Aberrant Crypt Focus Size Predicts Distal Polyp Histopathology

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Abstract

Aberrant crypt foci (ACF) are the earliest histopathologic lesion associated with colorectal cancer. ACFs are commonly used as a surrogate marker for colorectal cancer chemoprevention studies in rodents and, more recently, in humans. However, ACF prevalence in unselected populations is not known, nor which ACF features are important for predicting polyp histopathology. To address these questions, we did magnification chromo-colonoscopy on all patients undergoing routine colorectal cancer screening over a 31-month period. ACFs were classified by location, size (small, <20 crypts/ACF; medium, 20-100 crypts/ACF; large, >100 crypts/ACF), and whether they were elevated above the tissue plane. Overall, 802 magnification chromo-colonoscopies with ACF enumeration were done. Mean patient age was 58.6 ± 8.5 years, of whom 56% were female, 58% were African American, 21% were Caucasian, and 16% were Latino. Total ACF number, along with increasing ACF size and elevation, correlated with the presence of distal hyperplastic polyps and were higher in African Americans. In contrast, ever-smaller ACFs correlated with the presence of distal adenomas and were independent of age and race. The odds ratio for patients with ≥6 small ACFs and adenomas was 6.02 (95% confidence interval, 2.64-13.70) compared with patients with ≤5 small ACFs, whereas the odds ratio for patients with ≥1 large ACF and hyperplastic polyps was 5.88 (95% confidence interval, 3.00-11.67) compared with patients with none. Small flat ACFs correlate with the presence of distal adenomas, whereas large raised ACFs correlate with the presence of hyperplastic polyps. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1155–62)

Introduction

Aberrant crypt foci (ACF) are believed to be the earliest histopathologic lesion associated with malignant transformation in the colon. These structures were initially identified by Bird in 1987 in carcinogen-treated mice (1) and were characterized as ≥1 crypts that were larger and thicker and which stained more intently than adjacent normal structures (2). *Ex vivo* studies have identified more ACFs in patients with a genetic predisposition to polyp syndromes such as familial adenomatous polyposis (2, 3) or a prior history of colorectal cancer (2, 3) compared with patients undergoing colectomy for reasons unrelated to malignancy.

In 1997, Yokota et al. (4) first reported on the ability to identify ACFs in humans *in vivo* when using a special magnifying endoscope and staining the colonic mucosa with the vital dye methylene blue. Since then, several investigators have reported on ACF prevalence in highly selected populations, consistently observing increased ACF number in patients with adenomatous polyps and/or colorectal cancer (4, 5) or a personal (5, 6) or family history thereof (7). Additionally, Hurlstone et al. (8) found increased numbers of ACF in patients with flat adenomas and cancers and suggested that patients with increased numbers of ACFs might need to be surveyed more frequently than is otherwise recommended (9).

Because all studies to date have been done in highly selected populations (reviewed in refs. 10, 11), nothing is known as to ACF frequency in a general population, including as a function of race and sex. Additionally, most endoscopic studies to date have limited themselves to reporting on ACF present in the distal rectum, typically because of the technical difficulty associated with identifying these structures more proximally when ACF enumeration is done according to standard techniques. Finally, most clinical studies have analyzed ACFs as a function of the total number present, and none have investigated whether ACFs associate with nonadenomatous polyps, such as hyperplastic polyps. As a result, nothing is known about the importance, if any, of other characteristics, such as ACF size and elevation, and whether these variables are important for assessing their correlation with specific polyp histopathology.

In January 2005, the University of Illinois at Chicago (UIC) Colorectal Cancer Screening Clinic began performing all exams by magnification chromo-colonoscopy (MCC). This was made possible by virtue of our technical advance permitting magnification chromoscopy to be done as an adjunct to regular colonoscopy (12) and was pursued in light of studies strongly, suggesting that this technique improved the detection of flat adenomas and colorectal cancer (8), lesions not infrequently missed during regular endoscopy. In this study, we report on the prevalence of ACFs in patients in a nonselected urban population evaluated as a part of standard colorectal cancer screening. We herein show that total ACF number as well as the number of large elevated ACFs correlate with the presence of hyperplastic polyps and

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that small flat ACFs correlate with the presence of distal adenomas. Our observations have significant implications for using ACFs as a surrogate marker for colorectal cancer, provide insight into colorectal cancer pathogenesis, and show that screening colonoscopy by MCC with ACF enumeration can be readily incorporated into routine clinical practice.

Materials and Methods

Subjects. Patients were recruited from the UIC Colorectal Cancer Screening Clinic at the time of their initial visit and all information recorded into our ACF database. Subjects were excluded if they were not asymptomatic or otherwise had medical complaints referable to their gastrointestinal tract that would have indicated the need for diagnostic colonoscopy. All patients completed a questionnaire regarding their medications and cancer history that was verified by one of the physicians associated with this study during an individual interview. All patients underwent focused physical examination. This study was reviewed and approved by the UIC Institutional Review Board.

MCC and ACF Enumeration. Colonoscopy was done using a Fujinon XL-401 videoscope (Fujinon) providing ×40 magnification. As described previously (12), patients underwent colonic cleansing with a polyethylene glycol–based solution (that is, Golytely) supplemented with 30 mL glycerol to remove overlying mucus from the gastrointestinal epithelium. On the day of the exam, and after the recording of stable vital signs, patients received 25 μg fentanyl and 1 mg midazolam. Patients then were placed in the left lateral decubitus position and 250 mL of 0.05% methylene blue diluted in 0.45% normal saline was administered *per rectus* using a standard barium enema bag. Patients underwent vigorous abdominal massage and were placed in the supine position and their abdomens were again massaged vigorously. Patients were then returned to the left lateral decubitus position and excess methylene blue solution was removed. Additional sedation was provided as required and the endoscopic exam was started. Time of endoscopy was calculated from the time at which sedative-hypnotic agents were first administered to the time of endoscope withdrawal and includes the time of preparing the colon with methylene blue.

Endoscopy was done under standard magnification until the cecum was reached and was done under enhanced magnification during instrument withdrawal. ACFs were counted in a sequential fashion on a single withdrawal of the endoscope to guard against double counting. To be considered an ACF, each structure had to possess four of the following criteria originally defined by McLellan and Bird (13), including (*a*) crypts that were two to three times larger than normal, (*b*) a thickened layer of epithelial cells, (*c*) an increased pericryptal area, (*d*) possess a slit-shaped lumen, and/or (*e*) be microscopically elevated above the plane of normal crypts. ACF number was determined by location, with those found between the dentate line and the middle rectal fold classified as ''rectal'' and those found between the middle rectal fold and the splenic flexure as ''sigmoid,'' with the sum of both identified as ''left colon.'' For the last 346 MCC, ACFs were also characterized as large (>100 crypts/ACF), medium (21-99 crypts/ACF), or small (<20 crypts/ACF) and whether they were raised or flat with respect to the adjacent mucosa (Fig. 1).

Polyps. All resected specimens were evaluated by UIC clinical pathology for polyp histopathology determination. Each resected polyp was placed in its own formalin container for histopathologic assessment irrespective of size and location. Polyp sizing was done at the time of endoscopy by comparing it with the known diameter of a standard forceps biopsy in the closed or open position. Patients were considered to have advanced adenomas if they had a single adenoma ≥10 mm, had ≥3 adenomas removed, or had adenomas that were histologically villous or contained high-grade dysplasia. Polyp location was defined as proximal or distal based on their relationship to the splenic flexure.

Clinical Data Collection and Storage. All endoscopic reports were generated and maintained using cMore (ProVation Medical). Patients undergoing MCC were stored separately, whereas the total number of patients undergoing endoscopy for colorectal cancer screening was determined by sorting by procedure indication.

Inpatient and outpatient records were maintained using the Gemini system (Cerner). This system interacts with the endoscopic record-keeping system (cMore) as well as with the digital X-ray system used by the UIC Department of Radiology. This system allows for the ready screening of patient records enrolled in the ACF database.

Statistical Analysis. Data were evaluated using Stata 9.0 for Macintosh statistical software. Intraobserver and interobserver error in ACF enumeration was calculated as the half the observational range divided by the mean of all observations. For differences between groups in mean number of ACF total, sigmoid and rectum, ANOVA was used. Logistic regression was used to evaluate the association between the absence and the presence of hyperplastic polyps as well as adenomatous polyps. Multivariable logistic regression was done to compute odds ratios (OR) and 95% confidence intervals (95% CI). Eliminating individual variables from a multivariate model and assessing their effect on the OR of interest identified potential confounders. All significance tests were two sided. Data were graphed and trend lines were plotted using Excel (Microsoft). Data are expressed as mean ± SE, unless otherwise specified, with $P < 0.05$ considered significant.

Results

This study reports on data collected from patients seen from January 1, 2005 to December 24, 2007. During this timeframe, data collection was suspended by our institutional review board from May to September 2005 to resolve issues relating to the consent process. Hence, the data collected herein are for individuals seen over a ~31-month period.

All patients were initially seen in the UIC Colorectal Cancer Screening Clinic, the majority of whom were internal to the UIC clinic system and referred from General Internal Medicine or Family Practice clinics.

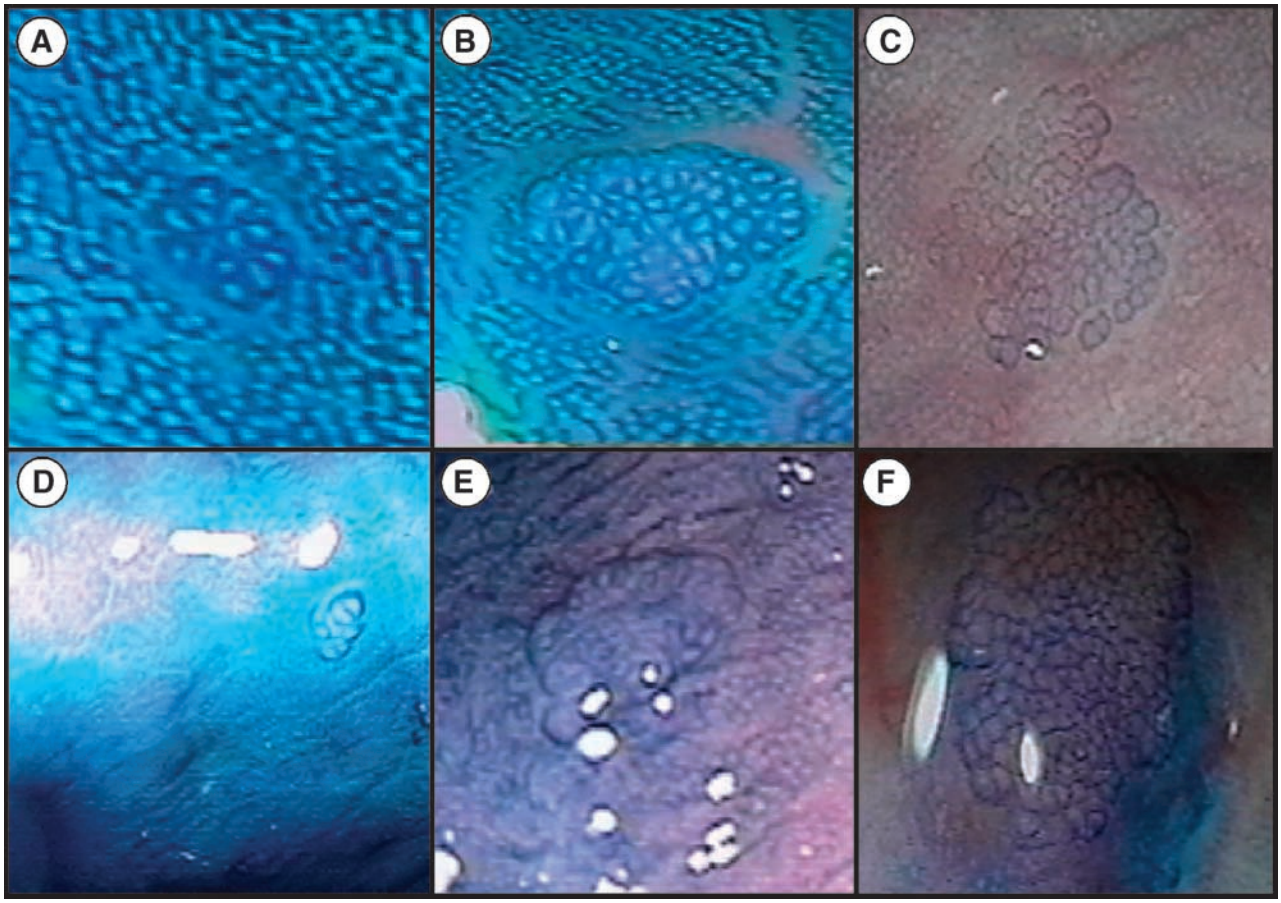

Figure 1. ACF types seen during MCC. The different types include those that are flat and are sized as small (<20 crypts; **A**), medium (20-100 crypts; **B**), or large (>100 crypts; **C**) and those that are raised and sized as small (**D**), medium (**E**), or large (**F**). Magnification, ×40.

Although the UIC Medical Center is a tertiary care referral hospital, it also serves as a *de facto* community hospital for the neighboring urban population such that ~50% of patients seen in the UIC Colorectal Cancer Screening Clinic live within 7 miles of the hospital.

Overall, 1,385 patients were seen in the UIC Screening Clinic, of whom 1,054 (76.1%) agreed to allow their information to be collected in our ACF database. Of these, 802 underwent MCC by December 24, 2007. During the same timeframe, an additional 561 patients underwent screening by standard colonoscopy by other members of the UIC faculty typically because of direct physician-to-physician referrals. Thus, patients evaluated by MCC and included in this article represent 58.8% of patients undergoing endoscopic screening for colorectal cancer over the timeframe covered by this study.

General. Our first evaluation was done on the entire cohort of patients seen over the course of this study (identified as group A; Table 1). Overall, patients ranged in age from 25 to 82 years (mean, 58.6 ± 8.5). The largest group of patients was African American (n = 463) followed by Caucasian (n = 165) and Latino (n = 125), with the remainder (n = 49) not classifiable in these groups. The main three groups of patients were similarly aged, but with there being more male Caucasians than for African Americans and Latinos (Table 1, group A). There was no significant difference in the prevalence of polyps and their histology as a function of race (Table 1, group A).

The time necessary to perform MCC, and the extent to which methylene blue dye was observed in the colon, was similar across groups. For the purposes of comparison, we determined the time of MCC for patients with colonic preps graded good or excellent (14) without biopsy or polypectomy to be 30.9 ± 12.0 min. There was no difference in MCC time across race or sex (data not significant). There was no difference on the extent to which successful staining of the colon using methylene blue was achieved by race or sex (data not significant), with staining to at least the mid-transverse colon was achieved in 51.2% and to the splenic flexure in all.

Overall, 8.0% had no ACF detected, 26.6% had 1 to 5 ACF, 17.2% had 6 to 10 ACF, 21.2% had 11 to 19 ACF, and 27.0% had ≥20 ACF. There were ~20% to 50% additional ACF detected between the splenic flexure and the middle rectal fold (that is, ''sigmoid'') compared with what was observed distal to the middle rectal fold (referred to as ''rectal''; Table 2). Overall, only 7 patients

had any ACF located proximally to the splenic flexure (range, 1-2).

We determined our interobserver and intraobserver error in identifying ACFs as follows. Intraobserver error was determined by performing three separate counts in 22 patients during the same endoscopy, yielding an error rate of 5.4 ± 1.0%, and in 17 patients who underwent MCC on two separate occasions (done <30 days apart as part of a separate study), yielding an error rate of 12.7 ± 0.2%. We assessed interobserver error by recording the entire endoscopy of 6 patients and then having these recordings scored independently by the three endoscopists associated with this study. In this manner, our interobserver error rate was determined to be 10.2 ± 3.4%.

Total Number of ACFs Does Not Correlate with Polyp Histopathology. All studies to date have reported on the total number of ACFs present, with the endoscopic studies restricting this reporting to those located within 10 to 20 cm of the dentate line. However, no study to date has determined what relationship these structures have as a function of polyp histology. We noted that ACFs present in the entire distal colon (referred to as ''left colon'') provided stronger statistical correlations than what was observed for ACFs detected distally to the middle rectal fold (that is, ''rectal''; Table 2). Thus, all analyses that follow are for ACFs identified in the entire distal colon (that is, ''left colon'').

Total ACF number correlated strongly with the presence of hyperplastic polyps located both distally ($P < 0.001$) and proximally ($P = 0.021$; Wilcoxon test). Surprisingly, total ACF number did not correlate with adenomatous polyps located either distally ($P = 0.117$) or proximally ($P = 0.604$; Table 2). Thus, total ACF number is not helpful in assessing the presence of adenomas. Although the preceding analysis was done on all 802 patients, these conclusions were apparent after an interim analysis was done on the first 457 MCC examinations. Thus, for the subsequent 345 exams, we also collected information relating to ACF size and whether each ACF was flat or raised with respect to the adjacent mucosa (Fig. 2).

ACF Size Predicts Polyp Histopathology. There was no significant difference in patient age, sex, or racial distribution in the 345 patients who underwent detailed ACF categorization (Table 1, group B) compared with the entire group of 802 patients (Table 1, group A). Likewise, the prevalence of adenomas, advanced adenomas, and hyperplastic polyps also was similar (Table 1). Thus, the group of 345 patients undergoing a more detailed ACF analysis was similar to the group for whom total ACF number was determined.

Similar to what we noted for total ACFs, ACFs characterized by size and present in the entire distal colon (that is, ''left colon'') generally provided stronger statistical correlations than what was observed for ACFs detected distally to the middle rectal fold (that is, ''rectal''). Thus, the analyses that follow pertain to ACFs identified in the entire distal colon (that is, ''left colon'').

Large ACFs correlated with the presence of distal ($P < 0.001$) but not proximal ($P = 0.703$) hyperplastic polyps nor adenomas irrespective of their location (Table 2). In contrast, the number of medium ACFs correlated with the presence of both proximally ($P = 0.024$) and distally located ($P < 0.001$) hyperplastic polyps as well as associated with the presence of distal, but not proximal, adenomas, but only for those located in the sigmoid colon. Finally, small ACFs correlated with the presence of distal adenomas ($P < 0.001$) but not proximal adenomas ($P = 0.793$). Importantly, this correlation was also significant when small ACFs were classified as distal or proximal to the middle rectal fold (Table 2). Because these observations suggested that ACF size correlated with polyp histopathology, we did multiple regression (trend) analysis. This analysis indicated that increasing ACF size correlated with the presence of distal

Table 1. Characteristics of patients enrolled in the ACF database

	Total		African American		Caucasian		Latino	
	Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
n	802	346	463	194	165	74	125	58
Age (y)	58.6 ± 8.5	58.4 ± 8.7	59.2 ± 8.3	58.1 ± 8.5	56.9 ± 8.8	56.1 ± 9.3	59.8 ± 8.6	60.6 ± 8.7
Male (%)	44	45	35	56	64	62	45	50
Personal history of polyps (%)	5	4	5	2	6	7	3	4
Family history of colorectal cancer (%)	13	14	12	13	19	24	7	5
Adenoma at MCC (%)	20	20	21	22	25	23	14	12
Advanced adenoma at MCC (%)	8	8	7	7	9	11	8	8
Hyperplastic at MCC (%)	20	24	21	25	23	28	14	12
Adenoma only at MCC (%)	16	14	15	14	22	19	13	9
Advanced adenoma only at MCC (%)	6	6	6	6	5	6	7	4
Hyperplastic only at MCC (%)	16	17	16	17	18	20	13	9
Adenoma and hyperplastic at MCC (%)	6	7	7	8	7	7	2	4
Advanced adenoma and hyperplastic at MCC (%)	4	4	4	3	5	6	4	6

NOTE: Group A includes all patients enrolled in the ACF database and includes enumeration of total ACF number, whereas group B represents the last 346 patients seen and for whom ACFs where further characterized by size and elevation. Adenoma only at MCC and advanced adenoma only at MCC indicate that the relevant patients had only adenomas and did not have any hyperplastic polyps, whereas hyperplastic only at MCC identifies patients having only hyperplastic polyps but no adenomas. Mean ± SE.

Table 2. Association of ACF by type with polyp by location and histology

ACF type	Hyperplastic-proximal			Hyperplastic-distal			Adenoma-proximal			Adenoma-distal		
	Absent	Present	*P*	Absent	Present	*P*	Absent	Present	*P*	Absent	Present	*P*
Number												
Left colon	14.3 ± 0.6	19.7 ± 2.3	0.021	13.0 ± 0.6	24.2 ± 2.1	<0.001	14.5 ± 0.6	15.5 ± 1.5	0.604	14.3 ± 0.6	17.3 ± 1.8	0.117
Sigmoid	5.2 ± 0.3	7.3 ± 1.0	0.033	4.8 ± 0.3	8.3 ± 1.0	<0.001	5.3 ± 0.3	5.5 ± 0.7	0.795	5.0 ± 0.3	8.0 ± 1.1	0.001
Rectal	4.0 ± 0.2	4.2 ± 0.7	0.8237	3.8 ± 0.2	5.4 ± 0.5	0.003	4.0 ± 0.2	4.0 ± 0.6	0.963	3.9 ± 0.2	5.2 ± 0.8	0.055
Small (<20 crypts)												
Left colon	5.4 ± 0.3	6.8 ± 1.0	0.150	5.5 ± 0.3	6.8 ± 0.8	0.080	5.6 ± 0.3	5.4 ± 0.7	0.793	5.2 ± 0.3	8.9 ± 1.0	<0.001
Sigmoid	4.3 ± 0.3	6.0 ± 1.0	0.065	4.3 ± 0.3	5.9 ± 0.8	0.030	4.5 ± 0.3	4.0 ± 0.6	0.507	4.1 ± 0.2	7.3 ± 1.1	<0.001
Rectal	3.6 ± 0.2	4.0 ± 0.7	0.630	3.5 ± 0.2	4.6 ± 0.5	0.089	3.7 ± 0.2	3.5 ± 0.6	0.855	3.5 ± 0.2	5.1 ± 0.8	0.013
Medium (20-100 crypts)												
Left colon	3.0 ± 0.2	4.8 ± 0.8	0.024	2.8 ± 0.2	5.2 ± 0.9	<0.001	3.1 ± 0.2	3.6 ± 0.6	0.494	3.1 ± 0.2	4.2 ± 0.8	0.131
Sigmoid	2.6 ± 0.2	4.7 ± 0.8	0.009	2.5 ± 0.2	4.6 ± 0.9	<0.001	2.7 ± 0.2	3.4 ± 0.6	0.313	2.7 ± 0.2	4.2 ± 0.8	0.036
Rectal	1.5 ± 0.1	1.2 ± 0.3	0.628	1.3 ± 0.1	2.3 ± 0.4	0.003	1.4 ± 0.1	1.4 ± 0.3	0.865	1.4 ± 0.1	1.5 ± 0.4	0.967
Large (>100 crypts)												
Left colon	1.3 ± 0.2	1.6 ± 0.4	0.703	0.9 ± 0.1	3.8 ± 0.8	<0.001	1.3 ± 0.2	1.5 ± 0.4	0.680	1.4 ± 0.2	1.1 ± 0.3	0.583
Sigmoid	1.2 ± 0.2	1.4 ± 0.4	0.721	0.8 ± 0.1	3.5 ± 0.8	<0.001	1.2 ± 0.2	1.4 ± 0.4	0.740	1.2 ± 0.2	1.1 ± 0.3	0.773
Rectal	0.4 ± 0.1	0.6 ± 0.3	0.281	0.3 ± 0.0	1.0 ± 0.2	<0.001	0.4 ± 0.1	0.4 ± 0.2	0.763	0.4 ± 0.1	0.1 ± 0.1	0.056

NOTE: Left colon refers to total ACFs seen distally to the splenic flexure, sigmoid refers to ACFs between splenic flexure and the middle rectal fold, and rectal refers to those between the middle rectal fold and the dentate line. Mean ± SE, with statistical significance determined by logistic regression analysis.

hyperplastic polyps ($P < 0.0001$), whereas decreasing ACF size correlated with the presence of distal adenomas ($P < 0.0001$).

The correlation between ACF size and histopathology also extended to polyp number (Fig. 2). Increased numbers of large ACFs were observed in patients as a function of the number of hyperplastic polyps present ($P < 0.001$; Fig. 2, *left*). In contrast, there was no correlation between the numbers of small ACF present as a function of the number of hyperplastic polyps present. The converse was true for adenomatous polyps. Whereas increased numbers of small ACFs were noted in patients with increased numbers of adenomatous polyps ($P < 0.001$), there was no correlation between the number of large ACFs present and the number of adenomatous polyps ($P = 0.935$; Fig. 2, *right*).

We next performed logistic regression analysis (Table 3) to evaluate which ACF variable correlated with the relevant distal polyp histology. Univariate analysis indicated that the OR for both small ACFs and large ACFs associated significantly with the presence of distally located hyperplastic polyps (Table 3A). However, multivariate analysis showed that only large ACFs remained significantly associated with the presence of hyperplastic polyps (OR, 1.30; 95% CI, 1.17-1.45). When ACF elevation was also considered in multivariate analysis, only large raised ACFs correlated with the presence of distal hyperplastic polyps (OR, 1.30; 95% CI, 1.15-1.47).

In contrast, univariate analysis showed that only small ACFs associated significantly with the presence of distally located adenomatous polyps (Table 3B). This association strengthened when elevation was also considered along with ACF size, such that only small flat ACFs correlated with the presence of distal adenomas (OR, 1.13; 95% CI, 1.06-1.21).

Because these data indicate that small ACFs, but not total ACF, correlate with the presence of distal adenomas, we performed reiterative regression analysis to determine the number of small ACF needed for maximum correlation with distal adenomas. We found that the presence of ≥6 small ACFs yielded the most significant correlation with the presence of distal adenoma (OR, 6.02; 95% CI, 2.64-13.70) compared with individuals with ≤5 small ACFs. Thus, a person with ≥6 small ACFs versus a person with <6 small ACFs is 6.02 times more likely to have a distal adenoma, with each

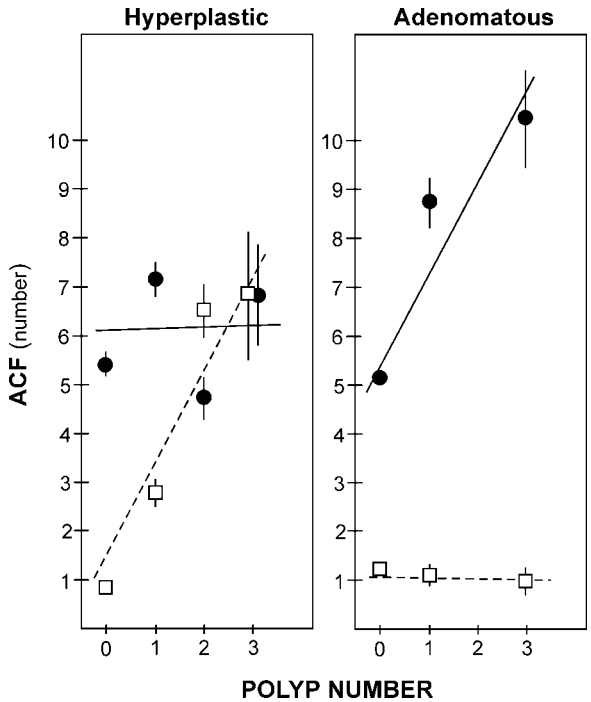

Figure 2. Correlation between polyp number as a function of histology and ACF number as a function of size. *Left,* number of large ACF (*open squares*; □) or small ACF (*solid circles*; ●) plotted versus number of hyperplastic polyps present. *Right,* number of large ACF (*open squares*; □) or small ACF (*solid circles*; ●) plotted versus the number of adenomatous polyps present. *Dashed lines,* trend lines for large ACF; *solid lines,* trend lines for small ACF. Mean ± SE.

Table 3.

Analysis	ACF type	OR (95% CI)
(A) Logistic analysis of distal hyperplastic polyps		
Univariate	ACF small	1.07 (1.02-1.13)
Univariate	ACF large	1.32 (1.18-1.47)
Multivariate	ACF small	1.06 (0.99-1.12)
	ACF large	1.30 (1.17-1.45)
Multivariate	Small and flat	1.04 (0.98-1.11)
	Small and raised	1.06 (0.97-1.16)
	Large and flat	1.01 (0.78-1.32)
	Large and raised	1.30 (1.15-1.47)
(B) Logistic analysis of distal adenomatous polyps		
Univariate	ACF small	1.13 (1.07-1.20)
Univariate	ACF large	0.96 (0.83-1.11)
Multivariate	ACF small	1.14 (1.07-1.21)
	ACF large	0.92 (0.78-1.09)
Multivariate	Small and flat	1.13 (1.06-1.21)
	Small and raised	1.01 (0.91-1.13)
	Large and flat	1.14 (0.81-1.58)
	Large and raised	0.87 (0.68-1.10)

additional small ACF increasing the likelihood of a distal adenoma by 13%.

We likewise performed reiterative regression analysis to determine the number of large ACF needed for maximum correlation with distal hyperplastic polyps. We found that the presence of ≥1 large ACF yielded the most significant correlation with the presence of distal hyperplastic polyps (OR, 5.88; 95% CI, 3.00-11.67) compared with individuals with none. Thus, a person with ≥1 large ACF versus a person with no large ACF is 5.88 times more likely to have a distal hyperplastic polyp, with each additional large ACF increasing the likelihood of a distal hyperplastic polyp by 30%.

ACFs as a Function of Aging and Race. Previous studies have suggested that ACF number increases with age (6, 15, 16), whereas nothing is known about ACFs as a function of race. We therefore assessed these variables in our population as a function of total ACF number as well as ACF size (Table 4). Overall, African Americans had increased total numbers of ACFs (16.1 ± 0.8) compared with Caucasians (13.6 ± 1.4) and Latinos (10.9 ± 1.1; $P = 0.006$, ANOVA; Table 4A). This racial difference was also observed for large ACFs ($P = 0.001$) but not for small ACFs ($P = 0.690$; Table 4A).

Total ACF number increased as a function of advancing age (Table 4B). Whereas individuals ages <50 years had 9.0 ± 2.4 ACF, those ages >70 years had 17.3 ± 2.2 ACF ($P = 0.015$). Although the total number of ACFs increased in African Americans and Caucasians as a function of age, this trended toward significance only for African Americans and was not statistically significant for Caucasians or Latinos (Table 4B). The increase in total ACF number with age may be due to an age-dependent increase in large ($P = 0.077$) but not small ($P = 0.446$) ACF. However, and similar to what was observed for total number of ACFs, the age-dependent increase in large ACF was not statistically significant when broken down by race (Table 4B).

Discussion

ACFs are an established biomarker for colorectal cancer in rodents, with >400 dietary modification and/or chemoprevention studies having been published using this lesion as a surrogate (17). Yet, ACFs have been much less studied in humans in part because of the difficulty associated with standard approaches for identifying these lesions during endoscopy. Hence, only about a dozen surgical or endoscopic studies have evaluated ACFs in humans (reviewed in refs. 10, 11): all have been restricted to highly selected populations, with all but two [one published (18) and one preliminary (19)] suggesting that they are present in increased numbers in patients with a personal or family history of colorectal cancer and/or adenoma. As a result, nothing is known about ACF prevalence in a diverse population undergoing routine screening. Finally, all clinical studies to date have been limited to studying ACFs in the distal rectum, whereas the importance of ACF size and correlation with polyp histopathology is completely unknown.

Table 4.

(A) ACF by size as a function of race

	All races	African American	Caucasian	Latino	*P*
Total ACF	14.7 ± 0.6	16.1 ± 0.8	13.6 ± 1.4	10.9 ± 1.1	0.006
Large ACF	1.3 ± 0.2	1.9 ± 0.3	0.8 ± 0.2	0.5 ± 0.1	0.001
Small ACF	5.5 ± 0.2	5.4 ± 0.3	5.9 ± 0.7	5.4 ± 0.6	0.690

(B) ACF by size and race as a function of age

	All races			African American			Caucasian		
	Total ACF	Large ACF	Small ACF	Total ACF	Large ACF	Small ACF	Total ACF	Large ACF	Small ACF
Age category									
<50	9.0 ± 2.4	0.5 ± 0.2	4.2 ± 1.4	11.6 ± 5.1	1.0 ± 0.3	7.4 ± 2.7	6.1 ± 1.4	0.1 ± 0.1	1.9 ± 0.3
50-59	13.6 ± 0.7	1.1 ± 0.2	5.4 ± 0.3	14.6 ± 1.0	1.6 ± 0.3	5.0 ± 0.4	12.5 ± 1.6	0.9 ± 0.2	6.0 ± 1.0
60-69	16.5 ± 1.1	1.8 ± 0.3	5.9 ± 0.5	17.6 ± 1.5	2.2 ± 0.5	5.3 ± 0.5	16.8 ± 3.1	1.1 ± 0.4	7.6 ± 1.3
>70	17.3 ± 2.2	1.6 ± 0.8	5.9 ± 0.8	20.4 ± 3.3	2.9 ± 1.5	7.0 ± 1.2	19.3 ± 6.9	0.3 ± 0.3	5.3 ± 2.0
P	0.015	0.271	0.566	0.084	0.430	0.182	0.128	0.400	0.201

NOTE: Mean ± SE, with statistical significance determined by one-way ANOVA.

In this study, we used our novel technique for MCC (12) to identify ACFs in the entire distal colon of patients undergoing routine colorectal cancer screening. We found that quantifying the number of ACFs present in the entire distal colon provided significantly more information compared with counting those present only in the distal rectum. Using this information, we then determined that small ACFs (that is, those composed of ≤20 crypts) correlated with distal, but not proximal, adenomas. In contrast, large ACFs (that is, those containing >100 crypts) correlated with the presence of hyperplastic polyps. This would suggest that at least two different ACF populations might exist.

The association of large ACFs with hyperplastic polyps may not be surprising because it is impossible to histologically differentiate the two structures. This is due to the fact that similar morphologic features, including a convoluted lumenal pattern of growth and growth by crypt fission, characterize both hyperplastic polyps and ACF (20). Furthermore, by definition, ACFs must be identified at low power in unembedded macroscopically normal mucosa (1) and thus must be identified at the time of endoscopy (1, 21). With increasing ACF size and elevation, the ability to differentiate this lesion from a small hyperplastic polyp becomes ever more difficult, particularly when using magnifying endoscopes. Thus, that a continuum exists between increasing ACF size and hyperplastic polyp formation is teleologically reasonable.

In contrast, small ACFs possibly include a second and distinct population destined to become adenomatous polyps. A recent report in F344 rats exposed to the carcinogen azoxymethane suggested that at least two types of ACF could be detected: the commonly found ''classic'' lesion that is raised and that rarely contains dysplasia and a significantly less frequent small flat lesion that the authors proposed as the actual precursor to colorectal cancer (22). Intriguingly, at the molecular level. β-Catenin mutations were prevalent in the flat lesions although were absent in the classic, larger, and raised ACF (23). This is interesting in light of our finding that flat small ACFs correlate with the presence of distal adenomas, whereas raised large ACFs correlate with the presence of distal hyperplastic polyps.

Molecular heterogeneity suggesting the presence of at least two different ACF populations exists in humans as well. For instance, only 2% to 3% of sporadic ACFs contain B-raf mutations (24), proposed as critical in the formation of larger hyperplastic polyps and serrated adenomas, whereas 60% of serrated ACF, a unique lesion described in familial cancers with associated serrated adenomas, possess this mutation (25). Similarly, APC mutations were found in 100% of ACF developing in patients with familial adenomatous polyposis, whereas β-catenin and APC mutations were absent (0%) in all non–familial adenomatous polyposis patients studied (26). Yet, the molecular pathway driving ACFs to becoming adenomas in cancer family syndromes may be unique and not applicable to what occurs in sporadic colon cancer. Thus, mutations that are as of yet uncharacterized may be important to the transformation of a sporadic ACF to polyp and cancer. Future studies will need to evaluate the relative frequencies of B-raf and APC/β-catenin mutations in both small and large ACFs occurring in populations not known to be at increased colorectal cancer risk to determine whether they indeed possess unique mutational profiles that predict their histological destiny.

Alternatively, it is possible that small ACFs are not a direct precursor to adenomatous polyps but rather reflect another process or processes associated with neoplastic transformation. For example, small ACFs may simply reflect the presence of a hyperproliferative epithelium, such as is known to exist in patients with colorectal cancer (27, 28). Mathematical modeling indicates that if a crypt within ACF fissions at a markedly higher rate than what is occurring in the background mucosa, the ACF will be large (29). In contrast, if ACF proliferation rates are similar to what is observed in the background mucosa, as would occur in a hyperproliferative epithelium, ACFs will be small (29). APC or β-catenin mutations causing adenoma formation could theoretically arise anywhere within the rapidly proliferating ''normal epithelium,'' making the small ACF an innocent bystander such that the initial development of this structure may not include any unique mutational signature.

The absence of ACF histopathology, or molecular characterization of key genes such as APC or B-raf, might be viewed as limitations of this study. Thus, our observations that that there are two separate and distinct ACF populations cannot be proven at the molecular level. Yet, an overarching purpose of this study was to determine if ACF identification and enumeration could be done in a manner that could conceivably be applied to routine clinical practice. Indeed, the ease with which we did MCC, along with the previously shown higher rate of this technique for identifying flat cancers (30), suggests that, as has been argued by others (30), chromoscopy should perhaps be the favored approach for colorectal cancer screening. As to whether ACF enumeration should likewise be routinely done is not clear and will require prospective studies to determine if the number of small ACFs present in a patient has prognostic significance with regard to future adenoma development.

Disclosure of Potential Conflicts of Interest

A. Arozullah: Takeda Global Research and Development employee. The other employees disclosed no potential conflicts of interest.

Acknowledgments

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