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ORIGINAL REPORT

Clinical Implications of the Colorectal Cancer Risk Associated With *MUTYH* Mutation

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose
Biallelic mutations in the base excision DNA repair gene *MUTYH* predispose to colorectal cancer (CRC). Evidence that monoallelic mutations also confer an elevated CRC risk is controversial. Precise quantification of the CRC risk and the phenotype associated with *MUTYH* mutations is relevant to the counseling, surveillance, and clinical management of at-risk individuals.

Methods
We analyzed a population-based series of 9,268 patients with CRC and 5,064 controls for the Y179C and G396D *MUTYH* mutations. We related genotypes to phenotype and calculated genotype-specific CRC risks.

Results
Overall, biallelic mutation status conferred a 28-fold increase in CRC risk (95% CI,17.66 to 44.06); this accounted for 0.3% of CRCs in the cohort. Genotype relative risks of CRC were strongly age dependent, but penetrance was incomplete at age 60 years. CRC that developed in the context of biallelic mutations were microsatellite stable. Biallelic mutation carriers were more likely to have proximal CRC ($P = 4.0 \times 10^{-4}$) and synchronous polyps ($P = 5.7 \times 10^{-9}$) than noncarriers. The performance characteristics of clinicopathologic criteria for the identification of biallelic mutations are poor. Monoallelic mutation was not associated with an increased CRC risk (odds ratio, 1.07; 95% CI, 0.87 to 1.31).

Conclusion
The high risk and the propensity for proximal disease associated with biallelic *MUTYH* mutation justify colonoscopic surveillance. Although mutation screening should be directed to patients with *APC*-negative polyposis and early-onset proximal MSS CRC in whom detection rates will be highest, the expanded phenotype associated with *MUTYH* mutation needs to be recognized. There is no evidence than monoallelic mutation status per se is clinically important.

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INTRODUCTION

Inherited predisposition is acknowledged to be a major risk factor for the risk of developing colorectal cancer (CRC), as it underlies approximately 35% of patient diagnoses[1]; in addition, a number of high-penetrant, autosomal dominant and recessive hereditary forms of CRC are recognized. Germline-inactivating mutations in the base excision DNA repair gene *MUTYH* cause recessive CRC,[2-4] and the two missense variants Y179C and G396D (previously designated Y165C and G382D), carried by approximately 1% of the population, account for greater than 80% of all pathogenic mutations in European populations.[3-5]

Since the association between *MUTYH* and CRC risk was first described, biallelic mutations have been shown to predispose to an attenuated form of familial adenomatous polyposis (FAP)[2] called *MUTYH*-associated polyposis (MAP), which is possibly responsible for up to 40% of *APC*-negative attenuated FAP.[3,4] In addition to *MUTYH* mutations that influence CRC through recessive inheritance, heterozygosity also has been proposed to confer an elevated risk,[6-13] although this assertion is controversial.

Precise quantification of the CRC risk and a description of the phenotype associated with *MUTYH* are relevant to the counseling, surveillance, and clinical management of at-risk individuals. To date, most of the information on *MUTYH* in CRC has been based on studies of polyposis[4,14] or familial CRC,[3] and as such is not optimal because of ascertainment bias, which inevitably leads to an overestimation of risk. Although a number of studies that are based on unselected patients have been conducted,[6-13,15-17] the large sample size required for a population-based analysis to robustly describe

the impact of *MUTYH* mutation on CRC has limited the value of studies. To address deficiencies in the knowledge of *MUTYH* on CRC risk, we have analyzed a population-based series of 9,268 patients with CRC and 5,064 controls.

METHODS

Patients

A total of 9,268 patients with CRC, age younger than 80 years at diagnosis, were ascertained between March 2003 and October 2007 through the National Study of Colorectal Cancer Genetics (NSCCG). The NSCCG is an ongoing, population-based study in the United Kingdom of patients with histologically proven CRC within 5 years of diagnosis that has no exclusion for known genetic susceptibility. Comprehensive details about NSCCG are provided in previously published material.[18]

Controls were the spouses of patients with malignancies, and were ascertained through the NSCCG (n = 1,856), the Genetic Lung Cancer Predisposition Study (n = 1,436),[19] and the Royal Marsden Hospital National Health Services Trust (RMHNHST) family history DNA database (n = 1,732). None of the controls had a personal history of malignancy at time of ascertainment.

All patients and controls were British residents and self reported a European ethnicity; there were no obvious differences in the demographics of patients and controls in terms of place of residence within the United Kingdom. Informed consent was obtained from all participants, and the study was carried out with ethical review board approval (Multi Research Ethics Committee [MREC] 02/0/097; MREC 98/2/67; RMHNHST-Centre for Clinical Research [CCR] 1552).

Molecular Analyses

DNA was extracted from EDTA-treated venous blood samples by using conventional methodologies, and it was quantified by using PicoGreen (Invitrogen Corp, Carlsbad, CA). Genotyping of Y179C and G396D was conducted by competitive, allele-specific polymerase chain reaction KASPar chemistry (KBiosciences Ltd, Hertfordshire, United Kingdom) that was implemented on an ABI7900HT platform (Applied Biosystems, Foster City, CA). Primers for Y179C were as follows: GAAGGT GACCAAGTTCATGCTCCGCCGGCCACGAGAATAG(T/C) and ATCC TACCCACAGGAGGTGAATCAA; primers for G396D were as follows: GAAGGTGACCAAGTTCATGCTCCCACAGTCCTGCCAGCAGA(T/C) and GCCTGGCTGCCCTCCCTCT. Genotyping quality control was evaluated by using duplicate DNA samples together with direct sequencing of 1% of samples. In addition, we compared the genotypes of 2,561 of the samples that had been typed for Y179C and G396D by using customized Illumina Sentrix Bead arrays (Illumina Inc, San Diego, CA) as part of a previous study.[20]

Microsatellite instability (MSI) in CRCs from 2,748 of the patients was determined, as previously described[18] by using BAT25 and BAT26, which are highly sensitive MSI markers.[21] Samples that showed novel alleles at either or both markers were assigned MSI status (which corresponded to high MSI).[22]

Statistical Analysis

Statistical analyses were principally conducted by using STATA (version 10; STATA, State College, TX). In all analyses, a two-sided *P* value of .05 was considered statistically significant. Differences between the distributions of categoric variables were assessed by means of either the χ^2 test or Fisher's exact test and by the Armitage trend test. Differences in the distribution of age at diagnosis were made by using a Wilcoxon-type test for trend.[23] CIs for the prevalence of *MUTYH* mutations were computed with the assumption that, given the relatively small prevalence, the number of mutations followed a Poisson distribution.

We estimated genotype relative risks (GRR) from the expected Hardy-Weinberg equilibrium proportion as obtained from the allele frequency in controls; these were assumed to be a representative sample of the general population. The 95% CIs for GRRs were obtained through bootstrapping 100,000 independent samples; the lower and upper 95% CIs corresponding to the 5,000th and 95,000th estimates, respectively. For monoallelic mutations, we also calculated odds ratios (ORs) and their associated 95% CIs by using logistic regression. Penetrance of biallelic mutations was estimated as previously described.[9] The probability of genotype given age was estimated by using logistic regression with age as a predictor of genotype, and the sex-averaged population incidence rates of CRC were obtained from the Office of National Statistics for 1998. CIs of penetrance estimates were obtained by bootstrapping.

Table 1. *MUTYH* Genotype Status and Colorectal Cancer Phenotype

				Sex				Location of CRC								MSI Status						No. of Affected FDRs			
		Age		Male		Female		Colon*		Rectum†		Proximal‡		Distal§		MSS		MSI		FH of CRC		1		≥ 2	
Carrier Status	No. of Patients	Mean	SD	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
NN	9,043	59.0	8.5	5,304	59	3,739	41	5,441	60	3,602	40	2,559	29	6,428	71	2,354	88	310	12	1,371	15	1,222	89	149	11
All M	225	58.9	8.5	128	57	97	43	148	66	77	34	84	39	134‖	62	78	93	6	7	46¶	20	39	85	7	15
MM	27	54.0‖	7.3	14	52	13	48	20	74	7	26	16	59	11#	41	9	100	—	—	7	26	7	100	—	—
Y179C/Y179C	4	49.5¶	6.0	4	100	—	—	2	50	2	50	3	75	1¶	25	1	100	—	—	1	25	1	100	—	—
Y179C/G396D	13	52.5‖	7.5	5	38	8	62	11	85	2	15	10	77	3#	23	6	100	—	—	3	23	3	100	—	—
G396D/G396D	10	57.9	6.2	5	50	5	50	5	50	5	50	3	30	7	70	2	100	—	—	3	30	3	100	—	—
MN	198	59.6	8.4	114	58	84	42	128	65	70	35	68	36	123¶	64	69	92	6	8	39	20	32	82	7	18
Y179C/–	70	58.4	7.8	38	54	32	46	50	71	20	29	28	40	42¶	60	25	96	1	4	9	13	8	89	1	11
G396D/–	128	60.2	8.5	76	59	52	41	78	61	50	39	40	33	81¶	67	44	90	5	10	30‖	23	24	80	6	20
Y179C	87	57.1¶	8.1	47	54	40	46	63	72	24¶	28	41	47	46#	53	32	97	1	3	13	15	12	92	1	8
G396D	151	59.4	8.7	86	57	65	43	94	62	57	38	53	37	91¶	63	52	91	5	9	36‖	24	30	83	6	17

Abbreviations: CRC, colorectal cancer; MSI, microsatellite instability; FH, family history; FDR, first-degree relative; MSS, microsatellite stable; SD, standard deviation; N, nonmutated allele; M, mutated allele.

*International Classification of Diseases (9th revision, clinical modification) codes 153.0-153.7 and 153.9.

†International Classification of Diseases (9th revision, clinical modification) codes 154.0 and 154.1.

‡International Classification of Diseases (9th revision, clinical modification) codes 153.0, 153.1, 153.4, 153.6, and 153.9.

§International Classification of Diseases (9th revision, clinical modification) codes 153.2, 153.3, 153.7, 154.0, and 154.1.

‖$P < .01$.

¶$P < .05$.

#$P < .001$.

To additionally explore the possibility that monoallelic mutation status might affect CRC, we also applied a kin-cohort approach[24] to compare risks in the 50,151 first-degree relatives (FDRs) of carriers and noncarriers. Age-specific, cumulative CRC distributions in FDRs were estimated by using a marginal likelihood approach, and a bootstrap estimate for the hazard ratio (HR) was used to calculate 95% CIs.

Meta-Analysis

To identify published studies of *MUTYH* variants in case-control series of CRC, we searched PubMed and EMBASE and used a combination of thesaurus and free-text terms (ie, *MYH*, *MUTYH*, or *MAP*) alone or in combination with colorectal cancer, case-control, or cohort. Meta-analysis was conducted by using standard methods.[25] We calculated Cochran's Q statistic to test for heterogeneity (P_{het}),[25] and we calculated the I^2 statistic[26] to quantify the proportion of the total variation caused by heterogeneity.

RESULTS

Analysis of MUTYH Y179C and G396D

Genotypes were obtained for all patients and controls. Concordance between duplicate samples was 100% and between genotyping platforms was 99.86%. Twenty-seven (0.3%) of the 9,268 patients had biallelic *MUTYH* mutations (95% CI, 0.2% to 0.4%; Table 1). Of these, four were homozygous for Y179C, 10 were homozygous for G396D, and 13 were compound heterozygotes. Monoallelic *MUTYH* mutations were identified in 198 patients (2.2%; 95% CI, 1.9% to 2.5%): 70 (0.8%) for Y179C, and 128 (1.4%) for G396D. Only one of the 5,064 controls were biallelic for Y179C/Y179C, and 101 (2.0%) were heterozygous (95% CI, 1.6% to 2.4%): 26 (0.5%) heterozygous for Y179C, and 75 (1.5%) for G396D. The frequencies of mutations, thus, are comparable to those documented in other European populations.[3-5,8,9,12-15,17] Demographic, phenotypic, and familial characteristics of the patients and controls by *MUTYH* genotype are detailed in Table 1.

Table 2. GRRs Associated With *MUTYH* Mutation

Carrier Status by Age Group (years)	Patients	Controls	GRR	95% CI
Overall				
MM	27	1	28.28	17.66 to 44.06
			14.82*	2.44 to 606.58*
MN	198	101	1.07	0.87 to 1.31
			1.08*	0.84 to 1.38*
NN	9,043	4,962	Reference group	—
< 40				
MM	0	0	—	—
MN	3	9	—	—
NN	254	247	Reference group	—
40-49				
MM	10	0	115.56	39.72 to 412.63
MN	25	12	1.40	0.80 to 2.69
			1.39*	0.69 to 2.78*
NN	921	613	Reference group	—
50-59				
MM	10	1	30.09	12.78 to 64.99
			4.99*	0.64 to 38.98*
MN	66	30	1.04	0.74 to 1.52
			1.10*	0.71 to 1.70*
NN	3,039	1,515	Reference group	—
60-69				
MM	7	0	15.21	5.53 to 33.71
MN	93	36	1.02	0.75 to 1.43
			1.01*	0.69 to 1.49*
NN	4,506	1,763	Reference group	—
≥ 70				
MM	0	0	—	—
MN	11	14	2.02	0.97 to 3.95
			2.00*	0.90 to 4.46*
NN	323	824	Reference group	—

Abbreviations: GRR, genotype relative risks; M, mutated allele; N, nonmutated allele.
*Asymptotic values.

Phenotypes Associated With MUTYH Mutations

Patients with biallelic *MUTYH* mutations were significantly younger than patients without mutations ($P = 1.4 \times 10^{-3}$; Table 1). A significant difference in age of onset was found between biallelic mutation genotypes ($P = .02$; Table 1); The mean age at diagnosis of CRC was 57.90 years for G392D homozygotes, 52.46 years for G396D/Y179C compound heterozygotes, and 49.50 years for Y179C homozygotes (Table 1).

All CRCs tested for MSI were microsatellite stable (MSS; Table 1). Biallelic mutation carriers were significantly more likely to have proximal CRC ($P = 4.0 \times 10^{-4}$; Table 1) and were more likely to have an FDR affected with CRC, albeit nonsignificantly (Table 1). Although biallelic mutation carriers were more likely to have synchronous polyps ($P = 5.7 \times 10^{-9}$; Appendix Table A1, online only), multiple polyposis was not universal (Appendix Table A2, online only). Furthermore, four of the carriers for whom we had reliable polyp information had no concurrent adenomas or metaplastic polyps. The performance characteristics and efficiency of each of these clinical criteria for the detection of biallelic mutations were not highly discriminatory (Appendix Table A3, online only). There were no other salient differences in terms of grade or stage at presentation of CRC between biallelic mutation carriers and noncarriers (Appendix Table A4, online only).

Monoallelic *MUTYH* mutation was not associated with an earlier age at diagnosis of CRC (Table 1). There was also no evidence of a relationship between heterozygosity and having an FDR with CRC; MSI status (Table 1); or the presence of polyps (Appendix Table A1). Although the proportion of monoallelic mutation carriers with colonic and rectal disease was not significantly different from noncarriers, there was evidence of over-representation of proximal disease ($P = .03$; Table 1).

Colorectal Cancer Risk Associated With MUTYH Mutation

The difference in frequency of mutations in patients and controls provides unequivocal evidence that biallelic *MUTYH* mutations confer a significant increase in risk of CRC. Overall, biallelic inactivation was associated with a 28.28-fold increase in risk (GRR, 28.28; 95% CI, 17.66 to 44.06; $P = 1.0 \times 10^{-4}$; Table 2). As for the combined data, there was evidence of a significantly increased risk associated with homozygosity for the individual risk variants (Table 1). Risks associated with Y179C homozygosity were greater than for G396D homozygosity (GRRs, 56.69 and 19.71, respectively), which is consistent with an earlier age of onset.

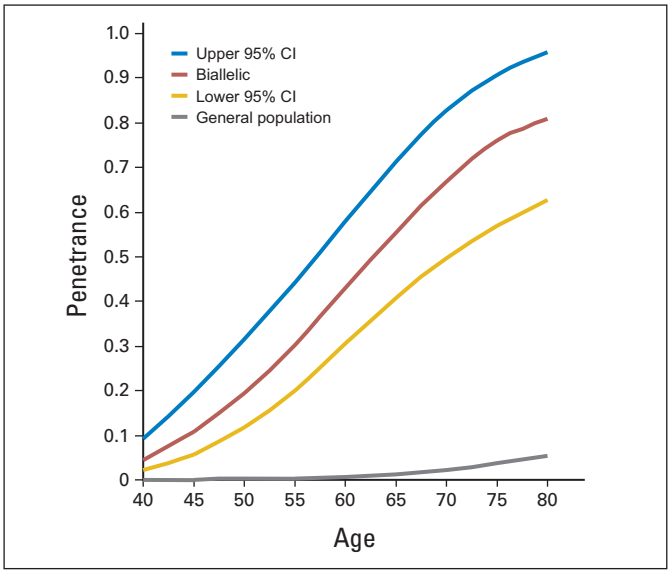

Fig 1. Penetrance curve of biallelic *MUTYH* mutation.

Although no biallelic mutations were identified among the 257 patients diagnosed before age 40 years, and 25% of biallelic patients were diagnosed after age 60 years, GRRs were strongly age dependent; GRRs for ages 40 to 49, 50 to 59, and 60 to 69 years were 115.56, 30.09, and 15.21, respectively ($P_{trend} = 4.6 \times 10^{-5}$; Table 2). Estimated penetrances at 50 and 60 years old were 19.47% (95% CI, 11.69% to 31.41%) and 42.89% (95% CI, 30.45 to 57.94%), respectively (Fig 1). These data establish that biallelic mutations are highly penetrant and that there is a substantially elevated CRC risk relatively early in life that is incomplete at age 60 years.

Overall, monoallelic *MUTYH* mutational status was not associated with an increased CRC risk (OR, 1.07; 95% CI, 0.87 to 1.31; $P = .55$; Table 2). No confounding with age was seen (Table 2). To additionally explore the possibility that monoallelic mutation status might affect CRC, we applied a kin-cohort approach to compare risks in FDRs of carriers and noncarriers. The HR for CRC associated with heterozygosity was 1.29 (95% CI, 0.25 to 1.95), which thereby provided no support for a dominant effect of *MUTYH* mutation.

To date, 11 case-control studies have reported on the frequency of *MUTYH* mutations in unselected patients with CRC and controls (Appendix Table A5, online only). To additionally investigate the possibility of an increased risk of CRC associated with heterozygosity, we performed a meta-analysis and pooled our data with previous studies, which provided data on a total of 18,610 patients and 12,822 controls (Fig 2). The OR for all monoallelic carriers was not significantly different from unity (OR, 1.14; 95% CI, 0.96 to 1.36; $P = .12$; $P_{het} = .81$; $I^2 = 0\%$). Our estimate of the risk associated with monoallelic carrier status is likely to be slightly inflated, because we restricted this analysis to Y179C and G396D mutations; hence, some individuals heterozygous for these variants may carry additional pathogenic variants. Incorporating these observations, the pooled OR associated with monoallelic mutations is 1.12 (95% CI, 0.94 to 1.33; $P_{het} = 0.83$, $I^2 = 0\%$).

DISCUSSION

To our knowledge, this study presents the largest survey of *MUTYH* mutations in a population-based series reported so far. The data demonstrates that biallelic mutation confers a significantly increased risk of developing CRC; however, the associated phenotype is far less distinctive than that previously reported by studies of familial CRC and polyposis.[3,4,14] We also found that the phenotypic effects of Y179C are more severe than G396D, which is consistent with recent clinical[14,15] and functional studies.[2,14,15,27,28]

Major strengths of this study are its size and its population-based design. We have previously shown that the familial CRC risk estimated from NSCCG data is identical to that obtained from meta-analyses of epidemiologic case-control and cohort studies,[29] and this makes preferential selection of familial CRC unlikely. Similarly, survivorship is

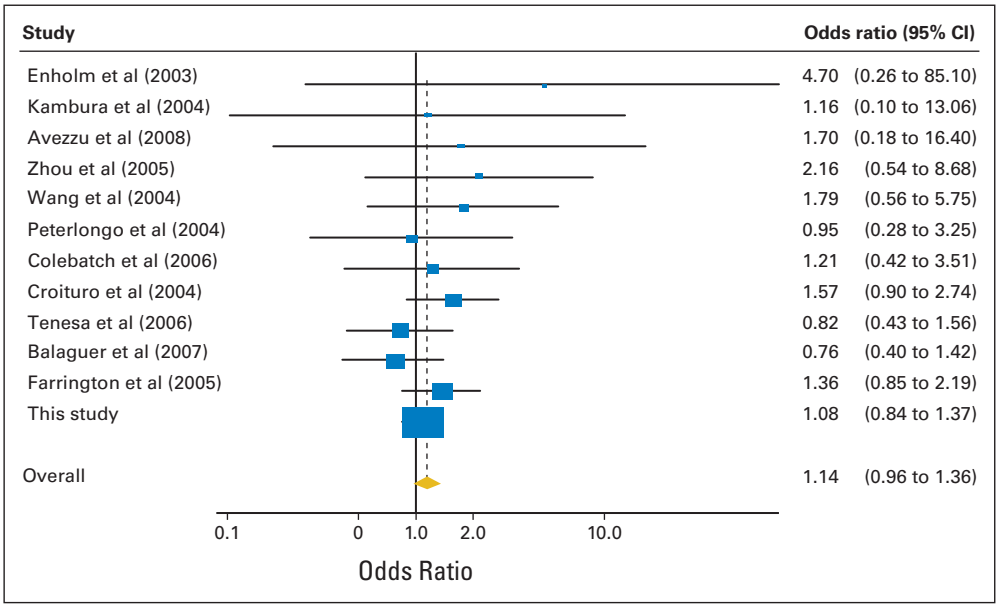

Fig 2. Forest plot of the odds ratio (OR) of colorectal cancer risk associated with monoallelic Y179C and G396D mutations. Boxes denote OR point estimates, their areas proportional to the inverse variance weight of the estimate. Horizontal lines represent 95% CIs.

unlikely to have influenced study findings, even though patient selection in NSCCG is biased to Duke's stages A and B CRC. Furthermore, this analysis is unlikely to be confounded by population stratification, as the analysis has been limited to individuals with self-reported European ethnicity. In this study, analysis was restricted to two risk mutations. Although this is a limitation, it mitigates against use of data on low frequency variants that require judgment calls on probable pathogenicity. Moreover, given that Y179C and G396D account for greater than 80% of pathogenic mutations in the United Kingdom population,[3-5] inability to screen for additional mutations only serves to marginally inflate CRC risks associated with heterozygosity.

In this study, the risk of CRC associated with biallelic mutations was apparent only in the fifth decade of life. Although this is consistent with previously published population-based series that have rarely reported patients with CRC who had biallelic mutations and who were age younger than 40 years (Appendix Table A2),[6-8,11,13,15,16] the power of this study to demonstrate an elevated risk before age 40 years was low. Accepting the caveat of ascertainment bias, data from surveys of *MAP* provide a rationale for advocating that colonoscopic surveillance of *MAP* should commence at 18 to 20 years of age.[14,30] In contrast to a recent study that suggested that biallelic mutations are fully penetrant by age 60 years,[9] a quarter of carriers in this series and in other studies[6-8,11,13,16] are diagnosed after age 60 years. Taken together, these data are compatible with biallelic mutations that confer a substantial risk, but a risk in which the profile is likely to be different from FAP and hereditary nonpolyposis colorectal cancer (HNPCC), which are characterized by much higher relative risks at earlier ages.[31,32] It is also apparent that the risk profile is influenced by the type of *MUTYH* mutation. Hence, genotypic stratification may be useful in additionally defining the clinical management of carriers.

Screening for *MUTYH* mutations is clinically important, as siblings of biallelic carriers have a 25% risk of being carriers by the nature of segregation of a recessive disorder. To date, there are no generally acknowledged screening criteria for instigating genetic testing. Testing for *MUTYH* mutations in the presence of multiple polyps and early-onset CRC has been suggested.[7,9,11,13] However the presence of polyps per se represents an insensitive marker because of the high frequency of individuals who do not have synchronous disease[7,9]; this view is supported by the data in this study. Similarly, the predictive value of family history has been questioned. The lack of agreement with respect to clinical recommendations for indicating *MUTYH* analyses was one of the primary reasons that this analysis was conducted.

In clinical practice, it is rational to screen all patients with CRC for a presumptive diagnosis of FAP, in which *APC* mutation can be excluded. These data support targeting screening for *MUTYH* variants to individuals with early-onset proximal disease and concomitant multiple polyps, especially if CRCs are MSS. Furthermore, an FDR affected with CRC provides an increased probability of detecting biallelic mutation carriers. Individually and collectively, these phenotypes are relatively insensitive predictors of detection of biallelic mutations. Hence, it is far less clear on what basis screening could be extended to other groups outside those with a presumptive diagnosis of attenuated FAP. Given that MSI testing is advocated for all patients in whom CRC is diagnosed before age 50 years,[33] testing *MUTYH* in patients with MSS in this context is attractive.

Some researchers have proposed an additional dominant effect for *MUTYH*.[6-9,11-13] If this were the case, this would have significance from a public health perspective—even without direct clinical importance—even if the effect were modest. On the basis that the population carrier frequency of *MUTYH* mutations in the United Kingdom population is approximately 2%, this analysis had 80% power to demonstrate an association that stipulated a significance level of 5% if the GRR was only approximately 1.3. The findings in this study and the meta-analysis we have performed of previously published case-control series strongly argue against heterozygosity conferring an increased CRC risk.

Colorectal cancers that arise from *MUTYH* inactivation are characterized by an increased frequency of somatic transversion[34] and are thought to progress through a district pathway that does not involve MSI.[35] The association between MSS and biallelic mutation status in this study and in previous studies supports this tenet. Data from knockout mouse models indicate that, on an $APC^{min/+}$ background monoallelic inactivation does not impact on tumor burden or on the signature of G-T transversions.[34] These data show no correlation between MSS and monoallelic mutation status and, thus, are in keeping with monoallelic mutation that has no functional consequences. Collectively, these data provide additional support that heterozygosity per se is not risk factor for CRC.

Genetic testing for FAP and HNPCC[33] and targeted screening to individuals at a heightened risk of developing CRC because of family history have been shown to be highly effective strategies for reducing the risk of developing CRC.[36] Although the contribution of biallelic mutations to the incidence of CRC is not high, the risk of disease is substantial and justifies regular colonoscopic surveillance. Although mutation screening should be directed to patients with *APC*-negative polyposis and early-onset proximal MSS CRC in whom detection rates will be highest, the expanded phenotype associated with *MUTYH* mutation needs to be recognized. Finally, given that screening for the common *MUTYH* mutations, which are predominant in European populations, is simple and cheap, there is considerable merit in performing generalized screening of all patients with CRC from a public health perspective, except for the limited predictive value of a negative test result.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Final approval of manuscript: Richard S. Houlston

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