Pan-Cancer Interrogation of *MUTYH* Variants Reveals Biallelic Inactivation and Defective Base Excision Repair Across a Spectrum of Solid Tumors

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ABSTRACT

PURPOSE Biallelic germline pathogenic variants of the base excision repair (BER) pathway gene *MUTYH* predispose to colorectal cancer (CRC) and other cancers. The possible association of heterozygous variants with broader cancer susceptibility remains uncertain. This study investigated the prevalence and consequences of pathogenic *MUTYH* variants and *MUTYH* loss of heterozygosity (LOH) in a large pan-cancer analysis.

MATERIALS AND METHODS Data from 354,366 solid tumor biopsies that were sequenced as part of routine clinical care were analyzed using a validated algorithm to distinguish germline from somatic *MUTYH* variants.

RESULTS Biallelic germline pathogenic *MUTYH* variants were identified in 119 tissue biopsies. Most were CRCs and showed increased tumor mutational burden (TMB) and a mutational signature consistent with defective BER (COSMIC Signature SBS18). Germline heterozygous pathogenic variants were identified in 5,991 biopsies and their prevalence was modestly elevated in some cancer types. About 12% of these cancers (738 samples: including adrenal gland cancers, pancreatic islet cell tumors, nonglioma CNS tumors, GI stromal tumors, and thyroid cancers) showed somatic LOH for *MUTYH*, higher rates of chromosome 1p loss (where *MUTYH* is located), elevated genomic LOH, and higher COSMIC SBS18 signature scores, consistent with BER deficiency.

CONCLUSION This analysis of *MUTYH* alterations in a large set of solid cancers suggests that in addition to the established role of biallelic pathogenic *MUTYH* variants in cancer predisposition, a broader range of cancers may possibly arise in *MUTYH* heterozygotes via a mechanism involving somatic LOH at the *MUTYH* locus and defective BER. However, the effect is modest and requires confirmation in additional studies before being clinically actionable.

ACCOMPANYING CONTENT

Appendix

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INTRODUCTION

Cells are constantly exposed to both endogenous and exogenous oxidative stress—the former from byproducts of cellular respiration, the latter from exposures to various xenobiotics. MUTYH is a critical component of the base excision repair (BER) pathway and mediates repair of oxidative DNA damage from both endogenous and exogenous sources. Oxidative damage to DNA can lead directly to mutagenesis or programmed cell death.[1,2] Because of this, cells have multiple mechanisms to detect and repair oxidative DNA damage.

The most common product of oxidative damage to DNA is 8-oxo-7, 8-hydroxyguanine (8-oxoG).[3] The average level of 8-oxoG is estimated to be 1-2 per 10^6 guanine residues in nuclear DNA and is approximately an order of magnitude higher in mitochondrial DNA owing to higher exposure to the reactive oxygen species formed during cellular respiration.[4] 8-oxoG can pair with both adenine and cytosine during DNA replication leading to G:C to T:A transversion mutations. MUTYH, by excising mismatched adenine, prevents these mutations and is a key part of the BER pathway. *MUTYH* is widely expressed and particularly active in tissue with both high exposure to oxidative damage and frequent cell divisions.[5]

Defects in the BER pathway are associated with both increased mutational burden and carcinogenicity.[6] Individuals with homozygous or compound heterozygous (biallelic)

CONTEXT

Key Objective

Although biallelic germline *MUTYH* mutations predispose carriers to colorectal cancers, the relationship between heterozygous variants and cancer predisposition remains unclear. This study investigated the prevalence and consequences of germline pathogenic *MUTYH* variants and *MUTYH* loss of heterozygosity (LOH) in a large pan-cancer analysis.

Knowledge Generated

Within this population, just under two percent of patients had a *MUTYH* mutation, with the majority being heterozygous. Twelve percent of the heterozygous patients showed *MUTYH* LOH, higher rates of chromosome 1p loss, and higher COSMIC SBS18 signature scores reflective of base excision repair (BER) deficiency. These cancers also have a modestly elevated tumor mutational burden (TMB).

Relevance

Therefore, in addition to the established role of biallelic pathogenic *MUTYH* variants in bowel cancer predisposition, a broader range of cancers may possibly arise in *MUTYH* heterozygotes via a mechanism involving somatic LOH at the *MUTYH* locus and defective BER. This opens the way for future studies to explore novel therapeutic strategies for BER-deficient cancers.

germline *MUTYH* mutations develop *MUTYH*-associated polyposis (MAP), an autosomal recessive condition that typically manifests with adenomatous colorectal polyps and a greatly increased risk of colorectal cancer (CRC). Individuals with this syndrome are estimated to have an excess risk factor of 93-fold[7,8] and the syndrome is a significant contribution to the incidence of CRC in individuals younger than 55 years. In the absence of surveillance and treatment, with age, penetrance may reach nearly 100%.[9]

Both colon polyps and normal intestinal tissue from individuals with MAP show a higher mutation burden than age-matched controls as well as a distinct tumor mutational signature, COSMIC signature SBS18,[10] which is dominated by G:C -> T:A transversions. Nonetheless, among individuals with MAP, there is variation both in terms of total mutation burden as well as the overall contribution of the SBS18 signature.[11]

Whether monoallelic germline *MUTYH* carriers have an increased risk for cancer has been the subject of much controversy. Although some studies have found that heterozygous *MUTYH* mutations may predispose to CRC later in life, other studies have not,[12] and the most recent analysis has indicated there was benefit for early surveillance for colon cancer in *MUTYH* heterozygotes in the presence of family history[13] while a large-scale meta-analysis found that *MUTYH* variants were among the genes that conferred significant risk.[14] A large case-control study focusing on a European population found no association between monoallelic *MUTYH* carrier status and risk for colon, breast, or endometrial cancer,[15] while other studies have found an overall increase for several cancers.[16]

One possible carcinogenetic mechanism in heterozygous *MUTYH* carriers is somatic loss of the wild-type (WT) *MUTYH* allele, which may manifest as loss of heterozygosity (LOH).[17] In individuals with germline mutations in tumor suppressor genes such as *RB1* in retinoblastoma or *BRCA1/2* in breast/ovarian and prostate cancer, LOH at the *MUTYH* locus represents the second hit in accordance with Knudson's two-hit hypothesis.[18,19] Across all cancers, it is estimated that 16% of gene loci undergo LOH, but genomewide LOH rates are tumor-dependent and typically reflect rates of homologous recombination deficiency, with the highest average rates seen in adenoid cystic carcinoma (45%) and the lowest (0.1%) in thyroid carcinoma.[20]

A previous study using a small data set showed that although most cancers from heterozygous (monoallelic) *MUTYH* carriers showed no increase in tumor mutational burden (TMB), those with LOH at the *MUTYH* locus showed both higher TMB and association with the COSMIC SBS18 signature.[6] A larger study, using allele frequency (AF) in sequenced tumors to infer germline mutation status, found a higher-than-expected prevalence of monoallelic germline *MUTYH* mutations in patients with cancer but confirmed that monoallelic mutation on its own was not associated with increased TMB or the COSMIC SBS18 signature, while cancers that also had somatic LOH at the *MUTYH* locus were associated with both features.[21] Crucially, cancers from heterozygous *MUTYH* carriers that had LOH showed distinctive patterns of mutations in oncogenes such as *KRAS* and *PIK3CA*, suggesting that *MUTYH* inactivation, while not necessarily a driver, has the potential to accelerate tumor development, and is not a mere passenger during carcinogenesis.[21]

Here, we use the somatic/germline zygosity (SGZ) algorithm to infer germline and somatic *MUTYH* status in a very large data set from 354,366 tumor samples that had undergone comprehensive genomic profiling (CGP) using an next-

generation sequencing–based hybrid capture–based assay. We confirm the association of inherited biallelic pathogenic *MUTYH* variants with CRC and, much less frequently, other cancers. We also observed the occurrence of acquired LOH at the *MUTYH* locus across a range of cancer types in association with a monoallelic inherited pathogenic *MUTYH* variant. These cancers showed slightly increased TMB and the COSMIC SBS18 signature, consistent with a late-stage two-hit mechanism underlying the development of some cancers in *MUTYH* heterozygotes.

MATERIALS AND METHODS

Data

The study cohort consisted of tissue biopsies from 354,366 patients with solid tumors submitted for CGP during routine clinical care between August 2014 and February 2022. Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act compliance, was obtained from the Western Institutional Review Board (protocol 20152817).

CGP

CGP was performed on formalin-fixed paraffin-embedded tissue biopsy sections using FoundationOne or FoundationOne CDx (median coverage: >860×), both of which cover the full coding sequence of *MUTYH*. The histologic diagnosis of each case was confirmed on routine hematoxylin and eosin–stained slides, and all samples contained a minimum of 20% tumor nuclei. The CGP platform is a validated hybrid capture–based assay performed in a Clinical Laboratory Improvement Amendment–certified, College of American Pathologists–accredited, New York State–approved commercial laboratory (Foundation Medicine, Cambridge, MA).[22,23] F1 and F1CDx interrogate a total of 311 and 324 cancer-related genes, respectively, for base substitutions, short insertions and deletions, copy-number amplifications and homozygous deletions, and large genomic rearrangements, as well as microsatellite instability, TMB, and genomic LOH.

SGZ Algorithm

The germline or somatic origin of a particular *MUTYH* variant was computationally inferred using a previously described and validated algorithm (called SGZ) that leverages read depth and the local variability of single-nucleotide polymorphism allele frequencies.[24] This algorithm also predicts the zygosity of a variant in the tumor: heterozygous, homozygous, or uncertain. SGZ classifies a variant as inferred to be somatic or germline on the basis of its AF, considering the tumor content (proportion of neoplastic nuclei), tumor ploidy, and the local copy number. At the sequencing depths in this study, the SGZ algorithm is expected to have an accuracy of approximately 85% in predicting germline or somatic status.[24] Samples with at least one germline pathogenic *MUTYH* mutation were classified as germline biallelic (if a second germline alteration was found), germline with somatic (if a somatic alteration was found—no sample contained more than two variants), germline monoallelic with LOH at the *MUTYH* locus, or germline monoallelic without LOH (hereafter termed heterozygous). Samples with somatic *MUTYH* mutations were considered separately. Samples without any *MUTYH* mutations were designated as WT.

COSMIC SBS18 Signature

In samples with at least five somatic single-base substitutions (SBSs), each assessable mutation was classified as one of 96 subtypes (six substitution classes with all possible permutations of bases immediately 5′ and 3′ of the mutated base). These mutations were resampled 1,000 times and then compared with the first 30 COSMIC SBS signatures using methods previously described in the study by Zehir et al.[25]

Tumor Mutational Burden

TMB was estimated as previously described.[23] All synonymous and nonsynonymous short variants present at ≥5% variant AF were tallied. Potential germline variants were filtered out using published databases of known germline polymorphisms (including dbSNP, gnomAD, and ExAC), as well as by inferred germline status prediction via the SGZ algorithm. The final mutation number was divided by the total coding region (0.8 or 1.1 megabases for the FoundationOne and FoundationOne CDx assays, respectively). The TMB is reported in units of mutations per megabase of DNA (mut/Mb).

RESULTS

Prevalence of *MUTYH* Genomic Alterations

In a cohort of tissue biopsies from 354,366 patients, 6,572 (1.9%) harbored a genomic alteration in *MUTYH*. Of these, 6,110 harbored a germline alteration: 119 with biallelic inheritance, nine with a germline alteration and a somatic alteration, and 5,982 with monoallelic inheritance. Of the 5,982 samples with monoallelic *MUTYH* mutations, 5,244 (88%) were predicted to be heterozygotes, while 738 (12%) were predicted to have lost the WT *MUTYH* allele (LOH; Figs 1A and 1B). The variant allele frequencies were highest in the LOH sample group and lowest in the group with somatic *MUTYH* mutations (Fig 1C).

The two most prevalent germline pathogenic *MUTYH* variants were p.G396D (rs36053993) and p.Y179C (rs34612342) and their distributions were similar in the sample groups with biallelic inheritance, heterozygous status, and LOH (Fig 1D). Other recurrent germline alterations included truncating mutations, splice site alterations, and rare pathogenic

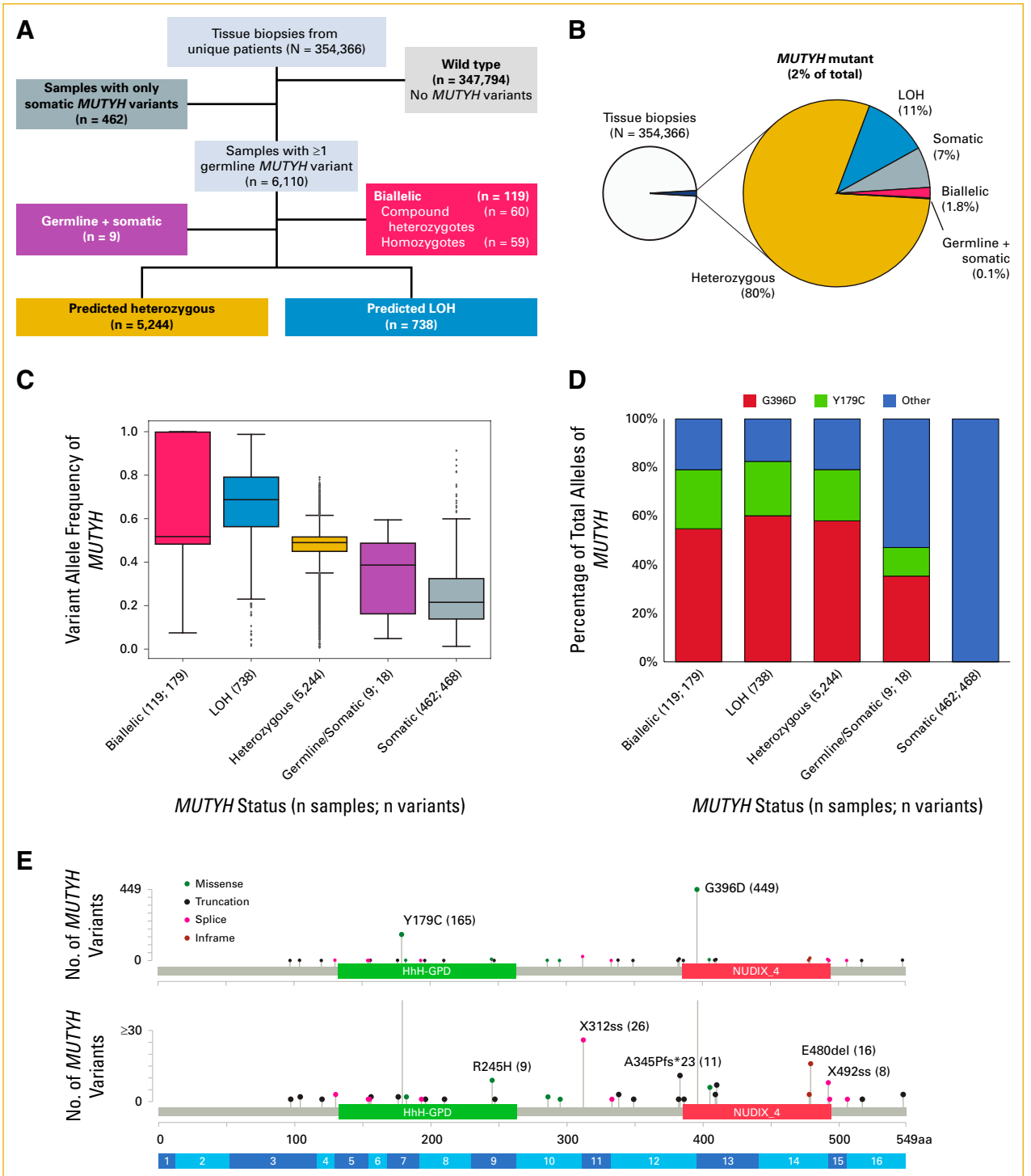

FIG 1. *MUTYH*-altered samples. (A) CONSORT diagram showing grouping of samples. Samples with somatic MUTYH alterations including pathogenic short variants, genomic rearrangements that result in truncations, and gene deletions. Samples with at least one MUTYH germline variant were further subdivided into samples with two germline variants (compound heterozygous) or one variant at AF of 100% (homozygous; biallelic), samples with one germline and one somatic variant (germline + somatic), and samples with one *MUTYH* variant that had undergone LOH at the *MUTYH* locus or not (heterozygous). Germline/somatic status and zygosity of each variant were predicted on the basis of the SGZ algorithm and heuristic criteria (see Materials and Methods). (B) The relative proportion of each group of the 6,572 *MUTYH*-altered samples. (C) The variant AF of all *MUTYH* mutations in each sample group: for biallelic, germline/somatic, and somatic, the numbers in parentheses are presented as samples:variants, as these samples can have more than one variant. (D) The relative proportions of the two most common mutations, G396D and Y179C, and other mutations in each sample group. (E) Lollipop plot (with zoom in) of the positions of the *MUTYH* variants predicted to be germline. AF, allele frequency; LOH, loss of heterozygosity; SGZ, somatic/germline zygosity.

changes such as splice site X312 (rs77542170), p.E480del (rs587778541), p.A345Pfs*23 (rs587778536), p.R245H (rs140342925), and splice site X492 (rs140288388) (Fig 1E).

Relationship Between *MUTYH* Mutation Status and Mutation Signatures

As expected, tumor samples with biallelic germline pathogenic variants of *MUTYH* had a dominant COSMIC SBS18 signature, characteristic of reactive oxygen species damage that is left unrepaired owing to a defective BER pathway (Fig 2A). However, COSMIC SBS18 scores were also significantly higher in samples with a germline *MUTYH* pathogenic variant that had undergone LOH at the *MUTYH* locus in comparison with samples that were heterozygous for *MUTYH* (Fig 2A); there was no significant difference between samples that were heterozygous, had germline and somatic mutations, had only somatic mutations, or were WT at the locus (Fig 2B). The same trends were observed for COSMIC SBS36,[26,27] another signature associated with the BER-deficient state (Appendix Fig A1). Examining the samples with one *MUTYH* germline variant that had undergone LOH, samples with the more deleterious Y179C had a higher median SBS18 score than those with G396D (Fig 2C), although the difference missed statistical significance.

Associations Between *MUTYH* Status, Chr 1p Loss, Genomic LOH, and TMB

Samples with *MUTYH* LOH also had a higher rate of Chr 1p loss (Fig 3A), suggesting that this is a common pathway by which *MUTYH* is inactivated. Genomic LOH (gLOH), which can be a consequence of homologous recombination deficiency, was higher in the sample group with LOH at the *MUTYH* locus, with a median gLOH of 10%, IQR of 5.3%-17%, while the heterozygous group had a median of 7.5%, IQR 3.5%-13% (P = 2.1E-18; Fig 3B), which did not differ significantly from WT. Thus, LOH at the *MUTYH* locus may arise through loss of an entire chromosome or homologous recombination repair deficiency without chromosome loss.

TMB was highest in the samples with germline and somatic variants or somatic variants only: median TMB 45 and 10 mutations/megabase, respectively (Fig 3C), suggesting that some of the somatic *MUTYH* variants are passenger mutations in high TMB samples. Nonetheless, 27% of samples with biallelic *MUTYH* variants met the 10 mutations/Mb threshold currently used as a pan-cancer biomarker predicting benefit from immune checkpoint inhibition.[28] The sample groups with heterozygous germline variants or LOH had a rate of TMB ≥10, similar to the WT samples (17%-18%; Fig 3D).

Relationship Between *MUTYH* Mutation Status and Cancer Type

Tumor samples with biallelic germline pathogenic variants of *MUTYH* were predominantly CRCs (78/119, 66%), but also included some small intestine and other cancers (Fig 4A). We observed only six cases with inherited biallelic pathogenic *MUTYH* variants in over 38,000 breast cancers, consistent with the negative findings in a case control study[29] that superseded initial reports of a possible increase in breast cancer risk in *MUTYH*-mutated patients.[30] Similarly, there were only two biallelic patients in over 15,000 patients with prostate cancer (Appendix Table A1).

At the same time, samples with monoallelic germline *MUTYH* pathogenic variants and somatic LOH were not normally distributed across cancer types (Fig 4A). Although 104/738 (14%) samples with both a monoallelic pathogenic variant and LOH were CRCs, we did not observe an enrichment of this genotype in the CRC cases, nor was there an excess of monoallelic-only pathogenic variants in CRCs.

Cancer types showing an apparent excess of germline *MUTYH* heterozygous alterations included many endocrine tumors: pancreas islet cell tumors (3.3% with germline variants), adrenal gland (3.0%), thyroid medullary (2.4%), thymus (2.3%), as well as germ cell tumors (3.0%), carcinoids (2.9%), small intestine (2.6%), and bone sarcomas (2.2%) compared with the *MUTYH* germline variant frequency in CRC (2.0%), the overall cohort (1.8%), and the estimate of *MUTYH* heterozygosity in the general population as derived from gnomAD (1.3%; Appendix Table A1). In general, the prevalence of monoallelic *MUTYH* variants was low for hormonally driven cancers (breast, prostate, uterine, endometrial, and ovarian, 1.3%-1.7%).

Notably, the highest rates of germline *MUTYH* variants plus somatic LOH, with prevalence of over 2%, were found in adrenal gland cancers and pancreatic islet cell tumors (Fig 4A). Some of the cancer types with more prevalent LOH were those with higher rates of chromosome 1p loss, with pancreas islet cell tumors, adrenal gland tumors, bone sarcomas, and GI stromal tumors all having high rates than the cohort (Fig 4B).

Examining the LOH samples' SBS18 scores by cancer type, we also observed adrenal gland tumors and pancreas cell tumors having high median scores (0.83 and 0.74, respectively). Esophageal cancer and CRC had median scores of 0.22 and 0.13, respectively. Cancer types with typically high mutational burden such as melanoma and bladder cancer had low SBS18 scores (Fig 5).

DISCUSSION

The findings from this large pan-cancer analysis suggest that LOH for *MUTYH* is seen in up to 12% of cancers originating in patients who carry a heterozygous germline pathogenic variant in *MUTYH*, an estimate that is in line with an earlier study on the basis of The Cancer Genome Atlas data.[21] The majority of germline mutations in this study were G396D, which retains partial functional activity, and Y179C, which demonstrates severely compromised BER function.[31]

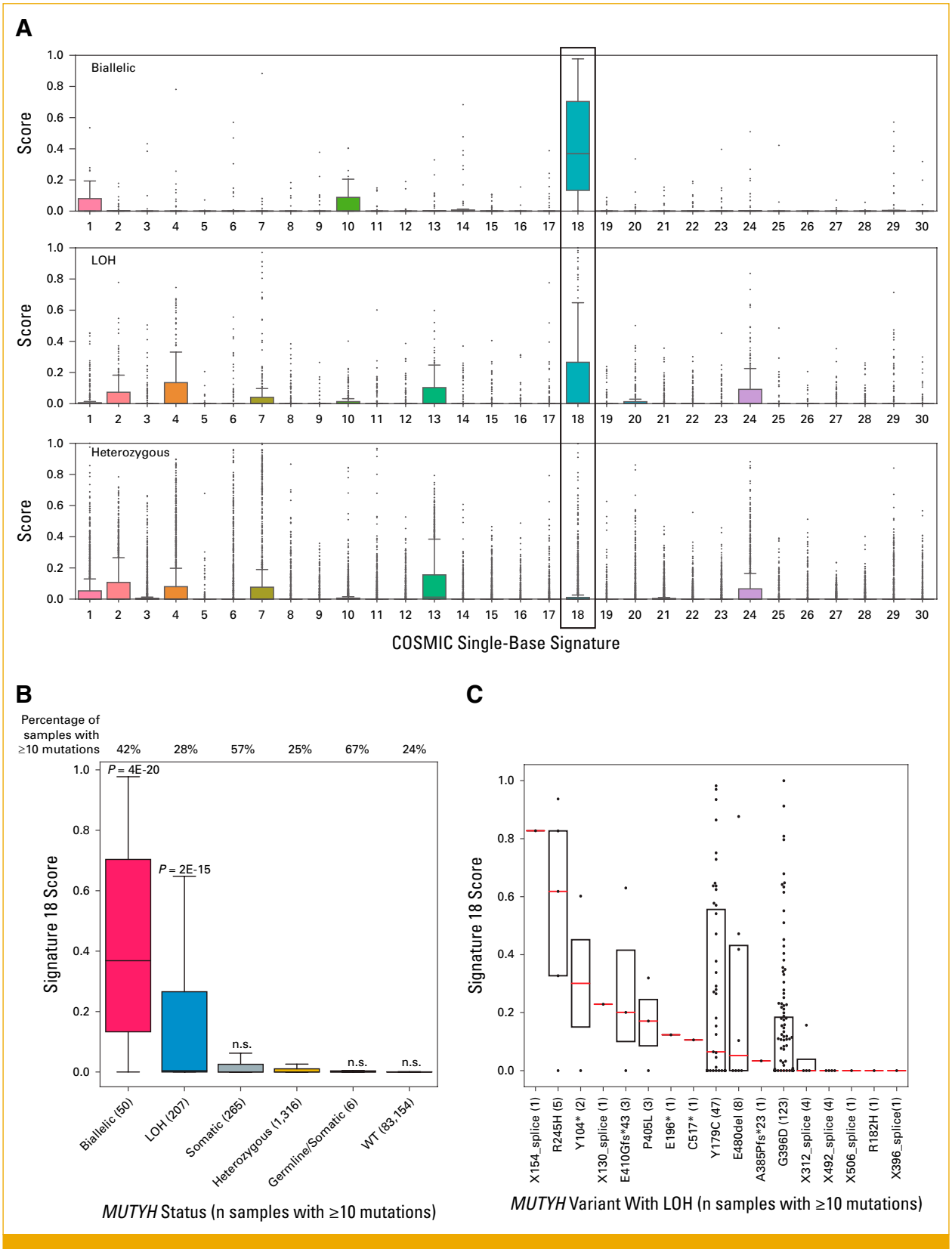

FIG 2. Single-base signatures in *MUTYH*-altered samples. (A) COSMIC SBS 1-30 scores in samples that are biallelic, LOH, and heterozygous at the *MUTYH* locus. Only samples with ≥10 assessable mutations (single-base substitutions) were included in the analysis. (B) COSMIC SBS 18 scores among all samples. The percentage of samples in each group that met the ≥10 assessable mutation threshold is indicated at the top. Each group was compared pairwise to the heterozygous group using a two-sided Mann-Whitney *U* test (*P* value above each boxplot when significant). Whiskers indicate 1.5× the IQR or maximum, whichever is smaller. (C) The COSMIC SBS18 scores in LOH samples with ≥10 assessable mutations, grouped by *MUTYH* variant, arranged in order of median score. LOH, loss of heterozygosity; n.s., nonsignificant; SBS, single-base substitution.

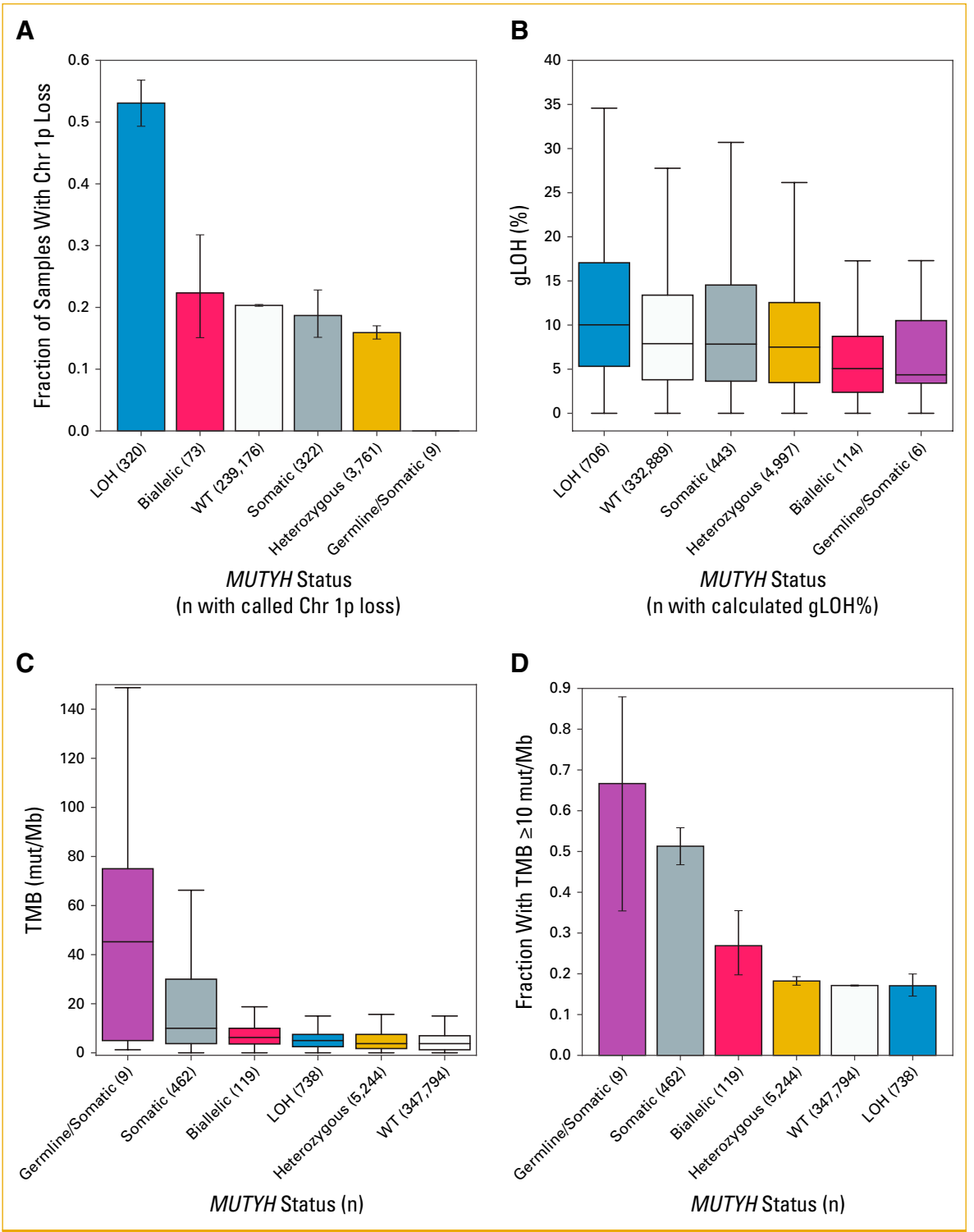

FIG 3. Associations of genomic features with *MUTYH* LOH. (A) For each sample group, the fraction of samples with detectable loss of chromosome 1p. The n in parenthesis indicates the number of samples where chromosome loss could be assessed. (B) For each sample group, gLOH scores presented as a boxplot, ordered by median. The n in parenthesis indicates the number of samples where gLOH could be assessed. (C) As in (B) but for tumor mutational burden (TMB) in mutations/megabase. (D) Similar to (C) but for each sample group, the fraction of samples with TMB ≥10 mutations/megabase. gLOH, genomic loss of heterozygosity; LOH, loss of heterozygosity.

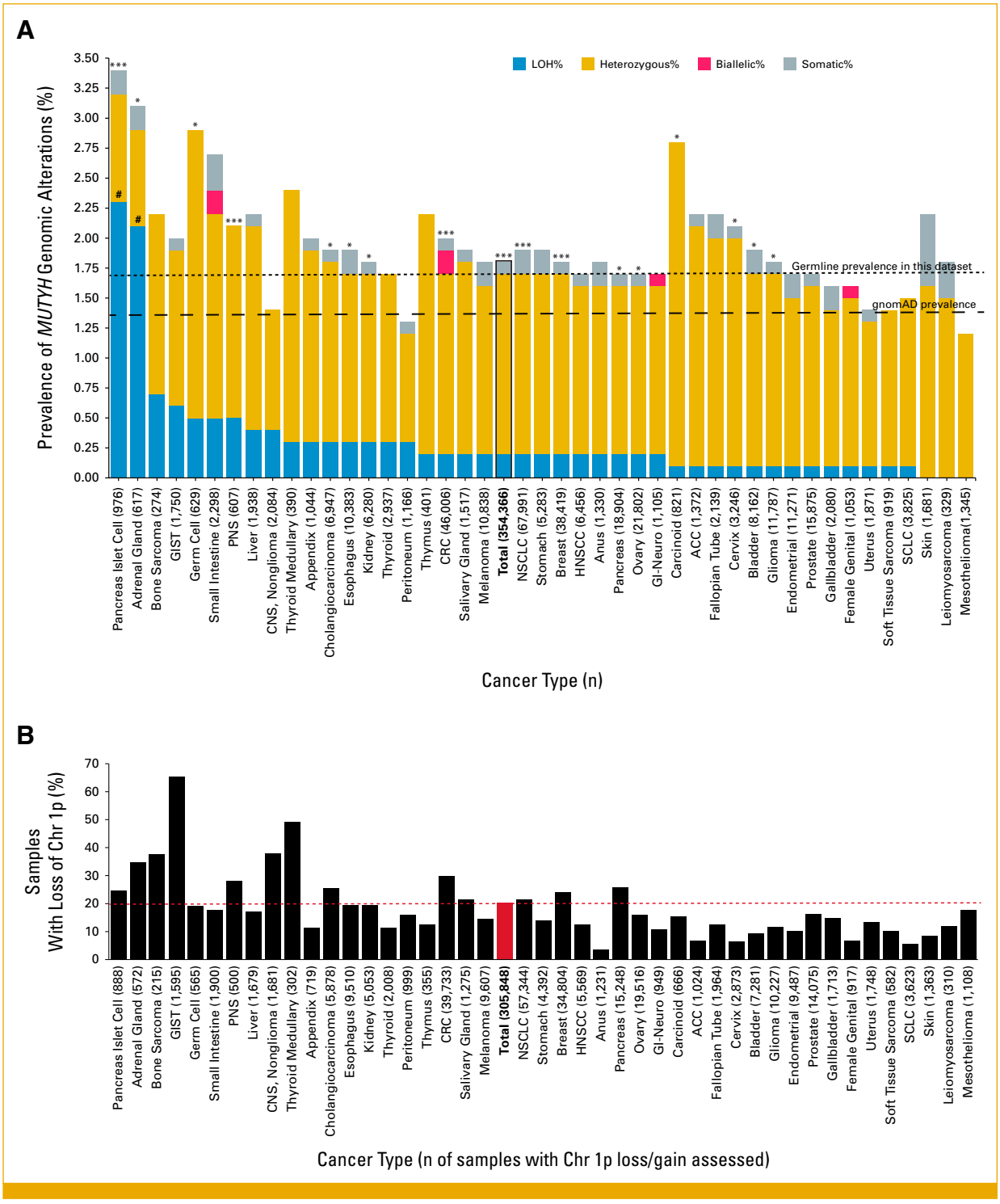

FIG 4. Cancer types with high rates of *MUTYH* LOH. (A) The prevalence of *MUTYH* alterations in each cancer type, comparing the number of *MUTYH* heterozygotes and those undergoing LOH at the *MUTYH* locus. Prevalence of the total cohort is provided in bold. Large dashed line denotes the prevalence of *MUTYH* variants included in this study in the general population of gnomAD. The thinner dashed line is the prevalence of germline *MUTYH* variants in the entire cohort. Asterisk denotes significant enrichment of LOH at the *MUTYH* locus in comparison with the number with heterozygote status within the monoallelic samples (Fisher exact test with Benjamini-Hochberg correction for multiple testing, false discovery rate <0.05). Cases with a somatic mutation at the *MUTYH* locus are labeled somatic in gray. More data are provided in Appendix Table A1. (B) Percentage of samples of each cancer type that had detectable loss of chromosome 1p. The n in parenthesis indicates the number of samples where chromosome loss could be assessed. Dashed line represents the fraction of samples in the total cohort with chromosome 1p loss. Significant enrichment of LOH compared with heterozygous prevalence: #FDR <.0001. Enrichment over gnomAD (continued on following page)

FIG 4. (Continued). prevalence: *FDR <.01; **FDR <.001, ***FDR <.0001. ACC, adenoid cystic carcinoma; CRC, colorectal cancer; FDR, false discovery rate; GIST, GI stromal tumor; HNSCC, head and neck squamous cell carcinoma; LOH, loss of heterozygosity; NSCLC, non–small-cell lung cancer; PNS, peripheral nerve sheath; SCLC, small-cell lung cancer.

Both are European founder variants and together account for approximately 80% of *MUTYH* pathogenic variants in individuals of European ancestry.[32] Although homozygosity for deleterious *MUTYH* variants is relatively rare, heterozygosity effects a considerably larger number of individuals and may predispose them to additional non-CRC malignancies.

Most studies of monoallelic *MUTYH* variants and cancer susceptibility have been hampered by relatively small data sets, owing both to the rarity of the genotype and the difficulty and expense of genotyping a sufficiently large number of patients for statistical significance. Studies on *MUTYH* heterozygosity and gene environment interactions are further limited, as capturing sufficiently granular data on potential exposures is difficult. However, *MUTYH* heterozygosity could, in tandem with other environmental exposures, increase oxidative DNA damage and increase DNA instability.

The SGZ algorithm employed here, by using tumor samples to infer germline mutation status, allows greater efficiency for larger population studies, and therefore may allow better understanding of how *MUTYH* variants affect tumorigenesis

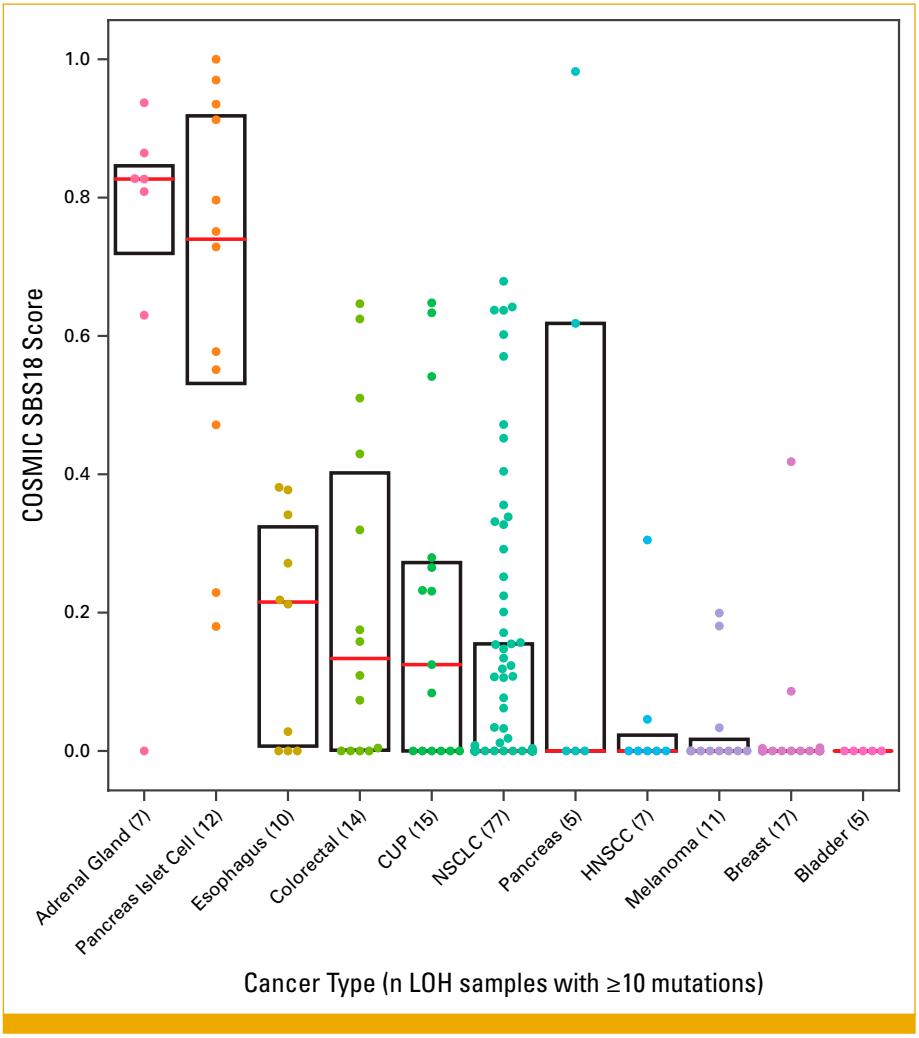

FIG 5. COSMIC SBS18 score in samples with *MUTYH* LOH across different cancer types. Samples with ≥10 assessable mutations, grouped by cancer type, arranged in order of median score. CUP, carcinoma of unknown primary; HNSCC, head and neck squamous cell carcinoma; LOH, loss of heterozygosity; NSCLC, non–small-cell lung cancer; SBS, single-base substitution.

while also informing future clinical studies to target tumors with altered MUTYH function.

Although our data do not necessarily imply that monoallelic *MUTYH* variants are at a markedly increased susceptibility for cancer overall, it does suggest, as have other studies, that there may be an increase in susceptibility to some types of cancer, but it does not appear to play a role in breast or prostate cancer. Within these cancers, LOH at the *MUTYH* locus is likely a turning point in so far as the BER pathway is markedly affected, as reflected in the higher SBS18 scores in the absence of increased TMB.

One obvious weakness of our data set was that the patients were selected for biopsy and sequencing of their tumors, which may have introduced a selection bias to more advanced/metastatic cancers, implying a potential overestimate of *MUTYH* alteration abundance, and because of this, it is difficult to draw strong conclusions about the risk *MUTYH* carriers face. In addition, using the SGZ algorithm, we could not detect the presence of copy-neutral LOH.

Some of the apparently contradictory evidence for the relationship between monoallelic *MUTYH* variants and cancer risk may reflect that a single functional copy of *MUTYH* suffices under most circumstances to prevent BER deficiency and an accumulation of oxidative damage, while somatic loss of the second allele will lead to BER deficiency. Our data herein suggest that germline *MUTYH* carriers have a modest increase in susceptibility to some cancers, such as adrenal cancers, pancreatic neuroendocrine tumors (PanNETs), and potentially to cancers characterized by a high incidence of LOH at chromosome 1p.

Interestingly, germline *MUTYH* mutations have been reported in the literature as being associated with PanNETs, which in turn harbor increased mutation burden and more pronounced COSMIC SBS18 signatures.[33] Some cancers, such as PanNETs[34] as well as astrocytoma, are more prone to have loss of chromosome 1p.[35] As our data confirm, chromosome 1p LOH (including copy-neutral LOH) happen at varying rates in different cancers and likely requires further study. The high rates of germline *MUTYH* alteration plus somatic LOH seen in adrenal gland tumors might be, in part, a consequence of the overall high rates of LOH in adrenal cancers. In our study, cancers with heterozygous *MUTYH* status had neither a significantly elevated TMB nor an increase in COSMIC 18 signature, consistent with a single functioning allele being sufficient for BER.[30]

Cancer cells depend on the BER pathway to overcome oxidative-induced DNA damage. Sensitivity and resistance to chemotherapies that act, at least in part, by causing oxidative DNA damage (eg, cisplatin, alkylating agents, and ionizing radiation) may theoretically be affected by alterations in BER function.[36,37] An intact BER pathway is required to overcome oxaliplatin- and cisplatin-induced transcription arrest[38] and BER inhibitors have been used as sensitizing agents to radiotherapy.[28] It is also possible that BER deficiency could be exploited to intentionally increase tumor mutational burden by use of DNA-damaging agents.[39] At the same time, it has been speculated on the basis of in vitro evidence that MUTYH may have evolved to be more than a BER enzyme and may be a multifunctional scaffold for rapid DNA damage response signaling,[40] playing an important role in tandem with the mismatch repair pathway to determine cell fate in the presence of DNA damage.[41]

Evidence from *Mutyh*$^{-/-}$ knockout mice indicates that lack of functional Mutyh only modestly increases the mutation rate, and less so than when there is loss of mismatch repair. Our observations in MUTYH-deficient human cancers aligns with these findings from mouse models. Paradoxically, however, in mice, a double knockout of *Msh2*$^{-/-}$ and *Mutyh*$^{-/-}$ led to higher mutation rates, yet lower tumor volume burdens.[42] Similar evidence from a case study in two siblings with MAP syndrome found that the individual with concurrent *MSH6* mutation had a milder phenotype.[43] This leaves the possibility that loss of MUTYH function may alter the progression of cancer in unpredictable ways depending on the concurrent presence or absence of an intact mismatch repair pathway and perhaps reflecting differences in the propensity for the resulting cancers to elicit a strong host immune response. There is much scope for clinical research to explore biologically informed approaches to the treatment of BER-deficient cancers, including both MAP-associated cancers and those arising in the context of an inherited pathogenic *MUTYH* variant and acquired LOH at that locus.

In conclusion, we present a large pan-cancer interrogation of MUTYH alterations in solid tumor malignancies using a real-world cohort and NGS analyses performed as part of routine clinical care. We show, unexpectedly, that biallelic *MUTYH* inactivation resulting from a germline heterozygous *MUTYH* mutation accompanied by somatic LOH occurs (at a low prevalence) in several noncolorectal malignancies including adrenal cancers, pancreatic islet cell tumors, and primary neurologic tumors (eg, PanNETs). Such cancers are characterized by deficient BER function manifesting as greater somatic mutation burden with significant contribution from the COSMIC SBS18 mutational signature, despite a rate of genomewide LOH below what is considered clinically actionable. This, in turn, may have therapeutic implications for checkpoint immunotherapy or synthetic lethality approaches that take advantage of defective base excision DNA repair.

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APPENDIX

TABLE A1. Pan Cancer Interrogation of MUTYH Variants by Cancer Type

Cancer Type	Total, n	Nongermline	Heterozygous	LOH	Germline/ Nongermline	Biallelic	% MUTYH Mutation	Odds Ratio LOH *v* het	FDR_bh_ LOH *v* het
Pancreas islet cell	976	2	9	22	0	0	3.50	17.872	0
Adrenal gland	617	1	5	13	0	0	3.18	18.788	0
Bone sarcoma	274	0	4	2	0	0	2.24	3.56	0.932
GIST	1,750	1	23	10	0	0	1.98	3.118	0.129
Germ cell	629	0	15	3	0	0	2.95	1.423	1
Small intestine	2,298	6	40	12	0	5	2.82	2.15	0.317
PNS	607	0	10	3	0	0	2.19	2.136	0.932
Liver	1,938	2	32	8	0	0	2.22	1.785	0.932
CNS, nonglioma	2,084	0	21	9	0	0	1.46	3.071	0.147
Thyroid medullary	390	0	8	1	0	0	2.36	0.888	1
Appendix	1,044	1	17	3	0	0	2.05	1.255	1
CCA	6,947	8	105	20	0	0	1.95	1.363	0.932
Esophagus	10,383	16	145	32	0	0	1.89	1.594	0.307
Kidney	6,280	6	90	18	1	1	1.88	1.432	0.932
Thyroid	2,937	1	41	9	0	1	1.80	1.567	0.932
Peritoneum	1,166	1	11	3	0	0	1.30	1.942	1
Thymus	401	0	8	1	0	0	2.30	0.888	1
CRC	46,006	56	704	104	1	78	2.09	1.058	1
Salivary gland	1,517	2	25	3	0	0	2.02	0.852	1
Melanoma	10,838	26	149	22	1	3	1.89	1.051	1
Total	354,366	462	5,244	738	9	119	1.89	nan	nan
NSCLC	67,991	111	989	129	2	7	1.85	0.911	1
Stomach	5,283	8	79	9	0	0	1.85	0.807	1
Breast	38,419	42	563	85	1	6	1.85	1.082	1
HNSCC	6,456	9	92	11	0	1	1.78	0.847	1
Anus	1,330	2	19	2	0	0	1.76	0.747	1
Pancreas	18,904	14	274	30	0	2	1.72	0.769	0.932
Ovary	21,802	22	305	39	0	3	1.72	0.903	1
GI-neuro	1,105	0	15	2	0	1	1.66	0.947	1
Carcinoid	821	0	22	1	0	0	2.88	0.322	1
ACC	1,372	1	28	2	0	0	2.31	0.506	1
Fallopian tube	2,139	4	40	2	0	0	2.20	0.354	0.932
Cervix	3,246	4	61	4	0	0	2.17	0.463	0.932
Bladder	8,162	17	134	10	0	0	2.01	0.524	0.459
Glioma	11,787	11	186	14	0	0	1.82	0.526	0.239
Endometrial	11,271	22	158	10	1	3	1.75	0.442	0.147
Prostate	15,875	14	237	9	0	2	1.68	0.261	0
Gallbladder	2,080	5	27	2	0	0	1.66	0.525	1
Female genital	1,053	0	15	1	0	1	1.64	0.473	1
Uterus	1,871	2	23	2	0	0	1.46	0.617	1
Soft tissue sarcoma	919	0	12	1	0	0	1.43	0.592	1
SCLC	3,825	0	52	2	0	0	1.43	0.271	0.469
Skin	1,681	10	27	0	1	0	2.31	0	0.515
Leiomyosarcoma	329	1	5	0	0	0	1.86	0	1
Mesothelioma	1,345	0	16	0	0	0	1.20	0	1

Abbreviations: ACC, adenoid cystic carcinoma; CCA, cholangiocarcinoma; CRC, colorectal cancer; FDR, false discovery rate (Benjamini-Hochberg corrected); GIST, GI stromal tumor; het, heterozygous; HNSCC, head and neck squamous cell carcinoma; LOH, loss of heterozygosity; NA, not applicable; NSCLC, non–small-cell lung cancer; PNS, peripheral nervous system; SCLC, small-cell lung cancer.

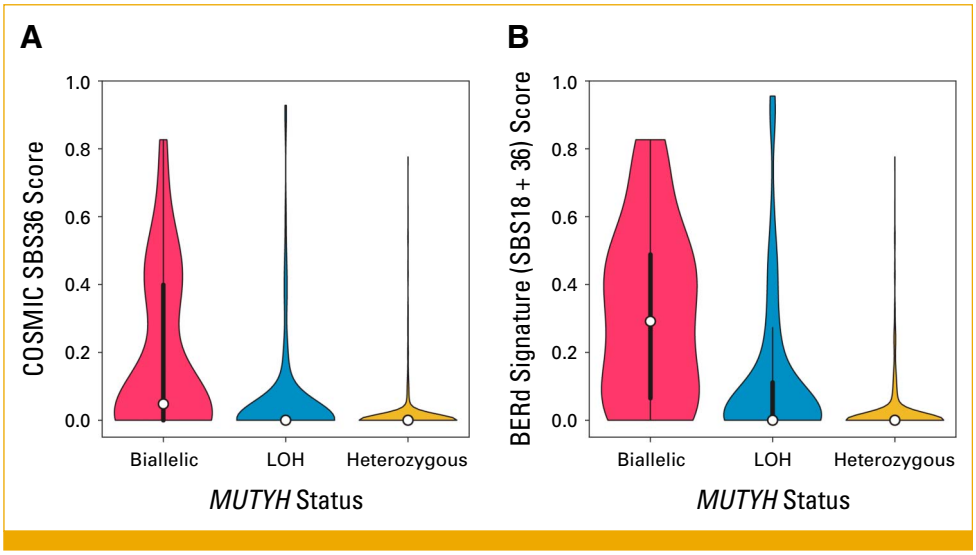

FIG A1. COSMIC signature 36. (A) COSMIC signature 36, indicative of defective base excision repair, including DNA damage due to reactive oxygen species, due to biallelic germline or somatic MUTYH mutations. It is similar to SBS18. SBS36 scores in each sample group, in samples with ≥10 assessable mutations. (B) Same as in (A) but adding up the SBS18 and SBS36 signature. LOH, loss of heterozygosity; SBS, single-base substitution.