

Gene panel testing detects important genetic alterations in ulcerative colitis-associated colorectal neoplasia

YOSHIFUMI SHIMADA^{1,2}, MAE NAKANO^{1,2}, KEN-ICHI MIZUNO³, JUNJI YOKOYAMA³, AKIO MATSUMOTO¹, KANA TANAKA¹, HIDEHITO OYANAGI¹, MASATO NAKANO¹, YUKI HIROSE¹, HIROSHI ICHIKAWA¹, JUN SAKATA¹, HITOSHI KAMEYAMA⁴, YASUMASA TAKII⁵, MIKA SUGAI⁶, YIWEI LING⁷, SHIHO TAKEUCHI⁷, SHUJIRO OKUDA^{7,8}, SHUJI TERAII³, YOICHI AJIOKA⁹ and TOSHIFUMI WAKAI^{1,2}

¹Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences;

²Medical Genome Center, Niigata University Medical and Dental Hospital; ³Division of Gastroenterology and Hepatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Niigata 951-8510;

⁴Department of Digestive Surgery, Niigata City General Hospital, Niigata, Niigata 950-1197; ⁵Department of Surgery, Niigata Cancer Center Hospital, Niigata, Niigata 951-8566; ⁶Division of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Niigata 951-8518; ⁷Medical AI Center/Bioinformatics Laboratory, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Niigata 951-8514; ⁸Center for Genomic Data Management, Niigata University Medical and Dental Hospital, Niigata, Niigata 951-8520; ⁹Division of Molecular and Diagnostic Pathology, Graduate School of Medical and Dental Sciences, Niigata, Niigata 951-8510, Japan

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Abstract. Ulcerative colitis-associated neoplasia (UCAN) harbors unique genetic alterations and mutational tendencies. The clinical application of gene panel testing enables precision medicine by tailoring treatment to individual gene alterations. We hypothesized that gene panel testing may detect clinically important genetic alterations in UCAN, with potential usefulness for the diagnosis and treatment of UCAN. In the present study, gene panel testing was used to identify genetic alterations in UCAN, and the possibility of clinical utility of gene panel testing in UCAN was investigated. The present study included 15 patients with UCAN, and gene panel testing was performed to identify genetic alterations associated with diagnosis and treatment. Genetic alterations of UCAN were compared with those of 203 patients with sporadic colorectal cancer (CRC). *APC* and *PTEN* mutations were less frequent, while *RNF43* frameshift or nonsense mutations were more frequent in UCAN compared with sporadic CRC. *TP53* mutations were identified in 13/15 patients (87%) with UCAN. Notably, 4/15 patients (27%) with UCAN had no genetic

alterations other than *TP53* mutation, while this occurred in 1/203 patients (0.5%) with sporadic CRC ($P<0.001$). Microsatellite instability-high was identified in 2/15 patients (13%) with UCAN. Mutational signature 3, which is associated with homologous recombination deficiency, was detected in 14/15 patients (93%) with UCAN, and enriched in UCAN compared with sporadic CRC ($P=0.030$). In conclusion, gene panel testing can detect important genetic alterations that can be useful for diagnosis and treatment in UCAN, and may provide clinicians with important information for tailored treatment strategies.

Introduction

The inflammatory bowel diseases, ulcerative colitis (UC) and Crohn's disease, are chronic inflammatory diseases of the gastrointestinal tract, and have become global diseases that are increasing in Westernized countries (1). UC is associated with an increased risk of colitis-associated neoplasia, which increases with extended and more active inflammation (2). Ulcerative colitis-associated neoplasia (UCAN) was first reported by Crohn and Rosenberg in 1925 (3), and it has been recognized as an important complication of UC. Surveillance colonoscopy is widely accepted as being important for the early detection and treatment of UCAN, and UCAN surveillance is recommended in many countries (4).

In this surveillance, clinicians need to distinguish between sporadic neoplasms and UCAN based on endoscopic and pathological findings. Generally, sporadic neoplasms and UCAN are treated differently. Endoscopic resection is usually applied if the lesion is diagnosed as a sporadic neoplasm; while total proctocolectomy with ileal pouch-anal anastomosis or ileal pouch-anal canal anastomosis is applied if the

Correspondence to: Dr Yoshifumi Shimada or Dr Toshifumi Wakai, Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Chuo-ku, Niigata, Niigata 951-8510, Japan
E-mail: shimaday@med.niigata-u.ac.jp
E-mail: wakait@med.niigata-u.ac.jp

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lesion is diagnosed as UCAN (4). The American College of Gastroenterology clinical guideline described subsequent surveillance colonoscopy should initially be performed at shortened intervals when dysplasia in the UC case in which discrete neoplasms are completely removed endoscopically refusing the total proctocolectomy (4). Therefore, the differential diagnosis between UCAN and sporadic neoplasm is important when determining the treatment strategy for neoplasms arising in long-standing UC. However, it is often difficult to distinguish between the two using endoscopic and histopathological findings.

UCAN chemotherapy regimens are usually selected from cytotoxic and molecular-targeted agents used for sporadic colorectal cancer (CRC), based on genetic testing for markers such as *RAS*, *BRAF*, and microsatellite instability (MSI) status (5). The genomic landscape of sporadic CRC has been fairly well studied using next-generation sequencing (NGS) technology. Despite previous analyses showing that UCAN harbors unique genetic alterations and mutational tendencies (6-20), chemotherapy regimens for UCAN continue to be extrapolated from those for sporadic CRC.

Clinical application of gene panel testing enables us to practice precision medicine by tailoring treatment to individual gene alterations, and we previously reported NGS-based gene panel testing for management of solid tumors (21). We assumed that gene panel testing would detect clinically important genetic alterations in UCAN, with potential utility in UCAN diagnosis and treatment. In this analysis, we aimed to identify genetic alterations of UCAN using gene panel testing, and investigate the possibility of clinical utility of gene panel testing in UCAN.

Materials and methods

Patients. We studied 15 patients with UCAN who had been treated between 2009 and 2021 at Niigata University Medical and Dental Hospital. We have previously reported on genetic alterations in Japanese patients with sporadic CRC using gene panel testing (22-27), but not in patients with UCAN. In this analysis, we identified genetic alterations in the 15 patients with UCAN, and compared them with those identified in Stage I-IV 203 patients with sporadic CRC according to the American Joint Committee on Cancer guidelines, 8th edition (28), who had undergone primary tumor resection between 2009 and 2015 at Niigata University Medical and Dental Hospital or Niigata Cancer Centre Hospital. Endoscopic diagnoses of UCAN were made by endoscopists specializing inflammatory bowel disease (K. M. and J. Y.) according to the SCENIC international consensus statement (29). Histopathological diagnoses of UCAN were made by a pathologist specializing in inflammatory bowel disease (Y. A.) according to the classification proposed by the Research Committee on Inflammatory Bowel Disease of the Japanese Ministry of Health and Welfare (30) and the Riddell's classification (31). p53 overexpression was assessed as an aid to histopathological diagnosis of UCAN (32). We included 15 UCAN diagnosed as UC-IV of the classification proposed by the Research Committee on Inflammatory Bowel Disease of the Japanese Ministry of Health and Welfare (30). UC-IV was defined as carcinoma including intramucosal carcinoma. The diagnosis of intramucosal carcinoma was to be made when

there was a high grade of cytological and structural atypia consistent with carcinoma (30). This retrospective analysis was performed in accordance with the Helsinki Declaration, and the Ethics Committee of the School of Medicine, Niigata University approved the study protocol (G2015-0816, G2020-0038). Written informed consent was obtained from the patients.

NGS for detecting genetic alterations. Formalin-fixed, paraffin-embedded biopsy or endoscopically/surgically resected samples were used for evaluating genetic alterations, as we have previously reported (22-27). Briefly, hematoxylin and eosin-stained sections were used to assess tumor content, ensuring >50% tumor content. Where applicable, unstained sections were macro-dissected to enrich for tumor content. DNA was extracted using a BioStic FFPE Tissue DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). All sample preparations, NGS, and bioinformatics analyses were performed in a Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP)-accredited laboratory. First, 50-150 ng DNA fragment libraries were prepared and enriched for CANCERPLEX version 3.0 (415-gene panel; KEW) or version 4.0 (435-gene panel; KEW). An average 500x sequencing depth was achieved using the Illumina MiSeq and NextSeq platforms. A 10% allelic fraction threshold for single nucleotide variants (SNVs) and insertions/deletions was used, as well as thresholds of >2.5-fold and 0.5-fold for gain and loss, respectively. MSI was tested based on an extended loci panel: in addition to the Bethesda panel, a collection of 950 regions consisting of tandem repeats of 1, 2 or 3 nucleotides with a minimum length of 10 bases was used. Tumor mutational burden was calculated as the number of non-synonymous mutations per megabase of sequence in the panel (panel size=1.3 Mb). Mutational signatures were analyzed as previously reported (27). Each SNV was classified in a matrix of the 96 possible substitutions, based on the sequence context comprising the nucleotides 5' and 3' to the position of the mutation. Mutational signatures were extracted using non-negative matrix factorization analysis with the SomaticSignatures R package (33) (The R Foundation, Vienna, Austria) and plotted with the ggplots R package (<http://ggplot2.org/>). We previously confirmed reliability of the mutational signatures at the gene panel level using data sets of gene panel testing and whole exome sequencing data (27).

Statistical analysis. Statistical analyses were performed with IBM SPSS Statistics 28 software (IBM Japan, Inc., Tokyo, Japan). The frequency of genetic alterations between UCAN and sporadic CRC were compared using Fisher's exact tests. The mutational signature ratio between UCAN and sporadic CRC was also compared using a Fisher's exact test. P-values of less than 0.05 were considered significant.

Results

Clinicopathological characteristics of UCAN. In this analysis of 15 patients with UCAN, UCAN was significantly associated with a lower T stage, N stage, and M stage compared with sporadic CRC (Table SI). The median disease duration of UC was 25 years (range, 9-45 years). Fourteen patients were diagnosed with UCAN caused by

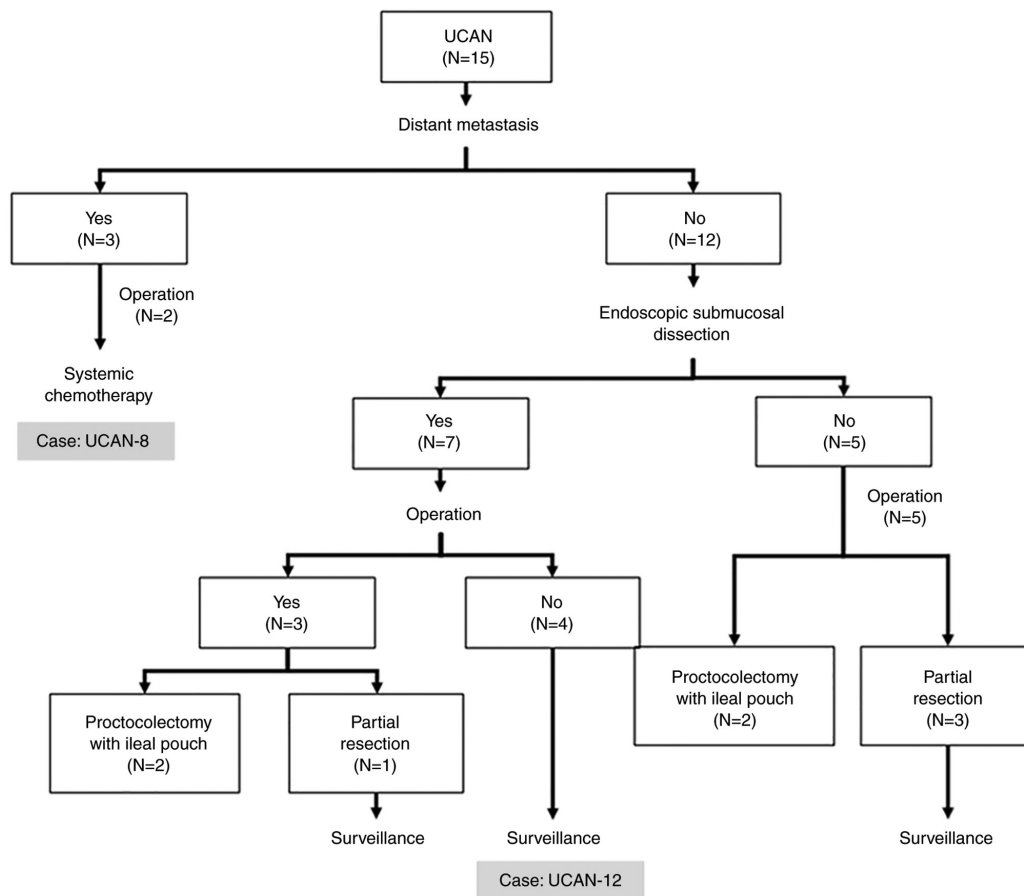


Figure 1. Flowchart of patients included in the analysis. UCAN, ulcerative colitis-associated neoplasia.

chronic inflammation of the large intestine, while one patient was diagnosed with UCAN caused by chronic pouchitis. Eight of 15 patients (53%) had UCAN in the rectum. Six patients had carcinoma in situ, while nine patients had a tumor invading to the submucosal layer or deeper. Detailed information for each patient is shown in Fig. 1 and Table I. Three patients had distant metastasis, and received systemic chemotherapy, as indicated for sporadic CRC. Four patients without distant metastasis underwent proctocolectomy with ileal pouch, while four patients without distant metastasis underwent partial resection of the large intestine because of the patients' request and have been followed-up using annual surveillance colonoscopy under informed consent. Seven patients who had a lesion localized in the mucosal layer or slightly invading into the submucosal layer underwent endoscopic submucosal dissection (ESD) for accurate diagnosis of UCAN. Four of the seven patients who underwent ESD have been followed-up using annual surveillance colonoscopy.

Gene panel testing of UCAN and sporadic CRC. Characteristic mutational tendencies in the WNT signaling pathway, including *APC* and *RNF43*, were identified in patients with UCAN. *APC* mutations were significantly less frequent in patients with UCAN compared with sporadic CRC ($P<0.001$), being identified in 2/15 patients (13%) with UCAN and 164/203 patients (81%) with sporadic CRC (Fig. 2). *RNF43* frameshift or nonsense mutations

were significantly more frequent in patients with UCAN compared with sporadic CRC ($P=0.025$), being identified in 4/15 patients (27%) with UCAN and 14/203 patients (7%) with sporadic CRC (Figs. S1 and 2). *PTEN* mutations were significantly less frequent in patients with UCAN compared with sporadic CRC ($P=0.014$), being completely absent in patients with UCAN (Fig. 2). *TP53* mutations were identified in 13/15 patients (87%) with UCAN, with most consisting of SNV, identified in the DNA-binding domain (Fig. S3). Interestingly, 4/15 patients (27%) with UCAN had no genetic alterations other than a *TP53* mutation, while this occurred in 1/203 patients (0.5%) with sporadic CRC ($P<0.001$) (Fig. S4).

MSI-H was identified in 2/15 patients (13%) with UCAN and 13/203 patients (6%) with sporadic CRC. Despite the reported clinical utility of immune checkpoint inhibitors (ICIs) in some patients with MSI-H CRC, no patients received ICIs in this cohort. Mutational signature 3, which is associated with failure of DNA double-strand break repair by homologous recombination deficiency (HRD), was detected in 14/15 patients (93%) with UCAN (Fig. 3), and enriched in UCAN compared with sporadic CRC ($P=0.030$) (Fig. S5). Among all the 15 patients with MSI-H in this cohort (two UCAN, 13 sporadic CRC), the two UCAN patients exhibited mutational signature 3 (Fig. S6). An oxaliplatin-based regimen, which is considered effective for tumors with failed DNA double-strand break repair, was used in the two patients with mutational signature 3, who had stage IV disease.

Table I. Clinicopathological characteristics of 15 patients with UCAN.

ID	Age, years	Sex	UC disease			TNM stage ^a	Histological classification ^b	p53 IHC overexpression	TMB (/Mb)	MSI status	APC	TP53	KRAS	RNF43	TP53 mutation alone	Treatment
			duration, years	Primary tumor location												
UCAN-1	67	M	45	Ascending	0	UC-IV + UC-III	Absent	26.94	MSI-H				p.G659fs			Partial resection
UCAN-2	66	F	35	Rectum	IIIB	UC-IV	NA	26.17	MSI-H		p.I195T			Yes		Partial resection
UCAN-3	67	M	40	Rectum	IVB	UC-IV	NA	17.7	MSS		p.S1198X	p.R273H, p.R248Q, loss				Systemic chemotherapy
UCAN-4	45	M	23	Ascending	I	UC-IV + UC-III	Absent	16.9	MSS	loss	p.C135F					Partial resection
UCAN-5	62	F	15	Transverse	I	UC-IV + UC-III	Present	14.6	MSS			p.P152L, loss	p.G12V			Proctocolectomy with ileal pouch
UCAN-6	37	M	18	Ascending	0	UC-IV + UC-III	Absent	13.9	MSS			loss		p.E742X, p.R132X		Proctocolectomy with ileal pouch
UCAN-7	61	M	25	Rectum	I	UC-IV + UC-III	Present	12.32	MSS		p.R248Q			Yes		ESD followed by partial resection
UCAN-8	43	F	26	Ileal pouch	IVA	UC-IV + UC-III	NA	11.55	MSS		p.G199V	p.G12D				Resection of ileal pouch followed by systemic chemotherapy
UCAN-9	42	M	17	Rectum	IVC	UC-IV	NA	8.5	MSS		p.Y234C			Yes		Partial resection followed by systemic chemotherapy
UCAN-10	53	M	15	Rectum	0	UC-IV + UC-III	Present	6.9	MSS		p.R248W	p.G13D				ESD
UCAN-11	67	M	27	Rectum	0	UC-IV + UC-III	Present	6.2	MSS		p.V173M					ESD followed by proctocolectomy with ileal pouch
UCAN-12	65	F	30	Sigmoid	0	UC-IV + UC-III	Present	6.2	MSS		Splice variant			Yes		ESD
UCAN-13	53	F	9	Ascending	0	UC-IV	Absent	2.3	MSS					p.G659fs		ESD

Table I. Continued. Clinicopathological characteristics of 15 patients with UCAN.

ID	UC				TNM stage ^a	Histological classification ^b	p53 IHC overexpression	TMB (/Mb)	MSI status	APC	TP53	KRAS	RNF43	TP53 mutation alone	Treatment
	Age, years	Sex	disease duration, years	Primary tumor location											
UCAN-14	49	F	15	Rectum	I	UC-IV + UC-III	NA	1.5	MSS		p.I254T	p.G13D			ESD followed by proctocolectomy with ileal pouch
UCAN-15	66	F	31	Rectum	I	UC-IV + UC-III	Present	0.8	MSS		p.R196X		p.D95fs		ESD

^aTNM stage was determined according to the American Joint Committee on Cancer Staging Manual, 8th Edition (28). ^bHistological classification was determined according to the classification proposed by the Research Committee on Inflammatory Bowel Disease of the Japanese Ministry of Health and Welfare (29). APC, adenomatous polyposis coli; UC-IV, carcinoma including intramucosal carcinoma; UC-III, neoplastic but not carcinomatous; ESD, endoscopic submucosal dissection; F, female; IHC, immunohistochemistry; M, male; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite stable; NA, not assessed; RNF43, ring finger protein 43; TMB, tumor mutational burden; UC, ulcerative colitis; UCAN, ulcerative colitis-associated neoplasm.

^aTNM stage was determined according to the American Joint Committee on Cancer Staging Manual, 8th Edition (28). ^bHistological classification was determined according to the classification proposed by the Research Committee on Inflammatory Bowel Disease of the Japanese Ministry of Health and Welfare (29). APC, adenomatous polyposis coli; UC-IV, carcinoma including intramucosal carcinoma; UC-III, neoplastic but not carcinomatous; ESD, endoscopic submucosal dissection; F, female; IHC, immunohistochemistry; M, male; MSI, microsatellite instability; MSS, microsatellite stable; NA, not assessed; RNF43, ring finger protein 43; TMB, tumor mutational burden; UC, ulcerative colitis; UCAN, ulcerative colitis-associated neoplasia.

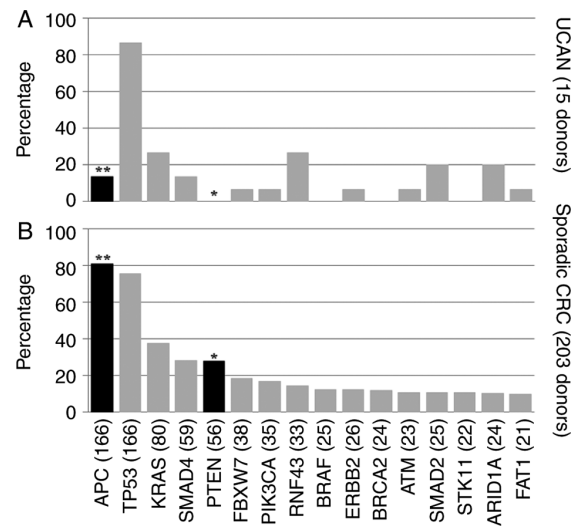


Figure 2. Distribution of genetic alterations in UCAN and sporadic CRC. Percentages indicate the mutation-positive rate of individual genes. (A) Distribution of genetic alterations in UCAN. (B) Distribution of genetic alterations in sporadic CRC. Dark bars indicate genes with a significant difference (P<0.05, Fisher's exact test). *P<0.05 (UCAN vs. sporadic CRC); **P<0.01 (UCAN vs. sporadic CRC). CRC, colorectal cancer; UCAN, ulcerative colitis-associated neoplasia.

Possibility of clinical utility of gene panel testing for diagnosis in UCAN. A 65-year-old-woman with a 30-year history of extensive UC was found to have a flat mucosal lesion in her sigmoid colon by surveillance colonoscopy (Fig. 4A). ESD was performed to diagnose UCAN (Fig. 4B), and the lesion was diagnosed as a well-differentiated adenocarcinoma (Fig. 4C). Gene panel testing revealed that the tumor had a *TP53* splice site variant, with no other mutations detected (Fig. 4D). The genome profile was considered to be characteristic of UCAN, and served an auxiliary role for diagnosis of UCAN. Although the patient was recommended proctocolectomy with an ileal pouch, she declined surgery and instead chose follow-up by annual surveillance colonoscopy. She has been treated with mesalazine, and her condition has been well controlled. No new lesion has been detected five years after ESD (Fig. 4A).

Possibility of clinical utility of gene panel testing for treatment in UCAN. A 43-year-old-woman received proctocolectomy with an ileal pouch for severe UC 26 years ago. She had a 13-year history of pouchitis, and had received various medical treatments for pouchitis (Fig. 5A). Pouchoscopy revealed an irregular ulcerative mass lesion in the ileal pouch near the anastomotic site, suggesting that the lesion might have developed at the remnant rectal tissue. Histopathological diagnosis of the biopsy specimen was poorly-differentiated adenocarcinoma. Taken together, she was diagnosed with UCAN arising from the ileal pouch (Fig. 5B). Abdominal computed tomography and magnetic resonance imaging revealed a solitary liver metastasis in S8 (Fig. 5C). She underwent resection of the ileal pouch, and gene panel testing of the primary tumor found *TP53*, *PIK3CA* and *KRAS* mutations. Moreover, mutational signature 3 was also identified (Fig. 5D). She received neoadjuvant chemotherapy including oxaliplatin (one course of CapeOx and four courses of mFOLFOX6), which seems to be effective for tumors with failure of DNA double-strand

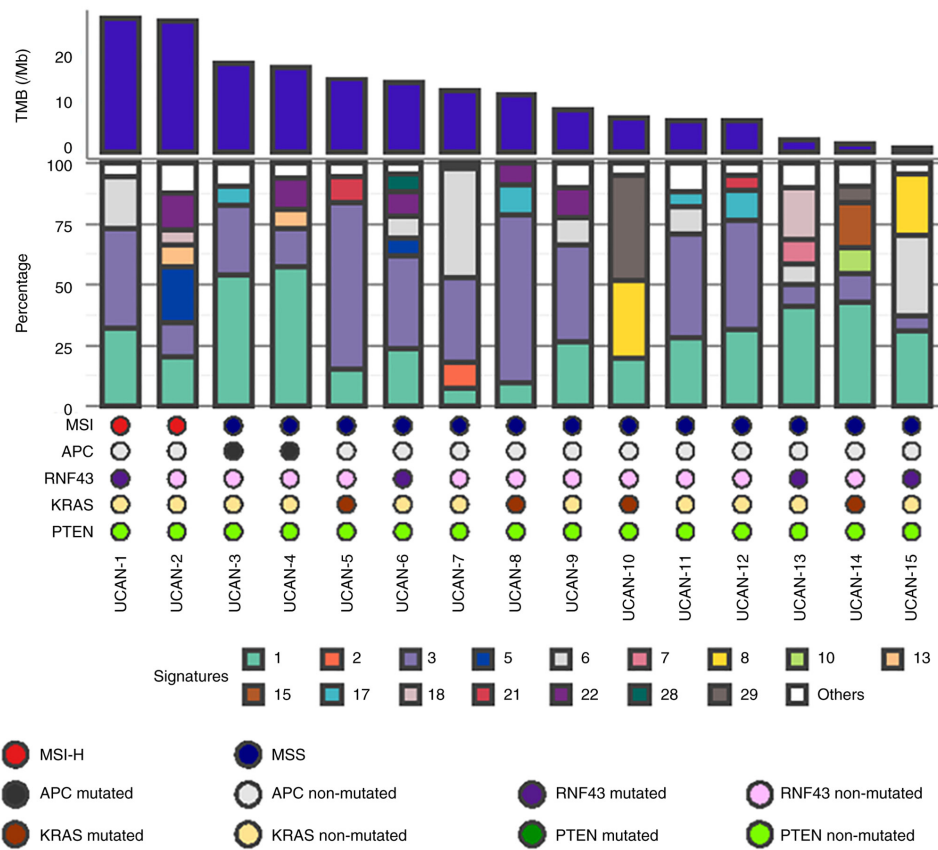


Figure 3. Mutational signature distributions of 15 patients with UCAN. APC, adenomatous polyposis coli; MSI-H, microsatellite instability-high; MSS, microsatellite stable; RNF43, ring finger protein 43; TMB, tumor mutational burden; UCAN, ulcerative colitis-associated neoplasia.

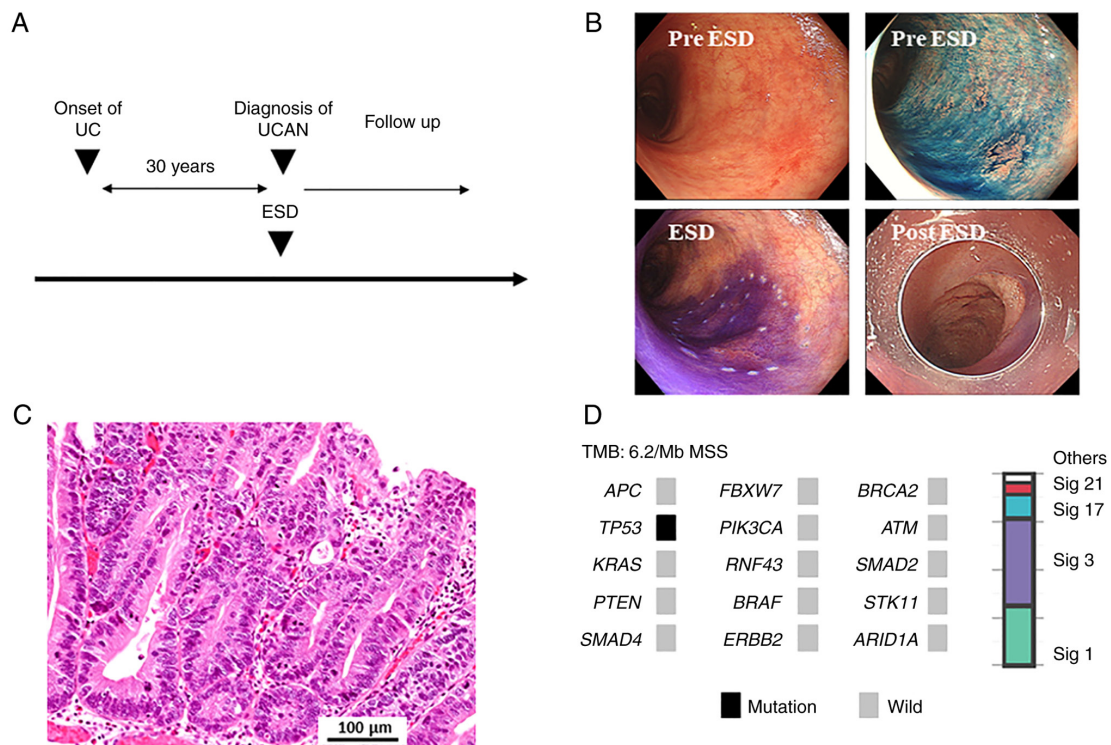


Figure 4. A patient (UCAN-12) with UCAN that underwent ESD followed by surveillance without proctocolectomy. (A) Clinical course of the patient. (B) ESD for UCAN in sigmoid colon. (C) Histopathological assessment, hematoxylin and eosin staining. Scale bar, 100 μ m. (D) Genome profile and mutational signature of the patient. APC, adenomatous polyposis coli; ARID1A, AT-rich interaction domain 1A; ATM, ataxia telangiectasia mutated; ERBB2, erb-b2 receptor tyrosine kinase 2; ESD, endoscopic submucosal dissection; FBXW7, F-box and WD repeat domain containing 7; MSS, microsatellite stable; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α ; RNF43, ring finger protein 43; Sig, signature; STK11, serine/threonine kinase 11; TMB, tumor mutational burden; UC, ulcerative colitis; UCAN, ulcerative colitis-associated neoplasia.

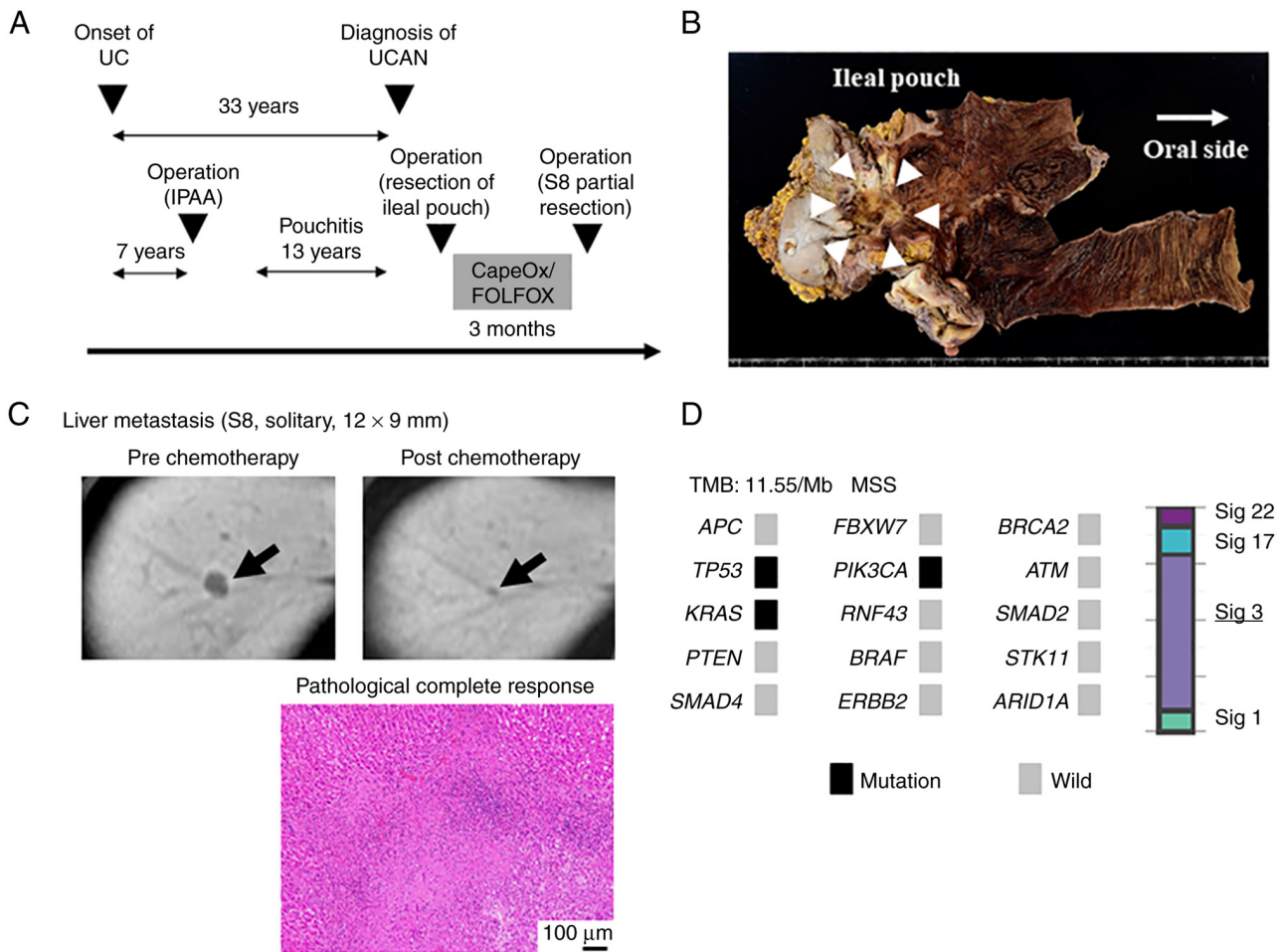


Figure 5. A patient (UCAN-8) with UCAN diagnosed as carcinoma arising from the ileal pouch with a solitary liver metastasis. (A) Clinical course of the patient. (B) Surgical specimen of the ileal pouch. (C) Radiological and histopathological assessment of liver metastasis. Scale bar, 100 μm. (D) Genome profile and mutational signature of the patient. Arrows indicate lesion in each state of pre and post chemotherapy. Hematoxylin and eosin staining demonstrates pathological complete response after chemotherapy. APC, adenomatous polyposis coli; ARID1A, AT-rich interaction domain 1A; ATM, ataxia telangiectasia mutated; ERBB2, erb-b2 receptor tyrosine kinase 2; FBXW7, F-box and WD repeat domain containing 7; FOLFOX, fluorouracil, leucovorin, oxaliplatin; IPAA, ileal pouch-anal anastomosis; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; RNF43, ring finger protein 43; Sig, signature; STK11, serine/threonine kinase 11; UC, ulcerative colitis; UCAN, ulcerative colitis-associated neoplasia.

break repair by HRD. After the chemotherapy, the liver metastasis was shrunk. Then, partial resection of the liver (S8) was performed, and no viable cancer cells were detected by histopathological assessment (Fig. 5C).

Discussion

This analysis of genetic alterations in UCAN using gene panel testing generated two main findings. First, we demonstrated UCAN has a distinct genomic profile compared with sporadic CRC. Second, we identified genetic alterations and mutational signature characteristics that may be associated with diagnosis and treatment in UCAN. These results indicate gene panel testing can be useful for differential diagnosis of UCAN and sporadic CRC, and for tailoring treatment to individual genome profiles in UCAN.

Genetic alterations in the WNT pathway, including *APC* and *RNF43*, are different in UCAN compared with sporadic CRC. *APC* is a negative regulator that controls beta-catenin concentrations and interacts with E-cadherin, which are involved in cell adhesion (34). Almost all sporadic CRC have genetic

alterations in *APC*, where it plays an important role in tumorigenesis (35). Conversely, previous reports have shown UCAN has fewer genetic alterations in *APC* compared with sporadic CRC (6-20), which is consistent with our findings (Table II). *RNF43* is thought to negatively regulate the WNT pathway, and loss of *RNF43* plays an important role in sporadic CRC through the enhancement of WNT signaling (36). We previously reported on the clinical significance of *RNF43* mutations in sporadic CRC, which were associated with aggressive tumor biology along with *BRAF* mutations in right-sided CRC (26). Fujita *et al* (9) reported that somatic mutation of *RNF43* is the driver genetic alteration that links chronic inflammation and cancer development in about 10% of patients with UCAN. In this analysis, we found *RNF43* frameshift or nonsense mutations, thought to be associated with functional loss of *RNF43*, were significantly more frequent in UCAN compared with sporadic CRC. These differences in WNT pathway genetic alterations may be due to differences in developmental mechanisms between sporadic CRC and UCAN, and are some of the most important genetic differences for the differential diagnosis of sporadic CRC and UCAN.

Table II. Previously published genomic analyses of colitis-associated cancer.

First author/s, year	Number of cases	Sequencing method	APC mutant, %	TP53 mutant, %	Hyper-mutated, %	MSI-H, %	Other findings	(Refs.)
Robles <i>et al</i> , 2016	31 (UC, 15; CD, 14; indeterminate, 2)	Whole-exome	13	65	6	3	High frequency of <i>SOX9</i> , <i>EP300</i> , <i>NRG1</i> and <i>IL16</i> mutations in CAC	(6)
Yaeger <i>et al</i> , 2016	47 (UC, 29; CD, 18)	Targeted	21	89	NA	NA	High frequency of <i>IHD1</i> R132 mutations in CD, and high frequency of <i>MYC</i> amplification in CAC	(8)
Fujita <i>et al</i> , 2017	90 (UC, 58; CD, 32)	Targeted	16	66	NA	NA	High frequency of <i>RNF43</i> mutations, and <i>RNF43</i> -mutated CACs had elevated expression of c-MYC	(9)
Tanaka <i>et al</i> , 2017	12 (UC, 12)	Targeted	17	58	NA	NA	<i>CDH1</i> and <i>FGFR2</i> became mutated at an early stage in colitic carcinogenesis	(10)
Din <i>et al</i> , 2018	31 (UC, 16; CD, 15)	Whole-exome	29	65	29	21	Hypermutated-CAC had increased numbers of predicted neo-peptides	(11)
Yan <i>et al</i> , 2019	9 (UC, 9)	Whole-exome	22	33	NA	NA	High frequency of <i>KMT2D</i> and <i>NCOA6</i> mutations	(12)
Baker <i>et al</i> , 2019	12 (UC, 9; CD, 3)	Exome	40 ^a	80 ^a	17	17	Precancerous clones bearing SNAs and CNAs at dysplastic and non-dysplastic mucosa	(13)
Alpert <i>et al</i> , 2019	35 (UC, 35; CD, 18; indeterminate colitis, 2)	Targeted	15	69	NA	NA	Potentially targetable <i>IDH1</i> R132 mutation was present in 7% of CAC cases	(14)
Wanders <i>et al</i> , 2020	25 (UC, 15; CD, 10)	Targeted	16	48	NA	NA	<i>FBXW7</i> mutation was more frequent in IBD-associated dysplastic lesions than in sporadic adenomas.	(15)
Hirsch <i>et al</i> , 2021	23 (UC, 23)	Targeted	22	87	NA	0	<i>TP53</i> mutation and chromosomal aneuploidies including gains of chromosome arm 5p	(16)
Matsumoto <i>et al</i> , 2021	36 (UC, 36)	Targeted	47	44	NA	NA	<i>KRAS</i> and <i>TP53</i> mutations were mutually exclusive in CAC	(17)
Mäki-Nevala <i>et al</i> , 2021	27 (UC, 27)	Targeted	11	52	37	4	Hypermutated was divided into two distinct subgroups: Hypermutated microsatellite-stable and hypermutated microsatellite-unstable	(18)
Rajamäki <i>et al</i> , 2021	31 (UC, 27; CD, 2; unclassified IBD, 2)	Whole-genome	22 ^b	63 ^b	NA	10	<i>AXIN2</i> and <i>RNF43</i> were strongly downregulated in CAC	(19)
Present study	15 (UC, 15)	Targeted	13	87	13	13	<i>TP53</i> mutation alone was a characteristic gene profile in CAC, and signature 3 was enriched in CAC	-

^aPercentage of 10 lesions with microsatellite-stable tumor. ^bPercentage of 27 lesions with microsatellite-stable tumor. APC, adenomatous polyposis coli; CAC, colitis-associated cancer; CD, Crohn's disease; CNA, copy number alteration; IBD, inflammatory bowel disease; MSI-H, microsatellite instability-high; NA, not assessed; SNA, single nucleotide alteration; UC, ulcerative colitis.

Previous reports suggested that genetic alteration in *TP53* is a late event in sporadic CRC, but an early event in UCAN (37). Most *TP53* mutations in UCAN occurred in the DNA-binding domain (8,9,18), and these mutations were considered to be oncogenic and, for some of the missense mutations, potentially gain-of-function (8). Our analysis found a characteristic genome profile for UCAN, where 4/15 patients (27%) had a *TP53* mutation alone, whereas only 1/203 patients (0.5%) with sporadic CRC had a *TP53* mutation alone. This provides both mechanistic insight into UCAN tumorigenesis, as well as the diagnostic potential of gene panel testing.

In this analysis, UCAN diagnoses were made by a pathologist specializing in inflammatory bowel disease, and p53 overexpression was assessed as an aid to UCAN diagnosis. We consider that p53 overexpression is not essential for the diagnosis of UCAN; hence, we included four cases which has no p53 overexpression (Table I). We speculate that the results of p53 immunohistochemical staining are unlikely to have resulted in selection bias and influenced the profile of genetic alterations in UCAN.

The clinical utility of ICIs has been observed in a subset of patients with MSI-H CRC. Clinical studies have demonstrated MSI status as an accepted response biomarker for ICIs with progression-free survival rates of up to 78% in MSI-H CRC compared with 11% in microsatellite stable (MSS) CRC (38). The rate and timing of MSI-H are similar in UCAN and sporadic CRC, as is the prevalence of *MLH1* hypermethylation and silencing (6). Schulmann *et al* (39) reported that 18/107 lesions (17%) showed MSI-H in UCAN, and the profiles of coding microsatellite mutations differed between MSI-H UCAN and MSI-H sporadic CRC. In this analysis, MSI-H was identified in 2/15 patients (13%) with UCAN. Although ICIs were not used in either patient, they might be potential candidates for ICIs. However, knowledge regarding the clinical and molecular events underlying UCAN with MSI-H are limited, and it is unclear whether ICIs have the same effect on MSI-H sporadic CRC and MSI-H UCAN.

Mutational signature 3, which is associated with failed DNA double-strand break repair, is one of the genomic features of HRD, in addition to loss of heterozygosity, telomeric allelic imbalance, and large-scale state transitions (40). Tumors with HRD show high sensitivity to platinum compounds and poly(ADP-ribose) polymerase inhibitors in several malignancies, such as breast (41), ovarian, prostate, and pancreatic cancers. However, only few data are available regarding the role of HRD alterations in CRC (42), so it is unclear whether CRC patients with HRD show high sensitivity to platinum compounds and poly(ADP-ribose) polymerase inhibitors. The TRIBE2 study reported that patients with MSS and HRD tumors showed longer overall survival than patients with MSS and homologous recombination proficient tumors (40.2 vs. 23.8 months; $P=0.04$) (43). The TRIBE2 study was designed to assign 679 patients with unresectable, previously untreated metastatic CRC to receive two first-line oxaliplatin-based regimens: FOLFOX plus bevacizumab or FOLFOXIRI plus bevacizumab (43). We consider these results might imply HRD tumors have high sensitivity to an oxaliplatin-based regimen compared with homologous recombination proficient tumors in CRC. In this analysis, we demonstrated that 14/15 patients (93%) with UCAN had signature 3, and that

signature 3 was enriched in UCAN compared with sporadic CRC. Moreover, we presented a rare case of UCAN arising from the ileal pouch, which showed a remarkable response to oxaliplatin-based chemotherapy. Taken together, we think that UCAN might respond well to an oxaliplatin-based regimen.

This study has several limitations. First, this study included a small number of patients, with only 15 patients with UCAN. Second, we did not compare UCAN with sporadic CRC in patients with UC because its number was limited as well as UCAN. Third, there was selection bias of sporadic CRC, which included more patients with distant metastasis compared with UCAN. Fourth, we did not treat any patients based on the results of gene panel testing. However, to the best of our knowledge, this is the first report that focused on the clinical utility of gene panel testing for UCAN. We have shown a potential role for gene panel testing in the diagnosis and treatment of UCAN, and the clinicians might be able to develop more effective strategy in UCAN based on message from gene panel testing.

In conclusion, gene panel testing can detect important genetic alterations that can be useful for diagnosis and treatment in UCAN, and may provide clinicians with important information for tailored treatment strategies for UCAN.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the informed consent obtained not including unrestricted disclosure of sequencing data but are available from the corresponding author on reasonable request.

Authors' contributions

YS, STe, YA and TW made substantial contributions to the design and interpretation of data, and drafting of the article. MaeN, KIM, JY, AM, KT, HO, MasN, YH, HI, JS, HK, YT and MS made substantial contributions to acquisition of clinical data and interpretation of data. YL, STa and SO made substantial contributions to statistical analysis of the data and creation of the figures. SO and YL confirm the authenticity of all the raw data. TW critically revised the work and provided final approval of article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective analysis was performed in accordance with the Helsinki Declaration, and the Ethics Committee of the School of Medicine, Niigata University (Niigata, Japan)

approved the study protocol (G2015-0816, G2020-0038). Written informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

SO received research funding from Denka Co., Ltd. TW received remuneration and research funding from Denka Co., Ltd. All other authors declare that they have no competing interests.

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