CANCER GENETICS

Prevalence and Characterization of Biallelic and Monoallelic *NTHL1* and *MSH3* Variant Carriers From a Pan-Cancer Patient Population

Erin E. Salo-Mullen, MS, MPH[1]; Anna Maio, BS[1]; Semanti Mukherjee, PhD[1]; Chaitanya Bandlamudi, PhD[2]; Jinru Shia, MD[3]; Yelena Kemel, MS, ScM[1]; Karen A. Cadoo, MD[1]; Ying Liu, MD, MPH[1]; Maria Carlo, MD[1]; Megha Ranganathan, MS[1]; Sarah Kane, MS[1]; Preethi Srinivasan, PhD[3]; Shweta S. Chavan, PhD[2]; Mark T. A. Donoghue, PhD[2]; Caitlin Bourque, BA[2]; Margaret Sheehan, MS[1]; Prince Rainier Tejada, BA[1]; Zalak Patel, BS[1]; Angela G. Arnold, MS[1]; Jennifer A. Kennedy, MS[1]; Kimberly Amoroso, MS[1]; Kelsey Breen, MS, MSc[1]; Amanda Catchings, MS[1]; Rosalba Sacca, PhD, MS[1]; Vanessa Marcell, MS[1]; Arnold J. Markowitz, MD[1]; Alicia Latham, MD[1]; Michael Walsh, MD[1]; Maksym Misyura, PhD[3]; Ozge Ceyhan-Birsoy, PhD[3]; David B. Solit, MD[1,2]; Michael F. Berger, PhD[2,3]; Mark E. Robson, MD[1]; Barry S. Taylor, PhD[2,4,5]; Kenneth Offit, MD, MPH[1]; Diana Mandelker, MD, PhD[3]; and Zsofia K. Stadler, MD[1]

abstract

PURPOSE *NTHL1* and *MSH3* have been implicated as autosomal recessive cancer predisposition genes. Although individuals with biallelic *NTHL1* and *MSH3* pathogenic variants (PVs) have increased cancer and polyposis risk, risks for monoallelic carriers are uncertain. We sought to assess the prevalence and characterize *NTHL1* and *MSH3* from a large pan-cancer patient population.

MATERIALS AND METHODS Patients with pan-cancer (n = 11,081) underwent matched tumor-normal sequencing with consent for germline analysis. Medical records and tumors were reviewed and analyzed. Prevalence of PVs was compared with reference controls (Genome Aggregation Database).

RESULTS *NTHL1*-PVs were identified in 40 patients including 39 monoallelic carriers (39/11,081 = 0.35%) and one with biallelic variants (1/11,081 = 0.009%) and a diagnosis of isolated early-onset breast cancer. *NTHL1*-associated mutational signature 30 was identified in the tumors of the biallelic patient and two carriers. Colonic polyposis was not identified in any *NTHL1* patient. *MSH3*-PVs were identified in 13 patients, including 12 monoallelic carriers (12/11,081 = 0.11%) and one with biallelic *MSH3* variants (1/11,081 = 0.009%) and diagnoses of later-onset cancers, attenuated polyposis, and abnormal MSH3-protein expression. Of the 12 *MSH3* carriers, two had early-onset cancer diagnoses with tumor loss of heterozygosity of the wild-type *MSH3* allele. Ancestry-specific burden tests demonstrated that *NTHL1* and *MSH3* prevalence was not significantly different in this pan-cancer population versus controls.

CONCLUSION *NTHL1* and *MSH3* germline alterations were not enriched in this pan-cancer patient population. However, tumor-specific findings, such as mutational signature 30 and loss of heterozygosity of the wild-type allele, suggest the potential contribution of monoallelic variants to tumorigenesis in a subset of patients.

JCO Precis Oncol 5:455-465. © 2021 by American Society of Clinical Oncology

INTRODUCTION

Mendelian colorectal cancer (CRC) predisposition syndromes, several of which are also associated with GI polyposis, explain approximately 5% of all CRC diagnoses. Beyond *MUTYH*-associated polyposis (MAP) syndrome, other autosomal recessive CRC predisposition syndromes in novel genes, including *NTHL1* and *MSH3*, have been described.[1,2] However, knowledge about the spectrum of neoplasia, medical management implications, and the risk of neoplasia in biallelic versus monoallelic germline variant carriers for these newly described genes remains limited.

MAP is caused by biallelic pathogenic variants (PVs) within the base excision repair gene *MUTYH*.[3,4] The clinical features of MAP include increased risk of CRC, polyposis, and possibly other cancers; however, the risk of neoplasia and polyposis in monoallelic *MUTYH* PV carriers has been an area of active research and debate. Although a number of prior publications suggested an increased risk of CRC for monoallelic *MUTYH* carriers, with one study also suggesting risks for several extracolonic-type cancers, a meta-analysis by Ma et al found that individuals carrying monoallelic *MUTYH* PVs had only a very small increased CRC risk (odds ratio [OR], 1.17; 95% CI, 1.01 to 1.34).[5–7] As such, the most recent NCCN and other guidelines suggest that this level of risk is not sufficient to warrant modification of current CRC screening guidelines in the absence of CRC in a first-degree relative.[8,9]

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on January 27, 2021 and published at ascopubs.org/journal/po on February 26, 2021: DOI https://doi.org/10.1200/PO.20.00443

CONTEXT

Key Objective

Germline genetic testing has led to the identification of individuals with monoallelic *NTHL1* and *MSH3* pathogenic variants (PVs), but research to date has focused on phenotypically enriched cohorts, such as those with histories of colorectal cancer and/or polyposis. In this study, we assessed the prevalence and characterize *NTHL1* and *MSH3* carriers from a population of more than 11,000 patients with pan-cancer.

Knowledge Generated

Monoallelic germline *NTHL1* and *MSH3* PVs are identified in < 0.5% of patients with pan-cancer. An enrichment of PVs was not identified when compared with reference controls from the Genome Aggregation Database, inclusive of disease-specific analyses for colorectal and breast cancer in the *NTHL1* population.

Relevance

Increased cancer surveillance for monoallelic *NTHL1* or *MSH3* carriers is not supported currently; however, our findings in a patient with biallelic *NTHL1* PVs support increased breast cancer surveillance. Additional research of *NTHL1* and *MSH3* monoallelic carriers, especially in more diverse patient populations, is needed.

NTHL1 and *MSH3* are two other autosomal recessive genes implicated in GI polyposis and cancer risk.[1,2] *NTHL1* belongs to the same base excision repair pathway as *MUTYH*. Biallelic mutations in *NTHL1* characterize *NTHL1* tumor syndrome (initially called *NTHL1* associated polyposis syndrome).[10,11] Although most cases reported in the literature are associated with the recurrent p.Gln90* PV, other PVs have also been reported.[12] Through the identification of several individuals harboring homozygous or compound heterozygous *NTHL1* PVs, a broad tumor spectrum has been illustrated, including breast, gynecologic, urothelial, prostate, and brain tumors, among others.[1,10,12–16] Reported polyp histology has also varied, including the presence of hyperplastic and serrated polyps, but with adenomatous polyps being the most common.[12] Tumors from individuals with biallelic *NTHL1* PVs have been described to have a preponderance of C>T transitions[1] and a distinct *NTHL1*-associated mutational signature, notably mutational signature 30 of the Catalogue of Somatic Mutations in Cancer.[12] Documented monoallelic and/or obligate carrier family members have occasionally been included in past reports focused on biallelic individuals. Descriptions of these relatives have ranged from being unaffected at various ages to having one or more primary cancers.[1,10,12,14] The identification of individuals with heterozygous *NTHL1* variants has also been reported in phenotypically enriched cohorts, such as two individuals from 523 (2/523 = 0.38%) cases with familial mismatch repair-proficient nonpolyposis CRC,[14] three individuals from 312 (3/312 = 0.96%) patients with personal and/or family history of multiple tumor types, and five of 488 (5/488 = 1.0%) patients with hereditary nonpolyposis CRC.[17]

MSH3 is a mismatch repair gene that works to identify and correct large insertion-deletion loops by forming a heterodimer, MutSβ, with *MSH2*.[18] Adam et al[2] described four individuals from two families with biallelic *MSH3* PVs and histories of GI cancer and adenomatous polyposis, and multiple extracolonic neoplasia. CRC and adenomatous polyps from the affected individuals were described to have absence of the MSH3 protein on immunohistochemical (IHC) staining and elevated microsatellite alterations at selected tetranucleotide repeats (EMAST).[19] In one family, a heterozygous sibling was described to be unaffected with a normal colonoscopy at 51 years of age and the obligate carrier father, deceased at 63 years of age, had a cancer of unknown primary.

As with the initial descriptions of MAP, whether individuals carrying monoallelic *NTHL1* or *MSH3* PVs are at an increased risk of neoplasia is a topic of interest and warrants additional investigation beyond the previous enriched phenotypic-specific populations. With the inclusion of *NTHL1* and *MSH3* on commercially available hereditary cancer multigene panels, individuals who harbor monoallelic PVs in these two genes are increasingly being identified, necessitating data and clinical guidance. In this study, we assess the prevalence and characterize *NTHL1* and *MSH3* variant carriers in a large pan-cancer patient population.

MATERIALS AND METHODS

Through an institutional review board-approved research protocol (NCT01775072), patients at Memorial Sloan Kettering Cancer Center (MSK) were prospectively offered matched tumor-normal sequencing via a custom next-generation sequencing assay known as MSK-IMPACT (Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets).[20] A subset of patients undergoing MSK-IMPACT somatic tumor analysis provided additional consent for a New York State Department of Health approved germline analysis as previously described.[21] Patients who had germline analyses between October 25, 2017, and December 31, 2019, were included in this study. PVs and likely PVs (inclusively referred to as PVs) were reported for 88 cancer predisposition genes,

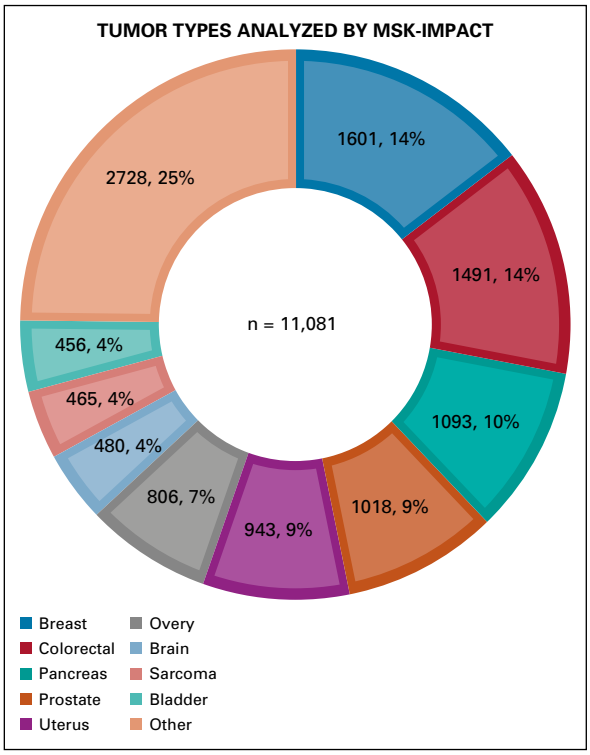

FIG 1. Distribution of tumor types in the germline MSK-IMPACT population (n = 11,081). MSK-IMPACT, Memorial Sloan Kettering Cancer Center-Integrated Mutation Profiling of Actionable Cancer Targets.

inclusive of *NTHL1* and *MSH3,* and all cancer-related genes recommended for reporting by the American College of Medical Genetics and Genomics.[22] Germline results were interpreted by MSK molecular geneticists and/or molecular pathologists with all patients identified to have a PV referred for genetic counseling with the MSK Clinical Genetics Service. Genetic variants of uncertain significance were not included in the clinical reports.

For patients identified to have PVs in the *NTHL1* or *MSH3* genes, clinical data, including cancer pathology, age at diagnosis, previous cancer and polyp history, ancestry, and family history, were extracted from the medical record. For patients who completed a Clinical Genetics Service consultation, family history of cancer and ancestry was obtained by a genetic counselor through construction of a three-generation pedigree.

The US Food and Drug Administration–authorized somatic MSK-IMPACT assay, inclusive of 468 cancer-related genes, was used to calculate tumor mutation burden and determine microsatellite-instability (MSI) status via MSI-Sensor. MSI-Sensor is a bioinformatic algorithm in which each tumor is assigned an MSI-Sensor score with scores $>$ 10 being consistent with MSI-High status.[23,24] Loss of heterozygosity (LOH) of the wild-type allele was identified via Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing (FACETS), an allele-specific copy number analysis.[25] Mutational signatures were inferred on all single-nucleotide somatic mutations in patients with total mutation counts of five or more. The proportion of somatic mutations attributable to each of the 30 established mutational signatures[26] was determined using a basin-hopping algorithm.[27] The Illumina methylationEPIC microarray was applied to selected tumor tissue for the comparison of methylation profiles between tumor and normal blood specimens.[28,29] For patients with *MSH3* PVs, IHC staining analysis of the MSH3 protein (antibody clone EPR4334) was performed on available cancer and colorectal polyp specimens. All results were interpreted by a specialized GI pathologist (J.S.). Exome sequencing was used in selected tumor and colorectal polyp tissue to evaluate for presence of EMAST.[30,31]

Allele frequencies of PVs in cases were compared with genome aggregate allele frequencies (Genome Aggregation Database [gnomAD] r2.1.1) in a population control. We used the gnomAD to assess the frequency of truncating germline variants and applied analogous variant pathogenicity annotation methods as used in our clinical pipeline to determine PVs, with $<$ 1% population allele frequency in the *NTHL1* and *MSH3* genes.[32,33] Population-stratified burden calculations were then performed with the frequency of PVs aggregated per gene in cases compared with the frequencies in controls. The risk estimates, reported as ORs and *P* values, were determined by two-sided Fisher exact test in R software version 3.4.2.

RESULTS

Patient Cohort

Between October 25, 2017, and December 31, 2019, 11,081 patients with pan-cancer consented to the matched tumor-normal sequencing analysis inclusive of germline analysis. Tumor distribution is detailed in Figure 1. Nearly 17% (1,861) of patients were found to have PVs on the 88-gene panel, with 170 individuals having more than one variant identified.

Patients With *NTHL1* Pathogenic Variants

Clinical and tumor characteristics for the 40 patients with *NTHL1* PVs are presented in Table 1 and Figure 2. One patient (1/11,081 = 0.009%) was found to be homozygous for the *NTHL1* c.268C>T (p.Gln90*) PV in the setting of invasive ductal adenocarcinoma of the breast at 36 years of age, but a negative colonoscopy at 37 years of age (Fig A1). The breast tumor was microsatellite stable (MSS), and a dominant mutational signature 30 was observed. Thirty-nine (39/11,081 = 0.35%) patients were found to have monoallelic PVs in the *NTHL1* gene, with 33 having the commonly reported c.268C>T (p.Gln90*) PV. Nine patients harboring the p.Gln90* heterozygous PV were also found to have heterozygous PVs in other, often high-penetrance, genes. Two patients with only monoallelic *NTHL1* p.Gln90* were found to have dominant mutational signature 30 in their tumors: a male with Gleason 9 prostate

TABLE 1. Baseline Characteristics of *NTHL1* and *MSH3* Patient Cohorts

Total Number of Patients	*NTHL1* = 40	*MSH3* = 13
Biallelic	1 (homozygous p.Gln90*)	1 (compound heterozygous)
Monoallelic	39 (33, 85% with p.Gln90*)	12
Sex		
Female	25 (62.5%)	9 (69.2%)
Male	15 (37.5%)	4 (30.8%)
Patients with a second germline finding		
In total	9 (22.5%)	1 (7.7%)
BRCA1/BRCA2	4 (1 *BRCA1*/ 3 *BRCA2*)	0
MSH2	1	0
RB1	1	0
SDHB	1	0
SMARCA4	1	0
ERCC3	1	0
YAP1	0	1
Primary cancer type		
Colorectal	7	1
Breast	7	1
Pancreas	3	2
Bladder	1	1
Prostate	3	1
Uterine	3	1
High-grade serous ovary	4	0
Cervical	1	0
Retinoblastoma	2	1
Cholangiocarcinoma	1	0
Melanoma	2	0
Sarcoma	3	1
Germ-cell tumor	1	0
Neuroblastoma	0	1
Mesothelioma	0	1
Ocular NOS	0	1
SCCOHT	1	0
Thyroid	0	1
Cancer of unknown primary	1	0
Median age (range) at primary cancer diagnosis		
NTHL1 or *MSH3* variant only	60 years (23 months-77 years)	50 years (10 months-71 years)
With another germline finding	33 years (5 months-63 years)	22 years (only 1 case)
Colorectal cancer history (at any time)		
NTHL1 or *MSH3* variant only	6	0
With another germline finding	2	1
Colorectal polyp history		
No colonoscopy	13	5
Negative colonoscopy	15	4
≤ 10 polyps	12	2
≥ 10 polyps	0	2

Abbreviations: NOS, not otherwise specified; SCCOHT, small-cell carcinoma of the ovary hypercalcemic type.

cancer at 70 years of age, and a female with high-grade serous ovarian cancer at 66 years of age. Upon further investigation of these tumors, the prostate tumor did not demonstrate LOH of the wild-type *NTHL1* allele and *NTHL1* promoter methylation was not identified, whereas the high-grade serous ovarian cancer did demonstrate LOH of the wild-type *NTHL1* allele. Two other high-grade serous ovarian cancers and one early-onset breast cancer (which occurred in the setting of a *BRCA2* germline PV) demonstrated LOH of the wild-type *NTHL1* allele, but these tumors did not demonstrate mutational signature 30. None of the tumors from patients with germline *NTHL1* PVs were found to have somatic *NTHL1* variants. Notably, in an assessment of > 22,000 tumors analyzed via MSK-IMPACT, the overall percentage of tumors that had a dominant mutational signature 30 was approximately 2%, even when broken down by malignancy type, indicating the rarity of this mutational signature.

Patients With *MSH3* Pathogenic Variants

Clinical and tumor characteristics for the 13 (13/11,081 = 0.12%) patients with *MSH3* PVs are presented in Table 1 and Figure 2. One patient was found to have biallelic *MSH3* variants (one PV identified upon the MSK-IMPACT secondary germline analysis and one variant of uncertain significance [VUS] identified upon clinical testing, proven to be *in trans* through family segregation analysis, Figure A1) with all others (12) being monoallelic carriers.

The patient with biallelic variants had undergone subsequent clinical germline genetic testing, which identified the *MSH3* VUS, based upon clinical features, including a history of > 29 adenomatous colorectal polyps first identified in her early-70s, followed by synchronous endometrial and lobular breast cancer diagnoses at 75 years of age. Upon tissue analysis, three separate tubular adenomas had loss of MSH3 expression upon IHC staining, and the endometrial cancer was noted to have equivocal expression of MSH3 as the stain appeared negative in the entire tissue except for a few tumor cells and a few nontumor cells demonstrating very weak or equivocal nuclear labeling (the external control was adequate) (Fig 3A). The endometrial cancer and one tubular adenoma were found to be MSS via MSI-Sensor analysis; and further, an exome-based MSI analysis was performed on the endometrial cancer tissue and a tubular adenoma with no evidence of EMAST being identified in 328 and 339 tetranucleotide sites in these specimens, respectively.

In the 12 monoallelic *MSH3* PV carriers, two early-onset tumors (a breast cancer and a pancreas adenocarcinoma) were identified to have LOH of the wild-type *MSH3* allele. Upon MSH3-IHC analysis, the breast tumor was noted to have intact expression; unfortunately, the pancreas tumor was not available for staining. Another *MSH3* PV carrier had very early-onset CRC and attenuated polyposis (> 5 sessile serrated polyps and > 8 hyperplastic polyps) at 22 years of age but MSH3-IHC staining analysis on the rectal cancer tissue and a sessile serrated polyp revealed intact expression (Fig 3B). None of the tumors from patients with germline *MSH3* PVs were found to have somatic *MSH3* variants and all were classified as MSS on MSI-Sensor.

Comparison of *NTHL1* and *MSH3* Variants to Controls

We next sought to assess the burden of *NTHL1* and *MSH3* PVs in our pan-cancer patient cohort with comparison to individuals from gnomAD. Ancestry cohorts are listed in Table 2; burden calculations were limited to Non-Finnish European and Ashkenazi Jewish populations because of sample size constraints in other populations. Neither *NTHL1* nor *MSH3* PVs were enriched in our Non-Finnish European and Ashkenazi Jewish pan-cancer populations compared with gnomAD Non-Finnish European and Ashkenazi Jewish controls (Table 3). For *NTHL1*, where biallelic PVs are associated with increased risks of CRC and breast cancer, we did not observe an excess of *NTHL1* PVs in Non-Finnish Europeans diagnosed with CRC (0.40%) or breast cancer (0.44%) compared with gnomAD Non-Finnish European controls (0.46%). Because of sample size limitations, disease-specific analyses for the *NTHL1* Ashkenazi Jewish and *MSH3* cohort were not possible.

DISCUSSION

Our systematic assessment of *NTHL1* and *MSH3* in a large pan-cancer population demonstrates that monoallelic PVs are identified in < 0.5% of patients with cancer, with patients harboring biallelic PVs in either of these genes being even more rare (0.009%). Importantly, an enrichment of *NTHL1* and *MSH3* PVs was not identified when compared with gnomAD Non-Finnish European and Ashkenazi Jewish controls, inclusive of disease-specific analyses for CRC and breast cancer in the *NTHL1* population. Nonetheless, our finding of *NTHL1*-associated tumor mutational signature 30 in two monoallelic *NTHL1* carriers is intriguing and raises the suspicion that, if present, these monoallelic PVs might contribute to tumorigenesis.

Our study expands upon the existing literature describing the rare, but important, germline biallelic *NTHL1* and *MSH3* phenotypes. Individuals with *NTHL1* tumor syndrome have often been described to have at least an attenuated GI polyposis phenotype and multiple malignant and benign neoplasms, including a high incidence of breast cancer.[12] Interestingly, our only patient with homozygous p.Gln90* PVs had no colorectal polyps identified upon baseline colonoscopy in her mid-30s, but had an early-onset breast cancer demonstrating the *NTHL1*-associated mutational signature 30. Increased CRC surveillance recommendations were provided to this patient, given her young age and apparent increased likelihood of developing polyposis in the future. Although an enrichment of C>T transitions associated with tumor mutational signature 30 has been described in tumors of patients with *NTHL1*

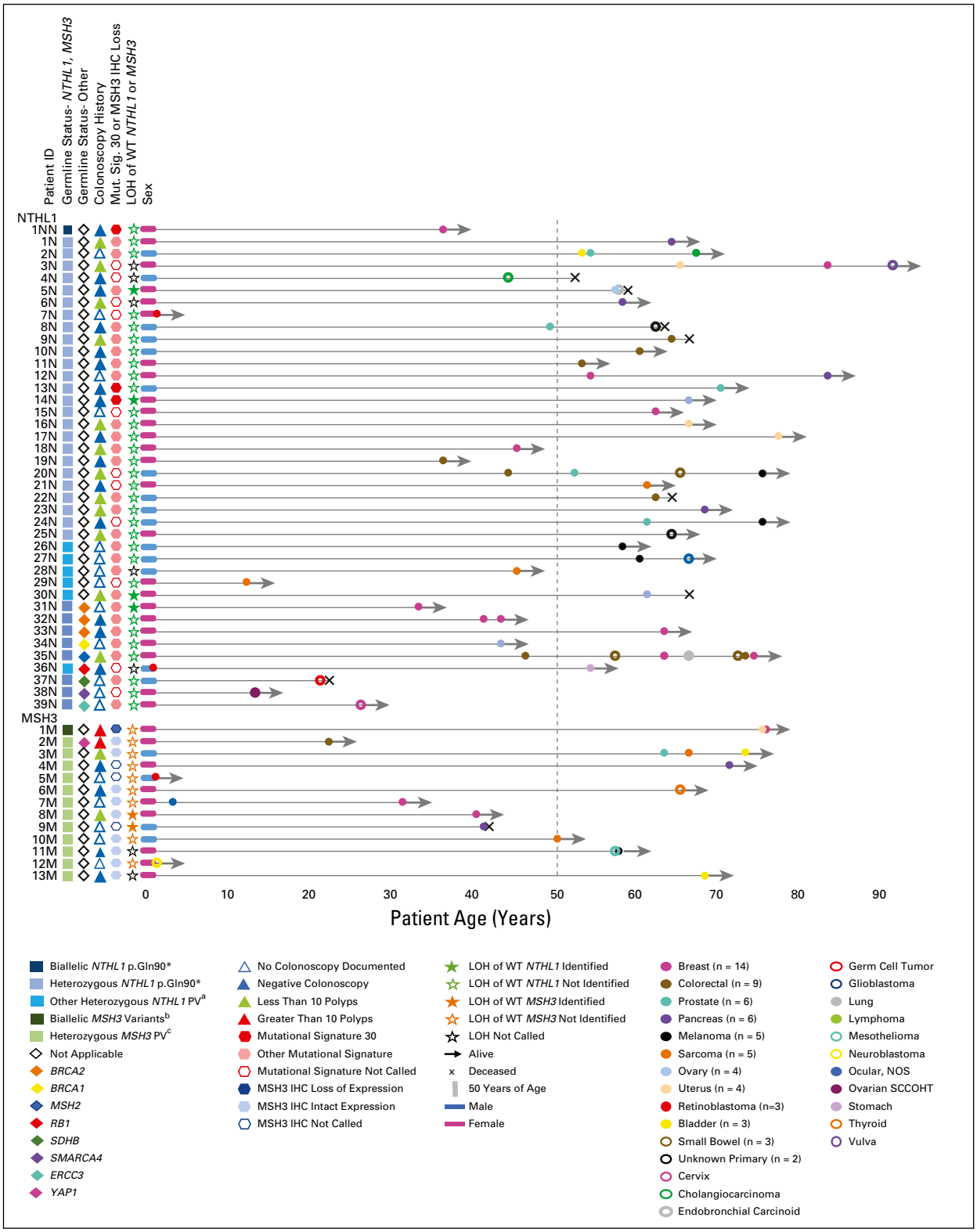

FIG 2. Germline, clinical, and tumor characteristics of *NTHL1* and *MSH3* patient cohorts. [a]26N, 27N: c.628G>T (p.Glu210*); 28N: c.718_719delAC (p.Thr240Alafs*32); 29N: c.806G>A (p.Trp269*); 30N: c.819delG (p.Glu273Aspfs*68); 36N: c.484delG (p.Asp162Thrfs*25). [b]1M: c.978_984delTTCCCGG (p.Phe326Leufs*3); c.3130+3A>G. [c]2M: c.1060dupG (p.Val354Glyfs*4); 3M: c.580-294_649del (p.?); 4M: c.802C>T (p.Arg268*); 5M: c.1341-1G>T; 6M: c.1625dupT (p.Leu542Phefs*12); 7M: c.2686G>T (p.Gly896*); 8M: c.2938_2941dupGGGA (p.Thr981Argfs*6); 9M: c.3001-2A>G; 10M: c.3046_3050delinsTCA (p.Glu1016Serfs*20); 11M: exons 3-24 deletion; 12M: deletion exons 4-8; 13M: whole gene heterozygous deletion. IHC, immunohistochemistry; LOH of WT, loss of heterozygosity of the wild-type allele; NOS, not otherwise specified; SCCOHT, small-cell carcinoma of the ovary hypercalcemic type.

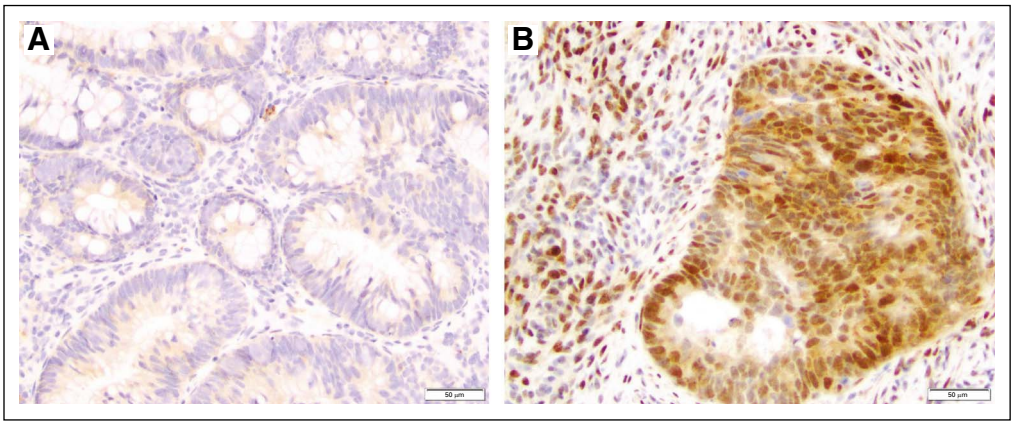

FIG 3. Immunohistochemical stains of the MSH3 protein; (A) case 1M—patient with biallelic *MSH3* variants and multiple colorectal polyps. Repeated immunohistochemistry attempts failed to demonstrate positive nuclear labeling in a tubular adenoma, whereas the external control was satisfactory; (B) case 2M—patient with early-onset rectal adenocarcinoma and multiple polyps. MSH3-immunohistochemistry revealed intact nuclear staining in the rectal tumor cells.

tumor syndrome, Drost et al[34] described an individual with monoallelic *NTHL1* p.Gln287* and LOH at *NTHL1* in a breast tumor resulting in mutational signature 30.[12,34] In our cohort, we identified a woman with high-grade serous ovarian carcinoma harboring monoallelic *NTHL1* p.Gln90* with corresponding LOH of the wild-type *NTHL1* allele in the tumor tissue resulting in a dominant mutational signature 30. Surprisingly though, a prostate cancer patient with monoallelic *NTHL1* p.Gln90* was also found to have a dominant mutational signature 30, but without corresponding LOH of the wild-type allele, somatic variant at *NTHL1*, or promoter hypermethylation. We hypothesize that there may be another, undetected, mechanistic force at work, perhaps epigenetic or an occult second germline variant, that may be contributing to this tumor's dominant mutational signature. Belhadj et al[17] recently analyzed eight heterozygous *NTHL1* cases for methylation of the *NTHL1* promoter, but evidence of constitutional CpG island methylation was not detected. Elsayed et al[35] also reported on a small number of *NTHL1* carriers and did not find any tumors with a dominant mutational signature 30. Although we observed LOH of the wild-type *NTHL1* allele in tumors from three other patients (two of whom also had high-grade serous ovarian cancer), alternative mutational signatures were noted and thus the significance of the LOH finding is unclear.

Our knowledge of the clinical phenotype associated with biallelic *MSH3* PVs to date is limited to four individuals with compound heterozygous *MSH3* PVs.[2] Adam et al identified loss of MSH3 upon IHC analysis in adenomatous polyps in addition to EMAST. Although we performed MSH3 IHC staining on multiple tissues from our *MSH3* cohort, the only tissues found to have abnormal staining were from a patient who had biallelic *MSH3* variants, specifically a PV and a currently classified VUS on the opposite allele. This class 3 variant has been identified in 4/251,110 chromosomes in gnomAD and has been reported in a CRC patient at 77 years of age.[36] Although the phenotypic, germline, and IHC findings were consistent with what one might expect in the setting of biallelic *MSH3* PVs, we did not identify EMAST upon assessment of > 300 tetranucleotide sites in the patient's endometrial cancer and colorectal tubular adenoma specimens. LOH of the wild-type *MSH3* allele was seen in an early-onset breast cancer and an early-onset pancreatic adenocarcinoma; and although this patient with breast cancer also had a history of colorectal polyps in her early-40s, the significance of the LOH finding in the presence of intact MSH3 protein expression warrants further investigation.

Limitations of the current study include a potential selection bias in that despite evaluation of > 11,000 patients with pan-cancer, our cohort reflects patients who were offered and accepted participation in the MSK-IMPACT-matched tumor-normal sequencing protocol and further elected to undergo germline analysis. Although parallel tumor-normal sequencing allowed for interrogation of corresponding cancerous and precancerous lesions, some tissue specimens were insufficient to provide information about mutational signatures, LOH, MSI, and/or IHC. Despite our large denominator, given the rarity of these germline PVs in the

TABLE 2. Ancestry Cohorts

Ancestry	Total Pan-Cancer Cohort	*NTHL1* Variants	*MSH3* Variants
Non-Finnish European	6,367	33	8
Ashkenazi Jewish	1,620	4	0
Other[a]	2,771	3	4
Unknown	323	0	1
Total	11,081	40	13

[a]Other included African, African American, East Asian, Finnish, Latino, Native American, and South Asian.

TABLE 3. Burden Test of *NTHL1* and *MSH3* Variants in Pan-Cancer Population Versus Control Population (gnomAD v2 Exome Cohort)

	Cases—Non-Finnish European			gnomAD—Non-Finnish European					
Patient Cohort	Cases With PV	Total Cases Tested	Carrier Frequency (%)	Controls With PV	Total Controls Tested	Carrier Frequency (%)	OR	95% CI	*P*
NTHL1 pan-cancer cohort	33	6,367	0.52	255	54,891	0.46	1.1	0.7 to 1.6	.5
MSH3 pan-cancer cohort	8	6,367	0.13	72	54,770	0.13	0.9	0.4 to 1.9	1
	Cases—Ashkenazi Jewish			gnomAD—Ashkenazi Jewish					
	Cases With PV	Total Cases Tested	Carrier Frequency (%)	Controls With PV	Total Controls Tested	Carrier Frequency (%)	OR	95% CI	*P*
NTHL1 pan-cancer cohort	4	1,620	0.25	7	4,956	0.14	1.7	0.38 to 6.9	.5
MSH3 pan-cancer cohort	0	1,620		3	4,907	0.06	—	—	—

Abbreviations: gnomAD, Genome Aggregation Database; OR, odds ratio; PV, pathogenic variant.

population, our case numbers remained too small in some instances to allow for certain ancestry-specific burden analyses. Indeed, further assessment of the role of these PVs in more diverse patient populations is necessary. Notwithstanding these limitations, to the best of our knowledge, the current study represents the first systematic assessment of *NTHL1* and *MSH3* PVs from a pan-cancer population and sheds further light on the significance of these findings in a clinical setting.

With inclusion of the *NTHL1* and *MSH3* genes on commercially available germline multigene cancer panels, determination of neoplasia risk for monoallelic *NTHL1* and *MSH3* carriers is of significant clinical interest. Current NCCN guidelines do not comment on management for monoallelic *NTHL1* or *MSH3* carriers but do suggest increased colonoscopy surveillance for individuals with biallelic *NTHL1* and *MSH3* PVs.[8] Our finding of an isolated early-onset breast cancer patient with biallelic *NTHL1* variants supports the suggestion by Grolleman et al[12] for at least moderately increased breast cancer surveillance in women with biallelic *NTHL1* variants. An increased prevalence of *NTHL1* and *MSH3* PVs compared with ancestry-specific control populations was not identified and, as such, at this juncture, increased cancer surveillance for monoallelic carriers is not warranted. By contrast, the question of whether some of the identified germline monoallelic variants contributed to some extent to carcinogenesis warrants further investigation. Additional research of *NTHL1* and *MSH3* monoallelic carriers, especially in more diverse patient populations, is needed to sufficiently assess the implications of these genetic alterations on cancer risk.

AFFILIATIONS

[1]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY
[2]Marie-Josee and Henry R. Kravis Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, New York, NY
[3]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY
[4]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY
[5]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY

CORRESPONDING AUTHOR

Zsofia K. Stadler, MD, Memorial Sloan Kettering Cancer Center, 1275 York Ave., Box 295, New York, NY 10065; e-mail: stadlerz@mskcc.org.

PRIOR PRESENTATION

Presented in part during the Presidential Plenary Abstract Session at the 22nd Annual Meeting of the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer in October 14, 2018, San Diego, CA.

SUPPORT

Supported in part through the Marie-Josée and Henry R. Kravis Center for Molecular Oncology at Memorial Sloan Kettering (tumor or germline sequencing); the Precision, Interception and Prevention Program at Memorial Sloan Kettering (tumor or germline sequencing); the Robert and Kate Niehaus Center for Inherited Cancer Genomics at Memorial Sloan Kettering (tumor or germline sequencing); and the National Institutes of Health National Cancer Institute Cancer Center Support Grant P30 CA008748 (infrastructure support).

AUTHOR CONTRIBUTIONS

Conception and design: Erin E. Salo-Mullen, Anna Maio, Sarah Kane, Margaret Sheehan, David B. Solit, Kenneth Offit, Zsofia K. Stadler
Financial support: David B. Solit, Kenneth Offit
Administrative support: Erin E. Salo-Mullen, Caitlin Bourque, David B. Solit, Kenneth Offit
Provision of study materials or patients: Erin E. Salo-Mullen, David B. Solit
Collection and assembly of data: Erin E. Salo-Mullen, Anna Maio, Chaitanya Bandlamudi, Jinru Shia, Yelena Kemel, Maria Carlo, Megha Ranganathan, Caitlin Bourque, Margaret Sheehan, Prince Rainier Tejada, Zalak Patel, Angela G. Arnold, Jennifer A. Kennedy, Kimberly Amoroso, Kelsey Breen, Amanda Catchings, Rosalba Sacca, Vanessa

Marcell, Alicia Latham, Michael Walsh, David B. Solit, Barry S. Taylor, Kenneth Offit, Diana Mandelker, Zsofia K. Stadler
Data analysis and interpretation: Erin E. Salo-Mullen, Anna Maio, Semanti Mukherjee, Chaitanya Bandlamudi, Jinru Shia, Karen A. Cadoo, Ying Liu, Shweta S. Chavan, Mark T. A. Donoghue, Arnold J. Markowitz, Michael Walsh, Maksym Misyura, Ozge Ceyhan-Birsoy, David B. Solit, Michael F. Berger, Mark E. Robson, Barry S. Taylor, Diana Mandelker, Zsofia K. Stadler
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted. I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Semanti Mukherjee
Stock and Other Ownership Interests: Regeneron

Karen A. Cadoo
Honoraria: OncLive
Consulting or Advisory Role: GlaxoSmithKline/Tesaro
Research Funding: AstraZeneca, Syndax
Travel, Accommodations, Expenses: AstraZeneca

Ying Liu
Research Funding: AstraZeneca

Maria Carlo
Consulting or Advisory Role: Pfizer
Other Relationship: Prostate Cancer Foundation, Robert Wood Johnson Foundation

Preethi Srinivasan
Stock and Other Ownership Interests: Guardant Health, Moderna Therapeutics

Kelsey Breen
Stock and Other Ownership Interests: Imago Pharma, Isabl Technologies
Consulting or Advisory Role: DarwinHealth, Imago Pharma, Karyopharm Therapeutics, Emendo
Patents, Royalties, Other Intellectual Property: Royalty from licensing agreements with MI Bioresearch

Alicia Latham
Other Relationship: Conquer Cancer Foundation

David B. Solit
Stock and Other Ownership Interests: Loxo, Scorpion Therapeutics, Inc
Consulting or Advisory Role: Pfizer, Loxo, Illumina, Vividion Therapeutics, Lilly, QED Therapeutics, BridgeBio Pharma, Scorpion Therapeutics, Inc

Michael F. Berger
Research Funding: Grail
Patents, Royalties, Other Intellectual Property: Provisional patent pending for "Systems and Methods for Detecting Cancer via cfDNA Screening"

Mark E. Robson
Consulting or Advisory Role: Change HealthCare
Research Funding: AstraZeneca, Abbvie, Pfizer, Merck
Travel, Accommodations, Expenses: AstraZeneca, Merck
Other Relationship: Research to Practice, Clinical Care Options, Physicians' Education Resource, Invitae, Pfizer
Uncompensated Relationships: Merck, Pfizer, Daiichi Sankyo, Epic Sciences
Open Payments Link: https://openpaymentsdata.cms.gov/physician/612669/summary

Barry S. Taylor
Employment: Loxo Oncology
Stock and Other Ownership Interests: Lilly
Consulting or Advisory Role: Boehringer Ingelheim, Loxo Oncology
Research Funding: Genentech

Zsofia K. Stadler
Consulting or Advisory Role: Allergan, Genentech/Roche, Regeneron, Optos, Adverum, Novartis, Regenxbio, Gyroscope Tx, Neurogene

No other potential conflicts of interest were reported.

REFERENCES

1. Weren RD, Ligtenberg MJ, Kets CM, et al: A germline homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer. Nat Genet 47:668-671, 2015
2. Adam R, Spier I, Zhao B, et al: Exome sequencing identifies biallelic MSH3 germline mutations as a recessive subtype of colorectal adenomatous polyposis. Am J Hum Genet 99:337-351, 2016
3. Al-Tassan N, Chmiel NH, Maynard J, et al: Inherited variants of MYH associated with somatic G: C-->T: A mutations in colorectal tumors. Nat Genet 30:227-232, 2002
4. Sieber OM, Lipton L, Crabtree M, et al: Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. N Engl J Med 348:791-799, 2003
5. 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 146:1208-1211.e1-5, 2014
6. Win AK, Reece JC, Dowty JG, et al: Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH. Int J Cancer 139:1557-1563, 2016
7. Ma X, Zhang B, Zheng W: Genetic variants associated with colorectal cancer risk: Comprehensive research synopsis, meta-analysis, and epidemiological evidence. Gut 63:326-336, 2014
8. National Comprehensive Cancer Network: Genetic/Familial High-Risk Assessment: Colorectal. Version 3. https://www.nccn.org/, 2019
9. Katona BW, Yurgelun MB, Garber JE, et al: A counseling framework for moderate-penetrance colorectal cancer susceptibility genes. Genet Med 20:1324-1327, 2018
10. Rivera B, Castellsague E, Bah I, et al: Biallelic NTHL1 mutations in a woman with multiple primary tumors. N Engl J Med 373:1985-1986, 2015

11. Kuiper RP, Nielsen M, De Voer RM, et al: NTHL1 Tumor Syndrome. https://www.ncbi.nlm.nih.gov/books/NBK555473/
12. Grolleman JE, de Voer RM, Elsayed FA, et al: Mutational signature analysis reveals NTHL1 deficiency to cause a multi-tumor phenotype. Cancer Cell 35:256-266.e5, 2019
13. Chubb D, Broderick P, Dobbins SE, et al: Rare disruptive mutations and their contribution to the heritable risk of colorectal cancer. Nat Commun 7:11883, 2016
14. Belhadj S, Mur P, Navarro M, et al: Delineating the phenotypic spectrum of the NTHL1-associated polyposis. Clin Gastroenterol Hepatol 15:461-462, 2017
15. Fostira F, Kontopodis E, Apostolou P, et al: Extending the clinical phenotype associated with biallelic NTHL1 germline mutations. Clin Genet 94:588-589, 2018
16. Altaraihi M, Gerdes AM, Wadt K: A new family with a homozygous nonsense variant in NTHL1 further delineated the clinical phenotype of NTHL1-associated polyposis. Hum Genome Var 6:46, 2019
17. Belhadj S, Quintana I, Mur P, et al: NTHL1 biallelic mutations seldom cause colorectal cancer, serrated polyposis or a multi-tumor phenotype, in absence of colorectal adenomas. Sci Rep 9:9020, 2019
18. Genschel J, Littman SJ, Drummond JT, et al: Isolation of MutSbeta from human cells and comparison of the mismatch repair specificities of MutSbeta and MutSalpha. J Biol Chem 273:19895-19901, 1998
19. Carethers JM, Koi M, Tseng-Rogenski SS: EMAST is a form of microsatellite instability that is initiated by inflammation and modulates colorectal cancer progression. Genes (Basel) 6:185-205, 2015
20. Zehir A, Benayed R, Shah RH, et al: Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 23:703-713, 2017
21. Mandelker D, Zhang L, Kemel Y, et al: Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. JAMA 318:825-835, 2017
22. Kalia SS, Adelman K, Bale SJ, et al: Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): A policy statement of the American College of Medical Genetics and Genomics. Genet Med 19:249-255, 2017
23. Niu B, Ye K, Zhang Q, et al: MSIsensor: Microsatellite instability detection using paired tumor-normal sequence data. Bioinformatics 30:1015-1016, 2014
24. Middha S, Zhang L, Nafa K, et al: Reliable pan-cancer microsatellite instability assessment by using targeted next-generation sequencing data. JCO Precis Oncol 10.1200/PO.17.00084
25. Shen R, Seshan VE: FACETS: Allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res 44:e131, 2016
26. Alexandrov LB, Nik-Zainal S, Wedge DC, et al: Signatures of mutational processes in human cancer. Nature 500:415-421, 2013
27. www.github.com/mskcc/mutation-signatures
28. Pidsley R, Zotenko E, Peters TJ, et al: Critical evaluation of the Illumina MethylationEPIC BeadChip microarray for whole-genome DNA methylation profiling. Genome Biol 17:208, 2016
29. Kling T, Wenger A, Beck S, et al: Validation of the MethylationEPIC BeadChip for fresh-frozen and formalin-fixed paraffin-embedded tumours. Clin Epigenetics 9:33, 2017
30. Chen MH, Chang SC, Lin PC, et al: Combined microsatellite instability and elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) might be a more promising immune biomarker in colorectal cancer. Oncologist 24:1534-1542, 2019
31. Venderbosch S, van Lent-van Vliet S, de Haan AF, et al: EMAST is associated with a poor prognosis in microsatellite instable metastatic colorectal cancer. PLoS One 10:e0124538, 2015
32. Wang Q, Pierce-Hoffman E, Cummings BB, et al: Landscape of multi-nucleotide variants in 125,748 human exomes and 15,708 genomes. Nat Commun 11:2539, 2020
33. Karczewski KJ, Francioli LC, Tiao G, et al: The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581:434-443, 2020
34. Drost J, van Boxtel R, Blokzijl F, et al: Use of CRISPR-modified human stem cell organoids to study the origin of mutational signatures in cancer. Science 358:234-238, 2017
35. Elsayed FA, Grolleman JE, Ragunathan A, et al: Monoallelic NTHL1 loss-of-function variants and risk of polyposis and colorectal cancer. Gastroenterology 159:2241-2243.e6, 2020
36. Kraus C, Rau TT, Lux P, et al: Comprehensive screening for mutations associated with colorectal cancer in unselected cases reveals penetrant and non-penetrant mutations. Int J Cancer 136:E559-E568, 2015

APPENDIX

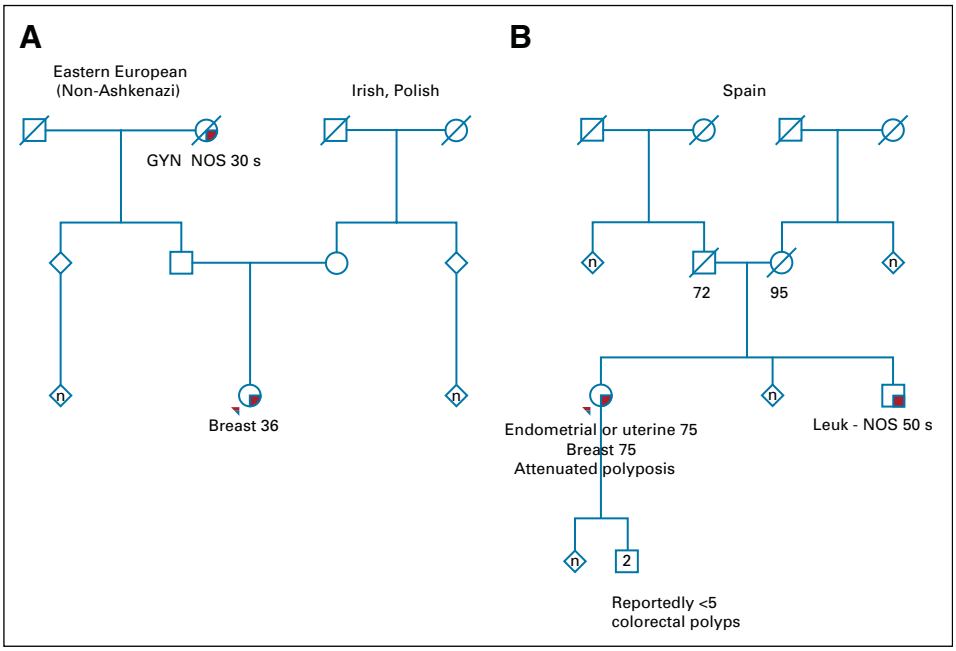

FIG A1. Three-generation pedigrees of identified biallelic *NTHL1* (A) and *MSH3* (B) patients. GYN NOS, gynecological not otherwise specified; NOS, not otherwise specified.