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Association between monoallelic *MUTYH* mutation and colorectal cancer risk: a meta-regression analysis

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Abstract

Whether people who inherit a mutation in *MUTYH* from only one parent (monoallelic mutation) are at increased risk of colorectal cancer (CRC) remains controversial. Most previous studies and meta-analyses have not found statistically significant associations but, given carriers are relatively rare, may be underpowered to detect small increased risks. We have conducted a systematic review and meta-regression analysis of previously published case–control studies to estimate the strength of association for monoallelic *MUTYH* mutation and CRC risk. Potential sources of heterogeneity were evaluated. We have compared the carrier frequency in cases with a family history of CRC to that of controls, as a novel and powerful design, to measure statistical evidence of an association but not the strength of association. The magnitude of the genotype-disease association, estimated from a pooled odds ratio comparing cases unselected for family history with controls, was 1.15 (95% CI = 0.98–1.36) and not substantially altered by adjustment for potential sources of heterogeneity. Monoallelic mutation carrier frequency was greater for cases ascertained due to a family history (3.3%; SE 0.9%) than for controls (1.4%; SE 0.3%) (P = 0.02). Monoallelic *MUTYH* mutation carriers are at increased risk of CRC but the average increase is small.

Keywords

MUTYH; Colorectal cancer; Family history

Introduction

People with a family history of colorectal cancer (CRC) are, on average, about twice likely to be diagnosed with CRC than those with no family history [1, 2]. All the causes for this excess risk are unknown as less than half of it is explained by rare mutations in the known major CRC susceptibility genes (mismatch repair genes [3] and adenomatous polyposis coli gene [4]) and the common, but not necessarily causal, variants associated with small increments in risks recently identified by genome wide association studies [5].

Human *MutY* homologue (*MUTYH*) gene is a base excision repair gene which repairs oxidative damage of DNA by excising adenines incorporated opposite 8-oxo-7,8-dihydro-2′-

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deoxyguanosine (8-oxoG) [6]. A number of studies [7-20] have confirmed that biallelic mutation carriers are at high risk of CRC. It remains uncertain, however, as to whether, and to what degree carriers of a mutation inherited from only one parent (monoallelic mutations) are at increased risk of CRC despite many case–control studies [10-24] and several meta-analyses [15-18,20, 25] that have been conducted to date. This lack of certainty is in part due to the rarity of monoallelic mutation carriers in the population and likely small increased risk. For example, assuming a population carrier frequency of 1.7% in Caucasian populations, a study of at least 34,000 cases and 34,000 controls is needed to have 90% power to detect an odds ratio of 1.2 or greater at the 0.05 level of significance.

Justifications for a further meta-analysis are: (1) the most recently published meta-analysis [20] did not include two unselected case–control studies (including the second largest study to date [19]); and (2) there has been no meta-analysis of familial case–control studies which is a powerful design to measure the statistical evidence of an association. Comparison of the genotype frequency for cases ascertained because they have a family history of disease with that for controls is a more powerful but seldom used design to address the *evidence* (i.e. *P* value) for an association [26, 27]. If monoallelic mutations are more common in CRC cases with a family history of disease compared to controls (with or without a family history of disease), then there is evidence that the monoallelic mutation is associated with disease risk. This is a more powerful design than the traditional case–control design, as the cases with a family history of CRC will be enriched for monoallelic mutation carriers *if* monoallelic mutations increase the risk of CRC. Note, however, that this "familial cases versus controls" design cannot be used to estimate the *magnitude* (i.e. odds ratio) of the association in the population.

One aspect of monoallelic *MUTYH* mutations under-studied to date is the role of the less commonly reported mutations. Almost all previous studies [11, 12, 14-16, 20,21] have restricted their analyses to the variants Y179C and G396D (previously known as Y165C and G382D) which account for more than 80% of all reported mutant alleles of *MUTYH* in Caucasian populations [9, 28, 29]. Previous meta-analyses have not included *MUTYH* sequence variants which are common in different ethnic groups; for example, E480X in Indian, Y104X in Pakistani [8, 28, 30], c.1437_1439delGGA in Italian [31], c. 1228_1229insGG in Portuguese [32], Q498H in German [33], and G25D and P18L in Chinese populations [34]. In this analysis we have assessed the combined role of all previously identified pathogenic variants in *MUTYH* mutations on the risk of CRC, and separately for Y179C and G396D.

We performed meta-analysis of case–control studies to estimate the *strength* of association between monoallelic mutations and CRC risk. We have compared the frequency of monoallelic mutation carriers in cases with a family history of CRC to that of controls to estimate the statistical *evidence* of the association.

Materials and methods

Identification of studies

MEDLINE, PUBMED (http://www.ncbi.nlm.nih.gov/) was searched for all relevant case–control studies of the association between germline monoallelic mutations in *MUTYH* and risk of CRC using the following combinations of key words: "colon or colorectal", "cancer or neoplasm or tumour" and "*MUTYH* or *MYH* or *hMYH* or human *MutY* or base excision repair gene". Search engines, such as "Google Scholar" and "SuperSearch" of the University of Melbourne library, were also used to search for related articles. No language restrictions were imposed. References from relevant articles, letters, reviews and previous meta-analyses were reviewed to identify any additional studies that were not indexed by the electronic

database. Studies were reviewed initially on the basis of title and abstracts, and then all full manuscripts for those that appeared relevant were obtained and checked for eligibility.

Eligibility criteria

case–control studies were eligible if they reported analyses of the associations between *MUTYH* mutations and CRC risk. Eligible studies needed to have presented the genotype data for cases and controls, the number of cases and controls tested, and to have described whether or not case ascertainment was independent of family history of CRC. Studies were excluded if they (1) defined cases by presence of multiple adenomas or polyposis, and not CRC, (2) did not state how CRC diagnoses were confirmed, (3) ascertained cases only because they had no family history, (4) recruited cases under more than one category of family history but did not provide numbers for each ascertainment method, (5) only reported on variants of *MUTYH* previously described as non-pathogenic, or (6) reported data included in a later publication.

Definitions

Exposure was defined as carrying a monoallelic germline mutation in *MUTYH* (as evidenced by testing DNA extracted from a blood sample). CRC cases were *MUTYH* genotyped people with a histologically confirmed colorectal adenocarcinoma and controls were *MUTYH* genotyped people without a previous diagnosis of CRC. In terms of groups, familial cases were defined as CRC cases ascertained because they had a family history of CRC. Unselected cases were defined as CRC cases who were ascertained irrespective of their family history.

Data extraction

For each study, the following data were extracted: first author's name, year of publication, country in which the study was performed, name of the study if given, study design, case ascertainment method, recruitment and selection criteria for cases and controls, number of individuals and their age and sex distributions for both cases and controls, and the number of monoallelic and biallelic carriers of *MUTYH* mutations by variant tested for both cases and controls in each study. Corresponding authors were contacted to confirm and request data if required. The names of *MUTYH* variants were provided using nomenclature according to the Leiden Open Variation Database of the *MUTYH* gene [35].

Statistical analysis

For familial case–control studies, we compared the pooled frequency of monoallelic mutations in cases to that of controls. For unselected case–control studies, the pooled odds ratio (OR) and its 95% confidence interval (CI) was estimated to compare the frequency of monoallelic *MUTYH* mutation carriers between cases and controls. Odds ratios were estimated for each genotype, and forest plots of the odds ratios were generated. We fitted linear meta-regression models to the log-transformed individual study odds ratios to create pooled estimates and to evaluate the role of several potential sources of heterogeneity; carrier frequency in controls, study site (Australia, Europe and North America), ascertainment method of controls (population-based or hospital-based), and the year of publication. To test the influence of individual studies on the pooled estimate, we omitted data from each study one at a time and repeated the analysis. Random and fixed effects models were fitted, and heterogeneity was tested using Cochran's *Q* statistic. Funnel plots and statistical tests for funnel plot asymmetry were performed to test the evidence of publication bias [36, 37].

All statistical tests were two-sided and, following convention, statistical significance for testing a predetermined null hypothesis was assessed by $P < 0.05$. All statistical analyses were conducted using Stata 10.0 [38].

Results

Of the 221 studies identified by the literature search, 16 studies [11-15, 17-21, 23, 24, 33, 39-41] passed the inclusion and exclusion criteria (Fig. 1). Tables 1 and 2 show that there were five studies using familial cases [14,24, 33, 39, 40] comprising a total of 363 cases and 1,698 controls, and 12 studies using unselected cases [11-15, 17-21, 23, 41] comprising a total of 21,369 cases and 14,639 controls. One study [14] contained both familial and unselected cases. For this study we included the relevant cases for each analysis and used all controls for both analyses.

Studies using familial CRC cases

All five studies attempted to exclude known or suspected cases due to mutation in a mismatch repair gene (Table 1). All studies recruited controls from the general population irrespective of a family history of CRC [14, 24, 33, 39] except for one study where controls had no family history of CRC and no sign of neoplasia in colonoscopy [40].

Table 3 shows that all but one study found that the monoallelic *MUTYH* mutation carrier frequency for familial cases was greater than for controls, though the difference was not statistically significant in any study (all $P \geq 0.05$). Overall, monoallelic *MUTYH* mutations were identified in 12 of 363 familial cases (3.3%; SE 0.9%) compared with 23 of 1,698 controls (1.4%; SE 0.3%) ($P = 0.02$).

Studies using unselected CRC cases

Table 2 shows that eight of the 12 studies [11, 12, 14, 18-21, 23] found a higher frequency of monoallelic mutation carriers in CRC cases compared with controls, but for all except one [19], the difference was not statistically significant. Overall, carriers of monoallelic *MUTYH* mutation were found in 411 of 21,369 cases (1.9%; SE 0.9%) and 243 of 14,639 controls (1.7%; SE 0.1%).

The association between being a monoallelic *MUTYH* mutation carrier for any variant and CRC was estimated as a pooled odds ratio of 1.15 (95% CI = 0.98–1.36) (Fig. 2). There was no evidence to reject the homogeneity of studies (Cochran's $Q = 8.88$, with 11 degrees of freedom; $P = 0.6$). The pooled odds ratio for the association between a monoallelic mutation and CRC was 1.35 (95% CI = 0.99–1.85) for Y179C; and 1.06 (95% CI = 0.88–1.28) for G396D.

Heterogeneity

From meta-regression analysis, association between monoallelic *MUTYH* mutation and CRC risk remained virtually unchanged after adjusting for: the monoallelic carrier frequency in controls ($P = 0.15$), the ascertainment method of controls ($P = 0.34$), study site ($P = 0.40$) or the year of publication ($P = 0.84$). There were no obvious differences in summary estimates when each study was omitted one at a time and analysis was repeated, consistent with there being no major influence of individual studies on the overall estimate.

Publication bias

Funnel plots did not reveal any evidence of publication bias of the studies included in either meta-analysis (Fig. 3). Begg's statistical tests also showed no evidence of publication bias; $P = 0.59$ for studies using familial cases and $P = 0.71$ for studies using unselected cases.

Discussion

We have shown that people who have inherited a mutation in the gene *MUTYH* from only one parent (monoallelic mutation carriers) are at increased risk of CRC but only by a small amount, about 15%. The novelty of this aspect of the analysis is that it includes the second largest casecontrol study to date, which was not included in the most recent meta-analysis. The statistical significance of the association is provided by our novel meta-analysis of studies using cases ascertained because they have a family history of the disease, which is more powerful in terms of sample size than unselected case–control studies for addressing this issue. This analysis allow us to reject the null hypothesis that the carrier frequency is the same for cases and controls ($P = 0.02$), as the cases with a family history of CRC will be enriched for monoallelic mutation carriers *if* monoallelic mutations increase the risk of CRC. The magnitude of the association is provided by the meta-analysis of standard case–control studies (OR = 1.15). The observed association is unlikely to be due to biallelic mutation carriers, as biallelic mutations were tested for both cases and controls by each study and we excluded them from our analysis.

The meta-analysis of studies in which cases were ascertained irrespective of a family history of CRC (unselected case–control studies) comprised a total pooled sample of 21,369 CRC cases and 14,639 controls, which is the largest study to date. We estimated the strength of association between monoallelic *MUTYH* mutations and CRC after adjusting for possible sources of heterogeneity including carrier frequency of controls, control ascertainment, study site and the year of publication, but the association was not changed. The estimate of the effect size for this analysis was similar to that of the recent meta-analysis of Lubbe et al. [20] (OR = 1.14). However, with the use of the familial cases we have been able to conclude that there was an statistically significant evidence that monoallelic carriers are at greater risk than non-carriers ($P = 0.02$), whereas the study of Lubbe et al. which did not utilize familial cases reported a P value of 0.12.

We did not find statistical significant evidence for an association between specific variants of *MUTYH* mutation and CRC risk separately for Y179C and G396D, but had little power to detect even moderate associations given the decrease in sample size from stratification by mutation type. Among previous case–control studies, the study of Clearly et al. [19] was the only one that screened for the nine most frequent variants of *MUTYH* mutations in cases and controls. Mutation testing in all other studies was restricted to the most common mutations Y179C and G396D in Caucasian populations, or only one or two additional variants. A comprehensive mutation screening approach might be required to observe a significant association with CRC risk, if present.

Houlston and Peto [27] have pointed out that standard case–control designs, in which cases are ascertained irrespective of a family history of the disease, have limited power to identify alleles with small associations with risk if the carrier frequency of the deleterious allele in population is low (e.g. *MUTYH* mutations). Antoniou and Easton [26] showed that the sample size required to detect a disease allele association was substantially reduced if the cases were selected because they had a family history of the disease. Therefore case–control studies using familial cases hold promise as more powerful to examine the question of the existence of an association between monoallelic *MUTYH* mutations and CRC risk, despite their typically low frequency in the population. Our analyses here demonstrate the importance of study design using cases selected for a family history to detect evidence for a role of genetic variants on disease risk.

Two family studies based on the relatives of monoallelic *MUTYH* carriers diagnosed with CRC did observe a significant association between monoallelic *MUTYH* mutation and CRC

risk. A kin-cohort study [25] of the relatives of *MUTYH* mutations carriers observed that monoallelic mutation carriers who had a relative diagnosed with CRC were at threefold risk of CRC (Hazard Ratio 2.9; 95% CI 1.2–7.0; $P = 0.02$) in addition to that expected due to their family history. A retrospective cohort analysis [42] of obligate carriers of monoallelic mutations in *MUTYH*, being the parents of biallelic carriers, estimated monoallelic carriers had twice the CRC risk of general population (Standardized Incidence Ratio 2.12; 95% CI 1.30–3.28; $P < 0.01$). It is important to note that the estimates of association from these studies (two to threefold increased risk) are relevant to monoallelic carriers who have a relative with CRC, whereas the result we have reported here (1.15-fold increased risk) is relevant to monoallelic carriers from the general population (irrespective of family history of CRC).

We have included all previously published unselected and familial case–control study in our meta-analyses. To assist readers evaluate the quality of and differences between different study designs we have provided extracted relevant details in Table 1. Meta-regression models might be able to resolve some of the inconsistencies across studies, within the limitations of power, but considerable residual heterogeneity for unmeasured modifiers of risk might remain.

When combined, all previous case–control studies of monoallelic *MUTYH* mutations and CRC suggest that the risk of CRC is increased for carriers, but only to a small degree, on average. Given the rarity of monoallelic mutations they account for only a trivial proportion of CRC. Therefore the clinical significance of increased risk of CRC for monoallelic mutation carriers is minimal. This study demonstrates the value of using familial cases for detecting rare, “low penetrance” cancer susceptibility alleles.

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Abbreviations

CRC	Colorectal cancer
HNPCC	Hereditary non-polyposis colorectal cancer
MUTYH	Human *MutY* homologue
MMR	Mismatch repair
FAP	Familial adenomatous polyposis
APC	Adenomatous polyposis coli
IBD	Inflammatory bowel disease
CI	Confidence interval
OR	Odds ratio
SE	Standard error

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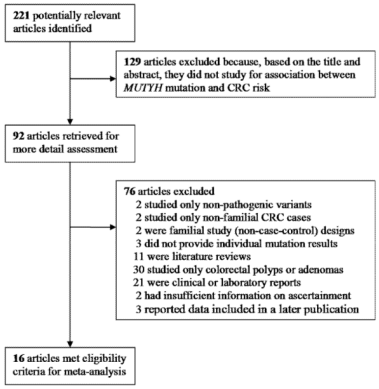

Fig. 1.
Flow diagram of the selection of studies

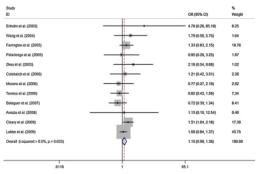

Fig. 2.

Meta-analysis of monoallelic MUTYH mutations for any variant and CRC risk in case–control studies where cases were ascertained independently of a family history of CRC. *Horizontal lines* represent 95% CIs. Each *box* represents the OR point estimate, and its area is proportional to the weight of study (the inverse variance of each study's effect). The *diamond* represents the combined summary estimated OR, with 95% CI given by the width of the *diamond*. The *unbroken vertical line* is at the null value (OR = 1)

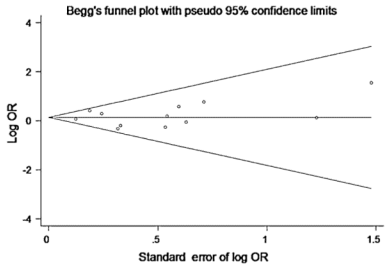

Fig. 3.

Begg's funnel plot for the meta-analysis of studies where cases were ascertained independently of a family history of CRC. The *horizontal line* in the funnel plot indicates the fixed-effects summary estimates (using inverse-variance weighting). The *sloping lines* indicate the expected 95% CIs for a given standard error assuming no heterogeneity between studies. Asymmetry in the funnel plot indicate a systematic under-reporting of smaller and negative studies (publication bias) or a systematic difference between smaller and larger studies that arises from inherent between-study heterogeneity

Table 1

Selection criteria for cases and controls in the studies included in the meta-analysis

Study	Year	Site	Selection of CRC cases	Selection of controls
Studies where cases were ascertained because of a family history of CRC				
Kambara et al. [24][a]	2004	Brisbane	Familial CRC cases not meeting the Bethesda guidelines; no evidence of *MMR* deficiency	Healthy blood donors
Zhou et al. [14]	2005	Sweden	Positive family history; excluded germline mutations in *MLH1, MSH2* and *MSH6*	Healthy blood donors
Gorgens et al. [33]	2006	Germany	Consecutive cases from HNPCC registry; fulfilled at least one criterion of the revised Bethesda guidelines; family history showing no vertical transmission of disease; microsatellite stable and normal protein expression for *MSH2, MLH1, MSH6* and *PMS2*	Healthy blood donors
Peterlongo et al. [39][a]	2006	New York	Families fulfilling Amsterdam I or II criteria or families with at least three individuals affected with CRC diagnosed at any age; no mutations in *MLH1, MSH2* and *MSH6*	Healthy blood donors and individuals unaffected by any cancer; recruited at hospitals and community centres; with comparable ethnic backgrounds
Grunhage et al. [40]	2008	Germany	Fulfilled at least two of the three criteria: (1) family history of at least three CRC cases in two generations, (2) one of the CRC cases a first degree relative of the index patient, (3) one of the cases with a first diagnosis at the age of 50 years or earlier; and normal expression of *MLH1, MSH2* and microsatellite stable	Individuals with no prior personal and family history of CRC or any other tumour; no sign of abnormal mucosal growth in colonoscopy; recruited at out patient clinics and private practice for colonoscopy
Studies where cases were ascertained independently of a family history of CRC				
Enholm et al. [11]	2003	Finland	CRC cases recruited at nine Finnish regional central hospitals	Healthy blood donors
Wang et al. [21]	2004	Minnesota	Individuals with CRC from Mayo Clinic; *APC* mutation negative	No polyp in screening colonoscopy; recruited at Mayo clinic
Farrington et al. [12]	2005	Scotland	CRC cases ascertained at surgical and pathology departments in Scotland; recruited shortly after diagnosis of CRC	Healthy controls recruited through central National Health Service; age and sex-matched
Peterlongo et al. [13]	2005	New York	CRC cases ascertained at oncology clinic at Memorial Hospital in New York; recruited regardless of ethnic origin and religion	Individual unaffected by any cancer; ages 30–69; age, ethnic group and religion group frequency matched, recruited at hospitals and blood donation centres
Zhou et al. [14]	2005	Sweden	CRC cases from surgery department in Uppsala University Hospital and pathology department in Linköping Hospital	Healthy blood donors
Colebatch et al. [23]	2006	Sydney	Individuals undergoing complete surgical resection of CRC; recruited at St. Vincent's Hospital; excluded known inflammatory bowel disease, FAP and	Healthy blood donors

Study	Year	Site	Selection of CRC cases	Selection of controls
			HNPCC	
Moreno et al. [41]	2006	Spain	Newly diagnosed CRC cases recruited at University Hospital in Barcelona	Patients with a new disease, exclude chronic diseases; recruited at University Hospital in Barcelona during the same period of time
Tenesa et al. [15]	2006	Scotland	CRC cases ascertained at surgical and pathology departments in Scotland; recruited shortly after diagnosis of CRC	Healthy controls recruited through central National Health Service; age and sex-matched
Balaguer et al. [17]	2007	Spain	Newly diagnosed CRC cases recruited at 25 Spanish hospitals Epicolon project; excluded patients with *FAP, APC, MSH2* and *MLH1* mutation carriers, or personal history of IBD	Individual unaffected by any cancer; age and sex-matched controls; recruited at outpatient clinics of orthopaedic surgery departments from 25 Spanish hospitals in Epicolon project
Avezzù et al. [18]	2008	Italy	CRC cases unselected for phenotype, unrelated and consecutive cases of CRC; recruited at Padova's hospital	Healthy blood donors and individuals with no sign of abnormal mucosal growth in colonoscopy who were recruited at Padova hospital; age-matched
Cleary et al. [19][c]	2009	Canada, Australia, USA	Known cases of FAP, non-incident cases, those diagnosed with in situ malignancies, *MLH1, MSH2* and *MSH6* mutations were excluded	Population-based controls; age and sex frequency matched controls
Lubbe et al. [20][b]	2009	UK	CRC cases diagnosed before age 80; identified through the National Study of Colorectal Cancer Genetics (NSCCG); did not exclude for known genetic susceptibility	Spouses of patients with malignancies from other cancer studies; unaffected by any cancer

Presented separately for studies where cases were selected because of a family history of CRC and studies where cases were selected independently of a family history of CRC

[a] Only familial CRC cases were used in this analysis

[b] Contains the data reported by Fleischmann et al. [22] and Webb et al. [16]

[c] Contains the data reported by Croitoru et al. [10]

Table 2

Summary of monoallelic and biallelic *MUTYH* mutation carriers in the studies included in the meta-analysis

Study	Year	Variant analysed	Cases							Controls							
			Total cases	Monoallelic					Biallelic		Total controls	Monoallelic					Biallelic	
				n	%	Y179C	G396D	Other	n	%		n	%	Y179C	G396D	Other	n	%
Studies where cases were ascertained because of a family history of CRC																		
Kambara et al. [24]	2004	*Y179C, G396D, Y104X, E480X*	19	1	5.26	1	0	0	0	0.00	53	1	1.89	1	0	0	0	0.00
Zhou et al. [14]	2005	*Y179C, G396D*	84	0	0.00	0	0	-	0	0.00	469	3	0.64	2	1	-	0	0.00
Gorgens et al. [33]	2006	*Y179C, G396D, Q498H*	50	2	4.00	0	1	1	0	0.00	116	2	1.72	1	1	0	0	0.00
Peterlongo et al. [39]	2006	*Y179C, G396D, c.1437_1439delGGA*	117	5	4.27	2	3	0	2	1.71	967	16	1.65	4	12	0	0	0.00
Grunhage et al. [40]	2008	*Y179C, G396D*	93	4	4.30	3	1	-	2	2.15	93	1	1.08	0	1	-	0	0.00
Total			**363**	**12**	**3.31**	**6**	**5**	**1**	**4**	**1.10**	**1,698**	**23**	**1.35**	**8**	**15**	**0**	**0**	**0.00**
Studies where cases were ascertained independently of a family history of CRC																		
Enholm et al. [11]	2003	*Y179C, G396D*	1,003	5	0.50	1	4	-	4	0.40	424	0	0.00	0	0	-	0	0.00
Wang et al. [21]	2004	*Y179C, G396D*	444	10	2.25	5	5	-	2	0.45	313	4	1.28	2	2	-	0	0.00
Farrington et al. [12]	2005	*Y179C, G396D*	2,217	45	2.03	14	31	-	12	0.54	1,822	28	1.54	8	20	0	0	0.00
Peterlongo et al. [13]	2005	*Y179C, G396D, c.1437_1439delGGA*	555	4	0.72	2	2	0	2	0.36	918	7	0.76	2	5	0	0	0.00
Zhou et al. [14]	2005	*Y179C, G396D*	438	6	1.37	4	2	-	0	0.00	469	3	0.64	2	1	-	0	0.00
Colebatch et al. [23]	2006	*Y179C, G396D, c.1437_1439delGGA*	872	11	1.26	3	8	0	2	0.23	478	5	1.05	1	4	0	0	0.00
Moreno et al. [41]	2006	*Y179C, G396D*	278	6	2.16	0	6	-	0	0.00	323	9	2.79	0	9	-	0	0.00
Tenesa et al. [15]	2006	*Y179C, G396D*	928	18	1.94	3	15	-	5	0.54	845	20	2.37	6	14	-	0	0.00
Balaguer et al. [17]	2007	*Y179C, G396D, c.l228_1229insGG, c.1147delC*	1,116	19	1.70	4	15	0	8	0.72	934	22	2.36	1	20	1	0	0.00
Avezzù et al. [18]	2008	*Y179C, G396D, c. 1437_1439delGGA, c.933 + 3A > C*	439	2	0.46	0	2	0	2	0.46	247	1	0.40	1	0	0	0	0.00

Study	Year	Variant analysed	Cases							Controls							
			Total cases	Monoallelic					Biallelic		Total controls	Monoallelic					Biallelic	
				n	%	Y179C	G396D	Other	n	%		n	%	Y179C	G396D	Other	n	%
Cleary et al. [19]	2009	*Y179C, G396D, Y104X, R274Q, c. 1147delC, c.933 + 3A > C, E480X, c.1437_1439delGGA, Q391X*	3,811	87	2.28	15	63	9	27	0.71	2,802	43	1.53	10	32	1	1	0.04
Lubbe et al. [20]	2009	*Y179C, G396D*	9,268	198	2.14	70	128	-	27	0.29	5,064	101	1.99	26	75	-	1	1.02
Total			**21,369**	**411**	**1.92**	**121**	**281**	**9**	**91**	**0.43**	**14,639**	**243**	**1.66**	**59**	**182**	**2**	**2**	**0.01**

Presented separately for studies where cases were selected because of a family history of CRC and studies where cases were selected independently of a family history of CRC

Table 3

Frequencies of monoallelic *MUTYH* mutation carriers among CRC cases who were ascertained because of a family history of CRC and controls

Study	Familial cases *n/N* (%)	Controls *n/N* (%)	*P* value
Kambara et al. [24]	1/19 (5.3%)	1/53 (1.9%)	0.44
Zhou et al. [14]	0/84 (0.0%)	3/469 (0.6%)	0.46
Gorgens et al. [33]	2/50 (4.0%)	2/116 (1.7%)	0.38
Peterlongo et al. [39]	5/117 (4.3%)	16/967 (1.7%)	0.05
Grunhage et al. [40]	4/93 (4.3%)	1/93 (1.1%)	0.17
Total	**12/363 (3.3%)**	**23/1,698 (1.4%)**	**0.02**

N = total number, *n* = number of monoallelic *MUTYH* mutation carriers