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Mismatch repair polymorphisms and risk of colon cancer, tumour microsatellite instability and interactions with lifestyle factors

P T Campbell[1], **K Curtin**[2], **C M Ulrich**[1,3], **W S Samowitz**[4], **J Bigler**[1], **C M Velicer**[1], **B Caan**[5], **J D Potter**[1,3], and **M L Slattery**[2]

[1] Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

[2] Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, Utah, USA

[3] Department of Epidemiology, University of Washington, Seattle, Washington, USA

[4] Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah, USA

[5] Division of Research, Kaiser Permanente Medical Care Program, Oakland, California, USA

Abstract

Background—Germline mutations in DNA mismatch repair (MMR) genes cause Lynch syndrome colon cancers. Less understood is the risk of colon cancer associated with common polymorphisms in MMR genes and the potential interacting role of lifestyle factors known to damage DNA.

Methods—A study was conducted to examine whether *MLH1* (*−93G>A* and *Ile219Val*) and *MSH6* (*Gly39Glu*) polymorphisms were associated with risk of colon cancer in data from 1609 colon cancer cases and 1972 controls. Genotype data were further stratified by microsatellite instability status, smoking, alcohol, Western diet, alcohol and obesity, to investigate potential heterogeneity.

Results—The *MSH6 39Glu* allele was associated with increased risk of colon cancer among men (*Gly/Glu* or *Glu/Glu* vs *Gly/Gly*, OR 1.27; 95% CI 1.04 to 1.54). Neither *MLH1* polymorphism was associated with colon cancer risk overall. When stratified by microsatellite stability status, however, the *MLH1 −93A* allele was associated with a more than doubling in microsatellite instability (MSI)-positive colon cancer risk (*AA* vs *GG*, OR 2.47; 95% CI 1.48 to 4.11); no associations were observed between the MMR polymorphisms examined and MSI-negative colon cancer. Statistically significant interactions were observed between: *MLH1 −93G>A* and smoking (MSI-negative colon cancer only, p value interaction: 0.005); and *MLH1 Ile219Val* and Western diet (p value interaction: 0.03).

Conclusions—The *MSH6 Gly39Glu* and *MLH1 −93G>A* polymorphisms were associated with risk of overall colon and MSI-positive colon cancers, respectively. Risk for colon cancer, stratified by MMR genotype, was further modified by smoking and Western diet.

Colon cancer is a major public health problem worldwide, and deficiency of DNA mismatch repair (MMR) has been causally linked to its aetiology.[1, 2] MMR enzymes identify and repair incompatible DNA base pairings that commonly occur during the replication of repetitive genomic tracts due to the slippage of DNA polymerase.[1, 2] The MMR system in humans involves a highly conserved series of proteins, including MSH2, MSH6, MSH3, MLH1 and

Correspondence to: Dr C Ulrich, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100, Fairview Ave N, M4-B402, Seattle, WA 98109-1024, USA;, nulrich@fhcrc.org.

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PMS2. Failure to correct DNA mispairings causes microsatellite instability (MSI), a phenomenon whereby a germline micro-satellite undergoes a somatic gain or loss in repeat length. The accumulation of such errors may inactivate genes that are critical to cell integrity, including tumour suppressor genes, and facilitate carcinogenesis. About 15% of colorectal cancers display MSI, the majority caused by somatic mutation and hypermethylation of the *MLH1* CpG island promoter region,[3] whereas a smaller portion is caused by Lynch syndrome, a familial cancer predisposition syndrome caused by auto-somal dominant inheritance of defective MMR genes.[1, 2]

There are large interindividual differences in DNA repair capacity,[4] and twin studies suggest that a genetic component explains part of this variation.[5] The *MLH1 −93G>A* polymorphism (rs1800734) involves a substitution in the promoter region of *MLH1*. Recent evidence from two colorectal cancer registries suggested that the *−93A* allele was associated with increased risk of MSI-high, but not microsatellite-stable, colorectal tumours.[6] The *MLH1 Ile219Val* (*665A>G*) polymorphism (rs1799977) is located in exon 8. The *219Val* allele was previously associated with refractory ulcerative colitis[7] and breast cancer[8]; other studies suggest no association between the allele and risk of colorectal polyps[9] or cancer.[6, 10] The *MSH6 Gly39Glu* (*116G>A*) polymorphism (rs1042821) is located in exon 1 and has been previously associated with risk of rectal, but not colon, cancer.[10]

Given that minor reductions in DNA repair capacity may alter the overall rate at which mutations accumulate, we hypothesised that polymorphisms in MMR genes (*MLH1* and *MSH6*) may be associated with altered risk of colon cancer, particularly for risk of MSI-positive tumours, and in combination with lifestyle factors including tobacco smoking, Western diet, alcohol and obesity. The associations between these lifestyle factors and colon cancer risk are well characterised,[11] yet whether or not they act as effect modifiers on a background of deficient DNA repair capacity is unknown. Further, unlike family history or age, these lifestyle exposures are modifiable. The rationale for investigating interactions between these exposures and MMR polymorphisms is strengthened by findings of increased oxidative stress and DNA damage from tobacco smoking,[12] as well as from Western diet,[13] alcohol[14] and obesity.[15] We investigated these hypotheses in a multicentre study of 3581 colon cancer cases and controls from the USA.

MATERIALS AND METHODS

Cases and controls

Participants were Caucasian, African-American or Hispanic subjects from the Kaiser Permanente Medical Care Program of Northern California, an eight-county area in Utah and the metropolitan Twin Cities area of Minnesota. Eligibility criteria for cases included: diagnosis of primary incident colon cancer (ICD-O second rubric: 18.0, 18.2–18.9) between 1 October 1991 and 30 September 1994; age 30–79 years at the time of diagnosis; and mentally competent to complete the interview. Cases were excluded if diagnosed with: adenocarcinoma or carcinoma of the rectosigmoid junction or rectum (defined as the first 15 cm from the anal opening), familial adenomatous polyposis, ulcerative colitis or Crohn's disease. Case eligibility was determined by the Surveillance Epidemiology and End Results Cancer Registries in Northern California and Utah, and the Minnesota Surveillance System. Seventy-six percent of cases contacted agreed to participate in the study. Controls with no personal history of colon or rectal cancer were randomly selected in proportion to geographic location of the cases. Controls were frequency matched to cases by sex and 5-year age group, with methods that were previously described in detail.[16] Sixty-four percent of the controls contacted participated in the study.

Data collection

In-person interviews were conducted by trained staff to collect diet and lifestyle data. Study quality control methods have been described elsewhere.[17] The referent period was approximately 2 years prior to the date of diagnosis for cases or the date of selection for controls. Information was collected on demographic factors such as age, sex, physical activity, adult height and weight during the referent period, regular use of aspirin and/or non-steroidal anti-inflammatory drugs (NSAIDs), alcohol use, cigarette smoking history, medical history, and family history of cancer in first-degree relatives. Participants were asked the usual number of cigarettes smoked in a day during the period they smoked regularly, together with when they started and stopped smoking. Dietary intake data were obtained during the interview with a computer-assisted diet history questionnaire based on the validated CARDIA diet questionnaire.[17, 18] Consumption of alcoholic beverages (beer, wine and liquor) was recorded for the referent period, and 10 and 20 years prior to interview to assess long-term use of alcohol (g/day, average of 10 and 20 years ago). Body mass index (BMI; kg/m^2) was calculated from self-reports of weight and height and categorised as non-obese (BMI <30 kg/m^2) or obese (BMI ≥ 30 kg/m^2).

Genotyping

MLH1 (*−93G>A, Ile219Val*) and *MSH6* (*Gly39Glu*) genotyping was conducted at the Molecular Epidemiology Laboratory, Public Health Sciences Division, Fred Hutchinson Cancer Research Center. Of the 4403 cases and controls with valid study data, 3680 (84% overall, 83% of cases and 85% of controls) additionally provided blood samples during the in-person interview. Of the 3680 who provided a blood sample, we were able to genotype successfully 3581 individuals for *MLH1 Ile219Val*, 3576 individuals for *MLH1 −93G>A* and 3571 individuals for *MSH6 Gly39Glu.* Those who could not be genotyped had insufficient DNA or DNA of poor quality, or opted out of laboratory testing. Genomic DNA was extracted using previously described methods.[19, 20] Genotyping was conducted with the technician blinded to case–control status, and each batch included approximately equal numbers of cases and controls.

The genotyping methods have been recently described in detail.[9] Briefly, the *MLH1 −93G>A* and *Ile219Val* polymorphisms and the *MSH6 Gly39Glu* polymorphism were detected by allelic discrimination using the 5′ nuclease assay on a 7900HT sequence detection system (Applied Biosystems, Foster City, California, USA). Validation of the 5′ nuclease assay was conducted among 92 individuals by comparing genotype with genotype from restriciton fragment length polymorphism (RFLP) or sequencing. There were no discrepancies between assays. Positive and negative controls were included on each plate. For additional quality control, genotyping of 94 randomly selected samples was repeated with no discrepancies between runs.

Microsatellite instability

Of 1609 cases with study data and MMR genotypes from blood DNA, we obtained tissue and tumour DNA from 1250 subjects (78%); 1211 had adequate tumour DNA to evaluate MSI. Definitions of MSI-positive and MSI-negative have been previously described in detail.[21, 22] The blood sample group (~84% of the whole study population) and MSI groups (~78% of the case group that also had DNA) were representative of the whole study population, with no discernible differences between consenters and non-consenters for age, sex or other demographic features.

Statistical analysis

χ^2 tests determined if the observed genotype frequencies differed from Hardy–Weinberg equilibrium among the >1900 controls. D' and r^2 were calculated as measures of linkage disequilibrium for the two *MLH1* polymorphisms.

Odds ratios (ORs) and 95% CIs were calculated from unconditional logistic regression models to estimate risk of colon cancer associated with genotype while adjusting for confounding variables: age at diagnosis or selection, sex, study centre and number of cigarettes smoked per day on a regular basis. We stratified the data by sex, tumour MSI, smoking, Western diet, alcohol and obesity to investigate potential heterogeneous effects of the genotypes. MLH1 polymorphisms were categorised into combined genotypes to investigate potential combined effects of the alleles; individuals who were homozygous for the common allele at both loci were the referent group. Assessment of interactions between genotypes and lifestyle factors were based on a multiplicative scale. Effect modification was determined by evaluating the improvement in fit (difference in −2 log likelihood values) of a model that included a multiplicative interaction term compared with a restricted model with no interaction term.

In the analysis of interactions between genotypes and lifestyle factors on the risk of colon cancer, the effect of a high-risk diet was assessed using a Western diet eating pattern, based on factor analysis as described previously.[23, 24] Red and processed meats, fast foods, eggs, high-fat dairy, refined grains and added sugar contributed heavily to the Western diet pattern. Categories to represent high-, intermediate- or low-risk Western diet were based on tertile distribution of factor scores among controls. All analyses were conducted using SAS version 9.1 (Cary, North Carolina, USA).

RESULTS

Characteristics of the study sample are presented in table 1. Cases and controls were ~65 years of age and 92% self-described themselves as non-Hispanic Caucasian. All control genotype frequencies were in Hardy–Weinberg equilibrium, stratified by race. The *MLH1 −93G>A* (rs1800734) and *Ile219Val* (rs1799977) polymorphisms were in linkage disequilibrium (non-Hispanic White, D'= 0.99, r^2 = 0.37; Hispanic, D'= 1.0, r^2 = 0.38; and Black, D'= 0.88, r^2 = 0.14).

Main associations between MMR genotypes and colon cancer risk are shown in table 2. Multivariable-adjusted ORs and CIs suggest no association between the *MLH1* polymorphisms and risk of colon cancer. Male participants heterozygous (*Gly/Glu*) or homozygous (*Glu/Glu*) for the *MSH6* variant were at 27% (95% CI 1.04 to 1.54) increased risk of colon cancer; while no associations were observed among females, the p value for heterogeneity between sexes was not statistically significant.

The association between MMR genotypes and risk of MSI-positive and MSI-negative colon cancer is shown in table 3. Because risks were similar between men and women, combined results are shown. There was a greater than twofold increased risk of MSI-positive colon tumours among carriers of the *MLH1 -93AA* genotype relative to the *GG* genotype (OR 2.47; 95% CI 1.48 to 4.11); no associations were observed between the *MLH1 −93A* allele and MSI-negative colon tumours. The p value for heterogeneity between MSI-positive and MSI-negative colon cancers with the *−93G>A* polymorphism was statistically significant (p = 0.01).

No statistically significant associations were observed between the *MLH1 Ile219Val* polymorphism and MSI-positive or MSI-negative colon cancer, although a non-statistically significant inverse association was observed among MSI-positive tumours and the *Val/Val* genotype. In dominant models for *MSH6 Gly39Glu* among males, risk was slightly stronger

for MSI-positive (*Glu/Glu* or *Glu/Gly* vs *Gly/Gly*, OR 1.74; 95% CI 1.11 to 2.73) than MSI-negative (*Glu/Glu* or *Glu/Gly* vs *Gly/Gly*, OR 1.22; 95% CI 0.98 to 1.53) colon cancers.

The risk of MSI-positive and MSI-negative colon cancer associated with MMR genotype stratified by smoking status is shown in table 4. Among MSI-positive tumours, no heterogeneity by smoking status and MMR genotype was detected; however, case–control comparisons suggested that carriers of the *MLH1 −93A* allele, who were recent smokers (within 2 years of the referent period), were at decreased risk of MSI-negative colon cancer relative to carriers of the *MLH1 −93G* allele who did not smoke (*GA/AA* vs *GG*, OR 0.60; 95% CI 0.42 to 0.87; p value for interaction 0.005). The associations between the *MLH1 Ile219Val* polymorphism and colon cancer risk differed by Western diet pattern (table 5). Among those who consumed a "high-risk" Western diet pattern, characterised by high red and processed meat intake, fast foods, eggs, high-fat dairy, refined grains and added sugar, a twofold increased risk of colon cancer was observed among carriers of two copies of the variant allele (*Val/Val*) relative to individuals with two copies of the common allele (*Ile/Ile*) who consumed a less Western diet (OR 2.47; 95% CI 1.63 to 3.74; p value for interaction 0.03).

No statistically significant heterogeneity was detected between alcohol and MMR genotype for risk of MSI-positive and MSI-negative tumours in case–control analyses (data not shown). Relative to non-obese carriers of the *MLH1 −93GG* genotype, obese carriers of the *AA* genotype experienced about a doubling in overall colon cancer risk (OR 1.94; 95% CI 1.02 to3.67) whereas non-obese carriers of the *AA* genotype experienced a risk similar to the referent group (OR 1.1; 95% CI 0.79 to 1.53), although the p value for the interaction was not statistically significant (p value 0.09).

Because age and family history are clearly important risk factors for colon cancer, we investigated these factors in stratified analyses with the MMR polymorphisms and saw no meaningful effect modification (data not shown). Further, we investigated associations between *MLH1* haplotypes and colon cancer risk; the analyses did not yield any additional insight.

DISCUSSION

The influence of common missense polymorphisms on colon cancer risk is poorly understood; however, relatively rare mutations in MMR genes are well understood to cause cancer in humans[1] and laboratory animals.[25] To our knowledge, this is the first study to report a statistically significant association between the *MSH6 Gly39Glu* variant and colon cancer. This study adds support to recent evidence[6] that the *MLH1 −93G>A* variant is more strongly linked with MSI-positive than MSI-negative colon cancer. Given the large sample size and wealth of lifestyle data in the current study, we were able to examine novel potential gene–lifestyle interactions relevant to DNA damage. We found that smoking and Western diet pattern modified the risk of colon cancer from some MMR polymorphisms studied. We recognise that the associations we observed in our investigation of specific study hypotheses may reflect chance because a number of comparisons were made; thus replication in other studies is important to confirm or disprove our results.

The *MLH1 −93G>A* polymorphism is located in the promoter of the gene[26]; two transcription binding sites exist in this region, NF-IL6 and GT-IIB.[26–28] Thus, the variant could plausibly reduce MLH1 transcription and expression, thereby reducing overall DNA repair capability. No differences in MLH1 gene expression, however, were noted between the *−93A* and *−93G* alleles by a luciferase assay.[29] Other work, however, has shown that the region harbouring this allele contains CpG islands,[30] susceptible to hypermethylation (ie, gene silencing),[30, 31] the relevance of which may have been missed by a luciferase assay.

Our finding that the *MLH1 −93G>A* variant is associated with MSI-positive, but not MSI-negative, colon cancer is supported by one recent study.[6] Raptis and colleagues described results from two population-based colorectal cancer registries in Ontario and Newfoundland, Canada.[6] The *MLH1 −93G>A* variant was not predictive of colorectal cancer risk overall; however, when stratified by tumour MSI status, the *MLH1 −93A* allele was predictive of MSI-high (similar to MSI-positive in the current study), but not MS-stable (similar to MSI-negative in the current study), colorectal cancer susceptibility. Collectively, our data and those from the Canadian study suggest that the *MLH1 −93G>A* polymorphism is specifically associated with microsatellite-unstable, and not microsatellite-stable, colon cancer.

In our study population we observed an inverse association between risk of MSI-negative colon cancer and jointly the high-risk genotype (*MLH1 −93AA*) and high-risk exposure (current smoking). Further, we noted the overall elevated risk of MSI-positive tumours in those with the rare allele. Together, these associations suggest strongly that the *MLH1 −93AA* allele, particularly in combination with smoking, specifically diminishes the likelihood of an MSI-negative phenotype.

The *MLH1 Ile219Val* polymorphism is in a conserved region of exon 8; it may represent a conservative amino acid change, as both alleles result in amino acids that are non-polar and pH-neutral. Functional analyses suggest that the variant has DNA repair efficiency[32, 33] and binding properties (to PMS2) similar to the wild type.[34] It must be noted, however, that functional assays are limited in their ability to detect MMR defects related to, for example, mRNA splicing or stability.[35] Homozygosity for the *219Val* variant was statistically significantly correlated with reduced MLH1 protein expression among sporadic colorectal cancer cases from Korea.[36] Further, as noted recently,[10] the *Ile219Val* polymorphism may be predictive of a haplotype linked with greater mutation frequency.[37, 38]

The *MLH1 Ile219Val* variant was not associated with risk of colon cancer in this study, consistent with recent work from Canada,[6] the USA[10] and Israel.[39] These reports are further supported by earlier results for colon adenoma.[9] There is evidence, however, that the *MLH1 Ile219Val* polymorphism is not entirely benign. The *MLH1 219Val* variant has been associated with an almost fivefold increased risk of ulcerative colitis,[7] a major risk factor for colon cancer. Other work found that, when combined with genotypes known to increase susceptibility to leukaemia, the *MLH1 219Val* variant increased the risk of acute lymphoblastic leukaemia by 6- to 16-fold, depending on diplotype combination.[40] We found that a high Western diet pattern modified the association between the *MLH1 219Val* variant and colon cancer risk, such that the variant genotype (*219Val/Val*) was associated with increased colon cancer risk only among those who also reported a high Western diet pattern. Berndt and colleagues recently reported that processed meat intake, a component of our Western dietary pattern, similarly modified risk of colon cancer from two common variants in another MMR gene, *MSH3* (*R940Q* and *T1036A*),[10] which has some overlap in function with *MSH6*.[2]

These observations of lifestyle (eg, diet) interacting on an MMR-deficient background are consistent with temporal changes in the cancer phenotype of a Lynch syndrome cancer family over the last century.[41, 42] The cancer family, first identified by Warthin in 1913, and later identified as a Lynch syndrome cancer family, had a strong predisposition to stomach and endometrial cancers in early generations,[41] similar to the general US population at the time.[43] As later described by Lynch in the 1980s, by the third and fourth generations the phenotype was more commonly expressed as colon and rectal cancers,[42] also similar to the general US population at the time.[43] These data suggest that while DNA repair is critical to cancer risk, the site of tumour development is greatly influenced by environmental factors.

Evidence from Western diet feeding experiments in normal[44, 45] and APC knock-out[46] mice largely support the role of Western diet in colorectal carcinogenesis. Few studies, however, have been conducted among MMR-deficient mice,[47] which would be more relevant to the current study. Tsao and colleagues investigated the effects of a high-fat/low-calcium diet compared with normal and calorie-restricted diets in MLH1+/− mice [48]; the high-fat/low-calcium diet group experienced significantly more adenomas per mouse and reduced lifespan relative to mice on the normal diet.

Among males in our study, the *MSH6 39Glu* variant allele was associated with increased risk of colon cancer. When stratified by MSI status, risk was slightly stronger for MSI-positive than MSI-negative colon cancers. Earlier work suggested slightly higher frequencies for the *MSH6 39Glu* allele among colorectal cancer cases (23%) than controls (15%),[49] although no formal statistical comparisons were made. To our knowledge, only one previous study examined the association between the *MSH6 Gly39Glu* variant and risk of colon or rectal cancer[10]: Berndt and colleagues reported that homozygosity for the *MSH6 39Glu* variant was associated with a more than tripling of rectal, but not colon, cancer. We are aware of no functional assays for this polymorphism; however, in silico analyses of predicted protein function suggested the variant was “benign” (Polyphen score: 1.43)[50] and “tolerated” (SIFT score: 0.23).[51]

In summary, we found that a common genetic variant in exon 1 of the *MSH6* gene, *Gly39Glu*, was associated with increased risk of colon cancer among men, but not among women. When the colon cancer outcome was further stratified by MSI status, homozygosity for the *MLH1 −93G>A* variant was associated with a more than doubling in risk of MSI-positive colon tumour among men and women, whereas the genotype was not associated with risk of MSI-negative colon tumour. We noted effect modification between MMR variants and lifestyle factors (Western diet, smoking) well known to increase colon cancer risk. This is consistent with time trends of colon cancer risk in Lynch syndrome cancer families that parallel the general US population. These data suggest that common variants in MMR genes influence susceptibility to colon carcinogenesis, particularly for tumours with a mutator phenotype, and jointly with relevant environmental exposures.

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Table 1

Characteristics of the study population

Characteristic	Cases No (%)	Controls No (%)	χ^2 p Value
All subjects	1609	1972	
Age*	64.9 (9.8)	65.0 (10.2)	0.77
Sex			
Male	901 (56.0)	1043 (52.9)	
Female	708 (44.0)	929 (47.1)	0.06
Race/ethnicity			
White, non-Hispanic	1470 (91.4)	1835 (93.1)	
Hispanic	62 (3.8)	79 (4.0)	
Black	74 (4.6)	56 (2.8)	
Other	3 (0.2)	2 (0.1)	0.02
Postmenopausal hormone use			
No	470 (75.6)	551 (69.5)	
Yes	144 (23.5)	242 (30.5)	0.003
Western diet†			
Low risk	401 (24.9)	668 (33.9)	
Intermediate risk	556 (34.6)	652 (33.1)	
High risk	652 (40.5)	652 (33.1)	<0.001
Number of cigarettes smoked regularly			
Never	661 (41.3)	925 (47.0)	
≤20/day	643 (40.2)	773 (39.3)	
>20/day	297 (18.6)	269 (13.7)	<0.001
Study centre			
Northern California	777 (48.3)	819 (41.5)	
Minnesota	579 (36.0)	805 (40.8)	
Utah	253 (15.7)	348 (17.6)	<0.001
Microsatellite instability			
MSI-positive	193 (15.9)		
MSI-negative	1018 (84.1)		
MLH1 −93G>A			
GG	958 (59.6)	1173 (59.6)	
GA	555 (34.5)	689 (35.0)	
AA	95 (5.9)	106 (5.4)	0.78
MLH1 Ile219Val			
Ile/Ile	767 (47.7)	939 (47.6)	
Ile/Val	682 (42.4)	846 (42.9)	
Val/Val	160 (9.9)	187 (9.5)	0.88
MLH1 −93G>A/Ile219Val			
GG and *Ile/Ile*	354 (22.0)	411 (20.9)	

	Cases	Controls	χ^2
Characteristic	**No (%)**	**No (%)**	**p Value**
GA and *Ile/Ile*	318 (19.8)	421 (21.4)	
GG and *Ile/Val*	444 (27.6)	576 (29.3)	
GA and *Ile/Val*	237 (14.7)	267 (13.6)	
AA and *Ile/Ile*	95 (5.9)	106 (5.4)	
GG and *Val/Val*	160 (10.0)	187 (9.5)	0.55
MSH6 Gly39Glu			
Gly/Gly	1042 (64.8)	1343 (68.4)	
Gly/Glu	517 (32.2)	559 (28.5)	
Glu/Glu	48 (3.0)	62 (3.2)	0.06

*Mean (SD and t test).

†Based on lower (low risk), middle (intermediate risk) and upper tertiles (high risk) of Western dietary pattern scores for control subjects; see Materials and methods section.

Table 2

Risk of colon cancer associated with mismatch repair genotype*

	Overall			Men			Women		
Genotype	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**
MLH1 −93G>A									
GG	952	1170	1 (referent)	542	633	1 (referent)	410	537	1 (referent)
GA	553	688	1.00 (0.87 to 1.16)	295	349	1.00 (0.83 to 1.22)	258	339	0.99 (0.81 to 1.22)
AA	95	105	1.12 (0.84 to 1.51)	58	58	1.19 (0.81 to 1.75)	37	47	1.03 (0.66 to 1.63)
MLH1 Ile219Val (655A>G)									
Ile/Ile	764	937	1 (referent)	403	496	1 (referent)	361	441	1 (referent)
Ile/Val	678	843	1.00 (0.87 to 1.15)	397	448	1.11 (0.91 to 1.34)	281	395	0.89 (0.72 to 1.09)
Val/Val	159	187	1.05 (0.83 to 1.33)	96	97	1.22 (0.89 to 1.68)	63	90	0.87 (0.61 to 1.25)
MLH1 −93G>A/Ile219Val†									
GG and *Ile/Ile*	352	410	1 (referent)	186	218	1 (referent)	166	192	1 (referent)
GA and *Ile/Ile*	317	421	0.90 (0.73 to 1.11)	159	219	0.88 (0.66 to 1.17)	158	202	0.91 (0.68 to 1.23)
GG and *Ile/Val*	441	574	0.92 (0.76 to 1.11)	260	319	0.99 (0.76 to 1.28)	181	255	0.85 (0.64 to 1.13)
GA and *Ile/Val*	236	266	1.07 (0.85 to 1.35)	136	129	1.29 (0.94 to 1.76)	100	137	0.87 (0.62 to 1.21)
AA and *Ile/Ile*	95	105	1.08 (0.79 to 1.48)	58	58	1.22 (0.80 to 1.85)	37	47	0.93 (0.58 to 1.51)
GG and *Val/Val*	159	187	1.01 (0.78 to 1.31)	96	97	1.19 (0.84 to 1.68)	63	90	0.84 (0.57 to 1.23)
MSH6 Gly39Glu (116G>A)									
Gly/Gly	1037	1340	1 (referent)	580	724	1 (referent)	457	616	1 (referent)
Gly/Glu	514	557	1.19 (1.03 to 1.38)	283	277	1.28 (1.05 to 1.56)	231	280	1.11 (0.90 to 1.38)
Glu/Glu	48	62	0.99 (0.67 to 1.46)	31	33	1.15 (0.69 to 1.91)	17	29	0.81 (0.44 to 1.51)
Gly/Glu or *Glu/Glu*	562	619	1.17 (1.02 to 1.35)	314	310	1.27 (1.04 to 1.54)	248	309	1.09 (0.88 to 1.34)

*Adjusted for age, sex, race, centre and usual amount smoked (cigarettes/day); observations vary slightly from table 1 due to missing data.

†Combined genotypes *GA* and *Val/Val*, *AA* and *Ile/Val*, and *AA* and *Val/Val* were not observed in the study population.

Table 3

Risk of colon cancer associated with mismatch repair genotype, by microsatellite instability (MSI) status*

	MSI-positive tumours				MSI-negative tumours			
Genotype	**Cases No**	**Controls No**	**OR (95% CI)**	**p Trend**	**Cases No**	**Controls No**	**OR (95% CI)**	**p Trend**
MLH1 −93G>A								
GG	104	1170	1 (referent)	0.01	616	1170	1 (referent)	0.61
GA	67	688	1.10 (0.79 to 1.52)		345	688	0.96 (0.82 to 1.14)	
AA	22	105	2.47 (1.48 to 4.11)		51	105	0.94 (0.66 to 1.34)	
MLH1 Ile219Val (655A>G)								
Ile/Ile	100	937	1 (referent)	0.19	480	937	1 (referent)	0.43
Ile/Val	80	843	0.92 (0.67 to 1.25)		424	843	1.00 (0.85 to 1.17)	
Val/Val	13	187	0.62 (0.35 to 1.18)		108	187	1.16 (0.89 to 1.51)	
MSH6 Gly39Glu (116G>A)								
Gly/Gly	121	1340	1 (referent)	0.27	655	1340	1 (referent)	0.13
Gly/Glu	67	557	1.30 (0.95 to 1.79)		326	557	1.19 (1.00 to 1.41)	
Glu/Glu	5	62	0.88 (0.35 to 2.25)		30	62	0.96 (0.61 to 1.51)	

*Adjusted for age, sex, race, centre and usual amount smoked (cigarettes/day).

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Table 4

Risk of colon cancer associated with mismatch repair genotype, by smoking and microsatellite instability (MSI) status*

Exposure	Never smoked, or not recently			Smoker ≤ 2 years of referent			
Genotype	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**	**p Interaction**
	MSI-positive tumours			**MSI-positive tumours**			
MLH1 −93G>A							
GG	81	1004	1 (referent)	23	168	1.78 (1.09 to 2.94)	0.27
GA	50	570	1.12 (0.77 to 1.61)	17	118	1.89 (1.08 to 3.32)	
AA	19	83	3.00 (1.72 to 5.22)	3	23	1.83 (0.53 to 6.29)	
MLH1 Ile219Val (655A>G)							
Ile/Ile	80	782	1 (referent)	20	156	1.30 (0.77 to 2.20)	0.56
Ile/Val	60	714	0.83 (0.58 to 1.18)	20	131	1.57 (0.92 to 2.65)	
Val/Val	10	164	0.59 (0.30 to 1.17)	3	23	1.31 (0.38 to 4.47)	
MSH6 Gly39Glu (116G>A)							
Gly/Gly	88	1120	1 (referent)	33	222	1.95 (1.27 to 3.00)	0.12
Gly/Glu	57	480	1.48 (1.04 to 2.10)	10	78	1.67 (0.83 to 3.37)	
Glu/Glu	5	52	1.25 (0.48 to 3.21)	0	10	0.00 Undefined	
	MSI-negative tumours			**MSI-negative tumours**			
MLH1 −93G>A							
GG	516	1004	1 (referent)	102	168	1.18 (0.90 to 1.54)	0.005
GA	307	570	1.06 (0.89 to 1.26)	39	118	0.64 (0.44 to 0.93)	
AA	46	83	1.09 (0.75 to 1.60)	5	23	0.43 (0.16 to 1.15)	
MLH1 Ile219Val (655A>G)							
Ile/Ile	417	782	1 (referent)	64	156	0.76 (0.56 to 1.05)	0.09
Ile/Val	365	714	0.97 (0.82 to 1.16)	61	131	0.88 (0.63 to 1.22)	
Val/Val	87	164	1.03 (0.77 to 1.38)	21	23	1.78 (0.97 to 3.27)	
MSH6 Gly39Glu (116G>A)							
Gly/Gly	556	1120	1 (referent)	101	222	0.90 (0.70 to 1.17)	0.99
Gly/Glu	286	480	1.19 (0.99 to 1.42)	41	78	1.06 (0.71 to 1.57)	

Exposure	Never smoked, or not recently			Smoker ≤ 2 years of referent			
Genotype	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**	**p Interaction**
Glu/Glu	26	52	0.97 (0.60 to 1.58)	4	10	0.79 (0.24 to 2.57)	

*Adjusted for age, sex, race and centre.

Table 5

Risk of colon cancer associated with mismatch repair genotype, by Western diet pattern*

Exposure	Western diet tertile 1			Western diet tertile 2			Western diet tertile 3			
Genotype	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**	**Cases No**	**Controls No**	**OR (95% CI)**	**p Interaction**
MLH1 −93G>A										
GG	230	406	1 (referent)	323	396	1.45 (1.16 to 1.80)	399	368	1.83 (1.47 to 2.28)	0.16
GA	137	228	1.07 (0.82 to 1.40)	199	218	1.61 (1.25 to 2.08)	217	242	1.56 (1.22 to 2.01)	
AA	32	32	1.72 (1.02 to 2.90)	32	36	1.65 (0.99 to 2.73)	31	37	1.44 (0.86 to 2.39)	
MLH1 Ile219Val (655A>G)										
Ile/Ile	193	311	1 (referent)	256	305	1.36 (1.06, 1.74)	315	321	1.54 (1.21 to 1.96)	0.03
Ile/Val	175	279	1.02 (0.79 to 1.33)	245	281	1.43 (1.11 to 1.83)	258	283	1.44 (1.12 to 1.85)	
Val/Val	31	77	0.66 (0.42 to 1.05)	53	64	1.37 (0.91 to 2.06)	75	46	2.47 (1.63 to 3.74)	
MSH6 Gly39Glu (116G>A)										
Gly/Gly	241	450	1 (referent)	362	437	1.56 (1.27 to 1.93)	434	453	1.71 (1.39 to 2.12)	0.45
Gly/Glu	141	195	1.35 (1.03 to 1.77)	178	189	1.74 (1.34 to 2.26)	195	173	2.04 (1.57 to 2.65)	
Glu/Glu	16	19	1.54 (0.78, 3.07)	13	23	1.01 (0.50 to 2.05)	19	20	1.74 (0.91 to 3.35)	

*Adjusted for age, sex, race, centre and amount smoked (cigarettes/day). Adjusting for long-term alcohol or body mass index did not affect the estimates. See Slattery *et al*[2324] for additional descriptions of the Western diet pattern.