.-33G>C
1093190267	*MUTYH*	c.37-7G>A
1093190269	*CDH1*	c.2440-6C>G
1093190269	*SMAD4*	c.1106A>G (p.Asn369Ser)
1093190273	*CDKN2A (P16)*	c.-2G>A
1093190279	*ATM*	c.2096A>G (p.Glu699Gly)
1093190280	*PMS2*	c.572A>G (p.Tyr191Cys)
1093190280	*PTEN*	c.802-51_802-14del
1093190280	*STK11*	c.970C>G (p.Pro324Ala)
1093190284	*ATM*	c.2608A>G (p.Asn870Asp)
1093190284	*ATM*	c.3993+5G>T
1093190284	*NBN*	c.797C>T (p.Pro266Leu)
1093190284	*TP53*	c.139C>T (p.Pro47Ser)
1093190285	*NBN*	c.1882G>A (p.Glu628Lys)
1093190285	*TP53*	c.851C>T (p.Thr284Ile)
1093190286	*ATM*	c.544G>C (p.Val182Leu)
1093190286	*PMS2*	c.2347G>A (p.Val783Ile)
1093190287	*BRCA2*	c.8360G>A (p.Arg2787His)
1095079850	*BRIP1*	c.550G>T (p.Asp184Tyr)
1095079852	*BARD1*	c.33G>T (p.Gln11His)
1095079854	*CDKN2A (P16)*	c.-33G>C
1095079855	*ATM*	c.5267C>G (p.Thr1756Arg)
1095079857	*MUTYH*	c.1037C>T (p.Ser346Leu)
1095079860	*BRIP1*	c.2236A>G (p.Ile746Val)
1095079862	*BRIP1*	c.1961G>T (p.Gly654Val)
1095079865	*RAD51C*	c.890T>C (p.Leu297Pro)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1095079866	*BARD1*	c.33G>T (p.Gln11His)
1095079869	*MSH2*	c.797C>T (p.Ala266Val)
1095079871	*APC*	c.5026A>G (p.Arg1676Gly)
1095079871	*APC*	c.7399C>A (p.Pro2467Thr)
1095079872	*CDH1*	c.88C>A (p.Pro30Thr)
1095079872	*CHEK2*	c.14C>T (p.Ser5Leu)
1095079878	*BRCA2*	c.6413T>A (p.Val2138Asp)
1095079878	*CDKN2A (P16)*	c.146T>C (p.Ile49Thr)
1095079879	*ATM*	c.3961A>G (p.Met1321Val)
1095079889	*ATM*	c.4424A>G (p.Tyr1475Cys)
1095079892	*PALB2*	c.1250C>A (p.Ser417Tyr)
1095079896	*PTEN*	c.802-51_802-14del
1095079899	*APC*	c.7570A>G (p.Lys2524Glu)
1095079904	*PMS2*	c.2108C>T (p.Thr703Met)
1095079907	*BRIP1*	c.890A>G (p.Lys297Arg)
1095079908	*CDKN2A (P16)*	c.-25C>T
1095079908	*MSH2*	c.114C>G (p.Asp38Glu)
1095079908	*PTEN*	c.802-51_802-14del
1095079908	*TP53*	c.139C>T (p.Pro47Ser)
1095079909	*ATM*	c.5071A>C (p.Ser1691Arg)
1095079913	*MLH1*	c.100G>A (p.Glu34Lys)
1095079914	*BMPR1A*	c.1141C>G (p.Leu381Val)
1095079917	*MUTYH*	c.700G>A (p.Val234Met)
1095079933	*APC*	c.2586C>G (p.Asn862Lys)
1095079933	*CDH1*	c.892G>A (p.Ala298Thr)
1095079934	*MSH2*	c.1897A>G (p.Ile633Val)
1095079934	*RAD51C*	c.376G>A (p.Ala126Thr)
1095079935	*NBN*	c.511A>G (p.Ile171Val)
1095079937	*CHEK2*	c.470T>C (p.Ile157Thr)
1095079939	*MSH2*	c.2105T>G (p.Val702Gly)
1095079941	*SMAD4*	c.1573A>G (p.Ile525Val)
1095079944	*CDH1*	c.2635G>A (p.Gly879Ser)
1095111827	*PALB2*	c.2816T>G (p.Leu939Trp)
1095112405	*APC*	Dup exon 13
1095112405	*ATM*	Dup exon 23
1095112405	*CDKN2A (P14ARF)*	c.217A>C (p.Ser73Arg)
1095112406	*MUTYH*	c.322A>G (p.Lys108Glu)
1095112412	*APC*	c.8276G>A (p.Arg2759His)
1095112412	*TP53*	c.845G>A (p.Arg282Gln)
1095112413	*BRIP1*	c.584T>C (p.Leu195Pro)
1095112417	*MSH6*	c.1180T>G (p.Ser394Ala)
1095112420	*PTEN*	c.235G>A (p.Ala79Thr)
1095112424	*ATM*	c.3787A>G (p.Ser1263Gly)
1095112425	*NBN*	c.37+5G>A
1095112426	*APC*	c.7352C>A (p.Thr2451Asn)
1095112426	*ATM*	c.2804C>T (p.Thr935Met)
1095112426	*SMAD4*	c.455-6A>G
1095112428	*CDK4*	c.409G>A (p.Val137Ile)
1095112430	*CDK4*	c.684-4A>T
1095112437	*APC*	c.8141G>A (p.Arg2714His)
1095112440	*ATM*	c.2932T>C (p.Ser978Pro)
1095112444	*BRIP1*	c.2220G>T (p.Gln740His)
1095112447	*MSH2*	c.1595T>C (p.Val532Ala)
1095112454	*NBN*	c.283G>C (p.Asp95His)
1095112458	*NBN*	c.511A>G (p.Ile171Val)
1095112461	*PALB2*	c.3307G>A (p.Val1103Met)
1095112463	*BRIP1*	c.1655T>C (p.Ile552Thr)
1095112463	*PALB2*	c.3191A>T (p.Tyr1064Phe)
1095112466	*APC*	c.848G>A (p.Arg283Gln)
1095112468	*ATM*	c.5653A>G (p.Thr1885Ala)
1095112471	*APC*	c.3161A>C (p.His1054Pro)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1095112475	*PALB2*	c.-5G>T
1095112476	*ATM*	c.8268+6T>A
1095112479	*CHEK2*	c.1607C>T (p.Pro536Leu)
1095112479	*PMS2*	c.1247C>G (p.Ser416Cys)
1095112480	*BRIP1*	c.430G>A (p.Ala144Thr)
1095112484	*BRIP1*	c.890A>G (p.Lys297Arg)
1095112485	*MSH6*	c.261-14C>A
1095112485	*MUTYH*	c.1255G>A (p.Ala419Thr)
1095112488	*APC*	c.3535T>A (p.Tyr1179Asn)
1095112488	*BMPR1A*	c.1433G>A (p.Arg478His)
1095112488	*NBN*	c.37+5G>A
1095112491	*ATM*	c.1229T>C (p.Val410Ala)
1095112492	*CHEK2*	c.1217G>A (p.Arg406His)
1095112493	*ATM*	c.3137T>C (p.Leu1046Pro)
1095112496	*BARD1*	c.1409A>G (p.Asn470Ser)
1095188497	*PTEN*	c.802-51_802-14del
1095188500	*CHEK2*	c.1525C>T (p.Pro509Ser)
1095188501	*BRIP1*	c.584T>C (p.Leu195Pro)
1095188508	*ATM*	c.6995T>C (p.Leu2332Pro)
1095188508	*TP53*	c.139C>T (p.Pro47Ser)
1095188510	*APC*	c.6512G>A (p.Gly2171Glu)
1095188512	*PMS2*	c.883C>T (p.Arg295Trp)
1095188513	*BRIP1*	c.139C>G (p.Pro47Ala)
1095188515	*MUTYH*	c.925C>T (p.Arg309Cys)
1095188515	*PALB2*	c.2674G>A (p.Glu892Lys)
1095188516	*TP53*	c.1150A>G (p.Met384Val)
1095188519	*PMS2*	c.1420G>T (p.Ala474Ser)
1095188520	*ATM*	c.5071A>C (p.Ser1691Arg)
1095188521	*RAD51D*	c.568G>A (p.Ala190Thr)
1095188521	*RAD51D*	c.481-7G>A
1095188532	*APC*	c.4088A>G (p.Lys1363Arg)
1095188541	*ATM*	c.186-7C>T
1095188541	*BRIP1*	c.2236A>G (p.Ile746Val)
1095188546	*APC*	c.2204C>T (p.Ala735Val)
1095188546	*ATM*	c.496+4T>C
1095188546	*MUTYH*	c.1276C>T (p.Arg426Cys)
1095188546	*RAD51C*	c.790G>A (p.Gly264Ser)
1095188547	*MSH6*	c.124C>T (p.Pro42Ser)
1095188547	*MSH6*	c.1932G>C (p.Arg644Ser)
1095188547	*RAD51D*	c.146C>T (p.Ala49Val)
1095188550	*STK11*	c.1283C>G (p.Ser428Trp)
1095188551	*CDH1*	c.88C>A (p.Pro30Thr)
1095188556	*BARD1*	c.709C>G (p.Gln237Glu)
1095188564	*ATM*	c.1744T>C (p.Phe582Leu)
1095188566	*MSH2*	c.490G>A (p.Gly164Arg)
1095188574	*ATM*	c.1810C>T (p.Pro604Ser)
1095188579	*ATM*	c.131A>T (p.Asp44Val)
1095188581	*STK11*	c.1211C>T (p.Ser404Phe)
1095188586	*ATM*	c.6988C>G (p.Leu2330Val)
1095188588	*BMPR1A*	c.1355A>G (p.Glu452Gly)
1095188588	*PALB2*	c.194C>T (p.Pro65Leu)
1095214770	*CDKN2A (P16)*	c.-33G>C
1095214770	*MLH1*	c.-43C>T
1095214818	*TP53*	c.97-6C>T
1095216809	*PMS2*	c.2174C>T (p.Ala725Val)
1095216810	*CHEK2*	c.410G>A (p.Arg137Gln)
1095216811	*MLH1*	c.1937A>G (p.Tyr646Cys)
1095216813	*NBN*	c.2146A>G (p.Asn716Asp)
1095216822	*CDKN2A (P16)*	c.206A>G (p.Glu69Gly)
1095216822	*CHEK2*	c.14C>T (p.Ser5Leu)
1095216825	*BRIP1*	c.890A>G (p.Lys297Arg)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1095216826	*PMS2*	c.163+4A>G
1095216830	*APC*	c.6236A>C (p.Asp2079Ala)
1095216830	*BRIP1*	c.2285G>A (p.Arg762His)
1095216833	*ATM*	c.7592T>C (p.Met2531Thr)
1095216833	*BARD1*	c.2282G>A (p.Ser761Asn)
1095216836	*BMPR1A*	c.1432C>T (p.Arg478Cys)
1095216850	*NBN*	c.2146A>G (p.Asn716Asp)
1095216850	*PMS2*	c.1556A>G (p.Tyr519Cys)
1095216850	*PMS2*	c.1559C>T (p.Ala520Val)
1095216851	*NBN*	c.832T>G (p.Ser278Ala)
1095216852	*NBN*	c.1882G>A (p.Glu628Lys)
1095216871	*ATM*	c.7912T>G (p.Trp2638Gly)
1095216871	*BRIP1*	c.430G>A (p.Ala144Thr)
1095216871	*MUTYH*	c.74G>A (p.Gly25Asp)
1095216871	*MUTYH*	c.53C>T (p.Pro18Leu)
1095216875	*APC*	c.4072G>A (p.Ala1358Thr)
1095216879	*ATM*	c.6067G>A (p.Gly2023Arg)
1095216882	*MSH2*	c.1760-7T>C
1095216883	*BRIP1*	c.790C>T (p.Arg264Trp)
1095216895	*CDH1*	c.1234G>A (p.Val412Ile)
1095216897	*RAD51C*	c.376G>A (p.Ala126Thr)
1095216899	*BARD1*	c.1738G>A (p.Glu580Lys)
1095216899	*NBN*	c.2146A>G (p.Asn716Asp)
1095216899	*PTEN*	c.802-51_802-14del
1097148993	*ATM*	c.7522G>A (p.Gly2508Arg)
1097148993	*CDH1*	c.2635G>A (p.Gly879Ser)
1097148999	*PMS2*	c.857A>G (p.Asp286Gly)
1097149000	*APC*	c.2222A>G (p.Asn741Ser)
1097149002	*ATM*	c.2289T>A (p.Phe763Leu)
1097149005	*MLH1*	c.739T>C (p.Ser247Pro)
1097149006	*CDH1*	c.2512A>G (p.Ser838Gly)
1097149006	*PALB2*	c.2903C>G (p.Ala968Gly)
1097149010	*ATM*	c.1810C>T (p.Pro604Ser)
1097149010	*ATM*	c.2494C>T (p.Arg832Cys)
1097149010	*RAD51D*	c.695G>A (p.Arg232Gln)
1097149011	*NBN*	c.456G>A (p.Met152Ile)
1097149013	*ATM*	c.6067G>A (p.Gly2023Arg)
1097149014	*APC*	c.7808A>G (p.Glu2603Gly)
1097149016	*BRIP1*	c.550G>T (p.Asp184Tyr)
1097149017	*TP53*	c.139C>T (p.Pro47Ser)
1097149018	*TP53*	c.97-6C>T
1097149019	*BRIP1*	c.3262C>T (p.His1088Tyr)
1097149019	*CHEK2*	c.470T>C (p.Ile157Thr)
1097149020	*MSH6*	c.1822A>G (p.Ile608Val)
1097149025	*BRCA2*	c.3581G>A (p.Gly1194Asp)
1097149030	*APC*	c.3323A>G (p.Asn1108Ser)
1097149030	*RAD51D*	c.629C>T (p.Ala210Val)
1097149031	*STK11*	c.336G>C (p.Gln112His)
1097149032	*BARD1*	c.668A>G (p.Glu223Gly)
1097149034	*ATM*	c.186-7C>T
1097149034	*BRIP1*	c.3378A>C (p.Glu1126Asp)
1097149043	*BMPR1A*	c.499A>G (p.Met167Val)
1097149045	*PALB2*	c.400G>A (p.Asp134Asn)
1097149046	*MSH6*	c.3758T>C (p.Val1253Ala)
1097149047	*APC*	c.6985A>G (p.Ile2329Val)
1097149048	*BARD1*	c.1738G>A (p.Glu580Lys)
1097149052	*BMPR1A*	c.1327C>T (p.Arg443Cys)
1097149054	*MSH6*	c.2926C>T (p.Arg976Cys)
1097149054	*NBN*	c.1489A>G (p.Thr497Ala)
1097149054	*PTEN*	c.802-51_802-14del
1097149055	*MSH6*	c.949A>G (p.Lys317Glu)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1097149056	*APC*	c.835G>T (p.Gly279Cys)
1097149056	*ATM*	c.1229T>C (p.Val410Ala)
1097149056	*BRIP1*	c.10A>G (p.Met4Val)
1097149056	*PALB2*	c.2027T>C (p.Ile676Thr)
1097149057	*ATM*	c.4414T>G (p.Leu1472Val)
1097149057	*BRCA2*	c.9442G>T (p.Ala3148Ser)
1097149059	*BMPR1A*	c.1439G>T (p.Arg480Leu)
1097149060	*BRIP1*	c.2236A>G (p.Ile746Val)
1097149060	*NBN*	c.797C>T (p.Pro266Leu)
1097149061	*ATM*	c.1744T>C (p.Phe582Leu)
1097149062	*PTEN*	c.349A>C (p.Asn117His)
1097149065	*PALB2*	c.821C>T (p.Thr274Ile)
1097149066	*BRIP1*	c.550G>T (p.Asp184Tyr)
1097149066	*TP53*	c.704A>G (p.Asn235Ser)
1097149067	*CHEK2*	c.538C>T (p.Arg180Cys)
1097149070	*CDH1*	c.892G>A (p.Ala298Thr)
1097149070	*MLH1*	c.1637A>G (p.Lys546Arg)
1097149072	*PALB2*	c.2201C>A (p.Thr734Asn)
1097149074	*BRIP1*	c.370A>G (p.Thr124Ala)
1097149083	*CDKN2A (P16)*	c.-2G>A
1097149085	*BRCA1*	c.655G>A (p.Asp219Asn)
1097149086	*MLH1*	c.1761G>A (p.Met587Ile)
1097149086	*NBN*	c.1489A>G (p.Thr497Ala)
1097162627	*PALB2*	c.653A>T (p.Glu218Val)
1097162628	*ATM*	c.2362A>C (p.Ser788Arg)
1097162628	*ATM*	c.6088A>G (p.Ile2030Val)
1097162633	*ATM*	c.544G>C (p.Val182Leu)
1097162633	*NBN*	c.797C>T (p.Pro266Leu)
1097162638	*MUTYH*	c.998-9C>T
1097162639	*ATM*	c.6919C>T (p.Leu2307Phe)
1097162639	*RAD51C*	c.146-8A>G
1097162641	*APC*	c.277C>G (p.Leu93Val)
1097162643	*ATM*	c.8968G>A (p.Glu2990Lys)
1097162646	*MSH6*	c.3762_3764del (p.Glu1254del)
1097162647	*ATM*	c.3077+4G>A
1097162647	*ATM*	c.544G>C (p.Val182Leu)
1097162650	*PALB2*	c.2135C>T (p.Ala712Val)
1097162653	*BMPR1A*	c.1204G>T (p.Val402Leu)
1097162653	*MUTYH*	c.56G>A (p.Arg19Gln)
1097162655	*BRIP1*	c.752G>A (p.Arg251His)
1097162656	*BMPR1A*	c.1327C>T (p.Arg443Cys)
1097162656	*CDKN2A (P16)*	c.-25C>T
1097162661	*CHEK2*	c.1076A>G (p.Glu359Gly)
1097162662	*ATM*	c.5071A>C (p.Ser1691Arg)
1097162668	*BARD1*	c.2191C>T (p.Arg731Cys)
1097162668	*BARD1*	c.1601C>T (p.Thr534Ile)
1097162668	*MSH2*	c.1690A>G (p.Thr564Ala)
1097162669	*MSH6*	c.3731T>C (p.Leu1244Ser)
1097162670	*RAD51C*	c.376G>A (p.Ala126Thr)
1097162672	*BRIP1*	c.1000G>T (p.Ala334Ser)
1097162675	*MLH1*	c.1649T>C (p.Leu550Pro)
1097162677	*MSH6*	c.3190G>C (p.Ala1064Pro)
1097162677	*TP53*	c.139C>T (p.Pro47Ser)
1097162678	*PALB2*	c.2135C>T (p.Ala712Val)
1097162680	*ATM*	c.1810C>T (p.Pro604Ser)
1097162680	*ATM*	c.4388T>G (p.Phe1463Cys)
1097162684	*ATM*	c.1229T>C (p.Val410Ala)
1097162686	*NBN*	c.2146A>G (p.Asn716Asp)
1097162686	*STK11*	c.894C>A (p.Phe298Leu)
1097162687	*BRCA2*	c.5270A>G (p.Tyr1757Cys)
1097162691	*BRCA2*	c.5284T>G (p.Tyr1762Asp)

Supplementary Table 2. Continued

Patient ID	Gene	HGVS alteration name
1097162695	*MUTYH*	c.1417G>A (p.Ala473Thr)
1097162696	*APC*	c.6068G>T (p.Arg2023Ile)
1097162699	*ATM*	c.4091A>G (p.Asp1364Gly)
1097162699	*CHEK2*	c.320-5T>A
1097162700	*ATM*	c.3925G>A (p.Ala1309Thr)
1097162704	*PTEN*	c.802-51_802-14del
1097162709	*MSH2*	c.2211-5T>G
1097162710	*ATM*	c.6067G>A (p.Gly2023Arg)
1097162712	*APC*	c.4018_4020dupTCT (p.Ser1341dup)
1097162713	*CDH1*	c.2104G>A (p.Glu702Lys)
1097162716	*MUTYH*	c.481G>C (p.Asp161His)
1097162717	*ATM*	c.5890A>G (p.Lys1964Glu)

Dup, duplication; HGVS, Human Genome Variation Society.