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Are the common genetic variants associated with colorectal cancer risk for DNA mismatch repair gene mutation carriers?

Aung Ko Win[1], **John L. Hopper**[1], **Daniel D. Buchanan**[2], **Joanne P. Young**[2], **Albert Tenesa**[3,4], **James G. Dowty**[1], **Graham G. Giles**[5], **Jack Goldblatt**[6], **Ingrid Winship**[7,8], **Alex Boussioutas**[9,10,11], **Graeme P. Young**[12], **Susan Parry**[13,14], **John A. Baron**[15], **David Duggan**[16], **Steven Gallinger**[17,18], **Polly A. Newcomb**[19], **Robert W. Haile**[20], **Loïc Le Marchand**[21], **Noralane M. Lindor**[22], and **Mark A. Jenkins**[1]

[1]Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, Parkville, Victoria, Australia [2]Cancer and Population Studies Group, Queensland Institute of Medical Research, Bancroft Centre, Herston, Queensland, Australia [3]The Roslin Institute, University of Edinburgh, Edinburgh, Scotland [4]Medical Research Council Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, Scotland [5]Cancer Epidemiology Centre, Cancer Council Victoria, Carlton, Victoria, Australia [6]Genetic Services of Western Australia and School of Paediatrics and Child Health, University of Western Australia, Perth, Australia [7]Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia [8]Genetic Medicine, The Royal Melbourne Hospital, Parkville, Victoria, Australia [9]Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Parkville, Australia [10]Cancer Genomics and Predictive Medicine, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia [11]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, Victoria, Australia [12]Flinders Centre for Innovation in Cancer, Flinders University, Adelaide, South Australia, Australia [13]New Zealand Familial Gastrointestinal Cancer Registry, Auckland City Hospital, Auckland, New Zealand [14]Department of Gastroenterology, Middlemore Hospital, Auckland, New Zealand New Zealand [15]Department of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA [16]Genetic Basis of Human Disease Division, Translational Genomics Research Institute (TGen), Phoenix, Arizona, USA [17]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada [18]Cancer Care Ontario, Toronto, Ontario, Canada [19]Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA [20]Department of Preventive Medicine, University of Southern California, Los Angeles, California, USA [21]University of Hawaii Cancer Center, Honolulu, Hawaii, USA [22]Department of Health Science Research, Mayo Clinic Arizona, Scottsdale, Arizona, USA

Corresponding author: Mark A. Jenkins, PhD, Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, Melbourne School of Population Health, Level 3, 207 Bouverie Street, The University of Melbourne VIC 3010, Australia, Tel: +61 3 8344 0902, Fax: +61 3 9349 5815, m.jenkins@unimelb.edu.au.

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Conflict of interest statement

The authors have no conflict of interest to declare with respect to this manuscript.

Abstract

Background—Genome-wide association studies have identified at least 15 independent common genetic variants associated with colorectal cancer (CRC) risk. The aim of this study was to investigate whether 11 of these variants are associated with CRC risk for carriers of germline mutations in DNA mismatch repair (MMR) genes.

Methods—A total of 927 MMR gene mutation carriers (360 *MLH1*, 442 *MSH2*, 85 *MSH6* and 40 *PMS2*) from 315 families enrolled in the Colon Cancer Family Registry, were genotyped for the SNPs: rs16892766 (8q23.3), rs6983267 (8q24.21), rs719725 (9p24), rs10795668 (10p14), rs3802842 (11q23.1), rs4444235 (14q22.2), rs4779584 (15q13.3), rs9929218 (16q22.1), rs4939827 (18q21.1), rs10411210 (19q13.1) and rs961253 (20p12.3). We used a weighted Cox regression to estimate CRC risk for homozygous and heterozygous carriers of the risk allele compared with homozygous non-carriers as well as for an additive per allele model (on the log scale).

Results—Over a total of 40,978 person-years observation, 426 (46%) carriers were diagnosed with CRC at a mean age of 44.3 years. For all carriers combined, we found no evidence of an association between CRC risk and the total number of risk alleles (hazard ratio [HR] per risk allele=0.97, 95% confidence interval [CI]=0.88–1.07, p=0.52).

Conclusions—We found no evidence that the SNPs associated with CRC in the general population are modifiers of the risk for MMR gene mutation carriers overall, and therefore any evidence of proven clinical utility in Lynch syndrome.

Keywords

genetic variant; colorectal cancer; Lynch syndrome; mismatch repair

INTRODUCTION

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers worldwide, with over one million diagnosed cases (9.8% of cancer diagnoses) and ~600,000 deaths (8.1% of all cancer deaths) in 2008(1). Approximately 3–4% of all CRC(2), and 5–15% of CRC diagnosed before age 50 years(3, 4), are Lynch syndrome cases caused by germline mutations in a DNA mismatch repair (MMR) gene; *MLH1, MSH2, MSH6* and *PMS2*(5). Average cumulative risk of CRC to age 70 years for MMR gene mutation carriers has been varyingly estimated between 20% and 70% depending on sex and the MMR gene that is mutated(6–8). Cancer risks for *MLH1* and *MSH2* mutation carriers vary greatly from family to family(9), consistent with the existence of multiple inherited genetic (polygenic) modifiers.

Several genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) in at least 15 independent loci associated with CRC risk (odds ratio ranging from 1.10 to 1.26 per risk allele)(10–12) (Supplementary Table 1). If these SNPs also predicted CRC risk in MMR gene mutation carriers, there would be a potential to use them to more accurately predict individual risk estimates for Lynch syndrome. Three studies observed two variants, 8q23.3 (rs16892766) and 11q23.1 (rs3802842), to be associated with increased risk of CRC in Lynch syndrome especially for females only(13, 14) or *MLH1* mutation carriers only(14, 15); however, another study(16) observe no associations. In this study of MMR gene mutation carriers, we have investigated associations of CRC with SNPs at 11 loci: 8q23.3 (rs16892766), 8q24.21 (rs6983267), 9p24 (rs719725), 10p14 (rs10795668), 11q23.1 (rs3802842), 14q22.2 (rs4444235), 15q13.3 (rs4779584), 16q22.1 (rs9929218), 18q21.1 (rs4939827), 19q13.1 (rs10411210) and 20p12.3 (rs961253).

MATERIALS AND METHODS

Study Sample

Subjects were heterozygote carriers of pathogenic mutations in MMR genes who were recruited from the Colon Cancer Family Registry (Colon CFR). Details of recruitment, data collection and mutation testing have been described in detail previously(17, 18). Written informed consent was obtained from all subjects, and the study protocol was approved by the institutional human ethics committee at each center of the Colon CFR.

Genotyping of the SNPs

Genotyping for SNPs was performed using Sequenom's iPLEX Gold. PCR and extension primers for these SNPs were designed using the MassARRAY Assay Design 3.0 software (Sequenom, Inc.). Extension product sizes were determined by mass spectrometry using Sequenom's Compact matrix-assisted laser desorption ionization-time of flight mass spectrometer. Resulting mass spectra were converted to genotype data using SpectroTYPER-RT software. Genotype data from 30 CEPH trios (Coriell Cell Repository, Camden, NJ) were used to confirm reliability and reproducibility of the genotyping. No errors of Mendelian inheritance were detected in the CEPH trios and genotypes for these subjects showed perfect concordance with genotypes from the International HapMap Project.

Statistical Analysis

Cox proportional hazards regression analysis was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the published CRC risk allele of each SNP to CRC risk for MMR gene mutation carriers. We estimated HRs separately for homozygous carriers of the risk allele (2 risk alleles) and heterozygous carriers of the risk allele (1 risk allele) versus homozygous carriers of the non-risk allele (0 risk allele); and we estimated HRs per risk allele, i.e. a linear association on the log scale. We also estimated the association with the total number of risk alleles over the SNPs, i.e. 0–22. (see Supplementary Table 1 for risk alleles).

Since some carriers were ascertained because they were diagnosed with CRC, the identification of MMR gene mutation carriers was not random with respect to CRC. To adjust for this non-random ascertainment, we used the weighted cohort approach(19). Previously estimated age-specific CRC incidence rates for MMR gene mutation carriers(20) were used to calculate sampling fractions to weight the proportion of CRC-affected and unaffected carriers in 5-year age stratum so the proportion of affected carriers in each age group equalled that expected for mutation carriers in the population.

Time-at-risk started at birth and ended at age at diagnosis of CRC (n = 426), any other cancer (n = 92), polypectomy (n = 132), death (n = 4) or last contact (n = 273), whichever occurred first. Proportional hazards assumption was tested by examining the relationship between the scaled Schoenfeld residuals and survival time (21). Associations between genetic variants and CRC risk were estimated stratified by gender and the MMR gene that was mutated after adjusting for country of recruitment and ascertainment source (clinic- or population-based). To allow for any correlation of risk between family members, the Huber-White robust variance correction was applied by clustering on family membership (22).

To reduce false discovery rate expected from the large number of associations investigated, the p-value cut-off for classifying a HR as statistically significant was determined using methods by Benjamini and Hochberg (23). This method controls the expected high false

discovery rate and can result in power gains over traditional multiplicity 'correction' methods such as the Bonferroni procedure(24).

A test of the null hypothesis (no association between any of the genetic variants and CRC risk) against the alternative hypothesis (associations with CRC risk in the same direction as in the general population) was conducted using Fisher's test of whether the distribution of one-sided p-values from fitting the additive per allele association on the log scale deviated from the uniform distribution on the interval [0, 1]. This was done by summing the $-2 \ln p_i$, where p_i is the p-value for the ith variant, across all SNPs and comparing with the χ^2 distribution with $2n$ degrees of freedom, where n is the number of SNPs(25). Statistical analyses were performed using Stata 11.0(26).

RESULTS

A total of 927 MMR gene mutation carriers from 315 families (117 *MLH1*, 136 *MSH2*, 41 *MSH6*, and 21 *PMS2*) were included in this study. Over 40,978 person-years observation, 426 (46%) carriers were diagnosed with CRC at a mean age of 44.3 (standard deviation, SD 11; median 44, range 17–80) years. Of all carriers, 738 (223 families) were recruited in Australia or New Zealand, 164 (77 families) in the USA and 25 (15 families) in Canada (Table 1). SNP genotype frequencies did not deviate from that expected under Hardy–Weinberg equilibrium except for rs3802842 (p=0.004) and rs6983267 (p=0.002) (Table 2).

We found no evidence of an increased CRC risk associated with any of the 11 SNPs overall or separately for male and female carriers (Figure 1 and 2). Also, there was no evidence of an association between the total number of risk alleles of the 11 SNPs (as a continuous factor) and CRC risk (HR per risk allele=0.97, 95%CI=0.88–1.07, p=0.52 all carriers combined; HR=0.99, 95%CI=0.86–1.14,p=0.87 for males; HR=0.96, 95%CI=0.84–1.10, p=0.56 for females; HR=0.98, 95%CI=0.82–1.18, p=0.86 for *MLH1*; HR=0.95, 95%CI=0.82–1.10, p=0.50 for *MSH2*, HR=1.03, 95%CI=0.85–1.26,p=0.74 for *MSH6*; HR=0.82, 95%CI=0.50–1.32, p=0.41 for *PMS2*). We also found no evidence of variation from a uniform distribution of CRC risk for the SNPs (p=0.53 for all carriers combined, 0.83 for males, 0.72 for females, 0.80 for *MLH1*, 0.61 for *MSH2*, 0.0005 for *MSH6* and 0.39 for *PMS2*).

For *PMS2* mutation carriers, carriers of the G-allele of rs10795668 (10p14) were at decreased risk of CRC (HR=0.07, 95%CI=0.01–0.40, p=0.003 for AG carriers; and HR=0.03, 95%CI=0.00–0.39, p=0.007 for GG carriers compared with AA carriers). For *PMS2* mutation carriers, carriers of the G-allele of rs992918 (16q22.1) were also at decreased risk of CRC (HR=0.14, 95%CI=0.03–0.61, p=0.009 for GA carriers; and HR=0.07, 95%CI=0.01–0.51, p=0.008 for GG carriers compared with AA carriers). For *MSH2* mutation carriers, homozygous carriers of the C allele of rs16892766 were at increased risk of CRC compared with homozygous carriers of the A allele (HR=10.74, 95%CI=2.24–51.39, p=0.003) (Table 3).

DISCUSSION

Our analyses provided no evidence to support the hypothesis that, overall, the SNPs associated with CRC risk for the general population are also associated with CRC risk for MMR gene mutation carriers, let alone having associations in the same direction. We found no evidence for SNP associations with CRC for all carriers combined, or when stratified by gender. Our estimate of CRC risk per allele for mutation carriers (HR=0.97, 95%CI=0.88–1.07) was lower (p=0.03) than reported for the general population (OR=1.09, 95%CI=1.05–1.13) (27).

These findings strongly suggest that the GWAS SNPs for CRC in the general population are not useful predictors for CRC in those with an inherited MMR gene mutation. Whatever the reason for the association between these SNPs and CRC, whether it be due to linkage disequilibrium with a common or rare causal genetic variant, protein binding site or promoter region, they do not appear to be having the same effect in carriers of high-risk mutations. Perhaps cancers with microsatellite instability, i.e. Lynch syndrome cancers, are not subject to the same slight effects that these SNPs have on the more common microsatellite stable cancers. Is this apparent lack of SNP and cancer association carriers of MMR gene mutations also seen for carriers of other high-risk cancer genes? Two of the six GWAS SNPs associated with breast cancer in the general population were also associated with breast cancer risk for carriers of *BRCA1* mutations, and five of the six SNPs were associated with breast cancer risk for *BRCA2* mutation carriers(28). It appears then, that the predictive utility of SNPs identified by GWAS using cancer in the general population, for predicting cancer in carriers of high-risk mutations, may depend on the specific high-risk gene; no utility for MMR genes; almost no utility for *BRCA1,* and some utility for *BRCA2*.

Further, as shown in Figure 1, for all carriers combined, only 3 of SNPs had a point estimate greater than one for the OR per allele for association with CRC. If these associations for MMR gene mutation carriers were consistent with studies for the general population, we would expect all of the SNPs to be positively associated with risk, i.e. OR>1. The apparent difference in estimates for SNP associations with CRC between MMR gene mutation carriers and the general population might be due to differences in the pathogenesis of these cancers, as exemplified by differences in tumor location in the large intestine. Proximal and distal colon have been different gene expression profiles and risk factors(29–31). Lynch syndrome-associated CRCs are more likely to present in the proximal colon compared with CRCs in the general population. In addition, differential effects for SNPs may also arise due to the differing mechanisms of carcinogenesis: Lynch syndrome, specifically progression via microsatellite instability, in contrast with the chromosomal instability which characterises population-based CRC.

This is the only study assessing the CRC SNPs for *PMS2* mutation carriers. We observed heterozygous and homozygous carriers of the G alleles for the rs10795668 and rs9929218 SNPs were at decreased CRC risk, i.e. the opposite direction to that observed for the general population. Although our study group consisted of only 40 *PMS2* mutation carriers, the observed associations were significant after correction for multiple testing—suggesting these findings are not spurious. However, further validation in larger sample set of *PMS2* mutation carriers is necessary. There was evidence suggesting that rs10795668 varied by tumor site, being more common in rectal than colonic tumors(32). In contrast, Lynch syndrome associated CRCs occur predominantly in the proximal colon and therefore, the findings from this study for rs10795668 in *PMS2* mutation carriers may reflect an indirect association related to tumor location.

The association between homozygous carriers of the C allele of rs16892766 and CRC risk for *MSH2* mutation carriers was in the same direction as reported for CRC from the general population(32). In the general population, the association was stronger for CRC diagnosed under age 60 years suggesting potential age modifying effects in MMR gene mutation carriers. This SNP has also been reported as a CRC risk modifier for MMR gene mutation carriers in three other studies(13–15); but not in one study(16). Talseth-Palmer et al.(14) did not observe a significant effect of rs16892766 alone on CRC risk but observed a trend of an increased risk of CRC for the pair-wise combination of SNPs rs3802842 and rs16892766 in *MLH1* mutation carriers. In contrast, Houlle et al.(16) reported a decreased risk of CRC (HR=0.27, 95%CI=0.08–0.86, p=0.03) for homozygous carriers of the C allele in mutation carriers overall. The lack of consistency in the results between our study and this study(16)

could be attributable to the small number of homozygous carriers of the C allele of rs16892766 identified in each study. We concluded a meta-analysis of combining data from our study with the four previous studies(13–16), as infeasible given our study and another one study(13) used a weighted approach(19) while others did not(14–16).

A limitation of our study was the possibility that the estimates of association were only generalizable to MMR gene mutation carriers with substantial survival as, to be included in the analysis, cases had to survive long enough to provide a blood sample for DNA testing. Another limitation is that we had limited data on the type of colorectal polyps, which were removed, and therefore we censored at the age of polypectomy instead of estimating post-polypectomy CRC risk. This could have resulted in underestimating the true cancer risk if some of the polyps were malignant. Finally, the study was underpowered to detect weak associations of the SNPs with CRC risk. Assuming the risk allele frequency of each SNP to be 45% in unaffected carriers, our study of 927 MMR gene mutation carriers had 68% power to detect a 20% increased or decreased risk of CRC, and 23% power to detect a 10% increased or decreased risk of CRC, at the 0.05 level of significance.

Future studies should include a further four SNPs not included in this analysis that have been confirmed as being associated with CRC (1q41, 3q26.2, 12q13.13 and 20q13.33)(12). In addition, our study chose SNPs *a priori* based on previous associations with CRC in GWAS from the general population. It is unclear whether any of the millions of other SNPs tested, but not previously associated with CRC in the general population, may predict CRC risk for MMR gene mutation carriers.

In conclusion, our findings suggest that 11 SNPs identified from previous GWAS that were known to be associated with CRC risk in those general populations studied do not substantially alter the CRC risk of MMR gene mutation carriers. Therefore we found no evidence of proven clinical utility for these SNPs for Lynch syndrome carriers. The search for the hypothesized genetic modifiers of cancer risk due to MMR mutations should therefore examine other SNPs. Genome-wide association studies of Lynch syndrome colorectal cancer cases and controls is needed to identify these SNP modifiers, but given the rarity of the syndrome, international collaborations would be required to gain sufficient statistical power to adequately address this question.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRC	colorectal cancer
Colon CFR	the Colon Cancer Family Registry
CI	confidence interval
HR	hazard ratio
MMR	mismatch repair
SD	standard deviation
SE	standard error
SNP	single nucleotide polymorphism

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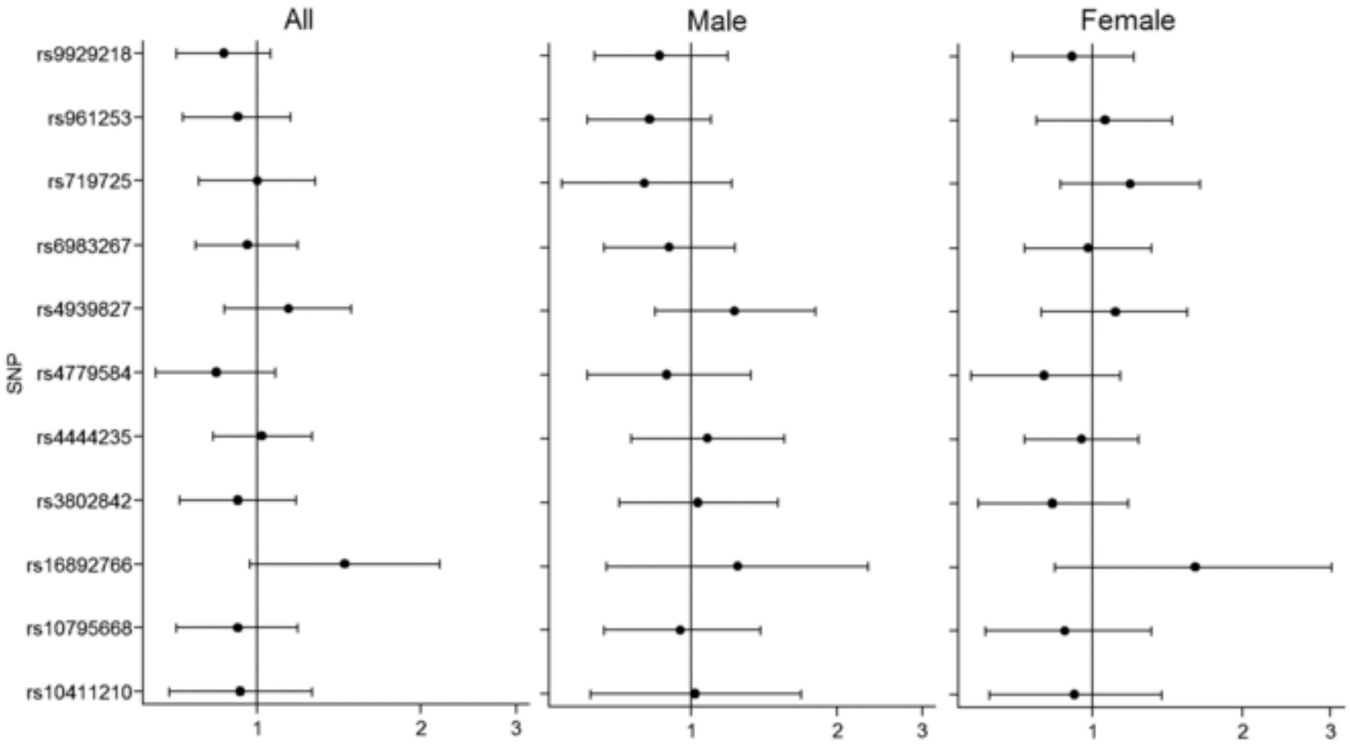

Figure 1.
Hazard ratios and 95% confidence intervals for associations between common genetic variants and colorectal cancer risk for mismatch repair gene mutation carriers.

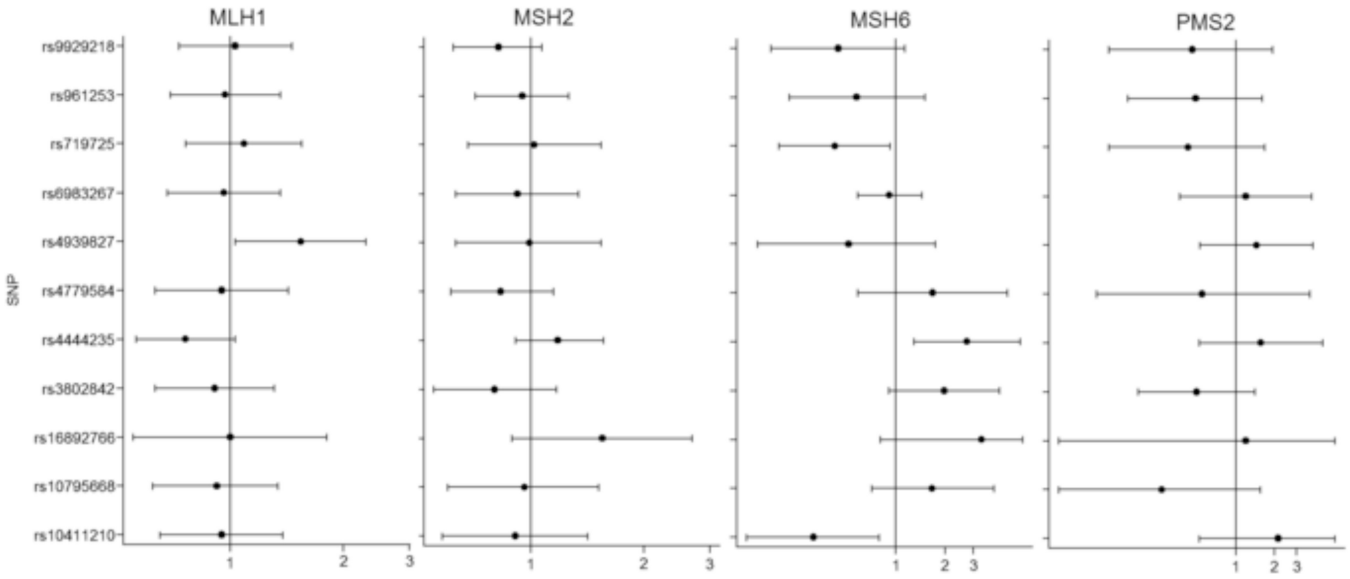

Figure 2.

Hazard ratios and 95% confidence intervals for associations between common genetic variants and colorectal cancer risk for carriers of mutations in specific mismatch repair gene. Note: Horizontal lines represent 95% confidence intervals. Each dot represents the point estimate for hazard ratio per allele. X-axis as log scale.

Table 1

Baseline characteristics of mismatch repair gene mutation carriers included in the study

		No colorectal cancer (n=501) N (%)	Colorectal cancer (n=426) N (%)	All (n=927) N (%)
Sex				
	male	204 (41)	204 (48)	408 (44)
	female	297 (59)	222 (52)	519 (56)
Country				
	Canada	6 (1)	19 (4)	25 (3)
	Australia or New Zealand	429 (86)	309 (73)	738 (80)
	USA	66 (13)	98 (23)	164 (17)
Ascertainment				
	Population-based	56 (11)	87 (20)	143 (15)
	Clinic-based	445 (89)	339 (80)	784 (85)
Gene mutated				
	MLH1	180 (36)	180 (42)	360 (39)
	MSH2	252 (50)	190 (45)	442 (87)
	MSH6	53 (11)	32 (8)	85 (9)
	PMS2	16 (3)	24 (5)	40 (4)
Age*	Mean (SD)	44.15 (14.17)	44.26 (11.05)	44.20 (12.82)

*Age at diagnosis for carriers with colorectal cancer; age at diagnosis of other cancer or polypectomy or death or last contact for carriers without colorectal cancer.

Table 2

Minor allele frequency of 11 SNPs included in the study

	No colorectal cancer	Colorectal cancer	All
rs10411210 [T]	0.12	0.13	0.12
rs10795668 [A]	0.30	0.30	0.30
rs16892766 [C]	0.06	0.08	0.07
rs3802842 [C]	0.28	0.29	0.29
rs4444235 [C]	0.47	0.48	0.48
rs4779584 [T]	0.23	0.22	0.22
rs4939827 [T]	0.46	0.48	0.49
rs6983267 [T]	0.45	0.48	0.47
rs719725 [C]	0.36	0.34	0.35
rs961253 [A]	0.38	0.36	0.37
rs9929218 [A]	0.29	0.30	0.29

Table 3

Hazard ratios and corresponding 95% confidence intervals for common genetic variants and colorectal cancer risk for mismatch repair gene mutation carriers by sex and the gene that was mutated

	All			Male			Female			*MLH1*			*MSH2*			*MSH6*			*PMS2*		
	n/N	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**
rs10411210																					
TT	12/21	*ref*		4/6	*ref*		8/15	*ref*		3/6	*Ref*		8/12	*ref*		0/1	*ref*		1/2	*ref*	
TC	76/167	0.87 (0.41–1.91)	0.73	34/70	0.62 (0.13–2.85)	0.54	42/97	1.11 (0.42–2.35)	0.90	35/67	1.25 (0.29–5.34)	0.77	26/70	0.56 (0.17–1.84)	0.34	10/21	-		5/9	0.87 (0.09–8.33)	0.90
CC	303/660	0.82 (0.41–1.71)	0.60	147/295	0.67 (0.16–2.86)	0.59	156/365	0.90 (0.41–1.98)	0.80	130/255	1.13 (0.31–4.31)	0.85	139/320	0.59 (0.21–1.68)	0.32	20/61	-		14/24	2.31 (0.13–40.25)	0.57
per allele	391/848	0.93 (0.69–1.26)	0.65	185/371	1.02 (0.62–1.69)	0.94	206/477	0.92 (0.62–1.38)	0.69	168/328	0.95 (0.65–1.38)	0.77	173/402	0.91 (0.58–1.42)	0.68	30/83	0.31 (0.12–0.79)	0.01	20/35	2.14 (0.51–8.97)	0.30
rs10795668																					
AA	42/79	*ref*		16/32	*ref*		26/47	*ref*		22/38	*Ref*		15/27	*ref*		2/11	*ref*		3/3	*ref*	
AG	108/274	0.65 (0.35–1.21)	0.18	55/117	1.14 (0.47–2.80)	0.77	53/157	0.42 (0.18–0.98)	0.05	40/96	0.91 (0.34–2.43)	0.85	48/134	0.35 (0.14–0.87)	0.02	15/35	3.31 (0.58–18.97)	0.18	5/9	0.07 (0.01–0.40)	0.003*
GG	168/367	0.73 (0.42–1.26)	0.25	79/166	0.98 (0.42–2.31)	0.97	89/201	0.56 (0.26–1.21)	0.14	71/144	0.84 (0.38–1.86)	0.66	77/177	0.55 (0.24–1.26)	0.16	11/30	1.75 (0.28–10.93)	0.55	9/16	0.03 (0.00–0.39)	0.007*
per allele	318/720	0.92 (0.71–1.19)	0.51	150/315	0.95 (0.66–1.39)	0.80	168/405	0.88 (0.61–1.31)	0.52	133/278	0.92 (0.62–1.34)	0.65	140/338	0.96 (0.60–1.52)	0.86	28/76	1.67 (0.71–3.98)	0.25	17/28	0.26 (0.04–1.55)	0.14
rs16892766																					
AA	348/776	*ref*		160/333	*ref*		188/443	*ref*		147/296	*Ref*		154/373	*ref*		26/74	*ref*		21/33	*ref*	
CA	53/104	1.40 (0.80–2.43)	0.24	31/54	1.32 (0.59–2.95)	0.50	22/50	1.38 (0.71–2.70)	0.36	25/44	1.24 (0.59–2.62)	0.57	22/48	0.93 (0.38–2.26)	0.87	5/9	4.93 (1.14–21.35)	0.03	1/3	1.19 (0.04–31.73)	0.92
CC	4/6	2.45 (0.49–12.25)	0.27	2/3	1.08 (0.39–3.02)	0.88	2/3	3.84 (0.36–40.72)	0.26	1/3	0.19 (0.05–0.81)	0.02	3/3	10.74 (2.24–51.39)	0.003*	0/0	-		0/0	-	
per allele	405/886	1.45 (0.97–2.17)	0.07	193/390	1.25 (0.67–2.32)	0.49	212/496	1.61 (0.84–3.02)	0.15	173/343	1.00 (0.55–1.81)	1.00	179/424	1.55 (0.89–2.70)	0.12	31/83	4.93 (1.14–21.35)	0.03	22/36	1.19 (0.04–31.73)	0.92
rs3802842																					
AA	209/469	*ref*		94/206	*ref*		115/263	*ref*		97/199	*Ref*		87/210	*ref*		13/43	*ref*		12/17	*ref*	
CA	139/304	0.71 (0.51–1.00)	0.05	67/129	0.84 (0.51–1.42)	0.52	72/175	0.64 (0.42–0.99)	0.04	54/101	0.81 (0.47–1.37)	0.42	64/155	0.63 (0.36–1.12)	0.12	15/34	1.41 (0.46–4.37)	0.55	6/14	0.26 (0.06–1.14)	0.07
CC	47/97	1.09 (0.66–1.79)	0.75	28/48	1.22 (0.56–2.64)	0.62	19/49	0.95 (0.48–1.89)	0.89	17/35	0.94 (0.41–2.21)	0.88	23/49	0.85 (0.44–1.66)	0.64	3/7	3.84 (0.92–16.04)	0.07	4/6	0.56 (0.10–3.05)	0.51

	All			Male			Female			*MLH1*			*MSH2*			*MSH6*			*PMS2*		
	n/N	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**
per allele	395/870	0.92 (0.72–1.18)	0.51	189/383	1.03 (0.71–1.51)	0.88	206/487	0.83 (0.59–1.18)	0.31	168/335	0.91 (0.63–1.31)	0.61	174/414	0.80 (0.55–1.17)	0.25	31/84	1.98 (0.90–4.33)	0.09	22/37	0.49 (0.17–1.41)	0.19
rs4444235																					
TT	120/261	*ref*		55/108	*ref*		65/153	*ref*		43/87	*Ref*		63/138	*ref*		7/21	*ref*		7/15	*ref*	
CT	188/421	0.99 (0.69–1.42)	0.95	89/190	0.89 (0.53–1.49)	0.66	99/231	1.04 (0.64–1.668)	0.87	80/160	0.81 (0.44–1.42)	0.44	84/205	1.15 (0.68–1.96)	0.60	14/40	4.92 (0.99–24.51)	0.05	10/16	0.71 (0.20–2.38)	0.56
CC	107/219	1.05 (0.69–1.59)	0.82	55/101	1.17 (0.58–2.36)	0.66	52/118	0.89 (0.51–1.54)	0.67	51/103	0.57 (0.31–1.06)	0.07	40/87	1.40 (0.81–2.43)	0.23	10/22	9.22 (1.64–51.79)	0.01	6/7	3.57 (0.54–23.66)	0.19
per allele	415/901	1.02 (0.83–1.26)	0.83	199/399	1.08 (0.75–1.56)	0.67	216/502	0.95 (0.73–1.24)	0.69	174/350	0.76 (0.56–1.03)	0.08	187/430	1.18 (0.91–1.56)	0.23	31/83	2.73 (1.28–5.82)	0.01	23/38	1.57 (0.51–4.84)	0.43
rs4779584																					
CC	245/531	*ref*		117/238	*ref*		128/293	*ref*		119/227	*Ref*		100/239	*ref*		18/51	*ref*		8/14	*ref*	
CT	115/281	0.71 (0.50–0.97)	0.03	54/120	0.76 (0.45–1.28)	0.31	61/161	0.68 (0.43–1.05)	0.08	42/99	0.80 (0.48–1.34)	0.40	58/143	0.67 (0.43–1.04)	0.08	7/22	1.24 (0.41–3.76)	0.71	8/17	0.19 (0.04–0.81)	0.03
TT	28/54	0.97 (0.52–1.81)	0.93	14/23	1.07 (0.41–2.80)	0.88	14/31	0.87 (0.36–2.09)	0.76	7/12	1.53 (0.48–4.91)	0.47	14/31	0.95 (0.46–2.11)	0.90	4/8	3.47 (0.41–30.35)	0.26	3/3	3.55 (0.55–23.15)	0.19
per allele	388/866	0.84 (0.65–1.08)	0.17	185/381	0.89 (0.61–1.33)	0.58	203/485	0.80 (0.57–1.14)	0.22	168/338	0.95 (0.63–1.43)	0.81	172/413	0.83 (0.61–1.15)	0.26	29/81	1.68 (0.58–4.86)	0.34	19/34	0.54 (0.08–3.84)	0.54
rs4939827																					
CC	74/192	*ref*		29/80	*ref*		45/112	*ref*		28/73	*Ref*		37/91	*ref*		6/22	*ref*		3/6	*ref*	
TC	165/373	1.08 (0.67–1.75)	0.74	81/165	1.34 (0.64–2.81)	0.44	84/208	0.99 (0.55–1.77)	0.96	66/143	1.24 (0.67–2.28)	0.50	74/180	0.99 (0.44–2.23)	0.97	14/33	0.26 (0.06–1.26)	0.09	11/17	4.42 (0.56–34.91)	0.16
TT	87/171	1.31 (0.77–2.21)	0.32	43/76	1.52 (0.69–3.36)	0.30	44/95	1.24 (0.65–2.35)	0.51	41/67	2.35 (1.11–4.96)	0.03	36/79	0.97 (0.41–2.33)	0.95	7/20	0.34 (0.06–2.11)	0.23	3/5	2.42 (0.15–37.92)	0.53
per allele	326/736	1.14 (0.87–1.49)	0.33	153/321	1.23 (0.84–1.81)	0.29	173/415	1.11 (0.79–1.55)	0.55	135/283	1.54 (1.03–2.30)	0.04	147/350	0.99 (0.63–1.54)	0.96	27/75	0.51 (0.14–1.74)	0.28	17/28	1.44 (0.52–4.03)	0.49
rs6983267																					
GG	87/199	*ref*		41/82	*ref*		46/117	*ref*		35/69	*Ref*		38/97	*ref*		11/28	*ref*		3/5	*ref*	
GT	199/397	1.31 (0.84–2.06)	0.23	90/174	1.25 (0.65–2.43)	0.51	109/223	1.48 (0.83–2.63)	0.19	90/163	1.27 (0.65–2.46)	0.49	87/188	0.96 (0.47–1.96)	0.92	*12/27*	1.06 (0.34–3.37)	0.92	10/19	2.11 (0.08–52.23)	0.65
TT	99/256	0.94 (0.58–1.53)	0.81	47/110	0.83 (0.42–1.63)	0.59	52/146	1.01 (0.52–1.93)	0.98	40/95	0.96 (0.45–2.02)	0.91	45/123	0.84 (0.39–1.83)	0.67	7/27	0.81 (0.32–2.09)	0.66	7/11	1.85 (0.09–3.81)	0.69

	All			Male			Female			*MLH1*			*MSH2*			*MSH6*			*PMS2*		
	n/N	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**	**n/N**	**HR (95%CI)**	**P**
per allele	385/852	0.96 (0.77–1.19)	0.69	178/366	0.90 (0.66–1.23)	0.52	207/486	0.98 (0.73–1.31)	0.89	165/327	0.96 (0.68–1.36)	0.83	170/408	0.92 (0.63–1.34)	0.65	30/82	0.91 (0.58–1.44)	0.70	20/35	1.19 (0.36–3.97)	0.77
rs719725																					
CC	40/92	*ref*		20/35	*ref*		20/57	*ref*		13/26	*Ref*		19/49	*ref*		6/14	*ref*		2/3	*ref*	
CA	145/337	1.08 (0.62–1.89)	0.79	72/152	0.86 (0.36–2.04)	0.73	73/185	1.28 (0.58–2.81)	0.55	72/152	1.48 (0.57–3.85)	0.42	54/145	0.78 (0.33–1.83)	0.57	12/31	1.53 (0.29–8.19)	0.62	7/9	0.35 (0.02–5.65)	0.46
AA	142/307	1.03 (0.61–1.79)	0.91	60/134	0.66 (0.227–1.59)	0.35	82/173	1.47 (0.69–3.10)	0.32	54/106	1.40 (0.54–3.67)	0.49	73/156	0.94 (0.42–2.11)	0.89	8/30	0.22 (0.03–1.59)	0.13	7/15	0.17 (0.01–2.55)	0.20
per allele	327/736	1.00 (0.78–1.28)	1.00	152/321	0.80 (0.54–1.21)	0.28	175/415	1.19 (0.86–1.65)	0.29	139/284	1.09 (0.76–1.55)	0.65	146/350	1.02 (0.68–1.54)	0.91	26/75	0.42 (0.19–0.92)	0.03	16/27	0.42 (0.10–1.68)	0.22
rs961253																					
CC	174/364	*ref*		88/164	*ref*		86/200	*ref*		79/149	*Ref*		65/154	*ref*		20/45	*ref*		10/16	*ref*	
CA	150/347	0.81 (0.58–1.14)	0.23	67/143	0.81 (0.47–1.38)	0.43	83/204	0.87 (0.55–1.39)	0.57	53/118	0.64 (0.41–0.98)	0.04	81/187	1.15 (0.69–1.91)	0.60	6/25	0.33 (0.10–1.09)	0.07	10/17	0.42 (0.08–2.27)	0.31
AA	67/142	0.91 (0.57–1.42)	0.64	31/68	0.67 (0.36–1.25)	0.21	36/74	1.22 (0.65–2.28)	0.53	35/65	1.11 (0.57–2.15)	0.75	27/65	0.80 (0.42–1.52)	0.50	3/9	0.82 (0.15–4.54)	0.82	2/3	0.27 (0.04–1.79)	0.17
per allele	391/853	0.92 (0.73–1.15)	0.45	186/375	0.82 (0.61–1.10)	0.19	205/478	1.06 (0.77–1.45)	0.73	167/332	0.97 (0.69–1.36)	0.86	173/406	0.95 (0.71–1.26)	0.70	29/79	0.57 (0.22–1.51)	0.26	22/36	0.48 (0.14–1.61)	0.23
rs9929218																					
AA	52/101	*ref*		25/49	*ref*		27/52	*ref*		20/40	*Ref*		29/56	*ref*		1/3	*ref*		2/2	*ref*	
GA	144/327	0.91 (0.56–1.44)	0.65	75/144	1.08 (0.53–2.21)	0.84	69/183	0.76 (0.39–1.48)	0.41	61/129	1.28 (0.58–2.82)	0.54	65/153	0.81 (0.41–1.55)	0.51	10/31	0.61 (0.06–5.95)	0.67	8/14	0.14 (0.03–0.61)	0.009 *
GG	216/473	0.76 (0.51–1.16)	0.21	96/201	0.82 (0.41–1.62)	0.56	120/272	0.76 (0.44–1.32)	0.33	92/177	1.17 (0.54–2.53)	0.68	91/224	0.66 (0.39–1.11)	0.12	21/51	0.25 (0.02–2.62)	0.25	12/21	0.07 (0.01–0.51)	0.008 *
per allele	412/901	0.87 (0.71–1.06)	0.17	196/394	0.86 (0.63–1.19)	0.38	216/507	0.91 (0.69–1.21)	0.51	173/346	1.03 (0.73–1.46)	0.86	185/433	0.82 (0.62–1.07)	0.13	32/85	0.44 (0.17–1.13)	0.09	22/37	0.45 (0.10–1.95)	0.29

Hazard ratios were adjusted for country of recruitment and source of ascertainment.

* p-values were classified as statistically significant, determined using methods by Benjamini and Hochberg (23)

n, total number of colorectal cancer; N, total number of mismatch repair gene mutation carriers