Intestinal phenotype of variable-weight cystic fibrosis knockout mice

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Canale-Zambrano JC, Poffenberger MC, Cory SM, Humes DG, Haston CK. Intestinal phenotype of variable-weight cystic fibrosis knockout mice. Am J Physiol Gastrointest Liver Physiol 293: G222–G229, 2007; doi:10.1152/ajpgi.00405.2006.—Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) knockout mice present the clinical features of low body weight and intestinal disease permitting an assessment of the interrelatedness of these phenotypes in a controlled environment. To identify intestinal alterations that are affected by body weight in CF mice, the histological phenotypes of crypt-villus axis height, goblet cell hyperplasia, mass cell infiltrate, crypt cell proliferation, and apoptosis were measured in a population of 12-wk-old (C57BL/6 × BALB/cj) F2 CFTR+/− (B6 CF) small intestine profile to include altered expression of genes of the innate immune system and involved in lipid metabolism (21).

An additional CF phenotype reflected in mice (9, 14) that is, in part, related to the intestinal defect is the low body weight of CF patients (16) relative to the non-CF population. A CF patient’s failure to thrive is due to a composite of factors, including nutrition level and environment, as well as fat malabsorption, which persists in this population even with pancreatic enzyme replacement therapy (22). Peretti et al. (22) proposed that fat malabsorption in CF may be the result of histological changes in the intestine or of altered regulation of transport or metabolic enzymes. The cause of the lower body weight of CF mice is unknown, but studies have revealed the mouse to have the clinical phenotype of fat malabsorption, possibly due to altered pH of the intestine (1), while severe pancreatic disease is not a feature of most CF mouse models (3). A bacterial presence in the intestine has also been reported to affect the growth of CF mice (20), and this phenotype may be due to the abnormal dissolution of Paneth cell granules in CF intestinal crypts (2). Knowledge of mechanisms governing the growth of CF patients is important as percent ideal body weight has been shown to be prognostic of both survival and lung disease severity (15, 25).

To investigate if the intestinal pathology of CF mice is dependent on their body weight we measured the ileal histology of variable weight C57BL/6 × BALB/cj F2 UNC CFTR+/− mice and compared this to non-CF mice. F2 mice were studied as the population presents a greater range of the body weight phenotype than does a congenic CF population (9). In addition, to identify a set of non-CFTR genes that may influence the development of intestinal complications (MI, DIOS, and related to growth), we assessed the ileal gene expression of CF and non-CF mice presenting high- or low-body weight phenotypes. These expression data were integrated with the histology and blood biochemistry results to propose specific genes and pathways leading to the intestinal phenotype in CF mice.

MATERIALS AND METHODS

Animals. The mice of the C57BL/6 (B6) CFTR+/− strain were provided by Dr. Danuta Radziwich of the Montreal General Hospital, and the BALB/cj (BALB) strain was purchased from the Jackson

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Laboratory. F2 CF mice were created from these strains as the B6 \textit{Cftr}+/− and BALB mice were crossed to create F1 mice, and the resultant F1 mice were intercrossed to create F2 CF−/− and \textit{Cftr}++ mice. The \textit{Cftr} genotype of the mice was determined using a previously described PCR assay (13), and genomic DNA was isolated from the tails of the mice, which were clipped at 16 days of age. All CF and control mice were maintained on liquid diet (Peptamen) from the age of 18 days until euthanization (1). Peptamen is a complete liquid enteral formulation composed principally of medium-chain triglycerides, essential fatty acids (0.16% linoleic and 0.023% linolenic acids), carbohydrates, and hydrolyzed protein (1). All mice were housed in microisolator cages in a specific pathogen-free room and handled according to standard husbandry of the animal facility at the McIntyre Building of McGill University and cared for under a protocol approved by the McGill University Animal Care Committee.

Mice were killed, by carbon dioxide exposure, at the age of 12 wk. At this time, their total body weight was measured, and blood was drawn by cardiac puncture. At dissection, a 2-cm portion of the terminal ileum was removed, its contents was eliminated, and 1 cm of the proximal ileum was immediately homogenized in 1 ml of TRIZol reagent (Sigma). The homogenate was kept on dry ice until it was stored at −85°C. A second 1 cm of the ileum was fixed in formalin and submitted for standard histological processing. Obvious obstructions observed upon inspection of the intestine at autopsy were recorded. The body weight of a total of 98 F2 CF (40 females and 58 males) mice was evaluated at 12 wk of age. Five mice were identified to have ileal obstructions and were not included in this study so that the reported body weight of a CF mouse was not influenced by the development of an intestinal obstruction.

\textbf{Histology.} Paraffin-embedded sections (5 μm) were stained with hematoxylin and eosiin for the evaluation of general histological structure. For each section, the crypt-villus axis (CVA) height was measured, and the numbers of goblet cells were counted for an average of 20 CVAs/section. The CVA height measurements included the entire length of the crypt and villus, and only complete and intact CVAs were measured using image analysis of the histological sections (Olympus BX51, Image-Pro Plus 5.1, Media Cybernetics). In addition, the goblet cells per CVA count was normalized to a linear length of 100 μm of epithelium along the CVA, and separate measurements of villus height and crypt depth were taken to calculate the villus-to-crypt ratio. From 3-μm sections stained with Toluidine blue, mast cell counts for each mouse were calculated as the average numbers of stained cells per millimeter squared (×400 magnification), for an average of 40 fields/section.

For these phenotypes, one section from each of 13 control mice (7 males and 6 females) fed the liquid diet and 18 CF mice (9 males and 9 females) selected to represent the weight range of the F2 population was scored. All sections were scored by an observer blinded to mouse genotype.

\textbf{Assessment of proliferation and apoptosis.} To visualize cells undergoing proliferation or apoptosis, paraffin-embedded tissue sections (5 μm thick) were immunostained with antibodies directed against PCNA (Santa Cruz Biotechnology, Santa Cruz, CA) and caspase-3 (Cell Signaling Technology), respectively. In brief, sections were initially deparaffinized and hydrated through graded ethanol. Antigen sites were unmasked by antigen retrieval treatment for 10 min at 98°C in a citrate buffer. Endogenous peroxidase activity was quenched using 3% (vol/vol) hydrogen peroxide in Tris-buffered saline (TBS) for 15 min. Nonspecific binding sites were blocked with 10% sheep serum (Cedarlane Laboratories, Hornby, ON, Canada) in TBS for 20 min at 37°C. Sections were then incubated overnight at 4°C with the primary antibody (PCNA, 1:50; and caspase-3, 1:100). After being washed, sections were incubated with 1:100 biotinylated sheep anti-rabbit secondary antibody (Serotec, Raleigh, NC) for 60 min at room temperature. Sections were then washed and incubated in an avidin-peroxidase solution (StreptABCComplex/HRP, DakoCytomation, Mississauga, ON, Canada) for 45 min. Sections were developed using 3,3-diaminobenzidine tetrahydrochloride (Liquid DAB Substrate Chromogen System, DakoCytomation), counterstained with hematoxylin, dehydrated, and then mounted. For negative control preparations, the primary antibody was replaced by TBS. Blinded scoring of 30–100 crypts/mouse was performed and presented as the average number of positively stained cells per crypt.

\textbf{Blood analysis.} Blood was collected by cardiac puncture on anesthetized animals, stored in plasma separator tubes containing EDTA, and centrifuged. The blood analysis was completed on a biochemistry analyzer Hitachi 911, and the levels of glucose, cholesterol, high-density lipoprotein, and triglycerides were determined.

\textbf{Phenotypic data analysis.} Tests for differences in mouse weight, blood biochemistry, and ileal morphology between groups were done by t-test. Association of body weight with histological features was assessed by regression analysis using Microsoft Excel software, where a value of \(P < 0.05\) was considered significant. To use all F2 CF mice in one test, the weights of the F2 female mice were increased by the relative mean weight of male mice to female mice, as in Haston et al. (9).

\textbf{Gene expression analysis.} Gene expression profiles were determined for groups of mice defined by \textit{Cftr} genotype, sex, and body weight. To ensure that potential differences in ileal gene expression by murine weight could be assessed, RNA from mice with body weights in the highest and lowest quartiles of the weight distribution was selected for hybridization as in Ref. 23. The gene expression of high-weight CF mice was assayed with five chips, three of which represent the RNA of an individual male mouse (mouse weights = 25.6, 25.9, and 26.0 g) per chip and two of the pooled RNA from five mice (5 males on 1 chip and 5 females on the second chip), with weights from the 4th quartile of the CF population. For low-weight CF mice, the gene expression profile was assayed from four chips, two of individual male mice (weights = 19.4 and 19.5 g), one individual female mouse (17.1 g), and one of RNA pooled from female mice with weights within the 1st quartile of the weight distribution. The gene expression profile of liquid diet-fed control mouse (\textit{Cftr}+/+) was assayed with two chips. Each represents pooled RNA of five mice (1 chip for males and 1 chip for females) that were not selected for extreme weight.

Total RNA was extracted, according to the manufacturer’s (Sigma) instructions, from the homogenate stored after tissue harvest. The quality of the isolated RNA was assessed and confirmed by Agilent Bioanalyzer readings (Agilent Technologies). Hybridization to the Murine MOE430 2.0 GeneChip (Affymetrix) was performed by the Affymetrix Gene Chip Core Facility at the McGill University and Genome Quebec Innovation Centre, as previously described (10). Lists of significantly differentially expressed genes were generated for comparisons between groups using routines from Bioconductor version 1.6 (http://www.bioconductor.org/) within the R version 2.1.0 statistical language (12) as in Ref. 10. The detection of significantly overrepresented Gene Ontology categories was performed using the GohyperG function in Bioconductor (8). Raw and normalized expression data are available from the National Center for Biotechnology Information GEO website.

\textbf{RESULTS}

\textbf{Body weight phenotype.} To study CF and non-CF control mice with variable weights, a population of B6 × BALB F2 mice was bred from F1 progenitors. The average weight (mean ± SE) of male CF mice at 12 wk of age was significantly lower than that of controls (23.2 ± 0.3 compared with 32.0 ± 1.8 g, \(P = 0.001\)), and this difference was also evident in females (20.3 ± 0.3 g for CF vs. 22.8 ± 0.6 g for control mice, \(P = 0.003\)).
Ileal histology. To document the pathological changes in the ileum of CF and non-CF mice, histological sections were made and subsequently scored for CVA height, number of goblet cells, and presence of mast cells. As shown in Fig. 1, the CVA height in CF mice exceeded that of liquid diet-fed control mice [355 ± 10 μm (means ± SE) vs. 185 ± 7 μm, P = 1.8 × 10^-13], and the ilea of CF mice contained more goblet cells than those of non-CF mice (goblet cells/CVA: 18.0 ± 0.6 vs. 5.7 ± 0.5, P = 4.3 × 10^-15; goblet cells/100 μm of CVA: 2.5 ± 0.1 vs. 1.6 ± 0.13, P = 8.3 × 10^-7), suggesting the presence of a hyperplastic state in CF mice. Intestinal sections from CF mice also featured more mast cells in the submucosa and muscularis externa layers than did the sections of non-CF mice (3.6 ± 0.4 vs. 0.02 ± 0.01 mast cells/mm², P = 0.0003). These histological observations are consistent with previous reports on CF mice, including B6 CF mice (2, 5, 20, 21).

Regression analyses were completed to determine if the histological changes in the CF intestine were dependent on mouse body weight. As shown in Fig. 2, CVA height of both male and female CF mice decreased with increasing body weight (r = -0.59, P = 0.01) while a directly proportional correlation of CVA height to weight was evident in control mice (r = 0.59, P = 0.03). Goblet cell numbers per linear length of CVA were identified to be independent of weight (r = 0.05, P > 0.05; see Fig. 2); correspondingly, numbers of goblet cells per CVA decreased with increasing body weight (r = -0.50, P = 0.03) in CF mice. These findings indicate that the ileum of a heavier CF mouse more closely resembles that of a control mouse than does the ileum of a lighter CF mouse, with the latter presenting more severe morphological alterations. Mast cell counts were independent of body weight in CF mice (r = 0.2, P > 0.05; data not shown).

To further investigate the relationship of CVA height to weight, the crypt depth and villus height of each mouse were measured. The ratio of villus height to crypt depth in CF mice was found to be significantly lower than that of control mice (1.6:1 ± 0.05 vs. 2.5:1 ± 0.13, P = 1 × 10^-7) and to increase with increasing body weight in CF mice (r = 0.5, P = 0.03). The average crypt depth of CF mice was approximately twice that of control mice (1.82 times), whereas the villi of CF mice were 1.15 times the length of those in non-CF mice. Therefore, the increase in CVA height is primarily due to an elongation of the crypts in CF mice.

Ileal crypt proliferation and apoptosis. Crypt cell proliferation and upward migration have been shown to be increased in the CF mouse small intestine (7), and our observation of increased CVA height is consistent with such changes. To further characterize the observed difference in crypt length between high- and low-body weight CF and control mice, crypt cell proliferation and apoptosis were measured with immunohistochemical staining. As shown in Fig. 3, there were significantly more PCNA-positive (proliferating) cells in the crypts of CF mice compared with the levels detected in non-CF controls (8.4 ± 0.8 PCNA-positive cells/crypt in CF mice vs. 4.5 ± 0.7 PCNA-positive cells/crypt in non-CF controls, P = 0.003). Due to the crypt elongation in CF mice, however, the numbers of proliferating cells per unit length of crypt did not differ between CF and control mice (Fig. 4A). Furthermore, the number of proliferating cells per crypt was found to be independent of body weight in CF mice (P = 0.6), indicating a lower rate of proliferation to exist in smaller CF mice, which present a CVA of increased length.

In contrast, numbers of apoptotic cells in the crypts of CF mice were not different from control levels (0.13 ± 0.02 positive cells/crypt in CF mice vs. 0.15 ± 0.01 positive cells/crypt in non-CF controls, P = 0.5). In this case, the increased crypt length in CF mice resulted in a lower number of apoptotic cells per unit length of tissue in CF compared

![Fig. 1. Sections of ileal tissue from 12-wk-old F2 control and F2 cystic fibrosis (CF) mice (stained with hematoxylin and eosin). A and C: F2 control mouse ileum. The crypt lumen was small, and goblet cells appeared compacted. B and D: F2 CF mouse ileum featuring greater crypt-villus axis (CVA) height, crypt elongation, and hyperplasia of goblet cells.](http://ajpgi.physiology.org/Downloadedfrom)
with control mice (Fig. 4B). Based on these observations, the change in crypt length in the CF mice is due to a relative reduction in numbers of apoptotic cells and an expansion of the proliferative zone. Both of these features are more pronounced in the smaller CF mice and thus may be associated with variable weight in this F2 CF mouse model.

**Blood biochemistry phenotype.** To investigate if the blood biochemistry of CF mice differed from that of non-CF mice, and if this was influenced by body weight, cardiac blood samples were collected from a cohort of mice at death. The blood of CF mice of both sexes (n = 11 males and 9 females), representing the high and low ends of the weight distribution, and of liquid diet-fed control mice (n = 9 males and 8 females) was tested. Blood biochemistry was not dependent on the sex of the mice within either the CF or non-CF groups (P = 0.14–0.97; data not shown), with the exception of a high glucose measure in male control mice relative to that of females (20.1 ± 1.3 vs. 16.5 ± 0.9 mmol/l, P = 0.036), and, therefore, these data were combined for a comparison of CF mice to non-CF mice. As shown in Table 1, triglyceride levels were significantly lower in CF mice than in control mice, as was the body weight, and no other differences in blood biochemistry levels by *Cftr* genotype were evident. Blood glucose levels did not differ between female CF and non-CF mice (P = 0.77), but male CF mice had lower glucose levels than male non-CF controls (15.8 ± 1.6 vs. 20.1 ± 1.3 mmol/l, P = 0.055). Blood triglyceride levels were not significantly correlated with weight in either of the CF (P = 0.56) or control mouse groups (P = 0.23; data not shown).

**CF intestinal gene expression profiles.** Eleven Affymetrix Gene Chip microarrays (45,101 probes sets/chip) were used to determine which genes were differentially expressed in the ilea of (B6 × BALB) F2 CF mice compared with (B6 × BALB) F2 non-CF liquid diet-fed control mice and how this differential expression was influenced by the sex and weight of the mice.

To identify the set of genes involved in the intestinal disease of CF mice, data from nine arrays (samples from 6 male *Cftr*+/− and 3 female *Cftr*+/− mice) were compared with data from two control arrays (from *Cftr*+/+ mice); 205 genes/expressed sequence tags (ESTs) were determined to be significantly differentially expressed in the ilea of (B6 × BALB) F2 CF mice compared with (B6 × BALB) F2 non-CF liquid diet-fed control mice and how this differential expression was influenced by the sex and weight of the mice.

To identify the set of genes involved in the intestinal disease of CF mice, data from nine arrays (samples from 6 male *Cftr*+/− and 3 female *Cftr*+/− mice) were compared with data from two control arrays (from *Cftr*+/+ mice); 205 genes/expressed sequence tags (ESTs) were determined to be significantly differentially expressed in the ilea of CF mice compared with control mice (fold > 2, P < 0.05; Table 2 and Supplementary Table 1).1 The genes most significantly differentially expressed in the CF intestinal response included complement component factor i, CD177 antigen, and fucosyltransferase 2, which were of increased expression in CF mice relative to controls, whereas *Cftr* and genes of oxidoreductase processes (cytochrome P-450 family members and retinol dehydrogenase 7) were of lower expression in CF mice. The ileal CF response also included 51 solute carrier family members and 9 genes for ATP binding cassette transporters. By

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1 Supplemental data for this article is available online at the American Journal of Physiology-Gastrointestinal and Liver Physiology website.
gene ontology analysis, among the biological processes most affected in the murine CF ileum were cellular physiological processes \( (P = 1.3 \times 10^{-08}) \); metabolism \( (P = 1.5 \times 10^{-06}) \), including the subcategories of cellular, macromolecule, protein, and organic acid metabolism; and DNA replication initiation (see Table 3 and Supplementary Table 2). In further analyses, completed by comparing data of CF male mice (6 chips) with those from CF female mice (3 chips), we determined the sex of the mouse to have a minimal influence on the intestinal gene expression as eight genes were found to be differentially expressed by sex \( (P < 0.05; \text{Supplementary Table 3}) \).

Finally, to ascertain if ileal gene expression differed with murine body weight, data from high-weight CF mice (weights > 75th percentile, assayed with 5 chips) were compared with data from low-weight CF mice (weights < 25th percentile, assayed with 4 chips). In this analysis, no probe sets were identified to be significantly differentially expressed \( (P < 0.05) \).

**DISCUSSION**

In this study, we demonstrated that the CF intestinal phenotype of increased CVA height decreases with increasing body weight in \( B6 \times BALB F2 \) CF mice, whereas mast cell infiltration and goblet cell hyperplasia did not change with mouse weight. We further showed the intestinal crypts from CF mice to have fewer cells undergoing apoptosis per unit length than did non-CF mice and to have an expanded proliferative zone. By gene expression analysis, DNA replication and metabolic biological processes were revealed to be affected in CF mice regardless of their weight. Finally, the CF defect of low blood triglyceride levels was shown to be strongly dependent on CF status and not on body weight.

Through histological evaluation, we showed the intestinal phenotype of \( B6xBALB F2 \) CF mice to be consistent with that previously reported for CF mice, which includes crypt elongation and dilatation as well as goblet cell hyperplasia \((3, 14, 20, 26)\). The evaluation of a group of \( F2 \) CF mice, which, on the genetically mixed \( B6 \times BALB \) background present a range in body weight \((9)\), revealed the known hyperplastic state of the CF intestine to decrease with increasing animal mass. The existence of the relatively increased crypt depth in smaller CF mice, which was due to both an expansion of the proliferative zone and a reduced rate of crypt cell apoptosis, suggests this phenotype to be associated with a more severe intestinal disease. These observations of crypt cell number changes are consistent with those of Gallagher et al. \((7)\), who identified an increased rate of epithelial cell proliferation, but no difference in apoptosis, within the intestinal crypts in \( Cfr\)-null mice relative to non-CF mice. In the present study, we also detected an increased number of PCNA-positive cells in the CF intestine and a similar number of apoptotic cells in CF and control mice. Differences between the studies occur based on normalization. In the work of Gallagher et al. \((7)\), numbers of positively stained cells within the first 10 cells following the Paneth cells in the crypts were evaluated, and the CVA height of CF and control mice was found not to differ. In this study, apoptotic cells per unit length of crypt decreased due to the difference in crypt depth between CF and non-CF mice. In addition, animals here were studied at 12 wk of age, whereas in Ref. 7, the age was not indicated; thus, events taking place earlier or later in mouse intestinal disease development might reflect in the reported differences. In our study, the increase in the proliferative zone and decrease in crypt cell apoptosis could have contributed to the disease in CF mice by altering the functional structure of the small intestine. Indeed, the increased numbers of goblet cells in smaller CF mice producing and secreting an increased or altered amount of mucus to the intestinal lumen could influence body weight through the creation of an extraphysical barrier to nutrient absorption. The histological

**Table 1. Blood biochemistry in CF and liquid diet-fed non-CF mice at 12 wk of age**

<table>
<thead>
<tr>
<th></th>
<th>CF</th>
<th>Non-CF</th>
<th>( P ) Value (CF vs. non-CF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/l</td>
<td>15.8 ± 1.1</td>
<td>18.4 ± 0.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.06 ± 0.12</td>
<td>1.69 ± 0.14</td>
<td>1.5 × 10^{-3}</td>
</tr>
<tr>
<td>High-density lipoprotein, mmol/l</td>
<td>2.66 ± 0.13</td>
<td>2.59 ± 0.12</td>
<td>0.48</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>3.31 ± 0.19</td>
<td>3.06 ± 0.17</td>
<td>0.34</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>22.0 ± 0.5</td>
<td>32.0 ± 1.0</td>
<td>4.6 × 10^{-9}</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 20 \) cystic fibrosis (CF) and 17 non-CF control mice. Body weights of the F2 female mice were increased by the relative mean weight of male to female mice, as described in MATERIALS AND METHODS.
changes in the lower-weight CF mice are also reminiscent of short bowel syndrome, which features CVA of increased height as an adaptation to a surgical or medical loss of intestinal length (18). As the entire length of the small intestine was not measured in this study, it is not known if the observed histological change occurred through a similar mechanism, i.e., as an adaptation to reduced length of the small intestine. Further study is required.

The intestinal phenotype of F2 CF mice was also assessed through gene expression profiling, and this revealed genes of cell proliferation, including DNA replication, to be among those differentially expressed in CF mice, supporting the histological observations. Furthermore, specific to the proliferative response, the gene for Eph receptor B2, which is a key regulator of epithelial cell migration and proliferation (11), was more highly expressed in CF mice than in controls.

The gene expression profile for the ileum of B6 × BALB F2 CF mice also agrees with, and extends, the previous report (21) made of the small intestine of B6 CF mice, in that 62% of the genes identified to be differentially expressed in the prior study were among those of altered expression in this report, and, as an additional, 609 genes were identified to be part of the F2 CF response, 92 of which had a fold change >2. By using the Affymetrix 430 2.0 chip, the expression of a greater number of genes was sampled, for an increased number of mice; thus, we were able to build on previous observations. For example, we confirm the observation of Norkina et al. (21) showing that components of the innate immune system are upregulated in the small intestine of CF mice, whereas genes of lipid metabolism are downregulated. In addition, we identified other innate response genes, such as S100 calcium-binding protein A11.

### Table 2. Genes most significantly differentially expressed in the ilea of 12-wk-old B6 × BALB F2 CF mice relative to non-CF mice

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene</th>
<th>Fold Change</th>
<th>UniGene Identifier</th>
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<tr>
<td>1600029D21 Rik</td>
<td>RIKEN cDNA 1600029D21 gene</td>
<td>9.2</td>
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<td>Tra2a</td>
<td>Transformer 2a homolog (Drosophila)</td>
<td>6.8</td>
<td>Mm.196598</td>
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<td>Cecam10</td>
<td>CEA-related cell adhesion molecule 10</td>
<td>5.4</td>
<td>Mm.30300</td>
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<td>Cd177</td>
<td>CD177 antigen</td>
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<td>Aquaporin 4</td>
<td>4.5</td>
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<td>Vip</td>
<td>Vasoactive intestinal polypeptide</td>
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<td>Fuc2</td>
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<td>Mm.290046</td>
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<td>Cfi</td>
<td>Complement component factor i</td>
<td>3.7</td>
<td>Mm.117180</td>
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<tr>
<td>Munc2d1</td>
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<td>Pla2g5</td>
<td>Phospholipase A2, group V</td>
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<td>Mm.23347</td>
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<td>Tmprss2</td>
<td>Transmembrane protease, serine 2</td>
<td>3.1</td>
<td>Mm.276145</td>
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<tr>
<td>Mfsd2</td>
<td>Major facilitator superfamily domain containing 2</td>
<td>3.0</td>
<td>Mm.331842</td>
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<td>C1galt1</td>
<td>Core 1 UDP-galactose:N-acetylgalactosamine-α-Rβ-1,3-galactosyltransferase</td>
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<td>Evl1</td>
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<td>Tlr4</td>
<td>Toll-like receptor 4</td>
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<table>
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<th>Symbol</th>
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<th>Fold Change</th>
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<tr>
<td>Cyp3a25</td>
<td>Cytochrome P-450, family 3, subfamily A, polypeptide 25</td>
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<td>Pdxk1</td>
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<td>Rdh67</td>
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<td>Mm.10034</td>
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<td>Cubulin (intrinsic factor-cobalamin receptor)</td>
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<td>Mm.313915</td>
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<td>Serpina1b</td>
<td>Serine (or cysteine) preptidase inhibitor, clade A, member 1b</td>
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<tr>
<td>Slc7a15</td>
<td>Solute carrier family 7 (cationic amino acid transporter, y+ system), member 15</td>
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</tr>
<tr>
<td>Treh</td>
<td>Trehalase (brush-border membrane glycoprotein)</td>
<td>6.2</td>
<td>Mm.45380</td>
</tr>
<tr>
<td>Arg2</td>
<td>Arginase type II</td>
<td>6.1</td>
<td>Mm.3506</td>
</tr>
<tr>
<td>Akr1b7</td>
<td>Aldo-keto reductase family 1, member B7</td>
<td>5.9</td>
<td>Mm.90151</td>
</tr>
<tr>
<td>Susd2</td>
<td>Sushi domain containing 2</td>
<td>5.8</td>
<td>Mm.247956</td>
</tr>
<tr>
<td>1300013J15 Rik</td>
<td>RIKEN cDNA 1300013J15 gene</td>
<td>5.7</td>
<td>Mm.100741</td>
</tr>
<tr>
<td>Aadac</td>
<td>Arylacetamid deacetylase (esterase)</td>
<td>5.4</td>
<td>Mm.24547</td>
</tr>
<tr>
<td>Cndp1</td>
<td>Carnosine dipeptidase 1 (metallopeptidase M20 family)</td>
<td>5.1</td>
<td>Mm.23278</td>
</tr>
<tr>
<td>Cftr</td>
<td>Cystic fibrosis transmembrane conductance regulator homolog</td>
<td>5.0</td>
<td>Mm.15621</td>
</tr>
<tr>
<td>Slc2a2</td>
<td>Solute carrier family 2 (facilitated glucose transporter), member 2</td>
<td>4.4</td>
<td>Mm.18443</td>
</tr>
</tbody>
</table>
independent of weight. These data also indicate that body weight. From the gene expression analysis, the re-
differential expression of mast cell genes, which may be due
to the relative density of mast cells to the entire tissue
sampled by gene expression or may reflect the fact that the
cells were positive for toluidine blue staining but were not
active and expressing secretory markers.

Our analysis of the gene expression data also revealed the profile of the ileal tissue of the heaviest CF mice not to
differ from that of the lightest mice. This finding is likely
due to histological change observed (increased crypt depth)
being only a fraction of the tissue composition or may
decrease and processed by 5 microarrays), that the changes in gene
expression by mouse weight are more subtle than those induced by nonfunctional Cftr. It is also possible that gene
expression profiles in the more proximal small bowel may differ in mice segregated by body weight due to the func-
tional differences of segments of the small intestine. Such
changes were not evaluated as in this investigation we
focused on alterations in the ileum as this is the site of
obstruction in the clinical CF intestinal phenotypes M1 and
DIOS. Importantly, the data of the gene expression profiles
suggest that lipid metabolism is similarly altered in high-
weight CF mice.

The intestinal phenotype of CF mice includes the altered expression of genes encoding solute and fatty acid transporters
and genes of lipid metabolism. Among these were genetic
factors implicated in the development of colitis or Crohn’s
disease (multidrug resistance 1a, Slc22a4, and Slc22a5) (24),
which may suggest the existence of an overlap in the pathways
leading to ileal inflammation and hyperplasia in these diseases.
Also of reduced expression in CF mice was the gene for
peroxisome proliferator-activated receptor-α, which is a tran-
scription factor regulating levels of enzymes of fatty acid
metabolism (19), as were fatty acid processing genes for
phospholipases A2 (Pla2g4a and Pla2g5), the altered ex-
pression of which may influence both lipid metabolism and the
development of the inflammatory component of the CF intes-
tinal phenotype (6).

Finally, the lower body weight phenotype of CF patients is
thought to be due in part to fat malabsorption (22). Our
analysis of absorption through blood biochemistry confirmed
the CF mice to have lower triglyceride levels than Peptamen-
fed non-CF mice, as reported in Ref. 1, and furthermore
showed triglyceride levels to depend on CF status and not
body weight. From the gene expression analysis, the re-
duced expression of the intracellular metabolic enzyme
diacylglycerol O-acyltransferase 1 (Dgat1) could partially
account for this phenotype, as Dgat1 is one of two known
enzymes that catalyze the final step in mammalian triglycer-
ide synthesis (22). Of significance, these data suggest that
blood triglyceride levels remain lower in near-normal
weight CF mice.

In summary, we demonstrated the CVA elongation pheno-
type of CF mice to feature an increase of the proliferative zone
and decreased crypt cell apoptosis and to be related to body
weight. The proliferative response to nonfunctional Cftr has
also been shown in CF lung disease in mice (10) and thus may
be mechanistically important to disease severity in CF.
Furthermore, we showed the expression of genes for trans-
porters and metabolic enzymes responsible for the uptake
and processing of nutrients from the lumen to be reduced in
the murine CF intestine, independent of weight, and blood
triglyceride levels to be influenced by CF status, and not
weight, in mice.

GRANTS

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Verkade HJ. Fat absorption in cystic fibrosis mice is impeded by


