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Leading Off

Genetic Profiling of Colorectal Carcinomas of Patients with Primary Sclerosing Cholangitis and Inflammatory Bowel Disease

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Background: Patients with primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD) run a 10-fold increased risk of developing colorectal cancer (CRC) compared to patients with IBD only. The aim of this study was to perform an extensive screen of known carcinogenic genomic alterations in patients with PSC-IBD, and to investigate whether such changes occur already in nondysplastic mucosa.

Methods: Archival cancer tissue and nondysplastic mucosa from resection specimens of 19 patients with PSC-IBD-CRC were characterized, determining DNA copy-number variations, microsatellite instability (MSI), mutations on 48 cancer genes, and CpG island methylator phenotype (CIMP). Genetic profiles were compared with 2 published cohorts of IBD-associated CRC (IBD-CRC; $n = 11$) and sporadic CRC (s-CRC; $n = 100$).

Results: Patterns of chromosomal aberrations in PSC-IBD-CRC were similar to those observed in IBD-CRC and s-CRC, MSI occurred only once. Mutation frequencies were comparable between the groups, except for mutations in *KRAS*, which were less frequent in PSC-IBD-CRC (5%) versus IBD-CRC (38%) and s-CRC (31%; $P = .034$), and in *APC*, which were less frequent in PSC-IBD-CRC (5%) and IBD-CRC (0%) versus s-CRC (50%; $P < .001$). Cases of PSC-IBD-CRC were frequently CIMP positive (44%), at similar levels to cases of s-CRC (34%; $P = .574$) but less frequent than in cases with IBD-CRC (90%; $P = .037$). Similar copy number aberrations and mutations were present in matched cancers and adjacent mucosa in 5/15 and 7/11 patients, respectively.

Conclusions: The excess risk of CRC in patients with PSC-IBD was not explained by copy number aberrations, mutations, MSI, nor CIMP status, in cancer tissue, nor in adjacent mucosa. These findings set the stage for further exome-wide and epigenetic studies.

Lay Summary

The excessive risk of colorectal carcinoma (CRC) in patients with both primary sclerosing cholangitis and inflammatory bowel disease (IBD) was not explained by an extensive screen of copy number aberrations, mutations, microsatellite instability, and CpG island methylator phenotype status when compared with patients with IBD-CRC and sporadic CRC.

Key Words: primary sclerosing cholangitis, inflammatory bowel disease, colorectal cancer

Introduction

Patients with longstanding inflammatory bowel disease (IBD) are at risk of developing colorectal cancer (CRC) that is 2–3 times higher than in the general population.[1] This risk increases substantially in cases of concomitant primary sclerosing cholangitis (PSC), yielding a 3- to 4-fold higher risk for CRC in patients with both PSC and IBD (PSC-IBD) compared to patients with IBD alone.[2] In a nationwide cohort study in

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the Netherlands, this risk was even higher, with a 10-fold risk of developing CRC in patients with PSC and ulcerative colitis (UC) compared to patients with UC alone, and a cumulative risk of CRC of 13% at 30 years since IBD diagnosis.[3]

Little is known regarding the mechanisms behind this increased CRC risk in patients with PSC-IBD compared to patients with IBD alone. When low-grade dysplasia is detected in patients with PSC-IBD, they are at higher risk of developing advanced CRC compared to patients with IBD without PSC, suggesting a more aggressive disease course.[4] Moreover, since the development of CRC in IBD is thought to be influenced by chronic inflammation with subsequent dysplasia, the PSC-IBD colonic phenotype, with a predominance of involvement of the right-sided colon, where most of the neoplasia occurs, is intriguing.[5] Notwithstanding, this phenotype is often characterized by a relatively mild disease course with less inflammation, which seems contradictory to the relation between the inflammatory burden and dysplasia.[5,6]

It is unknown which genomic alterations are associated with PSC-IBD-associated CRC (PSC-IBD-CRC) compared to IBD-associated CRC (IBD-CRC), giving rise to the high risk of patients with PSC-IBD to develop CRC. In general, 3 molecular carcinogenesis pathways have been identified in CRC; chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP).[7] In several aspects, the molecular pathways occurring in IBD-CRC differ from those observed in sporadic CRC (s-CRC), in which the adenoma-to-carcinoma pathway is well established.[5,8] Mutations in tumor suppressor gene *APC* and oncogene *KRAS* are less prevalent in IBD-CRC versus s-CRC.[9,10] Furthermore, IBD-associated dysplasia more frequently shows DNA copy number aberrations compared to sporadic adenomas.[11] Also, the timing of pathway disruption may be different in IBD-CRC compared to s-CRC. For instance, mutations in tumor suppressor gene *TP53* usually occur as a late event in s-CRC, whereas they occur early in IBD-CRC development or are even already present in nondysplastic mucosa.[8,12]

Previous studies have suggested that chronically inflamed mucosa of patients with IBD undergoes a so-called field change, causing molecular alterations associated with cancer in histologically still nondysplastic mucosa.[13] Adjacent tissue from patients with UC with high-grade dysplasia or cancer showed hypermethylation of adjacent, normal-appearing mucosa.[14] Mutations in the *TP53* gene were found in a subset of biopsy IBD samples without dysplasia.[12] Also, in patients with PSC-UC, aberrant expression of p53 was found to already be significantly increased in nondysplastic mucosa as compared to in the nondysplastic mucosa of patients with UC without PSC.[15]

Because of the increased CRC risk, patients with concomitant PSC-IBD need annual surveillance colonoscopies, leading to a high diagnostic burden. Identification of early changes in the gut mucosa, heralding the development of CRC in patients with PSC-IBD, could pave the way for development of biomarkers for early detection of the dysplasia-carcinoma sequence in PSC-IBD. This could help to stratify patients for short or long surveillance intervals.

In this retrospective study, our aim was 2-fold. First, we aimed to investigate molecular alterations associated with CRC in patients with concomitant PSC-IBD and compare this with patients with IBD-CRC and s-CRC. Secondly, we intended to investigate whether genomic alterations present in the cancer also could be identified in adjacent, nondysplastic mucosa.

Methods

Patient and Sample Selection

Cases were selected from the well-defined cohort of the 'Epi PSC PBC project', a large, population-based cohort to study cholestatic liver diseases (PSC and primary biliary cholangitis [PBC]) in the Netherlands.[3] Out of 590 patients, 25 patients with PSC and concurrent IBD developed CRC during a median follow-up of 92 months. Formalin-fixed, paraffin-embedded (FFPE) biopsies and resection specimens were requested via the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA).[16] Tissue of the cancer itself, as well as of adjacent, nondysplastic mucosa, was collected based on the pathology reports. Cases with high-grade dysplasia were reevaluated by an experienced gastrointestinal pathologist (J.V.), and were not included if no cancer was diagnosed. Dysplasia was absent in all adjacent mucosa samples. The diagnosis of PSC was established according to the guidelines of the European Association for the Study of the Liver, and the diagnosis of IBD was based on the Lennard-Jones criteria.[3] The Medical Research Involving Human Subjects Act did not apply for this study. Collection, storage, and use of tissue and patients' data were performed in compliance with the Code for Proper Secondary Use of Human Tissue in the Netherlands (available at: http://www.coreon.org). All data and tissue samples were handled coded-anonymously throughout the study.

Control Group

Two published series were used as control groups. The first series consisted of IBD-associated CRC samples (n = 13), of which 2 samples from patients with concomitant PSC were excluded.[11] The second series consisted of cases of sporadic adenocarcinomas (n = 100).[17]

DNA Isolation

One pathologist (J.V.) evaluated hematoxylin and eosin–stained sections of the tumor and adjacent mucosa in order to distinguish between carcinoma tissue and adjacent, nondysplastic mucosa. After macro-dissection from 10-µm FFPE sections, DNA was isolated using the AllPrep DNA/RNA FFPE isolation kit (Qiagen). Briefly, slides were deparaffinized in series of xylene and ethanol, after which either of the 2 different conditions (cancer mucosa or adjacent mucosa) were scratched out from the tissue sections. DNA was isolated after incubation with proteinase K (20 mg/ml) at 56 °C in lysis buffer for an overnight incubation period. Small samples were incubated for a 6-day incubation period. DNA concentrations and purity were measured with a Nanodrop ND-1000 spectrophotometer (Isogen), as well as with a Qubit 3.0 Fluorometer (Thermo Fisher Scientific) using the Qubit dsDNA HS Assay Kit to measure double-stranded DNA.

Microsatellite Instability Status

The MSI analysis was performed using the fluorescent multiplex polymerase chain reaction (PCR)–based method from Promega (MSI Analysis System, version 1.2, Promega), according to the manufacturer's instructions. When 2 or more of the markers did not show the normal pattern, samples were

considered to have MSI. All other samples were classified as being microsatellite stable.

DNA Copy Number Analysis

DNA copy number aberrations were analyzed with low-coverage, whole-genome sequencing (WGS).[18] Briefly, DNA was fragmented by sonication (Covaris S2) and run on the Illumina HiSeq 2500 (Illumina) on a 65-basepair, single-read modus using the KAPA HyperPrepKit (KAPA Biosystems, KK8504). The WGS reads were analyzed with Bioconductor R-package QDNAseq, using a workflow that was previously published.[19] For every nonoverlapping, fixed-sized region of 30 kb on the genome, the relative abundance of sequence reads was used to determine the aberration status. Copy number profiles were corrected for their mappability and the GC content, and germ-line-specific variations were removed.[18] Genomic waves, which may be caused by replication timing of proliferating cells, were smoothed using NoWaves.[20] Raw DNA copy number data have been deposited in the European Genome-Phenome Archive (EGA)[21] with the study ID: EGAS00001004497.

Mutation Analysis

For mutation analysis, DNA libraries were prepared using the KAPA HyperPrep Kit (KAPA Biosystems), according to the manufacture's protocol (KR0961, V5.16). A gene panel consisting of 48 cancer-related genes was used (Table S1). Target enrichment was performed as previously described,[22] using a custom 48-gene IDT Custom xGen Predesigned Gene Capture Pools (Integrated DNA Technologies), according to the Rapid Protocol for DNA Probe Hybridization and Target Capture using an Illumina TruSeq Library, version 2.1, with an extended hybridization reaction of 24 hours. Paired-end 65-basepair sequencing data were produced on an Illumina Hiseq 2500 (Illumina) with rapid run mode. Variants affecting noncoding sequences, as well as variants present in ≥1% in the population according to the Exome Aggregation Consortium exome data, were excluded.[23] Oncoprints of the mutations in the genes present in each patient were generated using R Bioconductor, package ComplexHeatmap in R Studio. To appraise whether the derived variants were pathological mutations or not, the mutations were compared with the publicly available variant list from OncoKB and the Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census.[24,25] National Center for Biotechnology Information's remap tool was used to convert from hg38 assembly to hg19 coordinates, followed by a comparison using "bcftools" intersect. Additionally, ClinVar was consulted when a mutation was not present in either of those databases. Raw DNA mutation data have been deposited in the EGA[21] with the study ID: EGAS00001004497.

CIMP Status

The CIMP status was analyzed using a panel of 5 loci (CACNA1G, NEUROG1, RUNX3, SOCS1, and IGF2), as defined by Weisenberger et al.[26] This CIMP panel was determined by nested methylation-specific PCR (MSP) using sodium bisulfite–modified genomic DNA (EZ DNA methylation kit; ZYMO Research Co.), as described before.[27] When 3 or more loci were methylated, the sample was considered to be CIMP-positive; samples with less than 3 methylated loci were considered to be CIMP-negative.

Statistical Analysis

Patient characteristics were analyzed using descriptive statistics. Differences between 2 or 3 independent groups were calculated with the Mann-Whitney U test and Kruskal-Wallis test for continuous variables and the Fisher's exact test for dichotomous variables. Paired data were compared with a McNemar test. Results were considered statistically significant at a P value <.05. For the comparison of copy number aberration frequencies between 2 groups, R package CGHtest was used, which runs a chi-square test. In order to correct for multiple testing, a correction to the P value was performed according to the Benjamini and Yekutieli false discovery rate (FDR) rule, using a cutoff for significance of FDR < 0.1.[28] For the unsupervised data analysis, a hierarchical cluster analysis of the copy number aberrations was performed using weighted clustering of called array Comparative Genomic Hybridization (aCGH) data, using Ward linkage.[29]

Results

Clinical Characteristics

Nineteen patients with PSC-IBD who developed CRC (PSC-IBD-CRC) were included, and their clinical characteristics are described in Table 1. All patients were diagnosed with large-duct PSC. The vast majority of patients were diagnosed with UC (18 [95%] with UC; 1 [5%] with Crohn's disease [CD]). The median age at CRC diagnosis was 38 years (interquartile range [IQR], 30–56 years), with a median IBD duration of 14 years (IQR, 10–23 years) and a median duration since diagnosis of PSC of 4 years (IQR, 0–13 years). In 3 cases, patients developed overt PSC only after the diagnosis of CRC (range, 5–20 years). Two patients developed cholangiocarcinoma after their diagnosis of CRC, but no other malignancies were reported. The control group of patients with IBD without a diagnosis of PSC (IBD-CRC) contained 11 CRCs in 9 patients.[11] The age at IBD diagnosis and duration of IBD at the time of the CRC diagnosis of patients with diagnosed PSC did not differ from those in the patients with IBD without PSC (Table 1). Patients with PSC-IBD developed CRC at a younger age than patients without PSC (median ages of 38 years and 48 years, respectively; P = .061). The s-CRC series consisted of 100 carcinoma samples from patients diagnosed with CRC between 2001 and 2010. Patients with hereditary CRC (ie, Lynch syndrome or polyposis syndromes) or a previous history of CRC or IBD were excluded from this cohort.[17] The age at CRC diagnosis was significantly higher in the s-CRC group compared with both the PSC-IBD-CRC and IBD-CRC groups (median, 72 years; P < .001; Table 1).

Comparison Between Molecular Profiles of PSC-IBD-CRC, IBD-CRC, and s-CRC

In order to assess whether CRCs of patients with PSC comprise a specific molecular phenotype, we compared the specimens of PSC-IBD-CRC with 2 control data sets (IBD and sporadic data sets). The MSI status was successfully determined for 17 cancers; in 1 sample the signal was too low to determine the MSI, due to an insufficient DNA yield, and in 1 sample not enough DNA was available (Figure S1). Only 1 out of the 17 cancer samples was MSI (6%). This patient was known to have UC for 9 years, but developed PSC 5 years after the CRC diagnosis. In both the IBD control group and cases of sporadic carcinomas, 9% were MSI (1/11 IBD-CRC and 9/96 s-CRC; Table 1).

Table 1. Patient characteristics of patients with PSC-IBD-CRC, IBD-CRC, and s-CRC.[a]

Characteristic	PSC-IBD-CRC *n* = 19	IBD-CRC, *n* = 9	s-CRC, *n* = 100	*P* value
Male, *n* (%)	9 (47)	4 (44)	43 (43)	.947
Age at PSC diagnosis, years, median (IQR)	35 (23–46)	-	-	-
Age at IBD diagnosis, years, median (IQR)	22 (17–39)	34 (22–40)	-	.562
IBD type, *n* (%)				.084
UC	18 (95)	6 (67)	-	
CD	1 (5)	3 (33)	-	
Age at CRC diagnosis, median (IQR)	38 (30–56)	48 (44–59)	72 (63–78)	<.001
Disease duration of PSC, years, median (IQR)	4 (0–13)	-	-	-
Disease duration of IBD, years, median (IQR)	14 (10–23)	16 (10–30)	-	.410
UC phenotype,[b] *n* (%)*				.494
Pancolitis	10 (77)	9 (100)		
Left sided	2 (15)	0 (0)		
Proctitis	1 (8)	0 (0)		
Post LTx, *n* (%)	1 (5)	-	-	-
MSI status,[c] *n* (%)				.673
MSI	1 (6)	1 (9)	9 (9)	
MSS	16 (94)	10 (91)	87 (91)	

[a] Statistical differences were calculated with a Fisher's exact test, Kruskal-Wallis test, or Mann-Whitney U test. A *P* value < .05 is considered statistically significant. Abbreviations: CD, Crohn's disease; CRC, colorectal cancer; IBD, inflammatory bowel disease; IBD-CRC, colorectal cancer associated with inflammatory bowel disease; IQR, interquartile range; LTx, liver transplantation; MSI, microsatellite instability; MSS, microsatellite stable; PSC, primary sclerosing cholangitis; PSC-IBD, both primary sclerosing cholangitis and inflammatory bowel disease; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease; s-CRC, sporadic colorectal cancer; UC, ulcerative colitis.
[b] The UC phenotype was known for 13 patients with PSC-IBD and 9 patients with IBD.
[c] The MSI was determined for 17 PSC-IBD samples, 11 IBD samples, and 96 sporadic samples.

Patterns of Chromosomal Aberrations in PSC-IBD-CRC are Similar to Those Observed in IBD-CRC and s-CRC

Good-quality DNA copy number profiles were obtained from all 19 cancer samples (Figure S1). Several DNA copy number gains and losses were observed in PSC-IBD-CRC, with an average of 9.8 gains (range, 0–16 gains) and 8.2 losses (range, 0–12 losses). Frequently observed gains were present in 5p, 6p, 7, 8q, 13q, 19q, and 20q. Frequently observed losses occurred in chromosome 4, 5q, 8p, 15q, 18, 21q and 22q (Figure 1A, middle panel). In the cancer sample of the only patient with an MSI cancer, almost no aberrations were present, as expected.

The patterns of chromosomal aberrations in PSC-IBD-CRCs were very similar to those of sporadic and IBD-associated CRCs, and the averages of gains and losses were similar between groups (Figure 1A; Table S1). The frequencies of different gains and losses were overall higher in the s-CRC and IBD-CRC groups compared with the PSC-IBD-CRC group (Figure 1A), but after correction for multiple testing, no significant differences were found between the groups. The IBD-CRCs showed significant losses in chromosomes 3p and 17p compared to the PSC-IBD-CRCs, yet this did not remain significant after correction for multiple testing (FDR > 0.1; Figure 1A, upper panel). Compared to the s-CRC group, the PSC-IBD-CRC group showed a significant gain in the q-arm of chromosome 19 and a significant loss in the q-arm of chromosome 5, though again this did not remain statistically significant after correction for multiple testing (FDR > 0.1; Figure 1A, lower panel). This loss in 5q was also present in the IBD-CRC group.

The Gene *TP53* is Mutated in the Majority of PSC-IBD-CRCs

Mutation analysis was successful in all 19 cancer samples (Figure S1). Targeted deep sequencing was performed using a panel of 48 genes that are known to be frequently mutated in cancer (Table S2). From the 48 genes analyzed, 24 were mutated in the PSC-IBD-CRCs (Figure 1B). *TP53* was the most frequently mutated gene, present in 68% (13/19) of the cancers (Figure 1B). The second most mutated gene was *NOTCH1*, in 21% (4/19) of the cancers. Both *KRAS* and *APC* mutations were only present in 1 patient (5% [1/19] and 5% [1/19], respectively), and no *BRAF* mutations were found. Table 2 gives an overview of the mutated genes in the cancer samples and their suspected pathogenicity. *TP53* detected mutations involved 10 different variants. The majority of these mutations were allocated as pathogenic. The mutation observed in the *NOTCH1* gene was predicted to be a benign mutation. The only *APC* mutation, present in 1 patient, was considered a benign mutation. More detailed information about the specific variants is depicted in Table S3.

Mutation statuses for the 48 genes analyzed in the 19 PSC-IBD-CRCs were compared with mutation statuses obtained from 8 IBD-CRCs and 80 s-CRCs.[11,17] Mutation frequencies of the majority of genes mutated in the PSC samples did not differ between the groups (Table 3). The highest mutated gene throughout all 3 groups was again *TP53*, of which the mutation frequency did not differ significantly between the PSC-IBD-CRCs as compared to the IBD-CRCs and the s-CRCs (68%, 75%, and 48%, respectively; *P* = .134). However, mutations in the *KRAS* gene were significantly less frequent in PSC-IBD-CRCs than in IBD-CRCs and s-CRCs

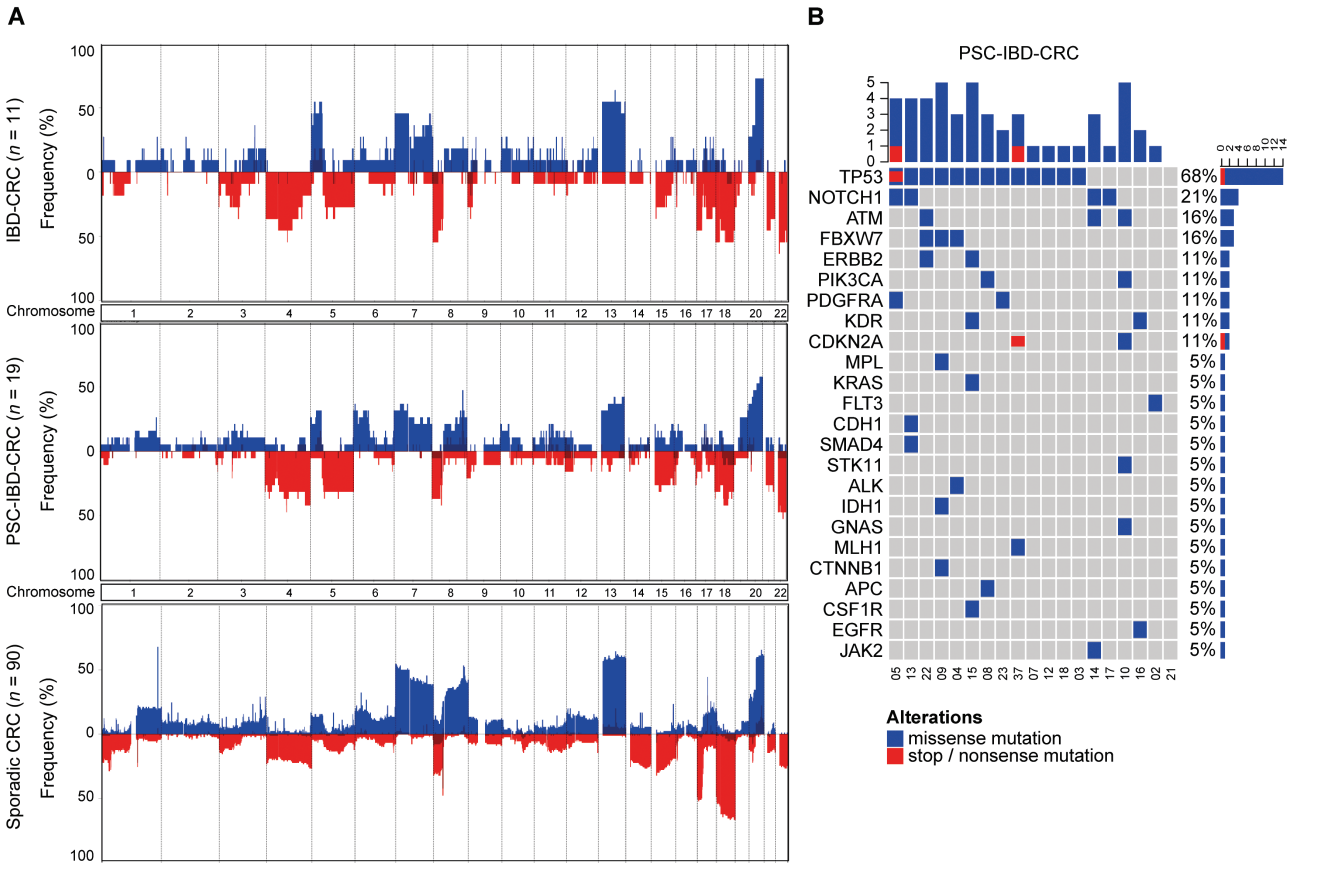

Figure 1. Copy number aberrations in PSC-IBD-CRC, IBD-CRC, and s-CRC and mutation frequencies in PSC-IBD-CRCs. A, Frequency plots of the copy number aberrations in cancer samples of IBD-CRC (upper panel; *n* = 11), PSC-IBD-CRC (middle panel; *n* = 19), and s-CRC (lower panel; *n* = 90). Copy number gains (blue) and amplifications (dark blue) or losses (red) and homozygous deletions (dark red) on the different chromosomes are shown. B, Oncoprint of the top 24 mutated genes from the 48-gene mutation panel in PSC-IBD-CRCs (*n* = 19). Abbreviations: CRC, colorectal cancer; IBD, inflammatory bowel disease; IBD-CRC, colorectal cancer associated with inflammatory bowel disease; PSC, primary sclerosing cholangitis; PSC-IBD, concomitant primary sclerosing cholangitis and inflammatory bowel disease; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease; s-CRC, sporadic colorectal cancer.

(5%, 38%, and 31%, respectively; $P = .034$). Mutations in the *APC* gene were less prevalent in the PSC-IBD-CRC and IBD-CRC groups as compared to the s-CRC group (5%, 0% and 50% respectively; $P < .001$). Mutations in the *NOTCH1* gene were present in 21% of the PSC-IBD-CRCs and none of the other 2 cancer types, yet these mutations were not allocated as being pathogenic in the COSMIC and OncoKB databases (Table 2).

A Large Proportion of PSC-IBD-CRCs has a CIMP-positive Phenotype

For a determination of CIMP status, DNA was available from 18 cancer samples. In 2 cases the test failed; hence, results could not be assessed (Figure S1). Seven out of 16 cancers (44%) showed a CIMP-positive phenotype. One CIMP-positive cancer also displayed an microsatellite instable profile. There were no differences in the age at CRC diagnosis, PSC or IBD duration, or cancer location between patients with a CIMP-positive or CIMP-negative cancer (Table S4). In the s-CRC series, 34 out of 100 (34%) of the cancers was CIMP positive, which was not significantly different from the result in the PSC-IBD group ($P = .574$). In the IBD-CRC group, 9 out of 10 (90%) cancers was CIMP positive, which was significantly higher than in the PSC-IBD-CRC group ($P = .037$).

No Clear Separation of PSC-IBD-CRCs and IBD-CRCs in Hierarchical Clustering Analysis

To provide an overview of the different molecular characteristics and to address whether this shows new insights contributing to the cancer formation in patients with IBD, with and without concomitant PSC, a hierarchical cluster analysis of the copy number aberrations was performed (Figure 2). There was no clear separation of the patients with and without PSC. Additionally, there was no separation evident based on the disease location, IBD duration, or PSC duration. Five cancers with only minimal chromosomal aberrations also showed fewer mutations in the *TP53* gene.

Comparison of PSC-IBD Cancers and Adjacent Mucosa

To assess potential early defects or changes in nondysplastic colonic mucosa of patients with PSC-IBD, DNA was isolated from mucosal tissue adjacent to the cancerous tissue. The copy number variations, mutation profile, and CIMP status were determined.

DNA Copy Number Aberrations are Present in Adjacent Mucosa

Good-quality DNA copy number profiles were obtained from 15 adjacent mucosa samples (Figure S1). The adjacent,

Table 2. Genes mutated in 19 PSC-IBD-CRC.[a]

Gene	Mutations, *n*	Mutated samples, *n* (%)	Mutations in COSMIC Cancer Gene Census	Mutations in OncoKB
TP53	10	13 (68)	10/10 pathogenic	6/10 likely oncogenic, 2/10 oncogenic
NOTCH1	3	4 (21)	-	-
ATM	3	3 (16)	2/3 pathogenic, 1/3 SNP	-
FBXW7	2	3 (16)	1/2 pathogenic	1/2 likely oncogenic
ERBB2	2	2 (11)	2/2 pathogenic	1/2 oncogenic
PIK3CA	2	2 (11)	2/2 pathogenic	1/2 oncogenic
PDGFRA	2	2 (11)	-	-
KDR	2	2 (11)	1/2 pathogenic	-
CDKN2A	2	2 (11)	2/2 neutral	-
MPL	1	1 (5)	-	-
KRAS	1	1 (5)	1/1 pathogenic	1/1 oncogenic
FLT3	1	1 (5)	1/1 pathogenic	-
CDH1	1	1 (5)	-	-
SMAD4	1	1 (5)	1/1 pathogenic	-
STK11	1	1 (5)	1/1 pathogenic	-
ALK	1	1 (5)	-	-
IDH1	1	1 (5)	-	-
GNAS	3	1 (5)	-	-
MLH1	1	1 (5)	-	-
CTNNB1	1	1 (5)	-	-
APC	1	1 (5)	-	-
CSF1R	1	1 (5)	-	-
EGFR	1	1 (5)	1/1 pathogenic	-
JAK2	2	1 (5)	1/1 pathogenic	-

[a] A hyphen (-) indicates that the value is not present in the database. Abbreviations: COSMIC, catalogue of somatic mutations in cancer; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease; SNP, single nucleotide polymorphism.

non-dysplastic mucosa was relatively quiescent in most PSC samples, showing only a few DNA copy number aberrations compared to the cancer mucosa samples (Figure 3). The average numbers of gains and losses in the adjacent mucosal samples were 3.2 (range, 0–15 gains) and 2.9 (range, 0–8 losses), respectively. In 5 out of 15 paired samples, 1 or more aberrations that were found in the cancer tissue were also present in nondysplastic, adjacent mucosa (Figure S2). In 2 cases, aberrations present in the adjacent mucosa were not present in the cancer tissues. This included a gain in chromosome 7q and losses in chromosomes 5q, 8q, and 12p in 1 patient, and a loss in chromosome 6p in another patient. All patients with mucosal aberrations were diagnosed with concomitant UC, and the majority were male (4/5; 80%). There was a trend towards a longer disease duration of PSC in patients with aberrations in the adjacent mucosa compared to patients with no changes in the mucosa (25 years vs 2 years, respectively; $P = .055$).

Mucosa Adjacent to the Cancer Also Shows Mutations

Paired samples were used to assess whether mutations present in the cancer tissue were also present in the non-dysplastic, adjacent mucosa, reflecting a widespread defect in mucosal tissue. Mutation profiles of 48 genes were addressed in mucosal, nondysplastic tissue adjacent to the cancer (proximal from the tumor when possible) and compared with the mutation profile in the cancer itself. Matched cancer and adjacent mucosal tissue were available from 11 patients. In 7 out of these 11 patients, at least 1 mutation was present in both the cancer and adjacent mucosa, and in total, 37% (95% CI, 22%–55%) of detected mutations occurred in both tissues. Identical mutations were present in genes *TP53* (2/11), *FBXW7* (1/11), *PDGFRA* (2/11), *NOTCH1* (2/11), *ATM* (1/11), *MPL* (1/11), *FLT3* (1/11), *IDH1* (1/11), *CTNNB1* (1/11), and *KDR* (1/11; Table 4). The 2 patients with mutations in the *TP53* gene in the adjacent mucosa shared 3 different *TP53* mutations, all characterized as pathogenic according to the COSMIC Cancer Gene Census. In the majority of mutations present in adjacent mucosa, the same mutation was also present in the cancer tissue (13/15; 87%; Figure S3). In the 2 cases in which this did not apply, the mutation was predicted to be either pathogenic (*TP53*, p.Trp146) or a mutation with unknown significance (*FBXW7*, p.Thr385Ile).

CIMP Phenotype is Variable Across Cancer and Adjacent Mucosa

For determination of CIMP status, DNA was available from 15 adjacent, nondysplastic mucosa samples. In 4 cases, results could not be assessed, as the test failed (Figure S1). A paired analysis was available in 10 patients. The cancers showed a CIMP-positive phenotype in 5/10 (50%) cases, and the majority (3/5; 60%) of adjacent mucosal samples in these cases also showed a CIMP-positive phenotype. In 3 patients, the cancer was CIMP negative, but the adjacent mucosa was CIMP positive. Overall, there was no significant relation

Table 3. Mutation frequencies in PSC-IBD-CRC (n = 19), IBD-CRC (n = 8), and s-CRC (n = 80).[a]

Gene	PSC-IBD-CRC, *n* (%)	IBD-CRC, *n* (%)	s-CRC, *n* (%)	*P* value
TP53	13 (68)	6 (75)	38 (48)	.134
NOTCH1	4 (21)	0 (0)	0 (0)	.001
ATM	3 (16)	0 (0)	4 (5)	.186
FBXW7	3 (16)	0 (0)	9 (11)	.657
ERBB2	2 (11)	0 (0)	1 (1)	.156
PIK3CA	2 (11)	2 (25)	14 (18)	.663
PDGFRA	2 (11)	0 (0)	2 (3)	.264
KDR	2 (11)	0 (0)	2 (3)	.264
CDKN2A	2 (11)	0 (0)	1 (1)	.156
MPL	1 (5)	0 (0)	0 (0)	.252
KRAS	1 (5)	3 (38)	25 (31)	.034
FLT3	1 (5)	0 (0)	0 (0)	.252
CDH1	1 (5)	0 (0)	4 (5)	1.000
SMAD4	1 (5)	1 (13)	9 (11)	.744
STK11	1 (5)	0 (0)	3 (4)	.694
ALK	1 (5)	0 (0)	0 (0)	.252
IDH1	1 (5)	0 (0)	0 (0)	.252
GNAS	1 (5)	0 (0)	0 (0)	.252
MLH1	1 (5)	0 (0)	1 (1)	.443
CTNNB1	1 (5)	0 (0)	0 (0)	.252
APC	1 (5)	0 (0)	40 (50)	<.001
CSF1R	1 (5)	0 (0)	0 (0)	.252
EGFR	1 (5)	0 (0)	1 (1)	.443
JAK2	1 (5)	0 (0)	0 (0)	.252

[a] The *P* values were calculated with a Kruskal-Wallis test. A *P* value < .05 is considered statistically significant. Abbreviations: IBD-CRC, colorectal cancer associated with inflammatory bowel disease; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease; s-CRC, sporadic colorectal cancer; UC, ulcerative colitis.

between the CIMP statuses of cancer and adjacent mucosal tissue (P = 1.000). The age at CRC diagnosis was significantly higher in those with CIMP-positive mucosa samples compared to those with CIMP-negative mucosa samples (median ages 54 years and 31 years, respectively; P = .038). Mutation data was available in 5/6 CIMP-positive mucosa samples. Of these, only in 2 patients mutations were present in the mucosa; a pathogenic *TP53* mutation and 2 likely benign *PDGFRA* and *NOTCH1* mutations in 1 case, and a likely pathogenic *ATM* mutation in the other case. From the CIMP-negative mucosa samples, mutations were detected in the *FDGFRA* and *FBXW7* genes in 1 patient (both of unknown significance) and in *FLT3* and *KDR* in 2 other patients (both likely pathogenic).

Discussion

Patients with concomitant PSC and IBD have a much higher risk of developing CRC than patients with IBD alone, but it is unclear whether the molecular phenotypes of these carcinomas are distinct. We performed the first extensive molecular characterization of PSC-IBD associated CRCs to date. DNA copy number changes in PSC-IBD-CRCs were similar to those of IBD-CRCs and s-CRCs. In case of a difference, mutation frequencies were overall lower in PSC-IBD-CRC compared to s-CRC. Mutation frequencies of PSC-IBD-CRC and IBD-CRC were rather comparable. A substantial percentage of PSC-IBD-CRCs showed a hypermethylated phenotype, suggesting that epigenetic changes may play an important role; however, this was less prominent than in IBD-CRCs.

The PSC-IBD-CRCs harbored many copy number aberrations that are well established in CRC, including losses on chromosomes 5 and 18, which are both linked to more aggressive disease.[9,30] The PSC-IBD phenotype was not associated with major differences in terms of DNA copy number patterns compared to both the IBD-CRC and s-CRC phenotypes. In the series analyzed, only 1 lesion was MSI, which is in line with previous studies showing that MSI is rare in colitis-associated CRC and nonmalignant lesions.[9,31]

The most frequently mutated gene in this cohort was *TP53*, a known early event in IBD-associated tumorigenesis. The mutation frequency was comparable to those in previous studies reporting *TP53* mutation frequencies ranging from 63% to 89% in IBD-CRCs, suggesting that this mutation is also an early event in PSC-IBD-CRC.[10,32] Mutations in the *APC* gene, which is located on the 5q arm, were present only in 1 patient (5%). This is in line with previous observations that IBD-CRCs have a lower frequency of APC mutations compared to s-CRCs.[10,32,33] We did observe a high frequency of loss of the 5q arm in our series, which could have led to a disruption of the *APC* gene. Interference with the WNT signaling pathway could therefore be caused by copy loss of *APC* rather than

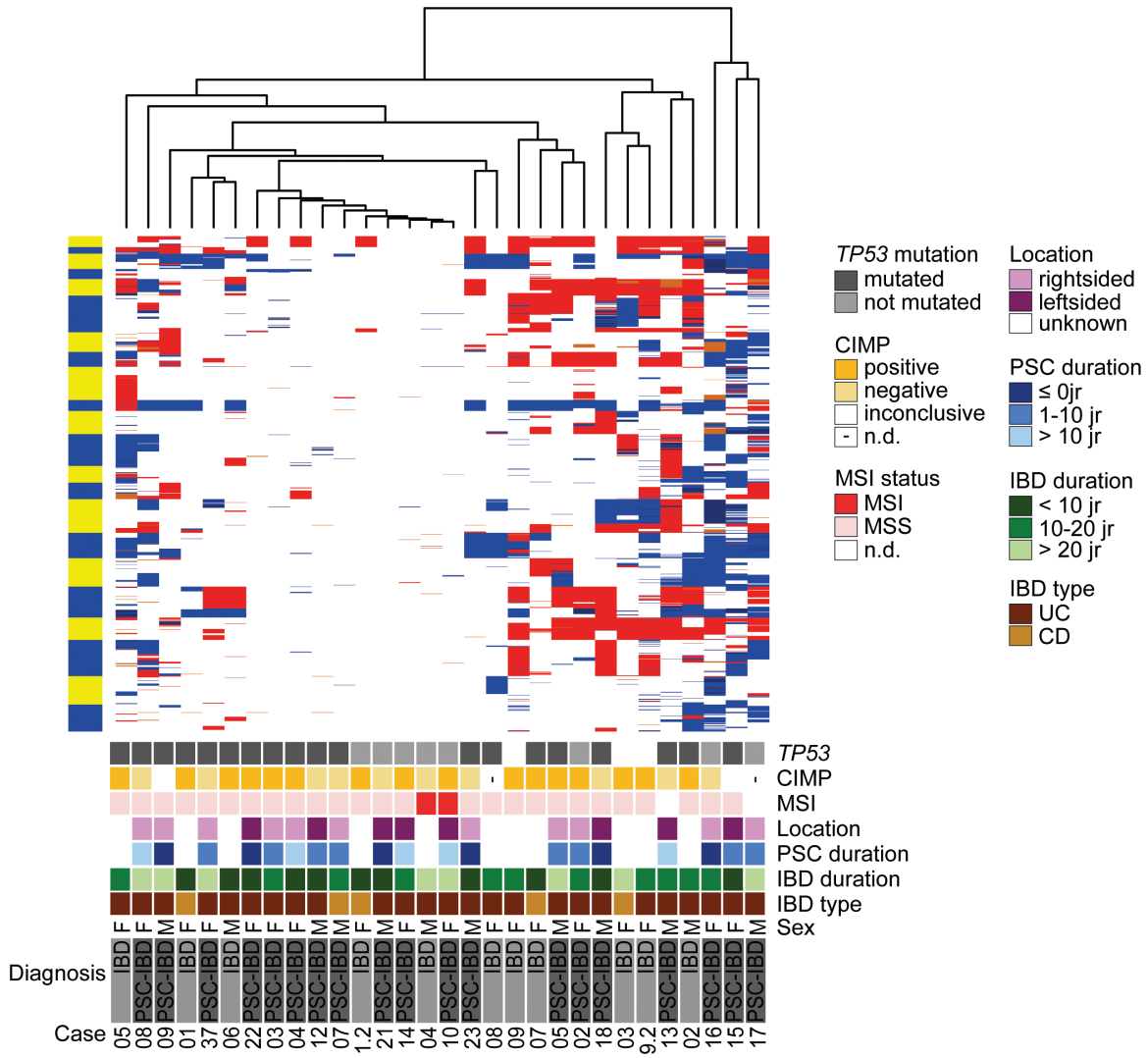

Figure 2. Hierarchical cluster analysis of PSC-IBD-CRC and IBD-CRC. Hierarchical cluster analysis of the copy number aberrations using weighted clustering of called aCGH data, with Ward linkage. Copy number gains (blue) or losses (red) on the different chromosomes are shown. Different chromosomes are depicted on the left in blue and yellow, with chromosome 1–22 listed from the bottom up. PSC-IBD-CRC (n = 19) and IBD-CRC (n = 11) were included. Mutations with a frequency >50% are shown. Abbreviations: CD, Crohn's disease; CIMP, CpG island methylator phenotype; IBD, inflammatory bowel disease; IBD-CRC, colorectal cancer associated with inflammatory bowel disease; MSI, microsatellite instability; MSS, microsatellite stable; n.d., not determined; PSC, primary sclerosing cholangitis; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease; UC, ulcerative colitis.

mutations, which was previously suggested in IBD-CRC.[9] Alternatively, 1 study showed that in IBD, methylation of WNT signaling genes occurs, thereby circumventing the need for *APC* mutations for a cancer to develop.[34]

DNA methylation interferes with the accessibility of a gene to transcription factors, and has been linked to silencing of mismatched repair genes and tumor suppressor genes in cancer.[27] A meta-analysis study has shown a shorter survival time in patients with CIMP-positive s-CRCs.[35] The role of hypermethylation in IBD-CRC remains rather unclear, with a varying prevalence between 5% and 15% reported amongst studies.[36,37] Interestingly, in our samples, 7/16 (44%) of the PSC-IBD-CRCs and 9/10 (90%) of the IBD-CRCs displayed a CIMP-positive phenotype, compared to 34/100 (34%) in the s-CRCs. This could suggest that especially in the IBD population, promotor methylation is an important player in tumorigenesis, which seems to be less the case in the PSC-IBD-CRCs. Nevertheless, we investigated the methylation status based on a specific panel of 5 loci, reflecting only a small proportion of possible epigenetic interference. Therefore, it would be of interest to further characterize the individual methylation profiles of the PSC-IBD-CRCs.

Overall, our data did not allow us to pinpoint the PSC-IBD-CRCs towards a specific tumor profile. The relatively high CIMP positivity could indicate a more serrated-like lesion phenotype.[38] However, we did not observe any *BRAF* mutations in the 19 cancers, and only 1 cancer showed MSI. Furthermore, the low abundance of *KRAS* mutations and the absence of *NRAS* mutations suggests that different mechanisms are in place for further progression from dysplasia to carcinoma. An alternative explanation could be that for PSC-IBD, the relevant mutations are not included in the panels we applied in this exploratory study. For example, the cancer stemness gene

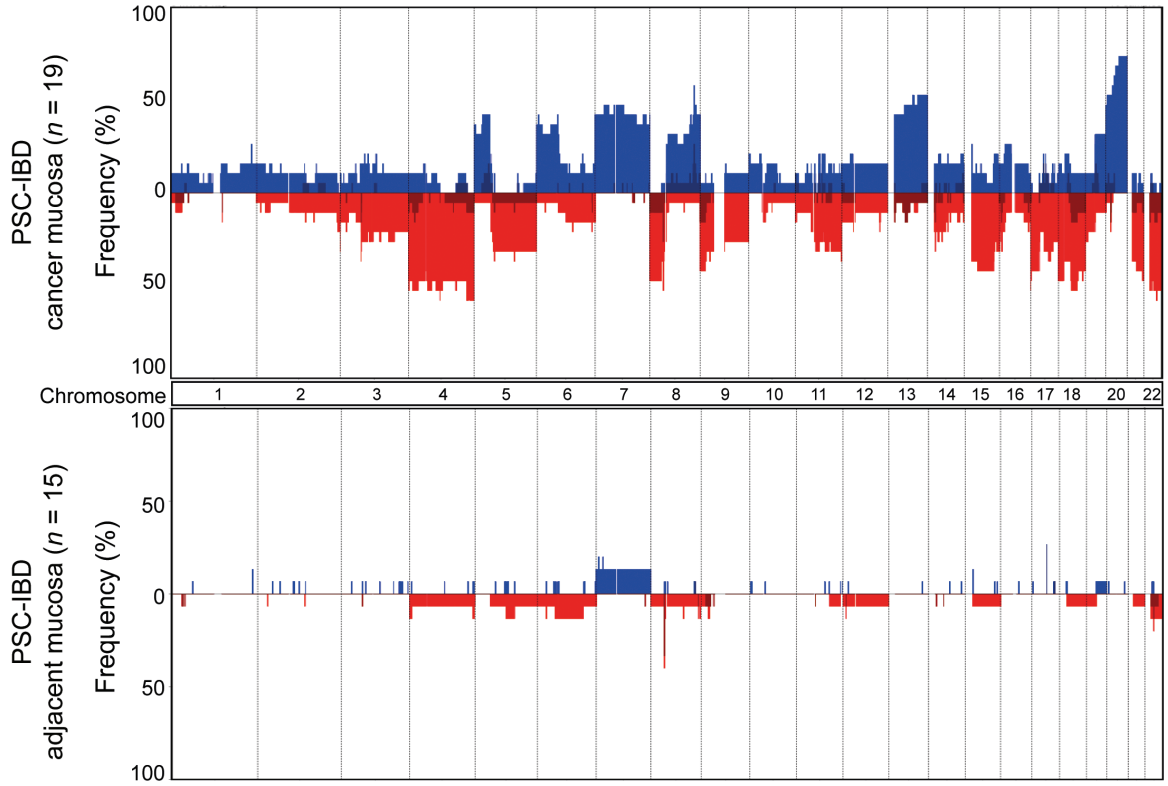

Figure 3. Copy number aberrations in cancer samples and adjacent mucosa of patients with PSC-IBD. Frequency plots of the copy number aberrations in 19 cancer samples of PSC-IBD-CRC (upper panel) and 15 adjacent mucosa samples (lower panel). Copy number gains (blue) and amplifications (dark blue) or losses (red) and homozygous deletions (dark red) on the different chromosomes are shown. Abbreviations: PSC-IBD, concomitant primary sclerosing cholangitis and inflammatory bowel disease; PSC-IBD-CRC, colorectal cancer associated with primary sclerosing cholangitis and inflammatory bowel disease.

OLFM4 was overexpressed in colonic biopsies of patients with PSC-UC.[39] Also, the recent study of Pleguezuelos-Manzano et al.[40] may be of particular interest. The authors showed a distinct mutational signature in CRC, induced via colibactin-producing *Escherichia coli* bacteria carrying the *pks* pathogenicity island.[40] Colonic dysbiosis has been firmly established in both IBD and PSC-IBD; however, the abundance of this specific *E. coli* strain is unknown.[41] Additionally, we did not investigate the potential role for changes associated with the bile acid metabolism giving rise to altered concentrations of potentially carcinogenic secondary bile acids.[41]

The second aim of this study was to address whether in patients with PSC-IBD, changes arise either from a field defect or from noxious DNA changes in the mucosa. Previous studies in UC have shown that field defects occur throughout the colons of patients with UC with dysplasia or cancer.[13] A recent study by Pekow et al.[42] showed fewer differences in cancers and adjacent tissues of patients with UC-CRC compared to cancers and adjacent tissues in patients with s-CRC, suggesting some changes were already present in the UC mucosa. Our findings suggest that only in a subset of patients, changes present in the cancer extend to a wider surface, exceeding the malignant mucosa.

Although mutations were present in the adjacent mucosa of a substantial amount of patients, the number of mutations present were small; between 0 and 2 mutations per patient, with the exception of 1 case with 6 genes mutated. We confirmed prior findings that mutations in tumor suppressor gene *TP53* can be present in nondysplastic mucosa in patients with PSC-IBD.[15] Previous studies have shown this in IBD mucosa, and showed an increased *TP53* mutation load in relation to inflammation.[12] We did also observe *TP53* mutations in the adjacent mucosa of our PSC-IBD series. However, in general, the degree of colonic inflammation in patients with PSC-IBD is relatively low, and in this study we could not evaluate inflammation, as we did not have information on the inflammatory status at the time of resection. The second most mutated gene in our mucosal samples was *FBXW7*, a tumor suppressor gene whose mutations are associated with a variety of cancer types, including CRC.[43] One of the *FBXW7* mutations, which was present in both cancer and adjacent mucosa in 1 patient (p.Arg465His), was described in cholangiocarcinoma as well as colon carcinoma in a study characterizing primary human tumors.[43] The other *FBXW7* mutation was only present in adjacent mucosa, which has also been described in a recent study by Lee-Six et al.[44] who showed that several driver mutations, including mutations in *FBXW7*, but also *PIK3CA* and *ERBB2*, appear in normal colonic crypts.

Of the adjacent mucosal samples, 60% showed a CIMP-positive phenotype. In UC, it has been shown that in cases of high-grade dysplasia or cancer, normal-appearing (non-dysplastic) mucosa can be highly methylated.[14] In our series, 3 cases showed a CIMP-positive phenotype in the normal mucosa, whereas the cancer from the same patient was not CIMP positive, suggesting that this hypermethylated status does not appear throughout the colonic mucosa.

This study has some limitations. Although this PSC-IBD-CRC series is unique, the number of samples was

Table 4. Mutations present in cancer mucosa samples and frequencies of the same mutation appearing in adjacent nondysplastic mucosa of 11 patients with PSC-IBD.[a]

Gene	Mutation	Cancer	Adjacent mucosa	Both cancer and mucosa	Not present
MPL	p.Arg102Pro	0/11	0/11	1/11	10/11
ATM	p.Ala220Val	1/11	0/11	0/11	10/11
	p.Asp1853Val	0/11	0/11	1/11	10/11
FLT3	p.Ser102Phe	0/11	0/11	1/11	10/11
TP53	p.Arg282Gln	0/11	0/11	1/11	10/11
	p.Arg280Thr	1/11	0/11	1/11	9/11
	p.Arg273His	1/11	0/11	0/11	10/11
	p.Val272Met	1/11	0/11	0/11	10/11
	p.Glu271	1/11	0/11	0/11	10/11
	p.Gly266Glu	1/11	0/11	0/11	10/11
	p.Arg248Gln	3/11	0/11	0/11	8/11
	p.Arg175His	1/11	0/11	0/11	10/11
	p.Trp146	0/11	1/11	0/11	10/11
ERBB2	p.Ser310Phe	1/11	0/11	0/11	10/11
STK11	p.Pro179Leu	1/11	0/11	0/11	10/11
ALK	p.Gly756Ser	1/11	0/11	0/11	10/11
IDH1	p.Asp220Gly	0/11	0/11	1/11	10/11
GNAS	p.Pro22Leu	1/11	0/11	0/11	10/11
	p.Glu192Asp	1/11	0/11	0/11	10/11
MLH1	p.Arg659Gln	1/11	0/11	0/11	10/11
CTNNB1	p.Ile607Val	0/11	0/11	1/11	10/11
PIK3CA	p.Asp725Asn	1/11	0/11	0/11	10/11
PDGFRA	p.Thr200Ser	0/11	0/11	1/11	10/11
	p.Arg293His	0/11	0/11	1/11	10/11
KDR	p.Ser112Leu	0/11	0/11	1/11	10/11
FBXW7	p.Arg465His	1/11	0/11	1/11	9/11
	p.Thr385Ile	0/11	1/11	0/11	10/11
	p.Trp321Cys	1/11	0/11	0/11	10/11
EGFR	p.Asn139Asp	1/11	0/11	0/11	10/11
CDKN2A	p.Arg128Gln	1/11	0/11	0/11	10/11
	p.Arg58	1/11	0/11	0/11	10/11
NOTCH1	p.Arg1350Leu	0/11	0/11	1/11	10/11
	p.Arg621His	0/11	0/11	1/11	10/11

[a] Abbreviation: PSC-IBD, concomitant primary sclerosing cholangitis and inflammatory bowel disease.

limited. Therefore, for some of the comparisons the power to detect statistically significant differences may be lacking. Notwithstanding, the large difference in cancer risk between patients with PSC-IBD, IBD, and patients who develop s-CRC suggests a strong contrast in any of the molecular alterations, should there be 1 or more responsible for the excess CRC risk in PSC-IBD cases. A recent study by Trivedi et al.[45] pointed out that a diagnosis of IBD at an age younger than 40 was associated with a greater CRC risk in patients with PSC-IBD. The majority (15/19; 79%) of patients included in our study were diagnosed with IBD at an age below 40, suggesting that we were indeed looking at this high-risk group. In addition, we also applied hierarchical clustering to look for combinations of several molecular alterations, together with phenotypic characteristics that may each have small individual effect sizes but together may explain the high cancer risk. Hierarchical clustering also did not reveal such a synergistic phenomenon. There was a significant difference in age between the s-CRC group and the other 2 groups. However, this is unavoidable since sporadic cancer develops at an older age. Three patients with IBD and PSC developed their CRC before they were diagnosed with PSC. The ages at CRC diagnosis were 26, 27, and 60 years. The young ages of 2 of the 3 patients suggest that the underlying PSC was indeed an effector. Nonetheless, another underlying cause cannot be excluded. A sensitivity analysis excluding these 3 patients did not change the outcomes. Because our data did not reveal a common denominator in either carcinoma or non-dysplastic adjacent tissue in the PSC-IBD-CRC group, we did not study earlier surveillance biopsies from these patients. Although unlikely, a neoplasia heralding pattern based on the read-outs used in our study is not fully excluded by our current findings.

Although we applied a large and well-established panel of 48 genes known to be involved in cancer, this may not cover all potentially carcinogenic mutations that could underlie the development of CRC in patients with PSC. Other,

more extensive approaches, like exome sequencing covering all exomes, could be a possibility to discover genetic variants or mutations contributing to carcinoma formation in the PSC setting. In s-CRC, polygenic risk scores have proven valuable for predicting the CRC risk.[46] In PSC, the attribution of genetic variation to carcinoma formation is unclear, and no genome-wide association studies studies have been performed yet. The ongoing formation of large-scale, prospective registries of patients with PSC-IBD could aid in collecting larger sample sizes to study CRC in PSC-IBD.

Biomarkers for a more personalized surveillance strategy are highly needed, since there are still patients with PSC-IBD who die from incurable CRC despite regular surveillance, while others are submitted to a high burden of colonoscopies but might not profit from them. In this first-ever exploratory study, we found no striking differences between PSC-IBD-CRC, IBD-CRC, and s-CRC, despite their contrasting phenotypical characteristics. The excess risk of CRC in patients with PSC-IBD is not explained by differences in copy number aberrations, the MSI or CIMP status, nor the mutational burden based on a large panel of 48 cancer genes. Our findings set the stage for exome-wide or epigenetic studies to better understand the development of CRC in PSC, and for a potential translation to clinical practice.

Supplementary data

Supplementary data are available at *Inflammatory Bowel Diseases* online.

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Author Contributions

Conceptualization of the study: M.d.K., B.C., C.Y.P., J.V., G.A.M. Tissue collection, DNA and RNA isolations, microsatellite instability assay, CpG island methylator phenotype analysis: M.d.K., J.V., A.S.B., P.M.D.-v.D., M.T., and M.v.E. Bioinformatics analyses and statistical analyses: C.R. and N.M. Interpretation of data and preparation of manuscript: M.d.K., B.C. and C.Y.P. Critical review of manuscript: all authors.

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Conflicts of Interest

M.d.K., C.R., A.S.B., P.M.D.-v.D., M.T., M.v.E, N.M., J.V.: none declared.

B.C. has several patent (applications) pending on biomarkers for colorectal cancer (CRC) early detection. R.M.M.B. received an unrestricted educational grant from Pentax Medical BV. E.D. has endoscopic equipment on loan from FujiFilm and has received a research grant from FujiFilm; honorarium for consultancy from FujiFilm, Olympus, Tillots, GI Supply, and CPP-FAP; and speakers' fee from Olympus, Roche, GI Supply and Norgine. A.A.M.M. has received a ZON MW, The Netherlands Organization for Health Research and Development, health care efficiency grant to evaluate the efficacy of peppermint oil in Irritable Bowel Syndrome (IBS); received an unrestricted research grant from Will Pharma S.A.; received research funding from Allergan and Grünenthal on IBS topics; given scientific advice to Bayer (topic: IBS), Kyowa Kirin (topic: constipation), and Takeda (topic: gastroparesis); received funding from Pentax Europe GmBH; and received funding from the Dutch Cancer Society related to endoscopy and to colorectal polyps. G.A.M. has several patent (applications) on biomarkers for CRC early detection and has a research collaboration with Exact Sciences. C.Y.P. has received research grants form Takeda; speaker's fees from Takeda, Tillotts, and Abbvie; and consultancy fees from Takeda and Pliant.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Lutgens MW, van Oijen MG, van der Heijden GJ, et al. Declining risk of colorectal cancer in inflammatory bowel disease: an updated meta-analysis of population-based cohort studies. *Inflamm Bowel Dis*. 2013;19:789–799.
2. Zheng HH, Jiang XL. Increased risk of colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease: a meta-analysis of 16 observational studies. *Eur J Gastroenterol Hepatol*. 2016;28:383–390.
3. Boonstra K, Weersma RK, van Erpecum KJ, et al. Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis. *Hepatology*. 2013;58:2045–2055.
4. Shah SC, Ten Hove JR, Castaneda D, et al. High risk of advanced colorectal neoplasia in patients with primary sclerosing cholangitis associated with inflammatory bowel disease. *Clin Gastroenterol Hepatol*. 2018;16:1106–1113.e3.
5. Breynaert C, Vermeire S, Rutgeerts P, Van Assche G. Dysplasia and colorectal cancer in inflammatory bowel disease: a result of inflammation or an intrinsic risk? *Acta Gastroenterol Belg*. 2008;71:367–372.
6. Boonstra K, van Erpecum KJ, van Nieuwkerk KM, et al. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:2270–2276.
7. Harada S, Morlote D. Molecular pathology of colorectal cancer. *Adv Anat Pathol*. 2020;27:20–26.
8. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61:759–767.
9. Baker AM, Cross W, Curtius K, et al. Evolutionary history of human colitis-associated colorectal cancer. *Gut*. 2019;68(6):985–995.
10. Robles AI, Traverso G, Zhang M, et al. Whole-exome sequencing analyses of inflammatory bowel disease-associated colorectal cancers. *Gastroenterology*. 2016;150:931–943.
11. Wanders LK, Cordes M, Voorham Q, et al. IBD-associated dysplastic lesions show more chromosomal instability than sporadic adenomas. *Inflamm Bowel Dis*. 2020;26(2):167–180.
12. Hussain SP, Amstad P, Raja K, et al. Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res*. 2000;60:3333–3337.

13. Leedham SJ, Graham TA, Oukrif D, et al. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. *Gastroenterology*. 2009;136:542–550.e6.
14. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res*. 2001;61:3573–3577.
15. Wohl P, Hucl T, Drastich P, et al. Epithelial markers of colorectal carcinogenesis in ulcerative colitis and primary sclerosing cholangitis. *World J Gastroenterol*. 2013;19:2234–2241.
16. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in the Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol*. 2007;29:19–24.
17. Bogie RMM, le Clercq CMC, Voorham QJM, et al. Molecular pathways in post-colonoscopy versus detected colorectal cancers: results from a nested case-control study. *Br J Cancer*. 2022;126(6):865–873.
18. Scheinin I, Sie D, Bengtsson H, et al. DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly. *Genome Res*. 2014;24:2022–2032.
19. Rausch C, Carvalho B, Fijneman RJ, et al. Qdnaseqflow: a computational analysis workflow of DNA copy number aberrations from low-coverage whole genome sequencing reads [version 1; not peer reviewed]. *F1000Research*. 2017;6:1414. doi:10.7490/f1000research.1114668.1
20. van de Wiel MA, Brosens R, Eilers PH, et al. Smoothing waves in array CgH tumor profiles. *Bioinformatics*. 2009;25:1099–1104.
21. Lappalainen I, Almeida-King J, Kumanduri V, et al. The European genome-phenome archive of human data consented for biomedical research. *Nat Genet*. 2015;47:692–695.
22. van Lanschot MCJ, Carvalho B, Rausch C, et al. Molecular profiling of longitudinally observed small colorectal polyps: a cohort study. *EBioMedicine*. 2019;39:292–300.
23. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285–291.
24. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer*. 2004;4:177–183.
25. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base [published online ahead of print May 16, 2017]. *JCO Precis Oncol*. doi:10.1200/PO.17.00011.
26. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet*. 2006;38:787–793.
27. Derks S, Postma C, Moerkerk PT, et al. Promoter methylation precedes chromosomal alterations in colorectal cancer development. *Cell Oncol*. 2006;28:247–257.
28. Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *Ann Stat*. 2001;116:5–88.
29. Van Wieringen WN, Van De Wiel MA, Ylstra B. Weighted clustering of called array CgH data. *Biostatistics*. 2008;9:484–500.
30. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330–337.
31. Lovig T, Andersen SN, Clausen OP, Rognum TO. Microsatellite instability in long-standing ulcerative colitis. *Scand J Gastroenterol*. 2007;42:586–591.
32. Yaeger R, Shah MA, Miller VA, et al. Genomic alterations observed in colitis-associated cancers are distinct from those found in sporadic colorectal cancers and vary by type of inflammatory bowel disease. *Gastroenterology*. 2016;151:278–287.e6.
33. Tanaka T, Kobunai T, Yamamoto Y, et al. Colitic cancer develops through mutational alteration distinct from that in sporadic colorectal cancer: a comparative analysis of mutational rates at each step. *Cancer Genomics Proteomics*. 2017;14:341–348.
34. Dhir M, Montgomery EA, Glockner SC, et al. Epigenetic regulation of WNT signaling pathway genes in inflammatory bowel disease (IBD) associated neoplasia. *J Gastrointest Surg*. 2008;12:1745–1753.
35. Juo YY, Johnston FM, Zhang DY, et al. Prognostic value of CpG island methylator phenotype among colorectal cancer patients: a systematic review and meta-analysis. *Ann Oncol*. 2014;25:2314–2327.
36. Olaru AV, Cheng Y, Agarwal R, et al. Unique patterns of CpG island methylation in inflammatory bowel disease-associated colorectal cancers. *Inflamm Bowel Dis*. 2012;18:641–648.
37. Sanchez JA, Dejulius KL, Bronner M, Church JM, Kalady MF. Relative role of methylator and tumor suppressor pathways in ulcerative colitis-associated colon cancer. *Inflamm Bowel Dis*. 2011;17:1966–1970.
38. Issa JP. Colon cancer: it's CIN or CIMP. *Clin Cancer Res*. 2008;14:5939–5940.
39. Neyazi M, Bharadwaj SS, Bullers S, et al. Overexpression of cancer-associated stem cell gene *OLFM4* in the colonic epithelium of patients with primary sclerosing cholangitis. *Inflamm Bowel Dis*. 2021;27(8):1316–1327.
40. Pleguezuelos-Manzano C, Puschhof J, Huber AR, et al. Mutational signature in colorectal cancer caused by genotoxic pks(+) *E. coli*. *Nature*. 2020;580(7802):269–273.
41. Torres J, Palmela C, Brito H, et al. The gut microbiota, bile acids and their correlation in primary sclerosing cholangitis associated with inflammatory bowel disease. *United European Gastroenterol J*. 2018;6:112–122.
42. Pekow J, Hernandez K, Meckel K, et al. IBD-associated colon cancers differ in DNA methylation and gene expression profiles compared to sporadic colon cancers. *J Crohns Colitis*. 2019;13(7):884–893.
43. Akhoondi S, Sun D, von der Lehr N, et al. Fbxw7/hCdc4 is a general tumor suppressor in human cancer. *Cancer Res*. 2007;67:9006–9012.
44. Lee-Six H, Olafsson S, Ellis P, et al. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature*. 2019;574:532–537.
45. Trivedi PJ, Crothers H, Mytton J, et al. Effects of primary sclerosing cholangitis on risks of cancer and death in people with inflammatory bowel disease, based on sex, race, and age. *Gastroenterology*. 2020;159:915–928.
46. Frampton MJ, Law P, Litchfield K, et al. Implications of polygenic risk for personalised colorectal cancer screening. *Ann Oncol*. 2016;27(3):429–434.