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Increased frequency of disease-causing *MYH* mutations in colon cancer families

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The genetic factors that cause clustering of colorectal cancers (CRCs) other than mutations in the mismatch repair (MMR) genes are not well understood. Clustering in families who lack MMR gene mutations may be attributable to low-penetrance mutations. Hypothetically, mono-allelic *MYH* mutations could contribute to the risk of CRC in these families. Using Fisher's exact test and logistic regression, we compared the frequency of the known disease-causing *MYH* mutations Y165C, G382D and 466delE in 137 probands (117 cases with CRC and 20 cases diagnosed on the basis of adenomatous polyps only) from families with three or more CRCs but negative for mutations in the MMR genes and in 967 healthy controls with comparable ethnic backgrounds. Of 137 cases, 6 (4.4%) carried mono-allelic *MYH* mutations compared with 16 of 967 (1.6%) controls. In addition, three bi-allelic *MYH* mutation carriers, who eventually developed *MYH*-associated polyposis, were also identified in families with pedigree structures consistent with dominant inheritance of CRC susceptibility. By Fisher's exact tests, there was a statistically different frequency of cases with any *MYH* mutation (mono- or bi-allelic carriers; *P*-value = 0.002) and of cases with mono-allelic *MYH* mutation (*P* = 0.04) compared with the controls. Using a logistic regression model, the unadjusted odds ratio associated with any *MYH* mutation was 4.14 (*P*-value < 0.001); for mono-allelic carriers, it was 2.79 (*P*-value = 0.04). Adjusting for ethnic backgrounds, gender and age, the odds ratio associated with any disease-causing *MYH* mutation was 3.23 (*P*-value = 0.01); for mono-allelic carriers, it was 1.99 (*P*-value = 0.20). Overall, the results support previous studies suggesting that mono-allelic mutations of *MYH* constitute low-penetrance CRC-causing alleles. These data further support a model in which low-penetrance alleles are enriched in MMR gene mutation-negative CRC families.

Abbreviations: CRCs, colorectal cancers; HNPCC, hereditary non-polyposis colorectal cancer; MMR, mismatch repair.

Introduction

Mutations that disrupt the function of the proteins encoded by the mismatch repair (MMR) genes *MLH1, MSH2, MSH6* and *PMS2* cause the Mendelian disease hereditary non-polyposis colorectal cancer (HNPCC) (1). Carriers of a germ-line mutation in one of these high-penetrance genes have a >70% lifetime risk of developing colorectal cancer (CRC). Germ-line mutation of one of the MMR genes accounts for ~3% of all CRCs; yet, family and twin studies have shown that heritable genetic factors play a role in as much as 35% of CRC cases (2). When family history criteria—Amsterdam Criteria (AC) I and AC II (3,4)—are used to ascertain families for genetic testing, approximately half of the families are found to carry a disease-causing MMR gene mutation (5,6). Thus, there are many families with strong histories of CRC that are genetically unexplained. We hereafter refer to these families as MMR gene mutation-negative families.

To explain the familial clustering in MMR gene mutation-negative families, a polygenic disease model, in which multiple, low-penetrance and interacting genetic factors are segregating, is highly attractive (7,8). In such a model, familial clustering would be explained by the segregation of an excess of low-penetrance alleles within the high-risk family. If this genetic model is correct, alleles that increase CRC risk should be relatively more frequent in familial cases compared with sporadic cases (7). For example, the *APC*c.3920T>A (I1307K) (9) and the *CHEK2*c.1100delC (10) mutations have been identified as low-penetrance mutations that confer a 1.5- to 3-fold increase in CRC and breast cancer risk, respectively, in case–control studies. Each of these mutations is two to three times more frequent in familial cases compared with sporadic cases (11,12).

To search for new low-penetrance CRC susceptibility alleles, then, it may be most efficient to sequence cases from MMR gene mutation-negative families. We sought to test the efficacy of this approach through the investigation of the *MYH* gene, the product of which excises 8-oxo-guanine (GO) lesions specifically from GO:A mismatches—a major oxidative lesion in DNA. Bi-allelic mutations of *MYH* are associated with adenomatous polyposis in persons who test negative for mutations in the *APC* gene (13,14). Importantly, carriers of bi-allelic *MYH* mutations are at greatly increased risk of developing CRC, because such carriers are ~100 times more frequent amongst consecutive CRC cases than expected (15–22). Carriers of mono-allelic *MYH* mutations also appear to be at increased risk of developing

CRC, because, in naively combined data from the published case–control studies (op. cit.), the frequency of carriers in consecutive CRC cases (109 carriers in 6612 cases = 1.65%) is significantly greater than in cancer-free controls from the same geographic areas (69 carriers in 5878 controls = 1.17%) (unadjusted odds ratio = 1.41, 95% CI: 1.03–1.94, $P = 0.03$).

Following the polygenic disease model, we would predict that disease-causing *MYH* mutations are more frequent in CRC cases with a strong family history of CRC than in consecutive CRC cases. In the present study, we have sequenced *MYH* and assayed for the three most common disease-causing *MYH* mutations (Y165C, G382D and 466delE) found in the Caucasian population in order to estimate the frequency of *MYH* mutations in MMR gene mutation-negative families and in healthy controls with comparable ethnic backgrounds.

Subjects and methods

Ascertainment of MMR gene mutation-negative HNPCC/HNPCC-like families

CRC families were ascertained at the cancer genetics clinics of three different cancer centers, namely, Memorial Hospital in New York City, Hospital Clinico San Carlos in Madrid and Istituto Nazionale Tumori in Milan. Probands were ascertained from families fulfilling Amsterdam I criteria, Amsterdam II criteria or our minimal criteria. The minimal criteria included families with at least three individuals affected with CRC, diagnosed at any age, with the CRCs occurring in the first- or second-degree relatives of an index case. Families meeting less stringent criteria were excluded with seven exceptions [see Ref. (23)]. Families were excluded in which a person was diagnosed with >20 adenomatous polyps. Within each family, persons with CRC or persons who developed adenomatous polyps were identified and available tumors were obtained for the analysis of microsatellite instability and of MLH1, MSH2 and MSH6 gene products by immunohistochemistry (testing was performed on one or more tumors in >90% of the families) (24). Blood samples from persons with CRC were obtained for DNA testing; if a person with CRC was not available (most often because all persons with CRC were deceased in the family), a blood sample was obtained from an individual diagnosed with one or more adenomatous polyps. The case tested for an MMR gene mutation was designated as the proband of the family for the purposes of this study.

Mutations in *MLH1, MSH2* and *MSH6* were identified by denaturing high performance liquid chromatography or by direct DNA sequencing (23). Probands with CRCs or adenomatous polyps that exhibited MSI but in which a disease-causing point mutation was not detected were also tested for large genomic deletions and insertions using fluorescent, semi-quantitative multiplex PCR. If no MMR gene mutation was detected in the proband but all the available colon tumors tested from the family exhibited MSI, then the proband was assumed to harbor an undetected MMR gene mutation and was excluded. Seven MMR gene mutation-negative probands were included in which MSI was detected in one tumor but not in other tumors tested in the family. A total of 137 probands were thereby ascertained—63 from Memorial Hospital, 38 from Hospital Clinico San Carlos and 36 from Istituto Nazionale Tumori. Of the 137 families, 57 fulfilled the Amsterdam I criteria, 10 fulfilled the Amsterdam II criteria and 70 fulfilled the minimal criteria for family history. These 70 families are hereafter referred to as HNPCC-like families.

Of the 137 probands, 117 were diagnosed with CRC and 20 were diagnosed on the basis of adenomatous polyps only. All but two of the patients included in the study on the basis of a diagnosis of adenomatous polyps but not CRC were ascertained at Memorial Hospital, where the majority of families entering the study were referred to the clinical genetics service from the endoscopy clinic and where a program of CRC prevention has for many years concentrated on the use of surveillance colonoscopy in persons with a family history of CRC (23). In persons with CRC, the distribution of the numbers of adenomatous polyps at initial ascertainment was as follows: 61 had no adenomatous polyps, 42 had 1–5, 4 had 6–10, 3 had 11–20 and no information was available for 7 patients. In persons with adenomatous polyps only, 15 had 1–5, 3 had 6–10 and 2 had 11–20. The mean age of all cases was 53.3 years (range: 28–89). The mean age of the CRC cases was 54.2 (range 28–89), and the mean age of the cases with adenomatous polyps only was 46.9 years (range 28–68).

Ascertainment of control individuals

A total of 967 cancer-free controls were analyzed in this study. A total of 621 controls were a subset of a larger series ascertained at hospitals and community centers that are geographically close to the Memorial Hospital in New York City. These controls had ethnic backgrounds that were comparable to the cases on the basis of race and religion. They had been screened previously for the Y165C, G382D and 466delE mutations as described previously (20). A total of 196 controls were unrelated healthy blood donors from the Blood Bank of Hospital Clinico San Carlos in Madrid. A total of 150 controls were blood donors consecutively collected from February to March 2004 at the Blood Bank of Istituto Nazionale Tumori in Milan, all aged >50 years and without a personal history of any neoplastic diseases. The mean age of the all controls together was 53.4 years (range 18–86, with data on age missing for eight controls).

Mutation analyses

We scanned for mutation in *MYH* by directly sequencing the entire coding region and the intron/exon boundaries of the gene. Primers for PCR amplification and sequencing were designed based on the *MYH* gene sequence (GenBank accession no. NT_004852.13). Sequence results were analyzed using Sequencer 4.2.2 software (Gene Code Corporation, Ann Arbor, MI, USA). Sequences of primers, PCR conditions and sequencing protocols are available upon request from the authors. The *MYH* gene was sequenced in its entirety in all mono-allelic mutation carriers, and they were also tested for large genomic deletions and insertions using fluorescent, semi-quantitative multiplex PCR and long-range PCR. We specifically genotyped for the Y165C, G382D and 466delE mutations as described previously (20).

Statistical analyses

MYH genotype frequencies in the MMR gene mutation-negative HNPCC/HNPCC-like cases and in controls were compared in 2×2 and 2×3 tables using a two-sided Fisher's exact test, and we tested for trend in frequencies of no mutation versus mono-allelic versus bi-allelic *MYH* mutations using the Cochran–Armitage test. Odds ratios and confidence intervals were calculated using logistic regression analysis in which case-control status was the dependent variable and mutation status was the predictor variable. Analyses were performed on the entire dataset in which mutation status was determined by the presence or absence of any mutation in *MYH* (mono-allelic or bi-allelic). Two subgroup analyses were performed: one in which bi-allelic mutation carriers were eliminated and another in which cases diagnosed with adenomatous polyps only were eliminated. In addition to the unadjusted logistic models, ethnicity/region, gender and age separately and together were also included as covariates in the logistic models. All analyses were conducted using SAS version 9.0 (SAS Institute Inc., Cary, NC).

Results

MYH mutations in MMR gene mutation-negative HNPCC/HNPCC-like families

To determine whether previously unrecognized disease-causing mutations might be present in our families, we sequenced the entire coding region of *MYH* in 80 probands—62 from New York, 5 from Madrid and 13 from Milan. A total of 21 DNA changes were identified (Table I)—11 in the non-coding regions and 10 in the coding regions of *MYH*. Two DNA changes were localized to the promoter region of *MYH*, and nine changes were intronic; none of these DNA changes affected splice sites. Of the 10 DNA changes in the *MYH* coding region, 1 was a previously reported silent mutation c.1389G>C; 3 were previously reported benign polymorphisms c.64G>A (p.Val22Met), c.972G>C (p.Gln324His) and c.1502C>T (p.Ser501Phe); 3 were the known disease-causing *MYH* mutations c.494A>G (p.Tyr165Cys, commonly referred to as Y165C), c.1145G>A (p.Gly382Asp, commonly referred to as G382D) and c.1395_1397delGGA (p.Glu466-del, commonly referred to as 466delE); and 3 were missense mutations c.658G>A (p.Val220Met), c.971A>G (p.Gln324-Arg) and c.1216C>A (p.Leu406Met). The c.971A>G and c.1216C>A mutations had not been reported previously. None of the previously unreported mutations was found in a

Table I. DNA changes in *MYH*[a] gene identified by direct DNA sequencing in probands from MMR gene mutation-negative HNPCC/HNPCC-like families

Mutation[b]	Individuals ($N = 80$)[c]		
	Normal	Heterozygote	Homozygote
Non-coding region			
c.−165C>T	79	1	0
c.−127C>T[d]	76	4	0
c.157+30A>G[d]	77	3	0
c.346+56G>A	78	2	0
c.462+35G>A[d]	63	16	1
c.648+21C>A	79	1	0
c.1145−27C>T[d]	78	2	0
c.1282−34C>T	79	1	0
c.1435−125C>A[d]	61	17	2
c.1435−40G>C[d]	63	16	1
c.1435−28G>A	79	1	0
Coding region			
c.64G>A (p.Val22Met)[d]	73	7	0
c.494A>G (p.Tyr165Cys)	78	2	0
c.658G>A (p.Val220Met)	79	1	0
c.971A>G (p.Gln324Arg)	79	1	0
c.972G>C (p.Gln324His)[d]	49	22	9
c.1145G>A (p.Gly382Asp)	75	4	1
c.1216C>A (p.Leu406Met)	79	1	0
c.1389G>C (p.-)	79	1	0
c.1395_1397delGGA (p.Glu466del)	79	1	0
c.1502C>T (p.Ser501Phe)	71	9	0

[a]GenBank accession no. NT_004852.13.
[b]Mutation nomenclature with 'c'. Symbol is based on cDNA sequence and nucleotide numbering is based on the A of the ATG translation initiation codon, i.e. nucleotide +1 in the reference sequence.
The p.Tyr165Cys mutation is also known as Y165C, p.Gly382Asp as G382D, and p.Glu466del as 466delE.
[c]Entries indicate the number of times each mutation was identified and its zygosity. The *MYH* gene was sequenced in 62 probands from New York, 13 from Milan and 5 from Madrid.
[d]Previously reported in dbSNP at http://www.ncbi.nlm.nih.gov/SNP/.

person with a known disease-causing *MYH* mutation; hence their significance is unclear. Because this analysis failed to identify any additional putative disease-causing mutations, the remaining 57 probands were tested only for the Y165C, G382D and 466delE mutations. Control individuals were tested only for these three mutations.

In the entire series of 137 cases, disease-causing *MYH* mutations were identified in 9 (6.6%) MMR gene mutation-negative HNPCC/HNPCC-like families (Figure 1 and Table II). The probands of families NY-1741, MD-9, MD-19 and MI-1484 were heterozygous for the G382D mutation, the probands of families NY-3571 and MD-152 were heterozygous for the Y165C mutation, and the probands of families NY-5998, MI-308 and MI-1317 were bi-allelic *MYH* mutation carriers with the genotypes Y165C/G382D, G382D/466delE and G382D/G382D, respectively. The *MYH* gene was sequenced in its entirety in the probands of families NY-1741, NY-3571, MI-1484, MD-9 and MD-152, and additionally they were screened for large genomic deletions and insertions using fluorescent, semi-quantitative multiplex PCR and long-range PCR. No additional *MYH* mutations were detected, making it unlikely that these probands harbor a second disease-causing *MYH* mutation. Insufficient DNA was available to perform these analyses in family MD-19. In the 967 cancer-free controls tested, we identified 16 mono-allelic *MYH* mutation carriers (1.6%; 4 Y165C heterozygotes and 12 G382D heterozygotes) and no bi-allelic *MYH* mutation carriers (Table II).

By Fisher's exact test of the 2×3 table for the comparison of the three genotypes or the 2×2 table for the comparison of any *MYH* mutation versus no mutation, the *P*-values were <0.001 and 0.002, respectively. Similarly, the *P*-value for trend by the Cochran–Armitage test was <0.001. Using a logistic regression model for any *MYH* mutation versus no mutation (Table III), we found an unadjusted odds ratio of 4.18 (95% CI: 1.81–9.65; *P*-value <0.001). Controlling for ethnic backgrounds, gender and age, we found an odds ratio of 3.23 (95% CI: 1.32–7.89; *P*-value = 0.01). Because the disease status of the cases who were diagnosed with polyps only is not as unambiguous as the cancer cases, we also performed analyses in which these probands were eliminated. In these logistic models, the unadjusted odds ratio was 3.75 (95% CI: 1.51–9.31; *P*-value = 0.004) and the fully adjusted odds ratio was 3.05 (95% CI: 1.17–7.98; *P*-value = 0.02). The reduction in the odds ratio in the CRC only analyses possibly reflects the association of bi-allelic *MYH* cases with colonic polyp development as the proband of family NY-5998, who is a bi-allelic *MYH* mutation carrier, is eliminated. Although age was significantly correlated in multivariate analysis of cases with CRC only, age was not correlated in the univariate analysis, perhaps reflecting the older ages of mono-allelic *MYH* mutation carriers in the Spanish CRC cases versus younger Spanish controls (Table I). In summary, with the bi-allelic mutation carriers included, the frequency of any *MYH* mutation in the cases was statistically different from the frequency in controls.

Bi-allelic MYH mutations in MMR gene mutation-negative HNPCC/HNPCC-like families

Finding bi-allelic *MYH* mutation carriers in HNPCC/HNPCC-like families (Figure 1B) was unexpected because the polyposis and CRC susceptibility associated with bi-allelic *MYH* mutations is thought to be recessive. The findings also raised the possibility that these cases have been mis-classified. Consequently, we sought additional clinical information on the patients and their relatives that might have accumulated in 2–5 years since the patients were seen in the genetics service.

The proband of family MI-308, who is a genetic compound for the G382D and 466delE mutations, was initially diagnosed with CRC and six adenomas at age 47. Since then, he has been diagnosed with an additional 69 adenomas. A brother of the patient was diagnosed with CRC at age 68, and we subsequently learned he had 38 adenomas. The patient's mother (an obligate *MYH* mutation carrier), two maternal aunts and two maternal cousins developed CRC, and the maternal side of the family is also remarkable for four stomach cancers. DNA was available from one maternal cousin with CRC, but the *MYH* genotype was normal. The proband of family MI-1317, who is homozygous for the G382D mutation, was initially diagnosed with CRC and 14 adenomas at age 43. Since then, he has been diagnosed with an additional 82 adenomas. The *MYH* genotype of the mother was heterozygous for the G382D mutation, as expected, but the genotypes of the maternal aunts with CRC were normal. The proband of family NY-5998, who is a genetic compound for the G382D and Y165C mutations, had been diagnosed with 16 tubular adenomas at ascertainment. He has had two colonoscopies since that time in which 13 and 8–23 tubular

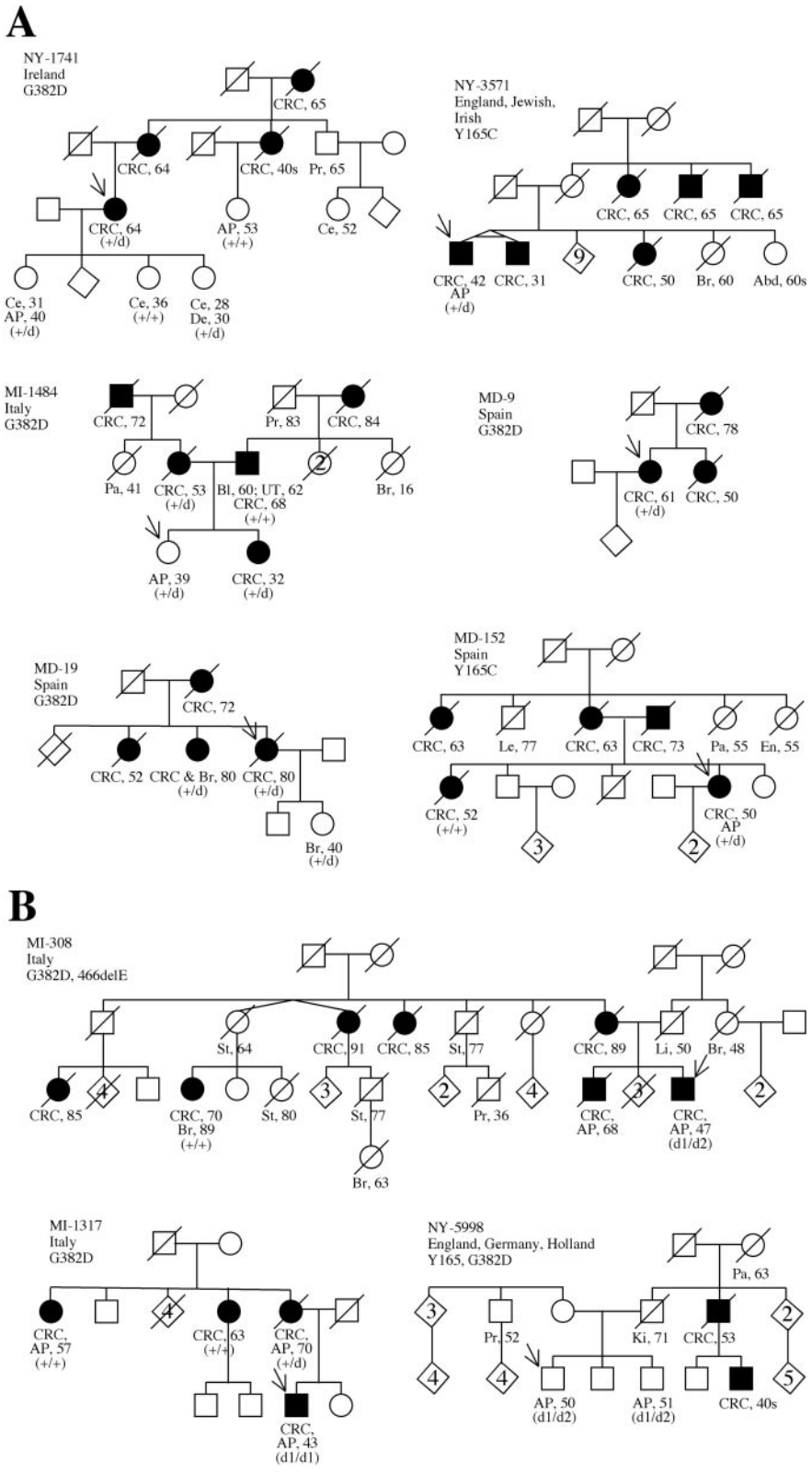

Fig. 1. Abbreviated pedigrees of the nine families with disease-causing mutations of *MYH*. (**A**) The six families in which mono-allelic *MYH* mutations were detected. (**B**) The three families in which bi-allelic *MYH* mutations were detected. Each of the probands in these families has since developed >20 adenomas. For each of the families, the geographic or ethnic origin and the mutation identified are indicated. NY, New York, USA; MD, Madrid, Spain; MI, Milan, Italy. Symbols: square, male; circle, female; diamond, gender unspecified; number in diamond, number of persons; diagonal bar, person deceased; arrow, designated proband; closed symbol, person diagnosed with CRC. *MYH* genotype is indicated under each person tested: (+/+), homozygous normal; (+/d), heterozygous; (d1/d1), homozygous mutant; (d1/d2), genetic compound. The abbreviation under each symbol indicates the diagnosis of a tumor, followed by the age of diagnosis: Abd, abdomen; AP, adenomatous polyps; Bl, bladder; Br, breast; Ce, cervix; De, Desmoid; En, endometrium; Ki, kidney; Le, leukemia; Li, liver; Pa, pancreas; Pr, prostate; St, stomach; UT, urinary tract.

Table II. Numbers of HNPCC/HNPCC-like cases and control individuals screened for the Y165C, G382D and 466delE disease-causing *MYH* mutations

Ascertainment[a]	HNPCC/HNPCC-like cases[b]				Controls		
	N	Mean age[c]	With mono-allelic mutations (%)	With bi-allelic mutations (%)	*N*	Mean age[d]	With mono-allelic mutations (%)[e]
New York non-Jewish	40	51.5	2 (5.0)	1 (2.5)	468	56.9	7 (1.5)
New York Jewish	23	51.7	0 (0.0)	0 (0.0)	153	62.4	0 (0.0)
Madrid	38	56.1	3 (7.9)	0 (0.0)	196	34.2	6 (3.1)
Milan	36	53.4	1 (2.8)	2 (5.6)	150	57.4	3 (2.0)
Total	137	53.3	6 (4.4)	3 (2.2)	967	53.4	16 (1.6)

[a]Because it was shown that frequency of disease-causing *MYH* mutations in Jewish was lower than in non-Jewish Caucasians (20), familial cases ascertained in New York were subdivided into Jewish and non-Jewish groups.
[b]The cases in this study were from families who were ascertained as having three of more CRCs inclusive of the proband and diagnosed at any age (HNPCC/HNPCC-like). Families with mutations in *MLH1*, *MSH2* or *MSH6* were excluded.
[c]Mean age refers to the age at diagnosis of CRC or adenomatous polyps of familial cases.
[d]Mean age refers to the age at which the sample was collected from a control.
[e]No bi-allelic mutations were identified in controls.

Table III. Analysis of the frequencies of mono-allelic and bi-allelic *MYH* mutations in HNPCC/HNPCC-like cases and control in unadjusted logistic regression models and models adjusting for ethnicity, gender and age

Cases	Model with covariate adjustment	*P*-value for covariate	Odds ratio for mutation	95% CI	*P*-value
Any (mono-allelic or bi-allelic) *MYH* mutation versus no mutation					
All cases	No covariates	—	4.18	1.81–9.65	<0.001
	Ethnicity, gender, age	NS, NS, NS	3.23	1.32–7.89	0.01
CRCs only	No covariates	—	3.75	1.51–9.31	0.004
	Ethnicity, gender, age	NS, NS, 0.001	3.05	1.17–7.98	0.02
Mono-allelic mutations only versus no mutation					
All cases	No covariates	—	2.79	1.07–7.25	0.04
	Ethnicity, gender, age	NS, NS, NS	1.99	0.70–5.69	0.20
CRCs only	No covariates	—	2.68	0.96–7.45	0.06
	Ethnicity, gender, age	NS, NS, 0.001	2.09	0.70–6.19	0.18

NS, not significant at the 0.05 level.

adenomas were diagnosed. In addition, a brother of the patient, who has the same genotype, was diagnosed with 14 adenomatous polyps. For all three bi-allelic carriers, the current clinical picture is consistent with a diagnosis of *MYH*-associated polyposis. However, the pedigree structures of these families are remarkable for the apparently dominant inheritance of CRC.

Mono-allelic MYH mutations in MMR gene mutation-negative HNPCC/HNPCC-like families

Because the bi-allelic carriers of *MYH* mutations were eventually diagnosed with *MYH*-associated polyposis, we excluded these three probands and repeated the statistical analyses of *MYH* mutations in HNPCC/HNPCC-like cases versus controls. There were 5 mono-allelic *MYH* mutation carriers in 115 cases with CRC and 1 mono-allelic *MYH* mutation carrier in 19 cases diagnosed only with polyps. The proband of family MI-1484, who when first ascertained had 12 adenomas, subsequently has developed a total of 103 adenomas. We decided to continue to include this family in the analysis because no one else in the family has developed multiple adenomas, and her sister who developed CRC at age 32 also carries a mono-allelic *MYH* mutation (Figure 1A). The *P*-value as determined by two-sided Fisher's exact test of a 2×2 table for the comparison of the carriers and non-carriers of an *MYH* mutation in the cases and controls was 0.04. As before, using a logistic regression model (Table III), we found an unadjusted odds ratio of 2.79 (95% CI: 1.07–7.25; $P = 0.04$). Controlling for ethnic differences, gender and age, we found an odds ratio of 1.99 (95% CI: 0.70–5.69; *P*-value $= 0.20$). In the CRC only analyses, the unadjusted odds ratio was 2.68 (95% CI: 0.96–7.45; *P*-value $= 0.06$) and the fully adjusted odds ratio was 2.09 (95% CI: 0.70–6.19; *P*-value $= 0.18$). The reduction in the odds ratio in the CRC only analyses reflects the exclusion of the proband of family MI-1484 (mentioned above). Again, in the CRC only analysis, age was correlated in the multivariate but not the univariate analysis (data not shown). In general, stratification of the data resulted in lower odds ratios and *P*-values, which was expected because the difference in the mean frequency of mutation carriers in cases and controls was the same in each of the strata. In summary, the CRC risk associated with *MYH* mutation carriers was reduced when considering the mono-allelic carriers only, and the association was statistically significant in the unadjusted but not in the adjusted logistic models.

We also tested for segregation of the *MYH* mutations in all available relatives of the mono-allelic *MYH* mutation carriers (Figure 1A). Of the 10 relatives tested, there were 6 carriers (1 person with an adenoma, 3 with CRC and 2 with other cancers) and 4 non-carriers (1 with one or more adenomas, 2 with CRC and 1 with another cancer). Of first-degree relatives with CRC, three were carriers and two were non-carriers. The segregation of *MYH* mutations was not

significantly different from what one would expect by chance, but admittedly the number of relatives tested is small.

Discussion

In the present study, we compared the frequencies of *MYH* mutations carriers in cases with CRC or with adenomatous polyps from MMR gene mutation-negative HNPCC/HNPCC-like families to the frequencies in healthy controls. The frequency of any (bi-allelic or mono-allelic) *MYH* mutation (6.6%) was significantly increased over the frequency of any mutation in controls (1.6%) in all models tested with odds ratios ranging from 3.05 to 4.15. The frequency of mono-allelic *MYH* mutation (4.4%) was increased over the frequency in controls, with odds ratios ranging from 1.99 to 2.79. However, the comparisons were only significant in the unadjusted logistic models, which was most likely a consequence of lost power due to stratification of the data.

Naively combining the data from published case–control series of consecutive CRCs, the frequency of mono-allelic *MYH* mutation was 1.65% in cases and 1.17% in controls (15–22), which is a 1.4-fold difference. In the present study, the fold difference between the familial cases and controls was 2.75. In a published meta-analysis of these previous studies, an odds ratio of 1.42 (95% CI: 1.02–1.96; *P*-value = 0.04) was obtained (25), which is smaller than the unadjusted odds ratio of 2.79 (95% CI: 1.07–7.25) obtained from the comparison of familial cases and controls in the present study. Although the difference between these two odds ratios is not significant because the 95% confidence intervals overlap, the results are consistent with a model in which CRC clustering in MMR gene mutation-negative HNPCC/HNPCC-like families is caused by an excess of low-penetrance genetic risk factors.

The ascertainment of three probands with bi-allelic *MYH* mutations and family histories consistent with HNPCC was an unexpected finding in the present study. After these probands were ascertained, additional polyps were diagnosed over time, allowing us to re-classify the probands as having an attenuated polyposis, which is a known consequence of bi-allelic *MYH* mutations. Consistent with these findings, a recent study of patients with attenuated polyposis found that an unexpectedly high number of bi-allelic *MYH* mutation carriers had strong family histories of CRC (26). Previous reports have also shown that CRC can develop in bi-allelic mutation carriers before the development of polyposis (18,21). Because the risk of CRC and polyposis associated with bi-allelic *MYH* mutations is $\sim$100 times the risk in controls (21), disease-associated bi-allelic *MYH* mutations follow a classical Mendelian recessive mode of inheritance. Thus, the strong family histories of CRC observed in the bi-allelic *MYH* families studied here are unexplained. Formally, a third grandparent in these families could carry an *MYH* mutation, but we were unable to find evidence supporting this possibility. From a genetic counseling perspective, we suggest that a vertical family history of CRC is not a counter indication to *MYH* genetic testing and MMR gene testing of other cases in the pedigree may be recommended when such families are encountered.

Evidence of an increased frequency of mono-allelic *MYH* mutations in familial CRC cancer compared with consecutive CRC cases has been sought in other studies. Croitoru *et al.* (17) showed in their study of 1238 consecutive Canadian cases that mono-allelic carriers of either the Y165C or G382D mutation were more likely to have a first- or second-degree relative affected with CRC compared to non-carriers. Further analysis of these data using a kin-cohort design showed that first-degree relatives of mono-allelic *MYH* mutation carriers had a 3-fold increased risk of developing CRC (25). Ashton *et al*. (27) showed in their study of MMR gene mutation-negative cases from families fulfilling the Amsterdam or Bethesda criteria that mono-allelic carriers of the Y165 and G382D mutations were more frequent in 233 Australian cases (2.1%) compared to 296 controls (1.4%), although the difference was not statistically significant. In this study, 71 persons in the case group were not diagnosed with CRC, which undoubtedly decreased the power to detect a significant association. Finally, Zhou *et al*. (22) failed to detect any carriers of mono-allelic *MYH* mutations in 84 unrelated familial Swedish CRC cases tested. However, 54 of the 84 tested families contained only 2 persons with CRC, and the frequency of the Y165 and G382D mutations in controls was only 0.64%. Both these factors would reduce the power to detect an association.

There is increasing support in the literature for the hypothesis that low-penetrance cancer susceptibility alleles are present at increased frequencies in CRC cases with strong family history of CRC compared with consecutive cases. In addition to the observations cited in the Introduction, another example is the six alanine (6A) variant of the *TGFβR1* gene (the normal allele, which has nine alanines, is called 9A) (28). In an analysis of 63 CRC cases from MMR gene mutation-negative HNPCC families, the frequency of the 6A allele was 18.8% compared with 9.5% in consecutive CRC cases (29). As in the present study, a smaller number of hereditary cases was used to detect a frequency difference.

Conclusions

The present data support previous work suggesting that mono-allelic *MYH* mutations are associated with an increased risk of developing CRC. We found the frequency of the known disease-causing *MYH* mutations is higher in MMR gene mutation-negative HNPCC/HNPCC-like families than in consecutive CRC cases. If the frequency of other CRC risk alleles is higher in familial than in consecutive cases, then, when sequencing candidate genes to look for potential CRC susceptibility alleles, familial cases would be more useful for the identification of rare risk alleles.

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