

# Risk of Colorectal Cancer in Monoallelic and Biallelic Carriers of *MYH* Mutations: A Population-Based Case-Family Study

Mark A. Jenkins,<sup>1</sup> Marina E. Croitoru,<sup>2</sup> Neerav Monga,<sup>4</sup> Sean P. Cleary,<sup>2</sup> Michelle Cotterchio,<sup>4</sup> John L. Hopper,<sup>1</sup> and Steven Gallinger<sup>3,4</sup>

<sup>1</sup>Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Victoria, Australia; <sup>2</sup>Samuel Lunenfeld Research Institute and <sup>3</sup>Department of Surgery, Mount Sinai Hospital; and <sup>4</sup>Cancer Care Ontario, Toronto, Ontario, Canada

## Abstract

Previous case-control studies have suggested that carriers of monoallelic germline mutations in the *MYH* gene may be at increased risk of colorectal cancer. We applied a kin-cohort design, using a modified segregation analysis, to estimate the colorectal cancer risk using 300 first-degree relatives of 39 colorectal cancer cases who were monoallelic or biallelic carriers of *MYH* mutations. We found that monoallelic carriers had a 3-fold increased risk of colorectal cancer (hazard ratio,

2.9; 95% confidence interval, 1.2-7.0;  $P = 0.02$ ) and biallelic carriers a 50-fold increased risk (hazard ratio, 53; 95% confidence interval, 14-200;  $P < 0.0001$ ). This analysis illustrates the potential of family analysis to estimate cancer risk for low-frequency mutations and, based on the proportion of relatives predicted to be carriers, we believe that this constitutes the largest study of monoallelic carriers to date. (Cancer Epidemiol Biomarkers Prev 2006;15(2):312-4)

## Introduction

A recessive colorectal cancer risk associated with mutations in the base excision repair gene *MYH* was first shown by Alt-Tassan et al. (1) and Sieber et al. (2). Since then, we (3) and several other groups (4-8) have observed a higher prevalence of monoallelic mutation carriers in colorectal cases compared with controls. Although no individual study alone found statistically significant evidence, when combined, these studies suggest an additional dominant effect (3). Most recently, Farrington et al. (9) found 45 monoallelic *MYH* mutation carriers in 2,239 colorectal cases and 28 monoallelic *MYH* mutation carriers in 1,845 controls, and concluded that monoallelic *MYH* mutation carriers may be at increased risk of colorectal cancer. However, whereas the increased risk was observed only in carriers over the age of 55 years [risk ratio, 1.68; 95% confidence interval (95% CI), 1.07-2.95], there was no difference between the increased risk in this age group and the risk in carriers 55 years old or younger.

We have applied a kin-cohort design to assess colorectal cancer risk in monoallelic and biallelic *MYH* mutation carriers, all of whom are a first-degree relative of a colorectal cancer case identified in a population-based study. We analyzed the cancer histories of ungenotyped first-degree relatives of the colorectal cancer cases we previously found to be carriers of *MYH* mutations (3).

## Materials and Methods

As explained in detail by Cotterchio et al. (10), the Ontario Familial Colorectal Cancer Registry identified individuals diagnosed with colorectal cancer (excluding familial adenomatous polyposis) of ages 20 to 74 years at diagnosis

(probands). Attempts were made to confirm all reports of colorectal cancer in relatives using clinical records and/or death certificates. All probands defined by strong family histories of colorectal cancer and age of diagnosis as being either at "high risk" (Amsterdam Criteria ref. 11) or "intermediate risk" of carrying a genetic mutation and 25% of the remaining "low-risk" cases were invited to provide a blood sample. As described by Croitoru et al. (3), *MYH* Y165C and G382D mutations were identified in the probands by denaturing high-performance liquid chromatography and confirmed by sequencing. In subjects heterozygous for the *MYH* Y165C and G382D mutations, the entire coding region of the *MYH* gene was analyzed by denaturing high-performance liquid chromatography to identify other pathogenic *MYH* mutations. This identified 41 mutation carrying probands. To exclude the possibility that a proportion of colorectal cancer in the probands, and hence in their relatives, was due to germline mutations in the mismatch repair genes, available tumors of probands were tested for microsatellite instability and loss of mismatch repair protein expression. If tumors showed high-frequency microsatellite instability and/or were immunodeficient for a mismatch repair protein, the germline was tested for mutations in mismatch repair genes *hMLH1*, *hMSH2*, and *hMSH6* by sequencing and multiplex ligation-dependent probe amplification. This led to the identification of two germline mismatch repair gene mutation carriers and these probands and their relatives were excluded from analysis. Of the remaining 39 probands, 27 were monoallelic carriers for Y165C ( $n = 8$ ) or G382D ( $n = 19$ ), 6 were homozygous carriers for Y165C ( $n = 2$ ) or G382D ( $n = 4$ ), and 6 were compound heterozygous carriers for G382D/Y165C ( $n = 3$ ), Y165C/Y90X ( $n = 1$ ), Y165C/891+3A>C ( $n = 1$ ), and G382D/891+3A>C ( $n = 1$ ). Of the 39 probands, 25 were from the 100% sampled high-risk or intermediate-risk group and 14 were from the 25% sampled low-risk group.

The observed incidence of colorectal cancer in relatives (number of relatives with colorectal cancer divided by the total person years of relatives) was compared with the expected incidence calculated by applying age- and sex-specific cancer incidence rates obtained for the Ontario population 1993-2002. Standardized incidence ratios and 95% CIs were calculated using STATA version 8.0 (12).

Received 10/14/05; revised 11/25/05; accepted 12/16/05.

**Grant support:** National Cancer Institute of Canada grant 16073 (S. Gallinger and M. Cotterchio).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Steven Gallinger, Department of Surgery, Mount Sinai Hospital, University of Toronto, Room 1225, 600 University Avenue, Toronto, Ontario, Canada. Phone: 416-586-8550; Fax: 416-586-8392. E-mail: sgallinger@rogers.com

Copyright © 2006 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-05-0793

**Table 1. Frequency of monoallelic MYH carriers colorectal cancer cases and controls from previous case-control studies**

Study	Frequency in cases* (%)	Frequency in controls* (%)
Farrington et al. (9)	45 of 2,205 (2.0%)	28 of 1,822 (1.5%)
Croitoru et al. (3)	29 of 1,226 (2.4%)	21 of 1,255 (1.7%)
Fleischmann et al. (5)	6 of 356 (1.7%)	2 of 354 (0.6%)
Peterlongo et al. (8)	4 of 553 (0.7%)	7 of 918 (0.8%)
Enholm et al. (4)	5 of 1,038 (0.5%)	0 of 424 (0.0%)
Kambara et al. (6)	2 of 92 (2.2%)	1 of 53 (1.9%)
Wang et al. (7)	10 of 442 (2.3%)	4 of 313 (1.3%)

\*Excluding biallelic carriers.

We estimated the average hazard ratio (the ratio of the age-specific incidence in carriers of a germline *MYH* mutation to that in noncarriers) by a modified segregation analysis fitted under maximum likelihood theory using the statistical package MENDEL (13-15). Biallelic carriers were assumed to have both alleles mutated and treated as homozygous carriers. The colorectal cancer incidence for each family member was assumed to follow a Cox proportional hazards model where the incidence depends on the genotype (carrier or noncarrier), colorectal cancer diagnosed in at least one parent, and colorectal cancer diagnosed in at least one sibling. The latter two covariates account for familial aggregation unrelated to the mutations. Specifically, based on unpublished control data from the same population, the Ontario Familial Colorectal Cancer Registry, the probability of having at least one parent with colorectal cancer was set at 0.08 (8% of population controls reported having at least one parent with colorectal cancer) and of having at least one sibling with colorectal cancer was set at 0.04 (4% of population controls reported having at least one sibling with colorectal cancer). We assumed that in the population, having at least one first-degree relative with colorectal cancer doubled colorectal cancer risk (16, 17). The population allele frequency of germline mutations was taken to be 0.0085 based on a 1.7% monoallelic carrier frequency in the control population (3). The likelihood of each family was expressed as a function of the colorectal cancer status and the ages at last contact, death, or diagnosis of family members. Recessive, dominant, and codominant modes of inheritance were compared using the likelihood ratio test.

To adjust for ascertainment, the likelihood for each family was conditioned on the colorectal cancer status and *MYH* genotype status of the proband, thus ensuring unbiased risk estimates. The incidence in noncarriers was approximated by the population incidence of colorectal cancer for Ontario from 1993-2002. The cumulative risk in carriers was calculated by integrating over the age-specific incidence in carriers (equal to the population age-specific incidence multiplied by the estimated average hazard ratio). To account for stratified sampling based on family history, the low-risk families were given a weight of 4.0 compared with a weight of 1.0 for the high-risk and intermediate-risk families. Standard errors for calculation of confidence intervals were estimated using the robust Huber-White sandwich estimator (18).

The overall evidence from previous studies for an association between monoallelic *MYH* mutation carriers and colorectal cancer was estimated by a meta-analysis on the data from seven case-control studies (3-9). These constituted all identified studies that tested for monoallelic and biallelic carriers of *MYH* mutations in colorectal cancer cases and in controls. Exposed cases were defined as colorectal cancer cases who were monoallelic carriers of *MYH* mutations and nonexposed cases were colorectal cancer cases with no germline mutations. Controls were subjects without colorectal cancer (see Table 1). Random and fixed effects models were fitted; heterogeneity was tested using Cochran's *Q*; and a Forrest plot of the odds

ratios was generated. Odds ratios, their 95% CIs, and *P* values were estimated using the software Comprehensive Meta-analysis (<http://www.meta-analysis.com>).

## Results

A total of 300 first-degree relatives of 39 mutation-carrying probands were identified (204 of the 27 monoallelic probands and 96 of the 12 biallelic probands). Of the 123 first-degree relatives of the high-risk or intermediate-risk monoallelic probands, 10 (8%) were diagnosed with colorectal cancer, an incidence which is 5.0 (95% CI, 2.6-9.3) times that expected based on their age distribution. Of the 66 first-degree relatives of the biallelic probands, 5 (8%) were diagnosed with colorectal cancer, which is 5.2 (95% CI, 2.1-12.5) times more than expected. Of the 81 first-degree relatives of the low-risk monoallelic probands, 5 (6%) were diagnosed with colorectal cancer, which is 3.0 (95% CI, 1.2-7.3) times that expected, and of the 30 first-degree relatives of the low-risk biallelic probands, 2 (7%) were diagnosed with colorectal cancer, which is 4.5 (95% CI, 1.1-18.1) times that expected.

A codominant model (homozygote risk is greater than heterozygote risk which is greater than wild-type risk) gave a better fit than a recessive model ( $P = 0.002$ ). Table 2 shows that monoallelic carriers were, on average, 2.9 (95% CI, 1.2-7.0) times more likely to be diagnosed with colorectal cancer compared with the general population ( $P = 0.02$ ). This hazard ratio translates to an 8% cumulative risk to age 70 years. Biallelic carriers were, on average, 53 (95% CI, 14-200) times more likely to be diagnosed with colorectal cancer compared with the general population ( $P < 0.0001$ ) and were almost certain to be diagnosed with colorectal cancer by age 70 years.

There was no difference in risk of colorectal cancer for monoallelic carriers between those over the age of 55 years (hazard ratio, 2.9; 95% CI, 1.0-8.2;  $P = 0.04$ ) and those 55 years old or younger (hazard ratio, 2.8; 95% CI, 0.5-17;  $P = 0.3$  and  $P$  for difference = 0.9).

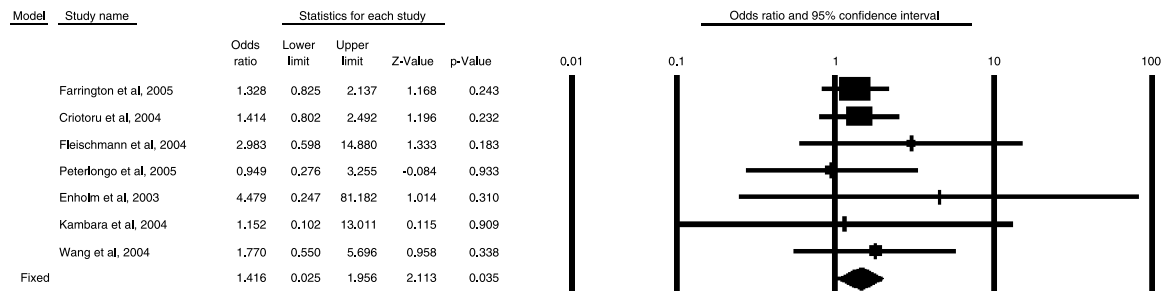
Figure 1 shows that whereas no single previous study showed conclusively that monoallelic carriers were at increased risk, the combined estimate from all studies was significant. The widths of the confidence intervals in Fig. 1 illustrate the limited statistical power for most of the individual studies. The pooled odds ratio estimate from the meta-analysis was 1.4 (95% CI, 1.0-2.0;  $P = 0.04$ ) with no evidence to reject homogeneity (Cochran's  $Q = 2.076$  with 6 degrees of freedom;  $P = 0.9$ ).

## Discussion

We have applied a kin-cohort design to assess colorectal cancer risk in monoallelic and biallelic *MYH* mutation carriers, all of whom are a first-degree relative of a colorectal cancer case. We analyzed the cancer histories of ungenotyped first-degree relatives of the 39 colorectal cancer cases we previously found to be carriers of *MYH* mutations from a population-based study (3). We found an ~3-fold increased risk of colorectal cancer in monoallelic carriers and a 50-fold

**Table 2. Hazard ratios of risk of colorectal cancer in MYH carriers compared with that in the general population, and associated cumulative risk to age 70 years, estimated using modified segregation analysis**

Carriers	Hazard ratio (compared with population risk)	<i>P</i>	Cumulative risk to age 70 y
Monoallelic	2.9 (1.2-7.0)	0.02	8% (4-19%)
Biallelic	53 (14-200)	<0.0001	80% (35-100%)



**Figure 1.** Meta-analysis of seven published case-control studies of monoallelic *MYH* mutation and colorectal cancer risk.

increased risk in biallelic carriers. This analysis illustrates the power of family-based data for the study of rare variants. It is more efficient than a case-control analysis because for every monoallelic carrier, on average, half their first-degree relatives will also be monoallelic carriers, and for every biallelic carrier (assuming mutations are on different alleles), both parents, all children, and, on average, half their siblings will be monoallelic carriers. Based on Mendelian inheritance, the number of monoallelic and biallelic probands, and the number of their parents, siblings, and offspring, we estimate that 185 relatives are monoallelic *MYH* mutation carriers, which is more than the total number in all previous studies (see Table 1). The statistical method we used takes into account that we do not know which relatives were carriers, and in effect weights them according to their relationship to the proband so as to derive maximum information from the family data (19).

This population-based family study, when combined with the meta-analysis of case-control studies, provides the clearest evidence yet that monoallelic *MYH* carriers are at increased colorectal cancer risk. Our meta-analysis suggested that monoallelic carriers have, on average, a 40% increased colorectal cancer risk although individually no single study showed a statistically significant increase, suggesting that individual studies were underpowered to detect an effect of this magnitude. Given the considerable overlap in confidence intervals (meta-analysis, 1.0-2.0; segregation analysis, 1.2-7.0), there is no evidence that these estimates differ from one another. Further larger studies are needed to provide a more precise estimate of the increased risk. Given that all carriers in our analysis were identified via a first-degree relative with colorectal cancer, these results may only be applicable to carriers with such a family history (i.e., the characteristic of individuals attending a family cancer clinic).

Similar to the analysis of Farrington et al. (9), we observed an increased risk in monoallelic carriers only in those over the age of 55 years, but as in the study of Farrington et al. (9), there was no significant difference between the two age groups; i.e., one should be careful about inferring modification of effect by age.

It is important when analyzing case-family data using a modified segregation analysis to allow for a proportion of familial aggregation of disease to be due to causes other than *MYH*, particularly when a large proportion of relatives have not been genotyped. We did this by including in the model effects representing the background familial aggregation of colorectal cancer (i.e., increased risks associated with having an affected close relative). Failure to do so results in overestimation of effects attributed to the mutation status of the proband that led to the family being included in the analysis. It is therefore unlikely that our estimates of increased risk in carriers were due to other familial risk factors.

In conclusion, using a family design, we have estimated colorectal cancer risk for monoallelic *MYH* mutation carriers. We found that, on average, such carriers were approximately three times the population risk for colorectal cancer. Further

studies using genotyped relatives will be necessary to refine these estimates for the *MYH* Y165C and G382D mutations, as well as for the less common pathogenic *MYH* mutations.

### Acknowledgments

We thank Dr. Antonis Antoniou and Prof. Douglas Easton for their programming and statistical advice.

### References

- Al-Tassan N, Chmiel NH, Maynard J, et al. Inherited variants of *MYH* associated with somatic G:C>T:A mutations in colorectal tumors. *Nat Genet* 2002;30:227-32.
- Sieber OM, Lipton L, Crabtree M, et al. Multiple colorectal adenomas, classical adenomatous polyposis and germ-line mutations in *MYH*. *N Engl J Med* 2003;348:791-9.
- Criotoru M, Cleary S, Di Nicola N, et al. Association between biallelic and monoallelic germline *MYH* gene mutations and colorectal cancer risk. *J Nat Cancer Inst* 2004;96:1631-4.
- Enholm S, Hienonen T, Suomalainen A, et al. Proportion and phenotype of *MYH*-associated colorectal neoplasia in a population-based series of Finnish colorectal cancer patients. *Am J Pathol* 2003;163:827-32.
- Fleischmann C, Peto J, Cheadle J, Shah B, Sampson J, Houlston RS. Comprehensive analysis of the contribution of germline *MYH* variation to early-onset colorectal cancer. *Int J Cancer* 2004;109:554-8.
- Kambara T, Whitehall VLJ, Spring KJ, et al. Role of inherited defects of *MYH* in the development of sporadic colorectal cancer. *Genes Chromosomes Cancer* 2004;40:1-9.
- Wang L, Baudhuin LM, Boardman LA, et al. *MYH* mutations in patients with attenuated and classic polyposis and with young-onset colorectal cancer without polyps. *Gastroenterology* 2004;127:9-16.
- Peterlongo P, Mitra N, Chuai S, et al. Colorectal cancer risk in individuals with biallelic or monoallelic mutations of *MYH*. *Int J Cancer* 2005;114:505-7.
- Farrington SM, Tenesa A, Barnetson R, et al. Germline susceptibility to colorectal cancer due to base-excision repair gene defects. *Am J Hum Genet* 2005;77:112-9.
- Cotterchio M, McKeown-Eyssen G, Sutherland H, et al. Ontario Familial Colon Cancer Registry: methods and first-year response rates. *Chronic Dis Can* 2000;21:81-6.
- Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. *Gastroenterology* 1999;116:1453-6.
- StataCorp. Stata statistical software: release 8.0. College Station (TX): Stata Corporation; 2003.
- Lange K, Boehnke M, Weeks D. Programs for pedigree analysis. Los Angeles: Department of Biomathematics, University of California at Los Angeles; 1987.
- Hopper JL, Southey MC, Dite GS, et al. Population-based estimate of the average age-specific cumulative risk of breast cancer for a defined set of protein-truncating mutations in *BRCA1* and *BRCA2*. Australian Breast Cancer Family Study. *Cancer Epidemiol Biomarkers Prev* 1999;8:741-7.
- Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;72:1117-30.
- Johns LE, Houlston RS. A systematic review and meta-analysis of familial prostate cancer risk. *BJU Int* 2003;91:789-94.
- Baglietto L, Jenkins MA, Severi G, et al. Two measures of familial aggregation from case-control and cohort studies: the example of colorectal cancer. *J Clin Epidemiol*. In press 2005.
- White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica* 1980;48:817-30.
- Hopper JL, Bishop DT, Easton DF. Population-based family studies in genetic epidemiology. *Lancet* 2005;366:1397-406.