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Single Nucleotide Polymorphisms within MicroRNAs, MicroRNA Targets, and MicroRNA Biogenesis Genes and Their Impact on Colorectal Cancer Survival

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Abstract

We have shown that single nucleotide polymorphisms (SNPs) in microRNA (miRNA) genes, miRNA target genes, and miRNA biogenesis genes minimally contribute to colon cancer risk. It is possible that these SNPs alter survival. We analyzed 565 SNPs in or adjacent to microRNAs, target genes, or biogenesis genes, using 1115 cases and 1173 controls; 837 cases had survival information. We tested SNPs for associations with colorectal cancer (CRC) survival using a Cox proportional hazard model adjusting for age, study center, gender, AJCC disease stage, and MSI tumor status. Multiple comparison adjustments were made using the step-down Bonferroni correction. SNPs associated with survival ($P_{raw} < 0.05$) also were assessed with messenger RNA (mRNA). Seven of the 565 SNPs analyzed were associated significantly with CRC survival after adjustment for multiple comparisons. Six of these increased risk of dying, and one, rs12140 (*ADAMTSL1*) decreased risk of dying from CRC (HRR=0.44, 95% CI (0.24, 0.83); $P_{Holm}=0.011$). Six SNPs altered colon cancer risk and five were associated with altered mRNA expression across genotypes. One SNP, rs2059691 (*PRKRA*), was associated with increased mRNA expression and worse survival, and one SNP, rs6598964 (*LIN28A*), decreased risk of developing colon cancer (OR=0.77 95% CI (0.61, 0.98)) and increased risk of dying from CRC (HRR=2.26 95% CI (1.52, 3.36). $P_{Holm}=0.003$). The few SNPs associated with CRC survival, colon cancer risk, or with mRNA expression, resided in genes that influence metastasis and angiogenesis.

Keywords

MiRNA; SNP; survival; polymorphism; colorectal; cancer

INTRODUCTION

MiRNAs are endogenous, single-stranded RNA molecules, approximately 22 nucleotides long that bind to mRNA to repress mRNA translation into protein, or degrade the mRNA

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Competing Interests

The authors have no competing interests to disclose.

Accession Numbers

GWAS data are available in dbGaP under the accession number phs001056.v1.p1.

molecule (Ambros, 2004; Macfarlane and Murphy, 2010; Murray et al., 2010; Arora et al., 2013). It is generally thought that miRNAs regulate mRNAs via the seed region—nucleotides 2–6 on the 5' end of the miRNA—binding the 3' UTR of the mRNA (Barrett et al., 2012). As a single miRNA is able to bind to multiple mRNAs, which in turn participate in a variety of biological responses, miRNAs have been implicated as key effector molecules in cancer development and progression (Baskerville and Bartel, 2005; Lagana et al., 2009; Macfarlane and Murphy, 2010).

MiRNAs undergo three major processing steps to generate the mature miRNA, and this process involves, directly and indirectly, dozens of proteins (Winter et al., 2009; Krol et al., 2010; Ha and Kim, 2014). Additionally, Single Nucleotide Polymorphisms (SNPs) within miRNA gene regions may alter miRNA transcription and SNPs within miRNA-targets may influence binding of the miRNA to a target, leaving the miRNA unbound within the cytoplasm and vulnerable to degrading nucleases (Krol et al., 2010). SNPs within miRNAs, miRNA-biogenesis genes, or in the 3' UTR of mRNA targets may alter mature miRNA levels by altering miRNA transcription, mRNA translation, or binding to miRNA target genes (Saunders et al., 2007); as such, it has been previously proposed that SNPs in these regions that have been found associated with colon cancer might be operating through altered levels of miRNA expression.

We previously investigated SNPs within miRNAs, within miRNA target genes, and within genes participating in miRNA biogenesis for associations with altered risk of developing colon cancer and we determined that few of these SNPs impacted colon cancer risk (Mullany et al., 2015). However others have shown that SNPs within miRNAs, while not altering risk of developing cancer, are associated with prognosis (Ryan et al., 2012). In this paper we investigate whether SNPs previously investigated with colon cancer risk have any impact survival after diagnosis with colon cancer. We hypothesize that these SNPs will be associated with CRC survival, even if they have not been previously associated with cancer risk.

MATERIALS AND METHODS

Study Population

The study population consisted of individuals previously enrolled in a study of Diet, Activity, and Lifestyle Study of colon cancer (Slattery et al., 1997) for whom Genome Wide Association Study (GWAS) data were available (Table 1). Study subjects included incident cases of colon cancer between the ages of 30 and 79 who were able to provide a signed informed consent prior to participation in the study. GWAS analysis included only non-Hispanic white individuals. AJCC tumor stage was centrally coded for cancers identified through local tumor registries. The study was approved by the University of Utah Institutional Review Board for Human Subjects; the committee numbers for this paper are IRB_00055877 and IRB_00002335.

GWAS Genotyping

GWAS data were obtained using Illumina HumanHap 550K, 610K as part of the GECCO study and has been described previously (Dunlop et al., 2012). Imputation to HapMap2 Release 24 was performed using MACH, which was imputed to HapMap Release 22 using BEAGLE. All SNP coordinates were defined using hg19/GRCh37. We excluded SNPs from consideration that failed Illumina quality measures or standard quality control procedures (Laurie et al., 2010). We further excluded SNPs that showed limited variability in our data (see statistical methods below).

Selection of SNPs

SNPs were previously identified from the literature as being associated with cancer risk and being either within a miRNA gene, a miRNA target gene, or a gene participating in miRNA biogenesis. UCSC (Karolchik et al., 2004) Table Browser was utilized to obtain all SNP coordinates; all coordinates are from the GRCh37 assembly. More detail on how these SNPs were obtained is available in our previous works (Mullany et al., 2015; Mullany et al., 2016).

RNA Processing, RNA-Seq Library Preparation and Sequencing and Data Processing

MRNA expression data were analyzed from 197 carcinoma and normal mucosa pairs. Total RNA was extracted from formalin-fixed paraffin embedded tissues and processed as previously described (Slattery et al., 2015a). Sequencing library construction was done with the Illumina TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero. The samples were then fragmented and primed for complementary DNA (cDNA) synthesis, adapters were then ligated onto the cDNA, and the resulting samples were then amplified using polymerase chain reaction (PCR); the amplified library was then purified using Agencount AMPure XP beads. A more detailed description of the methods can be found in our previous work (Slattery et al., 2015b). Of these, 175 passed QC based on acceptable number of sequence reads for both carcinoma tissue and normal mucosa. Of these, 71 subjects also had GWAS data available for comparison with mRNA expression data in carcinoma tissue, 67 for normal colonic mucosa, and 61 for the difference between normal colonic mucosa and carcinoma tissue.

Sequencing was done using an Illumina TruSeq v3 single read flow cell and a 50 cycle single-read sequence run was performed on an Illumina HiSeq instrument. Reads were then aligned to a sequence database containing the human genome (build GRCh37/hg19, February 2009 from genome.ucsc.edu) and alignment was performed using novoalign v2.08.01. Python and a pysam library were used to calculate counts for each exon and UTR of the genes using a list of gene coordinates obtained from <http://genome.ucsc.edu>. We dropped features that were not expressed in our data or for which the expression was missing for the majority of samples. A more detailed description of the methods can be found in our previous work (Slattery et al., 2015b).

Statistical Analysis

There were 448 unique SNPs from the 871 miRNA-SNP candidate pairs obtained from literature search for SNPs within miRNAs and miRNA target genes. After eliminating miRNAs whose nomenclature did not match those of miRNAs on our platform, there are

642 miRNA-SNP candidate pairs and 363 unique SNPs. An additional 218 SNPs were identified from the literature as residing in miRNA biogenesis genes, yielding a total of 581 SNPs selected for further assessment. Of those 581 SNPs selected, 565 were available in the GWAS dataset.

The GWAS dataset consisted of 2288 subjects (1115 cases and 1173 controls). There are 837 cases with survival data along with other adjustment variables such as AJCC stage and MSI status. We used a Cox proportional hazards model, implemented in SAS, to evaluate the SNPs associations with CRC survival adjusting for age at diagnosis, study center, AJCC stage, and gender and MSI status. CRC-specific survival was calculated from the date of diagnosis until death or the date of the last follow-up. An additive model was used for all SNPs except when the homozygote rare genotype had fewer than six people, at which point a dominant model was used. In some instances, where the recessive model appeared to be more appropriate we re-ran the analysis using the recessive model. We adjusted for multiple comparisons using the step-down Bonferroni correction (Holm) based on the effective number of independent SNPs per chromosomes, using spectral decomposition to determine the number of comparisons within each chromosome, as proposed by Nyholt (Nyholt, 2004) and modified by Li and Ji (Li and Ji, 2005).

For those SNPs that were significantly associated with survival prior to correction for multiple comparisons, we performed logistic regression analysis adjusting for age at diagnosis/selection, study center and gender to determine if there was any relationship between the significant targeted SNPs and colon cancer risk.

Twenty-nine of the 30 significant ($P_{\text{raw}} < 0.05$ for survival) SNPs had their respective gene in RNA-seq data. We used linear regression to determine if there was a trend in the mean value of the mRNA across the SNP levels adjusting for age, study center and gender. MRNA expression data were analyzed using the log2 of the Reads per Kilobase per Million (RPKM). We performed 10,000 bootstrap (Davison and Hinkley, 1997) re-samplings ('boot' package in R) of the residual of the null model to calculate a distribution of the F statistic and calculate the p-value. We looked at mean mRNA expression data for colonic carcinoma, normal colonic mucosa and the difference between carcinoma and normal colonic mucosa for the mRNA-SNP comparison.

Results

The study included 1173 controls and 1115 cases, and approximately 55% of the population were male and 45% were female; the average age at diagnosis/selection was approximately 65 years for both cases and controls (Table 1). The average follow-up months were 60.3.

Thirty SNPs were associated significantly with survival prior to adjustment for multiple comparisons and of these, seven SNPs in five genes (*ADAMTS1*, *ADAR*, *DROSHA*, *LIN28A* and *PRKRA*) remained significant after multiple comparison adjustment (Table 2). Six of these SNPs (rs2335230 in *ADAR*, rs10068052, rs13183642, and rs17408227 in *DROSHA*, rs6598964 in *LIN28A*, and rs2059691 in *PRKRA*) increased risk of dying from CRC, while rs12140 (*ADAMTS1*) decreased risk of dying from CRC (HRR=0.44, 95% CI (0.24, 0.83) $P_{\text{Holm}}=0.011$).

Of the 30 SNPs associated with survival prior to adjustment, four SNPs in three genes (*BRCA1*, *GDE1*, and *LIN28A*) were associated significantly with risk of developing colon cancer (Table 2). Of these, only rs6598964 (*LIN28A*) was significantly associated with survival after adjustment for multiple comparisons. Rs6598964 decreased risk of developing colon cancer (OR=0.77 95% CI (0.61, 0.98), however it increased risk of dying from CRC (HRR=2.26 95% CI (1.52, 3.36; $P_{\text{Holm}}=0.003$). Two SNPs in *BRCA1*, rs799917 and rs8176318, increased risk of developing colon cancer, while rs2521795 (*GDE1*) decreased risk of developing colon cancer.

Further evaluation of the 30 SNPs associated with CRC survival prior to adjustment for multiple comparisons showed that five of these SNPs were associated significantly with differential mRNA expression across genotypes (Table 3). One of these SNPs was associated with mRNA expression in colonic carcinoma tissue (*ASB6* rs1045647), two with normal colonic mucosa (*FADS2* rs174568 and *SF3A3* rs17465826), one with both carcinoma and normal colonic tissues (*PRKRA* rs2059691), and one with normal colonic mucosa and differential tissue expression (*HLA-DPBI* rs1042544). Of these five SNPs only one, (*PRKRA* rs2059691), was associated significantly with altered risk of dying from CRC after adjustment for multiple comparisons. This SNP, within Protein kinase, interferon-inducible double stranded RNA dependent activator (*PRKRA*) was not associated with risk of developing colon cancer.

DISCUSSION

The hypothesis that a SNP may not influence risk while still influencing survival is not without biological support. Lopez-Camarillo et al. (2012) wrote that certain genes, those participating in biological processes such as apoptosis resistance, migration, invasion, and angiogenesis induction, are more likely to cause metastasis than others; although these processes enable tumors to metastasize, they are not necessarily deleterious under normal cellular circumstances. We observed that, of the 565 SNPs analyzed, seven SNPs remained associated significantly with survival after adjustment for multiple comparisons ($P_{\text{holm}} < 0.05$) and four were associated significantly with risk of developing colon cancer. Only one SNP was associated significantly with survival after adjustment for multiple comparisons as well as with risk: rs6598964 (*LIN28A*), which decreased risk of developing colon cancer and increased risk of dying from CRC. Five SNPs were associated with mRNA expression in colonic carcinoma tissue, normal colonic mucosa, or differential between carcinoma tissue and normal mucosa; only one of these SNPs, rs2059691 (*PRKRA*), was also significantly associated survival ($P_{\text{holm}} = 0.042$ for survival).

Others also have found that SNPs in miRNAs, although not associated with risk, do influence survival. A study by Ryan et al. (2012) identified rs4919510, within hsa-miR-608, was not associated with risk of developing cancer but was associated with increased risk of dying from CRC in a Caucasian population. We analyzed this SNP with CRC survival in our population and found that it was not associated significantly with ($P_{\text{raw}} = 0.20$) when using a recessive model such as the one used by Ryan et al. (2012). We do report, however, that rs12140 (*ADAMTS1*), rs2335230 (*ADAR*), rs10068052 (*DROSHA*), rs13183642 (*DROSHA*), rs17408227 (*DROSHA*), and rs2059691 (*PRKRA*) had no impact on risk of

developing colon cancer but did influence survival. Additionally, rs6598964 (*LIN28A*) decreased risk of developing colon cancer but worsened survival.

We previously looked at SNPs within miRNAs, miRNA target genes, and genes participating in miRNA biogenesis. In our previous work we observed an association between rs8176318 (*BRCA1*) and colon cancer risk, OR_{AA} 1.34 95% CI 1.01, 1.78 (Mullany et al., 2015), and with rs2059691 (*PRKRA*) and increased mRNA expression in normal colonic mucosa (Mullany et al., 2016). We report here that *PRKRA* rs2059691 is also associated with survival, and that *PRKRA* mRNA expression increases with the variant allele (G < A) in colonic carcinoma and normal colonic mucosa. We also report here that rs8176318 (*BRCA1*) increases risk of developing colon cancer OR_{AA} 1.34 95% CI 1.01, 1.78, but does not affect survival.

Rs12140 (*ADAMTS1*) was included in this analysis because this gene was identified as a target for hsa-miR-140-3p in our previous work (Mullany et al., 2015). This SNP was not associated with risk of developing colon cancer but did reduce the risk of dying of CRC. The protein encoded by this gene has anti-angiogenic activity, may be involved in inflammatory processes, and is likely necessary for normal growth (O'Leary et al., 2016). While *ADAMTS1* was not differentially expressed by genotype, which implies that this SNP does not impact transcription of *ADAMTS1*, it is possible that rs12140, which is located in the 3' UTR of *ADAMTS1*, affects the binding of miRNA to this mRNA transcript and thereby reduces repression of this gene. As *ADAMTS1* has anti-angiogenic properties, it is possible that this gene would not impact colon cancer risk in a normal-functioning cell, however, once a tumor has formed it would reduce the tumor's ability to metastasize. Previously, we saw that hsa-miR-140-3p expression was reduced with the recessive genotype; as miRNA expression has been tied to the presence of its target (Sun et al., 2009; Kai and Pasquinelli, 2010), it is possible that this SNP causes *ADAMTS1* to not be targeted by hsa-miR-140-3p and therefore its anti-angiogenic effect is not diminished, improving a patient's chance of survival.

Only one SNP, rs6598964 (*LIN28A*), was associated with both risk of developing colon cancer and CRC survival. This SNP decreased risk of developing cancer but increased risk of dying from CRC. This SNP is an intronic variant, and we found no significant changes in expression of *LIN28A* in normal, carcinoma, or differential tissue, suggesting that this SNP does not impact transcription of this gene. This gene plays a role in miRNA biogenesis, recruiting ZCCHC11 to the miRNA for uridylation, inhibiting the processing of miRNAs, including the tumor suppressor let-7, by Dicer (Heo et al., 2009; Krol et al., 2010; Stefani et al., 2015). In other studies, overexpression of *LIN28* has been shown to contribute to tumorigenesis, by promoting angiogenesis and cell death resistance and increasing cell proliferation (Wang et al., 2015), and be associated with advanced malignancies (Hamano et al., 2012). It is intuitive that a gene regulating metastasis would negatively impact survival while not necessarily be detrimental under normal circumstances, thus not influencing risk. It is, however, somewhat perplexing that a SNP in a metastatic regulating gene would decrease risk of cancer while being detrimental to survival. In order to determine whether an outside factor was confounding our results, we re-ran the risk analysis adjusting for factors previously shown to influence risk of CRC, including age, sex, center, recent aspirin/NSAID

use, smoking status (current, former, or never), body mass index, and lifetime physical activity; this adjustment did not change the results. Zhang et al. (2014) report that rs221636, a SNP within the 3' UTR of *LIN28B*, a homolog of *LIN28A*, was associated with altered risk of oral cancer in a Chinese Han population, with decreased risk in subjects who did not drink with AT or TT genotypes compared to drinkers with the AA genotype. Zhang et al. (2014) suggested this finding was the result of a “synergistic effect” between drinking and the variant in *LIN28B*. We looked at long-term and current use of alcohol with rs6598964 and found that at lower levels of risk for moderate drinkers (GG/GA: OR 0.99 95% CI (0.80, 1.22); AA: OR 0.71 95% CI (0.47, 1.07)) and subjects who did not drink (AA: OR 0.64 95% CI (0.43, 0.93)) and had the AA genotype, with slightly higher non-significant risk of colon cancer for subjects who had high long-term and current alcohol use and the AA genotype (GG/GA: OR 1.00 95% CI (0.76, 1.32); AA: OR 1.29 95% CI (0.76, 2.17)). This suggests there may be a possible interaction between alcohol use and rs6598964 in regard to the development of colon cancer, although it was not statistically significant in our data. Lin28A and Lin28B transgenic animals have been shown to be more sensitive to insulin and have lower peripheral glucose levels (Thornton and Gregory, 2012); hyperinsulinemia and hyperglycemia have been linked to colon cancer development (Giovannucci, 2007). It is therefore possible that rs6598964 creates a more functional protein that is initially able to reduce glucose levels, potentially reducing risk of colon cancer, however once a tumor is formed, the angiogenic and metastatic properties of Lin28A prevail and we see worsened survival in subjects with the AA genotype.

The only SNP significantly associated with survival and mRNA expression was rs2059691 (*PRKRA*). This SNP increased risk of death from CRC (OR 1.87 95% CI 1.11, 3.13, $P_{\text{holm}}=0.042$) and increased mRNA expression with the homozygous rare genotype in both colonic carcinoma tissue (p-value=0.024) and normal colonic mucosa (p-value<0.001); mRNA expression was higher in colonic carcinoma tissue than in normal colonic mucosa. *PRKRA*, as a protein kinase, belongs to a group of genes that are considered metastatic initiation genes (Lopez-Camarillo et al., 2012). As rs2059691 (*PRKRA*) increased risk of death from CRC and was not associated with risk of developing colon cancer, it is likely that this SNP affects tumor progression and not initiation.

We used mRNA expression from RNA-Seq data in this analysis; it is possible that the SNPs we investigated in this study may not impact mRNA transcription, but do impact translation of the mRNA into protein or the tertiary structure of the protein product. If this were the case, the normal function of the protein would be altered and could be responsible for the altered risk of CRC or impact on survival; however, we would not detect this effect. Additionally, as we restrict our study sample to those samples for which we had mRNA expression data, it is possible that our reduced sample size limited our ability to detect weaker associations between SNPs and survival. We consider our inclusion of paired mRNA expression data an asset to the investigation and the field, and we encourage larger studies to replicate our findings.

We investigated whether SNPs previously looked at with colon cancer risk impact CRC survival. Few SNPs we analyzed were associated with either risk of colon cancer or survival, and only one was associated with both. The genes that negatively impact CRC survival, but

have a positive influence on risk or do not affect risk of colon cancer, appear to have a large role in biological processes that are involved in the metastatic process, even those that predominantly participate in miRNA biogenesis. It is unclear the exact mechanism by which these SNPs influence risk and survival, as many do not appear to affect mRNA transcription; we therefore encourage others to investigate the impact of these SNPs on gene translation and protein function.

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Table 1

Descriptive table of included subjects.

		Controls		Cases	
		N	%	N	%
Sex	Male	643	54.8	618	55.4
	Female	530	45.2	497	44.6
Center	Kaiser	290	24.7	356	31.9
	Minnesota	609	51.9	527	47.3
	Utah	274	23.4	232	20.8
Vital Status	Dead			330	31.7
	Alive			711	68.3
AJCC Stage	1			346	34.7
	2			291	29.2
	3			270	27.1
	4			91	9.1
Microsatellite	Stable			705	83.5
	Unstable			139	16.5
		Mean	SD	Mean	SD
Age		65.3	9.9	65.0	9.9
Follow up months				60.3	27.6

N, number of individuals; AJCC, American Joint Committee on Cancer; SD, standard deviation.

Table 2

Risk of developing colon cancer and risk of dying from CRC by SNP genotype.

SNPs	Risk of Developing Colon Cancer						Risk of Dying From CRC			
	Controls			Cases			Censored		Raw P-value ³	Holm P-value
	N	%		N	%	OR ¹	(95% CI)	N	N	
<i>ADAMTS1</i> (rs12140)	1068	91.0	1010	90.6	1.00			603	151	0.011*
	TC/CC	105	9.0	105	9.4	1.00	(0.75, 1.34)	72	11	0.44 (0.24, 0.83)
<i>ADAR</i> (rs2335230)	1122	95.7	1068	95.8	1.00			650	151	0.033
	CC	51	4.3	47	4.2	0.99	(0.66, 1.49)	25	11	2.99 (1.60, 5.59)
<i>ASB6</i> (rs1045647)	461	39.3	439	39.4	1.00			286	55	1.00
	CT	527	44.9	499	44.8	0.98	(0.82, 1.18)	292	74	1.18 (0.83, 1.68)
	TT	185	15.8	177	15.9	1.01	(0.79, 1.28)	97	33	1.72 (1.11, 2.65)
<i>BRCA1</i> (rs799917) ⁴	540	46.0	470	42.2	1.00			287	58	1.00
	GA	517	44.1	508	45.6	1.12	(0.94, 1.33)	315	71	1.21 (0.85, 1.72)
	AA	116	9.9	137	12.3	1.35	(1.02, 1.78)	73	33	1.70 (1.10, 2.62)
<i>BRCA1</i> (rs8176318) ⁴	560	47.7	484	43.4	1.00			297	60	1.00
	CA	504	43.0	504	45.2	1.15	(0.96, 1.36)	308	73	1.27 (0.90, 1.80)
	AA	109	9.3	127	11.4	1.34	(1.01, 1.78)	70	29	1.72 (1.10, 2.70)
<i>CSEIL</i> (rs17632)	421	35.9	364	32.6	1.00			213	59	1.00
	CT	540	46.0	554	49.7	1.19	(0.99, 1.43)	332	81	0.76 (0.54, 1.06)
	TT	212	18.1	197	17.7	1.08	(0.85, 1.37)	130	22	0.56 (0.34, 0.92)
<i>DGCR8</i> (rs2286926)	1104	94.1	1039	93.2	1.00			636	149	1.00
	CT/TT	69	5.9	76	6.8	1.16	(0.83, 1.63)	39	13	1.96 (1.10, 3.48)
<i>DGCR8</i> (rs17817767)	1102	93.9	1039	93.2	1.00			636	149	1.00
	GA/AA	71	6.1	76	6.8	1.13	(0.81, 1.58)	39	13	1.96 (1.10, 3.48)

SNPs	Risk of Developing Colon Cancer						Risk of Dying From CRC					
	Controls			Cases			Censored		HRR ² (95% CI)	Raw P- value ³	Holm P- value	
	N	%	N	%	OR ¹	(95% CI)	N	No				
<i>DROSHA</i> (rs524138)	GG	1115	95.1	1062	95.2	1.00		648	149	1.00	0.03	1.000
	GA/AA	58	4.9	53	4.8	0.93	(0.63, 1.37)	27	13	1.86	(1.05, 3.30)	
<i>DROSHA</i> (rs7447423)	GG	1117	95.2	1062	95.2	1.00		648	149	1.00	0.03	0.993
	GA/AA	56	4.8	53	4.8	0.97	(0.66, 1.43)	27	13	1.89	(1.06, 3.35)	
<i>DROSHA</i> (rs10068052)	AA/AG	1016	86.6	980	87.9	1.00		601	133	1.00	<0.01	0.040
	GG	157	13.4	135	12.1	0.89	(0.69, 1.14)	74	29	1.96	(1.31, 2.94)	
<i>DROSHA</i> (rs11748548)	GG	1121	95.6	1067	95.7	1.00		651	150	1.00	0.02	0.655
	GA/AA	52	4.4	48	4.3	0.95	(0.63, 1.42)	24	12	2.05	(1.12, 3.74)	
<i>DROSHA</i> (rs13183642)	GG/GT	1082	92.2	1048	94.0	1.00		640	145	1.00	<0.01	0.021
	TT	91	7.8	67	6.0	0.77	(0.55, 1.06)	35	17	2.48	(1.48, 4.14)	
<i>DROSHA</i> (rs16901109)	AA	1133	96.6	1090	97.8	1.00		662	155	1.00	0.04	1.000
	AC/CC	40	3.4	25	2.2	0.62	(0.37, 1.04)	13	7	2.24	(1.02, 4.93)	
<i>DROSHA</i> (rs17408227)	CC/CT	992	84.6	952	85.4	1.00		585	125	1.00	<0.01	0.038
	TT	181	15.4	163	14.6	0.93	(0.74, 1.17)	90	37	1.86	(1.28, 2.68)	
<i>DROSHA</i> (rs17409624)	CC	297	25.3	298	26.7	1.00		181	37	1.00	0.04	1.000
	CT	592	50.5	572	51.3	0.97	(0.79, 1.18)	357	87	1.22	(0.83, 1.80)	
<i>E2F2</i> (rs2075993)	TT	284	24.2	245	22.0	0.87	(0.69, 1.10)	137	38	1.63	(1.03, 2.57)	
	GG	310	26.4	279	25.0	1.00		162	52	1.00	<0.01	0.166
<i>FADS2</i> (rs174568)	GA	592	50.5	565	50.7	1.05	(0.86, 1.28)	336	81	0.70	(0.49, 1.00)	
	AA	271	23.1	271	24.3	1.09	(0.86, 1.38)	177	29	0.50	(0.32, 0.80)	
<i>FADS2</i> (rs174568)	CC	524	44.7	525	47.1	1.00		306	83	1.00	0.01	0.114
	CT/TT	649	55.3	590	52.9	0.90	(0.76, 1.06)	369	79	0.64	(0.47, 0.88)	
<i>GDE1</i> (rs2521795)	GG	827	70.5	837	75.1	1.00		515	115	1.00	0.02	0.436

SNPs	Risk of Developing Colon Cancer							Risk of Dying From CRC			
	Controls			Cases			Censored	HRR ²	(95% CI)	Raw P-value ³	Holm P-value
	N	%	N	%	N	%					
	GA/AA	346	29.5	278	24.9	0.79	(0.66, 0.95)	160	47	1.51	(1.07, 2.12)
<i>HLA-DPB1</i> (rs1042544)	AA	631	53.8	592	53.1	1.00		346	94	1.00	0.03
	AG	464	39.6	435	39.0	1.00	(0.84, 1.19)	272	54	0.80	(0.57, 1.12)
	GG	78	6.6	88	7.9	1.21	(0.88, 1.68)	57	14	0.55	(0.31, 0.98)
<i>IL6ST</i> (rs2228043)	GG	898	76.6	841	75.4	1.00		520	114	1.00	0.01
	GC	258	22.0	252	22.6	1.04	(0.85, 1.27)	142	43	1.48	(1.04, 2.12)
	CC	17	1.4	22	2.0	1.42	(0.75, 2.70)	13	5	2.69	(1.08, 6.71)
<i>LIN28A</i> (rs6598964)	GG/GA	995	84.8	980	87.9	1.00		615	131	1.00	<0.01
	AA	178	15.2	135	12.1	0.77	(0.61, 0.98)	60	31	2.26	(1.52, 3.36)
<i>MIR548AP</i> (rs2344843)	AA	472	40.2	467	41.9	1.00		283	78	1.00	0.03
	AG	529	45.1	499	44.8	0.97	(0.81, 1.16)	309	65	0.82	(0.59, 1.15)
	GG	172	14.7	149	13.4	0.87	(0.67, 1.12)	83	19	0.59	(0.35, 0.98)
<i>PDHX</i> (rs724714)	AA	776	66.2	722	64.8	1.00		450	96	1.00	0.04
	AG/GG	397	33.8	393	35.2	1.06	(0.89, 1.26)	225	66	1.38	(1.01, 1.90)
<i>PRKRA</i> (rs2059691)	GG	599	51.1	546	49.0	1.00		356	61	1.00	<0.01
	GA	478	40.8	458	41.1	1.05	(0.88, 1.24)	259	82	1.62	(1.16, 2.27)
	AA	96	8.2	111	10.0	1.25	(0.93, 1.68)	60	19	1.87	(1.11, 3.13)
<i>PRRC1</i> (rs400035)	TT	585	49.9	542	48.6	1.00		346	70	1.00	<0.01
	TC/CC	588	50.1	573	51.4	1.05	(0.89, 1.24)	329	92	1.62	(1.18, 2.22)
<i>RAN</i> (rs3809142)	CC	960	81.8	918	82.3	1.00		553	141	1.00	0.03
	CT/TT	213	18.2	197	17.7	0.96	(0.77, 1.18)	122	21	0.60	(0.37, 0.95)
<i>SF3A3</i> (rs17465826)	TT	1100	93.8	1025	91.9	1.00		630	145	1.00	0.03
	TG/GG	73	6.2	90	8.1	1.31	(0.95, 1.81)	45	17	1.74	(1.05, 2.90)

SNPs	Risk of Developing Colon Cancer						Risk of Dying From CRC				
	Controls			Cases			Censored			Raw P-value ³	Holm P-value
	N	%		N	%	OR ¹	(95% CI)	Yes	No		
<i>SUFU</i> (rs11594179)	CC	718	61.2	650	58.3	1.00		404	88	1.00	0.888
	CT	387	33.0	404	36.2	1.15	(0.97, 1.38)	241	61	1.24	(0.89, 1.73)
	TT	68	5.8	61	5.5	1.00	(0.69, 1.43)	30	13	1.86	(1.03, 3.37)
<i>UNC5B</i> (rs1060184)	CC/CG	1146	97.7	1093	98.0	1.00		662	155	1.00	<0.01
	GG	27	2.3	22	2.0	0.83	(0.47, 1.47)	13	7	3.31	(1.53, 7.16)

¹ adjusted for age at diagnosis/selection, study center and gender.

² adjusting for age at diagnosis, study center, gender, AJCC stage, and MSI status.

³ Based on a one degree of freedom test.

⁴ These SNPs are in high LD ($R^2=0.965$).

* Bolded terms are significant findings.

Messenger RNA expression differences by genotype in colonic carcinoma, normal colonic mucosa, and differential between colonic carcinoma tissue and normal colonic mucosa-.

Table 3

Gene	Genotype	Colonic Carcinoma						Normal Colonic Mucosa				Difference	
		Mean C ¹	Mean N ²	N ³	Beta ⁴	Raw P	N	Beta	Raw P-value	N	Beta	Raw P-value	
ADAMTS1 (rs12140)	TT	38.52	125.25	64	0.13	0.7799	61	-0.07	0.8151	55	0.01	0.9864	
	TC/CC	34.29	82.50	7			6			6			
ADAR (rs2335230)	AA/AC	682.28	374.98	69	0.92	0.1560	65	0.00	0.8738	59	0.99	0.1972	
	CC	814.00	231.50	2			2			2			
ASB6 (rs1045647)	CC	27.90	16.43	29	0.27	0.0288*	28	0.14	0.4468	25	0.14	0.4766	
	CT	26.00	15.11	30			28			26			
	TT	43.33	28.55	12			11			10			
BRCA1 (rs799917) ⁵	GG	140.56	39.34	36	0.09	0.6304	32	0.04	0.6970	30	0.06	0.7520	
	GA	130.16	31.92	25			25			22			
	AA	202.80	49.30	10			10			9			
BRCA1 (rs8176318) ⁵	CC	138.14	38.18	37	0.08	0.6721	33	-0.03	0.9403	31	0.06	0.7504	
	CA	139.85	37.52	26			27			23			
	AA	199.38	39.57	8			7			7			
CSEIL (rs17632)	CC	421.92	71.52	25	-0.30	0.0989	27	-0.22	0.2524	23	-0.11	0.5696	
	CT	198.24	40.60	34			30			28			
	TT	266.08	45.10	12			10			10			
DGCR8 (rs2286926)	CC	137.86	97.05	66	-0.33	0.3877	61	-0.59	0.1162	56	0.23	0.6614	
	CT/TT	97.00	29.33	5			6			5			
DGCR8 (rs17817767)	GG	137.86	97.05	66	-0.33	0.3828	61	-0.59	0.1202	56	0.23	0.6564	
	GA/AA	97.00	29.33	5			6			5			
DROSHA (rs524138)	GG	204.72	97.91	68	0.55	0.6592	64	0.62	0.2822	58	-0.24	0.7018	

Gene	Genotype	Colonic Carcinoma					Normal Colonic Mucosa			Difference		
		Mean C ^I	Mean N ²	N ³	Beta ⁴	Raw P	N	Beta	Raw P-value	N	Beta	Raw P-value
	GA/AA	170.00	74.33	3			3			3		
<i>DROSHA</i> (rs7447423)	GG	204.22	97.21	67	0.64	0.3908	63	0.83	0.0719	57	-0.26	0.6337
	GA/AA	187.00	91.25	4			4			4		
<i>DROSHA</i> (rs10068052)	AA/AG	189.10	92.39	62	0.50	0.4722	59	0.34	0.4566	53	0.11	0.7803
	GG	300.78	129.75	9			8			8		
<i>DROSHA</i> (rs11748548)	GG	204.72	97.91	68	0.55	0.6660	64	0.62	0.2830	58	-0.24	0.6989
	GA/AA	170.00	74.33	3			3			3		
<i>DROSHA</i> (rs13183642)	GG/GT	187.53	92.29	66	0.58	0.4867	62	0.27	0.8102	56	0.26	0.5899
	TT	410.80	153.40	5			5			5		
<i>DROSHA</i> (rs16901109)	AA	203.06	96.66	69	0.92	0.5320	65	0.97	0.1334	59	-0.38	0.6146
	AC/CC	210.00	103.00	2			2			2		
<i>DROSHA</i> (rs17408227)	CC/CT	201.17	97.67	60	0.28	0.5425	57	0.25	0.4451	51	0.06	0.8678
	TT	214.64	92.20	11			10			10		
<i>DROSHA</i> (rs17409624)	CC	142.53	92.00	19	0.27	0.1842	14	-0.01	0.8819	14	-0.07	0.7235
	CT	211.87	99.90	38			42			37		
	TT	262.29	91.36	14			11			10		
<i>E2F2</i> (rs2075993)	GG	27.88	20.47	17	0.04	0.8025	16	0.12	0.5273	15	0.02	0.9353
	GA	43.94	30.39	35			33			31		
	AA	27.26	33.89	19			18			15		
<i>FADS2</i> (rs174568)	CC	42.03	10.61	32	0.31	0.1187	33	0.52	0.0218	27	0.35	0.1837
	CT/TT	90.64	26.68	39			34			34		
<i>GDE1</i> (rs2521795)	GG	147.90	100.60	48	-0.08	0.5957	48	-0.14	0.4032	43	0.26	0.3578
	GA/AA	139.13	111.79	23			19			18		
<i>HLA-DPB1</i> (rs1042544)	AA	58.25	84.80	36	0.11	0.9598	30	-0.32	0.0338	28	0.46	0.0164

Gene	Genotype	Colonic Carcinoma					Normal Colonic Mucosa			Difference		
		Mean C ^J	Mean N ²	N ³	Beta ^J	Raw P	N	Beta	Raw P-value	N	Beta	Raw P-value
<i>IL6ST</i> (rs2228043)	AG	49.30	55.07	27			28			25		
	GG	83.13	37.00	8			9			8		
<i>IL6ST</i> (rs2228043)	GG	247.88	327.30	49	-0.01	0.9353	50	-0.18	0.7372	44	0.22	0.3743
	GC	270.75	323.33	20			15			15		
	CC	151.00	267.00	2			2			2		
<i>LIN28A</i> (rs6598964)	GG/GA	1.90	2.27	63	-0.12	0.7907	60	0.38	0.3725	55	-0.48	0.2551
	AA	0.75	3.00	8			7			6		
<i>PDHX</i> (rs724714)	AA	62.17	36.30	47	0.23	0.5037	44	-0.09	0.8549	40	0.13	0.6469
	AG/GG	65.78	29.18	24			23			21		
<i>PRKRA</i> (rs2059691)	GG	48.67	15.37	33	0.35	0.0407	30	0.56	0.0003	27	-0.14	0.4629
	GA	79.52	45.68	29			28			25		
	AA	120.22	68.56	9			9			9		
<i>PRRC1</i> (rs400035)	TT	163.37	120.48	35	0.15	0.7182	33	-0.15	0.3037	29	0.48	0.0734
	TC/CC	198.14	87.32	36			34			32		
<i>RAN</i> (rs3809142)	CC	206.02	55.09	55	0.23	0.3533	53	-0.20	0.5974	47	0.23	0.4831
	CT/TT	287.81	64.07	16			14			14		
<i>SF3A3</i> (rs17465826)	TT	101.70	48.43	67	-0.04	0.9161	63	0.94	0.0281	57	-0.87	0.1063
	TG/GG	69.25	66.25	4			4			4		
<i>SUFU</i> (rs11594179)	CC	39.30	29.64	47	-0.17	0.3504	47	-0.23	0.2709	43	-0.09	0.7090
	CT	41.85	40.75	20			16			14		
	TT	17.25	12.50	4			4			4		
<i>UNC5B</i> (rs1060184)	CC/CG	88.49	65.56	70	1.14	0.2120	66	-0.19	0.9387	60	1.36	0.1988
	GG	215.00	59.00	1			1			1		

^J Colonic carcinoma tissue mean mRNA expression displayed in count data (rather than in RPKM).

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² Normal colonic mucosa mean mRNA expression displayed in count data (rather than in RPKM).
³ Number of individuals analyzed with the specified genotype and tissue mRNA expression data.
⁴ adjusted for age, AJCC stage and gender.
⁵ These SNPs are in high LD ($R^2=0.965$).
* Bolded terms are significant findings.