Gene Expression Changes Accompanying the Duodenal Adenoma-Carcinoma Sequence in Familial Adenomatous Polyposis

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OBJECTIVES: Duodenal cancer in familial adenomatous polyposis (FAP) arises from adenomas. Differentially expressed genes (DEGs) in the duodenal adenoma-carcinoma pathway have been identified in murine FAP models, but similar data in patients with FAP are limited. Identifying such changes may have significance in understanding duodenal polyposis therapies and identifying cancer biomarkers. We performed a genome-wide transcriptional analysis to describe the duodenal adenoma-carcinoma sequence and determine changes distinguishing patients with FAP with and without duodenal cancer.

METHODS: Transcriptional profiling was performed with the Affymetrix Human Transcriptome Array 2.0 on duodenal biopsies from 12 FAP patients with duodenal cancer (FAP cases) and 12 FAP patients without cancer (FAP controls). DEGs were compared between cancer-normal, adenoma-normal, and cancer-adenoma in FAP cases and between adenomas from FAP cases and FAP controls. Significant results at $P < 0.05$ were filtered using fold change $> 2$.

RESULTS: Two hundred twenty-four DEGs were identified at an absolute fold change $> 2$. In adenoma-normal, downregulation of DEGs involved in metabolism of brush border proteins (LCT), lipids (APOB/A4), reactive oxygen species (GSTA2), and retinol (RBP2) was observed. In the cancer-adenoma comparison, upregulation of DEGs involved in cell invasion/migration (POSTN, SPP1) and downregulation of DEGs involved in Paneth differentiation (DEF5A5/6) were observed. In the adenoma-adenoma comparison, downregulation of several DEGs (CLCA1, ADH1C, ANXA10) in FAP case adenomas was observed. DEGs with therapeutic potential include SPP1, which is involved in both cyclooxygenase and epidermal growth factor receptor pathways targeted by the sulindac/erlotinib combination for duodenal polyposis.

DISCUSSION: We describe DEGs in the human duodenal adenoma-carcinoma sequence in FAP, which may have prognostic and therapeutic significance. Validation studies are needed to confirm these findings.

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A51

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant condition caused by loss-of-function in the adenomatous polyposis coli (APC) gene. The APC gene product inhibits Wnt/β-catenin signaling (1). In FAP, loss-of-function of APC results in promotion of β-catenin’s tumorigenic effects and development of hundreds to thousands of intestinal adenomas. Resulting colorectal carcinoma (CRC) is nearly inevitable without early surgical intervention (2).

Duodenal cancer arises from duodenal adenomas and is a leading cause of death in FAP (3). Although the lifetime risk of duodenal polyposis in FAP approaches 100%, the cumulative
incidence of cancer is 4.5% by the age of 57. Chemoprevention with the cyclooxygenase 2 (COX-2) inhibitor celecoxib and with a combination of the nonselective COX inhibitor sulindac and the epidermal growth factor receptor (EGFR) inhibitor erlotinib have shown promise in decreasing polyp burden although long-term effect on cancer risk is unknown. Prophylactic duodenectomy is most effective at preventing cancer but is associated with significant morbidity and mortality.

The Spigelman stage (SS) of duodenal polyposis (I-IV) is the only known tool to determine duodenal cancer risk and is used to guide endoscopic surveillance and need for prophylactic duodenectomy in FAP. Despite the prognostic value of SS, up to 40% of FAP patients with duodenal cancer do not have advanced SS polyposis and develop cancer while under surveillance. Therefore, it is clear that additional predictive factors must be identified.

Molecular characteristics of duodenal adenomas may aid in determining duodenal cancer risk. This is supported by gene expression studies on APCMin/+ mice, which, like patients with FAP, have a germline APC mutation but predominantly develop small intestinal polyposis. In these mice, normal intestine, adenoma, and carcinoma are distinguished by differentially expressed genes (DEGs) suggesting that transcriptional changes herald malignant change of duodenal polyps in FAP. A recent study investigated gene expression changes between normal and adenomatous duodenal tissue in patients with FAP and found abnormalities in the Wnt/β-catenin, EGFR, and prostaglandin E2 (PGE2) pathways. However, no genome-wide investigation investigating the adenoma-carcinoma sequence in patients with FAP has been published. As a result, predictive and therapeutic targets to prevent duodenal cancer are largely unknown.

In this study, we first characterized the duodenal adenoma-carcinoma sequence in FAP by performing gene expression profiling on normal duodenum, adenoma, and cancer tissue from FAP patients with duodenal cancer (FAP cases). Next, we determined DEGs differentiating patients with duodenal cancer by comparing transcriptional profiles of adenomas from FAP cases with adenomas from FAP patients without cancer (FAP controls). Our ultimate objective was to uncover potential biomarkers for progression and therapeutic targets.

**METHODS**

**Patient selection**

Using the David G. Jagelman Inherited Colorectal Cancer Registry’s Institutional Review Board-approved Cologne database and the Cleveland Clinic Anatomic Pathology database, we identified FAP patients with duodenal polyposis. Clinical and endoscopic characteristics were obtained from electronic and paper medical records. Pathology specimens were obtained from Anatomic Pathology archives.

We identified 12 FAP patients with duodenal cancer (FAP cases) between 1988 and 2013 and 269 FAP patients with duodenal polyposis without cancer (FAP controls) undergoing upper endoscopic surveillance between 2005 and 2013. From this pool of FAP controls, we randomly selected 12 patients with similar age characteristics (mean, median, range) as our FAP cases (Figure 1). Clinical characteristics from FAP cases and FAP controls were collected, including age, gender, race, and sulindac or celecoxib use at the time of surveillance. Endoscopic characteristics were also collected, including polyp number (0–5, 6–20 or >20), size (0–5, 6–10 or >10 mm), histology (tubulous, tubulovillous, or villous) and dysplasia (low-grade or high-grade dysplasia) in the duodenum. Polyp histology and dysplasia information were taken from adenoma specimens obtained from FAP cases and FAP controls.

**Gene expression profiling**

Upper endoscopic surveillance of patients with FAP was performed with a systematic approach. In each endoscopy, a forward-viewing and, when needed to view the papilla, side-viewing endoscope was used. Biopsies were performed on representative duodenal polyps and the papilla and specimens were preserved as formalin-fixed paraffin-embedded (FFPE) or Hollande’s fixed samples. RNA extraction was performed with the Qiagen RNA precipitation method.
samples were retained in the data set. Transcription results have
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drolysis probes, Hs009590101_m1 (1-Step Master Mixtures Kit with primers and monocolor hy-
d incubated with the HTA to allow hybridization of cDNA fragments
to array oligonucleotides. Following hybridization, arrays under-
ried automated washing and fluorescent staining before collection of fluorescent signal intensities. At each step in this process, the same amount of RNA/cDNA from each sample was
used to reduce batch-to-batch effects.

After raw data collection, each image file was visually inspected; no crude blemishes or grid misalignment was ob-
served. Affymetrix proprietary algorithms featuring robust multi-
chip analysis normalization was applied to all samples during data spreadsheet generation. A custom report monitoring 14
different QC metrics was generated using Affymetrix Expression Console. Principal among these was area under the curve (AUC),
which indicated ease with which signal may be distinguished
from background noise. AUC values range from 0 (imperfect) to 1
(perfect) and Affymetrix recommends values greater than 0.8. All
48 samples exceeded this metric; AUC values ranged from 0.899
to 0.979. Further corroboration of quality was indicated by con-
sistency of other QC parameters, including perfect match mean and background mean. There were no outlier values, indicating no significant batch-to-batch discrepancy. Therefore, all 48 samples were retained in the data set. Transcription results have been deposited to NCBI GEO submission #GSE111156.

Verification by quantitative PCR
qPCR was performed in triplicate using a TaqMan RNA-to-CT
1-Step Master Mixtures Kit with primers and monocolor hy-
drosis probes, Hs009590101_m1 (SPP1), Hs00356112_m1 (SI), Hs00166361_m1 (APOA4), Hs00944023_M1 (CEA-
MS5), Hs01105012_m1 (ANXA10), and Hs00187842_m1 (B2M) (Applied Biosystems, Foster City, CA). qPCR was performed on an ABI PRISM 7500 real-time PCR system, according to the manufacturer’s instructions. For all genes, qPCR cycling conditions were 48 °C for 15 minutes, 95 °C for 10 minutes, 50 cycles of 95 °C for 15 seconds, 60 °C for 1 minute, and 37 °C for 1 minute. PCR products were subjected to electrophoresis on an agarose gel to confirm absence of nonspecific PCR products. For each sample, the crossing threshold point (Ct) for the amplification curves was determined by the second derivative maximum method. Ab-
solute quantitation was performed with an in-run standard curve. The reference gene Beta-2 microglobulin (B2M) was
l used for separation of control populations and all results were
ormalized against calibrator RNA from MCF7. ΔCT was defined as Ct (candidate gene) − Ct (B2M). ΔΔCT was defined as ΔCT (candidate gene in sample RNA) − ΔCT (candidate gene in calibrator RNA). Relative value, or power, for each candidate gene in each sample was calculated as 2−ΔΔCT.

Statistical analysis
Analysis of transcriptional data was performed using the Affy-
metrix Expression Console Software package (version 1.3), R
(version 3.2.0), and SAS (version 9.4). Raw data were processed using the Expression Console and further normalized with a cy-
clic loess approach. In comparisons, results for DEGs were
expressed as either a positive fold change (FC), indicating upre-
gulation or negative FC, indicating downregulation in the more
advanced sample compared to the less advanced sample.

Within each FAP case, we performed comparisons between
cancer and normal tissue (cancer-normal), between cancer and
adenoma tissue (cancer-adenoma), and between adenoma and
normal tissue (adenoma-normal) (Figure 1). For each pairwise
comparison, we tested for significant differences at P < 0.05 using a nonparametric paired Wilcoxon test. To control for potential false positive results, we filtered pairwise results using a false discovery rate (FDR) < 0.10 and an absolute FC > 2 criteria. We then performed an unpaired comparison between adenosas from FAP cases and adenosas from FAP controls (adenoma-
adenoma). We tested for significant differences at P < 0.05 using a nonparametric unpaired Wilcoxon test and filtered results using a FDR < 0.10 and an absolute FC > 2 criteria. Among DEGs in each comparison, we chose “representative” DEGs, which we
defined as genes that have previously been implicated in FAP
studies in mice or humans or in the development of other spor-
adical intestinal cancers.

Candidate DEGs were validated by quantitative polymerase chain reaction (PCR). For each comparisons, we tested for significant differences at P < 0.05 using nonparametric Wilcoxon tests.

RESULTS
Sample characteristics
The median age of FAP cases and FAP controls was 48.5 years
(range 34–70 years). Clinical and endoscopic characteristics in
FAP cases and FAP controls are described in Tables 1 and 2. Of 12
FAP cases, 4 had ampullary and 8 had nonampullary cancer. FAP
cases and FAP controls did not differ with regard to age, gender,
sale, sulindac/celecoxib use nor did they differ with regard to polyp
type, size, histology, or dysplasia (Table 2).

Overview of DEGs
One hundred seventy-eight DEGs were identified with a FDR <
0.10 and an absolute FC > 2 in at least one comparison. Sup-
plemental Table 1 (see Supplementary Digital Content 1, http://
links.lww.com/CTG/A51) lists DEGs in each comparison and Supplemental Table 2 (see Supplementary
digital Content 1, http://links.lww.com/CTG/A51) lists DEGs in
each comparison. Protein-coding DEGs were classified into one
of 9 groups according to cellular function/pathway of gene
products. Table 3 shows representative DEGs within each group
and FC in each comparison. Hierarchical clustering of DEGs in
each comparison is shown in Figure 2.

Transition from normal duodenal to adenoma in FAP
In the adenoma-normal comparison, 19 protein-coding DEGs were identified. Neoplastic processes involving 8 representative
DEGs are shown in Table 4.

Enterocyte dedifferentiation
Enterocyte dedifferentiation can be determined by examining
expression along the crypt-villus axis and in the Caco-2 cell line,
which spontaneously differentiates into mature small intestine (17). In adenoma-normal, we found downregulation of DEGs involved in brush-border metabolism. Among these, expression of LCT (18) and TMPRSS15 (19) increases during the crypt-villus axis, while expression of LCT (20) increases with Caco-2 differentiation. We observed downregulation of APOA4 and APOB, which encode apolipoproteins whose expression increases during Caco-2 cell differentiation (21). The downregulation of these brush border and lipid metabolism DEGs indicates enterocyte dedifferentiation. In adenoma-normal, we found upregulation of SLC12A2, which encodes the basolateral ion transporter NKCC1. NKCC1 expression decreases in the crypt-villus axis (22), suggesting that its upregulation further implicates enterocyte dedifferentiation.

**Warburg effect**

During the transition from normal to adenoma, certain DEGs implicate the Warburg effect, in which proliferating tumor cells prefer glycolysis over gluconeogenesis and aerobic respiration. We observed downregulation of APOA4 and APOB, which encode apolipoproteins whose expression increases during Caco-2 cell differentiation (21). The downregulation of these brush border and lipid metabolism DEGs indicates enterocyte dedifferentiation. In adenoma-normal, we found upregulation of SLC12A2, which encodes the basolateral ion transporter NKCC1. NKCC1 expression decreases in the crypt-villus axis (22), suggesting that its upregulation further implicates enterocyte dedifferentiation.

**Decreased production of all-trans retinoic acid**

In adenoma-normal, RBP2 was downregulated. In small intestine, the retinol binding protein 2 (RBP2) mediates Vitamin A (retinol) uptake. Retinol is oxidized to all-trans-retinaldehyde by alcohol dehydrogenase and then to all-trans retinoic acid (ATRA) by aldehyde dehydrogenase (23). ATRA suppresses tumorigenesis in part by blocking induction of COX-2 (24). Therefore, downregulation of RBP2 indicates that decreased ATRA production may play a role in the transition of normal duodenum to adenoma. Of note, decreased ATRA production is implicated in

### Table 1. Pathology of duodenal specimens from FAP cases and FAP controls

| Cancer patient (CP#) | Cancer location | Adenoma histology + degree of dysplasia | Most recent SS | Noncancer patient (NCP#) | Adenoma histology + degree of dysplasia | Most recent SS |
|----------------------|-----------------|--------------------------------------|---------------|-------------------------|--------------------------------------|---------------|
| CP1                  | Duodenum        | TA + LGD                             | III           | NCP1                    | TA + LGD                             | II            |
| CP2                  | Ampullary       | TA + LGD                             | III           | NCP2                    | TVA + LGD                            | III           |
| CP3                  | Ampullary       | TA + LGD                             | 0            | NCP3                    | TA + LGD                             | II            |
| CP4                  | Duodenum        | VA + LGD                             | III           | NCP4                    | TVA + LGD                            | III           |
| CP5                  | Ampullary       | TVA + HGD                            | I             | NCP5                    | TA + LGD                             | I             |
| CP6                  | Duodenum        | TVA + HGD                            | 0            | NCP6                    | TA + LGD                             | II            |
| CP7                  | Duodenum        | VA + HGD                             | IV            | NCP7                    | TA + LGD                             | II            |
| CP8                  | Duodenum        | TA + LGD                             | I             | NCP8                    | TVA + LGD                            | II            |
| CP9                  | Duodenum        | TA + LGD                             | IV            | NCP9                    | TA + LGD                             | III           |
| CP10                 | Duodenum        | TVA + HGD                            | IV            | NCP10                   | TVA + LGD                            | IV            |
| CP11                 | Duodenum        | TA + LGD                             | IV            | NCP11                   | TA + LGD                             | III           |
| CP12                 | Duodenum        | TA + LGD                             | NA           | NCP12                   | TVA + LGD                            | III           |

CP, cancer patient; FAP, familial adenomatous polyposis; HG, high-grade dysplasia; LD, low-grade dysplasia; NCP, noncancer patient; SS, Spigelman stage; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma.

*Only had one upper endoscopy, in which ampullary cancer with no other duodenal polyposis was diagnosed.

*No upper endoscopy reports available.

### Table 2. Clinical and endoscopic characteristics of FAP cases and FAP controls

|                         | FAP cases (n = 12) | FAP controls (n = 12) | P   |
|-------------------------|--------------------|-----------------------|-----|
| Age                     | 48.9 ± 11.4        | 49.7 ± 11.7           | 0.875|
| Male gender             | 7 (58%)            | 4 (33%)               | 0.414|
| White                   | 11 (92%)           | 11 (92%)              | 1    |
| Sulindac/celecoxib use  | 3 (25%)            | 5 (42%)               | 0.667|
| Polyp histology         | 0.68               |                       |      |
| TA                      | 6 (50%)            | 7 (58%)               |      |
| TVA                     | 4 (33%)            | 5 (42%)               |      |
| VA                      | 2 (17%)            | 0 (0%)                |      |
| Polyp dysplasia         | 0.093              |                       |      |
| LGD                     | 8 (67%)            | 12 (100%)             |      |
| HGD                     | 4 (33%)            | 0 (0%)                |      |
| Polyp number            | 0.648              |                       |      |
| 0–5                     | 4 (36%)            | 5 (42%)               |      |
| 6–20                    | 1 (9%)             | 3 (25%)               |      |
| >20                     | 6 (55%)            | 4 (33%)               |      |
| Polyp size (mm)         | 0.358              |                       |      |
| 0–5                     | 2 (18%)            | 4 (33%)               |      |
| 6–10                    | 2 (18%)            | 4 (33%)               |      |
| >10                     | 7 (64%)            | 4 (33%)               |      |

FAP, familial adenomatous polyposis; HG, high-grade dysplasia; LD, low-grade dysplasia; TA, tubular adenoma; TVA, tubulovillous adenoma; VA, villous adenoma.

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APC<sup>Min</sup>+/mice adenomas, which show downregulation of Adh1 (14).

**Impaired reactive oxygen species/carcinogen defense**
In adenoma-normal, we found downregulation of GSTA1 and GSTA2, which encode members of the α class of glutathione-S-transferase enzymes. These enzymes have glutathione peroxidase activity, which protects cells from reactive oxygen species (ROS). GSTA1 downregulation is seen in normal duodenum from patients with FAP compared to non-FAP controls (25). Furthermore, downregulation of Gsta4 is seen in intestinal adenomas of APC<sup>Min</sup>/1 mice (14). This suggests that diminished antioxidant defense plays a role in duodenal adenoma development in FAP.

**Transition from duodenal adenoma to cancer in FAP**
In the cancer-adenoma comparison, there were 26 protein-coding DEGs. Neoplastic processes involving 8 representative DEGs are shown in Table 5.

**Goblet and Paneth cell dedifferentiation**
In cancer-adenoma, we found downregulation of CLCA1, which encodes a chloride channel expressed in intestinal goblet cells. CLCA1 expression increases with Caco-2 differentiation (26), suggesting that its downregulation may indicate goblet cell dedifferentiation.

In cancer-adenoma, we also found downregulation of DEFA5 and DEFA6, which encode α-defensins and are primarily expressed in Paneth cells of the small intestine (27). Both are upregulated in colon adenomas and cancer compared to normal tissue (28), indicating abnormal Paneth cell differentiation in colon tumors (29). Given these findings, DEFA5/6 downregulation may indicate Paneth dedifferentiation during the evolution of duodenal adenoma to cancer in FAP.

**Decreased production of ATRA**
In cancer-adenoma, we found downregulation of ADH1C, which again implicates decreased ATRA production in the progression of duodenal neoplasia in FAP.

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**Table 3. Representative DEGs grouped by cellular function/pathway**

| Pathway                             | DEG    | FC          |
|-------------------------------------|--------|-------------|
| Brush-border digestion/absorption  | SI     | —5.1        |
|                                     | LCT    | —2.5        |
|                                     | TMPRSS15 | —4.8       |
| Non-brush border ion homeostasis    | CLCA1  | —2.1        |
|                                     | SLC12A2 | 3.4         |
| Lipid metabolism                    | APOA4  | —5.5        |
|                                     | APOB   | 3.4         |
| Carbohydrate metabolism             | ALDOB  | —6.7        |
|                                     | PKC1   | —2.4        |
|                                     | GBA3   | 2.2         |
| Vitamin A metabolism                | ADH1C  | —2.6        |
|                                     | ADH4   | —2.3        |
|                                     | RBP2   | 2.2         |
| Phase I/II metabolism               | CYP2C9 | —2.3        |
|                                     | GSTA1  | —3.2        |
|                                     | GSTA2  | 2.8         |
|                                     | UGT2B17 | 2.5         |
| Cell adhesion/ECM interactions      | COL12A1 | 4.4        |
|                                     | FN1    | 5.1         |
|                                     | SPP1   | 7.4         |
|                                     | MMP1   | 4.2         |
|                                     | MMP7   | 2.7         |
|                                     | POSTN  | 4.9         |
|                                     | CEACAM5 | 8.5        |
|                                     | CECAM6 | 6.0         |
|                                     | SULF1  | 4.3         |
| Defensins                           | DEFA5  | —3.2        |
|                                     | DEFA6  | —2.9        |
| Other                               | IL8    | 2.6         |
|                                     | CD44   | 2.1         |
|                                     | ANX1A10 | —2.1       |

Negative FC indicates downregulation and positive FC indicates upregulation in the more advanced sample.

Ad-Ad, adenoma tissue from FAP cases vs adenoma tissue from FAP controls; Ad-No, adenoma tissue vs normal tissue from FAP cases; Ca-Ad, cancer tissue vs adenoma tissue from FAP cases; Ca-No, cancer tissue vs normal tissue from FAP cases; DEG, differentially expressed gene; FC, fold change.
Increased tumor invasiveness

In cancer-adenoma, upregulation of several DEGs involved in cell adhesion and extracellular matrix interactions was observed, including upregulation of \textit{COL12A1}, which encodes for type XII collagen and is involved in the desmoplastic reaction between cancer cells and associated fibroblasts, which drives colon cancer metastases (30). Cancer tissue also exhibited upregulation of \textit{FN1} and \textit{SPP1}. \textit{FN1} encodes fibronectin 1, which promotes cell proliferation and invasion by interacting with α5β1 integrin (31). \textit{SPP1} encodes osteopontin (OPN), which mediates cell migration partially through interactions with αvβ3 integrin (32). In cancer-adenoma, \textit{POSTN} and \textit{IL8}, which encode the pro-angiogenesis factors periostin (33) and interleukin-8 (34), respectively, were also upregulated.

**DEGs with predictive potential in FAP**

Among our representative DEGs, several have potential as tissue or serum biomarkers for progression of duodenal neoplasia.

**Potential tissue biomarkers for duodenal cancer in FAP**

We identified 13 protein-coding DEGs that distinguished FAP case and FAP control adenomas, all of which were downregulated.
in FAP cases (Supplemental Table 1, see Supplementary Digital Content 1, http://links.lww.com/CTG/A51). Of these DEGs, CLCA1, ADH1C, and ANXA10 have particular significance as potential tissue biomarkers.

CLCA1 encodes a chloride channel expressed in goblet cells, whereas ADH1C encodes an alcohol dehydrogenase enzyme involved in retinol oxidation. Studies have shown CLCA1 downregulation in CRC (26) and ADH1C downregulation in gastric cancer (23). In this study, CLCA1 and ADH1C are downregulated in cancer compared to adenoma and in adenoma from FAP cases compared to FAP controls, indicating that downregulation of these DEGs within adenomas may indicate increased likelihood of neoplastic progression.

ANXA10 encodes annexin 10, a calcium- and phospholipid-binding protein normally expressed in gastric mucosa that inhibits tumorigenesis by causing growth suppression and stimulation of apoptosis (35). Decreased ANXA10 expression is seen in gastric cancer (35). In this study, ANXA10 expression followed a unique pattern. Within FAP cases, ANXA10 does not differ in cancer-normal but is upregulated in adenoma-normal (FC 2.3, FDR 0.30) and downregulated in cancer-adenoma (FC 2.1, FDR < 0.10) comparisons. Furthermore, ANXA10 is significantly downregulated in adenoma from FAP cases compared to FAP controls (FC 2.1, FDR < 0.10). Considering the aforementioned roles of ANXA10, it is possible that the upregulation of ANXA10 in duodenal adenomas indicates a protective “gastric programming.” Downregulation during the transition from FAP control to FAP case adenoma and from FAP case adenoma to cancer may reflect a loss in the tumor suppressive function of ANXA10. Given these findings, determining tissue expression of ANXA10 may predict the likelihood that a duodenal adenoma progresses to cancer in FAP.

Potential serum biomarkers for duodenal cancer in FAP
Among the DEGs identified, SPP1 and CEACAM5 have potential as serum biomarkers for duodenal cancer in FAP. SPP1 encodes OPN. SPP1 expression is 27-fold higher in sporadic ampullary cancer compared to normal duodenum and serum OPN progressively increases from healthy controls to patients with ampullary adenoma to patients with sporadic ampullary cancer (36). CEACAM5 encodes membrane-bound and secreted carcinoembryonic antigen (CEA). For CRC, serum CEA is an independent prognostic factor for recurrence and survival after curative resection (37). In this study, CEACAM5 is the only DEG upregulated in the adenoma-normal and cancer-adenoma comparisons (Table 3). Together, these findings suggest that

### Table 4. Transcriptional changes in adenoma-normal comparison

| Neoplastic change | Cellular function/pathway | Expression change (DEG) | Rationale |
|-------------------|--------------------------|------------------------|-----------|
| Enterocyte dedifferentiation to immature crypt phenotype | Brush border metabolism | D (LCT, TMPRSS15) | Expression increases in the crypt-villus axis |
| Lipid metabolism | D (APOA4, APOB) | Expression decreases in the crypt-villus axis |
| Non-brush border metabolism | U (SLC12A2) | |
| Warburg effect<sup>a</sup> | Carbohydrate metabolism | D (ALDOB) | Glucose regulatory enzyme |
| Decreased production of all-trans-retinoic acid (ATRA)<sup>b</sup> | Vitamin A metabolism | D (RBP2) | Transports dietary vitamin A into enterocytes for conversion to ATRA |
| Impaired ROS/carcinogen defense | Phase I/II metabolism | D (GSTA1/2) | Metabolize carcinogens by glutathione-S-transferase activity and protect cells from ROS by glutathione peroxidase activity |

<sup>a</sup>Warburg effect refers to tumor cell preference for glycolysis over gluconeogenesis and aerobic respiration.

<sup>b</sup>ATRA suppresses tumorgenesis in part by blocking COX-2 induction.

### Table 5. Transcriptional changes in cancer-adenoma comparison

| Neoplastic change | Cellular function/pathway | Expression change (DEG) | Rationale |
|-------------------|--------------------------|------------------------|-----------|
| Goblet cell dedifferentiation | Non-brush border metabolism | D (CLCA1) | Highly and selectively expressed in goblet cells |
| Paneth cell dedifferentiation | Defensin signaling | D (DEFA5/6) | Exclusively expressed in small intestinal Paneth cells |
| Decreased production of all-trans-retinoic acid (ATRA)<sup>a</sup> | Vitamin A metabolism | D (ADH1C) | Oxidizes retinol into all-trans retinaldehyde, which is converted into ATRA in enterocytes |
| Increased tumor invasiveness | Cell Adhesion/ECM interactions | U (COL12A1) | Stimulates desmoplastic reaction |
| | | U (FN1, SPP1) | Functions in integrin-mediated cell adhesion |
| | | U (POSTN) | Pro-angiogenesis factors |
| | | U (IL8) | |

<sup>a</sup>ATRA, all-trans retinoic acid; D, downregulated; DEG, differentially expressed gene; ECM, extracellular matrix; U, upregulated in cancer tissue vs adenoma tissue from FAP cases.

<sup>b</sup>ATRA suppresses tumorgenesis in part by blocking COX-2 induction.
serum OPN and CEA may help determine development of duodenal polyposis and progression to duodenal cancer in FAP.

**DEGs with therapeutic potential in FAP**

Certain DEGs may have significance in existing and novel chemopreventive therapies for duodenal polyposis.

Both celecoxib (5) and the sulindac/erlotinib combination (38) decrease duodenal polypl burden in FAP. We found upregulation of *SPP1* in cancer-normal and cancer-adenoma comparisons. *SPP1* is a Wnt/β-catenin target gene (39) and administration of the COX-2 inhibitor parecoxib to APCmin mice, which display a FAP phenotype, downregulates *SPP1* by inhibition of Wnt/β-catenin while decreasing intestinal tumor load and mice morality (40). OPN is an upstream activator of the EGFR pathway (41). In non-small-cell lung cancer cell lines, the radiosensitizing effect of erlotinib is abolished after OPN depletion (42). Given its role as a target of PGE2 signaling and an activator of EGFR signaling, tissue levels of OPN may be of particular significance in predicting response to the sulindac/erlotinib combination regimen.

*CEACAM6* upregulation in the adenoma-normal and cancer-normal comparisons was also noted. *CEACAM6* encodes a membrane-bound cell adhesion molecule, which confers resistance to anoikis, the apoptosis induced by lack of correct cell/extracellular matrix attachment (43). This allows for cancer cell survival and invasiveness. Accordingly, *CEACAM6* overexpression is seen in CRC (44) and pancreatic cancer (45). In a murine model of pancreatic cancer, administration of a CEACAM6-specific monoclonal antibody conjugated with immunotoxin increases tumor apoptosis and decreases tumor growth (46). In nonhuman primates, this antibody-drug conjugate has minimal toxicity, with a dose-dependent, reversible neutropenia (47). These findings implicate *CEACAM6* as a potential novel therapeutic target in the treatment of duodenal polyposis in FAP.

**PCR verification**

We determined expression levels of candidate genes *SPP1*, *CEACAM5*, *SI*, *APOA4*, and *ANXA10* by PCR. In certain samples from certain patients, PCR could not be successfully performed and expression levels were undefined (Supplemental Table 3, see Supplementary Digital Content 1, http://links.lww.com/CTG/A51).

For cancer-normal and cancer-adenoma comparisons, the sample size for comparison of SI expression was very low (n = 5). Therefore, we decided to exclude PCR results from SI expression. For the remaining genes, FAP cases 10 and 11 consistently did not yield results on PCR. Both FAP cases 10 and 11 had samples preserved with Hollande’s fixative (Supplemental Table 4, see Supplementary Digital Content 1, http://links.lww.com/CTG/A51), which can affect RNA yield and quality (48) and therefore may explain the failure of PCR expression analysis in these samples.

Table 6 shows HTA and PCR results. For every comparison, direction of FC mirrored HTA findings. Specific magnitude of FC and statistical significance is detailed below.
1. SPP1: Gene expression differences in SPP1 were fully verified by PCR.
2. CEACAM5: PCR analyses verified no difference in adenoma-adenoma comparison. As in HTA analysis, PCR analysis showed upregulation in cancer-normal and cancer-adenoma, but each comparison had a trend toward significance.
3. APOA4: PCR analysis verified downregulation in all comparisons; of note, for adenoma-normal, PCR analysis showed a trend toward downregulation.
4. ANXA10: PCR analysis verified ANXA10 upregulation in adenoma-normal, downregulation in cancer-adenoma, and the lack of significant difference in cancer-normal. In adenoma-adenoma, PCR analysis showed a trend toward downregulation, which mirrored significant HTA results.

DISCUSSION
Duodenal cancer is a leading cause of death in FAP after colec- tomy. SS IV duodenal polyposis is a risk factor for duodenal cancer, yet many FAP patients have no history of SS IV polyposis (4,9,10), indicating a need for additional predictors of cancer risk. In APC<sup>Min<sup>- mice, gene expression changes accompany the evolution of small intestinal neoplasia (13,14). To date, no such genome-wide investigation has been performed in patients with FAP. In this study, we described the duodenal adenoma-carcinoma sequence in FAP by comparing normal, adenoma, and cancer tissue of 12 duodenal cancer cases. In the transition from normal duodenum to adenoma, we found potential roles for enterocyte dedi-

Several DEGs distinguished FAP case from FAP control adenomas. ANXA10 is unique in that it is upregulated from normal to adenoma in FAP cases but downregulated from FAP case adenoma to cancer and from FAP control adenoma to FAP case adenoma. Given its function, ANXA10 expression in adenomas may indicate a protective “gastric programming” that suppresses neoplastic evolution. We also identified DEGs upregulated in cancer compared to adenoma that may have utility as biomarkers for neoplastic progression, including SPP1 and CEACAM5 (36,49,50).

Delker et al. (15) performed gene expression analysis on normal duodenum and adenoma in patients with FAP who were either treated with sulindac/erlotinib or with placebo. In the placebo group, they performed an adenoma-normal comparison similar to the one performed in this study. Genes involved in Wnt, PGE2, and EGFR signaling were differentially expressed in the placebo group but not in the sulindac/erlotinib group, indicating a beneficial inhibition of these pathways (15). Duodenal polyps in this study also exhibited upregulation of CD44, a cancer stem cell marker associated with PGE2 signaling (51), and MMP7, which encodes a matrix metalloproteinase and is a Wnt/Beta-catenin signaling target (52). In our study, CD44 and MMP7 were both upregulated in our cancer-normal comparisons (Table 3). Furthermore, MMP1, which is also a WNT/Beta-catenin target (53), was upregulated in our adenoma-normal and cancer-normal comparisons (Table 3).

We also identified DEGs with therapeutic potential in FAP. We found upregulation of SPP1, which plays a role in both the tumorigenic effect of PGE2 (40) and in activation of EGFR signaling (41). Given its relation to both pathways, determining SPP1 expression may help predict response to sulindac/erlotinib therapy. We also identified CEACAM6 as a potential novel therapeutic target for duodenal polyposis control in FAP. CEACAM6 has been successfully targeted in animal models of pancreatic cancer (45,47).

Several limitations merit further discussion. Our RNA extraction and gene expression profiling procedures were specific for FFPE and Hollande’s fixatives and all RNA samples met QC checkpoints for HTA profiling. However, during PCR verification, several samples, particularly Hollande’s fixed samples, yielded undefined results. As a result, PCR comparisons involved lower sample sizes and, while FCs matched our HTA results for 4 candidate genes, P values in some comparisons did not reach statistical significance. This indicates the importance of future validation studies with independent cohorts. Another limitation is the potential for false positives. To address this, we applied a FDR < 0.10 cutoff for our DEGs. Although there is still potential for false positives despite this cutoff, it should be noted that of 52 DEGs that differed in 2+ comparisons, all 52 differed in the same direction (upregulation/downregulation) in each comparison. Similarly, of 8 DEGs that differed in 3+ comparisons, all differed in the same direction in each comparison.

In summary, we have conducted the first ever genome-wide expression analysis of duodenal neoplasia in FAP. Future validation studies with immunohistochemical staining or Western Blot analysis are needed to verify protein expression of candidate genes. Furthermore, for genes whose expression may predict response to celecoxib or sulindac/erlotinib therapy, gene knock-in or knock-out in APC<sup>Min<sup>- mice can be performed to determine effect on therapeutic response. Effect of the CEACAM6 antibody-drug conjugate on APC<sup>Min<sup>- mice can also be investigated, and if this shows therapeutic benefit and low toxicity, targeting CEACAM6 may emerge as a viable option for duodenal polyposis control in FAP.

CONFLICTS OF INTEREST
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Specific author contributions: Study concept and design: S.S.T. and C.A.B. Acquisition of data (retrospective identification and selection of patients): S.S.T., R.L., M.O. and L.L. Acquisition of data (identification and preparation of archived tissue samples for transcriptional profiling): S.S.T. and K.P. Acquisition of data (transcriptional profiling): M.L.V. and C.L. Acquisition of data (PCR verification): S.S.T., Z.W., and B.S. Analysis and interpretation of the data: S.S.T., R.L., Y.C., J.S.B., and C.A.B. Drafting of the manuscript: S.S.T. Critical revision of the manuscript for important intellectual content: C.A.B., J.B.S., M.L.V., B.S., and Z.W. All authors have reviewed the final submitted draft.
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Study Highlights

WHAT IS KNOWN

✓ Murine models of FAP have identified DEGs in the duodenal adenoma-carcinoma sequence.
✓ This has not been studied in patients with FAP.

WHAT IS NEW HERE

✓ Transition from normal duodenum to adenoma is characterized by abnormal metabolism of brush border proteins, lipids, ROS, and retinol and transition from adenoma to cancer was characterized by upregulation of DEGs involved in cell invasion and migration.
✓ Certain DEGs differed between adenomas from cancer patients and controls.
✓ Several DEGs have potential therapeutic significance in existing chemopreventive regimens, including the sulindac/erlotinib combination for duodenal polyposis in FAP.

TRANSLATIONAL IMPACT

✓ In the future, physicians may be able to use differential expression of certain genes in order to determine progression of duodenal adenoma to cancer in FAP.
✓ In the future, physicians may be able to target novel and existing chemopreventive pathways to prevent progression of duodenal polyposis and development of cancer in FAP.

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