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IBD-Associated Dysplastic Lesions Show More Chromosomal Instability Than Sporadic Adenomas

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Background: Patients with longstanding inflammatory bowel disease (IBD; ie, ulcerative colitis and Crohn's disease) have an increased risk of colorectal cancer (CRC). Due to ongoing inflammation, IBD-associated dysplastic lesions can develop. These lesions have an increased risk to progress to cancer compared with sporadic adenomas, which are also found in these patients. Differentiating between these 2 types of dysplasia remains challenging, both clinically and histologically, while treatment strategies may differ. Therefore, the aim of this study was to investigate molecular alterations associated with colorectal dysplasia to cancer progression in IBD and evaluate to what extent these alterations differ from sporadic adenomas.

Methods: DNA copy number aberrations and mutation analyses of 48 genes were performed by next-generation sequencing in 43 IBD-associated dysplastic lesions, 30 of which were dysplastic and 13 of which were cancers. Results were compared with existing DNA copy number and mutation data from 118 sporadic adenomas and 24 sporadic cancers.

Results: Inflammatory bowel disease–associated dysplastic lesions harbor patterns of DNA copy number aberrations comparable to carcinomas, which are rare in sporadic adenomas. *TP53* mutation was the most frequent mutation observed in IBD-associated dysplastic lesions and in cancers. *FBXW7* was mutated significantly more often in IBD-associated dysplastic lesions than in sporadic adenomas.

Conclusions: Inflammatory bowel disease–associated dysplastic lesions show more DNA copy number aberrations than sporadic adenomas. *TP53* and *FBXW7* mutations appear to be involved in the development of IBD-associated dysplastic lesions and cancer. These findings indicate that IBD-associated dysplastic lesions are more genomically unstable, possibly reflecting a faster progression toward cancer.

Key Words: IBD-associated dysplasia, colorectal cancer, DNA copy number, mutation, inflammatory bowel disease

INTRODUCTION

Patients with inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are affected by lifelong relapsing inflammation of the intestinal mucosa. One of the most severe complications of longstanding IBD is the development of colorectal cancer (CRC). Inflammatory bowel disease patients carry about 2–3-fold greater CRC risk than the general population.[1, 2] Therefore,

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clinical guidelines recommend colonoscopic surveillance in all IBD patients with longstanding colonic disease.[3, 4]

During colonoscopic surveillance, both sporadic adenomas, which are not related to chronic inflammation, and IBD-associated dysplastic lesions can be encountered.[5] Inflammatory bowel disease–associated dysplastic lesions are thought to carry a higher CRC risk than sporadic adenomas and may reflect a field defect throughout the colonic mucosa, which increases the risk of synchronous and/or metachronous cancer.[6]

The distinction between sporadic adenomas and IBD-associated dysplastic lesions is clinically relevant, as therapeutic options may differ, ranging from endoscopic polypectomy for sporadic adenomas to a more radical and aggressive approach like proctocolectomy in the case of nonpolypoid IBD-associated dysplastic lesions.[3, 4] In the latter, lesions might be more difficult to delineate and endoscopically resect, and synchronous and metachronous lesions are frequently detected as a result of a field defect that causes multifocal disease. However, this differentiation based on both endoscopic and histological characteristics is often challenging, and interobserver agreement between expert endoscopists in IBD is poor.[7, 8] This underlines the need for better markers for distinguishing these 2 types of lesions. To this end, more insight into the tumor biology of IBD-associated dysplasia is needed.

Although sporadic adenomas, when progressing to cancer, follow the well-established adenoma–carcinoma pathway, the molecular pathway by which IBD-associated dysplasia progresses to cancer remains largely obscure and different hypotheses exist.[9, 10] From a molecular perspective, several inflammatory-related pathways are thought to be responsible for the development of CRC, which can roughly be divided into 4 groups: (1) genetic alterations (eg, chromosomal and microsatellite instability and DNA promoter hypermethylation), (2) mucosal inflammatory mediators (eg, COX-2, interleukin [Il]-6, Il-23, tumor necrosis factor alpha), (3) altered expression of epithelial cell surface receptors, and (4) oxidative stress, mucosal breakdown, and intestinal microbiota.[11] A common theme during progression from dysplasia to cancer is the accumulation of genomic changes, including DNA copy number aberrations, microsatellite instability (MSI), and mutations.[12]

The aim of the present study is to investigate DNA copy number aberrations, MSI status, and mutations in IBD-associated dysplastic lesions and evaluate to what extent these aberrations differ from sporadic adenomas.

METHODS

Material

Formalin-fixed paraffin-embedded (FFPE) tumor samples from patients who had undergone colectomy due to suspicion of IBD-associated dysplasia, operated in either the Academic Medical Center (AMC) or VU University Medical Center (VUMC) in Amsterdam between 2000 and 2014, were retrieved. All patients had had a biopsy-confirmed diagnosis of ulcerative colitis or Crohn's disease. The degree of neoplasia varied from low-grade dysplasia (LGD; n = 26) to high-grade dysplasia (HGD; n = 4) to invasive carcinoma (colorectal cancer [CRC]; n = 13). The 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 (http://www.federa.org). All data and tissue were coded anonymously for handling throughout the study.

Case Selection

Three IBD expert endoscopists in IBD (E.D., G.R.A.M.d.H., N.K.H.d.B.) independently assessed selected cases of potentially colitis-associated dysplastic lesions to define whether a dysplastic lesion was colitis-associated or not, based on their expert opinion. They reviewed the clinical information of the extent and duration of the colitis and the presence or absence of primary sclerosing cholangitis (PSC). Colonoscopy and pathology reports were assessed, and high-quality endoscopic images of the lesions and other parts of the colon were also evaluated. In case of discrepancy, consensus had to be reached between the IBD expert endoscopists. Only in the case of consensus were lesions considered colitis-associated and included. Histology of all lesions included was re-evaluated by 2 expert pathologists (N.C.T.v.G. and S.D.d.V.) based on the consensus criteria of the European Society of Pathology (ESP) guidelines.[13, 14] Lesions were excluded when the degree of neoplasia was unclear or the quantity of tissue was considered insufficient.

Control Group

As a control group, a published cohort[15] was used consisting of sporadic adenomas with polypoid or nonpolypoid morphology (n = 118) and carcinomas (n = 24) without associated IBD or any known hereditary risk for CRC.

DNA Isolation

DNA from FFPE material was isolated after macrodissection (>70% dysplastic cells) as previously described.[16] Good-quality DNA was obtained from 43 IBD-associated dysplastic lesions, which included both adenomas and cancers.

DNA Copy Number Analysis

DNA copy number changes were analyzed with low-coverage whole-genome sequencing (WGS).[17] Briefly, DNA was fragmented by sonication (Covaris S2, Woburn, MA, USA) and run on the Illumina HiSeq 2000 (Illumina, San Diego, CA, USA) on a 50-bp single-read modus using the Illumina Truseq Nano kit (Illumina, San Diego, CA, USA).

Low-coverage WGS copy number data were analyzed using QDNAseq.[17] Raw sequence reads were uniquely aligned to the human reference genome build GRCh37/hg19 with Burrows-Wheeler Alignment (BWA).[18] Reads with mapping qualities lower then Q37 and polymerase chain reaction (PCR)

duplicated were filtered out. QDNAseq was used to divide the human reference genome into nonoverlapping fixed-sized bins of 30 kb, and for each sample, estimates of the copy number were determined by counting the number of reads in each bin. Copy number profiles were corrected by a 2-dimensional Loess fit for mappability and GC content. Also, problematic genomic regions and common copy number variants were filtered out by a blacklist generated using germ-line sequence data from the 1000 Genomes Project.[19] Genomic waves caused by replication timing were smoothed using NoWaves.[20]

The DNA copy number data from the control group (sporadic colorectal adenomas and carcinomas) were obtained by high-resolution (180K) array comparative genomic hybridization (aCGH).[15] To allow for comparison with the low-coverage whole-genome sequencing data obtained from the IBD-associated dysplasia samples, the chromosomal coordinates of the sporadic aCGH data were converted from GRCh36/hg18 to GRCh37/hg19. To create a comparable data set from both sequencing and aCGH DNA copy number data, log2 ratios of the aCGH probes were divided into 30-kb bins according to their chromosomal position, using the Bioconductor packages IRanges and GenomicRanges. Binned copy number data from sequencing and aCGH were segmented using the circular binary segmentation algorithm.[21] CGHcall is a Bioconductor/R package used to discretize the log2 ratios of the segments back to 3 states: loss, normal, gain.[22] By using CGHregions, the data were further reduced to common regions, with exclusion of regions smaller then 5 Mbp.[23]

Raw DNA copy number data is made available in the European Genome-Phenome Archive (EGA; (https://www.ebi.ac.uk) under the Study ID dataset: EGAS00001003767.

Mutation Analysis

Targeted deep sequencing using the TruSeq 48-Gene Cancer Panel from Illumina (TSACP; Illumina, San Diego, CA, USA) was used for mutation analysis. Briefly, specific target mutations were detected by amplification of the corresponding exons by PCR. Polymerase chain reaction products were sequenced on a MiSeq Personal Sequencer (Illumina, San Diego, CA, USA). Of the 43 IBD-associated lesions, DNA from 25 IBD-associated lesions (16 dysplastic lesions and 9 carcinomas) was available for mutation analysis.

Raw DNA mutation data is made available in the European Genome-Phenome Archive (EGA; https://www.ebi.ac.uk) under the Study ID dataset: EGAS00001003767.

Mutation data from the control group (sporadic colorectal adenomas and carcinomas) were obtained using the MALDI-TOF mass spectrometer (Spetro-READER, Sequenom, San Diego, CA, USA) for genes *CTNNB1*, *KRAS*, *BRAF*, *PIK3CA*, *NRAS*, *EGFR*, *FBXW7*, *PTEN*, *STK11*, *MAP2K4*, *SMAD4*, *PIK3R1*, and *PDGFRA*, or by PCR and sequencing (3500 Genetic Analyser, Applied Biosystems, Foster City, CA, USA) in the case of the *APC* gene.[24]

MSI Status Analysis

Microsatellite instability analysis was performed using the multiplex marker panel from Promega (MSI Multiplex System, version 1.2, Promega, Madison, WI, USA) as previously described.[15] When 2 or more markers were unstable, the sample was interpreted as microsatellite unstable (MSI); all other samples were classified as microsatellite stable (MSS).

Statistical Analysis

Patient characteristics were analyzed with descriptive statistics. To compare differences between the IBD-associated dysplastic lesions and sporadic adenomas regarding their clinical-pathologic features, either the Mann-Whitney *U* test for age distribution or chi-square test (or Fisher exact test, when applicable) for all categorical features was applied. *P* values <0.05 were considered significant.

To analyze the DNA copy number changes between selected groups of patients, CGHMultiArray was used.[25] *P* values were calculated by performing a chi-square test with 10,000 permutations. Separate analyses were run to test for gains and losses. This test procedure includes a permutation-based false discovery rate (FDR) correction for multiple testing. Aberrations occurring <5% were a priori excluded, and an FDR <0.1 was considered statistically significant. For unsupervised data analysis, hierarchical cluster analysis of the regions of gains and losses using Weighted Clustering of Called aCGH data was performed using Ward linkage.[26]

Comparison of differences in mutations between the different groups of lesions was done using the chi-square test (or Fisher exact test, when appropriate). *P* values <0.05 were considered significant.

Ethical Considerations

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. All data and tissue were coded anonymously for handling throughout the study.

RESULTS

Clinical Characteristics

Clinico-histological features of IBD-associated lesions do not differ from sporadic lesions except for age of diagnosis

The clinical features of patients and their respective lesions for both cohorts (IBD-associated lesions and sporadic lesions) are described in Table 1. The IBD-associated cohort contained

TABLE 1. Patient and Lesion Characteristics for Cases (IBD-Associated) and Controls (Sporadic)

		IBD	Sporadic	*P*
Patients, No.		27	135	
CD/UC, No.		9/18	n.a.	-
Age of IBD onset, mean (range), y		31.0 (16–63)	n.a.	-
Age of detection of dysplasia, mean (range), y		53.2 (38–75)	70.2 (28–90)	<0.001
Sex, No. (%)	Male	17 (63)	73 (59)	0.630
	Female	10 (37)	53 (41)	
Lesions, No.		43	142	
MSI status, No. (%)	MSI	2 (4.7)	1 (0.7)	
	MSS	34 (79.1)	132 (93.0)	
	n.d.	7 (16.3)	9 (6.3)	0.115
Morphology, No.	Nonpolypoid	n.d.	83 (adenomas) + 15 (CRC)	
	Polypoid	n.d.	35 (adenomas) + 9 (CRC)	-
Type of lesion, No.	Adenomas	30	118	
Histology, No. (%)	TA	14 (47)	65 (55.1)	
	TVA	16 (53)	41 (34.7)	
	VA	0	7 (5.9)	
	Serrated	0	5 (4.2)	0.138
Grade of dysplasia, No. (%)	LGD	26 (86.7)	100 (84.7)	
	HGD	4 (13.3)	18 (15.3)	1.0
Type of lesion, No.	Carcinomas	13	24	
Differentiation, No. (%)	Well	0	5 (20.8)	
	Moderate	11 (84.6)	19 (79.2)	
	Poor	1 (7.7)	0	
	Mucinous	1 (7.7)	0	0.092

P value significant at $P < 0.05$. In case of absence of a variable or category in a comparison, statistics could not be calculated (indicated by "-").
Abbreviations: n.a., not applicable; n.d., not determined.

43 dysplastic lesions (30 dysplasia and 13 carcinomas) from 27 different patients. The sporadic cohort included 142 dysplastic lesions (118 adenomas and 24 carcinomas) from 135 different patients. Of the 118 sporadic adenomas, 83 were nonpolypoid and 35 were polypoid.

Approximately two-thirds of the IBD patients were diagnosed with ulcerative colitis (UC), and one-third with Crohn's disease (CD). The average age of onset of IBD was 31 years, with a range from 16 to 63 years. For IBD patients, the average age at which IBD-associated dysplasia was diagnosed was 53.2 years. In the non-IBD control group, the age of detection of sporadic adenomas was significantly higher, at an average of 70.2 years ($P < 0.001$). No significant differences were found between IBD-associated dysplasia and sporadic adenomas regarding type of histology and grade of dysplasia. Also, no significant differences in differentiation grade were observed between IBD-associated carcinomas and sporadic carcinomas.

Molecular Profiling

Good-quality DNA copy number profiles were obtained for all 43 IBD DNA samples (30 adenomas and 13 carcinomas). In the control data set, DNA copy number profiles were available for 142 cases.[15] Mutation status data for the 48 genes analyzed could be obtained for 25 of the 43 IBD-associated dysplastic lesions (16 dysplastic lesions and 9 carcinomas) where DNA was still available. In the control series, mutation data were available for only 12 of the 48 genes analyzed in the IBD-associated lesions, and the number of cases successfully analyzed varied per gene.[24] Microsatellite instability status was available in 36 out of 43 IBD lesions and in 133 out of 142 sporadic lesions. The frequency of MSI lesions was higher in IBD than sporadic lesions, 4.7% (2/36) and 0.7% (1/133), respectively. However, this difference was not statistically significant (Table 1).

Mutations of TP53 are more frequent in IBD-associated cancer than in IBD-associated dysplastic lesions

Inflammatory bowel disease–associated dysplastic lesions harbor many DNA copy number aberrations that are also commonly observed in cancer (Fig. 1). In IBD-associated dysplasia, the average number of DNA copy number gains and losses (range) was 4.3 (0–23) and 3.2 (0–13), respectively.

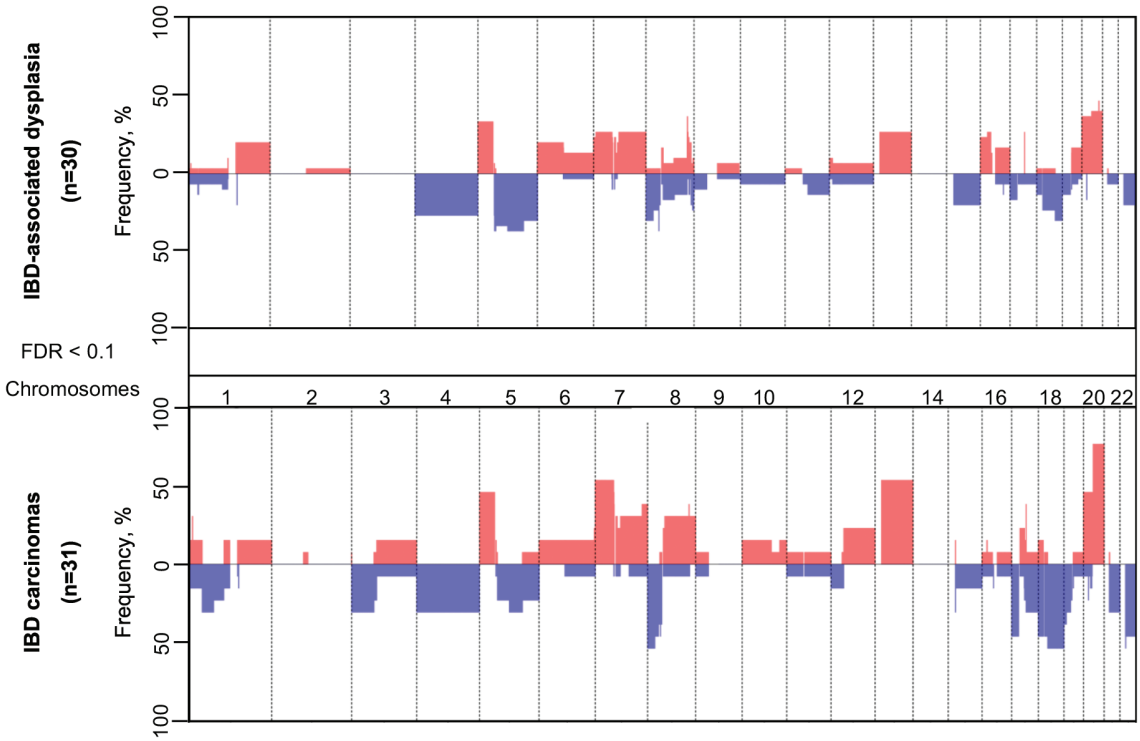

FIGURE 1. Copy number aberrations in IBD-associated dysplastic lesions and IBD-associated carcinomas. Frequency plots of copy number gains (red) and losses (blue) in 30 IBD-associated dysplastic lesions (upper panel) and 13 IBD-associated carcinomas (lower panel). Significant differences (FDR < 0.1) between the 2 types of lesions are depicted in black for losses and in gray for gains.

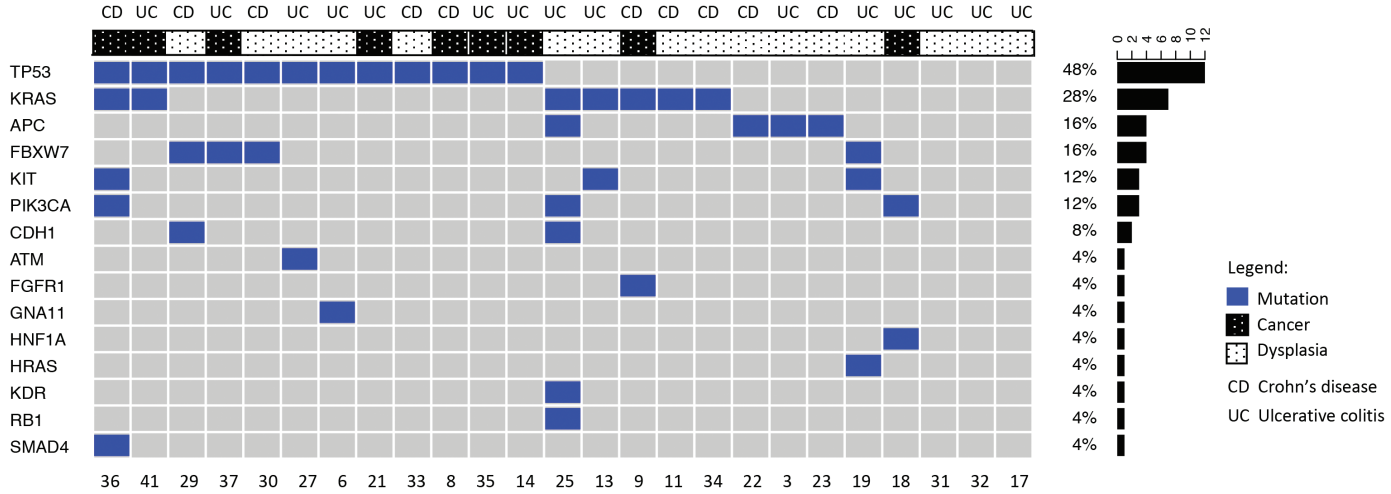

FIGURE 2. Oncoprint of mutations in IBD-associated dysplasia and cancer. Frequency of mutations in IBD-associated dysplasia (n = 16) and IBD-associated cancer (n = 9). In dark gray, mutation present; in dotted black, carcinomas; in dotted white, adenomas.

Inflammatory bowel disease–associated cancers showed an average (range) of 5.1 (0–13) gains and 7.7 (0–18) losses. The patterns of DNA copy number aberrations are very similar between these 2 types of lesions, with the exception of chromosome 3p, the loss of which is only observed in IBD-associated cancers ($P < 0.006$). However, this finding did not remain significant after correction for multiple testing (FDR = 0.2) (Fig. 1).

From the 48 genes analyzed, 15 showed mutations in IBD lesions (Fig. 2; Supplementary Table 1), with frequencies of 4% (*ATM*, *FGFR1*, *GNA11*, *HNF1A*, *HRAS*, *KDR*, *RB1*, *SMAD4*), 8% (*KIT*, *CDH1*), 12% (*PIK3CA*), 16% (*APC*, *FBXW7*), 28% (*KRAS*), and 48% (*TP53*) (Fig. 2).

Frequencies of mutations did not differ between dysplastic lesions and cancers, except for the *TP53* mutation, which

TABLE 2. Mutation Frequencies in IBD-Associated Lesions (Dysplasia and Cancer)

		IBD, % (No.)					IBD, % (No.)		
Gene		Dysplasia	Cancer	*P*	Gene		Dysplasia	Cancer	*P*
APC	WT	75 (12/16)	100 (9/9)		KDR	WT	94 (15/16)	100 (9/9)	
	Mutation	25 (4/16)	0 (0/9)	0.260		Mutation	6 (1/16)	0 (0/9)	1.0
ATM	WT	94 (15/16)	100 (9/9)		KIT	WT	88 (14/16)	89 (8/9)	
	Mutation	6 (1/16)	0 (0/9)	1.0		Mutation	12 (2/16)	11 (1/9)	1.0
BRAF	WT	100 (16/16)	100 (9/9)		KRAS	WT	75 (12/16)	67 (6/9)	
	Mutation	0 (0/16)	0 (0/9)	-		Mutation	25 (4/16)	33 (3/9)	0.673
CDH1	WT	88 (14/16)	100 (9/9)		MET	WT	100 (16/16)	100 (9/9)	
	Mutation	12 (2/16)	0 (0/9)	1.0		Mutation	0 (0/16)	0 (0/9)	-
CTNNB1	WT	100 (16/16)	100 (9/9)		NRAS	WT	100 (16/16)	100 (9/9)	
	Mutation	0 (0/16)	0 (0/9)	-		Mutation	0 (0/16)	0 (0/9)	-
FBXW7	WT	81 (13/16)	89 (8/9)		PIK3CA	WT	94 (15/16)	78 (7/9)	
	Mutation	19 (3/16)	11 (1/9)	1.0		Mutation	6 (1/16)	22 (2/9)	0.530
FGFR1	WT	100 (16/16)	89 (8/9)		RB1	WT	94 (15/16)	100 (9/9)	
	Mutation	0 (0/16)	11 (1/9)	0.360		Mutation	6 (1/16)	0 (0/9)	1.0
GNA11	WT	94 (15/16)	100 (9/9)		SMAD4	WT	100 (16/16)	89 (8/9)	
	Mutation	6 (1/16)	0 (0/9)	1.0		Mutation	0 (0/16)	11 (1/9)	0.360
HNF1	WT	100 (16/16)	89 (8/9)		TP53	WT	69 (11/16)	22 (2/9)	
	Mutation	0 (0/16)	11 (1/9)	0.360		Mutation	31 (5/16)	78 (7/9)	**0.041**
HRAS	WT	94 (15/16)	100 (9/9)						
	Mutation	6 (1/16)	0 (0/9)	1.0					

P value significant at $P < 0.05$. In case of absence of a variable or category in a comparison, statistics could not be calculated (indicated by "-").

was more common in IBD-associated cancers than in IBD-associated dysplastic lesions (78% vs 31%; $P = 0.04$) (Table 2).

IBD-associated dysplastic lesions show higher levels of chromosomal instability than sporadic adenomas

DNA copy number profiles of sporadic adenomas show relatively fewer aberrations, with an average number of gains and losses of 1.5 and 0.5, respectively. On the other hand, the IBD-associated dysplastic lesions show considerably more aberrations, with an average number of gains and losses of 4.3 and 3.2, respectively (Fig. 3). When comparing IBD-associated dysplasia and sporadic adenomas, significant differences between the 2 groups are losses in chromosomes 1q (FDR = 0.001), 4 (FDR < 0.001), 5q (FDR < 0.001), 7p (FDR = 0.008), 8 (FDR < 0.05), 9p (FDR = 0.008), 11q (FDR = 0.04), 12q (FDR = 0.05), 15q (FDR < 0.001), 16q (FDR < 0.05), 18 (FDR < 0.06), 19 (FDR < 0.05), 20p (FDR < 0.02), and 22 (FDR < 0.001) and gains in chromosome 1q (FDR < 0.02), 5p (FDR < 0.001), 6 (FDR < 0.08), 8q (FDR < 0.06), 16 (FDR < 0.02), 19q (FDR = 0.08), and 20q (FDR = 0.005).

In IBD-associated dysplasia, the average number of mutations observed per lesion (range) was 1.6 (0–6), and in sporadic adenomas 0.9 (0–3). There were no significant differences in the frequency of mutations per gene between the 2 groups, except for the *FBXW7* gene. It was more frequently mutated in IBD-associated dysplastic lesions than in sporadic adenomas (19% vs 2%; $P = 0.029$) (Table 3).

IBD-associated dysplastic lesions share molecular features with both nonpolypoid and polypoid sporadic adenomas. Among the sporadic adenomas, nonpolypoid adenomas have been considered a particular subtype, and it has been hypothesized that these lesions could potentially be associated with inflammation.[15] Previously, sporadic nonpolypoid adenomas have been reported to show significantly more 5q loss than sporadic polypoid adenomas.[15] In the present study, 5q loss was seen in even higher frequency in the IBD-associated dysplastic lesions (Fig. 3). Loss of chromosome 18, on the other hand, was frequently observed both in IBD-associated dysplastic lesions and polypoid sporadic adenomas, in contrast to sporadic nonpolypoid adenomas. Supplementary Tables 2 and 3 show an overview of the comparisons of IBD-associated dysplastic lesions vs nonpolypoid adenomas and IBD-associated dysplastic lesions vs polypoid adenomas, respectively.

Regarding mutation patterns, IBD-associated dysplastic lesions were significantly more often mutated on the *FBXW7* gene than sporadic nonpolypoid adenomas (19% vs 0%; $P = 0.011$), but not when compared with sporadic polypoid adenomas (19% vs 6%; $P = 0.320$). *APC* was significantly more often mutated in sporadic polypoid adenomas than in

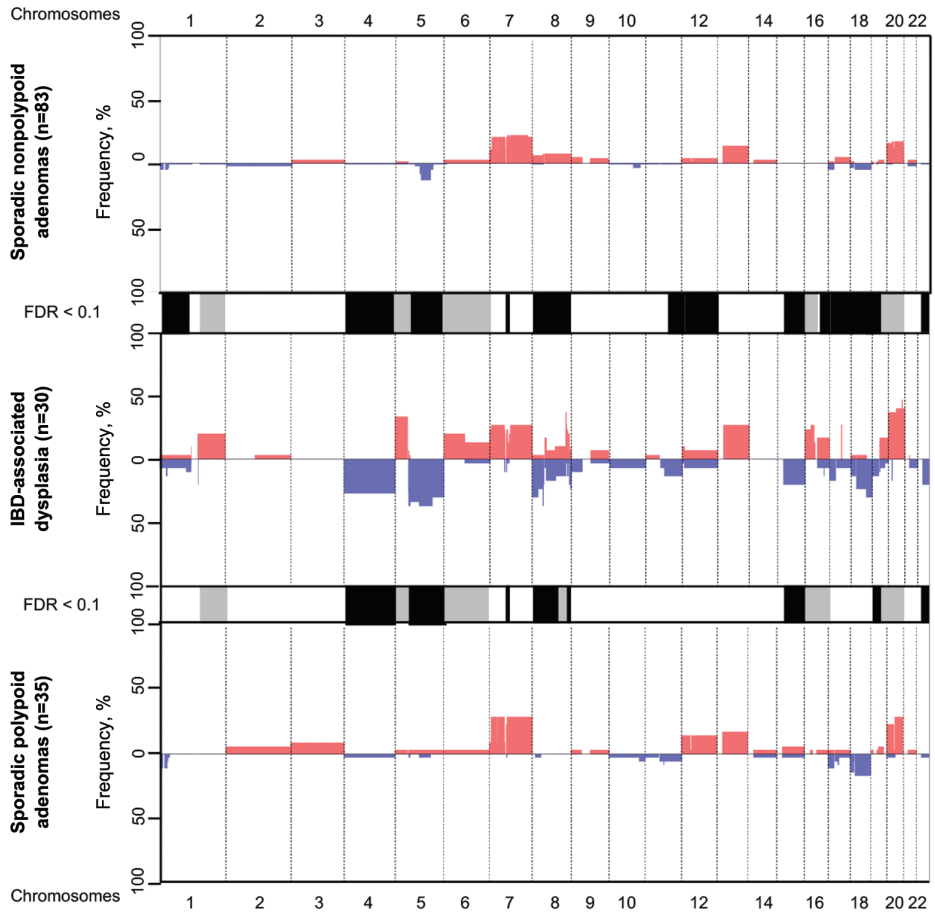

FIGURE 3. Frequency plots of copy number aberrations of IBD-associated dysplastic lesions (middle panel), sporadic nonpolypoid adenomas (upper panel), and sporadic polypoid adenomas (lower panel). Frequency plots of copy number gains (red) and losses (blue) in 83 sporadic nonpolypoid adenomas (upper panel), 30 IBD-associated dysplasia (middle panel), and 35 sporadic polypoid adenomas (lower panel). Significant differences (FDR < 0.1) between the 2 types of lesions are depicted in black for losses and in gray for gains.

IBD-associated dysplastic lesions (63% vs 25%; $P = 0.017$). This difference was not observed when comparing IBD-dysplastic lesions and sporadic nonpolypoid adenomas (25% vs 41%; $P = 0.265$) (Table 3).

IBD-associated cancers do not differ molecularly from sporadic cancers

Inflammatory bowel disease–associated carcinomas and sporadic carcinomas largely showed similar DNA copy number profiles. The average number of gains and losses observed in IBD-associated cancers was 5.1 and 7.7, respectively, and in sporadic cancers it was 10.1 and 8.5, respectively. Both types of carcinomas carry gains of chromosome 7, 8, 13, and 20 and show losses of chromosome 1p, 4, 5, 17p, and 18. In addition, gains of chromosome 16p and losses of chromosome 14 were observed in sporadic carcinomas but not in IBD-associated carcinomas (Fig. 4).

Mutations were observed in *TP53* (78%; 7/9), *KRAS* (33%; 3/9), *PIK3CA* (22%; 2/9), *FBXW7* (11%; 1/9), *FGFR1* (11%; 1/9), *HNF1A* (11%; 1/9), *KIT* (11%; 1/9), and *SMAD4* (11%; 1/9) in IBD-associated cancers. In sporadic cancers, mutations were observed in *KRAS* (43%; 3/7) and *CTNNB1* (14%; 1/7). For *KRAS*, *PIK3CA*, *FBXW7*, and *CTNNB1*, no statistically significant differences in mutation frequencies were found between IBD-associated cancers and sporadic cancers, whereas for *FGFR1, HNF1A, KIT, SMAD4*, and *TP53*, no data were available in the sporadic group (Table 4).

Integration of Molecular Features With Clinical Features

DNA copy number changes and mutations in IBD-associated lesions are not associated with any particular clinico-histological feature

Hierarchical cluster analysis of all cases (IBD-associated and sporadic) considering DNA copy number changes showed that 2 main clusters are formed, 1 of cases with hardly any aberrations (cluster 1) and another containing

TABLE 3. Mutation Frequencies in IBD-Associated Dysplasia and Sporadic Adenomas (Nonpolypoid and Polypoid Adenomas)

		IBD, % (No.)	Sporadic, % (No.)		IBD, % (No.)	Sporadic, % (No.)		IBD, % (No.)	Sporadic, % (No.)	
Gene		Dysplasia	Dysplasia	*P*	Dysplasia	Flat Adenoma	*P*	Dysplasia	Polypoid Adenoma	*P*
APC	WT	75 (12/16)	51 (50/98)		75 (12/16)	59 (37/63)		75 (12/16)	37 (13/35)	
	Mutation	25 (4/16)	49 (48/98)	0.104	25 (4/16)	41 (26/63)	0.265	25 (4/16)	63 (22/35)	**0.017**
ATM	WT	94 (15/16)	-		94 (15/16)	-		94 (15/16)	-	
	Mutation	6 (1/16)	-	-	6 (1/16)	-	-	6 (1/16)	-	-
BRAF	WT	100 (16/16)	98 (83/85)		100 (16/16)	96 (48/50)		100 (16/16)	100 (35/35)	
	Mutation	0 (0/16)	2 (2/85)	1.0	0 (0/16)	4 (2/50)	1.0	0 (0/16)	0 (0/35)	-
CDH1	WT	88 (14/16)	-		88 (14/16)	-		88 (14/16)	-	
	Mutation	12 (2/16)	-	-	12 (2/16)	-	-	12 (2/16)	-	-
CTNNB1	WT	100 (16/16)	99 (77/78)		100 (16/16)	100 (48/48)		100 (16/16)	97 (29/30)	
	Mutation	0 (0/16)	1 (1/78)	1.0	0 (0/16)	0 (0/48)	-	0 (0/16)	3 (1/30)	1.0
FBXW7	WT	81 (13/16)	98 (81/83)		81 (13/16)	100 (52/52)		81 (13/16)	94 (29/31)	
	Mutation	19 (3/16)	2 (2/83)	**0.029**	19 (3/16)	0 (0/52)	**0.011**	19 (3/16)	6 (2/31)	0.320
FGFR1	WT	100 (16/16)	-		100 (16/16)	-		100 (16/16)	-	
	Mutation	0 (0/16)	-	-	0 (0/16)	-	-	0 (0/16)	-	-
GNA11	WT	94 (15/16)	-		94 (15/16)	-		94 (15/16)	-	
	Mutation	6 (1/16)	-	-	6 (1/16)	-	-	6 (1/16)	-	-
HNF1A	WT	100 (16/16)	-		100 (16/16)	-		100 (16/16)	-	
	Mutation	0 (0/16)	-	-	0 (0/16)	-	-	0 (0/16)	-	-
HRAS	WT	94 (15/16)	-		94 (15/16)	-		94 (15/16)	-	
	Mutation	6 (1/16)	-	-	6 (1/16)	-	-	6 (1/16)	-	-
KDR	WT	94 (15/16)	-		94 (15/16)	-		94 (15/16)	-	
	Mutation	6 (1/16)	-	-	6 (1/16)	-	-	6 (1/16)	-	-
KIT	WT	88 (14/16)	-		88 (14/16)	-		88 (14/16)	-	
	Mutation	12 (2/16)	-	-	12 (2/16)	-	-	12 (2/16)	-	-
KRAS	WT	75 (12/16)	59 (45/76)		75 (12/16)	64 (28/44)		75 (12/16)	53 (17/32)	
	Mutation	25 (4/16)	41 (31/76)	0.273	25 (4/16)	36 (16/44)	0.541	25 (4/16)	47 (15/32)	0.213
MET	WT	100 (16/16)	99 (76/77)		100 (16/16)	100 (47/47)		100 (16/16)	97 (29/30)	
	Mutation	0 (0/16)	1 (1/77)	1.0	0 (0/16)	0 (0/47)	-	0 (0/16)	3 (1/30)	1.0
NRAS	WT	100 (16/16)	98 (52/53)		100 (16/16)	96 (25/26)		100 (16/16)	100 (27/27)	
	Mutation	0 (0/16)	2 (1/53)	1.0	0 (0/16)	4 (1/26)	1.0	0 (0/16)	0 (0/27)	-
PIK3CA	WT	94 (15/16)	100 (85/85)		94 (15/16)	100 (51/51)		94 (15/16)	100 (34/34)	
	Mutation	6 (1/16)	0 (0/85)	0.158	6 (1/16)	0 (0/51)	0.239	6 (1/16)	0 (0/34)	0.320
RB1	WT	94 (15/16)	-		94 (15/16)	-		94 (15/16)	-	
	Mutation	6 (1/16)	-	-	6 (1/16)	-	-	6 (1/16)	-	-
SMAD4	WT	100 (16/16)	-		100 (16/16)	-		100 (16/16)	-	
	Mutation	0 (0/16)	-	-	0 (0/16)	-	-	0 (0/16)	-	-
TP53	WT	69 (11/16)	-		69 (11/16)	-		69 (11/16)	-	
	Mutation	31 (5/16)	-	-	31 (5/16)	-	-	31 (5/16)	-	-

P value significant at $P < 0.05$. In case of absence of a variable or category in a comparison, statistics could not be calculated (indicated by "-").

cases with many copy number aberrations (cluster 2) (Fig. 5). When integrating clinical features with DNA copy number aberrations, no specific distribution of the IBD-associated cases over the 2 clusters was observed. The majority of IBD-associated dysplasia (22/30) and approximately half of IBD cancers (6/13) are present in cluster 1, but both groups of lesions are also present in cluster 2, dysplastic lesions (8/30) and cancers (7/13), and these differences are not statistically significant ($P = 0.09$). Sporadic lesions are also distributed over both clusters, although the majority of adenomas (91/118) are in cluster 1 and the majority of cancers (17/24) are in cluster 2 ($P < 0.001$). Inflammatory bowel

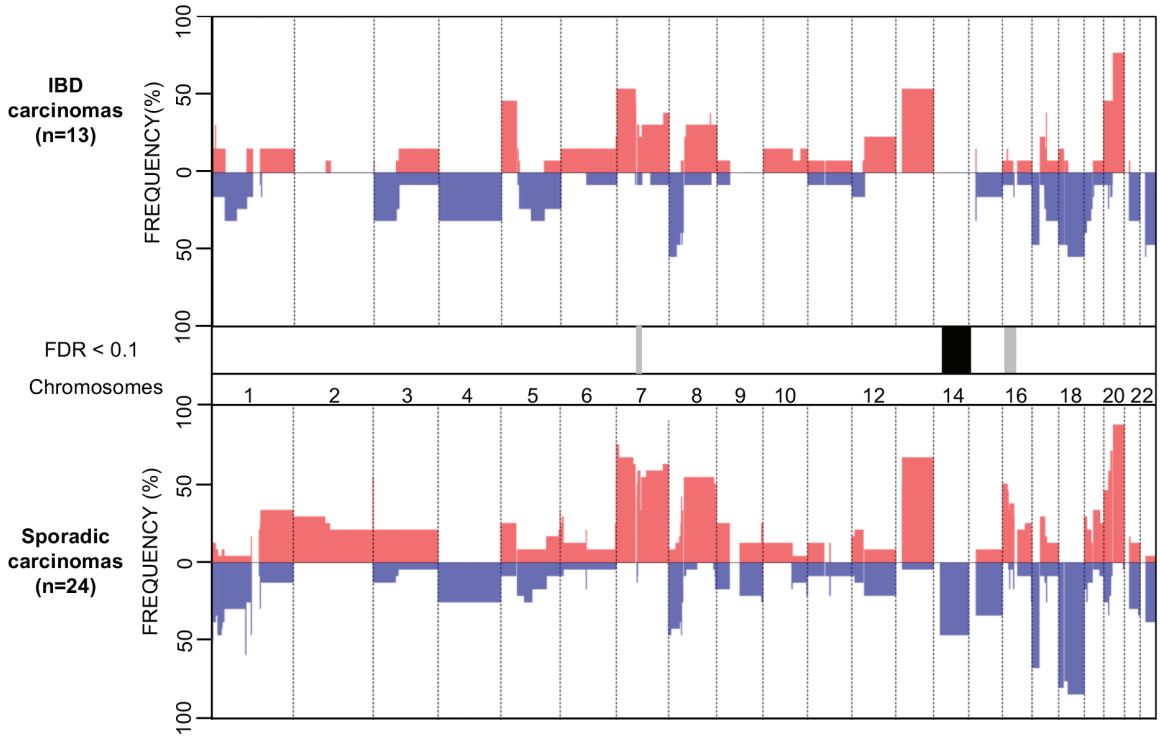

FIGURE 4. Frequency plot of copy number aberrations of IBD-associated carcinomas (upper panel) and sporadic carcinomas (lower panel). Frequency plots of copy number gains (red) and losses (blue) in 13 IBD-associated carcinomas (upper panel) and 24 sporadic carcinomas (lower panel). Significant differences (FDR < 0.1) between the 2 types of lesions are depicted in black for losses and in gray for gains.

TABLE 4. Mutation Frequencies in IBD-Associated and Sporadic Cancers

		IBD, % (No.)	Sporadic, % (No.)				IBD, % (No.)	Sporadic, % (No.)	
Gene		Cancer	Cancer	*P*	Gene		Cancer	Cancer	*P*
APC	WT	100 (9/9)	100 (4/4)		KDR	WT	100 (9/9)	-	
	Mutation	0 (0/9)	0 (0/4)	-		Mutation	0 (0/9)	-	-
ATM	WT	100 (9/9)	-		KIT	WT	89 (8/9)	-	
	Mutation	0 (0/9)	-	-		Mutation	11 (1/9)	-	-
BRAF	WT	100 (9/9)	100 (8/8)		KRAS	WT	67 (6/9)	57 (4/7)	
	Mutation	0 (0/9)	0 (0/8)	-		Mutation	33 (3/9)	43 (3/7)	1.0
CDH1	WT	100 (9/9)	-		MET	WT	100 (9/9)	100 (7/7)	
	Mutation	0 (0/9)	-	-		Mutation	0 (0/9)	0 (0/7)	-
CTNNB1	WT	100 (9/9)	86 (6/7)		NRAS	WT	100 (9/9)	100 (4/4)	
	Mutation	0 (0/9)	14 (1/7)	0.438		Mutation	0 (0/9)	0 (0/4)	-
FBXW7	WT	89 (8/9)	100 (8/8)		PIK3CA	WT	78 (7/9)	100 (8/8)	
	Mutation	11 (1/9)	0 (0/8)	1.0		Mutation	22 (2/9)	0 (0/8)	0.471
FGFR1	WT	89 (8/9)	-		RB1	WT	100 (9/9)	-	
	Mutation	11 (1/9)	-	-		Mutation	0 (0/9)	-	-
GNA11	WT	100 (9/9)	-		SMAD4	WT	89 (8/9)	-	
	Mutation	0 (0/9)	-	-		Mutation	11 (1/9)	-	-
HNF1A	WT	89 (8/9)	-		TP53	WT	22 (2/9)	-	
	Mutation	11 (1/9)	-	-		Mutation	78 (7/9)	-	-
HRAS	WT	100 (9/9)	-						
	Mutation	0 (0/9)	-	-					

P value significant at $P < 0.05$. In case of absence of a variable or category in a comparison, statistics could not be calculated (indicated by "-").

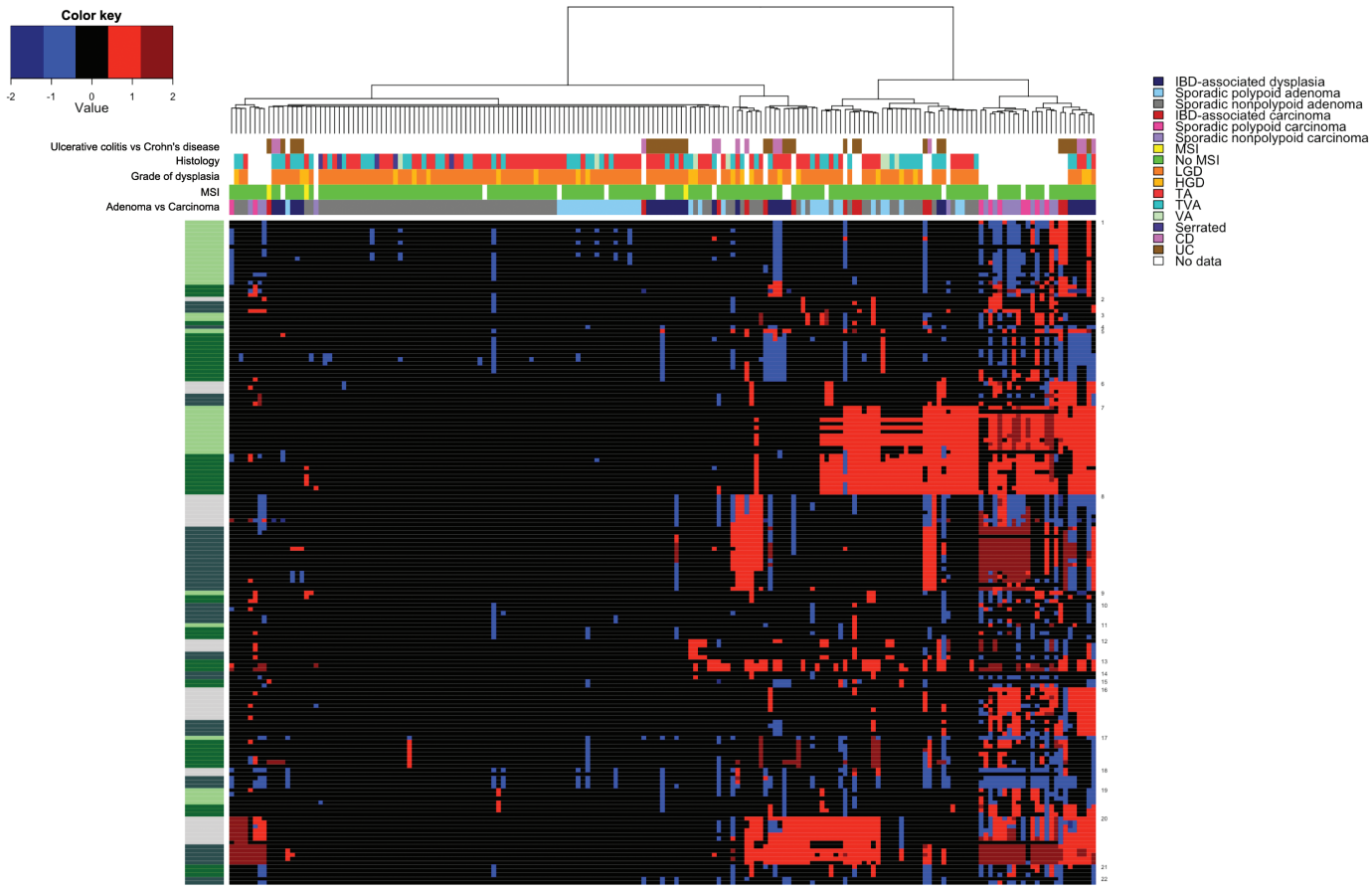

FIGURE 5. Hierarchical cluster analysis of both IBD-associated lesions and sporadic lesions based on DNA copy number aberrations. Heatmap displaying DNA copy number gains and losses on chromosomes 1–22 (lines) in all cases (columns). Weighted Clustering of Called aCGH data was performed using Ward linkage. In the horizontal upper bar, clinical features are described: type of IBD, histology, grade of dysplasia, microsatellite instability, and type of lesion. Red, copy number gain; blue, copy number loss; black, no copy number alteration.

disease–associated dysplastic lesions and nonpolypoid sporadic adenomas do not cluster together. All MSI cases are in cluster 1. Of the high-grade dysplasia cases, 73% (16/22) are in cluster 1 and 27% (6/22) are in cluster 2, but these differences are not statistically significant ($P = 0.1$).

Both clusters contain tubular adenomas (TAs), along with tubulovillous adenomas (TVAs) and villous adenomas (VAs). Most of the TAs are included in cluster 1 (65/79), along with all serrated lesions, although this distribution is not significantly different between the 2 clusters ($P = 0.08$). Also, UC and CD patients are distributed in both clusters, without significant differences ($P = 0.7$).

Regarding associations of specific mutations with clinical-histological features, in IBD-associated lesions, no significant differences were observed between different histological types, different grades of dysplasia, or MSI status for any type of mutation (Table 5). Comparing the 2 types of IBD, no significant differences were found in any specific mutation, showing that the same pathways are affected in both UC and CD (Table 5, Fig. 6).

DISCUSSION

In the present study, we compared the molecular profiles of IBD-associated dysplastic lesions and cancers with sporadic adenomas and cancers. We focused on whole-genome DNA copy number aberrations and mutations in recurrently altered genes in CRC. Inflammatory bowel disease–associated dysplastic lesions already harbor many of the DNA copy number aberrations that are typically observed in both IBD-associated cancers and sporadic cancers. This pattern of DNA copy number aberrations is much less common in sporadic adenomas, indicating that IBD-associated dysplastic lesions are more advanced in their progression toward cancer than sporadic adenomas. Fifteen of the 48 analyzed genes showed mutations in IBD-associated lesions, with *TP53* being the most frequently mutated gene in IBD-associated dysplastic lesions and cancers. *FBXW7* was significantly more often mutated in IBD-associated dysplastic lesions than in sporadic adenomas, especially when compared with nonpolypoid sporadic adenomas. On the other hand, *APC* was significantly less frequently mutated in IBD-associated dysplastic lesions compared with

TABLE 5. Mutation Frequencies in IBD-Associated Lesions Regarding Clinico-Histologic Features

		IBD, % (No.)			IBD, % (No.)			IBD, % (No.)			IBD, % (No.)		
Gene		TA (n = 10)	TVA (n = 6)	*P*	LGD (n = 13)	HGD (n = 3)	*P*	MSI (n = 2)	MSS (n = 23)	*P*	UC (n = 15)	CD (n = 10)	*P*
APC	WT	70 (7/10)	83 (5/6)		77 (10/13)	67 (2/3)		100 (2/2)	83 (19/23)		87(13/15)	80 (8/10)	
	Mutation	30 (3/10)	17 (1/6)	1.0	23 (3/13)	33 (1/3)	1.0	0 (0/2)	17 (4/23)	1.0	13 (2/15)	20 (2/10)	1.0
ATM	WT	100 (10/10)	83 (5/6)		92 (12/13)	100 (3/3)		100 (2/2)	96 (22/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	8 (1/13)	0 (0/3)	1.0	0 (0/2)	4 (1/23)	1.0	7 (1/15)	0 (0/10)	-
BRAF	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	100 (23/23)		100 (15/15)	100 (10/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	0 (0/23)	-	0 (0/15)	0 (0/10)	-
CDH1	WT	90 (9/10)	83 (5/6)		92 (12/13)	67 (2/3)		100 (2/2)	91 (21/23)		93 (14/15)	90 (9/10)	
	Mutation	10 (1/10)	17 (1/6)	1.0	8 (1/13)	33 (1/3)	0.350	0 (0/2)	9 (2/23)	1.0	7 (1/15)	10 (1/10)	1.0
CTNNB1	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	100 (23/23)		100 (15/15)	100 (10/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	0 (0/23)	-	0 (0/15)	0 (0/10)	-
FBXW7	WT	80 (8/10)	83 (5/6)		85 (11/13)	67 (2/3)		50 (1/2)	87 (20/23)		87(13/15)	80 (8/10)	
	Mutation	20 (2/10)	17 (1/6)	1.0	15 (2/13)	33 (1/3)	0.489	50 (1/2)	13 (3/23)	0.300	13 (2/15)	20 (2/10)	1.0
FGFR1	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	96 (22/23)		100 (15/15)	90 (9/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	4 (1/23)	1.0	0 (0/15)	10 (1/10)	0.400
GNA11	WT	100 (10/10)	83 (5/6)		100 (13/13)	67 (2/3)		100 (2/2)	96 (22/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	0 (0/13)	33 (1/3)	0.188	0 (0/2)	4 (1/23)	1.0	7 (1/15)	0 (0/10)	1.0
HNF1A	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		50 (1/2)	100 (23/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	50 (1/2)	0 (0/23)	0.080	7 (1/15)	0 (0/10)	1.0
HRAS	WT	100 (10/10)	83 (5/6)		92 (12/13)	100 (3/3)		50 (1/2)	100 (23/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	8 (1/13)	0 (0/3)	1.0	50 (1/2)	0 (0/23)	0.080	7 (1/15)	0 (0/10)	1.0
KDR	WT	100 (10/10)	83 (5/6)		92 (12/13)	100 (3/3)		100 (2/2)	96 (22/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	8 (1/13)	0 (0/3)	1.0	0 (0/2)	4 (1/23)	1.0	7 (1/15)	0 (0/10)	1.0
KIT	WT	100 (10/10)	67 (4/6)		85 (11/13)	100 (3/3)		50 (1/2)	96 (22/23)		87 (13/15)	90 (9/10)	
	Mutation	0 (0/10)	33 (2/6)	0.125	15 (2/13)	0 (0/3)	1.0	50 (1/2)	4 (1/23)	0.230	13 (2/15)	10 (1/10)	1.0
KRAS	WT	90 (9/10)	50 (3/6)		69 (9/13)	100 (3/3)		100 (2/2)	70 (16/23)		80 (12/15)	60 (6/10)	
	Mutation	10 (1/10)	50 (3/6)	0.118	31 (4/13)	0 (0/3)	0.529	0 (0/2)	30 (7/23)	1.0	20 (3/15)	40 (4/10)	0.378
MET	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	100 (23/23)		100 (15/15)	100 (10/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	0 (0/23)	-	0 (0/15)	0 (0/10)	-
NRAS	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	100 (23/23)		100 (15/15)	100 (10/10)	
	Mutation	0 (0/10)	0 (0/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	0 (0/23)	-	0 (0/15)	0 (0/10)	-
PIK3CA	WT	100 (10/10)	83 (5/6)		92 (12/13)	100 (3/3)		50 (1/2)	91 (21/23)		87(13/15)	90 (9/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	8 (1/13)	0 (0/3)	1.0	50 (1/2)	9 (2/23)	0.230	13 (2/15)	10 (1/10)	1.0
RB1	WT	100 (10/10)	83 (5/6)		92 (12/13)	100 (3/3)		100 (2/2)	96 (22/23)		93 (14/15)	100 (10/10)	
	Mutation	0 (0/10)	17 (1/6)	0.375	8 (1/13)	0 (0/3)	1.0	0 (0/2)	4 (1/23)	1.0	7 (1/15)	0 (0/10)	1.0
SMAD4	WT	100 (10/10)	100 (6/6)		100 (13/13)	100 (3/3)		100 (2/2)	96 (22/23)		100 (15/15)	90 (9/10)	
	Mutation	0 (0/10)	0 (6/6)	-	0 (0/13)	0 (0/3)	-	0 (0/2)	4 (1/23)	1.0	0 (0/10)	10 (1/10)	0.4
TP53	WT	70 (7/10)	67 (4/6)		77 (10/13)	33 (1/3)		100 (2/2)	48 (11/23)		53 (8/15)	50 (5/10)	
	Mutation	30 (3/10)	33 (2/6)	1.0	23 (3/13)	67 (2/3)	0.214	0 (0/2)	52 (12/23)	0.480	47 (7/15)	50 (5/10)	1.0

P value significant at $P < 0.05$. In case of absence of a variable or category in a comparison, statistics could not be calculated (indicated by "-").

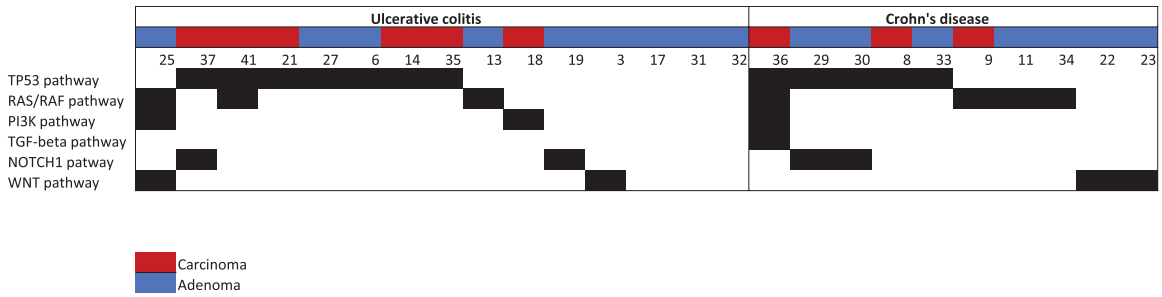

FIGURE 6. Signaling pathways affected in UC and CD. Genes mutated were assigned to curated pathways based on Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/pathway.html). In dotted black, carcinoma; in dotted white, dysplasia.

polypoid sporadic adenomas. Of note, IBD-associated dysplastic lesions showed significantly more losses of 5q, where *APC* is located (Fig. 3). These results indicate that IBD-associated dysplasia may follow different molecular pathways and/or use different mechanisms to disrupt crucial pathways in the progression to cancer, like the wnt-signaling pathway, when compared with sporadic adenomas.

Not unexpectedly, there was a significant difference in the age of the patients at the time of detection between the IBD patients with dysplastic lesions and the patients with sporadic adenomas (53.2 vs 70.2; $P < 0.001$).[2] However, when looking at the degree of dysplasia and histology of both IBD-associated dysplastic lesions and sporadic adenomas, no statistically significant differences were observed. Also, MSI status showed no significant difference between the 2 groups, although the frequency of MSI was very low, thereby precluding reliable analysis of any associations. Overall, we can observe a similar distribution of histological characteristics between IBD-associated dysplastic lesions and sporadic adenomas.

Inflammatory bowel disease–associated lesions, independently of being dysplastic or cancer, showed frequently complex profiles of copy number aberrations (Fig. 1). Ten of the 26 IBD low-grade dysplasia (LGD) lesions (38%) showed complex copy number patterns (Fig. 5). This is in agreement with a previously published study that showed that 40.5% of IBD-related LGD lesions displayed aneuploidy.[27] No significant DNA copy number differences were observed between the dysplastic lesions and cancers. However, at the mutation level, *TP53* was the most frequently mutated gene, and it was significantly more often mutated in IBD-associated cancers than in dysplastic lesions. *TP53* is a well-known tumor suppressor gene known to play an important role in the malignant transformation of sporadic adenomas,[28, 29] and our results indicate that this may also be the case in the IBD setting. Nonetheless, the fact that these TP53 mutations are already present in IBD-associated dysplastic lesions (31%) is consistent with a "reverse" Vogelstein model and could explain why these lesions have a high level of aneuploidy.

One of the main findings of this study is that IBD-associated dysplastic lesions show a much higher frequency of DNA copy number aberrations than sporadic adenomas. This indicates that chromosomal instability occurs earlier and/or at a higher frequency in IBD-associated dysplastic lesions than sporadic adenomas. This is consistent with earlier observations and may reflect a faster progression to cancer in at least a subset of IBD-associated dysplastic lesions.[6] In a recently published study, it was shown that LGD IBD-associated dysplastic lesions already showed significantly increased DNA copy number changes, which increased even more in HGD lesions. The latter had a total burden of DNA copy number alterations similar to IBD-associated cancers. Altogether, these data suggest an important role of DNA copy numbers in the progression to cancer.[30]

Inflammatory bowel disease–associated dysplastic lesions did not differ from the sporadic adenomas regarding gene mutation patterns, except for *FBXW7*, which was significantly more often mutated in IBD-associated dysplastic lesions (Table 3). *FBXW7* is a known tumor suppressor gene that encodes a protein that targets ubiquitination of several oncogenic proteins, like Cyclin E. Mutations leading to loss function of *FBXW7* can lead to impaired elimination of these oncogenic proteins and therefore contribute to uncontrolled cell proliferation. Colorectal cancers with mutations in this gene have been reported to have a worse prognosis.[31] In a recent study,[32] mutations in the *FBXW7* gene were also more common in a younger sporadic CRC cohort (<45 years), indicating that this gene might play a role in the early development of CRC in the IBD population.

Between all sporadic adenomas, morphology also seems to be of importance, as nonpolypoid adenomas and polypoid adenomas are molecularly different.[15, 33] As nonpolypoid adenomas could potentially be associated with inflammation,[15] comparison of IBD-associated dysplastic lesions with nonpolypoid and polypoid sporadic adenomas was conducted separately. Inflammatory bowel disease–associated lesions showed similarities with both polypoid and nonpolypoid dysplastic lesions. Sporadic nonpolypoid adenomas showed loss of chromosome 5q, which occurred even more frequently in the IBD-associated

dysplastic lesions but not in the sporadic polypoid adenomas. Loss of 5q has previously been associated with metastases and therefore can be considered a marker for aggressive lesions.[34] Also, the sporadic polypoid adenomas and IBD-associated dysplastic lesions showed similarities: Loss of chromosome 18q was present in both sporadic polypoid adenomas and IBD-associated dysplastic lesions, but not in sporadic nonpolypoid adenomas. This loss has previously been described to be involved in the adenoma-to-carcinoma progression.[12] Loss of 18q is the most frequently observed aberration in CRC and was also the most common aberration observed in the small number of carcinomas analyzed in this study (both sporadic cancers and IBD-associated cancers).[35] This might indicate that chromosome 18 loss is a crucial event in the development of cancer, independent of which molecular pathway is involved. Interestingly, a study on IBD-associated dysplastic lesions and cancers using fluorescence in situ hybridization and comparative genomic hybridization also detected the loss of chromosome 18 in both dysplastic lesions and cancers.[36] Three years later, the same group, showed, in addition to 18q loss, losses of 5q.[37]

When integrating clinical features and DNA copy number aberrations, IBD-associated dysplastic lesions do not seem to cluster with either polypoid or nonpolypoid adenomas. These results therefore do not support that IBD-associated dysplastic lesions are more similar to nonpolypoid adenomas.

At last, we compared both IBD-associated cancer and sporadic cancer. Both show typical DNA copy number aberrations observed in CRC.[35] In a recent study investigating the genetic landscape of IBD-associated cancer and non-neoplastic tissues from 31 IBD patients by whole-exome sequencing,[38] it was shown that *TP53* was the most common mutation observed in IBD-associated cancers, in agreement with our findings (Table 4). In the same study,[38] *TP53* mutation had a similar prevalence in both groups, but *APC* and *KRAS* mutations were significantly less frequent in IBD-associated cancers.[38] Another study comparing mutation patterns in IBD-associated cancers and sporadic cancers also showed that *APC* and *KRAS* were significantly less frequently mutated in IBD-associated cancers.[39] A meta-analysis on *KRAS* and *TP53* mutations showed that *TP53* was more common in IBD-associated cancers, whereas *KRAS* was less common.[40] Our observations are consistent with these reports.

A few limitations should be noted. First, IBD-associated dysplastic lesions are rare, which makes it difficult to collect large numbers of cases. Second, there is no gold standard for the diagnosis of IBD-related lesions. Therefore, we only included lesions that clinically were most evidently IBD-associated (most "severe" cases), and we may therefore have excluded a significant number of potentially IBD-associated dysplastic lesions. However, if a tendency toward advanced lesions were to exist, one would expect to observe mostly histologically high-grade lesions, and that was not the case.

CONCLUSIONS

In conclusion, the present study shows that IBD-associated dysplastic lesions have more chromosomal instability (CIN) than sporadic adenomas, suggesting a faster progression to cancer for IBD-associated dysplastic lesions. Our results indicate that IBD-associated dysplasia follows its unique pathway in progression to cancer on top of following, at least in part, similar progression pathways as both nonpolypoid adenomas and polypoid adenomas.

As not all IBD-associated dysplastic lesions display DNA copy number changes, screening for the presence of CIN in these lesions could be of help in deciding surveillance strategies for IBD patients. From the mutation analysis, although the sample size was limited, it appears that *FBXW7*, next to *TP53*, seems to play an important role in the progression of IBD-associated lesions.

SUPPLEMENTARY DATA

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

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