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Genetic Variants in *XRCC2*: New Insights Into Colorectal Cancer Tumorigenesis

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Abstract

Polymorphisms in DNA double-strand break repair gene *XRCC2* may play an important role in colorectal cancer etiology, specifically in disease subtypes. Associations of *XRCC2* variants and colorectal cancer were investigated by tumor site and tumor instability status in a four-center collaboration including three U.K. case-control studies (Sheffield, Leeds, and Dundee) and a U.S. case-control study of cases from high-risk Utah pedigrees (total: 1,252 cases and 1,422 controls). The 14 variants studied were tagging single nucleotide polymorphisms (SNP) selected from National Institute of Environmental Health Sciences/HapMap data supplemented with SNPs identified from sequencing of 125 cases chosen to represent multiple colorectal cancer groups (familial, metastatic disease, and tumor subsite). Monte Carlo significance testing using Genie software provided valid meta-analyses of the total resource that includes family-based data. Similar to reports of colorectal cancer and other cancer sites, the rs3218536 R188H allele was not associated with increased risk. However, we observed a novel, highly significant association of a common SNP, rs3218499G>C, with increased risk of rectal tumors (odds ratio, 2.1; 95% confidence interval, 1.3-3.3; $P_{\chi 2}$ = 0.0006) versus controls, with the largest risk found for female rectal cases (odds ratio, 3.1; 95% confidence interval, 1.6-6.1; $P_{\chi 2}$ = 0.0006). This difference was significantly different to that for proximal and distal colon cancers ($P_{\chi 2}$ = 0.02). Our investigation supports a role for *XRCC2* in colorectal cancer tumorigenesis, conferring susceptibility to rectal tumors. (Cancer Epidemiol Biomarkers Prev 2009;18(9): 2476–84)

Introduction

The X-ray repair complementing defective repair in Chinese hamster cells 2 (*XRCC2*) gene, located on 7q36.1, is an essential part of the homologous recombination repair pathway and a functional candidate for involvement in tumor progression (1-3). DNA double-strand breaks (DSB) trigger a response pathway via activation of *ATM* and the MRN complex (comprising *MRE11A, NBS1*, and *RAD50*), which is thought to initiate the DNA repair process (4). *ATM* phosphorylates *CHEK2* (leading to cell cycle arrest) and the breast cancer proteins BRCA1 and BRCA2 and also activates TP53 (4-6). The DSB can then be repaired by two alternative pathways: homologous recombination repair and the more error-prone nonhomologous end joining (4, 7). DNA DSBs induce a S-phase colocalization to nuclear foci of BRCA1 and BRCA2 with RAD51, which is central to homologous recombination (8). Other members of the RAD51-related protein family, XRCC2 and XRCC3, are also essential for homologous recombination repair (9, 10), and are required for correct chromosome segregation and the apoptotic response to DSBs (1, 11). Accurate repair of DSBs arising during DNA replication or from DNA-damaging agents is necessary to maintain genomic stability. Failure of these processes to repair DSBs can lead to mutations, apoptosis, tumor predisposition, and carcinogenesis (2, 4, 12); inherited deficiencies in several genes involved in the DSB pathway can confer an increased risk of cancer and may be predictive of later mortality (1, 12, 13).

Although a defective mismatch repair (MMR) is known to cause hereditary nonpolyposis colorectal cancer or Lynch syndrome, much less is known about the DSB pathway in colorectal cancer etiology and the role of DSB-related genes has only recently begun to be investigated (14). Common variants within *XRCC2*, particularly a coding single nucleotide polymorphism (SNP) in exon 3 (R188H, dbSNP ID rs3218536), have been identified as potential cancer susceptibility loci in recent studies, although association results are mixed. The *XRCC2* R188H polymorphism has been proposed to be a genetic modifier for smoking-related pancreatic cancer (15) and was associated with an increased risk of pharyngeal cancer (16), and

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the rs2040639 SNP was reported to contribute to oral cancer risk (17). In contrast, R188H and three SNPs in the 5′ promoter region have been associated with reduced bladder cancer risk (18). A large multiethnic study of epithelial ovarian cancer showed an association between R188H and reduced risk (19), although validation studies could not provide confirmation (20, 21). Studies have implicated *XRCC2* R188H in breast cancer (22-25); however, the Breast Cancer Association Consortium (26) and other subsequent studies found no association between R188H and breast cancer risk (27, 28) or evidence of a modest protective association (29, 30). Only a very limited number of studies of *XRCC2* specific to colorectal lesions have been conducted to date. In a large nested case-control study of colorectal adenomas, no association with R188H was found (31). In a hospital-based study and a large colorectal cancer candidate gene SNP scan, Moreno et al. and Webb et al. observed no association of R188H and colorectal cancer, respectively (14, 32).

Based on their meta-analysis of these two studies, Vineis et al. reported a nominally significant, modest increased risk of colorectal cancer with R188H [odds ratio (OR), 1.16; 95% confidence interval (95% CI), 1.01-1.34; $P = 0.034$], which they characterize as weakly credible in a comprehensive analysis of associations reported between several variants in DNA repair genes across cancer sites (33). To our knowledge, no study has examined *XRCC2* variants, other than R188H, in relation to colorectal cancer.

Colorectal tumors fall into two main groups, those exhibiting chromosomal instability and those exhibiting microsatellite instability (MSI). The latter group is deficient in DNA MMR; this can be caused by inherited mutations in genes encoding MMR proteins MLH1 and MSH2, as occurs in hereditary nonpolyposis colorectal cancer, or by loss of expression of these proteins (usually MLH1) in sporadic tumors. There are marked differences between MMR-proficient and MMR-deficient tumors in their etiology and progression. MMR-proficient tumors show chromosomal instability but not MSI, tend to be distally located in the colon, and carry mutations in genes such as *K-ras* and *TP53* (34, 35). MMR-deficient tumors exhibit MSI but not chromosomal instability, tend to be proximally located, carry mutations in genes such as *TGFBR2, BAX, MSH3*, and *MSH6*, and have a better prognosis (36-38). There are also differences in epidemiologic risk factors between chromosomal instability and MSI tumor types (39). Defects in DSB repair genes might therefore be predicted to confer a chromosomal instability phenotype (40), leading to the hypothesis that genetic differences exist between these two etiologic pathways. Thus, in addition to evaluating overall risk of colorectal cancer, we examined MMR-proficient and MMR-deficient cancers separately, because the genetic risk factors may differ between the two types.

Our investigation is the first to genotype a comprehensive set of tagging-SNPs (tSNP) in a meta-genetic association study of colorectal cancer in a large, combined resource that included three U.K. case-control cohorts (Sheffield, Leeds, and Dundee) and U.S.-Utah cases from high-risk pedigrees and matched controls.

Materials and Methods

Study Population. In Sheffield, colorectal cancer cases were identified from subjects residing in Sheffield, United Kingdom, and undergoing surgery for a primary colorectal tumor at the Royal Hallamshire or Northern General Hospitals, Sheffield, between March 2001 and June 2005. Control subjects, age- and sex-matched to cases, were identified from Sheffield General Practice registers and recruited between October 2001 and December 2005. In Leeds and Dundee, incident colorectal cancer cases were identified between 1997 and 2000 from examination of pathology records at the Leeds and Dundee Teaching Hospitals NHS Trust, and age- and sex-matched controls were identified from the records of general practitioners of cases as described previously (41-43). In addition to 1:1 matched controls, an additional 198 Dundee controls with *XRCC2* genotypes were available for analysis. In Utah, colorectal cancer cases were selected from 252 high-risk cancer pedigrees: 1 case per pedigree from 161 pedigrees (161 independent colorectal cancer cases) and ≥2 cases from 91 pedigrees (294 related colorectal cancer cases).

A high-risk pedigree was defined as one containing a statistical excess of individuals with cancer as assessed using the Utah Population Database. The Utah Population Database is a genealogic resource that is record linked to the Utah Cancer Registry and Utah vital records; it includes a subset of ~2.3 million individuals with extensive pedigree information from which high-risk families are identified. Utah controls, which represent a convenience sample not specifically ascertained for this study, were selected to be cancer-free and were matched by sex- and 5-year birth cohort to the prevalent cases. As age of Utah controls represents their age at ascertainment for prior studies, age at diagnosis for cases and age at selection for controls do not necessarily correspond; however, cases and controls were well matched for age based on birth cohort (see footnote 2 of Table 1). Study subjects in all centers were of north European descent. The total resource included 1,252 cases and 1,422 controls that were genotyped for SNP variants in *XRCC2*. Proximal colon site was defined as tumors of the cecum through transverse colon. Distal colon was defined as tumors of the splenic flexure, descending, and sigmoid colon. Rectal cancer was defined as tumors of the rectosigmoid junction and rectum.

tSNP Selection. Usually, small, neutral discovery panels (a set of individuals unselected for disease with dense genotyping or sequence data) are used to select tSNPs to study. Recently, it has been shown that diseased discovery panels can be superior to neutral panels for selecting tSNPs that are more powerful to detect rarer genetic variants in common, complex disease (44). We therefore sequenced *XRCC2* in a large, disease-based discovery panel and incorporated these results in addition to using publicly available sequence and map data to determine a more comprehensive set of tSNPs to study. Publicly available SNP data included that derived from sequence data for >90% of all nucleotides across *XRCC2* in 24 Caucasian samples available from the National Institute of Environmental Health Sciences SNPs Program[5] and map data of 60 CEU samples available from HapMap.[6] We supplemented this with SNPs identified from the sequencing of exons and ~500 bp of the promoter region in 125 Caucasian colorectal cancer cases chosen to represent

[5] http://egp.gs.washington.edu/
[6] http://www.hapmap.org/

Table 1. Description of case and control subjects with *XRCC2* genotypes

	All centers		Utah		Sheffield		Leeds		Dundee	
	Controls	Cases	Controls	Cases*	Controls	Cases	Controls	Cases	Controls	Cases
	n (%)									
Total subjects	1,422 (100.0)	1,252 (100.0)	449 (100.0)	455 (100.0)	419 (100.0)	419 (100.0)	224 (100.0)	258 (100.0)	330 (100.0)	120 (100.0)
Independent	1,422 (100.0)	958 (76.5)	449 (100.0)	161 (35.4)	419 (100.0)	419 (100.0)	224 (100.0)	258 (100.0)	330 (100.0)	120 (100.0)
Related	—	294 (23.5)	—	294 (64.6)	—	—	—	—	—	—
Men	759 (53.4)	700 (55.9)	250 (55.7)	250 (54.9)	206 (49.2)	232 (55.4)	130 (58.0)	146 (56.6)	173 (52.4)	72 (60.0)
Women	654 (46.0)	551 (44.0)	199 (44.3)	205 (45.1)	207 (49.4)	187 (44.6)	94 (42.0)	111 (43.0)	154 (46.7)	48 (40.0)
Unknown	9 (0.6)	1 (0.1)	0 (0.0)	0 (0.0)	6 (1.4)	0 (0.0)	0 (0.0)	1 (0.4)	3 (0.9)	0 (0.0)
Family history of colorectal cancer	1,414 (100.0)	1,237 (100.0)	449 (100.0)	455 (100.0)	419 (100.0)	419 (100.0)	224 (100.0)	249 (100.0)	322 (100.0)	114 (100.0)
None	1,275 (90.2)	701 (56.7)	420 (93.5)	62 (13.6)	378 (90.2)	344 (82.1)	199 (88.8)	208 (83.5)	278 (86.3)	87 (76.3)
1 relative	123 (8.7)	385 (31.1)	25 (5.6)	264 (58.0)	35 (8.4)	63 (15.0)	24 (10.7)	36 (14.5)	39 (12.1)	22 (19.3)
≥2 relatives	16 (1.1)	151 (12.2)	4 (0.9)	129 (28.4)	6 (1.4)	12 (2.9)	1 (0.4)	5 (2.0)	5 (1.6)	5 (4.4)
Age at onset or selection (y)†	772 (100.0)	1,234 (100.0)	—	442 (100.0)	416 (100.0)	415 (100.0)	224 (100.0)	257 (100.0)	132 (100.0)	120 (100.0)
≤50	23 (3.0)	83 (6.7)	—	54 (12.2)	9 (2.2)	16 (3.9)	10 (4.5)	9 (3.5)	4 (3.0)	4 (3.3)
50-59	153 (19.8)	211 (17.1)	—	69 (15.6)	100 (24.0)	82 (19.8)	30 (13.4)	40 (15.6)	23 (17.4)	20 (16.7)
60-69	289 (37.4)	372 (30.1)	—	134 (30.3)	157 (37.7)	109 (26.3)	83 (37.1)	88 (34.2)	49 (37.1)	41 (34.2)
≥70	307 (39.8)	568 (46.0)	—	185 (41.9)	150 (36.1)	208 (50.1)	101 (45.1)	120 (46.7)	56 (42.4)	55 (45.8)
Site‡	—	1,287 (100.0)	—	484 (100.0)	—	425 (100.0)	—	258 (100.0)	—	120 (100.0)
Proximal colon	—	364 (28.3)	—	158 (32.6)	—	110 (25.9)	—	76 (29.5)	—	20 (16.7)
Distal colon	—	383 (29.8)	—	147 (30.4)	—	113 (26.6)	—	80 (31.0)	—	43 (35.8)
Colon, not otherwise specified	—	21 (1.6)	—	16 (3.3)	—	0 (0.0)	—	5 (1.9)	—	0 (0.0)
Rectal	—	438 (34.0)	—	115 (23.8)	—	172 (40.5)	—	96 (37.2)	—	55 (45.8)
Colorectal, unknown	—	81 (6.3)	—	48 (9.9)	—	30 (7.1)	—	1 (0.4)	—	2 (1.7)
MMR capacity	—	468 (100.0)	—	—	—	112 (100.0)	—	236 (100.0)	—	120 (100.0)
Proficient	—	410 (87.6)	—	—	—	95 (84.8)	—	199 (84.3)	—	116 (96.7)
Deficient	—	58 (12.4)	—	—	—	17 (15.2)	—	37 (15.7)	—	4 (3.3)

*252 high-risk cancer families; 1 case from 161 pedigrees (161 colorectal cancer cases) and ≥2 cases from 91 pedigrees (294 colorectal cancer cases).
†Controls in Utah were matched on year of birth cohort to prevalent cases (see Materials and Methods); mean ± SE: cases 1922 ± 0.52 and controls 1923 ± 0.53.
‡Includes multiple primary cancers: Utah 29 and Sheffield 6; cases with a previous diagnosis of any cancer were excluded in Leeds and Dundee.

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Table 2. *XRCC2* MAF in case and control subjects

dbSNP	bp (minus strand)	Location	Major/minor allele	Controls		Cases	
				n	MAF	*n*	MAF
rs3218373	152,005,096	5′-Untranslated region	G/T	1,366	0.10	1,192	0.09
rs3218374	152,005,067	5′-Untranslated region	C/G	1,387	0.46	1,198	0.46
rs3218385	152,004,166	5′-Untranslated region	T/G	1,370	0.05	1,190	0.06
rs3218395	152,001,162	Intron 1	C/T	1,392	0.05	1,210	0.06
rs3218400	152,000,622	Intron 1	C/A	1,354	0.11	1,200	0.10
rs3218402	152,000,235	Intron 1	A/G	1,372	0.03	1,205	0.03
rs3218418	151,996,253	Intron 1	G/A	1,384	0.05	1,210	0.05
rs3218454	151,991,353	Intron 1	A/T	1,366	0.09	1,189	0.09
rs3218472	151,988,810	Intron 1	C/T	1,361	0.002	1,192	0.001
rs3218499	151,983,072	Intron 2	G/C	1,392	0.23	1,210	0.24
rs3218501	151,982,850	Intron 2	C/G	1,396	0.04	1,201	0.04
Novel	151,977,053	Exon 3	C/G	1,055	0.002	1,083	0.001
rs3218536*	151,976,940	Exon 3	G/A	1,380	0.08	1,209	0.08
Novel	151,976,692	Exon 3	A/G	1,049	<0.001	1,081	<0.001

*R188H polymorphism.

multiple groups (familial, sporadic, and metastatic disease) and tumor site (proximal colon, distal colon, and rectum) in a collection of U.K. and U.S. samples. Using a principal components method (45) and no restriction on minor allele frequency (MAF), we selected 14 tSNPs accounting for >93% of the intragenic variation in *XRCC2*. The average pairwise r^2 between selected tSNPs and the unselected SNPs they were chosen to represent was 0.88. We identified a total of four supplemental variants from our sequencing of the disease-based panel: two that were not represented in the neutral National Institute of Environmental Health Sciences/HapMap data as well as two rare, novel variants (Table 2). These 4 SNPs, plus the set of 14 tSNPs, were selected from a total of 93 possible *XRCC2* SNPs. Of these, 12 tSNPs and the two novel variants were successfully genotyped in the combined four-study resource of case and control subjects in the United Kingdom and the United States.

Genotyping. Genotyping was carried out at the Sheffield, United Kingdom center in 384-well plates using the Applied Biosystems SNPlex system, which allows multiplex analysis of up to 48 SNPs.[7] At least 5% of samples were duplicated in the plates to assess the reproducibility of the genotype calls. For each SNP, duplicate concordance, call rate, and test for compliance with Hardy-Weinberg equilibrium in controls separately for each study site are shown in Supplementary Table S1. We required a duplicate concordance of at least 95%, a call rate of at least 90%, and Hardy-Weinberg equilibrium in controls ($P > 0.05$) for a SNP to pass quality control. Two tSNPs (rs2106776 and rs3218455) failed quality control and two of the four supplemental SNPs failed primer design and were dropped from further analysis. However, tSNPs rs3218374 and rs3218536 adequately represented the omitted tSNPs ($r^2 = 0.7$ and 1.0, respectively). The remaining 14 SNPs (12 tSNPs and 2 novel variants) were taken forward to analysis.

MMR Capacity. Tumor samples in the U.K. studies, Sheffield, Leeds, and Dundee, were assessed for MMR capacity as measured by immunohistochemistry of the MLH1 and MSH2 proteins using antibodies raised against MLH1 (G168-15; BD Biosciences) and MSH2 (Ab-2; Oncogene) as described previously (38, 46). MMR deficiency was defined as loss of MLH1 or loss of MSH2; conversely, MMR proficiency was the expression of MLH1 and MSH2. An assessment of MMR capacity was available for 468 of 797 cases in the U.K. data with *XRCC2* genotypes.

Statistical Analysis. All analyses were conducted using Genie 2.6.2, a freely available software package.[8] Genie provides valid genetic association, Hardy-Weinberg equilibrium, and homogeneity testing in cases and controls that include related individuals using Monte Carlo significance testing (47). Specifically, Genie allows for valid meta-association testing, where constituent studies can include a mixture of family-based and independent individuals. In such situations, using standard statistical software to perform methods such as logistic regression is invalid. The meta-association capabilities and validity of Genie are described elsewhere in detail (47) and have been applied previously in candidate gene meta-association studies (48, 49). We performed meta-χ^2 tests for trend, ORs, and empirical 95% CIs using Cochran-Mantel-Haenszel techniques for each SNP. We repeated our Cochran-Mantel-Haenszel analyses also controlling for sex, early or late age at diagnosis, and family history in addition to study center. These did not differ substantively and are therefore not shown.

The primary statistical test employed throughout is a trend test together with heterozygote and homozygote ORs to indicate effect size; however, a dominant model was used for SNPs with insufficient homozygote counts to maintain statistical validity (MAF < 0.05). If the ORs indicated a recessive model, then this was also analyzed because a recessive model is not well represented by a trend test (50). Stratified analyses by sex, age at diagnosis, family history, and tumor site were performed. A cut point of 60 years (~25th percentile of the distribution of diagnosis age in the cases) was used to determine early or late onset. As controls in Utah were age matched by

[7] www.appliedbiosystems.com

[8] http://www-genepi.med.utah.edu/Genie/index.html

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5-year birth cohort to cases, age was stratified by younger or older birth cohort to approximate a cut point of age 60 years. Cochran's *Q* test was conducted to assess homogeneity of effect size across studies. Statistical heterogeneity was considered present if $P < 0.05$. All *P* values were empirically derived based on 10,000 simulations in the Genie null distribution as described (47, 51). The haplotype-mining hapConstructor module of Genie was used to comprehensively analyze multi-locus *XRCC2* haplotypes and combined genotypes (52).

Results

A description of the four study populations in the U.K. and U.S. centers and the combined resource is shown in Table 1. Cases from Utah had a higher proportion of first-degree relatives with colorectal cancer than cases in the U.K. cohorts ($P_{ANOVA} < 0.0001$), and a higher proportion of early-onset cases (age ≤59 years; $P_{ANOVA} = 0.002$), as would be expected for colorectal cancer cases selected from high-risk cancer pedigrees. Utah also had a lower proportion of rectal cancer ($P_{ANOVA} < 0.001$). This is also as expected because the colorectal cancer high-risk pedigrees were ascertained primarily for excess of colon cancers, and the relative incidence of rectal cancer to colon cancer is higher in the United Kingdom than in the United States (53). In Table 2, we describe the *XRCC2* tSNPs selected. All SNPs were in Hardy-Weinberg equilibrium; pairwise linkage disequilibrium between the SNPs studied is shown in Supplementary Table S2.

Meta-genetic associations of each tSNP with colorectal cancer are shown in Table 3. No results exhibited significant statistical heterogeneity across studies. Only one SNP indicated significant association with colorectal cancer. Individuals who were homozygous for the rs3218499C risk allele had a 60% increased risk of colorectal cancer compared with the GG genotype (OR_{meta}, 1.6; 95% CI, 1.1-2.2). There was no increased risk for individuals who were GC heterozygotes; thus, the C allele appeared to have a recessive mode of inheritance ($P_{\chi 2} = 0.009$). Risk estimates were somewhat higher in the three U.K. studies for rs3218499 (Supplementary Table S3), although there was no statistically significant evidence for heterogeneity across the four studies ($P_{homogeneity} = 0.90$). No significant haplotype associations were found.

We evaluated whether the observed association for rs3218499G>C differed by tumor site, sex, age at onset, and family history. In Table 4, the meta-association results for rs3218499, stratified by each characteristic, are shown. For tumor site, we compared proximal colon, distal colon, and rectal cases to controls. We observed that the increased risk for cancer differed substantially by tumor site. In cases with rectal tumors, the association was highly significant compared to controls (CC versus GC/GG, recessive: OR_{meta}, 2.1; 95% CI, 1.3-3.2; $P_{\chi 2} = 0.0006$). Furthermore, the increased risk for rectal cancer was

Table 3. Association of *XRCC2* with colorectal cancer in meta-analysis of U.K. and U.S. studies

SNP	Genotype	*n*		$P_{homogeneity}$*	Meta-OR (95% CI)	$P^{\dagger}$
		Controls	Cases			
rs3218373	GG	1,119	983		1 (Reference)	
	GT	232	193		0.9 (0.8-1.2)	
	TT	15	16	0.19	1.2 (0.5-2.9)	0.79
rs3218374	CC	411	370		1 (Reference)	
	CG	677	559		0.9 (0.8-1.1)	
	GG	299	269	0.86	1.0 (0.8-1.3)	0.94
rs3218385	TT	1,226	1,052		1 (Reference)	
	TG or GG	144	138	0.45	1.1 (0.8-1.4)	0.57
rs3218395	CC	1,255	1,081		1 (Reference)	
	CT or TT	137	129	0.35	1.1 (0.8-1.4)	0.51
rs3218400	CC	1,068	974		1 (Reference)	
	CA	275	210		0.8 (0.7-1.0)	
	AA	11	16	0.51	1.6 (0.7-3.7)	0.36
rs3218402	AA	1,302	1,130		1 (Reference)	
	AG or GG	70	75	0.89	1.2 (0.8-1.7)	0.35
rs3218418	GG	1,245	1,103		1 (Reference)	
	GA or AA	139	107	0.11	0.8 (0.6-1.1)	0.23
rs3218454	AA	1,140	999		1 (Reference)	
	AT	206	167		0.9 (0.7-1.2)	
	TT	20	23	0.07	1.3 (0.5-3.4)	0.89
rs3218472	CC	1,355	1,190		1 (Reference)	
	CT or TT	6	2	0.22	0.4 (0.0-2.7)	0.22
rs3218499	GG	823	712		1 (Reference)	
	GC	504	414		1.0 (0.8-1.1)	
	CC	65	84	0.89	1.6 (1.1-2.2)	0.23
	CC vs GC or GG			0.90	1.6 (1.1-2.2)	0.009
rs3218501	CC	1,296	1,117		1 (Reference)	
	CG or GG	100	84	0.24	1.0 (0.7-1.4)	0.93
rs3218536 (R188H)	GG	1,167	1,014		1 (Reference)	
	GA	204	185		1.0 (0.8-1.3)	
	AA	9	10	0.60	1.3 (0.4-3.8)	0.87

NOTE: Case-control comparison, reference genotype is homozygous for major allele; for MAF ≤ 0.05, genotypes were combined because of few subjects (results could not be determined for both novel variants due to very low allele frequencies).
*Empirical *Q* test to assess homogeneity, $P_{homogeneity}$ based on 10,000 simulations.
†Empirical Cochran-Mantel-Haenszel P_{trend} (additive model) or $P_{\chi 2}$ (dominant or recessive models) based on 10,000 simulations.

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Table 4. Association of *XRCC2* rs3218499G>C with colorectal cancer characteristics in a meta-analysis of U.K. and U.S. studies

Characteristics	*n*						Meta-OR (95% CI)			*P**
	Controls			Cases			GC vs GG	CC vs GG	CC vs GC/GG	
	GG	GC	CC	GG	GC	CC				
Overall	823	504	65	712	414	84	1.0 (0.8-1.1)	1.6 (1.1-2.2)	1.6 (1.1-2.2)	0.009
Tumor site										
Proximal colon	—	—	—	205	125	19	1.0 (0.8-1.3)	1.2 (0.8-2.0)	1.2 (0.8-2.0)	0.44
Distal colon	—	—	—	226	123	19	0.9 (0.7-1.2)	1.1 (0.7-1.8)	1.1 (0.7-1.8)	0.63
Rectal	—	—	—	239	149	38	1.0 (0.8-1.3)	2.1 (1.3-3.3)	2.1 (1.3-3.2)	0.0006
Rectal vs colon (case only)							1.2 (0.9-1.5)	1.9 (1.1-3.2)	1.7 (1.0-3.0)	0.02
Sex										
Men	441	259	38	402	231	45	1.0 (0.8-1.3)	1.3 (0.8-2.1)	1.3 (0.8-2.1)	0.24
Women	375	243	27	309	183	39	0.9 (0.7-1.2)	1.9 (1.1-3.2)	2.0 (1.2-3.4)	0.01
Women vs men (case only)							1.0 (0.8-1.3)	1.1 (0.7-1.9)	1.1 (0.7-1.9)	0.61
Age (y)										
<60	167	92	13	155	87	17	1.0 (0.7-1.4)	1.4 (0.6-3.2)	1.4 (0.6-3.1)	0.38
≥60	552	327	77	547	332	43	1.0 (0.8-1.2)	1.5 (1.0-2.3)	1.6 (1.1-2.3)	0.03
≥60 vs <60 (case only)							1.0 (0.8-1.4)	1.1 (0.5-2.2)	1.1 (0.5-2.1)	0.82
Family history of colorectal cancer										
No	735	458	56	419	212	46	0.8 (0.7-1.0)	1.6 (1.0-2.4)	1.7 (1.1-2.6)	0.02
Yes	83	44	8	287	197	38	1.1 (0.7-1.7)	1.8 (0.6-5.8)	1.8 (0.6-5.7)	0.17
Yes vs no (case only)							1.2 (0.9-1.7)	1.4 (0.7-2.8)	1.3 (0.7-2.6)	0.29

NOTE: Case-control comparison, unless otherwise indicated; reference genotype is homozygous for major allele.
*Empirical Cochran-Mantel-Haenszel χ^2 test for recessive model based on 10,000 simulations.

significantly higher than for proximal and distal colon cancer ($P_{\chi 2}$ = 0.02). Nominally significant results were also observed for other characteristics; however, none of the other subgroups were statistically different in case-case comparisons. When sex and colorectal cancer site were considered together, the risk was highest for female rectal cases compared to controls (CC versus GC/GG: OR_{meta}, 3.1; 95% CI, 1.6-6.1; P = 0.0006) and was significant in a case-case comparison to female colon cases ($P_{\chi 2}$ = 0.02, data not shown). However, the risk conferred by the rs3218499C allele in female rectal cases was not statistically significantly different compared to the risk in the male rectal cases ($P_{\chi 2}$ = 0.21; data not shown).

We inspected the association between rs3218499 and rectal cancer risk specifically in the four study sites. There was no statistically significant evidence for heterogeneity across the four studies for rs3218499 ($P_{homogeneity}$ = 0.42) and all risk estimates were in the same direction; however, higher and more significant risk estimates were found in the three U.K. studies (CC versus GC/GG): $OR_{UK\text{-}Sheffield}$ (95% CI), 1.8 (0.8-3.9); $OR_{UK\text{-}Leeds}$ (95% CI), 3.7 (1.1-10.4); $OR_{UK\text{-}Dundee}$ (95% CI), 2.0 (0.7-5.4); and $OR_{US\text{-}Utah}$ (95% CI), 1.4 (0.7-3.0). In female rectal cancer cases, statistical homogeneity was maintained ($P_{homogeneity}$ = 0.80) and associations were more consistent across studies: $OR_{UK\text{-}Sheffield}$ (95% CI), 2.4 (0.7-8.1); $OR_{UK\text{-}Leeds}$ (95% CI), 5.8 (0.9-31.8); $OR_{UK\text{-}Dundee}$ (95% CI), 2.6 (0.6-11.4); and $OR_{US\text{-}Utah}$ (95% CI), 3.1 (1.0-9.4; data not shown).

Rectal cancers in this study were less likely to exhibit MSI (3% of rectal tumors assessed for MMR capacity were deficient) than proximal colon (30%) or distal colon (6%) cancers, as observed elsewhere (35, 54), and differences in genetic and epidemiologic risk factors between colon and rectal tumor subsites have been suggested to exist (55-59). An exploratory analysis of *XRCC2* tSNP associations by MMR-deficient and MMR-proficient tumor status, in a subset of cases in the three U.K. studies, was thus done; no difference in risk by MMR status was observed for the rs3218499 SNP. However, one rarer *XRCC2* intronic SNP, rs3218402A>G (MAF = 0.03), was found to be nominally associated with MMR-deficient colorectal cancer in both a case-control ($P_{\chi 2}$ = 0.01) and a case-case comparison for a dominant model (MMR-deficient versus MMR-proficient, $P_{\chi 2}$ = 0.04; data not shown). Carriage of the G allele conferred an increased risk of MMR-deficient colorectal cancer in comparison to controls (AG/GG versus AA: OR_{meta}, 2.9; 95% CI, 1.1-7.4; data not shown). No increased risk of colorectal cancer was observed for MMR-proficient tumors (AG/GG versus AA: OR_{meta}, 1.2; 95% CI, 0.7-2.0; data not shown). Haplotype analyses based on MMR status suggested a haplotype of G-G across rs3218402 and rs3218385 was nominally associated with MMR-deficient tumors when compared to a reference wild-type haplotype of A-T ($P_{\chi 2}$ = 0.002). As several comparisons were made and only a subset of tumors in the overall resource had MMR status available, these results should be considered preliminary.

Discussion

Our study represents the first comprehensive genetic characterization of the role of *XRCC2* in colorectal cancer in a meta-analysis of three U.K. case-control studies and a U.S. family-based study. We used both publicly available data and results from sequencing a panel of colorectal cancer samples, well characterized for tumor type or genetically loaded from high-risk pedigrees, to select *XRCC2* tSNPs for further study. Valid analyses were made possible via Genie, which is designed to analyze both related and independent individuals. A major strength of our investigation was the sample size of the combined resource, which allowed increased power to examine associations including subphenotypes of interest such as colorectal cancer subsite.

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We observed no association between the putatively functional rs3218536 R188H SNP and colorectal cancer. Our most significant finding was a common *XRCC2* intron 2 variant (rs3218499G>C; MAF = 0.23) that appeared to be strongly associated with increased risk of rectal tumors (OR_{meta}, 2.1; $P = 0.0006$) and female rectal cancer in particular (OR_{meta}, 3.1; $P = 0.0006$). This is a finding that has not been previously reported to our knowledge, and no known functional studies of this SNP (or a SNP in high linkage disequilibrium with rs3218499) exist.[9] Genome-wide association studies on cancer including colorectal cancer have not reported any highly statistically significant findings for common DNA repair gene variants, including polymorphisms in *XRCC2* (33); however, these studies have not focused on associations in rectal cancers specifically.

The magnitude of risk estimates for *XRCC2* rs3218499 and rectal cancer were notably stronger in the U.K. cohorts. There was no evidence of statistical heterogeneity across studies; however, the *Q* test can be insensitive when a small number of studies are included. It is possible that the rectal tumors and *XRCC2* are interacting with environmental factors that may negatively affect DNA DSB repair (e.g., cigarette smoking). Environmental differences, particularly smoking and alcohol consumption, are similar in the three U.K. sites and differ with the US-Utah site, which is composed predominantly of members of the Church of Jesus Christ of Latter-day Saints (or Mormon), many of whom abstain from alcohol and tobacco use, and may be responsible for the differential in risks observed across the studies. However, environmental differences could not be assessed directly as the relevant data were not available for this study. Potential heterogeneity in phenotype origin is another plausible explanation for differences observed between U.K. and US-Utah studies. Utah cases from high-risk pedigrees could be influenced by yet undiscovered high-risk alleles and therefore less influenced by low-risk *XRCC2* alleles, although it is pertinent to note that colorectal cancer cases in the pedigrees were screened for hereditary nonpolyposis colorectal cancer variants and Amsterdam-type criteria, and none were found to be responsible for the clustering.

It is of note that female rectal cases were at highest risk (although not statistically significantly higher than male rectal cases; $P_{\chi 2} = 0.21$) and that risk estimates were more consistent across study sites for this subset of disease. This observation may argue instead for an etiology that involves interactions with hormone factors. It has been suggested that exogenous estrogens may reduce risk of sporadic colorectal cancer (54, 55), although associations with specific types of hormones have been inconsistent and it is unclear whether some tumor types differ in risk (39, 56). Hence, the potential role of environmental factors as well as endogenous and exogenous hormones should be assessed in future studies of *XRCC2* and colorectal cancer.

An exploratory investigation of colorectal cancer MMR status and *XRCC2* identified SNP rs3218402; additionally, a haplotype across this SNP and rs3218385 was nominally associated with MMR-deficient tumors. The latter SNP was identified in our disease-panel sequencing and would not have been examined had the study relied solely on publicly available data, suggesting the potential importance of supplementing tSNP selection with sequence data from disease-based samples. As several tests were conducted in a small subset of cases (37%) in the overall study resource, these results may be due to chance or selection bias and should be considered preliminary pending confirmation in other studies. Another limitation of our investigation of MMR status is our assessment of MMR capacity by immunohistochemistry of MLH1 and MSH2 proteins. Immunohistochemistry can be a valid tool to identify patients at risk for hereditary nonpolyposis colorectal cancer or Lynch syndrome and patients with sporadic microsatellite unstable colorectal cancer (57). However, it has been suggested in recent studies that adding PMS2 and MSH6 to immunohistochemical detection of MMR protein in screening colorectal cancer tumors has greater sensitivity (comparable to MSI testing) than immunohistochemical detection of MLH1 and MSH2 alone (58). Thus, it is possible that tumors evaluated as MMR-deficient in this investigation may be mischaracterized.

It has been shown that disease-based panels for tSNP selection can improve detection of rarer variants (MAF = 0.01-0.05) in subsequent association studies (44). Better characterization of such variants is due to their increased frequency and linkage disequilibrium structure that may vary in disease panels relative to neutral resources. Consistent with this, we found that there were loci identified in sequencing of our disease panel of 125 individuals that were not evident in the publicly available panels. However, our meta-investigation, which included a large collection of >2,500 subjects, was still underpowered to detect associations of very rare variants (MAF < 0.005), pointing to the need for continued large collaborations in studies of common, complex disease. It should also be noted that the increased power gained to detect association by including familial cases is accompanied by an overestimate of the effect size as measured by the OR for the general population (59). Tests of the null hypothesis (effect size or independence) remain valid with the combined populations; the Utah site contains predominantly familial cases, and as such, although our significance values are valid, our meta-OR estimates may be inflated. In our hypothesis-based investigation, we analyzed multiple SNPs and performed stratified analyses, including tumor site, gender, and MMR-proficient or MMR-deficient subgroups. As several comparisons were made, the possibility of observing a chance finding exists, and *P* values that achieve nominal significance should be interpreted with caution. Therefore, it is important that these association findings are replicated in other investigations for confirmation.

In summary, we present evidence that a common variant in *XRCC2* is associated with increased risk of colorectal cancer, an association that is particularly strong with regard to rectal cancer in women. Preliminary findings suggest that *XRCC2* may also play a role in MMR-deficient colorectal cancer.

[9] rs3218499 tSNP represents SNPs rs3218384, rs3218408, rs3218410, rs3218417, rs3218425, rs3218461, and rs3218560; average pairwise $r^2 = 0.92$.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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