

## **DNA repair and cancer in colon and rectum: novel players in genetic susceptibility**

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## **Abbreviation list**

BSGoF, binomial sequential goodness of fit;  
CCFR, Colon Cancer Family Registry;  
CI, confidence interval;  
eQTL, expression quantitative trait loci;  
FDR, false discovery rate;  
GECCO, Genetics and Epidemiology of Colorectal Cancer;  
GWAS, genome-wide association studies;  
HNPCC, hereditary nonpolyposis colorectal cancer;  
HR, homologous recombination;  
HRC, Haplotype Reference Consortium;  
htSNPs, haplotype tagging SNPs;  
HWE, Hardy-Weinberg Equilibrium;  
LD, linkage disequilibrium;  
MAF, minor allele frequency;  
MMR, mismatch repair pathway;  
MSI, microsatellite instability;  
NES, normalized effect size;  
OR, odds ratio;  
PARP, poly(ADP-ribose) polymerase;  
PCs, principal components;  
SNPs, single-nucleotide polymorphisms.

## Abstract

Inter-individual differences in DNA repair systems may play a role in modulating the individual risk of developing colorectal cancer.

To better ascertain the role of DNA repair gene polymorphisms on colon and rectal cancer risk individually, we evaluated 15,419 single nucleotide polymorphisms (SNPs) within 185 DNA repair genes using GWAS data from the Colon Cancer Family Registry (CCFR) and the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), which included 8,178 colon cancer, 2,936 rectum cancer cases and 14,659 controls.

Rs1800734 (in *MLH1* gene) was associated with colon cancer risk (p-value= $3.5 \times 10^{-6}$ ) and rs2189517 (in *RAD51B*) with rectal cancer risk (p-value= $5.7 \times 10^{-6}$ ). The results had statistical significance close to the Bonferroni corrected p-value of  $5.8 \times 10^{-6}$ . Ninety-four SNPs were significantly associated with colorectal cancer risk after Binomial Sequential Goodness of Fit (BSGoF) procedure and confirmed the relevance of DNA mismatch repair (MMR) and homologous recombination pathways for colon and rectum cancer, respectively.

Defects in MMR genes are known to be crucial for familial form of colorectal cancer but our findings suggest that specific genetic variations in *MLH1* are important also in the individual predisposition to sporadic colon cancer. Other SNPs associated with the risk of colon cancer (e.g. rs16906252 in *MGMT*) were found to affect mRNA expression levels in colon transverse and therefore working as possible cis-eQTL suggesting possible mechanisms of carcinogenesis.

## Novelty & Impact Statements

In a large cohort of colorectal cancer cases we found that *RAD51* rs2189517 was associated with rectal cancer risk while *MLH1* rs1800734 was confirmed to be associated with colon cancer risk. These results suggest that genetic variations within DNA repair genes significantly affect the risk of colon and rectal carcinoma independently with a significant impact not only, as known, for the familial forms but also for the sporadic ones.

## Keywords

Colon cancer, rectal cancer, DNA repair, single nucleotide polymorphisms, cancer susceptibility, genome-wide association studies.

## Introduction

Cancer is the consequence of the complex interactions between genetic susceptibility and environmental factors. Among the genes playing a role in cancer susceptibility, DNA repair genes are important candidates since cancer is associated with inherited deficiencies of DNA repair<sup>1</sup>. Defects in DNA repair cause genetic instability leading to increased rates of somatic mutations, providing the biological bases of this phenomenon<sup>2</sup>. Concerning the gastro-intestinal tract, the Lynch syndrome, which is most commonly clinically manifested as hereditary nonpolyposis colorectal cancer (HNPCC), is one of the most characterized inherited forms bound to defects in the DNA mismatch repair (MMR) pathway and it accounts for about 1-5% of all colorectal cancer cases<sup>3</sup>. According to a multistep model of carcinogenesis<sup>4</sup>, unrepaired mismatched bases (e.g. arising during DNA replication) cause a progressive accumulation of somatic mutations, predisposing replicating tissues with high turnover (such as the colon epithelium) to the malignant transformation<sup>5</sup>. The role of surveillance operated by MMR seems pivotal for colonocytes, as deficiencies within this pathway are observed also at somatic level in 7-10% of the sporadic forms conferring the so-called “microsatellite instability” (MSI) phenotype<sup>3,6</sup>.

On the basis of the observations in HNPCC families, it has been hypothesized that moderate inter-individual differences in the activity of DNA repair systems could also play a role in modulating the individual risk to develop sporadic form of colorectal cancer in the general population<sup>7-9</sup>. Thus, various hypothesis-driven case-control studies have been carried out to evaluate the association between the risk of sporadic colorectal cancer and polymorphisms within candidate genes such as *OGG1*, *APEX*, *POLB*, *XRCC1*, and *MUTYH* (base excision repair, BER), *ERCC1*, *ERCC2*, *XPC*, and *ERCC5* (nucleotide excision repair, NER), *XRCC2* and *XRCC3* (double-strand breaks repair, DSB), and Poly(ADP-ribose) polymerase (*PARP*)<sup>9,10</sup>. Positive associations were described for single-nucleotide polymorphisms (SNPs) within *APEX*, *ERCC1*, *MUTYH*, *OGG1*, *XPC*, *XPG*, *XRCC1*, and *XRCC3* genes<sup>11-16</sup>, but some results were either discordant or not replicated<sup>9,11,16-19</sup> likely as the consequence of a limited statistical power. Genome-wide association studies (GWASs) could not confirm most of the positive associations within the DNA repair genes previously described<sup>20-23</sup>. Similarly, GWASs carried out on other types of cancer detected only few DNA repair SNPs (see the GWAS catalog <https://www.ebi.ac.uk/gwas/home>) such as in breast cancer the rs999737 near *RAD51L1*, likely affecting the DSB DNA repair<sup>24</sup>. Most probably, the low number of disease-associated DNA repair SNPs in GWAS could be due to the very small effect of each SNP or to moderately penetrant, rare, and population-

specific alleles having various extents of linkage disequilibrium (LD) with the polymorphisms typically analyzed using commercial microarrays. Moreover, the effect of each SNP could be diluted in typical GWAS of overall colorectal cancer cases including tumors with different tumor molecular pathologies, as each risk allele is conceived to differentially influence specific carcinogenic mechanisms<sup>25</sup>. However, a previous study with adequate statistical power showed that the set of DNA repair SNPs, as a whole, could be associated with colorectal cancer risk<sup>20</sup>. Meta-analyses suggested also positive associations for rs1052133 and rs861539, respectively within *hOGG1*<sup>26</sup> and *XRCC3*<sup>27</sup> genes. These associations were observed only in specific ethnic groups, indirectly confirming the hypothesis of the moderately penetrant population-specific alleles<sup>28</sup>. In summary, further investigations are needed, in particular in large populations. In order to overcome the limitations imposed by the statistical power and in the attempt to draw more robust conclusions, we evaluated available SNPs within the full set of DNA repair genes in a large number of cases and controls combining data from two consortia: the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), and the Colon Cancer Family Registry (CCFR)<sup>29</sup>. We hypothesize that specific DNA repair pathways could be relevant in better describe risk association for colorectal cancer with particular care for cancer site subtypes. The large sample size allowed in fact to better investigating the role of DNA repair genes by stratifying for colon and rectal cancer separately.

## Material and methods

### ***Study population and genotyping***

We included 14 studies from the CCFR and GECCO consortia as described previously and in the **Supplementary Material (Text S1) and Table 1**<sup>29-31</sup>. All colorectal cancer cases were defined as colon or rectal adenocarcinoma and confirmed by medical records, pathologic reports, cancer registries, or death certificates. All analyses were restricted to individuals of European ancestry.

Methods of array-based genotyping, quality assurance/quality control and imputation, average sample and SNP call rates, and concordance rates for blinded duplicates have been previously published<sup>32</sup>. In brief, for quality insurance SNPs were excluded based on call rate (<98%), lack of Hardy-Weinberg Equilibrium (HWE) among controls (setting a threshold of  $p < 10^{-4}$ ), and low minor allele frequency (MAF) <0.05. We imputed the autosomal SNPs of all studies to the Northern Europeans from Utah (CEU population) in HapMap II. List of SNPs was restricted based on per-study minor allele count > 5 and imputation accuracy ( $r^2 > 0.3$ ). After imputation and quality-control exclusion, approximately 2.7M SNPs were available as complete genotype dataset. Imputations were done using the Haplotype Reference Consortium (HRC) r1.0 reference panel and Michigan Imputation Server, with phasing option set to ShapeIT v2.r790<sup>33-35</sup>.

### ***Selection of candidate genes and SNPs***

To evaluate the association between polymorphic DNA repair genes and risk of colon and rectal cancer, we initially selected genes involved in many aspects of DNA repair processing as listed in: <https://www.mdanderson.org/documents/Labs/Wood-Laboratory/human-dna-repair-genes.html>. A total of 185 genes (**Supplementary Table 1; Figure 1**) were retrieved and for each of them all known SNPs reported for the gene region (including 5' and 3' near regions, as classified and reported in dbSNP) were evaluated. As one example, see *MLH1* at URL:

[https://www.ncbi.nlm.nih.gov/projects/SNP/snp\\_ref.cgi?showRare=on&chooseRs=all&go=Go&locusId=4292](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?showRare=on&chooseRs=all&go=Go&locusId=4292)

The complete list of 15,419 SNPs is reported in **Supplementary Table 1**.

### ***In silico analyses***

In order to evaluate possible biological effects of specific SNPs, computational predictions were performed with the use of bioinformatics tools (**Figure 1**). First, we analyzed the presence of blocks of LD ( $r^2 > 0.8$ ) by using “LD TAG SNP selection” available at



<http://archive.broadinstitute.org/mpg/snap/ldsearch.php> and Haploreg V4.1 (<http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>). The latter is based on the ENCODE database and provides information for the analysis of the non-coding genome. Candidate regulatory SNPs were displayed together with their associated chromatin status and with the annotation of their protein binding sites (from the Roadmap Epigenomics and ENCODE projects). The information was also completed with the estimates of sequence conservation between mammals and the effects on the regulatory motifs and gene expression (from expression quantitative trait loci, eQTL, studies). Finally, for each SNP we examined gene expression levels as quantitative trait loci (cis-eQTLs), as available in GTEx Portal (<https://www.gtexportal.org>) for intestinal tissues (i.e. transverse, n=246 and sigmoid, n=203).

### ***Statistical analysis***

The association between SNPs and colon or rectal cancer risk was estimated using multiple logistic regression model with log-additive genetic effect. The model was adjusted for sex, age, genotype phase, batch effect, and principal components (PCs) for ancestry. The adjustment for multiple testing was initially approached by employing the Bonferroni's correction considering that, because of the presence of LD, about 4,300 independent haplotype-tagging SNPs (using a LD threshold with an  $r^2 \geq 0.8$ ) could recapitulate the whole genetic variability contained in the full set of SNPs. The novel threshold of statistical significance was, then,  $5.8 \times 10^{-6}$  (considering 2 sets of statistical tests, one for colon and one for rectum)

Moreover, as an alternative hypothesis-generating approach, we also tested the Binomial Sequential Goodness of Fit (BSGoF) method for multiple test adjustment. BSGoF (described in <sup>36</sup>) provides a good balance between false discovery rate (FDR) and power, particularly when the number of tests is large and the effect level is weak to moderate. We applied the BSGoF function to the total number of SNPs included in the study (n=15,419) to the p-value for SNP effect data ( $\alpha=0.05$ ,  $\gamma=0.05$ ).

### ***Ethics statement***

All participants provided informed consent and studies were approved by their respective Institutional Review Boards. The overall project was reviewed and approved by the Fred Hutchinson Cancer Research Center Institutional Review Board (approval number: 1177).

Each study was approved by the local IRB [University of Hawaii Human Studies Program (Colo23, Hawaii CCFR, and MEC); University of Utah Institutional Review Board (DALs); Partners Human Research Committee (NHS and PHS); Harvard School of Public Health Institutional Review Board (HPFS); Fred Hutchinson Cancer Research Center Institutional Review Board (PMH-CCFR, Seattle CCFR, VITAL, overall study); CSMC Institutional Review Boards (Cedars-Sinai CCFR); Cleveland Clinic Institutional Review Board (Cleveland Clinic CCFR); Mayo Clinic Institutional Review Board (Mayo Clinic CCFR); Mount Sinai Hospital Research Ethics Board (Ontario CCFR (OFFCR)); University of Melbourne Health Sciences Human Ethics Sub-Committee (Australasia CCFR); Ethics Committee of the Medical Faculty of the University of Heidelberg (DKFZ); NCI Special Studies Institutional Review Board (PLCO)]. For each participating study, participants or the next of kin in the case of deceased volunteers, provided either written informed consent to participate (the following CCFR sites: Australasia, Cedars-Sinai, Cleveland Clinic, Hawaii, Mayo Clinic and Ontario CCFRs), Colo23, DACHS, DALs, MEC, PHS, PLCO, VITAL, WHI) or they provided implied written consent by the return of the mailed questionnaires (NHS, HPFS) or the completion of telephone questionnaires (Seattle CCFR, PMH-CCFR). Additional consent to review medical records was obtained through signed written consent.

## Results

In this work, we included 14 studies from CCFR and GECCO consortia as described in the **Supplementary Material (Text S1) and Table 1** and elsewhere<sup>29-31</sup>. Overall, 15,419 SNPs within the 185 DNA repair genes were tested for different genotype distributions between 14,659 controls, 8,178 colon and 2,936 rectum cancer cases. The complete set of results is reported in **Supplementary Table 2**, whereas extracts concerning the htSNPs with the lowest p-values of association are showed grouped by gene in **Tables 2 and 3** for colon and rectum, respectively.

The SNP rs1800734 in *MLH1* was significantly associated with the risk of colon cancer after Bonferroni's adjustment (OR=1.13, 95%CI= 1.07-1.18,  $p=3.5 \times 10^{-6}$ ; Table 2). Other two htSNPs within *MLH1*, i.e. rs6784088 (OR=0.94, 95%CI= 0.90-0.98,  $p= 3.3 \times 10^{-3}$ ) and rs9855475 (OR=0.94, 95%CI= 0.90-0.98,  $p=3.4 \times 10^{-3}$ ), were associated with the risk of colon cancer when BSGoF was applied (**Table 2**). These two latter were mildly in LD each other ( $r^2=0.71$ ), whereas the strongest signal rs1800734 had a weak LD with them ( $r^2 = 0.33$  and  $= 0.30$ , respectively). This SNP showed also an association with the risk of colorectal cancer, although at a lesser extent (OR=1.09; 95%CI= 1.04-1.14;  $p=5.6 \times 10^{-5}$ ).

Concerning rectal cancer, the strongest signal was found for rs2189517 within *RAD51B* (OR=1.15, 95% CI=1.08-1.22,  $p\text{-value}=5.7 \times 10^{-6}$ ), a gene involved in Homologous recombination repair (HR), statistically significant also following the Bonferroni's correction (**Table 3**). Interestingly, other 14 htSNPs were found associated at a lesser extent with the risk of rectal cancer, being statistically significant only when BSGoF was applied. The list of these SNPs includes rs12587232, rs187645011, rs7350713, rs6573841, rs111611396, rs1989974, rs117544253, rs77726787, rs8016488, rs11628293, rs113020754, rs80085210, rs113300322, and rs74933543. The significance levels ranged from  $4.37 \times 10^{-5}$  to  $1.6 \times 10^{-3}$  with the highest risk (OR=0.71 corresponding to 1.41 for the common versus the rare allele) for rs187645011. Rs2189517 was not in LD with the others ( $r^2 < 0.3$ ) with the exception of rs12587232, having  $r^2$  of 0.77. The 14 htSNPs were not in LD each other as well (max  $r^2 < 0.6$ ). Rs2189517 was associated also with colorectal cancer risk (OR= 1.05, 95%CI= 1.02-1.09,  $p=1.7 \times 10^{-3}$ ) although statistically significant only following BSGoF correction.

When more exploratory and hypothesis-generating analyses were performed by considering statistically significant SNPs following BSGoF adjustment, several genes had multiple htSNPs associated with the risk of colon carcinoma, such as *ATM* (rs11212592, rs61915066), *FANCA* (rs2238526, rs3743860), *FANCE* (rs6907678, rs10947550), and *LIG1*

(rs1971775, rs73054038). Because htSNPs are mostly independent each other, the presence of multiple signals provides a more robust indication for the role of the gene in the susceptibility to the disease. Other genes, such as *EXO1*, *FEN1*, *PMS2*, *RBBP8*, and *TP53BP1*, had only one positive htSNPs (Table 2). For rectal carcinoma, multiple hits were found within *BLM* (rs2518967, rs35787687), *PMS1* (rs1233258, rs1233262) and *RAD51B* (14 hits). Single hits were found for *ATM* and *DCLRE1C* (Table 3).

Bonferroni's-positive htSNPs were also evaluated as potential cis-eQTL by investigating *in silico* their association with the gene expression using GTex portal. Rs1800734 was associated with *MLH1* expression in colon transverse but the statistical significance did not reach the genome-wide level ( $p=9.9 \times 10^{-4}$ , normalized effect size, NES, of 0.12). On the other hand, rs2189517 lacked completely any association with the expression of *RAD51B* in colonic tissues as well as in all other tissues available in GTex portal. To further investigate the role of these SNPs as eQTL or any functional annotation we have searched other databases (<http://www.exsnp.org/>; <http://www.scandb.org/newinterface/about.html>; and <http://bioinfo.life.hust.edu.cn/PancanQTL/> ). However, no additional information were retrieved since data on these SNPs are largely missing.

## Discussion

In the present study, we comprehensively analyzed variations in 185 DNA repair genes in over 27,000 individuals<sup>29</sup> to ascertain their implication for colon and rectal cancer risk. Two SNPs in MMR and HR pathways (i.e. rs1800734 in *MLH1* and rs2189517 in *RAD51B*) were associated in a statistically significant way with increased risk for cancer in colon and rectum, respectively.

Differences in the activity of DNA repair systems could play a role in modulating individual cancer risk according to tumor location in the gut<sup>7-9</sup>. Mutations within *MLH1* (MMR) predispose to HNPCC type-2<sup>37</sup>. Somatic mutations as well as hyper-methylation of the gene promoter were frequently observed also in sporadic colorectal cancer tissues associated with a MSI phenotype<sup>38</sup>. Rs1800734 encodes for a G to A transition at -93 from the transcription start site within the promoter region and it falls within NF-IL6 and GT-IIB transcription factor binding sites. The polymorphism has been associated with promoter methylation and gene silencing<sup>39,40</sup> and a meta-analysis by Wang and colleagues<sup>41</sup> reported that carriers of the A-allele are at increased risk of colorectal cancer, in agreement with the present results. The association was even stronger among cases positive for MSI. However, according to another recent meta-analysis results were not conclusive<sup>42</sup>. Our results, carried out on a very large series of patients, suggest that rs1800734 plays a role particularly in colon, perhaps causing a decreased activity of the MMR in this tissue<sup>39,43</sup>. This hypothesis is corroborated by the data from GTEx reporting that rs1800734 could act as a cis-eQTL by affecting mRNA expression levels in colon transverse ( $p=9.9 \times 10^{-4}$  with NES of 0.12). Discrepancies among past studies could be ascribed to statistical limitations or to differences in the composition of colon/rectal cancer patients and to variable proportions of patients with MSI phenotypes.

Concerning the second positive association (i.e. rs2189517), it is important to observe that *RAD51B* is an important gene within the HR pathway. Interestingly, previous studies reported that various *RAD51B* SNPs in LD with those reported in our study were associated with the susceptibility to prostate and breast cancer<sup>44</sup>. Finally, rs2189517 has been recently related to the risk of prostate cancer in a GWAS<sup>45</sup>. Furthermore, it should be stressed that other SNPs within the last intron of *RAD51B*, and not in LD with those presented here, were involved in the susceptibility to breast cancer in males<sup>46</sup>, and females<sup>47</sup>. Germline mutations within *RAD51B* were also found to confer predisposition to familial breast and ovarian cancer<sup>48</sup>, and cutaneous melanoma<sup>49</sup>.

Subsequently, we also investigated the potential, although minor, involvement of other SNPs in DNA repair by a hypothesis-generating approach, which means a less conservative adjustment for multiple testing as applies for Bonferroni adjustment. Various htSNPs resulted significantly associated after BSGoF correction for multiple testing and confirmed and provided further evidence for our hypothesis of the relevance of DNA MMR and HR pathways for colon and rectal subtypes, respectively. In fact, MMR showed more signals such as rs12112229 in *PMS2*, rs4658549 in *EXO1*, and rs72812338 in *MSH2* associated with increased colon cancer risk. It should be also noted that *PMS2* forms heterodimers with *MLH1* to generate MutL-alpha complex. This last, together with MutS heterodimers, is pivotal for MMR to correct small insertion-deletion mispairing formed during DNA replication or recombination. Interestingly, *MSH2* and *EXO1* are also physically interacting each other for MMR activity<sup>50</sup>. Additionally, together with the *RAD51B* SNPs found in the present study, other htSNPs within the same gene resulted associated with risk of rectal cancer being the association of rs2189517 with the lowest P-value while that of rs187645011 with the highest OR. In summary, according to all these observations, *RAD51B* and HR appear as pivotal in the individual susceptibility to various types of tumors, including rectal carcinoma.

## **Conclusions**

All findings hereby presented suggest the importance of genetic variations within MMR genes (in particular those that could physically interact with each other for intact MMR activity) in the predisposition to non-inherited forms of colon cancer. In contrast, for rectal carcinoma, the strongest associations were observed for a SNP within *RAD51B*, a gene involved in HR. Thus, our results show that genetic variations within DNA repair genes, in particular MMR and HR, significantly affect the risk of colon and rectal carcinoma independently with a significant impact not only, as known, for the familial forms but also for the sporadic ones.

**Table 1** Description of study populations included in the Colon Cancer Family Registry (CCFR) and the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO).

Study*	Total**	Sex		Controls N=14662	Cases N=11898	Cancer site	
		Females	Males			Colon (proximal / distal)	Rectum
ASTERISK	1839	763	1076	947	892	622 (249 / 373)	260
CCFR Set 1	2016	1010	1006	978	1038	700 (317 / 375)	448
CCFR Set 2	717	389	328	386	331	237 (97 / 127)	135
Colo 2&3	211	94	117	124	87	59 (35 / 24)	27
DACHS Set 1	3409	1393	2016	1702	1707	1037 (548 / 487)	668
DACHS Set 2	1164	435	729	498	666	385 (210 / 175)	281
DALS Set 1	1411	612	799	709	702	702 (329 / 358)	0
DALS Set 2	863	410	453	461	402	410 (209 / 185)	0
HPFS Set 1	456	0	456	229	227	158 (82 / 76)	48
HPFS Set 2	348	0	348	172	176	111 (54 / 57)	40
HPFS_AD	656	0	656	343	313	n/a	n/a
MEC	672	311	361	346	326	241 (155 / 86)	81
NHS Set 1	1165	1165	0	774	391	305 (175 / 123)	82
NHS Set 2	339	339	0	181	158	112 (67 / 44)	35
NHS_AD	1090	1090	0	577	513	n/a	n/a
OFCCR	1116	579	537	522	594	396 (204 / 164)	188
PHS	764	0	764	389	375	286 (122 / 121)	84
PLCO Set 1	2496	664	1832	1972	524	516 (323 / 193)	5
PLCO Set 2	889	379	510	414	475	320 (213 / 102)	161
PMH-CCFR	398	398	0	122	276	206 (132 / 72)	64
VITAL	566	267	299	287	279	215 (143 / 69)	66
WHI Set 1	1991	1991	0	1523	468	456 (308 / 147)	14
WHI Set 2	1984	1984	0	1006	978	704 (482 / 222)	249

Numbers may not add up to 100% of available subjects because of missing information; n/a information not available

\*For the complete list and description of the studies, see Supplementary materials. ASTERISK, Colo2&3, DALS Set 2, DACHS Set 1, PMH-CCFR, MEC, PLCO Set 2, WHI Set 2 and VITAL were genotyped on the Illumina CytoSNP BeadChip. WHI Set 1 was genotyped using Illumina 550K, 550K duo, and 610K platforms (only 550K and 550K duo if not utilizing hip fracture controls). PLCO Set 1 was genotyped using Illumina 550K and 610K platforms (also the 550K Duo platform if using the PLCO rematch set). DALS Set 1 was genotyped using Illumina 610K and 550K platforms. OFCCR was genotyped using Affymetrix GeneChip Human mapping 100K and 500K Array Set and a 10K non-synonymous SNP chip. CCFR was genotyped using Illumina Human1M and Human1M-Duo platforms. DACHS Set 2, HPFS, NHS, and PHS were genotyped on the OmniExpress platform. \*\* Sample sizes based on GECCO GIGSv3/HRCv1 data.

**Table 2** htSNPs with the lowest p-values for the association with risk of colon cancer, grouped by gene

Colon (proximal+distal)				
ht SNP ID	OR (95% CI)	p-value for SNP effect	BSGoF-Adjusted p-value	
<b>ATM</b> <sup>(DSBR)</sup>				
rs11212592	0.92 (0.87-0.97)	3.30X10 <sup>-03</sup>	0.021	
rs61915066	1.13 (1.04-1.24)	4.81X10 <sup>-03</sup>	0.021	
<b>EXO1</b> <sup>(MMR, EPN)</sup>				
rs4658549	1.06 (1.02-1.11)	5.33X10 <sup>-03</sup>	0.021	
<b>FANCA</b> <sup>(DCLR)</sup>				
rs2238526	0.94 (0.91-0.98)	3.98X10 <sup>-03</sup>	0.021	
rs3743861	0.94 (0.91-0.98)	6.13X10 <sup>-03</sup>	0.021	
<b>FANCE</b> <sup>(DCLR)</sup>				
rs6907678	0.94 (0.90-0.98)	1.79X10 <sup>-03</sup>	0.020	
rs10947550	0.94 (0.90-0.98)	2.32X10 <sup>-03</sup>	0.020	
<b>FEN1</b> <sup>(BER, EPN)</sup>				
rs4246215	0.93 (0.89-0.97)	1.59X10 <sup>-03</sup>	0.020	
<b>LIG1</b> <sup>(NER, BER)</sup>				
rs1971775	1.06 (1.02-1.11)	4.38X10 <sup>-03</sup>	0.021	
rs73054038	0.92 (0.87-0.98)	5.32X10 <sup>-03</sup>	0.021	
<b>MLH1</b> <sup>(MMR)</sup>				
rs1800734	1.13 (1.07-1.18)	<b>§3.48X10<sup>-06</sup></b>	0.019	
rs6784088	0.94 (0.90-0.98)	3.28X10 <sup>-03</sup>	0.020	
rs9855475	0.94 (0.90-0.98)	3.42X10 <sup>-03</sup>	0.021	
<b>PMS2</b> <sup>(MMR)</sup>				
rs12112229	1.07 (1.02-1.13)	3.15X10 <sup>-03</sup>	0.020	
<b>RBBP8</b> <sup>(HR)</sup>				
rs113047993	1.15 (1.07-1.25)	2.36X10 <sup>-04</sup>	0.019	
<b>TP53BP1</b> <sup>(NHEJ)</sup>				
rs17782975	0.88 (0.82-0.96)	1.91X10 <sup>-03</sup>	0.020	

§ statistically significant after Bonferroni's correction



**Table 3** htSNPs with the lowest p-values for the association with risk of rectal cancer, grouped by gene

Rectum				
	ht SNP ID	OR(95% CI)	p-value for SNP effect	BSGoF-Adjusted p-value
<b><i>ATM</i></b> ( <i>DSBR</i> )				
	rs11212592	0.87 (0.80-0.94)	1.67X10 <sup>-03</sup>	2.86X10 <sup>-03</sup>
<b><i>BLM</i></b> ( <i>DSBR</i> )				
	rs2518967	1.14 (1.06-1.24)	5.97X10 <sup>-04</sup>	1.35X10 <sup>-03</sup>
	rs35787687	1.14 (1.05-1.23)	1.07X10 <sup>-04</sup>	2.32X10 <sup>-03</sup>
<b><i>DCLRE1C</i></b> ( <i>NHEJ</i> )				
	rs7920514	1.12 (1.04-1.21)	1.37X10 <sup>-03</sup>	2.50X10 <sup>-03</sup>
<b><i>PMS1</i></b> ( <i>MMR</i> )				
	rs1233258	0.87 (0.80-0.93)	2.37X10 <sup>-04</sup>	7.22X10 <sup>-04</sup>
	rs1233262	0.89 (0.83-0.95)	1.21X10 <sup>-04</sup>	2.32X10 <sup>-03</sup>
<b><i>RAD51B</i></b> ( <i>HR</i> )				
	rs2189517	1.15 (1.08-1.22)	§5.73X10 <sup>-06</sup>	1.24X10 <sup>-05</sup>
	rs12587232	1.13 (1.06-1.20)	4.37X10 <sup>-05</sup>	6.56X10 <sup>-04</sup>
	rs187645011	0.71 (0.61-0.84)	7.53X10 <sup>-05</sup>	6.90X10 <sup>-04</sup>
	rs7350713	0.74 (0.63-0.87)	2.47X10 <sup>-04</sup>	7.44X10 <sup>-04</sup>
	rs6573841	0.86 (0.80-0.93)	3.43X10 <sup>-04</sup>	7.59X10 <sup>-04</sup>
	rs111611396	0.74 (0.63-0.87)	3.53X10 <sup>-04</sup>	7.66X10 <sup>-04</sup>
	rs1989974	0.76 (0.65-0.88)	4.20X10 <sup>-04</sup>	7.81X10 <sup>-04</sup>
	rs117544253	0.72 (0.60-0.86)	4.70X10 <sup>-04</sup>	8.69X10 <sup>-04</sup>
	rs77726787	0.74 (0.63-0.87)	4.76X10 <sup>-04</sup>	9.30X10 <sup>-04</sup>
	rs8016488	1.15 (1.06-1.25)	5.27X10 <sup>-04</sup>	1.31X10 <sup>-03</sup>
	rs11628293	1.12 (1.05-1.20)	6.28X10 <sup>-04</sup>	1.37X10 <sup>-03</sup>
	rs113020754	0.75 (0.64-0.88)	6.50X10 <sup>-04</sup>	1.54X10 <sup>-03</sup>
	rs80085210	0.74 (0.62-0.88)	9.30X10 <sup>-04</sup>	2.30X10 <sup>-03</sup>
	rs113300322	0.77 (0.66-0.90)	9.49X10 <sup>-04</sup>	2.30X10 <sup>-03</sup>
	rs74933543	1.11 (1.04-1.19)	1.59X10 <sup>-03</sup>	2.85X10 <sup>-03</sup>

§ statistically significant after Bonferroni's correction

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