Rectal Aberrant Crypt Foci in Humans Are Not Surrogate Markers for Colorectal Cancer Risk

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INTRODUCTION: **Over the past 20 years, aberrant crypt foci (ACF) have emerged as potential precursors and biomarkers for colorectal cancer (CRC). However, data regarding their molecular pathogenesis, as well as their endoscopic and histological identification, remain inconsistent.**

METHODS: **A wide cohort of ACF from 100 control subjects and 100 case patients, including patients with adenoma and CRC, were characterized for endoscopic, morphologic, and molecular features.**

RESULTS: **We observed that among all the endoscopic features evaluated, only the number of large ACF correlated with CRC risk ($P = 0.003$), whereas the histological classification, as assessed by 2 different pathologists, was inconsistent and did not differ between control and case patients. Moreover, only a few *APC* and *BRAF* mutations and no microsatellite instability were detected in our samples. *KRAS* mutations were detected in 16.3% of ACF samples, which also exhibited increased *MGMT* hypermethylation. However, none of those events were found to be predictive of CRC risk.**

DISCUSSION: **Although ACF might be preneoplastic lesions of the colon, they are not suitable biomarkers for assessing CRC progression.**

Clinical and Translational Gastroenterology 2019;10:e-00047. https://doi.org/10.14309/ctg.0000000000000047

INTRODUCTION

Aberrant crypt foci (ACF) are the earliest visible lesions in the colorectum and are considered potential precursors of colorectal cancer (CRC). They are defined as crypts with altered luminal openings, thickened epithelium, and larger in size than normal crypts. In addition, although ACF can arise in both the proximal and distal colon, they are mostly observed in the distal colon and rectum (1). ACF were first detected in the colon of rodents treated with colon-specific carcinogens (2–4), being later identified in human patients at a high risk of CRC (5).

The association of the size and number of ACF with CRC risk in humans is somehow controversial, with studies for and against these findings (6–12). These lesions also exhibit dysplasia, an increased proliferative index and some genetic alterations such as *KRAS*, *APC*, and *BRAF* mutations, commonly observed in adenomas and carcinomas (13). Nevertheless, the frequency and distribution of these alterations vary substantially between studies and among CRC risk groups (6,14,15), complicating the clinical utility of ACF as CRC biomarkers.

The most widely accepted histological approach is to classify ACF as hyperplastic and dysplastic, as recommended by the World Health Organization (16). The frequency of dysplastic ACF is low (10,11,17,18); however, it seems to have potential for malignant degeneration (6,19–22). In addition, studies of hyperplastic ACF have suggested that these, too, might have malignant potential, albeit *via* the serrated pathway of carcinogenesis (23).

High-magnification chromoendoscopy (CE) permits the direct observation of ACF and allows for the identification of several features that have been correlated with ACF histology and CRC risk (6,19,23). In fact, the most common definition of ACF is based on endoscopy crypt patterns after staining with methylene blue. ACF are clusters of crypts that are stained darker than the surrounding mucosa, have larger diameters, often with oval or slit-like lumens and thicker epithelial linings (6). Several human studies have demonstrated that ACF can be identified and characterized by conventional and electronic CE using magnification and high-definition scopes (6,7,11,19,20,24). Nevertheless, the data reported to date remain inconsistent (25), and the rate of agreement between the presence of ACF and their histologic confirmation varies substantially (6,7), thus further hindering their utility as surrogate markers for CRC. Indeed, most ACF

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Received July 25, 2018; accepted February 22, 2019; published online May 15, 2019

reportedly remain in a dormant state or even regress and disappear (26,27), and according to 1 multicenter study, rectal ACF were difficult to reidentify during follow-up examination (11).

Despite evidence supporting the notion that ACF are precancerous lesions, there are many inconsistences in the data regarding their molecular pathogenesis, as well as its endoscopic and histological identification. Therefore, the aim of this study was to determine whether rectal ACF are biomarkers of CRC risk by characterizing the endoscopic, morphologic, and molecular features of ACF samples collected from subjects without colonic lesions (controls), with adenoma and CRC.

METHODS

Subjects

This study was approved by the Ethics Committee at the Hospital Clinic of Barcelona, and all participants provided their written consent. Individuals were prospectively recruited from the regular patient agenda of the Endoscopy Unit at the Hospital Clinic of Barcelona. During the inclusion period, colonoscopy reports in the Endoscopy Unit were reviewed daily. Individuals with a colonoscopy not reaching cecum (or ileocolonic anastomosis, if applicable) and/or with a poor bowel preparation in any colonic segment were excluded. Subjects were invited to participate in the study *via* phone call a few days after the colonoscopy. They were selected and divided into 2 groups based on their endoscopic findings: (i) Control group: individuals with a normal colonoscopy and without a personal history of adenomas or CRC (n = 100) and (ii) Case group: patients with a personal history of CRC or current CRC (n = 50) or patients with ≥1 current colonic adenomas (n = 50). Patient exclusion criteria are detailed in Figure 1. For ACF detection, a different examiner, who was blinded to each patient's study group, performed a rectoscopy. The interval between the colonoscopy and the rectoscopy was less than 1 month.

Endoscopy assessment

A systematic examination of the distal 10 cm of the rectum was performed with a high-definition colonoscope (Olympus H180, Evis Exera II processor, Olympus Europe) in all patients. ACF were defined as crypts with a larger diameter than the normal mucosa, a thicker epithelial lining, and a dilated crypt lumen. ACF that raised >2 mm were considered polyps. The number of ACF per patient was categorized as less than 5, 5 to 15, or more than 15. The rectum was examined clockwise, proximal to distal, to record ACF features and their location, first with narrow-band imaging (NBI) and then with methylene blue 0.5% CE. CE was considered the gold standard for ACF detection. The size of ACF, as assessed by CE, was classified as small (<20 crypts per ACF), medium (20–40 crypts per ACF), or large (>40 crypts per ACF). The shape of the crypt lumens, as visualized by CE, was characterized as semicircular-oval, asteroid-like, or irregular. ACF vascular pattern intensity (VPI) was described as weak, normal, or strong in comparison with the appearance of the surrounding mucosa, as visualized by NBI.

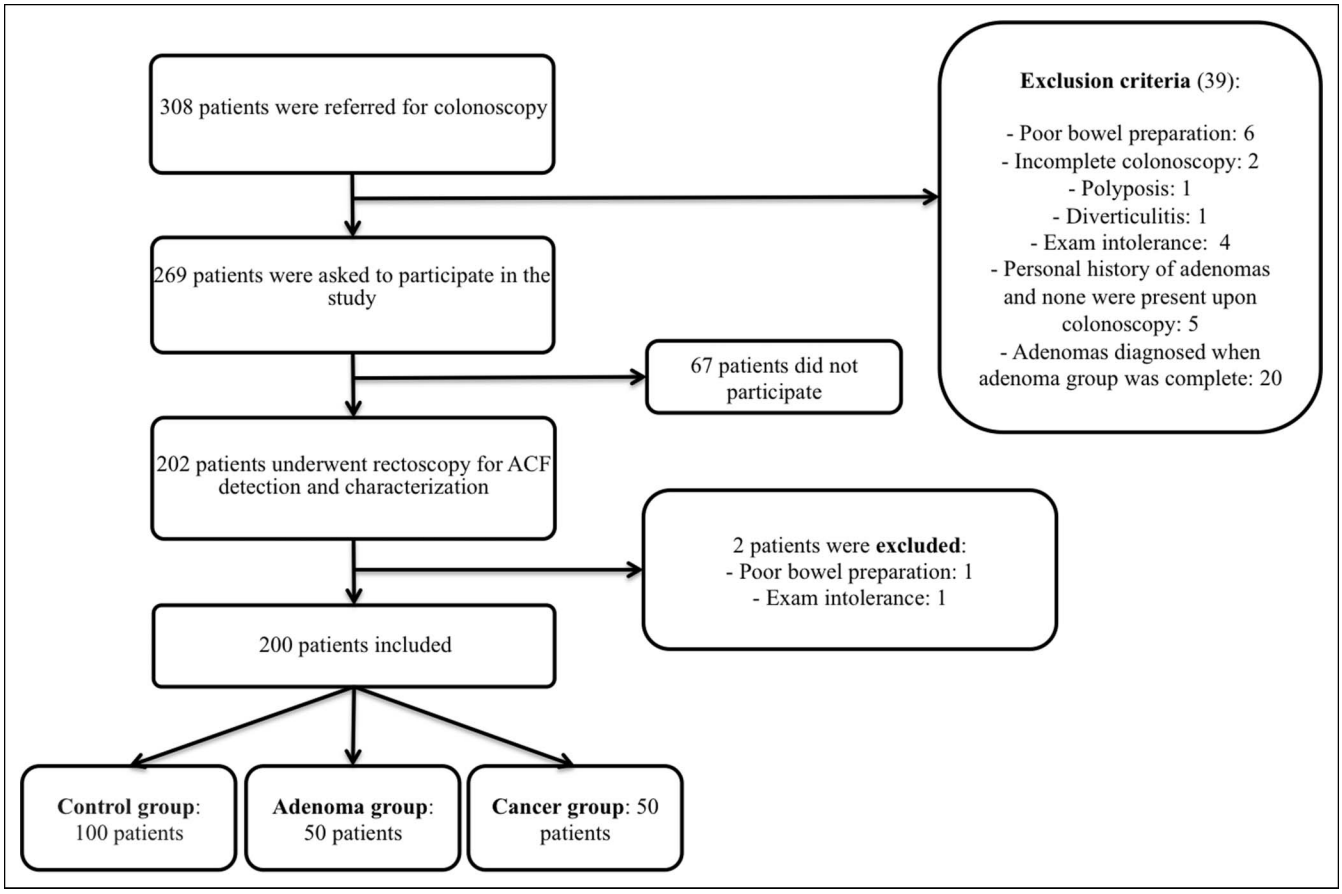

Figure 1. Patients' flowchart.

Pathological assessment

Normal mucosa and the 5 largest ACF were biopsied for each patient. ACF were immediately immersed in tissue freezing medium (OCT) and stored at −80 °C, whereas the normal mucosa was conserved in PBS at −80 °C. The tissue sections were stained with hematoxylin and eosin and analyzed by light microscopy. All samples were evaluated twice over a period of 6 months by 2 different pathologists (A and B) who were blinded to the endoscopic classification and to each other's diagnoses. Discordant diagnoses were reviewed to reach a consensus diagnosis. Histological findings were classified as inadequate sample, normal mucosa, hyperplastic ACF, and dysplastic ACF, according to the WHO classification (16). The "serrated morphology" category was incorporated into our final diagnosis to evaluate the possible role of ACF in the serrated pathway.

Molecular analysis

For feasibility reasons, molecular characterization was only performed for the first 3 histologically confirmed ACF samples from each patient. DNA from ACF and normal mucosa was extracted using the All Prep DNA/RNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's recommendations and quantified using a NanoDrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Waltham, MA).

KRAS *mutational analysis.* A fragment of the *KRAS* gene spanning codons 12 and 13 was amplified by COLD-PCR using the following primers: F, 5'-GCCTGCTGAAAATGACTGAA-3', and R, 5'-AGAATGGTCCTGCACCAG TAA-3'.

APC *mutational analysis.* *APC* mutations were analyzed from 2 amplified fragments (A and B) that spanned the majority (82.6%) of the *APC* gene mutations. Primer sequences were (i) A-F (5'-CA GTGAGAATACGTCCACACCT-3') and B-F (5'-TTTGAGA GTCGTTCGATTGC-3') and (ii) A-R (5'-CATTCCACTGCA TGGTTCAC-3') and B-R (5'-TGATGACTTTGTTGGCA TGG-3').

BRAF *mutational analysis.* *BRAF* V600E mutation genotyping was performed by Real-Time Taqman PCR using primers and probes designed by Applied Biosystems Custom Genotyping Assay Service.

Microsatellite instability analysis. The microsatellite instability (MSI) Analysis System, consisting of 5 nearly monomorphic mononucleotide markers (*BAT-25*, *BAT-26*, *NR-21*, *NR-24*, and *MONO-27*) and 2 polymorphic pentanucleotide markers (Penta C and Penta D) were used according to the manufacturer's guidelines (Promega, Wisconsin).

Methylation analysis. *MGMT* gene promoter methylation levels were investigated using pyrosequencing-based methylation analysis, as described previously (28).

Statistical analysis

CE and the consensus histological diagnoses were considered the gold standard for ACF detection and diagnostic confirmation, respectively. SPSS statistical software (IBM 2012, IBM SPSS Statistics, Version 20.0. Armonk, NY) was used for data analysis. Results for continuous variables were summarized using mean and SD or median and interquartile range for skewed data. Frequencies (%) were used to summarize categorical variables, and 95% confidence intervals were calculated when relevant. Student *t* or Mann-Whitney tests were used to compare the distribution of continuous variables by their outcome. Pearson χ^2 or Fisher exact tests were used to test for any association between categorical variables and outcome. All analyses were exploratory, and 2-tailed tests with a significance level of 5% were used throughout. The association between endoscopic features and study group were adjusted by age and sex using binary logistic regression. Paired analyses were performed when comparing molecular changes in normal mucosa and ACF. Inter- and intra-pathologist concordance for histological diagnosis were calculated by Weighed k-statistics and defined as follows: fair, 0.21–0.40; moderate, 0.41–0.60; good, 0.61–0.80; and very good, 0.81–1.00.

RESULTS

Patient baseline characteristics

Two hundred patients (56% female, age 62.9 ± 13.8 years) were included in this study. The control group was composed of more females, of younger age, than the study group (66% vs 46%, $P = 0.05$ and 57.62 ± 15.58 years vs 68.15 ± 9.26 years, $P < 0.001$, respectively). Among the patients with adenoma, 29 (58%) had ≥1 advanced adenoma. In the CRC group, 22 patients (44%) had a previous CRC, and 28 (56%) had a current CRC. Inclusion and exclusion criteria are shown in Figure 1, and colonoscopy indications are detailed in Table S1 (see Supplementary Digital Content 1, http://links.lww.com/CTG/A46).

Endoscopic features of ACF

CE detected the presence of at least 1 ACF in 176/200 (88%) patients. Forty-three individuals (21.5%) exhibited less than 5 ACF, 94 (47.0%) had between 5 and 15 ACF, and 63 (31.5%) had more than 15 ACF. A total of 1,103 ACF were characterized by CE, whereas 768 were characterized by NBI. Size evaluation determined that 305 ACF (27.2%) were small, 366 (33.2%) were medium, and 432 (39.2%) were large. The shape of the crypt lumens was semicircular-oval in 80.1% of the ACF, asteroid-like in 10.1%, and irregular in 9.8%. Finally, ACF VPI was weak in 525 ACF (68.4%), normal in 220 ACF (28.6%), and strong in only 23 ACF (3%).

As is shown in Tables 1 and 2, only the presence of large ACF was related to the CRC risk group. In fact, the number of large ACF increased progressively toward CRC risk (control, 6.06%; adenoma, 20%; CCR, 28%). Conversely, neither the number of ACF per subject, the lumen morphology, nor VPI was related to CRC risk.

ACF histology

Although 686 ACF were detected endoscopically, only 553 were confirmed by histology (38 inadequate samples, 95 normal mucosa) (see Figure S1, Supplementary Digital Content 1, http://links.lww.com/CTG/A46). According to the consensus diagnosis, 553 ACF samples were classified as hyperplastic ACF (71.6%), serrated ACF (10.1%), or dysplastic ACF (18.3%) (Figure 2). Consequently, the diagnostic yield of endoscopy (histologically confirmed ACF/ACF detected with CE) was 80.6%. Pathologist A showed a weighed K for intraobserver concordance of 0.59, and pathologist B 0.71 ($P \leq 0.001$). The best weighed K for interobserver concordance was only 0.25 ($P < 0.001$). Interestingly, the histology of ACF was not related to gender, sex, or CRC risk group (Table 2). Although dysplastic and serrated ACF exhibited an irregular shape more frequently than hyperplastic ACF, their size and VPI were not associated with ACF histology (Table 3).

Table 1. Analysis of individuals and ACF features predictive of CRC risk adjusted per patient's age and sex

	Control, N = 100	Case, N = 100	Adjusted *P* value	OR
Female (%)	66 (66.0)	46 (46.0)	0.005	0.32
Age (yr ± SD)	57.62 ± 15.58	68.15 ± 9.26	0.0001	1.08
No. of ACF per patient	23/48/29	20/46/34	n.s	
Categories: <5/5–15/>15 (%)	(23.0/48.0/29.0)	(20.0/46.0/34.0)		
Patients with:				
• At least 1 large ACF (%)	62 (62.0)	78 (78.0)	0.046	2.03
• More than 1 large ACF (%)	41 (41.0)	66 (66.0)	0.002	2.70
• More than 4 large ACF (%)	6 (6.0)	24 (24.0)	0.003	3.47
• One irregular ACF (%)	26 (26.0)	35 (35.0)	n.s	
• At least 1 dysplastic ACF (%)	32 (32.0)	40 (40.0)	n.s	
• More than 1 dysplastic ACF (%)	10 (10.0)	11 (11.0)	n.s	
• At least 1 serrated ACF (%)	19 (19.0)	26 (26.0)	n.s	

ACF, aberrant crypt foci.

Molecular analysis of ACF

The first 3 histologically confirmed ACF from each patient were used for molecular analysis, totaling 294 ACF samples. Of these, 128 were from 67 control subjects (47 female (70%); age 58.3 ± 15.2 years) and 166 were from 81 case patients, of which 40 were from patients with adenoma and 41 were from patients with CRC (32 female (39%); age 67.7 ± 9.9 years). Of this cohort of ACF samples, 146/294(49.7%) were categorized as large lesions. Histological examination revealed that 197 ACF (67.0%) were hyperplastic, 35 (12.0%) serrated, and 62 (21.0%) dysplastic lesions (see Figure S1, Supplementary Digital Content 1, http://links.lww.com/CTG/A46).

APC mutation and MSI analysis in ACF

APC mutations were determined for 285/294 (96.9%) ACF samples. The *APC* variant E1317Q was found in 5/285 (1.7%) samples (Table 4); however, these ACF belonged to patients who exhibited the same variant in their normal mucosa, indicating

Table 2. Analysis of ACF features predictive of CRC risk adjusted by age and sex

	Control, N = 100	Case, N = 100	Adjusted *P* value	OR
Large size	32.1%	45.0%	0.001	1.61
(Large ACF/ACF evaluated by chromoendoscopy)	(159/496)	(273/607)		
Irregular shape	7.4%	11.9%	n.s	
(Irregular ACF/ACF evaluated by chromoendoscopy)	(35/470)	(65/547)		
Large or irregular	36.8%	54.1%	0.0001	1.76
(Large or irregular ACF/ACF evaluated by chromoendoscopy)	(173/470)	(296/547)		
Intensive vascular pattern	2.2%	3.8%	n.s	
(Hypervascular ACF/ACF evaluated by NBI)	(8/369)	(15/399)		
ACF histologically confirmed	75.6%	84.8%	0.018	1.69
(ACF histologically confirmed/ACF biopsied)	(235/311)	(318/375)		
Dysplastic ACF	19.6%	17.3%	n.s	
(Dysplastic ACF/histologically confirmed ACF)	(46/235)	(55/318)		
Serrated ACF	10.2%	10.1%	n.s	
(Serrated ACF/histologically confirmed ACF)	(24/235)	(32/318)		
Hyperplastic ACF	70.2%	72.6%	n.s	
(Hyperplastic ACF/histologically confirmed ACF)	(165/235)	(231/318)		

ACF, aberrant crypt foci.

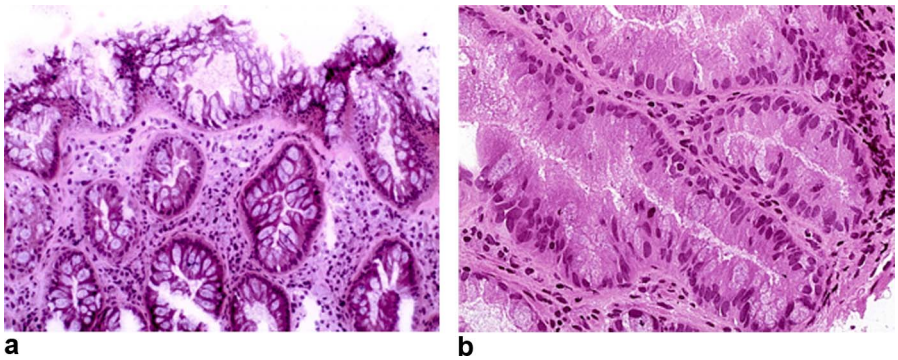

Figure 2. Histological sections of 2 aberrant crypt foci, with hyperplastic change, showing nondysplastic distorted architecture of the surface epithelium with widening and hyperplastic contour of the luminal end of the crypts (**a**) (H&E, ×100), and dysplastic change with hyperchromatic, cigar-shaped nuclei, pseudostratification, dense eosinophilic cytoplasm, mitotic figures, and loss of cytoplasmic mucin (**b**) (H&E, ×200).

that it was germline and not related to ACF formation. MSI status was evaluated for 276/294 (94.2%) ACF samples. All samples analyzed were MSS.

BRAF and *KRAS* mutations in ACF

BRAF and *KRAS* mutations were examined in 290/294 (98.6%) and 289/294 (98.3%) ACF samples, respectively. The *BRAF V600E* mutation was detected in only 6/290 (2.1%) ACF samples, whereas *KRAS* was mutated in 48/289 (16.3%) ACF samples. Moreover, as described previously, no ACF sample simultaneously exhibited *BRAF* and *KRAS* mutations (29).

MGMT methylation status analysis in ACF

As *KRAS* mutations have been previously associated with the serrated alternative pathway of carcinogenesis (30), we determined the *MGMT* methylation status in 33 *KRAS*-mutated and 75 *KRAS* wild-type ACF samples, as well as in their surrounding normal mucosa. Overall, the level of methylation was significantly higher in ACF samples compared with their corresponding normal mucosa (5.54 ± 3.70 vs 4.23 ± 2.31, $P = 0.004$). However, there were no differences in the levels of methylation among the control and case groups or between those ACF samples with mutated or wild-type *KRAS* status (see Figure S2A, B and C, Supplementary Digital Content 1, http://links.lww.com/CTG/A46). Next, we analyzed the methylation results as a categorical variable using the mean levels in the normal mucosa methylation as the cutoff for ACF hypermethylation (mean ± 2 SD, which corresponded to 8.66%). Interestingly, by using this cutoff, we observed significantly more hypermethylated samples in the set of *KRAS* mutated ACF than in the set of ACF displaying wild-type *KRAS* (21% vs 5.71%, $P = 0.02$). Nevertheless, we did not detect any difference in the distribution of the hypermethylated ACF among the control and case groups or according to their histology (Tables 4 and 5).

DISCUSSION

The present case-control study uses a translational approach to evaluate the role of rectal ACF as a precursor lesion of CRC and its clinical application as an intermediate end point in CRC carcinogenesis. First, we performed *in vivo* and *in situ* detection and characterized ACF by advanced endoscopy in 100 individuals without colonic lesions and 100 patients at risk of CRC. Second, 2

Table 3. Analysis of ACF features related to histology

	Hyperplastic	Serrated	Dysplastic	*P* value
Large size	59.6%	50.5%	58.9%	n.s
(Large ACF/histologically confirmed ACF)	(236/396)	(33/56)	(51/101)	
Irregular shape	10.1%	20.4%	20.6%	0.006
(Irregular ACF/histologically confirmed ACF)	(39/385)	(11/54)	(20/97)	
Asteroid-like or irregular shape	20.8%	25.9%	35%	0.012
(Asteroid-like or irregular ACF/histologically confirmed ACF)	(80/385)	(14/54)	(34/97)	
Intensive vascular pattern	3.9%	5.1%	5.6%	n.s
(Hypervascular ACF/histologically confirmed ACF)	(12/306)	(2/39)	(4/71)	
Control group	41.7%	42.9%	45.5%	n.s
(ACF from the control group/histologically confirmed ACF)	(165/396)	(24/56)	(46/101)	
Case group	58.3%	57.1%	54.5%	n.s
(ACF from the study group/histologically confirmed ACF)	(231/396)	(32/56)	(55/101)	

ACF, aberrant crypt foci.

Table 4. ACF molecular features related to the CRC risk group

	Control	Case	*P* value
KRAS mutations	17/124 (13.7%)	30/165 (18.2%)	n.s
BRAF mutations	3/128 (2.3%)	3/162 (1.9%)	n.s
APC mutations	0/127 (0%)	5/158 (3.2%)	n.s
MGMT hypermethylation	6/37 (16.2%)	6/71 (8.5%)	n.s

ACF, aberrant crypt foci.

different pathologists evaluated a set of 686 ACF samples twice to reach a consensus diagnosis on the basis of standardized criteria. Last, a molecular profile of common CRC alterations was assessed for these lesions. Our results showed that rectal ACF may be preneoplastic lesions and play a role in CRC carcinogenesis; however, they are not reliable biomarkers because their morphological and molecular findings were not consistent and there was no association with the risk group.

Previous studies have reported the diagnostic yield of endoscopy when using histology as gold standard to be between 60% and 68% (11,31). In our cohort, 80% of the lesions identified by endoscopy as potential ACF were corroborated by their histology, thus indicating that high-definition CE was a suitable technique for detecting these subtle lesions (32).

Previously, it has been reported that there is a stepwise increase in the number and prevalence of rectal ACF in relation to the CRC risk group (18,24). However, in accordance with data from multicenter studies (10,11), we did not find the number of ACF to be associated with CRC. On the other hand, although none of the other endoscopic features were predictive of CRC risk, we found that the number of large ACF was associated with the CRC risk group. Unfortunately, this endoscopic feature was not associated with a specific histopathological diagnosis or molecular pathway, rendering this observation of dubious clinical utility.

Previous data showed that dysplastic ACF were specially related to CRC risk (19). In addition, elongated and asteroid-like lumens are characteristic of dysplasia and hyperplasia, respectively (20,33). However, we only found irregular lumens to be associated with dysplastic and serrated ACF. This is in accordance with a previous study that showed that endoscopic features were inconsistent across endoscopists and did not accurately predict histology (31).

Histological characterization of ACF remains a challenge. There is significant variability across numerous studies in terms of the tissue sampling procedures and ACF classification (34,35). Two pathologists examined all specimens, twice, in random order, yet neither the interobserver nor the intraobserver concordances were satisfactory. Considering that we are evaluating very subtle changes, the small size of the samples (average of 2 mm) and the lack of orientation during sampling may have influenced these poor results. Nonetheless, this limitation reflects the difficulties in classifying ACF morphologically and suggests the necessity of a more careful and objective standardization among pathologists, such as examining different ACF sections to accurately assess dysplasia in these lesions, which may be focally represented. Our cohort of ACF samples was mainly composed of hyperplastic lesions, accounting for only 18.5% of dysplastic ACF, which is in agreement with the previously reported low prevalence of dysplasia. In fact, some studies did not even detect dysplastic ACF (10,17). Furthermore, as the appearance of sessile serrated polyps has been mainly associated with the proximal colon (36,37), we were only able to detect a modest percentage of serrated ACF. Nevertheless, we did not find any histological category to be predictive of CRC risk.

Several previous studies have stated that ACF harbor genetic alterations that might lead to malignant transformation, but these data remain controversial (14,15,38). *APC* mutations are considered an early event in CRC carcinogenesis; however, several studies have reported that *APC* mutations are found in ACF lesions from patients with familial adenomatous polyposis, whereas they are infrequent in sporadic ACF (15,38,39). Similarly, in our cohort of ACF samples, we found *APC* alterations to be rare. In addition, although mutations in *BRAF* are commonly associated with serrated lesions from the proximal colon, including ACF (40–42), others have detected them in only 2% of sporadic rectal ACF (14). In agreement with these findings, we observed *BRAF* mutations in only 2% of our ACF samples. In addition, although MSI has been detected in ACF from patients with Lynch syndrome (43), sporadic ACF are known to exhibit a lower frequency of MSI (42,44,45). Accordingly, we did not observe MSI in any of the ACF samples analyzed (35).

Last, several investigations have consistently reported a high but variable incidence of *KRAS* mutations in sporadic ACF (15,38). The discrepancies regarding the frequency of *KRAS* mutations among studies could be due to the different types and sizes of study populations. Accordingly, we detected *KRAS* mutations in 16.3% of ACF samples; however, there was no association with the case group, similar to what has been observed previously (6,15). Moreover, *MGMT* promoter hypermethylation and its association with *KRAS* mutations have been described in the early stages of CRC (46,47). We also observed an association between *KRAS* mutation and *MGMT*

Table 5. ACF molecular features related to histology

	Hyperplastic	Serrated	Dysplastic	*P* value
KRAS mutations	33/195 (16.9%)	2/35 (5.7%)	12/59 (20.3%)	n.s
BRAF mutations	3/193 (1.6%)	1/35 (2.9%)	2/62 (3.2%)	n.s
APC mutations	2/190 (1.1%)	1/35 (2.9%)	2/60 (3.3%)	n.s
MGMT hypermethylation	10/76 (13.2%)	0/9 (0%)	2/23 (8.7%)	n.s

ACF, aberrant crypt foci.

promoter hypermethylation in our ACF samples, although those ACF were not predictive of CRC risk.

Before reaching definitive conclusions, some limitations should be acknowledged. First, the cross-sectional design of this study precludes us from raising any associations of causality. Second, the study cohort was composed of more males, of older age, both variables associated with CRC risk; however, we introduced them in the multivariate analysis. Another potential limitation is the presence in the control group of subjects with gastrointestinal symptoms (see Table S1, Supplementary Digital Content 1, http://links.lww.com/CTG/A46), which could be a hallmark of colonic lesions, despite a complete normal recent colonoscopy. This possibility is remote because those patients underwent a high-quality colonoscopy just before inclusion. Last, the frozen and crush artifact, orientation of the sample and the small size of the biopsies may result on scarce or no representation of the lesion, even to little alteration of the morphology and thus to the difficult pathological interpretation or limitation of an accurate histological diagnosis of some of the early lesions. In addition, by examining only the rectum, we may have limited the type of ACF samples obtained and affected our rates of prevalence. Nevertheless, in previous studies, the vast majority of these lesions are detected in the distal colon, and rectal ACF have been described as representative of the rest of the colon (10). In fact, the evaluation of proximal ACF remains limited and has resulted in the detection of few gene mutations other than *KRAS* or *BRAF,* such as *EGFR* or*FLT3* (1,41). In addition, if a firm relationship between rectal ACF features and CRC risk would have been proven, we could use rectoscopy as a tool for identifying those patients at risk of CRC that would clearly benefit from screening with a complete colonoscopy. In particular, subjects with no rectal ACF would not need any more tests because they would be at very low risk, whereas those with dysplastic rectal ACF would need a complete colonoscopy to rule out colonic lesions. Last, by performing microdissection of our samples, we could have been able to slightly increase the frequency of the genetic alterations we observed; however, other studies have not used this technique (11,21,31,42), and the large number of samples limited the feasibility of accomplishing this with our resources. Furthermore, we considered that the methodology of our molecular analysis was sensitive enough for our purpose.

In conclusion, this large-scale study of ACF demonstrated that there is no consistent morphological characteristic that would enable us to recommend ACF as biomarkers for CRC risk. Our molecular analysis found that *KRAS* mutations and *MGMT* promoter hypermethylation might be responsible for ACF formation. Nevertheless, as none of the genomic alterations observed in ACF correlated with the CRC risk group, our results indicate that ACF might merely be preneoplastic lesions, but not suitable as an intermediate end point for CRC carcinogenesis.

CONFLICTS OF INTEREST

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Specific author contributions: I. Quintanilla and M. López-Cerón contributed equally to this work. Study concept and design: M.P. Acquisition of clinical data: M.L.C., C.R.M., M.Z., L.M., and J.L. Pathological analysis: M.C. and M.J. Molecular analysis: I.Q., J.M., and V.A. Statistical analysis and interpretation of the data: M.L.C., I.Q., M.J., and M.P. Drafting of the manuscript: I.Q., M.C., M.J., M.L.C., and M.P. Critical revision of the manuscript for important intellectual content: J.C., F.B., A.C., M.C., and M.P.

Funding Support: This work was supported by a grant from Fondo de Investigaciones Sanitarias (FIS) del Instituto de Salud Carlos III (FIS Proyectos de Evaluación de Tecnologías Sanitarias PS09/00669). Maria López-Cerón was a research fellow from the FIS grant (Rio Hortega contract). Isabel Quintanilla is funded by the Ministerio de Educación, Cultura y Deporte (FPU12/01351). Francesc Balaguer is funded by Instituto de Salud Carlos III (PI13/00719). Maria Pellisé is funded by Instituto de Salud Carlos III (PI12/01481), Fundación Científica Asociación Española Contra el Cáncer (GCB13131592CAST), and Agència de Gestió d'Ajuts Universitaris i de Recerca (2014SGR135). Jordi Camps was awarded with a grant from the Asociación Española Contra el Cáncer, "Cofinanciado por el Fondo Europeo de Desarrollo Regional (FEDER), Unión Europea, Una manera de hacer Europa". Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD) is funded by Instituto de Salud Carlos III. Work is supported by the Banc de Tumors-Biobanc Hospital Clinic-IDIBAPS and Xarxa de Bancs de Tumors de Catalunya (XBTC).

Potential competing interests: Maria Pellisé is a consultant for Norgine Iberia. The other authors do not have any conflict of interest.

Study Highlights

WHAT IS KNOWN

- ✓ ACF have been hypothesized to be the potential precursors of colonic neoplastic lesions and have been proposed as early biomarkers for colonic carcinogenesis.
- ✓ They can be identified by high-definition or magnification CE as clusters of colonic crypts with a thicker epithelial lining and a dilated crypt lumen.
- ✓ There are many inconsistencies in the data regarding their molecular pathogenesis and their endoscopic and histological identification.

WHAT IS NEW HERE

- ✓ Endoscopic and molecular features were investigated in a large cohort of rectal ACF in patients at different risk of CRC.
- ✓ No ACF endoscopic characteristic was consistently associated with CRC risk.
- ✓ The molecular analysis found that *KRAS* mutations and *MGMT* promoter hypermethylation might be responsible for ACF formation. Nevertheless, none of the genomic alterations observed in ACF correlated with the CRC risk group.

TRANSLATIONAL IMPACT

- ✓ ACF might merely be preneoplastic lesions, but not suitable as an intermediate end point for CRC carcinogenesis.

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