Spectrum of Somatic Cancer Gene Variations Among Adults With Appendiceal Cancer by Age

Andreana N. Holowatyj, PhD, MS; Cathy Eng, MD; Wanqing Wen, MD; Kamran Idrees, MD, MS, MMHC; Xingyi Guo, PhD

Abstract

IMPORTANCE The incidence of appendiceal cancer (AC) is rising, particularly among individuals younger than 50 years (early-onset AC), with unexplained etiologies. The unique spectrum of somatic cancer gene variations among patients with early-onset AC is largely undetermined.

OBJECTIVE To characterize the frequency of somatic variations and genomic patterns among patients with early-onset (age <50 years) vs late-onset (age ≥50 years) AC.

DESIGN, SETTING, AND PARTICIPANTS This cohort study included individuals aged 18 years and older diagnosed with pathologically verified AC. Cases with clinical-grade targeted sequencing data from January 1, 2011, to December 31, 2019, were identified from the international clinicogenomic data-sharing consortium American Association for Cancer Research Project Genomics Evidence Neoplasia Information Exchange (GENIE). Data analysis was conducted from May to September 2020.

EXPOSURES Age at clinical sequencing.

MAIN OUTCOMES AND MEASURES Somatic variation prevalence and spectrum in AC patients was determined. Variation comparisons between early-onset and late-onset AC were evaluated using multivariable logistic regression with adjustment for sex, race/ethnicity, histological subtype, sequencing center, and sample type.

RESULTS In total 385 individuals (mean [SD] age at clinical sequencing, 56.0 [12.4] years; 187 [48.6%] men; 306 [79.5%] non-Hispanic White individuals) with AC were included in this study, and 109 patients (28.3%) were diagnosed with early-onset AC. Race/ethnicity differed by age at sequencing; non-Hispanic Black individuals accounted for a larger proportion of early-onset vs late-onset cases (9 of 109 [8.3%] vs 11 of 276 [4.0%]; P = 0.04). Compared with patients aged 50 years or older at sequencing, patients with early-onset AC had significantly higher odds of presenting with nonsilent variations in PIK3CA, SMAD3, and TSC2 (PIK3CA: odds ratio [OR], 4.58; 95% CI, 1.72-12.21; P = .002; SMAD3: OR, 7.37; 95% CI, 1.24-43.87; P = .03; TSC2: OR, 12.43; 95% CI, 1.03-149.59; P = .047). In contrast, patients with early-onset AC had a 60% decreased odds of presenting with GNAS nonsilent variations compared with patients with late-onset AC (OR, 0.40; 95% CI, 0.21-0.76, P = .006). By histological subtype, young patients with mucinous adenocarcinomas of the appendix had 65% decreased odds of variations in GNAS compared with late-onset cases in adjusted models (OR, 0.35; 95% CI, 0.15-0.79; P = .01). Similarly, patients with early-onset nonmucinous appendiceal adenocarcinomas had 72% decreased odds of presenting with GNAS variations vs late-onset cases, although these findings did not reach significance (OR, 0.28; 95% CI, 0.07-1.14; P = .08). GNAS and TP53 variations were mutually exclusive in ACs among early-onset and late-onset cases (P < .05).

(continued)
CONCLUSIONS AND RELEVANCE  In the study, AC among younger individuals harbored a distinct genomic landscape compared with AC among older individuals. Development of therapeutic modalities that target these unique molecular features may yield clinical implications specifically for younger patients.

Introduction

Appendiceal cancer (AC) is a rare neoplasm, with an age-adjusted incidence rate of 0.12 per 100,000 person-years.\(^1,2\) The rarity of AC has presented challenges in understanding disease pathogenesis and in developing clinical management guidelines for AC. Definitive treatment for early-stage AC is surgery, and cytoreductive surgery (CRS) as well as the consideration of heated intraperitoneal chemotherapy (HIPEC) may also yield long-term survival benefit for select patients. However, most patients will present with distant metastatic disease with significant tumor burden in the peritoneum, placing them at higher risk for bowel obstruction and increased morbidity and mortality. For most patients with AC, CRS and HIPEC are not feasible, and systemic chemotherapy will be provided only for palliative intent. Currently, the National Comprehensive Cancer Network guidelines recommend treatment of AC cases with systemic therapy according to colon cancer guidelines.\(^3\) This is largely because of lack of robust data for AC, and treatment regimens are extrapolated from clinical studies related to colon cancer. However, emerging evidence reveals distinct molecular features between colorectal cancer (CRC) and AC.\(^4-7\) Recent genomic profiling of AC has begun to shed light on distinct variant profiles among patients of all ages, given that GNAS (OMIM 139320) and TP53 (OMIM 191170) variations were associated with overall survival.\(^8\) However, earlier studies reported contradictory findings because GNAS variations were not associated with survival among patients with appendiceal mucinous neoplasms.\(^9\) In the absence of prognostic and predictive biomarkers and new therapeutic targets specific to AC, therapeutic advances in this malignant neoplasm remain very limited.

Given the rarity of AC, little is also known regarding risk factors and the epidemiology of this disease. Incidence rates of individuals of all ages with malignant AC have risen 232% between 2000 and 2016 in the United States.\(^10,11\) However, rates of appendectomies—where many AC cases are detected as incidental findings\(^12,13\)—remained stable during this period.\(^11\) Given that AC incidence rates also continue to rise in older and younger patients,\(^11\) these findings have raised the question of what causes underlie the rising burden of AC among patients diagnosed younger than 50 years (ie, early-onset AC). Our recent findings\(^14\) have shed light on the clinicopathologic and demographic patterns of early-onset AC, noting disparities in survival among young patients by race/ethnicity and sex. However, to our knowledge, no studies to date have compared molecular phenotypes of AC by age. Given the known molecular phenotypes unique to early-onset vs late-onset CRC,\(^15,16\) we hypothesized that distinct etiologies also underlie the growing AC burden among young patients. The purpose of this study, comprised of patients from the international clinicogenomic data-sharing consortium American Association of Cancer Research (AACR) Project Genomics Evidence Neoplasia Information Exchange (GENIE),\(^17\) was to characterize distinct putative driver variations and genes between patients diagnosed with early-onset and late-onset AC.
Methods

Data Sources and Study Population

The AACR GENIE project has generated next-generation clinical sequencing data in tumor tissues and associated pathology reports from multiple cancer centers in the United States, Canada, and Europe. This study has been granted data access through Database of Genotypes and Phenotypes (dbGap) project #24541. Somatic variation and clinical data from AC cases were downloaded from the GENIE project via Synapse (release 7). This study was exempt from institutional review board approval and informed consent because deidentified GENIE data are publicly available to the entire scientific community. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. A total of 385 pathologically confirmed AC cases with a unique patient record and matched clinical and variation data sequenced between January 1, 2011, and December 31, 2019, were included in our study.

Clinicopathologic and Demographic Features

Demographic variables examined included patient sex, age at clinical sequencing, race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic/Spanish/Latino, Asian or Pacific Islander, or other), and sequencing center. The use of age at clinical sequencing likely carries temporal proximity to age at cancer diagnosis, as the clinical workflow for next-generation sequencing in oncology is applied after diagnosis of cancer and is used for clinical management/actionability. Clinical and pathological variables examined included histological subtype (nonmucinous adenocarcinoma, mucinous adenocarcinoma, goblet cell carcinoid, and signet ring cell carcinoma) and sample type (primary tumor or metastatic site).

Somatic Cancer Gene Variations

Somatic variation data in tumor tissues have been generated using clinical-grade targeted gene panel sequencing approaches from different sequencing centers. Median sequencing depth (pooled median read depth, 500X) by sequencing center is outlined in eTable 1 in the Supplement. To ensure consistent somatic variation calling in tumor tissues and to minimize artifacts and germline events, GENIE has applied a stringent filtering pipeline to remove putative germline variants (eg, using pooled blood samples as controls, existing databases of known artifacts, and common germline variants from the 1000 Genomes Project or Exome Sequencing Project with allele frequencies >0.1%). We restricted our analysis to nonsilent variants, including missense, frameshift, nonframeshift, splicing, nonsense, and truncating variations, defined as frameshift, splicing, and nonsense variations. Nonsilent variation events (eg, bin variable) and variant frequencies were calculated based on study participants harboring at least 1 nonsilent variation, as we have previously described. A recurrent variation was defined as a nonsilent variant observed in at least 3 patients within our cohort.

Statistical Analysis

To assess clinical and demographic features between patients diagnosed with early-onset AC (age <50 years at sequencing) and late-onset AC (age ≥50 years at sequencing), features were compared by age group using χ² or Fisher exact tests for categorical variables and t tests for continuous variables. The significance levels of cooccurrence and mutual exclusivity for a pair of variant genes were calculated by the Mutual Exclusivity Modules statistical method from cBioportal. Variant comparisons by age group were evaluated using multivariable logistic regression analysis with an adjustment for patient sex, race/ethnicity, histological subtype, sequencing center, and primary sample type, in which all covariates were used as fixed effects and the reference outcome category was individuals diagnosed with late-onset AC. In addition, we performed similar analysis stratified by histological subtype. All tests were 2-sided, and P < .05 was considered.
statistically significant. All analyses were conducted using R software version 3.3.3 (R Project for Statistical Computing).

Results

A total of 385 individuals diagnosed with AC were identified from 12 international institutions within the AACR Project GENIE Consortium during the 9-year study period (Table 1). Approximately 30% of the population was diagnosed with early-onset AC (109 patients [28.3%]), and mean (SD) age at clinical sequencing was 56.0 (12.4) years. A total of 187 men (48.6%) were in the sample, and the proportion of men did not differ between early-onset vs late-onset AC cases (54 [49.5%] vs 133 [48.2%]; \( P = .81 \)). Approximately 4 of every 5 patients was a non-Hispanic White individual (306 [79.5%]). Race/ethnicity differed by age group; non-Hispanic Black patients accounted for a larger proportion of early-onset vs late-onset cases (9 of 109 [8.3%] vs 11 of 276 [4.0%]; \( P = .04 \)). By histological subtype, 177 patients (44.4%) were diagnosed with nonmucinous adenocarcinoma, 156 (40.5%) had mucinous adenocarcinoma, 32 (8.3%) had goblet cell appendiceal carcinoma, and 26 (6.8%) had signet ring cell appendiceal carcinoma (Table 1). However, histological subtype did not statistically significantly differ by age group in this cohort.

Table 1. Clinical and Demographic Characteristics of Patients With Appendiceal Cancer From the American Association of Cancer Research Project Genomics Evidence Neoplasia Information Exchange, 2011 to 2019

| Characteristic                  | No. (%) | Age at clinical sequencing, y | \( P \) value* |
|--------------------------------|---------|------------------------------|---------------|
|                                | Total (N = 385) | <50 (n = 109) | ≥50 (n = 276) |               |
| Age at clinical sequencing, y  |         |                              |               |
| <30                            | 9 (2.3)  | 9 (8.3)  | 0             |               |
| 30-39                          | 26 (6.8) | 26 (23.9) | 0             |               |
| 40-49                          | 74 (19.2)| 74 (67.9) | 0             |               |
| 50-59                          | 125 (32.5)| 0         | 125 (45.3)   | NA            |
| 60-69                          | 95 (24.7)| 0         | 95 (34.4)    |               |
| 70-79                          | 48 (12.5)| 0         | 48 (17.4)    |               |
| ≥80                            | 8 (2.1)  | 0         | 8 (2.9)      |               |
| Mean (SD)                      | 56.0 (12.4)| 41.2 (7.3)| 61.9 (8.4)  | NA            |
| Race/ethnicity                 |         |                              |               |
| Non-Hispanic                   |         |                              |               |
| White                          | 306 (79.5)| 81 (74.3) | 225 (81.5)   |               |
| Black                          | 20 (5.2) | 9 (8.3)  | 11 (4.0)     |               |
| Hispanic, Spanish, or Latino   | 13 (3.4) | 3 (2.8)  | 10 (3.6)     | .04           |
| Asian or Pacific Islander      | 9 (2.3)  | 6 (5.5)  | 3 (1.1)      |               |
| Other                          | 3 (0.8)  | 1 (0.9)  | 2 (0.7)      |               |
| Unknown                        | 34 (8.8) | 9 (8.3)  | 25 (9.1)     |               |
| Sex                            |         |                              |               |
| Women                          | 198 (51.4)| 55 (50.5)| 143 (51.8)   | .81           |
| Men                            | 187 (48.6)| 54 (49.5)| 133 (48.2)   |               |
| Histological subtype           |         |                              |               |
| Adenocarcinoma                 |         |                              |               |
| Nonmucinous                    | 171 (44.4)| 45 (41.3)| 126 (45.7)   | .31           |
| Mucinous                       | 156 (40.5)| 48 (44.0)| 108 (39.1)   |               |
| Goblet cell                    | 32 (8.3) | 6 (5.5)  | 26 (9.4)     |               |
| Signet ring cell               | 26 (6.8) | 10 (9.2) | 16 (5.8)     |               |
| Sample type                    |         |                              |               |
| Primary tumor                  | 165 (42.9)| 45 (41.3)| 120 (43.5)   |               |
| Metastasis                     | 205 (53.2)| 62 (56.9)| 143 (51.8)   | .53           |
| Unknown                        | 15 (3.9) | 2 (1.8)  | 13 (4.7)     |               |

Abbreviation: NA, not applicable.
* \( P \) value calculations did not include unknown values.
A total of 39 genes in AC had a variation frequency of greater than 2% among all patients (Figure). More than half of all ACs (198 [51.4%]) had a KRAS variation (OMIM 190070), consistent with previous reports (Figure, A).22-24 TP53 and GNAS were altered in more than one-quarter of all ACs (105 [27.3%] and 101 [26.2%], respectively) (Figure, A). Other genes commonly altered in at least 5% of AC cases included SMAD4 (OMIM 600993), APC (OMIM 61731), PIK3CA (OMIM 171834), KMT2D (OMIM 602113), SOX9 (OMIM 608160), and ATM (OMIM 607585). Patterns of significant gene cooccurrence and mutual exclusivity by age group are described in Figure, B. Among both early-onset and late-onset AC cases, GNAS and TP53 variations were mutually exclusive (P < .05) (Figure, B). Among young patients with AC, SOX9 and KRAS variations as well as SOX9 and TP53 variations were also mutually exclusive pairs (P < .05). The frequency and type of variations for the top 10 frequently altered genes among ACs in patients diagnosed with early-onset and late-onset disease are presented in Figure, C and D, respectively. In particular, GNAS and PIK3CA harbored...
distinct variation frequencies between early-onset and late-onset ACs. A total of 21 of 109 young patients (19.3%) had ACs with GNAS variations, whereas nearly one-third of late-onset cases (80 of 276 [29.0%]) had variations in GNAS (Figure, C). In contrast, nearly 1 in 8 young patients had ACs with PIK3CA variants (13 [11.9%]), while only 13 tumors (4.7%) among patients aged 50 years and older at clinical sequencing had variants in PIK3CA (Figure, D).

Baseline variation probabilities among all AC patients and by early-onset vs late-onset AC are presented in Table 2. Next, we sought to characterize somatic alterations unique to patients with early-onset vs late-onset ACs. Among all patients with AC, young patients had significantly higher odds of presenting with nonsilent PIK3CA, SMAD3, and TSC2 somatic variations in ACs compared with

| Gene symbol | Baseline variant probability| Baseline variant probability by age at clinical sequencing, y | OR (95% CI) | P value |
|-------------|-----------------------------|-------------------------------------------------------------|--------------|---------|
| KRAS        | 0.5143                      | 0.5229                                                      | 0.98 (0.58-1.66) | .94     |
| TP53        | 0.2734                      | 0.3303                                                      | 1.49 (0.87-2.55) | .15     |
| GNAS        | 0.2630                      | 0.1927                                                      | 0.40 (0.21-0.76) | .006    |
| SMAD4       | 0.1328                      | 0.1193                                                      | 0.95 (0.45-2.04) | .90     |
| APC         | 0.0805                      | 0.0734                                                      | 0.95 (0.38-2.38) | .91     |
| SDX9        | 0.0772                      | 0.0889                                                      | 1.59 (0.61-4.12) | .34     |
| PIK3CA      | 0.0649                      | 0.1193                                                      | 4.58 (1.72-12.21) | .002    |
| KMT2D       | 0.0538                      | 0.0762                                                      | 2.16 (0.71-6.54) | .17     |
| TGFB2       | 0.0524                      | 0.0330                                                      | 0.54 (0.13-2.02) | .39     |
| SMAD2       | 0.0510                      | 0.0667                                                      | 1.38 (0.47-4.07) | .56     |
| ARID1A      | 0.0510                      | 0.0381                                                      | 0.66 (0.19-2.25) | .50     |
| ATM         | 0.0469                      | 0.0734                                                      | 1.81 (0.61-5.43) | .29     |
| FAT1        | 0.0418                      | 0.0333                                                      | 0.77 (0.19-3.18) | .72     |
| RNF43       | 0.0402                      | 0.0619                                                      | 1.75 (0.50-6.13) | .38     |
| CDH1        | 0.0365                      | 0.0183                                                      | 0.41 (0.07-2.52) | .34     |
| FBXW7       | 0.0339                      | 0.0367                                                      | 1.05 (0.29-3.81) | .94     |
| NOTCH1      | 0.0286                      | 0.0275                                                      | 1.03 (0.19-5.57) | .97     |
| BRF         | 0.0286                      | 0.0183                                                      | 0.41 (0.06-2.84) | .36     |
| ARID2       | 0.0283                      | 0.0095                                                      | 0.28 (0.03-2.36) | .24     |
| SMAD3       | 0.0257                      | 0.0556                                                      | 7.37 (1.24-43.87) | .03     |
| EP300       | 0.0255                      | 0.0190                                                      | 0.55 (0.08-3.86) | .55     |
| ATRX        | 0.0254                      | 0.0286                                                      | 1.40 (0.26-7.57) | .70     |
| KDM6A       | 0.0254                      | 0.0286                                                      | 1.32 (0.27-6.33) | .73     |
| NOTCH3      | 0.0248                      | 0.0515                                                      | 4.08 (0.88-18.87) | .07     |
| PLCG2       | 0.0248                      | 0.0309                                                      | 1.13 (0.24-5.31) | .88     |
| BCR         | 0.0246                      | 0.0103                                                      | 0.24 (0.02-2.83) | .26     |
| RB1         | 0.0234                      | 0.0275                                                      | 0.75 (0.13-4.18) | .74     |
| ALK         | 0.0234                      | 0.0092                                                      | 0.21 (0.02-2.14) | .19     |
| SETD2       | 0.0227                      | 0.0476                                                      | 4.03 (0.84-19.41) | .08     |
| ASXL1       | 0.0219                      | 0.0280                                                      | 1.82 (0.32-10.33) | .50     |
| TCF7L2      | 0.0217                      | 0.0323                                                      | 1.90 (0.35-10.48) | .46     |
| MED12       | 0.0216                      | 0.0412                                                      | 3.22 (0.59-17.44) | .17     |
| Nras        | 0.0208                      | 0.0183                                                      | 1.12 (0.21-6.13) | .89     |
| TSC2        | 0.0198                      | 0.0381                                                      | 12.43 (1.03-149.59) | .047  |
| CARD11      | 0.0198                      | 0.0190                                                      | 0.98 (0.18-5.31) | .98     |
| FLT1        | 0.0198                      | 0.0095                                                      | 1.54 (0.13-18.30) | .73     |
| ERBB2       | 0.0182                      | 0.0367                                                      | 3.27 (0.55-19.45) | .19     |
| CTNNB1      | 0.0182                      | 0.0183                                                      | 1.34 (0.23-7.75) | .74     |
| AKT1        | 0.0182                      | 0.0092                                                      | 0.31 (0.03-3.53) | .34     |

Abbreviations: AC, appendiceal cancer; OR, odds ratio.

Table 2. Baseline Variation Probability and Differential Expression of Somatic Variants Between Patients With Early-Onset and Late-Onset AC

a Genes ranked by baseline probability of variation occurrence.
b ORs and 95% CIs were calculated for genes from models adjusted for patient sex, race/ethnicity, histological subtype, sequencing center, and sample type. Reference outcome category was individuals with late-onset AC.
late-onset AC cases after adjustment for sex, race/ethnicity, histological subtype, sequencing center, and sample type \( \text{PIK3CA: odds ratio [OR], 4.58; 95\% CI, 1.72-12.21; } P = 0.002; \text{SMAD3: OR, 7.37; 95\% CI, 1.24-43.87; } P = 0.03; \text{TSC2: OR, 12.43; 95\% CI, 1.03-149.59; } P = 0.047 \) (Table 2). In contrast, young AC patients had 60% decreased odds of presenting with nonsilent GNAS variations compared with late-onset cases in adjusted models \( \text{OR, 0.40; 95\% CI, 0.21-0.76; } P = 0.006 \). Moreover, we observed dominant recurrent nonsilent variations for both \text{PIK3CA} and GNAS, providing additional evidence of their putative role in appendiceal carcinogenesis (Table 2). Notably, our main findings for \text{PIK3CA} remained statistically significant after adjustment for multiple testing among highly altered (ie, >4%) genes (data not shown).

To further explore age-related somatic cancer gene variation patterns, we evaluated baseline variant probability among individuals aged younger than 50, 50 to 59, 60 to 69, and 70 years or older at clinical sequencing (eTable 2 in the Supplement). Concordant with our findings, baseline variation probabilities of \text{PIK3CA}, \text{SMAD3}, and \text{TSC2} were highest for AC patients age younger than 50 years at sequencing across all age groups. Similarly, baseline GNAS variation probability also remained lowest among early-onset AC cases. Additional comparison of somatic cancer gene variation patterns specifically among adults diagnosed with AC and younger than 50 years at clinical sequencing vs those aged 70 years or older revealed consistent findings, given that AC cases among those younger than 50 years at sequencing had 74% decreased odds of presenting with nonsilent GNAS variations \( \text{OR, 0.26; 95\% CI, 0.11-0.63; } P = 0.003 \) compared with adults aged 70 years or older (eTable 2 in the Supplement). Similarly, early-onset AC cases had significantly higher odds of presenting with nonsilent \text{PIK3CA} variations compared with those aged 70 years or older \( \text{OR = 11.69; 95\% CI, 1.37-99.82; } P = 0.02 \).

Stratification of patients by histological subtype revealed that young patients with mucinous adenocarcinomas of the appendix had 65% decreased odds of nonsilent variations in GNAS \( \text{OR, 0.35; 95\% CI, 0.15-0.79; } P = 0.01 \) compared with late-onset cases in adjusted models (eTable 3 in the Supplement). Similarly, for patients with non-mucinous appendiceal adenocarcinomas, young individuals had 72% decreased odds of presenting with GNAS variations compared with late-onset cases, although these findings were not statistically significant \( \text{OR, 0.28; 95\% CI, 0.07-1.14; } P = 0.08 \) (eTable 3 in the Supplement).

**Discussion**

The genomic landscape of 385 appendiceal neoplasms provides novel insight into molecular differences of AC by age at sequencing and identifies potential biomarkers associated with AC diagnosed at younger ages that may help unravel distinct etiologies underlying the increasing incidence of early-onset AC. Most striking are differences in the variation patterns of GNAS, \text{PIK3CA}, \text{TSC2}, and \text{SMAD3} between early-onset and late-onset AC cases. Compared with cases age 50 years and older at clinical sequencing, younger patients had higher odds of presenting with somatic variations in \text{PIK3CA}, \text{SMAD3}, and \text{TSC2}, whereas younger patients had decreased odds of presenting with somatic variations in GNAS. Differences in GNAS by age group were also noted in stratified analyses for cases diagnosed with mucinous adenocarcinomas of the appendix. Moreover, GNAS and \text{TP53} variations were mutually exclusive for ACs among patients with early-onset and late-onset disease.

Pathogenesis of AC is driven by the accumulation of genetic and epigenetic alterations, which remain largely unknown. Somatic variations of GNAS, a heterotrimeric G protein a subunit that activates adenylyl cyclase downstream of activated G protein-coupled receptors in response to hormones and a plethora of extracellular signals,\(^{25}\) have been identified in many gastrointestinal diseases, including neoplasms of the pancreas,\(^{26-29}\) and stomach\(^{30}\) as well as adenomas of the colorectum.\(^{31,32}\) However, GNAS variation patterns in ACs remain incompletely understood. To date, studies have reported conflicting evidence on the prevalence of GNAS variants by tumor histological subtype among ACs.\(^{4,9,33}\) In a 2018 study of 703 AC samples,\(^8\) GNAS variations were reported in 22%
of nonmucinous adenocarcinomas and in 49% of mucinous adenocarcinomas of the appendix. In the present study, we observed that approximately 1 in every 4 appendiceal tumors carried a GNAS variation. Among ACs diagnosed in patients with early-onset and late-onset disease, GNAS variations were also found to be mutually exclusive with TP53 variations. Moreover, we reported that younger patients with AC had 63% decreased odds of presenting with GNAS variations compared with late-onset cases, patterns that persisted among patients with mucinous adenocarcinomas of the appendix. Given that previous studies have revealed that most high-grade ACs are GNAS wild-type tumors and also that GNAS and TP53 variations tend to be mutually exclusive,7,8 these findings suggest that a subset of early-onset ACs may be more likely to occur de novo rather than progressing from low-grade tumors—emphasizing that distinct pathways may contribute to early-onset AC. Given this mutual exclusivity for GNAS and TP53 variations and reduced likelihood for young patients with AC to have somatic GNAS variations compared with late-onset cases, future studies are also warranted to examine germline TP53 variants and hereditary syndromes among young patients diagnosed with AC.

**PIK3CA** encodes the p110 catalytic subunit of phosphatidylinositol-3-kinase (PI3K), among the key kinases in PI3K/AKT and the mammalian target of rapamycin (mTOR) (PI3K/AKT/mTOR) signaling,34,35 and promotes malignant cell growth and invasion.36 PIK3CA is among the most commonly altered genes across various cancer types, including CRC and gastric tumors.37 A 2019 comparison of PIK3CA variation frequencies between appendiceal adenocarcinoma and CRC cases revealed lower variation rates in appendiceal neoplasms (6% vs 17%-22%).4 Similar to these and other findings,4,6,38,39 PIK3CA variations were reported in 6.8% of AC cases in our cohort. However, in contrast to previous results from variation frequencies between patients diagnosed with early-onset vs late-onset CRCs that did not identify differences in PIK3CA variation rates,40 here we observed distinct PIK3CA variation patterns by age group for AC, which persisted after adjustment for multiple testing among highly altered genes. Compared with late-onset AC cases, young patients had a 4.7-fold increased odds of presenting with PIK3CA variations in ACs, findings that persisted after adjustment for multiple testing. These findings provide initial insight to suggest that mechanisms of early-onset appendiceal carcinogenesis may be distinct from early-onset colorectal carcinogenesis. Moreover, as alpelisib—a PIK3CA inhibitor—became FDA-approved last year for PIK3CA-altered, hormone receptor–positive advanced breast cancer,41 this study reveals that 12% of early-onset AC cases could potentially benefit from targeting this variation and merits further study. Moreover, as studies have posited that adolescents and young adults (AYAs; age 18-39 years) harbor a distinct biology of cancer42-44; additional investigation of variation patterns within the AYA population are needed in larger cohorts.

Currently, the roles of TSC2 and SMAD3 in appendiceal carcinogenesis remain unexplored. TSC2 is a target of RAS/ERK signaling, and direct phosphorylation of tuberous sclerosis complex 2 (TSC2) by ERK leads to suppression of tumor-suppressive functions.45 A study of 63 colon carcinomas46 showed that approximately one-third of colon carcinomas were positive for phosphorylated TSC2. Moreover, reduced expression of TSC2 was also found to be associated with shorter disease-free survival among 50 patients with CRC.47 Notably, TSC2 was shown to positively regulate expression of mucin2, a marker of goblet cell differentiation in intestinal cells.48,49 TSC2 inactivation altered differentiation throughout the intestinal epithelium, with a marked decrease in goblet cell lineages.50 As goblet cell carcinoïd tumors accounted for less than 10% of cases in this cohort, we were unable to assess genomic differences of AC by age at clinical sequencing specific to this histological subtype. Nevertheless, as young patients had higher odds of presenting with TSC2 variations, these findings posit a potential role for targeting the mTOR network51 in AC therapy, particularly for young patients.

SMAD genes are key mediators of transforming growth factor β (TGF-β) signals that, on inactivation, enhance tumor growth.52,53 Previous studies have reported that SMAD3 variations are infrequent in CRCs (<5% of sporadic tumors and colorectal liver metastases).52,54-56 Consistent with these reports, we observed SMAD3 variations in fewer than 5% of AC cases. Moreover, SMAD3 variations had higher odds of occurrence in ACs of young patients, positing a potential distinct role

---

**JAMA Network Open. 2020;3(12):e2028644. doi:10.1001/jamanetworkopen.2020.28644**

---

Downloaded From: https://jamanetwork.com/ by a Non-Human Traffic (NHT) User on 09/08/2021
for SMAD3 as well as TSC2 in early-onset appendiceal carcinogenesis. Given the relatively low somatic variation frequency in TSC2 and SMAD3 in our cohort, further investigations are warranted to explore the mechanistic role of these genes and related pathways, particularly in early-onset AC.

Strengths and Limitations
The use of data from the GENIE clinicogenomic data-sharing consortium is a strength of this study because it allowed for pathologically verified cases with clinical-grade sequencing data to be identified from 12 institutions worldwide. However, we also acknowledge that our study has limitations. Our analyses were conducted using GENIE data from a large number of patients with AC; however, GENIE does not record information about cancer stage, metastasis sites, pseudomyxoma peritonei, or tumor grade (eg, low-grade appendiceal mucinous neoplasms). As such, we were unable to assess for differences in these tumor characteristics by age at clinical sequencing or to investigate whether these differences were associated with distinct genomic patterns of early-onset AC. Similar to previous studies, specimens submitted for sequencing in GENIE derived from primary ACs and metastatic sites. Given that half of all tumors in this study derived from metastases—with similar proportions for early-onset and late-onset AC cases—these findings are indicative that most patients in this study had stage IV disease. However, primary AC tissue may have been sequenced in cases that presented with metastatic disease, which does not allow us to rule out that the molecular patterns reported in this study may be in part related to AC stage. In addition, because all somatic variations were not systematically evaluated within GENIE, the true prevalence of somatic variations in our cohort may be even higher. Risk of potential bias also exists in our study due to overfitting variations that occur with a small probability. GENIE also lacks detailed information regarding individual-level characteristics, including family history of cancer, and does not provide any data about germline genetic features, cancer treatments, or prognostic outcomes for patients with AC. Importantly, GENIE does not collect information on patient age at cancer diagnosis. Given that the date of clinical sequencing is likely to have occurred after the date of AC diagnosis, early-onset AC patients in our study were assigned to the early-onset group. However, a few patients with AC may have been misclassified into the late-onset AC group, or patients may not have undergone clinical sequencing until disease relapse. Notwithstanding this limitation, findings from our additional comparison of somatic cancer gene variation patterns specifically among adults diagnosed with early-onset AC vs those aged 70 years or older were consistent findings and further support our study results.

Conclusions
To our knowledge, this international consortium study is the first to examine molecular features of AC by age. This study found a distinct spectrum of somatic variations among early-onset AC cases, as younger patients had higher odds of presenting with PIK3CA, SMAD3, and TSC2 somatic variations and decreased odds of presenting with GNAS variations compared with late-onset AC cases. These findings demonstrate that ACs identified among young individuals harbor a distinct molecular phenotype compared with late-onset ACs and yield clinical actionability in future studies that should aim to elucidate distinct molecular phenotypes and mechanisms of early-onset AC and to develop and test personalized therapeutic modalities tailored to young patients diagnosed with AC.

ARTICLE INFORMATION
Accepted for Publication: October 13, 2020.
Published: December 9, 2020. doi:10.1001/jamanetworkopen.2020.28644
Correction: This article was corrected on March 26, 2021, to fix the incorrect reporting of the age variable collected by the American Association for Cancer Research (AACR) Project Genomics Evidence Neoplasia Information Exchange (GENIE).

Open Access: This is an open access article distributed under the terms of the CC-BY License. © 2020 Holowatyj AN et al. JAMA Network Open.

Corresponding Author: Andreana N. Holowatyj, PhD, MS (andreana.holowatyj@vumc.org), and Xingyi Guo, PhD (xingyi.guo@vumc.org), Department of Medicine, Vanderbilt University Medical Center, Ste 334-G (Dr Holowatyj), Ste 330 (Dr Guo), Nashville, TN 37203.

Author Affiliations: Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee (Holowatyj, Eng, Wen, Guo); Vanderbilt-Ingram Cancer Center, Nashville, Tennessee (Holowatyj, Idrees, Guo); Department of Surgery, Vanderbilt University Medical Center, Nashville, Tennessee (Idrees).

Author Contributions: Drs Holowatyj and Guo had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Holowatyj, Guo.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Holowatyj, Idrees, Guo.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Holowatyj, Wen, Guo.

Obtained funding: Holowatyj.

Administrative, technical, or material support: Holowatyj.

Supervision: Holowatyj, Eng, Guo.

Conflict of Interest Disclosures: Dr Wen reported receiving grants from the National Institutes of Health during the conduct of the study. No other disclosures were reported.

Funding/Support: This study was supported by the Vanderbilt University Medical Center (Drs Holowatyj and Guo). Dr Holowatyj was also supported by the grant K12 HD043483 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development. This work was also supported by grant R37 CA227130 from the National Cancer Institute to Dr Guo.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We would like to acknowledge the AACR and its financial and material support in the development of the AACR Project GENIE registry as well as members of the consortium for their commitment to data sharing.

REFERENCES
1. McCusker ME, Coté TR, Clegg LX, Sobin LH. Primary malignant neoplasms of the appendix: a population-based study from the surveillance, epidemiology and end-results program, 1973-1998. Cancer. 2002;94(12):3307-3312. doi:10.1002/cncr.10589
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30. doi: 10.3322/caac.21590
3. National Comprehensive Cancer Network. Colon cancer (version 3.2020). Accessed June 4, 2020. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf
4. Tokunaga R, Xiu J, Johnston C, et al. Molecular profiling of appendiceal adenocarcinoma and comparison with right-sided and left-sided colorectal cancer. Clin Cancer Res. 2019;25(10):3096-3103. doi:10.1158/1078-0432.CCR-18-3388
5. Johncilla M, Stachler M, Misdraji J, et al. Mutational landscape of goblet cell carcinoids and adenocarcinoma ex goblet cell carcinoids of the appendix is distinct from typical carcinoids and colorectal adenocarcinomas. Mod Pathol. 2018;31(6):989-996. doi:10.1038/s41379-018-0003-0
6. Borazanci E, Millis SZ, Kimbrough J, Doll N, Von Hoff D, Ramanathan RK. Potential actionable targets in appendiceal cancer detected by immunohistochemistry, fluorescent in situ hybridization, and mutational analysis. J Gastrointest Oncol. 2017;8(1):164-172. doi:10.21037/jgo.2017.01.14
7. Alakus H, Babicky ML, Ghosh P, et al. Genome-wide mutational landscape of mucinous carcinomatosis peritonei of appendiceal origin. Genome Med. 2014;6(9):43. doi:10.1186/gm559
8. Ang CS-P, Shen JP, Hardy-Abeloos CJ, et al. Genomic landscape of appendiceal neoplasms. JCO Precis Oncol. 2018;2(2):1-18.
9. Singh AD, Davison JM, Choudry HA, et al. GNAS is frequently mutated in both low-grade and high-grade disseminated appendiceal mucinous neoplasms but does not affect survival. Hum Pathol. 2014;45(8):1737-1743. doi:10.1016/j.humpath.2014.04.018
10. Marmor S, Portschy PR, Tuttle TM, Virnig BA. The rise in appendiceal cancer incidence: 2000-2009. J Gastrointest Surg. 2015;19(4):743-750. doi:10.1007/s11605-014-2726-7
11. Singh H, Koomson AS, Decker KM, Park J, Demers AA. Continued increasing incidence of malignant appendiceal tumors in Canada and the United States: a population-based study. Cancer. 2020;126(10):2206-2216. doi:10.1002/cncr.32793
12. Steiner CA, Karaca Z, Moore BJ, Imshaug MC, Pickens G. Surgeries in hospital-based ambulatory surgery and hospital inpatient settings, 2014: statistical brief #223. In: Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Agency for Healthcare Research and Quality; 2017.
13. Fingar KR, Stocks C, Weiss AJ, Steiner CA. Most frequent operating room procedures performed in U.S. hospitals, 2003-2012: statistical brief #186. Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Agency for Healthcare Research and Quality; 2014.
14. Holowatyj AN, Washington KM, Salaria SN, Lieu CH, Idrees K, Eng C. Early-onset appendiceal cancer survival by race or ethnicity in the United States. Gastroenterology. 2020;159(4):1605-1608. doi:10.1053/j.gastro.2020.06.011
15. Archambault AN, Su YR, Jeon J, et al. Cumulative burden of colorectal cancer-associated genetic variants is more strongly associated with early-onset vs late-onset cancer. Gastroenterology. 2020;158(5):1274-1286.e12. doi:10.1053/j.gastro.2019.11.012
16. Holowatyj AN, Gigic B, Herpel E, Scalbert A, Schneider M, Ulrich CM; MetaboCCC Consortium; ColoCare Study. Distinct molecular phenotype of sporadic colorectal cancers among young patients based on multiomics analysis. Gastroenterology. 2020;158(4):1155-1158.e2. doi:10.1053/j.gastro.2019.11.012
17. AACR Project GENIE Consortium. AACR project GENIE: powering precision medicine through an international consortium. Cancer Discov. 2017;7(8):818-831. doi:10.1158/2159-8290.CD-17-0151
18. GENIE. What is AACR Project Genie. Accessed November 12, 2020. https://www.synapse.org/#!Synapse:syn7222066/wiki/405659
19. Gagan J, Van Allen EM. Next-generation sequencing to guide cancer therapy. Genome Med. 2015;7:80. doi:10.1186/s13073-015-0203-x
20. Chen Z, Wen W, Beeghly-Fadiel A, et al. Identifying putative susceptibility genes and evaluating their associations with somatic mutations in human cancers. Am J Hum Genet. 2019;105(3):477-492. doi:10.1016/j.ajhg.2019.07.006
21. cBioPortal for Cancer Genomics. OncoPrinter. Accessed November 6, 2020. https://www.cbioportal.org/oncoprinter
22. Zauber P, Berman E, Marotta S, Sabbath-Solitare M, Bishop T. K-ras gene mutations are invariably present in low-grade mucinous tumors of the vermiform appendix. Scand J Gastroenterol. 2011;46(7-8):869-874. doi:10.3109/00365521.2011.565070
23. Liao X, Vavinskaya V, Sun K, et al. Mutation profile of high-grade appendiceal mucinous neoplasm. Histopathology. 2020;76(3):461-469. doi:10.1111/his.13986
24. Pai RK, Hartman DJ, Gonzalo DH, et al. Serrated lesions of the appendix frequently harbor KRAS mutations and not BRAF mutations indicating a distinctly different serrated neoplastic pathway in the appendix. Hum Pathol. 2014;45(2):227-235. doi:10.1016/j.humpath.2013.10.021
25. O’Hayre M, Vázquez-Prado J, Kufareva I, et al. The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. Nat Rev Cancer. 2013;13(6):412-424. doi:10.1038/nrc3521
26. Molin MD, Matthaei H, Wu J, et al. Clinicopathological correlates of activating GNAS mutations in intraductal papillary mucinous neoplasm (IPMN) of the pancreas. Ann Surg Oncol. 2013;20(12):3802-3808. doi:10.1245/s10434-013-3096-1
27. Wu J, Matthaei H, Maitra A, et al. Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development. Sci Transl Med. 2011;3(92):92ra66. doi:10.1126/scitranslmed.3002543
28. Komatsu H, Tanji E, Sakata N, et al. A GNAS mutation found in pancreatic intraductal papillary mucinous neoplasms induces drastic alterations of gene expression profiles with upregulation of mucin genes. PLoS One. 2014;9(2):e87875. doi:10.1371/journal.pone.0087875
29. Furukawa T, Kuboki Y, Tanji E, et al. Whole-exome sequencing uncovers frequent GNAS mutations in intraductal papillary mucinous neoplasms of the pancreas. Sci Rep. 2011;1:161. doi:10.1038/srep00161

30. Nomura R, Saito T, Mitomi H, et al. GNAS mutation as an alternative mechanism of activation of the Wnt/β-catenin signaling pathway in gastric adenocarcinoma of the fundic gland type. Hum Pathol. 2014;45(12):2488-2496. doi:10.1016/j.humpath.2014.08.016

31. Yamada M, Sekine S, Ogawa R, et al. Frequent activating GNAS mutations in villous adenoma of the colorectum. J Pathol. 2012;228(1):113-118. doi:10.1002/path.4012

32. Liu C, McKeone DM, Walker NI, Bettington ML, Leggett BA, Whitehall VLJ. GNAS mutations are present in colorectal traditional serrated adenomas, serrated tubulovillous adenomas and serrated adenocarcinomas with adverse prognostic features. Histopathology. 2017;70(7):1079-1088. doi:10.1111/his.13180

33. Nishikawa G, Sekine S, Ogawa R, et al. Frequent GNAS mutations in low-grade appendiceal mucinous neoplasms. Br J Cancer. 2013;108(4):951-958. doi:10.1038/bjc.2013.47

34. Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006;7(8):606-619. doi:10.1038/nrg1879

35. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129(7):1261-1274. doi:10.1016/j.cell.2007.06.009

36. Samuels Y, Díaz LA Jr, Schmidt-Kittler O, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. Cancer Cell. 2005;7(6):561-573. doi:10.1016/j.ccr.2005.05.014

37. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304(5670):554. doi:10.1126/science.1096502

38. Liu X, Mody K, de Abreu FB, et al. Molecular profiling of appendiceal epithelial tumors using massively parallel sequencing to identify somatic mutations. Clin Chem. 2014;60(7):1004-1011. doi:10.1373/clinchem.2014.225565

39. Zhu X, Salhab M, Tomaszewicz K, et al. Heterogeneous mutational profile and prognosis conferred by TP53 mutations in appendiceal mucinous neoplasms. Hum Pathol. 2019;85:260-269. doi:10.1016/j.humpath.2018.11.011

40. Kohari N, Teer JK, Abbott AM, et al. Increased incidence of FBXW7 and POLE proofreading domain mutations in young adult colorectal cancers. Cancer. 2016;122(18):2828-2835. doi:10.1002/cncr.30082

41. André F, Ciriules E, Rubovszky G, et al; SOLAR-1 Study Group. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380(20):1929-1940. doi:10.1056/NEJMoa1813904

42. Holowatyj AN, Lewis MA, Pannier ST, et al. Clinicopathologic and racial/ethnic differences of colorectal cancer among adolescents and young adults. Clin Transl Gastroenterol. 2019;10(7):e00059. doi:10.14309/ctg.0000000000000059

43. Holowatyj AN, Viskochil R, Ose D, et al. Diabetes, body fatness, and insulin prescription among adolescents and young adults with cancer. J Adolesc Young Adult Oncol. 2020. doi:10.1089/jyao.2020.0071

44. Bleyer A, Barr R, Hayes-Lattin B, Thomas D, Ellis C, Anderson B; Biology and Clinical Trials Subgroups of the US National Cancer Institute Progress Review Group in Adolescent and Young Adult Oncology. The distinctive biology of cancer in adolescents and young adults. Nat Rev Cancer. 2008;8(4):288-298. doi:10.1038/nrc2349

45. Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP. Proofreading and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell. 2005;121(2):179-193. doi:10.1016/j.cell.2005.02.031

46. Ma L, Teruya-Feldstein J, Bonner P, et al. Identification of S664 TSC2 phosphorylation as a marker for extracellular signal-regulated kinase mediated mTOR activation in tuberous sclerosis and human cancer. Cancer Res. 2007;67(15):7106-7112. doi:10.1158/0008-5472.CAN-06-4798

47. Vendrell E, Ribas M, Valls J, et al. Genomic and transcriptomic prognostic factors in R0 Dukes B and C colorectal cancer patients. Int J Oncol. 2007;30(5):1099-1107. doi:10.3892/ijo.30.5.1099

48. Zhou Y, Wang Q, Weiss HL, Evers BM. Nuclear factor of activated T-cells 5 increases intestinal goblet cell differentiation through an mTOR/Notch signaling pathway. Mol Biol Cell. 2014;25(18):2882-2890. doi:10.1091/mbc.e14-05-0998

49. Zhou Y, Wang Q, Guo Z, Weiss HL, Evers BM. Nuclear factor of activated T-cell c3 inhibition of mammalian target of rapamycin signaling through induction of regulated in development and DNA damage response 1 in human intestinal cells. Mol Biol Cell. 2012;23(15):2963-2972. doi:10.1091/mbc.e12-01-0037

50. Zhou Y, Rychahou P, Wang Q, Weiss HL, Evers BM. TSC2/mTORC1 signaling controls Paneth and goblet cell differentiation in the intestinal epithelium. Cell Death Dis. 2015;6(2):e1631. doi:10.1038/cddis.2014.588

51. Wang XW, Zhang YJ. Targeting mTOR network in colorectal cancer therapy. World J Gastroenterol. 2014;20(15):4178-4188. doi:10.3748/wjg.v20.i15.4178
52. Riggins GJ, Kinzler KW, Vogelstein B, Thiagalingam S. Frequency of SMAD gene mutations in human cancers. Cancer Res. 1997;57(13):2578-2580.

53. Nakao A, Imamura T, Souchelnytskyi S, et al. TGF-beta receptor-mediated signalling through SMAD2, SMAD3 and SMAD4. EMBO J. 1997;16(17):5353-5362. doi:10.1093/emboj/16.17.5353

54. Fleming NI, Jorissen RN, Mouradov D, et al. SMAD2, SMAD3 and SMAD4 mutations in colorectal cancer. Cancer Res. 2013;73(2):725-735. doi:10.1158/0008-5472.CAN-12-2706

55. Lang H, Baumgart J, Heinrich S, et al. Extended molecular profiling improves stratification and prediction of survival after resection of colorectal liver metastases. Ann Surg. 2019;270(5):799-805. doi:10.1097/SLA.0000000000003527

56. Arai T, Akiyama Y, Okabe S, Ando M, Endo M, Yuasa Y. Genomic structure of the human SMAD3 gene and its infrequent alterations in colorectal cancers. Cancer Lett. 1998;122(1-2):157-163. doi:10.1016/S0304-3835(97)00384-4

57. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49(12):1373-1379. doi:10.1016/S0895-4356(96)00236-3

58. Concato J, Feinstein AR, Holford TR. The risk of determining risk with multivariable models. Ann Intern Med. 1993;118(3):201-210. doi:10.7326/0003-4819-118-3-199302010-00009

SUPPLEMENT.

eTable 1. Median Read Depth for Clinical-Grade Targeted Sequencing Data From Tumor Tissues and AC Case Counts by Sequencing Center
eTable 2. Baseline Variation Probability and Differential Expression of Somatic Cancer Gene Variations by Age at Clinical Sequencing (<50 vs ≥70 Years) Among Adults Diagnosed With Appendiceal Cancers
eTable 3. Differential Expression of Nonsilent GNAS Variations Between Early-Onset (Age <50 Years) and Late-Onset (age ≥50 Years) Cases Diagnosed With Mucinous and Nonmucinous Adenocarcinomas of the Appendix