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Risk of extracolonic cancers for people with biallelic and monoallelic mutations in *MUTYH*

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Additional Supporting Information may be found in the online version of this article.

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Germline mutations in the DNA base excision repair gene *MUTYH* are known to increase a carrier's risk of colorectal cancer. However, the risks of other (extracolonic) cancers for *MUTYH* mutation carriers are not well defined. We identified 266 probands (91% Caucasians) with a *MUTYH* mutation (41 biallelic and 225 monoallelic) from the Colon Cancer Family Registry. Mutation status, sex, age and histories of cancer from their 1,903 first- and 3,255 second-degree relatives were analyzed using modified segregation analysis conditioned on the ascertainment criteria. Compared with incidences for the general population, hazard ratios (HRs) (95% confidence intervals [CIs]) for biallelic *MUTYH* mutation carriers were: urinary bladder cancer 19 (3.7–97) and ovarian cancer 17 (2.4–115). The HRs (95% CI) for monoallelic *MUTYH* mutation carriers were: gastric cancer 9.3 (6.7–13); hepatobiliary cancer 4.5 (2.7–7.5); endometrial cancer 2.1 (1.1–3.9) and breast cancer 1.4 (1.0–2.0). There was no evidence for an increased risk of cancers at the other sites examined (brain, pancreas, kidney or prostate). Based on the USA population incidences, the estimated cumulative risks (95% CI) to age 70 years for biallelic mutation carriers were: bladder cancer 25% (5–77%) for males and 8% (2–33%) for females and ovarian cancer 14% (2–65%). The cumulative risks (95% CI) for monoallelic mutation carriers were: gastric cancer 5% (4–7%) for males and 2.3% (1.7–3.3%) for females; hepatobiliary cancer 3% (2–5%) for males and 1.4% (0.8–2.3%) for females; endometrial cancer 3% (2%–6%) and breast cancer 11% (8–16%). These unbiased estimates of both relative and absolute risks of extracolonic cancers for people, mostly Caucasians, with *MUTYH* mutations will be important for their clinical management.

What's new?

People who have a mutation in the *MUTYH* gene have an increased risk of colorectal cancer. But are they also at higher risk for other types of cancer? In this study, the authors found that people with one mutated copy of *MUTYH* (monoallelic) have an increased risk of gastric, liver, breast and endometrial cancers, while people with two mutated copies (biallelic) have an increased risk of bladder and ovarian cancers. This information will be useful for risk assessment in patients and families who carry *MUTYH* mutations.

Germline mutations in the base excision repair gene, *MUTYH* (MIM# 604933), cause increased risks of colorectal adenomas and carcinomas,[1] presumably due to an increase in unrepaired, 8-oxoG-induced somatic G:C to T:A transversions in tumor suppressor genes.[2]

Biallelic (compound heterozygous or homozygous) *MUTYH* mutations, *i.e.*, inherited from both parents, occurring in 0.01–0.04% of the Caucasian population, are associated with a 18- to 100-fold increased risk of colorectal adenomas and cancer compared with the general population[3–6]; commonly known as *MUTYH*-associated polyposis (MAP) (OMIM #608456).[1,7] The risks of extracolonic cancers for biallelic mutation carriers, however, are not well defined.[8–16] Several studies have reported an increased risk of duodenal cancer for biallelic mutation carriers.[7,12,17,18] Some studies have also reported an increased risk of bladder, ovarian, skin,[12] breast[12,19] and endometrial[10,13] cancer for biallelic mutation carriers; while other studies failed to confirm associations with breast[11,16,20] or endometrial[12,21] cancer. Case reports of cancers of the sebaceous glands,[9,10,22] hair follicles,[23] stomach[18] and thyroid[9,10,12,15,18] in biallelic mutation carriers have been documented but these studies did not

determine whether the incidence of these cancers exceeded the expected population risk.

Monoallelic (heterozygous) *MUTYH* mutations, *i.e.*, inherited from only one parent, occurring in 1–2% of the Caucasian population,[24] are associated with a moderately increased risk of colorectal cancer.[3,5,6,25] Previous studies have reported an increased risk of gastric, liver and endometrial[26] and breast cancer[27–29] for monoallelic mutation carriers while other studies did not find statistical evidence for an increased risk of breast[11,20] or liver[30] cancer.

Given the rarity of biallelic and monoallelic *MUTYH* mutation carriers, most of the previous studies have been underpowered to provide reliable estimates for the risks of extracolonic cancers. Clarity on these cancer risks is important for the clinical management of *MUTYH* mutation carriers. In the current study, using a large dataset from the Colon Cancer Family Registry, we estimated extracolonic cancer risks for people with biallelic and monoallelic *MUTYH* mutations.

Material and Methods

Study sample

The study sample was from the Colon Cancer Family Registry that has been described in detail elsewhere[31] and at www.coloncfr.org. Between 1997 and 2012, the Colon Cancer Family Registry recruited families *via*: population-based probands who were recently diagnosed colorectal cancer cases from state or regional population cancer registries in the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina, and Hawaii), Australia (Victoria) and Canada (Ontario); and clinic-based probands who were enrolled from multiple-case families referred to family cancer clinics in the USA (Mayo Clinic, Rochester, Minnesota and Cleveland Clinic, Cleveland, Ohio), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney) and New Zealand (Auckland). Probands were asked for permission to contact their relatives to seek their enrolment in the Cancer Family Registry. For population-based families, first-degree relatives of probands were recruited and recruitment was extended to more distant relatives by some registries. For clinic-based families, recruitment was based on availability but attempts were made to recruit up to second-degree relatives of affected individuals (detailed by Newcomb *et al.*[31]). Informed consent was obtained from all study participants, and the study protocol was approved by the institutional research ethics review board at each registry.

Data collection

Information on demographics, personal characteristics, personal and family history of cancer, cancer-screening history, history of polyps, polypectomy and other surgeries was obtained by questionnaires from all probands and participating relatives. Participants were followed approximately every 5 years after baseline to update this information. For the present study, each individual's lifetime cancer history was based on the most recent data (baseline or most recent follow-up). Reported cancer diagnoses and age at diagnosis were confirmed using pathology reports, medical records, cancer registry reports and death certificates, where possible. We collected family history of cancer from all participants, thus may obtain multiple reports on a single individual. If so and reports conflict or vary, we used the specific protocol of algorithms for selecting reports of cancer. For example, in Australia reports of cancer were selected over reports of no cancer, detailed dates of death/birth are selected over estimates and relative sources were selected as follows (in the hierarchy): self-report; the spouse or partner; a parent or adult son/daughter; brother or sister; grandparent or grandchild; aunt, uncle, nephew or niece; cousin; other. The tumor anatomic location and histology were coded and stored using the International Classification of Diseases for Oncology (ICD-O).[32] We attempted to obtain blood samples from all participants and tumor tissue samples from participants affected with colorectal cancer.

MUTYH mutation testing

As previously described by Cleary *et al.*[3] genomic DNA extracted from each proband was tested for 12 previously identified *MUTYH* variants: c.536A > G p.(Tyr179Cys), c.1187G > A p.(Gly396Asp), c.312C > A p.(Tyr104Ter), c.821G > A p.(Arg274Gln), c.1438G > T p.(Glu480Ter), c.1171C > T p.(Gln391Ter), c.1147delC p.(Ala385ProfsTer23), c.933 + 3A > C p.(Gly264TrpfsX7), c.1437_1439delGGA p.(Glu480del), c.721C > T, p.(Arg241Trp), c.1227_1228dup p.(Glu410GlyfsX43) and c.1187-2A > G p.(Leu397CysfsX89) using the MassArray MALDI-TOF Mass Spectrometry (MS) system (Sequenom, San Diego, CA). To confirm the *MUTYH* mutation and identify additional mutations, screening of the entire *MUTYH* coding region, promoter and splice site regions was performed on all samples exhibiting MS mobility shifts using denaturing high-performance liquid chromatography (Transgenomic Wave 3500HT System; Transgenomic, Omaha, NE). All MS-detected variants and WAVE mobility shifts were submitted for sequencing for mutation confirmation (ABI PRISM 3130XL Genetic Analyser). That is, if a heterozygous *MUTYH* mutation was identified, then the *MUTYH* gene was screened for any additional mutations not captured by the Sequenom genotyping screen to ensure all potential compound heterozygous carriers were identified. The relatives of probands with a pathogenic *MUTYH* germline mutation underwent testing for the specific variant identified in the proband.

Statistical analysis

The median, range, mean and standard deviation of the ages at cancer diagnoses were calculated using Stata 13.0 (StataCorp, College Station, TX, 2013). Hazard ratios (HRs), *i.e.* the age-, sex- and country-specific cancer incidence for carriers divided by that for the general population, were estimated for each cancer site. Age- and sex-specific cancer incidences in 1988–1992 for each country (the USA, Canada and Australia) were obtained from Cancer Incidence in Five

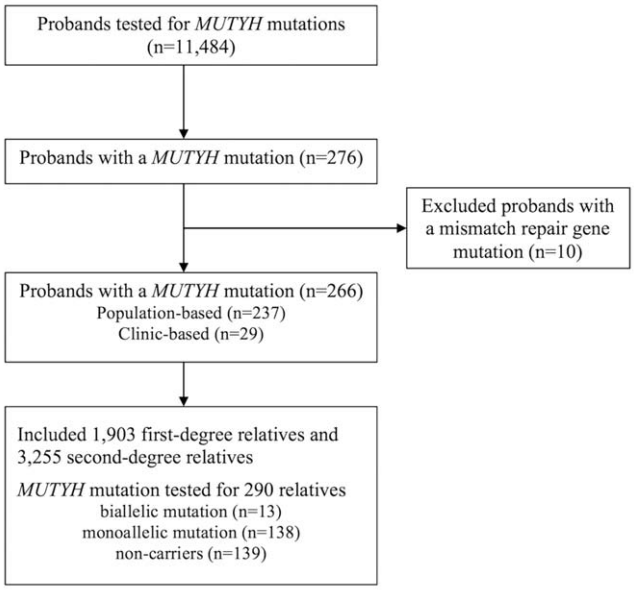

Figure 1. Selection of study sample.

Continents.[33] The period of 1988–1992 was selected for analysis because it was the closest available dataset to the mean calendar year of cancer diagnoses in the sample. We used a modified segregation analysis,[34,35] (as described in detail in the Appendix in the work by Dowty *et al.*[36]). This analytical method is not subject to population stratification, can be rigorously adjusted for ascertainment and uses data on all study participants, whether genotyped or not, thereby maximizing statistical power. Models were fitted by the method of maximum likelihood with the statistical package MENDEL 3.2.[37]

For each cancer site, the age at cancer diagnosis was modeled as a random variable whose hazard was the relevant population incidence rate multiplied by a site-specific HR. Observation time for each individual started at birth and ended at first diagnosis of any cancer, last follow-up or death, whichever occurred first. Where age at diagnosis of a cancer was not reported (22% of all cancer cases), we assumed the age of diagnosis to be the median age at that cancer diagnosis for the general population obtained from SEER Cancer Statistics Review (1975–2008).[38]

Estimates were appropriately adjusted for the clinic- and population-based ascertainment of families using a combination of retrospective likelihood and ascertainment-corrected joint likelihood,[35,39,40] in which each pedigree's data was conditioned on the proband's genotype, cancer status and age of onset (for population-based families) or on the proband's genotype and the affected statuses and ages of onset of all family members at the time of ascertainment, *i.e.* when the proband was found to be a *MUTYH* mutation carrier (for clinic-based families). Our estimates are therefore the parameter values which maximize the conditional likelihood of the observed data conditioned on the relevant ascertainment criteria. Our estimates are unaffected by the selective ascertainment of families into our study.

Table 1. Numbers and mean ages at diagnosis of extracolonic cancers in the first- and second-degree relatives (combined) of probands

	Male ($n = 2,552$)		Female ($n = 2,606$)	
Site of cancer	*N*	Mean age (SD)	*N*	Mean age (SD)
Stomach	27	68.4 (9.39)	26	73.2 (12.5)
Hepatobiliary tract	15	62.1 (12.9)	10	72.9 (8.37)
Brain	10	57.9 (12.7)	12	54.0 (12.1)
Urinary bladder	4	66.0 (10.7)	5	78.4 (12.3)
Renal pelvis/Kidney	6	64.7 (11.3)	9	66.4 (12.0)
Pancreas	4	64.1 (10.3)	6	72.0 (11.2)
Small bowel	0		1	52
Ureter	1	59	0	
Thyroid	1	29	4	41.0 (17.3)
Pharynx	9	62.7 (6.71)	6	69.0 (9.54)
Esophagus	7	63.8 (8.22)	2	72.0 (1.41)
Lung	46	63.7 (12.6)	15	62.7 (10.0)
Bone	7	67.3 (18.7)	4	55.5 (19.7)
Ovary			10	52.7 (12.8)
Endometrium			22	66.1 (14.0)
Breast			106	60.7 (12.0)
Cervix			5	37.4 (12.1)
Prostate	63	69.4 (8.41)		

N, total number of affected relatives; SD, standard deviation.

Estimated cumulative risks (penetrance) of cancers to age 70 years and corresponding 95% CIs for *MUTYH* mutation carriers were calculated for each sex from the HR estimates and the age- and sex-specific USA population incidences incidence_i at age i using the formula:

$$1-e^{-\sum_{i=0}^{70}\text{HR}\times\text{incidence}_i}$$

The total number of carriers was estimated by summing *MUTYH* carrier probabilities for all individuals, as calculated from Mendel's laws of inheritance, the known genetic relationship of each individual to his or her genotyped relatives and a population allele frequency of 0.0085 (but not any affected statuses).[26] These calculations were performed using R 2.15.0[41] and a modified version of Mendel 3.2.[37]

Results

We identified 276 probands who were known to carry germline mutations in the *MUTYH* gene from the Colon Cancer Family Registry. We excluded 10 probands who were also known to carry a pathogenic germline mutation in a DNA mismatch repair gene (Lynch syndrome).[42] Of the remaining 266 probands, 41 (15%) were biallelic *MUTYH* mutation carriers and 225 (85%) were monoallelic *MUTYH* mutation carriers. Of

Table 2. Hazard ratios and corresponding cumulative risks % to age 70 years of extracolonic cancers for carriers of germline monoallelic and biallelic mutations in *MUTYH*

		Cumulative risk % (95% CI)[2]	
Site of cancer	HR (95% CI)[1]	Males	Females
Biallelic carriers			
Urinary bladder	19 (3.7–97)	25 (5.4–77)	7.6 (1.5–33)
Ovary	17 (2.4–115)		14 (2.2–65)
Monoallelic carriers			
Stomach	9.3 (6.7–13)	5.0 (3.6–6.9)	2.3 (1.7–3.3)
Hepatobiliary tract	4.5 (2.7–7.5)	2.9 (1.7–4.7)	1.4 (0.8–2.3)
Endometrium	2.1 (1.1–3.9)		3.3 (1.8–6.2)
Breast	1.4 (1.0–2.0)		11 (8.3–16)
Ovary	0.4 (0.1–2.6)		
Prostate	0.5 (0.3–1.0)		
Brain	2.1 (0.9–4.9)		
Renal pelvis/ Kidney	2.3 (0.1–3.1)		
Pancreas	2.3 (0.2–4.1)		

CI, confidence interval; HR, hazard ratio.
[1]HR was provided for both males and females combined given that HRs were not different by sex.
[2]Cumulative risks were estimated only for cancers that were significantly associated with *MUTYH* mutations. These cumulative risks were calculated for carriers of germline monoallelic and biallelic mutations in *MUTYH* living in USA. See Supporting Information Table 3 for cumulative risks for carriers living in Canada and Australia.

these, 91% ($n = 241$) were Caucasians and 9% ($n = 25$) were others (9 African Americans, 5 Latinos, 2 Native Americans, 1 Portuguese and 8 unknown). Two hundred thirty-seven (89%) probands were ascertained *via* population-based resources (Fig. 1). There were 140 (53%) families recruited from the USA, 81 (30%) from Canada and 45 (17%) from Australia and New Zealand. The *MUTYH* variants of the probands are shown in Supporting Information Table 1. Of the 12 *MUTYH* variants examined, 73% of biallelic *MUTYH* mutations were compound heterozygous or homozygous p.(Tyr179Cys) or p.(Gly396Asp) mutations. Similarly, 92% of monoallelic *MUTYH* mutations were either p.(Tyr179Cys) or p.(Gly396Asp).

We obtained data on a total of 1,903 (929 female) first-degree relatives and 3,255 (1,623 female) second-degree relatives of the 266 probands. *MUTYH* mutation status was tested for 290 relatives (13 were found to be biallelic mutation carriers, 138 were monoallelic mutation carriers and 139 were non-carriers). We estimated that there were additional 40 biallelic and 1,874 monoallelic mutation carriers among non-genotyped relatives, giving a total estimated number of 53 biallelic and 2,012 monoallelic mutation-carrying relatives in our study sample.

Table 1 shows the numbers and mean ages of diagnoses of cancers at various sites in the affected first- and second-degree relatives (combined) of the probands. Of these cancer diagnoses in the relatives, 17% were verified by pathology report, medical clinical records, cancer registry reports and/or death certificates (Supporting Information Table 2).

Biallelic *MUTYH* mutation carriers had urinary bladder cancer incidence 19 (95% CI, 3.7–97) times and ovarian cancer incidence 17 (95% CI, 2.4–115) times higher than the general population. Monoallelic *MUTYH* mutation carriers had gastric cancer incidence 9.3 (95% CI, 6.7–13) times and hepatobiliary cancer incidence 4.5 (95% CI, 2.7–7.5) times higher than the general population. Monoallelic *MUTYH* mutation carriers also had a slightly higher incidence of endometrial cancer (HR, 2.1; 95% CI, 1.1–3.9) and breast cancer (HR, 1.4; 95% CI, 1.0–2.0). We did not find evidence for an increased risk of cancers at the other sites that we were able to estimate HRs (kidney, pancreas, brain and prostate) (Table 2). For cancers at some sites (*e.g.*, small bowel, thyroid, ureter), we were not able to estimate reliable HRs. A sensitivity analysis excluding all relatives with missing age at cancer diagnosis showed results similar to those of the main analysis (details not shown).

The estimated cumulative risks to age 70 years of specific cancer sites for carriers from the USA are provided in Table 2. It is estimated that 25% (95% CI, 5–77%) and 8% (95% CI, 2–33%) of male and female biallelic *MUTYH* mutation carriers, respectively, will be diagnosed with urinary bladder cancer by the age of 70 years, whereas 14% (95% CI, 2–65%) will be diagnosed with ovarian cancer. For monoallelic *MUTYH* mutation carriers, 5% (95% CI, 4–7%) and 2.3% (95% CI, 1.7–3.3%) of males and females, respectively, will be diagnosed with gastric cancer while 3% (95% CI, 2–5%) and 1.4% (95% CI, 0.8–2.3%), respectively, will be diagnosed with hepatobiliary cancer. Of female monoallelic *MUTYH* mutation carriers, 3% (95% CI, 2–6%) will develop endometrial cancer and 11% (95% CI, 8–16%) will develop breast cancer (Table 2). The corresponding cumulative risks for carriers living in Canada and Australia are given in Supporting Information Table 3.

Discussion

We have estimated the risk of extracolonic cancers for biallelic and monoallelic *MUTYH* mutation carriers, mostly Caucasians, using one of the world's largest resources of these carriers.

We estimated that biallelic *MUTYH* mutation carriers had a 19-fold increased risk of urinary bladder cancer and a 17-fold increased risk of ovarian cancer, compared with the general population. This is consistent with a previous study by Vogt *et al.*,[12] which found an increased risk of urinary bladder cancer (standardized incidence ratio [SIR], 7.2; 95% CI, 2.0–18.4) and ovarian cancer (SIR, 5.7; 95% CI, 1.2–16.7). These estimates are not statistically different from our estimates ($p = 0.34$ and $p = 0.37$, respectively, based on the method proposed by Altman and Bland[43]). Cancer screening guidelines for carriers of biallelic mutations in *MUTYH* currently only address cancer of the colon and upper gastrointestinal tracts.[44–46] Although our study confirms the previous report by Vogt *et al.*[12] of increased risks of bladder and

ovarian cancers for biallelic mutation carriers, it may be too early to advise clinicians to consider implementing early detection at these sites given the wide confidence intervals around our estimates as well as the lack of evidence for the efficacy of screening methods for these cancers.[47,48] Further, our study was unable to examine previous suggestions that biallelic mutations in *MUTYH* increase the susceptibility to duodenal,[7,12,17,18] breast,[12,19] endometrial[10,13] and gastric[18] cancer, possibly because of the small numbers of cases of these cancers in our study sample.

For monoallelic *MUTYH* mutation carriers, we found an increased risk of gastric and liver cancers, as well as a slightly increased risk of endometrial and breast cancers. In the current analysis, we observed only a slightly elevated risk of breast cancer, consistent with previous reports.[27–29] For example, in a population-based case–control study of Jewish descendants of North African origin, Rennert *et al.* reported an elevated risk of breast cancer for carriers of a *MUTYH* p.(Gly396Asp) variant (odds ratio [OR] = 1.86, 95% CI 1.02–3.39).[28] In a Chinese case–control study, Zhu *et al.* reported an association between AluYb8 insertion in *MUTYH* and a modest increased risk of breast cancer (OR = 1.26, 95% CI 1.01–1.56) although we did not test for this variant in our study.[29] Wasielewski *et al.* also reported a higher frequency of monoallelic *MUTYH* mutations in families with both breast and colorectal cancer compared with the population (4.1% *vs.* 1.9%).[27] Other studies failure to find evidence for an increased risk of breast cancer for monoallelic mutation carriers may be due to the lack of power given the modest increase in the risk of breast cancer and the small sample sizes.[11,20]

The wide confidence intervals for some cancer risks observed in the present study are due to limited sample size and/or variability in risk due to genetic heterogeneity or the influence of environmental risk factors. This is especially seen in our estimates for urinary bladder and ovarian cancer risks for biallelic mutation carriers. In a previous study, we have shown a substantial variation in colorectal cancer risks using a polygenic model that mimics the effect of a large number of cancer-susceptibility loci, in addition to the *MUTYH* mutation effect.[6] To our knowledge, thus far the only study conducted to investigate environmental modifiers of colorectal cancer risk for *MUTYH* mutation carriers was on the relationship with hormone replacement therapy,[5] which reported no evidence of interaction between hormone replacement therapy and *MUTYH* mutations.

The strengths of the study are the relatively large sample size (although the numbers of the biallelic mutation carriers were small), established registry with up to 15 years of follow-up and its multinational populations (increasing generalizability). This study avoided potential survival bias as deceased cases were represented using methods that estimated carrier probabilities based on the genetic relationship of the deceased and untested individuals to their confirmed carrier and non-carrier relatives. Furthermore, ascertainment bias (due to inclusion of relatives' cancers that resulted in ascertainment of the family) was avoided in this study as estimates were appropriately adjusted for the clinic- and population-based ascertainment of families.

A potential limitation of our study is that self-reported unverified cancer cases in the relatives (83%) may affect the accuracy of estimates. However, the majority of our families were recruited from population cancer registries (89%) and we tested their *MUTYH* mutation status after surveying family history. Therefore, any measurement error (under- or over-reporting) of family history of cancer will be non-differential with respect to mutation status and our results comparing cancer risks for carriers with the general population is likely to be attenuated. Further, previous studies showed a high probability of agreement between proband-reported cancer status in first-degree relatives and the validated report; for example, 95.4% (95% CI, 92.6–98.3) for female breast cancer, 83.3% (95% CI, 72.8–93.8) for ovarian cancer; and 79.3% (95% CI, 70.0–88.6) for prostate cancer.[49] We systematically attempted to estimate HR for each cancer site separately for monoallelic and biallelic mutation carriers. However, for many sites there were insufficient numbers of cancer diagnosis to generate reasonable estimates of HR. Because of many more relatives of monoallelic mutation carriers compared with biallelic mutation carriers (225 *vs.* 41 families), we were able to estimate the risk of more cancer sites for monoallelic mutation carriers while we were only able to estimate the cancer risk of two sites (urinary bladder and ovary) for biallelic mutation carriers. Further, we did not have sufficient power to examine cancer risks associated with specific variants of the *MUTYH* gene in our study. Some studies have reported associations of specific *MUTYH* variants with particular disease types and severity. For example, monoallelic or biallelic mutation carriers of a *MUTYH* p.(Tyr179Cys) variant had a higher risk of colorectal cancer than carriers of a *MUTYH* p.(Gly396Asp) variant.[5,6] Our results might have limited relevance for non-Caucasian populations, since our cohort was comprised mainly of individuals with *MUTYH* variants that commonly occur in Caucasians, and there are ethnic and geographical differences in *MUTYH* variants.[50] Finally, most of the relatives included in the study (including those affected with a cancer) were not tested for their mutation status, leading to less precise estimates than if every relative was genetically tested, so our estimates should be replicated in larger studies to obtain more precise estimates.

In summary, we found that biallelic *MUTYH* mutation carriers are at increased risks of developing urinary bladder and ovarian cancers and monoallelic carriers are at increased risks of gastric, liver, breast and endometrial cancers. Further studies investigating cancer risks and disease characteristics associated with specific *MUTYH* mutation variants are warranted.

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