Re

Re: Association Between Biallelic and Monoallelic Germline MYH Gene Mutations and Colorectal Cancer Risk

We read with interest the paper by Croitoru et al. *(1)*, in which they examined the role of germline MYH gene mutations in colorectal cancer and proposed an autosomal dominant weakly penetrant mode of inheritance. Previous evidence has suggested a recessive mode of inheritance for colorectal polyposis *(2)*. Hence, the novel proposal by Croitoru et al. would be of major clinical and scientific importance and must be fully justified by the data. We have major concerns about their findings and respectfully refute their interpretation of their data.

The authors compared case patients and control subjects with no MYH gene mutations (genotype Wt/Wt) with case patients and control subjects with either one (genotype Wt/Mut) or two (genotype Mut/Mut) MYH gene mutations. However, the authors' data do not support a dominant mode of inheritance. Individuals with a Wt/Mut genotype had no statistically significant excess colorectal cancer risk compared with those with a Wt/Wt genotype (odds ratio = 1.4; 95% confidence interval = 0.8 to 2.5) *(1)*. Therefore, the authors' decision to pool Wt/Mut individuals with homozygous Mut/Mut case patients for their analyses is not justified. Table 1 summarizes the authors' data from Table 1 *(1)*. We used these data and Fisher's exact test to examine associations between the frequency of germline MYH gene mutations and colorectal cancer risk among case patients and control subjects with different MYH genotypes. Our results—$P = .25$ for Wt/Wt versus Wt/Mut, $P = 2.1 \times 10^{-4}$ for Wt/Wt versus Mut/Mut, and $P = 5.3 \times 10^{-3}$ for Wt/Mut versus Mut/Mut—suggest that only a recessive model of inheritance is tenable. Thus, the only valid pooling strategy supported by the authors' data is to pool individuals with Wt/Mut and Wt/Wt genotypes (i.e., assume a recessive mode of inheritance).

Croitoru et al. *(1)* also compared the number of affected first- and second-degree relatives of heterozygous and homozygous MYH gene mutation carriers with that of noncarriers. This approach does not test whether people who inherit one mutant allele are at equivalent risk to those who inherit two mutant alleles. The modest effects detected by the authors that they ascribe to a dominant effect are no more consistent with a dominant than a recessive mode of inheritance. In fact, the offspring of Wt/Mut case patients have an increased risk of disease compared with offspring of Wt/Wt case patients under both fully dominant and fully recessive modes of inheritance. Thus, this evidence in itself does not support a dominant mode of inheritance.

Croitoru et al. *(1)* also presented loss of heterozygosity (LOH) data for tumors from Wt/Mut and Mut/Mut case patients.

Table 1. Summary of data in Table 1 of Croitoru et al. *(1)**

	MYH genotype			
	Wt/Wt	Wt/Mut	Mut/Mut	Total
Case patients	1197	29	12	1238
Control subjects	1234	21	0	1255
Total	2431	50	12	2493

*MYH = MutY human homologue gene; Wt = wild type; Mut = mutant.

However, LOH at chromosome 1p in tumors is highly variable and subject to confounding. Thorstensen et al. *(3)* detected LOH in chromosome 1p in 50% of primary carcinomas, 33% of local recurrences, and 64% of metastases from colorectal cancer patients, whereas Ogunbiyi et al. *(4)* and Zhou et al. *(5)* detected LOH in chromosome 1p in 26% and 22% of colorectal cancers, respectively. Rashid et al. *(6)* found that LOH in chromosome 1p varied with the anatomic location of hyperplastic polyps. Given such variability and the small numbers presented by Croitoru et al. and the fact that their samples were not matched for confounding variables, the difference in LOH frequency at chromosome 1p is best explained by chance.

Albert Tenesa
Susan M. Farrington
Malcolm G. Dunlop

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Notes

Affiliations of authors: Colon Cancer Genetics Group, University of Edinburgh, and MRC Human Genetics Unit, Western General Hospital, Edinburgh.

Correspondence to: Albert Tenesa, BSc, School of Molecular and Clinical Medicine, The University of Edinburgh, Colon Cancer Genetics Group, 14th Floor, MRC Human Genetics Unit, Western General Hospital, Crewe Road South, Edinburgh EH4 2XU, UK (email: albert.tenesa@ed.ac.uk).

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RESPONSE

Dominant and recessive transmission applies to traits or phenotypes and not to genes themselves. A good example of this terminology is sickle cell disease: homozygous carriers have the *disease* and heterozygous carriers have the sickle cell *trait*, a milder phenotype of the disease *(1,2)*. It is, of course, true that published reports to date clearly support a recessive mode of inheritance for MYH-associated polyposis, a phenotype associated with biallelic mutations in MYH *(3,4)*. We decided a priori to examine the incidence of MYH gene mutations as a way to validate the association between biallelic MYH gene mutations and colorectal cancer risk in a large North American population-based case–control series and to analyze the risk of colorectal cancer associated with the heterozygous state.

Although Tenesa et al. used Fisher's exact test to demonstrate, as have we, that biallelic mutations were more common in the colorectal cancer case patients than in the control subjects, their results failed to adequately support their argument that heterozygous carriers had no increased risk of colorectal cancer. We found a non–statistically significant increased risk of colorectal cancer among MYH heterozygotes (odds ratio = 1.4; 95% confidence interval = 0.8 to 2.5) *(5)*. However, our study was inadequately powered to rule out this level of risk. Furthermore, by using the pooled cumulative MYH genotype data available from the literature, we demonstrated a convincing association between mild increased colorectal cancer risk and heterozygous MYH mutations, and although this observation has now been corroborated by another group *(6)*, the need for larger studies to address this issue is obvious.

Our results suggested a statistically significant increased risk of colorectal cancer in first- and second-degree family members of both monoallelic MYH mutation carriers and in all mutation carriers. We disagree with Tenesa et al., who suggest that a purely recessive model is the only possible explanation for this result and continue to assert that the result we obtained by Poisson regression (relative risk = 1.57, 95% confidence interval = 1.05 to 2.36) provides additional evidence for increased colorectal cancer risk in monoallelic carriers. Given the frequency of MYH mutations in the general population and in the absence of consanguinity, we feel we can safely assume that the observed increased risk in family members is due to monoallelic carriers of the mutation present in the proband. Similarly, for biallelic carriers, 25% of siblings of the proband would be expected to carry biallelic mutations, whereas 50% of siblings as well as other first-degree relatives would be expected to carry monoallelic mutations. Thus, the magnitude of this association is too strong to be fully explained by a purely recessive model, and the increased risk must be due to monoallelic carriers.

The purpose of our loss of heterozygosity (LOH) experiments was to examine whether the modest increased risk of colorectal cancer in heterozygous MYH mutation carriers was consistent with Knudson's "two-hit" tumorigenesis model *(7)*. We agree with Tenesa et al. about the variability of published allelotype data, not only for chromosome 1p, but for many chromosomal loci in many tumor types. We confirmed our microsatellite marker LOH results by analyzing the autoradiographic densities corresponding to wild-type and mutant nucleotides (data not shown) and found no internal variability in our results.

The critical difference between different modes of inheritance relies on whether a phenotype is present in heterozygous carriers. In conclusion, our MYH gene mutation case–control frequency data, results of Poisson regression analyses, and LOH data and phenotypic observations, as well as results of pooled analyses by us and by Peterlongo et al. *(6)*, suggest that an increased risk of colorectal cancer conferred by the heterozygous state cannot be ruled out. Homozygous MYH gene mutation carriers are obviously at much greater risk for colorectal cancer than heterozygotes and, as we state in our article, studies with larger sample sizes are clearly necessary to accurately quantify risk of colorectal cancer in heterozygotes.

Sean P. Cleary
Marina E. Croitoru
Robert Gryfe
Michael Manno
Michelle Cotterchio
Julia Knight
Steven Gallinger

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Affiliations of authors: Samuel Lunenfeld Research Institute (MEC, SPC, MM, JK, RG, SG) and Department of Surgery (RG, SG), Mount Sinai Hospital, Toronto, ON, Canada; Ontario Familial Colorectal Cancer Registry, Cancer Care Ontario, Toronto, ON, Canada (MM, MC, JK, SG).

Correspondence to: Steven Gallinger, MD, MSc, FRCS, Rm. 1225, Mount S inai Hospital, 600 University Ave., Toronto, ON, Canada M5G 1X5 (e-mail: sgallinger@mtsinai.on.ca).

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