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**Brief Report - Clinical**

# Clonal Patterns Between Pouch Neoplasia and Prior Colorectal Neoplasia in Inflammatory Bowel Disease Patients: An Exploratory Cohort Study

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## Lay Summary

Prior colorectal neoplasia is the strongest predictor of pouch neoplasia in inflammatory bowel disease, but the underlying mechanism is unknown. We observed clonality between colorectal and pouch neoplasia in 30% of patients, indicating that most pouch neoplasia develops clonally independent from prior colorectal lesions.

## Introduction

Patients with ulcerative colitis have an increased colorectal cancer (CRC) risk. This is attributed to chronic intestinal inflammation through a dysplasia-carcinoma sequence. In case of endoscopically irresectable neoplasia, a proctocolectomy is recommended by international guidelines, with ileal pouch–anal anastomosis (IPAA) as the preferred restorative procedure.[1] Although proctocolectomy significantly reduces CRC risk, pouch neoplasia can still arise, and up to 3.3% of IPAA patients develop pouch carcinoma after 20 years of follow-up.[2]

The pathogenesis of pouch neoplasia is unclear. Prior colorectal neoplasia is the strongest predictor of pouch neoplasia, with a 4.4- to 15.0-fold increased risk.[1] It is difficult to assess whether pouch neoplasia is newly developed or qualifies as recurrence due to factors such as positive resection margins or premalignant colonic islets that result in clonally related pouch lesions.[2,3] This distinction is currently based on histology and spatiotemporal relations between the lesions. DNA copy number aberrations (CNAs), resulting from deletion and amplification of genomic regions, play a driving role in cancer development. Therefore, analyses of DNA CNAs can determine whether 2 lesions share a common origin or whether they have arisen independently.[4,5] Various methods for CNA analysis are available, including microarrays (both comparative genomic hybridization and single nucleotide polymorphism analyses) and next-generation whole-genome sequencing.[6] A recent study showed multiple advantages of shallow whole-genome sequencing compared with array-based CNA analysis, including an improved signal-to-noise ratio and lower costs.[7] Hence, similar copy number profiles between lesions could reliably indicate clonality and may clarify the relation between prior colorectal neoplasia and subsequent pouch neoplasia in IPAA patients with inflammatory bowel disease (IBD).[5]

In this exploratory study, we aimed to determine the clonal origin of pouch neoplasia in IBD patients with prior colorectal neoplasia through (1) identification of CNA in pouch neoplasia by shallow whole-genome sequencing, (2) assessment of the incidence of clonally related pouch neoplasia to prior colorectal neoplasia, and (3) comparison of the result with currently used indicators for recurrence.

## Methods

### Study Design and Patients

This is an exploratory retrospective cohort study using a Dutch nationwide IBD cohort with IPAA that has previously been identified with the Dutch Nationwide Pathology Databank.[2] This cohort included 13 patients with colorectal neoplasia and subsequent pouch neoplasia with available histopathological specimen. In this subgroup, we assessed clonality between pouch neoplasia and prior colorectal

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neoplasia.[2] This study was approved by the ethics board of the Dutch Nationwide Pathology Databank (PALGA) (2014/306).

### Data Extraction

Clinical and histological data were extracted from pathology reports and pseudonymized patient medical charts including IBD, surgery, and neoplasia characteristics.

### Tissue Analyses

All available tissue slides from both the primary colorectal neoplasia and pouch neoplasia were assessed by an expert pathologist (I.D.N.). The presence of neoplastic tissue, grade of neoplasia (indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, CRC), tumor subtype, and pouchitis were assessed. For copy number analysis, DNA was isolated from formalin-fixed sections. Shallow whole-genome sequencing and processing of the sequencing data was performed as previously described.[5,7] For the validation of clonality, we applied non–polymerase chain reaction–based, clinically frequently used techniques. Immunohistochemistry for mismatch repair (MMR), p16, human epidermal growth factor receptor 2 (HER2), and fluorescence in situ hybridization (FISH) for HER2 were performed. In addition, results were compared with histological and spatiotemporal indicators of recurrence including positive resection margins and presence of metastatic disease.

### Statistical Analyses

CNA profiles were compared by calculation of Pearson correlation coefficients. A correlation coefficient of >0.60 was considered as a strong correlation. Subsequent unsupervised hierarchical clustering of the correlation matrix using the "complete linkage" clustering method was used for comparison of the CNA profiles. Additionally, a statistical test strategy was applied, using a conditional likelihood model and the null hypothesis that 2 tumors are of independent origin as implemented in the R package "Clonality" as described previously.[8,9] Positive log-likelihood ratios were considered as likely clonally related.

## Results

### Patient Characteristics

We included 13 IBD patients with both a prior colorectal neoplasia (prior dysplasia: n = 7, 54%; prior CRC: n = 6, 46%) and pouch neoplasia (pouch cancer: n = 9, 69%; pouch dysplasia: n = 4, 31%). Pouchitis was observed in 8 (62%) of 13 histological pouch specimen. The median IBD duration until IPAA construction was 17 (interquartile range, 13.5-20.5) years. After IPAA, the median duration until pouch neoplasia was 8 (interquartile range, 3.5-12.5) years) (Table 1).

### Clonality Analyses

The copy number profiles of all samples were included in the analyses; however, the profiles of patient 8 had high observed-to-expected ratios, and the sample pairs from patient 5 and 13 showed minimal deviation from 0, making it difficult to draw conclusions about clonality for these patients. For 3 sample pairs consisting of a colorectal and a pouch sample of the same patient, a correlation coefficient above 0.60 was obtained (patients 4, 6, and 10: n = 3 of 10, 30%) (Figure 1A), and 7 had a positive log-likelihood ratio (patients 1 and 4-9: n = 7 of 10, 70%). The sample pairs for patients 2, 4, 6, and 10 (n = 4 of 10, 40%) are each in the same branch of the dendrogram obtained by clustering of the correlation matrix (data not shown). Furthermore, visual inspection shows in the profile of patient 4 clear shared alterations on chromosomes 2, 7, 15, and 17 (Figures 1B, C). For patients 6 and 10, the most obvious matches are at chromosomes 9 and 17, respectively. Taken together, for patients 4, 6, and 10 (n = 3 of 10, 30%), clonality between samples is most supported, whereas for patient 2 and the other 6 patients clonality is unlikely.

### Validation of Shared CNAs and Assessment of Field Effects

Immunohistochemistry for the 4 MMR proteins was performed on the colorectal and pouch neoplasia samples of patients 4, 6, and 10. All samples were MMR proficient. Additionally, for patient 6, p16 immunohistochemistry was selected because the samples shared a clear loss on chromosome 9 of the CDKN2A gene region encoding for p16. Immunohistochemistry of the p16 protein showed concordant negative staining of the neoplastic areas, supporting CNA results. Conversely, in the directly surrounding mucosa with histologically normal appearance concordant positive staining was observed. Patient 10 had an amplification of chromosome 17q12 encompassing the HER2 gene. Therefore, immunohistochemistry and FISH for HER2 were performed for validation, showing clear protein expression and high DNA amplification (Figures 1D-F). Normal and inflamed surrounding mucosa did not show immunohistochemistry expression of HER2. For patient 4, there were no other useful markers available for validation.

### Clinical and Histopathological Classification vs CNA Profiling

For patient 4, pouch carcinoma was detected 2 years after IPAA construction. Patient 6 developed pouch carcinoma after 1 year without clear resection margins. For patient 10, tumor growth through the posterior vagina wall adjacent to the pouch was found 2 years after IPAA construction, resulting in a clinical diagnosis of recurrence of the prior CRC.

Patients 4, 6, and 10 all showed concordant histology between sample pairs pre- and post-IPAA. Patient 4 had in both samples an adenocarcinoma with mucinous components. The samples of patients 6 and 10 were predominantly classical adenocarcinoma (Table 1).

## Discussion

In this exploratory study, we aimed to determine the clonal origin of pouch neoplasia after colorectal neoplasia in IBD. We were able to assess clonality by CNA analysis in 10 patients (n = 10 of 13, 77%) and determined matching CNA profiles between pouch and prior colorectal neoplasia in 3 patients (n = 3 of 10, 30%). Moreover, we confirmed the clonality with frequently used clinical techniques.

Our findings underline the feasibility of CNA analysis in pouch neoplasia, in line with studies on sporadic CRC with metastasis and multifocal breast cancer.[4,5] It should be noted that profiles with very few alterations, which are more likely

**Table 1.** Patient characteristics

| Patient | IBD type | Age of IBD diagnosis (y) | IBD duration (y) | Type of neoplasia before IPAA | Location of neoplasia before IPAA | Type of anastomosis | Pouch duration until neoplasia (y) | Type of pouch neoplasia and CRC stage | Location of pouch neoplasia |
|---|---|---|---|---|---|---|---|---|---|
| 1 | UC | 17 | 15 | TVA with LGD | Rectum | Stapled | 4 | AC NOS *T4N2M1* | ATZ |
| 2 | UC | 17 | 17 | Serrated AC | Colon NOS | Stapled | 3 | Serrated AC *T4N1M1* | ATZ |
| 3 | CD | 5 | 28 | Mucinous AC | Ascending colon | Stapled | 9 | Mucinous AC *T2N0Mx* | ATZ |
| 4 | UC | 29 | 27 | Mucinous AC | Rectum | Stapled | 2 | AC with SCRs and mucinous components *T4N2M0* | ATZ |
| 5 | UC | 26 | 11 | TA with LGD | Sigmoid colon | Hand-sewn | 0 | TA with LGD | Pouch |
| 6 | UC | 33 | 14 | AC NOS | Sigmoid colon | Hand-sewn | 1 | AC with adenosquamous component and clear cells *T3N1Mx* | ATZ |
| 7 | UC | 29 | 20 | TVA with HGD | Descending colon | Stapled | 12 | TVA with LGD | Pouch |
| 8 | UC | 18 | 17 | VA with both LGD and HGD | Sigmoid colon | Stapled | 17 | TA with LGD | Pouch and in random ATZ biopsies |
| 9 | UC | 34 | 15 | VA with LGD | Rectum | Stapled | 7 | TA with LGD | Pouch |
| 10 | UC | 28 | 18 | AC with clear cell component | Rectum | NOS | 2 | AC NOS *T3N1Mx* | In pouch wall and dorsal vaginal wall |
| 11 | UC | 23 | 7 | TA with LGD | Sigmoid colon | NOS | 8 | AC NOS *T1N0Mx* | Pouch |
| 12 | UC | 39 | 10 | AC NOS | Rectum | NOS | 16 | AC NOS *T2N0Mx* | Pouch |
| 13 | UC | 5 | 17 | Serrated polyp with HGD | Rectum | NOS | 12 | Mucinous AC *T3N0M1* | In pouch and sacral cavity |

Abbreviations: AC, adenocarcinoma; ATZ, anal transitional zone; CRC, colorectal carcinoma; HGD, high-grade dysplasia; IPAA, ileal pouch–anal anastomosis; LGD, low-grade dysplasia; SCR, signet ring cell; NOS, not otherwise specified; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma.

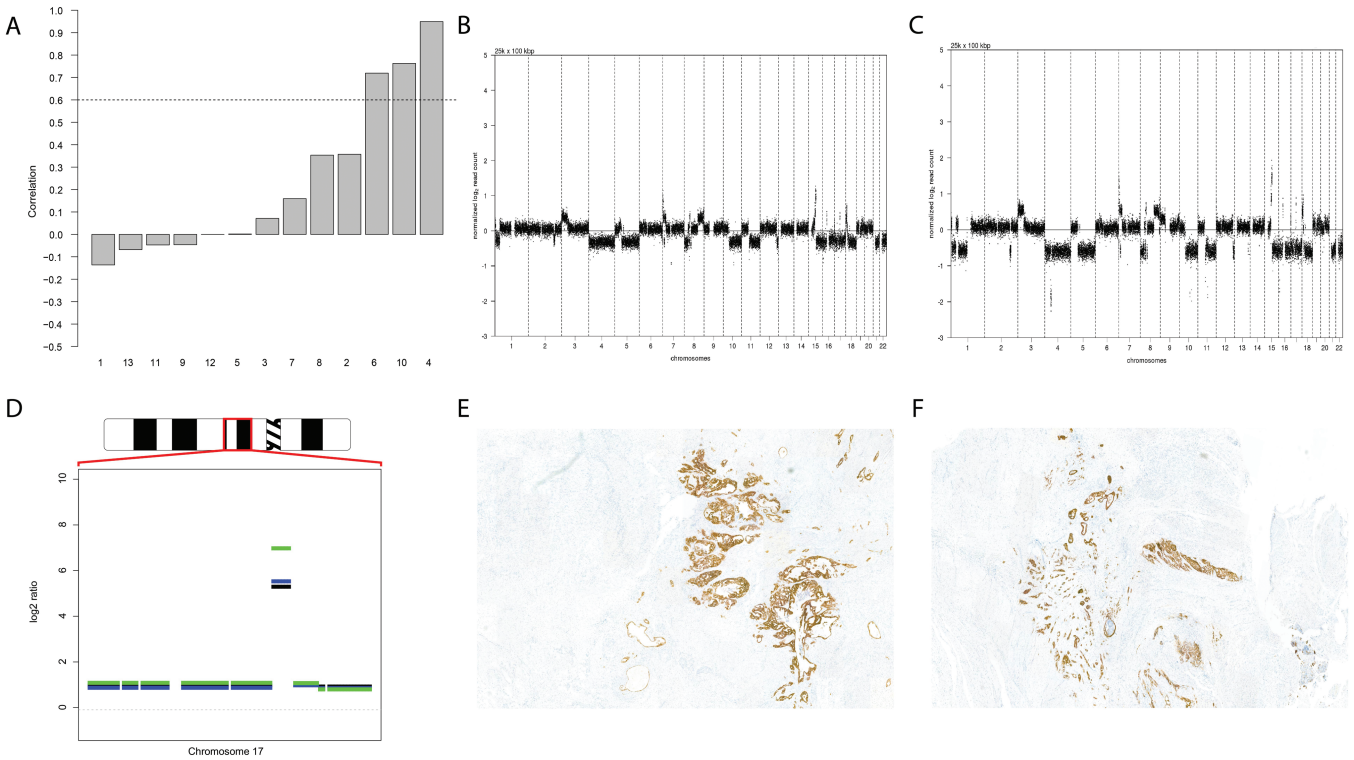


**Figure 1.** A, Correlation coefficients (y-axis) of colorectal and pouch neoplasia for individual patients (x-axis). Patients 6, 10, and 4 have coefficients above 0.6, indicating clonality. B and C, Clonally related copy number aberration profiles for patient 4 of colorectal neoplasia (B) and pouch neoplasia (C). The profiles are overall very similar, in line with correlation testing. The x-axis displays 100-kbp genomic bins sorted by chromosomal position. The boundaries between chromosomes are indicated by dotted lines. The y-axis displays the normalized log2 read counts. Positive values represent gains; negative values represent losses. D, DNA copy number profile of chromosome 17 of the colorectal cancer sample of patient 10. Zoom in on the cytobands q12, q21.1, and q21.2 of chromosome 17 (red square) for the DNA copy number profiles of patient 10 (colorectal cancer = black; pouch sample 1 = blue; pouch sample 2 = green). A large region including the human epidermal growth factor receptor 2 (HER2) gene is amplified. This amplification is detected in all 3 samples. E and F, Representative images of HER2 immunohistochemistry of the colorectal neoplasia (E) and pouch neoplasia (F) of patient 10. HER2 expression (brown staining) are clearly present in tumor cells, confirming the results from the copy number aberration analysis.

in early lesions, are less suited for determining clonality.[10] The 3 patients (n = 3 of 10, 30%) with matching clonality patterns had a short time between IPAA construction and pouch neoplasia (<2 years) compared with patients without clonally related neoplasia (up to 16 years), which may support the presence of recurrence instead of newly developed lesions. Moreover, 2 out of 3 patients had histological and clinical signs of colorectal neoplasia recurrence, with positive resection margins or metastatic disease.

The larger number of patients (n = 7 of 10, 70%) with no clonal match suggests a role of prior colorectal neoplasia independent of positive resection margins or local metastasis.[5,11] Previous studies suggested that widespread premalignant alterations in colorectal mucosa (field cancerization) might result in early carcinogenesis in remaining colonic islets after mucosectomy. Indeed, most pouch neoplasia is found at the anal transition zone harboring some remnant colonic mucosa.[2,3] Therefore, field cancerization could underlie clonally related neoplasia in our study, although the immunohistochemistry and FISH analyses for patients 6 and 10 did not indicate a field effect. Other factors that may increase both the colorectal and pouch neoplasia risk include pro-oncogenic microbiome changes, tumor-promoting and suppressive microenvironment, chronic inflammation, and presence of genetic factors.[12] Indeed, most included pouch specimens showed pouchitis.

Our cases were selected from one of the largest IBD-IPAA cohorts.[2] Nevertheless, this study is limited by the modest sample size as a result of the low incidence of pouch neoplasia in IBD, which may affect generalization. Moreover, there is a considerable intervariability between CNA analysis methods, especially in formalin-fixed tissue.[13] Nonetheless, our sequencing approach has shown to provide robust results in both colon cancer and other tumors.[5,14]

In conclusion, most pouch neoplasia in our cohort seemed to have had an independent clonal origin relative to the prior colorectal neoplasia. This may underline the importance of other pathophysiological mechanisms underlying prior colorectal neoplasia as risk factor for pouch neoplasia.

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## Conflict of Interest

The authors state no conflict of interest.

## Data Availability

All data underlying this article will be shared upon reasonable request to the corresponding author.

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