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GATA4 is essential for jejunal function in mice

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Abstract

Background and Aims—Although the zinc finger transcription factor GATA4 has been implicated in regulating jejunal gene expression, the contribution of GATA4 in controlling jejunal physiology has not been addressed.

Methods—We generated mice in which the *Gata4* gene was specifically deleted in the small intestinal epithelium. Measurements of plasma cholesterol and phospholipids, intestinal absorption of dietary fat and cholesterol, and gene expression were performed on these animals.

Results—Mice lacking GATA4 in the intestine displayed a dramatic block in their ability to absorb cholesterol and dietary fat. Comparison of the global gene expression profiles of control jejunum, control ileum, and GATA4 null jejunum by gene array analysis revealed that GATA4 null jejunum lost expression of 53% of the jejunal-specific gene set and gained expression of 47% of the set of genes unique to the ileum. These alterations in gene expression included a decrease in mRNAs encoding lipid and cholesterol transporters as well as an increase in mRNAs encoding proteins involved in bile acid absorption.

Conclusion—Our data demonstrate that GATA4 is essential for jejunal function including fat and cholesterol absorption and confirm that GATA4 plays a pivotal role in determining jejunal versus ileal identity.

It is well established that spatiotemporal gradients of transcription factor expression exist in the small intestine.¹ The complement of transcription factors present in a given region results in the activation and repression of specific genes that define the functional capacity of the duodenum, jejunum, and ileum. GATA4, a zinc-finger transcription factor, is expressed in the epithelium of the duodenum and the jejunum but is absent from the ileum suggesting that GATA4 has a specific role in controlling duodenal and jejunal function.^{2, 3} Supporting the proposal that GATA4 contributes to intestinal function, GATA4 has been shown to activate the expression of intestinal genes including *Lactase (Lct)*, *Fatty acid binding protein 1 (Fabp1)*, and *Sucrase isomaltase (Si)*.^{4–6} Moreover, Bosse *et al.*,² using a tamoxifen-inducible Cre recombinase to eliminate GATA4 specifically from the intestinal epithelium of adult mice,

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Transcript profiling: Microarray data from this study have been deposited into NCBI Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo>) and are accessible through GEO series accession number GSE1194.

demonstrated decreased expression of *Lct* and *Fabp1* in mutant jejunum whereas expression of two ileal-specific genes, *Solute carrier family 10, member 2* (*Slc10a2*) and *Fatty acid binding protein 6* (*Fabp6*), was induced. They proposed that elimination of GATA4 from the jejunal epithelium caused a shift in jejunal identity toward that of the ileum. While these data are intriguing because they suggest that a single transcription factor is sufficient to determine the functional fate of a specific region of the small intestine, it was unclear whether loss of GATA4 resulted in a global change in tissue identity or whether GATA4 was responsible for controlling a limited set of jejunal genes. Recent work addressing the role of GATA factors in transcriptional control of *C. elegans* intestinal development, however, supports the proposal that a single GATA factor controls intestinal function.⁷ In this analysis, examination of 74 promoters driving intestine-specific gene expression revealed that all contained highly similar GATA motifs, which were found in fewer than 5% of randomly selected promoters.

Taken together, these studies led us to propose that elimination of GATA4 from the intestinal epithelium would have a dramatic impact on jejunal function. Moreover, in light of the finding of Bosse *et al.*² that the expression of two important ileal-specific genes is activated in GATA4 conditional knockout jejunum, we further predicted that ileal functions would be induced in GATA4 null jejunum. Therefore, to determine the physiological consequences resulting from the loss of GATA4 in the jejunum as well as to investigate on a global scale the status of jejunal identity in GATA4 null tissue, we used a conditional knockout approach to eliminate GATA4 from the intestinal epithelium. Here we show that lipid and cholesterol absorption, two jejunal-specific functions, were severely disrupted in mice lacking GATA4 in the intestinal epithelium. We found decreased expression of several genes implicated in lipid and cholesterol uptake, transport, and metabolism in jejunum lacking GATA4. Moreover, we found a large set of genes associated with bile acid absorption, a function associated with the ileum, to be expressed at high levels in GATA4 null jejunum compared with control jejunum. Comparison of the global gene expression profile of GATA4 null jejunum with that of control jejunum and control ileum revealed that mutant jejunum lost expression of a large set of jejunal-specific genes and gained expression of genes normally expressed only in the ileum. Our studies show that GATA4 is an essential regulator of jejunal function and confirm that in the absence of GATA4 the jejunum adopts an ileal character.

Materials and Methods

Animals

Derivation of *Gata4*^{loxP} (*Gata4*^{tm1Sad}), *Gata4*⁻ (*Gata4*^{tm1Eno}), and *VilCre* (Tg(*Vil-cre*) 997Gum) mice has been previously described.^{8–10} The *Gata4*^{loxP} allele contains *loxP* sites flanking exons 3–5, which encode the DNA binding and nuclear localization domains. No protein was detected using an antibody recognizing an epitope in exon 7. In all experiments, duodenum (1cm adjacent to pyloric sphincter), jejunum (10cm from pyloric sphincter), and ileum (1cm from cecum) were harvested from adult (6–8 week) mice. The MCW IACUC approved all animal procedures.

RT-PCR

RT-PCR was carried out as previously described¹¹ using total RNA (RNeasy, Qiagen) isolated from adult male jejunum, ileum, or liver. Gene expression fold changes were calculated using a phosphorimager scanner. Samples were normalized to the level of *Polr2a*. Table 2 lists primers (supplemental information).

Histochemistry and Immunohistochemistry

Histochemistry and immunohistochemistry were performed following standard procedures using a minimum of 5–10 sections from at least 4 control and 4 mutant animals. Table 3 lists antibodies (supplemental information).

Metabolic analyses

Adult male mice were acclimated in DietMax chambers (Accuscan Instruments) for 3 days with body weights taken daily. A 24-hour meal pattern was recorded on day 4. Plasma levels of glucose, cholesterol, and phospholipids were determined following standard procedures. Dietary fat absorption was determined following the Jandacek *et al.*¹² protocol (Research Diets D12451 modified to 16% fat (safflower oil) of which 5% was sucrose polybehenate). Fecal pellets from day 4 on the experimental diet were analyzed. To measure cholesterol absorption, ¹⁴C-cholesterol and ³H-sitostanol were gavaged in an emulsion diet (vegetable oil, non-fat dry milk, water) into adult male mice that were fasted overnight. Fecal samples taken 24 hours post-gavage were saponified, extracted with hexane, extracts measured for ¹⁴C and ³H radioactivity by scintillation counting, and dietary and fecal ratios of the isotopes were calculated.

Oligonucleotide array analysis

Three Mouse Genome 430 2.0 arrays (Affymetrix) were hybridized with fragmented cRNA for each tissue. To be considered down-regulated in mutant jejunum, expression had to be called present in all control jejunal samples using presence/absence calls generated by GeneChip Operating Software. To be considered up-regulated, expression was called present in all mutant samples. Log-transformed expression values were determined using dChip 2007.¹³ We selected a cutoff of 2.0 fold, $p \leq 0.05$. Ingenuity Pathway Analysis was used for biological functional analysis and for network generation and network functional analysis. We generated sets of expressed genes for control jejunum, control ileum, and GATA4 null jejunum - only probe sets that had unique Entrez gene identifiers and had signal values called as present in all three arrays hybridized for the tissue of interest were retained.

Results

Gata4 is efficiently eliminated from the intestinal epithelium of *Gata4*^{loxP/-}-*VilCre* mice

Immunohistochemical staining for GATA4 along the anterior-posterior axis of the mouse small intestine confirmed its presence in the epithelium of the duodenum and the jejunum and its exclusion from the ileal epithelium (Figure 1A). Such differential expression of GATA4 between jejunum and ileum implicates GATA4 as a transcription factor with a potential role in demarcating jejunum versus ileum. To test our hypothesis that GATA4 is required to establish and maintain the physiological integrity of the jejunum as well as to investigate on a global scale the status of jejunal identity in GATA4 null tissue, we eliminated *Gata4* from the developing intestinal epithelium via excision of a conditional allele by *Cre recombinase* driven by a 12.4-kb region of the *Villin* promoter.^{8–10} Control *Gata4*^{loxP/+}-*VilCre* and conditional knockout *Gata4*^{loxP/-}-*VilCre* offspring were obtained in the expected ratio demonstrating that expression of GATA4 in the fetal intestine is dispensable for the completion of embryogenesis. To confirm *Gata4* deletion in adult *Gata4*^{loxP/-}-*VilCre* intestine, we harvested jejunal tissue from control and experimental animals and examined GATA4 protein distribution by immunohistochemistry and mRNA levels by RT-PCR. In contrast to control animals, both GATA4 protein and mRNA were undetectable in *Gata4*^{loxP/-}-*VilCre* jejunum (Figures 1B,C and Figure 7 (supplemental information)). We also examined the extent to which loss of GATA4 in the intestinal epithelium affected the expression of *Gata5* and *Gata6*, two closely related GATA factors expressed in the intestinal epithelium, and found comparable amounts

of both *Gata5* and *Gata6* transcripts in control and *Gata4^{loxP/-}VilCre* jejunal samples (Figure 1C). Although this result demonstrated that GATA4 is not required for the expression of these family members in the jejunum, it raises the possibility that the continued expression of GATA5 and GATA6 compensates for some aspects of GATA4 function in *Gata4^{loxP/-}VilCre* intestines.

Villous morphology is altered in GATA4 null jejunum

Examination of GATA4 null jejunum using histochemical stains as well as immunohistochemical stains for markers of the lamina propria (anti-laminin), the muscularis (anti- α -smooth muscle actin), the enteric nervous system (anti-acetylated tubulin), and the vasculature (anti-PECAM-1) revealed that GATA4 mutant tissue was virtually indistinguishable from that of controls with the exception of villus length and width (Figure 2A). Alcian blue staining identified numerous goblet cells in both control and mutant jejunum (Figure 2A). The relative number of goblet cells per villus in mutant (n=9 mice, 90 villi) versus control (n=6 mice, 60 villi) jejunum (expressed as a percentage of the total number of epithelial cells per villus), however, was modestly lower with mutant villi consisting of $7.6 \pm 0.32\%$ and control villi consisting of $8.8 \pm 0.33\%$ ($p < 0.05$). Comparison of H&E stained jejunal tissue sections from control and conditional knockout mice revealed that villi of GATA4 null jejunum were shorter and wider than those of control jejunum (Figure 2A). We measured the length and width of villi from control (n=7 mice, 151 villi) and *Gata4^{loxP/-}VilCre* (n=12 mice, 250 villi) jejunum and determined that jejunal villi of the mutant mice were 19% shorter and 10% wider than that of control mice (Figure 2B). The overall length and weight of the small intestine, however, did not differ (length = control, 46.5 ± 1.16 cm, n=10; mutant, 47.8 ± 0.74 cm, n=14 ($p > 0.1$); weight = control, 1.62 ± 0.15 g n=3; mutant, 1.99 ± 0.13 g n=7 ($p > 0.1$)).

Fat and cholesterol metabolism are defective in mice lacking GATA4 in the intestine

We observed that *Gata4^{loxP/-}VilCre* animals were smaller than control littermates to the extent that it predicted genotype (Figure 3A). To quantify this visually apparent size difference, we monitored the weight of a cohort of *Gata4^{loxP/+}VilCre* and *Gata4^{loxP/-}VilCre* mice over a 10-week period spanning from 3 weeks (weanlings) to 12 weeks (adults) of age. We found that both male and female *Gata4^{loxP/-}VilCre* mice consistently weighed approximately 1–2 grams less. Although reproducible, this difference was only statistically significant ($p < 0.05$) at early time points (Figure 3B). We reasoned that the observed size difference could result from malfunction of the intestinal epithelium, a change in food intake, or a combination of both. We therefore compared food consumption between male control and *Gata4^{loxP/-}VilCre* animals and found that loss of GATA4 in the intestinal epithelium had no impact on the amount of food consumed (Figure 4A). The observation that *Gata4^{loxP/-}VilCre* mice fed normally suggested that the diminutive nature of such mice more likely resulted from a disruption to intestinal physiology.

Given that the predominant role of the small intestine is nutrient absorption, we measured the levels of glucose, cholesterol, and phospholipids in the plasma of control and *Gata4^{loxP/-}VilCre* mice to initially address whether loss of GATA4 compromised gut function. Glucose levels were unchanged in conditional knockout animals compared with controls (Figure 4B). However, the levels of both cholesterol (Figure 4C) and phospholipids (Figure 4D) were significantly reduced in the plasma of animals lacking GATA4 in the small intestine. Plasma harvested from *Gata4^{loxP/-}VilCre* mice (n=7) contained 32% less cholesterol and 24% less phospholipids compared with control mice (n=7). Although defects in cholesterol and fat metabolism by peripheral organs such as the liver could potentially account for such changes, we favored the hypothesis that misregulated uptake of dietary nutrients caused the phenotype because GATA4 was specifically deleted in the intestinal epithelial cells in mutant mice. Therefore, to determine the ability of intestinal epithelia lacking GATA4 to absorb dietary fat,

we used a non-invasive, non-radioactive approach¹² to measure fat uptake in control and *Gata4*^{loxP/+}-*VilCre* mice. We found that control mice absorbed 97% dietary fat, whereas mice lacking GATA4 in the intestinal epithelium absorbed only 77%, representing a 26% reduction in dietary fat absorption (Figure 4E). Using a fecal dual-isotope ratio method to compare cholesterol uptake between control and *Gata4*^{loxP/+}-*VilCre* mice, we found that mice lacking intestinal GATA4 expression absorbed only 4% cholesterol compared with 60% absorbed by control mice (Figure 4F). Values for control animals (n=6) ranged from 32%–79% cholesterol absorption while, quite strikingly, eight of the ten *Gata4* conditional knockout mice examined failed to absorb any measurable amount of cholesterol. Based on these data, we concluded that GATA4 has an integral role in controlling the ability of the intestine to absorb dietary cholesterol and fats.

Expression of lipid metabolism genes is altered in jejunum lacking GATA4

The fact that *Gata4* encodes a transcription factor suggested that the defects observed in intestinal physiology resulted from changes in the regulation of gene expression in the intestinal epithelium. Therefore, to uncover the molecular mechanism through which GATA4 controls intestinal absorption, we used Affymetrix oligonucleotide arrays to identify genes with altered expression in GATA4 null jejunum. We focused on the jejunum because fat and cholesterol absorption occurs predominantly in this region. To avoid complications associated with sex- or age-specific gene expression, we limited our analysis to jejuna harvested from control *Gata4*^{loxP/+}-*VilCre* and experimental *Gata4*^{loxP/-}-*VilCre* adult male mice. Using GeneChip Operating Software (GCOS) and dChip software,¹³ we identified 213 genes with expression down-regulated ≥ 2.0 fold ($p \leq 0.05$) and 224 genes with expression up-regulated ≥ 2.0 fold ($p \leq 0.05$) in *Gata4*^{loxP/-}-*VilCre* jejunum compared with control jejunum (Table 4, supplemental information). To gain insight into how these aberrantly expressed genes caused disruption of intestinal function, we used Ingenuity Pathways Analysis (IPA) software to categorize the genes by biological function and to generate interaction networks among them. Such analysis identified lipid metabolism as the molecular/cellular function most affected by loss of GATA4 in the intestinal epithelium followed by small molecule biochemistry and molecular transport. Figure 5 shows the highest scoring IPA-generated network of interacting genes, which accordingly had the functions of lipid metabolism, small molecule biochemistry, and molecular transport most strongly associated with it. Finding these functional categories as principally affected corresponded well with the physiological differences we observed between control and *Gata4* conditional knockout mice. In all, IPA annotated more than 75 of the differentially expressed genes as encoding proteins with roles in various aspects of lipid metabolism and transport (Table 1).

To validate the gene expression changes found through array analysis, we selected 20 transcripts listed in Table 1 and compared the steady-state mRNA between control and GATA4 null jejunum using RT-PCR. In all cases, the gene expression changes predicted by dChip agreed with those determined by RT-PCR (Figure 6A). Among the set of down-regulated genes assayed by RT-PCR were several encoding proteins functioning in cellular uptake, transport, and metabolic processing of lipids and cholesterol including *Cd36 antigen (Cd36)*, *Scavenger receptor class B, member 1 (Scarb1)*, *Solute carrier family 27 (fatty acid transporter), member 2 (Slc27a2)*, *Apolipoprotein c3 (Apoc3)*, *Fatty acid binding protein 1 (Fabp1)*, *Cytochrome P450, family 2, subfamily b, polypeptide 10 (Cyp2b10)*, and *Cytochrome P450, family 27, subfamily a, polypeptide 1 (Cyp27a1)*. The transcription factor *Nuclear receptor subfamily 1, group I, member 3 (Nr1i3)*, also known as *Car*, shown to play multiple roles in lipid metabolism, was also confirmed as down-regulated in jejunum from conditional knockout animals (Figure 6A). In addition to finding decreased expression of these key components of lipid and cholesterol metabolism, we found several genes critical for intestinal bile acid absorption, a process occurring predominantly in the ileum, to be increased in GATA4 null

jejunum compared with control tissue, and we assayed these by RT-PCR. Expression of *Solute carrier family 10, member 2 (Slc10a2)*, which encodes the apical sodium-dependent bile acid transporter (ASBT), was dramatically increased in GATA4 null jejunum compared with control jejunum (Figure 6A). Moreover, *Nuclear receptor subfamily 1, group h, member 4 (Nr1h4)* as known as *Fxr*, a central transcriptional regulator of bile acid homeostasis, as well as several FXR target genes including *Fibroblast growth factor 15 (Fgf15)*, *Fatty acid binding protein 6 (Fabp6)*, *Organic solute transporter alpha (Osta)*, *Organic solute transporter beta (Ostb)*, and *Bile acid-Coenzyme A: amino acid N-acyltransferase (Baat)* were more abundant in GATA4 null jejunum compared with control tissue (Figure 6A). Two additional transcription factors cooperating with FXR in regulating bile acid metabolism, *Nuclear receptor subfamily 0, group b, member 2 (Nr0b2)* also known as *Shp* and *Nuclear receptor subfamily 5, group a, member 2 (Nr5a2)* also known as *Lrh-1*, were also found to have increased expression in conditional knockout jejunum compared with controls (Figure 6A).

Enterohepatic signaling is disrupted in mice lacking GATA4 in the intestine

Induction of *Fgf15* expression in GATA4 null jejunum suggested that enterohepatic signaling was altered in *Gata4* conditional knockout animals. In response to bile acid uptake, ileal enterocytes synthesize and secrete the growth factor FGF15. Binding of FGF15 to its receptor on the hepatocyte cell surface activates a signaling cascade resulting in decreased expression of *Cytochrome P450, family 7, subfamily a, polypeptide 1 (Cyp7a1)*, the rate-limiting enzyme in the conversion of cholesterol to bile acids.¹⁴ To determine if increased *Fgf15* expression by GATA4 null jejunal enterocytes corresponded with a change in enterohepatic physiology, we isolated RNA from livers of adult male control and *Gata4* intestine-specific knockouts and performed RT-PCR to measure the steady-state level of *Cyp7a1* mRNA. As predicted, livers from mice lacking GATA4 in the intestine expressed less *Cyp7a1* than livers from control mice (Figure 6B).

Loss of GATA4 in the intestine results in compromised jejunal identity and expansion of the ileal domain

The finding that jejunal functions were lost and ileal functions were gained in GATA4 null jejunum suggested a crucial role for GATA4 in establishing and maintaining the functional identity of the jejunum. This agrees with Bosse et al.² who first suggested such a role for GATA4 in the intestine based on changes in expression of a limited set of jejunal- and ileal-specific genes. To address the hypothesis that GATA4 is required to establish and to maintain the functional identity of the jejunum on a genome-wide scale, we used Affymetrix arrays to compare the gene expression profiles of control *Gata4^{loxP/+}VilCre* jejunum, control *Gata4^{loxP/+}VilCre* ileum, and conditional knockout *Gata4^{loxP/-}VilCre* jejunum. Arrays were performed in triplicate using samples collected from independent animals. To be considered as expressed in a given tissue, the probe set had to have an expression call of present in each array hybridized for that tissue and a unique Entrez gene identifier. Comparison of the probe sets expressed in control jejunum with that of control ileum revealed that jejunal and ileal gene expression profiles were quite similar with 89% of expressed genes common to both (Figure 6C). We identified 746 probe sets as expressed solely in jejunum and 568 as unique to ileum. Although a small percentage of the total gene expression profile, these sets are important because such variations likely provide for functional differences between these regions of the gut. Comparison of the GATA4 null jejunal expression profile with the control jejunal and ileal profiles revealed that GATA4 null jejunum expressed only 53% of jejunal-specific gene set and gained expression of 265 genes normally expressed only in the ileum, representing 47% of the ileum-specific set (Figure 6C).

Discussion

Taken together, the data presented here demonstrate that GATA4 has a crucial role in controlling jejunal physiology. The first indicator of disrupted intestinal function in GATA4 mutant mice was the finding that such mice were consistently smaller than control littermates. Considering that lipid absorption was impaired in mice lacking GATA4 in the intestinal epithelium, the fact that the weight difference did not reach statistical significance at later time points may reflect the change in diet at weaning from a high-fat diet (milk) to a low-fat diet (standard rodent chow). Indeed, we compared control and mutant pups at 1 week and 2 weeks of age and found that mutant pups weighed less at both timepoints (1wk control 5.35 ± 0.22 g, n=16; mutant 4.11 ± 0.24 g, n=6, ($p < 0.01$); 2wk control 8.25 ± 0.21 g, n=6; mutant 6.66 ± 0.19 g, n=5, ($p < 0.01$)). In further support of this difference resulting from a metabolic cause, we showed that mutant animals consumed the same amount as control animals ruling out the possibility that *Gata4* conditional knockout animals were smaller simply because they consumed less food.

The reductions in circulating cholesterol and phospholipids and in dietary fat and cholesterol uptake in *Gata4* conditional knockout animals compared with controls indicated that both lipid and cholesterol metabolism were severely disrupted in GATA4 mutant mice. Although we observed a small but statistically significant ($p < 0.05$) decrease in goblet cells, we do not believe that this would contribute to a defect in absorption since nutrient uptake is accomplished exclusively by enterocytes. In contrast, our observation of altered villous morphology in GATA4 null jejunum raises the possibility that reduced villous length resulted in decreased lipid and cholesterol absorption. However, *protein tyrosine kinase 6* (*Ptk6*) null mice, which have jejunal villi that are twice the length of control villi, failed to absorb any more fat than wild-type animals.¹⁵ Expression of *Ptk6* was increased 3.6 fold in GATA4 mutant jejunum compared with control jejunum providing a potential explanation for the shorter jejunal villi observed in the absence of GATA4 (Table 4, supplemental information). Increased jejunal expression of *Ptk6*, which is more abundant in the ileum compared with the jejunum,¹⁵ further supports our conclusion that the identity of GATA4 null jejunum shifted toward that of the ileum.

The exact mechanism through which loss of GATA4 so dramatically affects lipid and cholesterol uptake likely involves both direct and indirect mechanisms. Based on our gene array data, however, we believe it is reasonable to propose that GATA4 plays an essential role in the regulation of a plethora of genes whose combined action is required for normal jejunal cholesterol and fat absorption. Decreased expression of genes encoding fat and cholesterol receptors and transporters such as *Slc27a2*, *Cd36*, and *Scarb1* provide one molecular mechanism to explain the observed phenotype. The *Slc27a2* gene encodes a fatty acid transporter that caused increased fat uptake when over-expressed in cell lines.¹⁶ Although defining the role that the receptors CD36 and SCARB1 play in intestinal lipid and cholesterol uptake has been somewhat controversial because *Cd36* and *Scarb1* knockout mice failed to show a conclusive defect in intestinal fatty acid and cholesterol uptake,^{17, 18} new studies support the conclusion that both receptors directly participate in these processes. Specifically, transgenic mice over-expressing *Scarb1* showed accelerated lipid and cholesterol absorption by the intestine,¹⁹ and purified brush border membrane vesicles incubated with an antibody against SCARB1 failed to bind cholesterol with high affinity.²⁰ Moreover, both Nassir *et al.*²¹ and Drover *et al.*²² recently demonstrated that CD36 serves as a receptor for dietary fat and cholesterol absorption in the proximal intestine.

Bile acid homeostasis also plays a key role in lipid and cholesterol metabolism.²³ Bile acids function in the jejunum to emulsify lipids and cholesterol thereby enabling absorption of these nutrients. Most intestinal bile acid absorption occurs in the ileum, a site normally lacking

GATA4. We found that loss of GATA4 in the jejunum, however, creates a molecular environment in which genes encoding multiple critical components of the bile acid absorption pathway are improperly induced. We detected high-level expression of *Slc10a2*, which encodes the primary ileal bile acid transporter (ASBT), in GATA4 null jejunum suggesting that bile acids were prematurely absorbed in the jejunum of mutant mice. Uptake of bile acids results in activation of an FXR-driven pathway that culminates in repression of bile acid biosynthesis by the liver.^{24, 25} Although *Nr1h4* (*Fxr*) expression was up-regulated only 2 fold in GATA4 null jejunum compared with controls, its activity was strongly induced as was demonstrated by increased expression of FXR target genes including *Fabp6*, *Fgf15*, *Osta*, and *Ostb*. In fact, *Fabp6* and *Fgf15* were undetectable in control jejunum. Moreover, down-regulation of *Cyp7a1* expression in livers of GATA4 intestinal-specific knockout mice compared with controls provides further evidence that loss of GATA4 in the jejunum disrupted enterohepatic signaling.

In summary, elimination of GATA4 from the intestinal epithelium severely disrupted jejunal physiology. We propose that both decreases in expression of key fat and cholesterol transporters and the activation of bile acid absorption in jejunal enterocytes lacking GATA4 cause the dramatic decrease in lipid and cholesterol absorption observed. In future experiments, it will be important to determine the extent to which changes in receptor and transporter gene expression and changes in bile acid homeostasis each contribute to the observed defect in lipid metabolism. Our data agree with and expand upon the work of Bosse *et al.*² who suggested that GATA4 is required to maintain jejunal-ileal identities based on changes in expression of a limited set of jejunal and ileal specific genes. The data we present definitively demonstrate that the absence of GATA4 in the jejunum results in a loss of jejunal function and a concomitant gain in ileal function. In addition to physiological changes indicative of a loss of the jejunal domain and an extension of the ileal domain, comparison of the global gene expression profiles among control jejunum, control ileum, and GATA4 null jejunum demonstrated a wide-scale shift in gene expression in mutant mice from that specifically associated with jejunum to that of ileum. Future studies in which GATA4 is ectopically expressed in the ileum should provide insight into the extent to which GATA4 plays a dominant role in conferring jejunal character upon the intestine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

GATA4 is efficiently eliminated from the intestinal epithelium. (A) Nuclear GATA4 protein (brown) was detected by IHC in adult mouse duodenum and jejunum but not in ileum. Scale bar = 50 μ m. (B) IHC showed elimination of GATA4 protein in jejunum from adult *Gata4* conditional knockout (cKO) mice. Boxed regions are shown at higher magnification below original images. Arrows indicate positive nuclear staining (brown) in control tissue and negative nuclear staining (blue) in cKO tissue. Scale bar = 50 μ m (C) RT-PCR showed the absence *Gata4* mRNA in jejunum of *Gata4* cKO mice. *Gata5* and *Gata6* mRNA levels were unchanged in cKO jejunum compared with controls. *Polr2a* was used to normalize cDNA concentrations between samples.

Figure 2.

Villous morphology is abnormal in GATA4 null jejunum. (A) H&E stained jejunum harvested from control and *Gata4* conditional knockout (cKO) adult mice showed that GATA4 null villi are shorter and wider than those of controls. Alcian blue staining (deep blue) identified comparable numbers of goblet cells in the jejunum of control and mutant mice. IHC staining (brown) for acetylated tubulin (AT), α -smooth muscle actin (SMA), PECAM-1, and laminin (LAM) revealed no differences between control and GATA4 null jejunum. Scale bar = 50 μ m. (B) The length and width (μ m) of villi from control (n=7 mice, 151 villi) and *Gata4* cKO mice (n= 12 mice, 250 villi) were determined using H&E stained micrographs of jejunum and NIH ImageJ software. Mean villus length and width were compared using a two-sample Student *t* test. Error bars represent standard error of the mean (SEM). * $p \leq 0.01$; ** $p \leq 0.0001$

Figure 3.

Mice lacking GATA4 in the intestinal epithelium are smaller than control mice. (A) Image of two 7 week old male littermates showing that the *Gata4*^{loxP/-} *VilCre* mouse (right) is smaller than the control mouse (left). (B) The size difference between control and mutant mice was quantified by weighing mice over a 10 week period spanning from weaning (3 weeks) to 12 weeks of age. Black lines (diamonds) in each graph show the mean weight (g) of control mice (n= 6 males, 7 females). Gray lines (squares) show the mean weight of *Gata4*^{loxP/-} *VilCre* mice (n=6 males, 12 females). Error bars represent SEM. A two-sample Student *t* test was used to determine p-values. *p<0.05; **p<0.01

Figure 4.

Lipid and cholesterol metabolism are disrupted in mice lacking GATA4 in the intestinal epithelium. (A) Food consumption was measured over a 24-hour period for control (n=6) and *Gata4* conditional knockout (cKO, n=10) adult male mice using the DietMax system. White bars, black bars, and gray bars show mean consumption during the light cycle, during the dark cycle, and in total, respectively. (B–D) Plasma isolated from control (n=7) and *Gata4^{loxP/-}VilCre* (n=7) adult male mice was assayed for glucose (mg/dL), cholesterol (mg/dL), and phospholipids (mg/dL). The average concentration of each is represented by black bars for controls and by gray bars for mutants. The mean level of plasma glucose (B) was not changed between control and GATA4 mutant mice. The mean levels of both plasma cholesterol (C) and plasma phospholipids (D) were significantly lower in *Gata4^{loxP/-}VilCre* mice compared with controls. (E) Dietary fat absorption was measured in control (n=7) and *Gata4^{loxP/-}VilCre* (n=8) male mice using a non-invasive, non-radioactive method. Mutant mice (gray bar) absorbed less dietary fat compared with controls (black bar). (F) Cholesterol absorption was measured in control (n=6) and *Gata4^{loxP/-}VilCre* (n=10) male mice using a fecal dual-isotope ratio method. Mutant mice (gray bar) absorbed less cholesterol compared

with controls (black bar). Error bars show SEM. A two-sample Student *t* test was used to determine p-values (*p=0.013; **p<0.01; ***p<0.0001).

Figure 5.

Ingenuity Pathway Analysis (IPA) of microarray data revealed lipid metabolism, small molecule biochemistry, and molecular transport as the functions most affected by loss of GATA4 in the jejunum. Analysis of genes differentially expressed ($\pm \geq 2.0$, $p \leq 0.05$) in GATA4 null intestines compared with controls by IPA identified the network shown, which involved the most genes from our array data, as functioning in lipid metabolism, small molecule biochemistry, and molecular transport. Because IPA limits each network to 35 nodes, we selected the 30 nodes representing up- and down-regulated genes and expanded the network to be maximally inclusive of interactions between differentially expressed genes. This uncovered eight additional interacting, differentially expressed genes. Blue circles/ovals represent down-regulated genes; yellow circles/ovals represent up-regulated genes. Solid lines/arrows represent direct interactions; dashed lines/arrows represent indirect interactions.

Figure 6.

Loss of GATA4 in the jejunum resulted in a wide-scale shift in the jejunal gene expression profile from that characteristic of jejunum toward that of ileum. (A) Expression of genes encoding proteins involved in lipid metabolism was altered in GATA4 null jejunum. RT-PCR analysis of jejunum from three independent *Gata4^{loxP/+}VilCre* and three independent *Gata4^{loxP/-}VilCre* adult male mice confirmed that the mRNA levels of 20 genes classified as lipid metabolism genes by IPA were changed in mutant jejunum compared with control jejunum. Analysis of the level of *Gata4* mRNA verified that mutant mice expressed no detectable *Gata4*. *Polr2a* was used to normalize cDNA concentrations between samples. An asterisk (*) denotes those genes that exceeded our dChip p-value threshold of $p \leq 0.05$ (*Apoc3*, $p = 0.05681$; *Fabp1*, $p = 0.05287$; *Scarb1*, $p = 0.05497$). (B) Enterohepatic signaling was disrupted in mice lacking GATA4 in the intestine. RT-PCR analysis of livers from three independent control *Gata4^{loxP/+}VilCre* and three independent mutant *Gata4^{loxP/-}VilCre* adult male mice confirmed that the level of *Cyp7a1* mRNA was decreased in *Gata4* intestine-specific conditional knockout mice compared with control mice. *Polr2a* was used to normalize cDNA concentrations between samples. (C) We determined the gene sets expressed by control jejunum, control ileum, and conditional knockout (cKO) jejunum by microarray analysis. Comparison of control jejunum with control ileum revealed that although the majority of expressed genes were common to both tissues, there was a subset of jejunal-specific genes and a subset of ileal-specific genes. Comparison of the cKO expression profile revealed a large decrease in expression of jejunal genes and the induction of expression of ileal genes. J, jejunum; I, ileum.

Table 1

Genes annotated by IPA as involved in lipid metabolism and transport with either increased or decreased expression in GATA4 null jejunum compared to control jejunum.

INCREASED EXPRESSION					DECREASED EXPRESSION				
GENE	FOLD	GENE	FOLD	GENE	FOLD	GENE	FOLD	GENE	FOLD
<i>Fabp6</i>	1217.7	<i>P2ry2</i>	2.8	<i>Slco2a1</i>	2.1	<i>Cyp2b10</i>	-84.4	<i>Sdh</i>	-2.8
<i>Slc10a2</i>	195.4	<i>Tlr2</i>	2.8	<i>B4gal4</i>	2.0	<i>Cd36</i>	-14.3	<i>Itch</i>	-2.5
<i>Pla2g4c</i>	33.1	<i>Cd9</i>	2.6	<i>Bid</i>	2.0	<i>BC089597</i>	-6.9	<i>Ppap2a</i>	-2.5
<i>Fgl15</i>	32.7	<i>Fkbp1a</i>	2.6	<i>Ldlr</i>	2.0	<i>Adh4</i>	-4.8	<i>Sgpl1</i>	-2.4
<i>Saa1</i>	16.3	<i>Osta</i>	2.6	<i>Nr5a2</i>	2.0	<i>Nfe2l2</i>	-4.0	<i>Apoc3*</i>	-2.3
<i>Hao3</i>	15.6	<i>Syt12</i>	2.6	<i>Sgms1</i>	2.0	<i>Rdh7</i>	-3.8	<i>Ltb4dh</i>	-2.3
<i>Nr0b2</i>	7.4	<i>Foxa1</i>	2.5			<i>Ang</i>	-3.6	<i>Ugt2b5</i>	-2.3
<i>Scd2</i>	6.5	<i>Sdc1</i>	2.5			<i>Slc27a2</i>	-3.5	<i>Maob</i>	-2.2
<i>Lbp</i>	6.1	<i>Slc25a4</i>	2.5			<i>Cyp27a1</i>	-3.4	<i>Dgat2</i>	-2.1
<i>Pyy</i>	5.9	<i>Cd38</i>	2.3			<i>Gip</i>	-3.3	<i>Dlk1</i>	-2.1
<i>Thrsp</i>	5.6	<i>Plcd1</i>	2.3			<i>Esr1g</i>	-3.2	<i>Ephx2</i>	-2.1
<i>Angptl4</i>	3.9	<i>Hla-dmb</i>	2.3			<i>Fabp1*</i>	-3.2	<i>Faah</i>	-2.1
<i>Ostb</i>	3.9	<i>Sqle</i>	2.3			<i>Asah2</i>	-3.1	<i>Casp3</i>	-2.0
<i>Mfhas1</i>	3.5	<i>Baat</i>	2.2			<i>Afp</i>	-3.0	<i>Degs1</i>	-2.0
<i>Nr1h4</i>	3.5	<i>Cln8</i>	2.2			<i>Cyp2c44</i>	-3.0	<i>Rdh5</i>	-2.0
<i>Acm3</i>	3.1	<i>Acot4</i>	2.1			<i>Nr1i3</i>	-3.0	<i>Scarb1*</i>	-2.1
<i>Gsn</i>	3.0	<i>Fads2</i>	2.1			<i>Papss2</i>	-2.9	<i>Slc3a2</i>	-2.0
<i>Itgav</i>	2.9					<i>Dio1</i>	-2.8	<i>Slc37a4</i>	-2.0
<i>Hsd11b2</i>	2.8					<i>Ppargc1a</i>	-2.8		

* P-value exceeds threshold of $p \leq 0.05$ (*Apoc3*, $p = 0.05681$; *Fabp1*, $p = 0.05287$; *Scarb1*, $p = 0.05497$)