Ulcerative colitis neoplasia is not associated with common inflammatory bowel disease single-nucleotide polymorphisms

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Background. *Neoplasia complicating ulcerative colitis (UC-neoplasia) is a problem that is poorly addressed by present surveillance techniques. The association of greater than 300 single nucleotide polymorphisms (SNPs) with inflammatory bowel disease (IBD) suggests the possibility that certain genetic polymorphisms might identify patients with UC destined for malignant degeneration. This present study tested the hypothesis that presently known IBD-associated SNPs may correlate with UC-neoplasia.*

Materials and methods. *A total of 41 patients with UC-neoplasia (mean age 56 ± 2.1 years) were identified from our divisional IBD Biobank (low-grade dysplasia* n = *13, high-grade dysplasia* n = *8, colorectal cancer [CRC]* n = *20). These patients were individually age, sex, and disease duration matched with UC patients without neoplasia. Primary sclerosing cholangitis and family history of CRC were recorded. Patients were genotyped for 314 of the most commonly IBD-associated SNPs by a custom SNP microarray. Logistic regression and Fischer exact test were used for statistical analysis.*

Results. *After Bonferroni correction, none of the 314 IBD-associated SNPs correlated with UC-neoplasia when compared with matched UC controls. The incidence of primary sclerosing cholangitis was greater in the UC-neoplasia group (10/41, 24% vs 3/41, 7%;* P = *.03) compared with UC controls. The severity of neoplasia (low grade dysplasia versus high grade dysplasia versus CRC) correlated with disease duration (7.9 vs 13.4 vs 20.7 years, respectively).*

Conclusion. *The lack of correlation between well-known IBD-associated SNPs and UC-neoplasia demonstrated in this study suggests that the development of neoplasia in patients with UC is associated with genetic determinants other than those that predispose to inflammation or results from post-translational modifications or epigenetic factors rather than germline polymorphisms. (Surgery 2014;156:253-62.)*

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PATIENTS WITH ULCERATIVE COLITIS (UC) have increased rates of colorectal carcinoma (CRC) compared to the general population, with risk most closely correlating with disease extent and duration.[1-6] The current inflammatory bowel disease (IBD)-CRC model involves the progression of healthy tissue from inflamed to low-grade dysplasia (LGD) to high-grade dysplasia (HGD) to carcinoma.[7] However, importantly, this model can only be thought of as a very rough guide because progression from normal mucosa to cancer is not always observed in the previously stated order. Patients with UC not uncommonly have HGD or CRC discovered without previous evidence of LGD and often UC-CRC develops at an accelerated pace compared with sporadic CRC.

UC-CRC risk increases markedly with duration of UC with a cumulative CRC probability of 2% within 10 years, 8% by 20 years, and 18% by 30 years.[8] Additionally, patients with a concomitant

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diagnosis of primary sclerosing cholangitis (PSC) or a family history of CRC are at a particularly high risk.[9] This cancer risk is significant as approximately 15% of IBD patients die from CRC compared with approximately 2% of the non IBD population.[10-12]

Surveillance techniques for CRC and its precursor, dysplasia, are currently improving but remain inadequate.[13] Presently, such colonoscopic surveillance is recommended at least every 1–2 years starting 8 years after the onset of UC symptoms. However, this is burdensome for the patient and has been demonstrated to be very imperfect with a high percentage of missed lesions, less-than-ideal correlation between colonoscopic biopsy pathology and final colectomy pathology, and wide interobserver variation.[14,15] Alarmingly, 20% to 50% of patients diagnosed preoperatively with only dysplasia are in fact found to have cancer on final pathology, highlighting the gross deficiency of the present surveillance system. In addition, compared with sporadic CRC, the endoscopic appearance of IBD-related neoplasia is different, with inflammation-associated dysplasia commonly being less "mass-like" and more easily missed on colonoscopy. As a result, it is not uncommon for lesions to be found unexpectedly in a specimen resected for medically refractive IBD without previous diagnosis of dysplasia or carcinoma.[12,16]

Despite the demonstrated increased risk of UC-associated CRC and the poor performance of colonoscopic surveillance protocols, no genetic or serological marker has been identified or shown to be effective in clinical practice. Since the advent of genome-wide association studies, more than 300 single nucleotide polymorphisms (SNPs) and more than 160 genetic loci have been associated with IBD.[17] We propose that a genetic marker, possibly in the form of one of these SNPs, may be able to identify IBD patients at a greater risk of developing CRC and thus would improve surveillance routines. For example, colonoscopic surveillance frequency can be adjusted according to a patient's genetically defined predisposition to CRC. In the extreme, those with a genetic signature destined to develop CRC, a prophylactic colectomy could be planned prior to the development of cancer.

Few previous studies have looked at SNPs possibly associated with UC-CRC, and these few studies have been limited by numerous factors, including not comparing groups with similar risk factors and not matching patients by disease duration. Therefore, the aim of the present study was to identify IBD-associated SNPs that could be potential markers for UC-associated neoplasia by comparing groups of UC patients with and without neoplasia matched for sex, disease duration, and age of diagnosis.

MATERIALS AND METHODS

Patient selection. All recruited ulcerative colitis patients were identified from the Hershey Medical Center, Penn State College of Medicine Division of Colon and Rectal Surgery's Internal Review Board approved IBD Biobank. This divisional Biobank, created in 1998, contains patient demographic and clinical data, DNA, serum, immortalized B cells,[18] specimen photographs, and tissue samples from more than 1,800 patients with sporadic and familial IBD and controls in the form of diverticular disease, colon, and rectal cancer and healthy individuals. Patients undergoing colonic resection for UC-associated dysplasia or colorectal cancer (UC-neoplasia) between January 1996 and June 2012 were then identified from this group. A diagnosis of CRC, HGD, or LGD was confirmed on final colectomy pathology reports. Details including sex, duration of disease, age at diagnosis, family history of IBD, family history of CRC, concomitant diagnosis of PSC, and smoking status were recorded for this cohort. These patients were then individually matched with UC patients of the same sex without neoplasia with similar or greater disease duration and similar or younger age at diagnosis of IBD.

Genotyping. We isolated DNA from patients' blood or immortalized B cells by using a QIAGEN DNA Blood Midi kit (QIAGEN Inc. Valencia, CA), following the manufacturer's recommended protocol. The Hershey Medical Center Division of Colon and Rectal Surgery's custom-designed IBD Illumina Bead Express SNP chip containing 384 IBD-associated SNPs (Illumina, San Diego, CA) was used for genotyping. This chip was developed within our laboratory through an extensive review of the literature. Since its creation, it has undergone several revisions to remain reflective of current international IBD genetic research.

DNA concentrations were quantified with a spectrophotometer, and working stocks of 10 ng/μL were prepared in 10 mM Tris-HCl. For specimen optimization, dsDNA concentrations were quantified using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA). The customized chip was run on an Illumina BeadXpress Reader in Penn State Hershey Medical Center's Functional Genomics Core Facility.

Statistical analysis. Only SNPs for which greater than 90% of patients had a definitive genotype call

when analyzed by the Illumina reader and SNPs with a minor allele frequency greater than 5% were included for statistical evaluation for the current project. Thus the final number of SNPs analyzed for the current project was 314 (Table I). Logistic regression with a Bonferroni correction to correct for multiple comparisons of these 314 SNPs was used for the genetic analysis. Because the two cohorts were matched by sex, duration of disease, and age at diagnosis, the only variables that differed between the groups were family history of CRC and presence of PSC. R software[19] was used for all analyses. Values are given with standard error (SE) where appropriate.

RESULTS

Patient demographics. Patient demographics are listed in Table II. The UC-neoplasia cohort was predominately male (73.2%), and 51.2% were nonsmokers. A family history of IBD was present in 26.8% (11 of 41). The control group of UC patients without neoplasia was slightly younger at diagnosis, ie, 31.35 ± 2.36 years (range, 3.1–65.2) and had a slightly longer duration of disease duration, ie, 22.96 ± 1.87 years (range, 5.14–43.66). A family history of CRC was present in six of the patients with neoplasia and three of those without, but this was not statistically different ($P = .40$). More patients with neoplasia had a concomitant diagnosis of PSC (24.4% vs 7.3% in the non-neoplasia group, $P = .03$). All patients were Caucasian. The majority of neoplasia was found in the colon compared with rectosigmoid or rectum (Table III).

When subdivided by LGD, HGD and CRC, a trend towards longer disease duration as being associated with more severe neoplasia was seen. LGD patients had a mean duration of disease of 7.9 ± 3.6 years, HGD patients had a mean of 13.4 ± 3.5 years, whereas patients with CRC had the longest mean disease duration at 20.7 ± 5.1 years. However, this difference was not statistically different ($P > .05$).

Genetic analysis. Two comparisons were performed. First, all UC-neoplasia (CRC + HGD + LGD) patients were compared with the UC without neoplasia cohort. Although several SNPs initially were found to be associated with neoplasia, none of the 314 SNPs retained statistical significance after Bonferroni correction (Table IV).

The patients with dysplasia were then eliminated from the comparison, and UC-CRC only patients were compared with their matched UC without neoplasia controls. The number of SNPs initially associated with CRC was less than that seen

Table I. SNPs on HMC Division of Colon and Rectal Surgery's custom IBD SNP chip

SNP	*Most likely gene association*	*Disease association: CD, UC, or both*
rs10045431	IL12B	Both
rs1004819	IL23R	Both
rs10077785	IBD5	Both
rs10181042	C2orf74	Both
rs10495903	THADA	Both
rs10500264	gene desert	Both
rs1059276	MEP1A	Both
rs10758669	JAK2	Both
rs10761659	ZNF365	Both
rs10781499	CARD9/INPP5E/SNAPC4	Both
rs10883365	NKX2-3	Both
rs10889677	IL23R	Both
rs10995271	ZNF365	Both
rs1108542	CSDA	Both
rs11175593	LRRK2	Both
rs11190140	NXK2.3	Both
rs1126647	IL8	Both
rs11465804	IL23R	Both
rs11554257	TNFSF15 (TL1A)	Both
rs11564258	MUC19, LRRK2	Both
rs11584383	KIF	Both
rs11747270	PTGER4	Both
rs11805303	IL23R	Both
rs11807930	F11R-Diseasome	Both
rs11871801	STAT3/MLX	Both
rs12261843	CCNY	Both
rs12420	IL8	Both
rs1248634	DLG5 R30Q	Both
rs1248696	DLG5	Both
rs1250550	ZMIZ	Both
rs12529198	LYRM4	Both
rs12704036	U7	Both
rs12720356	TYK2/ICAM1/3	Both
rs1297265	gene desert	Both
rs13003464	PUS10	Both
rs13112910	IL8	Both
rs13361189	IRGM	Both
rs1363670	IL12B	Both
rs1456896	intergenic between C7orf72 and IKZF1	Both
rs1544705	CPA5	Both
rs1545620	MYO9B	Both
rs1547832	CGN	Both
rs1551398		Both
rs1679647	Zo-1	Both
rs17085007	GPR12	Both
rs17164867	CPA5	Both
rs17293632	SMAD3	Both
rs17309827	C6orf85	Both
rs1736135	Intergenic-between NRIp1 and CYCSP4	Both
rs1738074	TAGAP	Both

(*continued*)

Table I. (*continued*)

SNP	*Most likely gene association*	*Disease association: CD, UC, or both*
rs17582416	CCNY	Both
rs1801274	FCGR2A/2B	Both
rs1803205	IL8	Both
rs181359	YDJC	Both
rs1847472	BACH2	Both
rs1879039	ZO-3	Both
rs1893217	PTPN2	Both
rs1954260	RAB3B	Both
rs1992660	PTGER4	Both
rs1992662	PTGER4	Both
rs2039785	TJP2	Both
rs2058660	IL18RAP	Both
rs2066844	NOD2	Both
rs2066845	NOD2	Both
rs2066847	NOD2	Both
rs2076756	NOD2	Both
rs2166066	CSDA	Both
rs2188962	IBD5	Both
rs2228226	GLI1	Both
rs2234237	TREM1	Both
rs2241880	ATG16L1	Both
rs2243639	SFTPD	Both
rs2274910	ITLN1	Both
rs2279627	LOC729449 TNFSF14	Both
rs2297441	SLC2A4RG/STMN3/ ZBTB46	Both
rs2301436	CCR6	Both
rs2310173	IL1R2	Both
rs2315008	TNFSF6B	Both
rs2412973	HORMAD	Both
rs2413583	MAP3K	Both
rs2522057	IBD5	Both
rs2542151	PTPN22	Both
rs260526	ZO1	Both
rs2797685	PER2	Both
rs2836878	PSMG1	Both
rs2838519	*ICOSLG*	Both
rs2872507	ORMDL3	Both
rs2907748	NOD1	Both
rs3197999	MST1	Both
rs3212227	IL12B	Both
rs3732923	CLDN1	Both
rs3737240	ECM1	Both
rs3763313	*BTNL2, SLC26A3, HLA-DRB1, HLA-DQA1*	Both
rs3764147	C13orf31	Both
rs3806308	RNF186	Both
rs3810936	TNFSF15	Both
rs3828309	ATG16L1	Both
rs390017	KFL2	Both
rs3936503	CCNY	Both
rs41313262	IL23R	Both
rs4263839	TNFSF15	Both
rs4304218	CLDN4	Both
rs4379776	PARD3	Both
rs4409764	NXK2.3	Both
rs4613763	PTGER4	Both
rs4676410	CAPN10/GPR35	Both
rs4728142	IRF5/TNP03	Both
rs4807569	GPX4	Both
rs4833103	TLR1,TLR10,TRL6	Both
rs5771069	IL17R	Both
rs615969	ZO-3	Both
rs6451493	PTGER4	Both
rs6460055	CLDN3	Both
rs6488321		Both
rs6556412	*IL12B*	Both
rs6568421	PRDM1	Both
rs6584283	NXK2.3	Both
rs6822844	IL2	Both
rs6853	MY88	Both
rs6871626	*IL12B*	Both
rs6887695	*IL12B*	Both
rs6908425	CDKAL1	Both
rs6911490	PDRM	Both
rs6920220	gene desert	Both
rs6927210	U6	Both
rs6962966	MAGI2	Both
rs7081330	*NKX2.3*	Both
rs709932	SERPINA	Both
rs7134599	gene desert	Both
rs736289	gene desert	Both
rs744166	STAT3	Both
rs7554511	C1orf106/KIF21B	Both
rs7608910	PUS2	Both
rs762421	ICOSLG	Both
rs77050787	NXK2.3	Both
rs7746082	HLA REGION	Both
rs780093	GCKR	Both
rs780094	GCKR	Both
rs7848647	TNFSF15	Both
rs7849191	JAK2	Both
rs7869487	TNFSF15	Both
rs790055	F11R	Both
rs7923172	MST1	Both
rs7927894	C11orf30	Both
rs7927997	*C11orf30*	Both
rs8005161	GALC	Both
rs8049439	IL27	Both
rs8098673	gene desert	Both
rs8106955	SYMPK	Both
rs812761	CSDA	Both
rs8137602	NCF4	Both
rs8798	CLDN1	Both
rs888208	NXK2.3	Both
rs907611	LSP1	Both

(*continued*)

Table I. (*continued*)

SNP	*Most likely gene association*	*Disease association: CD, UC, or both*
rs916977	HERC2	Both
rs917997	IL18	Both
rs9292777	gene desert	Both
rs941823	LINC00598	Both
rs951199	NELL1	Both
rs9647373	CLDN1	Both
rs9809713	CLDN1	Both
rs9822268	MST1/UBA7/APEH/ AMIGO3/GMPPB,BSN	Both
rs9858542	MST1	Both
rs9911804	CCL2, CCL7, CCL11, ccl8	Both
rs6478108	TNFSF15	Both
rs10489629	IL23R	Both
rs17164838	CPA5	Both
rs2201841	IL23R	Both
rs41267765	TAGAP	Both
rs5743293	NOD2	Both
rs6478109	TNFSF15	Both
rs721917	SFTPD	Both
rs746713	NCF4	Both
rs7808907	IRF5	Both
rs3024505	IL10	Both
rs1801282	PPAR y	Both
rs652162	UBE2K	CD
rs1000113	IRGM	CD
rs102275	FADS1	CD
rs1050152	SLC22A4	CD
rs10733113	NLRP3	CD
rs10748643	ENTPD1, UGT3A1	CD
rs11167764	NDFIP1	CD
rs11362	DEFB1	CD
rs12037606	TNFSF14	CD
rs12521868	SLC22	CD
rs12722489	IL2RA	CD
rs13073817	Gene Island	CD
rs13428812	DNMT3A	CD
rs1457092	MYO9B	CD
rs151181	CLDN3	CD
rs17221417	NOD2	CD
rs17234657	PTGER4	CD
rs1793004	NELL1	CD
rs1799964	LTA/HLA DQA2/TNF/ LST1/LTB	CD
rs1800471	TGFB1	CD
rs1800795	IL6	CD
rs1800872	IL10	CD
rs1800896	IL10	CD
rs1800972	DEFB1	CD
rs1819658	UBE2D1	CD
rs1998598	DENND1B	CD
rs2062305	TNFSF11	CD
rs2075818	NOD1	CD
rs2075822	NOD1	CD
rs212388	TAGAP	CD
rs224136	ZNF365/EGR2 flanking region	CD
rs2279002	MYO9B	CD
rs2305764	MYO9B	CD
rs2476601	PTPN22	CD
rs2542152	PTPN22	CD
rs2549794	ERAP2/LRAP	CD
rs2631367	MIR4750, SLC22A5	CD
rs281379	MAMSTR	CD
rs2844480	DRAP	CD
rs3091315	CCL7/CCL2	CD
rs3130501	POU5F1	CD
rs34255737	TAGAP	CD
rs35105682	TAGAP	CD
rs35873774	xbp1	CD
rs359457	CPEB4	CD
rs363617	gene desert	CD
rs3792109	ATG16L1	CD
rs4077515	CARD9	CD
rs41267765	TAGAP	CD
rs4129267	IL6R	CD
rs415890	gene desert	CD
rs4656940	CD24/MUC1	CD
rs4809330	RTEL1/TNFSF6B	CD
rs4871611	gene desert	CD
rs4902642	ZFP36L1	CD
rs4958847	IRGM	CD
rs4986790	TLR4	CD
rs4986791	TLR4	CD
rs5051	AGT	CD
rs6651252	Gene Island	CD
rs6738825	PLCL1	CD
rs694739	prdx5/esrra	CD
rs713875	MTMR3	CD
rs7219780	TRPV3	CD
rs7423615	SP140	CD
rs7517810	TNFSF18/TNFSF4/ FASLG	CD
rs7702331	TMEM17	CD
rs7714584	BETWEEN IRGM AND ZNF300	CD
rs7720838	PTGER4	CD
rs7753394	*TNFAIP3*	CD
rs7807268	C7ORF33	CD
rs9469220	HLAII	CD
rs9471535	TREM1	CD
rs10439163	ATG4D	CD
rs11730582	SPP1	CD
rs12721602	PXR/NR1I2	CD
rs1373692		CD
rs17109951	FNBP1L	CD
rs2304165	ATG4D	CD

(*continued*)

Table I. (*continued*)

SNP	Most likely gene association	Disease association: CD, UC, or both
rs2728127	OPN	CD
rs2853744	OPN	CD
rs3814055	PXR/NR1I2	CD
rs3814057	PXR/NR1I3	CD
rs4754	SPP1	CD
rs5973822	ATG4A	CD
rs6596075		CD
rs6601764		CD
rs7248026	ATG4D	CD
rs7705189	IGR	CD
rs1049414	BRD	UC
rs1062633	mst1r	UC
rs10753575	IBD7	UC
rs10763976	PARD3	UC
rs10870077	CARD9	UC
rs11676348	IL8RA/SLC11A1/IL8R/ AAMP/ARPC	UC
rs11739663	EXOC3	UC
rs12242110	CREM	UC
rs12612347	ARPC2	UC
rs12661812	NOX3	UC
rs13294	ECM1	UC
rs1558744	IFN y/IL26/IL2	UC
rs17388568	ADAD1	UC
rs2305480	ORMDL3	UC
rs2395185	BTNL2/HLA-DQB	UC
rs254560	Gene Island	UC
rs2647025	HLADQB	UC
rs267939	DAP	UC
rs28435656	C2	UC
rs2844677	MUC21	UC
rs3130559	PSORS1C1	UC
rs3135391	HLA-DRA	UC
rs3194051	IL7R	UC
rs35675666	TNFSF9/UTS2/ERFI1	UC
rs440454	SKIV2L	UC
rs4510766	gene desert	UC
rs4676406	GPR35	UC
rs6017342	HNF4A	UC
rs6426833	1p36	UC
rs6499188	ZNF90	UC
rs660895	HLA-DRB1*0401	UC
rs6933763	HLA-DQA/B2	UC
rs734999	LOC100506589	UC
rs7524102	Gene Island	UC
rs7712957	S100Z	UC
rs7772982	ORV	UC
rs7809799	SMURF1/KPNA7	UC
rs836518	DAGLB	UC
rs886774	LAMB1	UC
rs900569	ULK4	UC
rs915654	NFKB/IL1/LTA	UC
rs9268480	BTNL2	UC
rs9268853	HLADRB9	UC
rs9273363	HLA-DQA1/B1	UC
rs943072	GENE DESERT	UC
rs9548988	gene desert	UC
rs1728785	CDH1	UC
rs6457740	IP6K3	UC
rs798502	GNA12	UC

CD, Crohn's disease; *HMC*, Hershey Medical Center; *SNP*, single-nucleotide polymorphism; *UC*, ulcerative colitis.

in the UC-neoplasia comparison. Similarly, no SNP associations retained significance after Bonferroni correction for all 314 IBD associated genes (Table V).

We then looked only at the UC-specific SNPs on the chip ($n = 49$). Again, no significance was seen after correction in either the all UC-neoplasia compared with UC or UC-CRC compared with UC comparisons.

DISCUSSION

The present study evaluated 314 SNPs previously identified as being associated with IBD. The genes corresponding to these SNPs are found in several IBD-associated pathophysiologic pathways,[20] namely innate immunity/autophagy, barrier function, antigen recognition, T-cell activation/differentiation, and proinflammatory cytokine and chemokine signaling. Several of these genes have roles in more than one of these pathways. Although this is a negative study, it provides important data because the lack of association of any of these SNPs with UC-neoplasia after careful disease duration matching and the use of the Bonferroni correction for multiple comparisons suggests that 1) perhaps genes associated with inflammation do not specifically causatively relate to carcinogenesis in UC patients, 2) perhaps SNPs that predispose patients to developing cancer are separate and distinct from those associated with UC, 3) the development of UC-neoplasia may result from posttranslational modification or epigenetic factors rather than germline polymorphisms, and/or 4) UC-neoplasia may be the result of rare variants, identifiable on whole-genome or exome sequencing. It is conceivable that the association between the SNPs that were initially found to be associated with neoplasia could retain significance after a multiple observation correction if a larger number of patients were studied. However, even after we used a less-rigorous correction, looking at 49 as

Table II. Patient demographics

	UC with dysplasia/CRC, n = *41*	*UC no dysplasia/CRC,* n = *41*	P *value*
Male	30 (73.2%)	30 (73.2%)	NSD
Family history of IBD	11 (26.8%)	12 (29.2%)	NSD
Family history of CRC	6 (14.6%)	3 (7.3%)	NSD
Primary sclerosing cholangitis	10 (24.4%)	3 (7.3%)	.03
Current smoker:ex smoker:never smoked:unknown smoking status	6:12:21:2	3:18:20:0	NSD
Age at UC diagnosis (y, SE)	34.49 ± 2.6 mean 9.8–76.8 range	31.35 ± 2.4 mean 3.1–65.2 range	NSD
Disease duration (y, SE)	21.6 ± 1.73 mean 3.14–44 range	23.0 ± 1.9 mean 5.14–43.7 range	NSD
Duration from IBD diagnosis to CRC/dysplasia surgery (y, SE)	14.10 ± 1.63 mean 0–40.9 range	NA NA	

CRC, Colorectal carcinoma; *IBD*, inflammatory bowel disease; *NA*, not applicable; *NSD*, no significant difference; *UC*, ulcerative colitis.

Table III. Location of neoplasia

Anatomic location	*Number of patients*
Colon	27
Rectum	15
Rectosigmoid	4
Synchronous lesions	3
Metachronous lesions	2

Table IV. All UC-neoplasia compared with UC without neoplasia

Gene	*SNP*	*Raw* P *value*	*Bonferroni corrected* P *value*
ITLN1	rs2274910	.006	1.8
Gene desert	rs1551398	.017	5.6
Gene desert	rs4871611	.023	7.1
FCGR2A	rs1801274	.024	7.1
S100Z	rs7712957	.029	9.1

SNP, Single-nucleotide polymorphism; *UC*, ulcerative colitis.

Table V. UC-CRC compared with UC without neoplasia

Gene	*SNP*	*Raw* P *value*	*Bonferroni corrected* P *value*
ITLN1	rs2274910	.029	9.1
Gene desert	rs4871611	.041	12.9
IRGM	rs4958847	.05	15.7
YDJC	rs181359	.05	15.7

CRC, Colorectal cancer; *SNP*, single-nucleotide polymorphism; *UC*, ulcerative colitis.

opposed to 314 SNPs, we found that significance remained elusive. These first-pass genes have a known role in autophagy (IRGM) and bacterial recognition (ITLN1) but have not yet been associated with carcinogenesis.

Previous studies on the genetics of CRC in IBD. Several genes have been shown to be up- or down-regulated in UC-neoplasia. Using gene expression chips to analyze more than 50,000 genes and transcripts in the rectal mucosa of 43 UC and 10 UC-CRC patients, a Japanese group has identified 40 such genes.[21,22] A subsequent, similar study using rectal biopsy samples in 5 normal controls, 4 UC patients, and 11 UC patients with neoplasia found more than 400 genes to be up-regulated and more than 500 to be down-regulated in UC-neoplasia patient tissue.[23] Such alterations in gene regulation are probably largely epiphenomenon, and such findings have not been translated into clinically relevant markers of disease. Other studies of the genetics involved in the progression to CRC in UC also have had small patient numbers and focused on studying the genetics of IBD-associated CRC as a means to better understand the pathogenesis of the disease rather than finding a genetic marker that might relate to risk assessment in a clinical environment. Several studies used animal models and, as such, imperfectly replicate the development of UC and CRC in the human condition.[24-27] Other studies looking for markers of neoplasia in UC have been tissue based and thus would require invasive methods for obtaining potential markers.[28-32] A genetic marker would have the advantage of being present from birth and easily measured using DNA from a cheek swab or blood draw. Given the lack of association between known IBD-associated SNPs and neoplasia in this cohort, short-term future research avenues include genotyping these UC-neoplasia patients for CRC-associated SNPs and comparing the results with sporadic UC patient

genotyping results. Ideally, whole-genome sequencing of these UC, UC-neoplasia, and a group of sporadic neoplasia patients would identify overlapping and unique mutations between the groups for further study. Currently, whole-genome sequencing of large patient cohorts is prohibitively expensive. However, in the very near future this will become a real possibility.

Of the few studies that looked at germline SNPs, a variant in the *OCTN1* gene was found to be associated with CRC in a cohort of Italian UC patients. However, only two genes (OCTN1 and OCTN2) were examined. This study evaluated 200 UC patients without neoplasia and 59 UC patients with CRC, 200 patients with sporadic CRC, and 200 healthy controls. The same OCTN1 SNPs associated with UC-CRC development also were found to be associated with early onset of sporadic CRC. OCTN1is an organic cation transporter, and a possible mechanism for its role in the development of CRC has not yet been elucidated.[33] Both SNPs investigated in this study, rs1050152 and rs2631367, were included in our SNP chip for this project. Even before Bonferroni correction, we did not find these SNPs to be associated with UC-neoplasia.

A genetic basis for cancer degeneration in UC-affected mucosa may still exist through epigenetic phenomenon. DNA methylation alterations have been noted in the setting of both inflammation and cancer associated with inflammation.[34] Mouse models of colitis have demonstrated the presence of DNA methylation before tumor development. Additionally, methylation levels have been demonstrated to be greater in human UC-CRC compared with sporadic cancer.[27] One study in which authors compared DNA from patients with sporadic CRC versus UC-CRC found hypermethylation present in the promoter region of the e-cadherin gene. This molecule is involved in epithelial cell to cell junctions.[35] Interestingly, known key mediators of IBD-associated inflammation, such as tumor necrosis factor-α, interleukin-6, and interferon-γ, have also been shown to induce DNA methylation,[27] which may explain why risk of cancer increases with duration of UC-inflammation.

Limitations. This study has several limitations. First, the two groups were not matched by PSC status, a known risk factor for the development of CRC.[36] Although, rates of PSC are greater in IBD patients compared with the general population, rates of PSC in the IBD population are still low overall. Therefore, numbers of UC patients without neoplasia with PSC in our Biobank are low. We prioritized matching patients by disease duration, the strongest risk factor. For the same reason, we were unable to match patients by disease extent. However, all non-neoplasia patients had severe UC, necessitating surgical resection. The majority of our neoplasia patients had left sided colitis, and the majority of our control patients had pancolitis. Although not matched exactly, no control patient had a disease extent less than their matched neoplasia patient. Similar to the slightly longer disease duration found in the control patients compared with their matched controls, this ensures that there was not a bias towards the development of neoplasia in the neoplasia group. Additionally, medication history was not included in this study because of ambiguity resulting from medical treatment by outside gastroenterologists and thus the lack of accurate records for many of the patients. However, the link between anti-inflammatory medication and its protection from UC-CRC is debated. A recent meta-analysis of 608 cases and 2,177 controls in four studies have suggested that 5-aminosalicylic acid, the most commonly prescribed drug class in UC, is not protective of CRC, for example.[21,37]

Another potential criticism is the inclusion of LGD patients in this analysis because some findings suggest that LGD inconsistently and infrequently progresses to CRC. However, many studies have shown that LGD is both a precursor and marker for CRC. This association is so strong that, in the surgically fit patient, colectomy is the recommended practice when LGD is found and confirmed on biopsy.[38,39] Our separate analysis that excluded both HGD and LGD and compared only UC-CRC patients with matched patients without neoplasia demonstrated that there was still no significant SNPs found to be associated with CRC.

In conclusion, in the present study, none of 314 studied IBD-associated SNPs were greatly associated with UC-neoplasia. This is the first study in patients were matched by age at diagnosis, sex, and disease duration for the evaluation of potential SNP markers for malignant degeneration. Duration of UC was greater in patients with more severe dysplasia and CRC. These results suggest a possible important role for epigenetic or posttranslational phenomena in the development of UC-neoplasia or that the development of UC-neoplasia may be the result of genetic mutations in molecular pathways other than those that predispose to inflammation. Further studies centered on these concepts are warranted.

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